AN INVESTIGATION OF THE CARDIOVASCULAR ACTIONS OF ANGIOTENSIN IN MAN

A THESIS

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by

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PREFACE

Angiotensin is a polypeptide present in measurable amounts in the blood of man and animals.

Its role in the control of aldosterone secretion is well-recognized. However less is known with regard to the mechanism of its powerful vasoconstrictor action.

This thesis describes a series of investigations designed to further elucidate this mechanism in man.

CONTENTS

DECLARATION AND ACKNOWLEDGEMENTS

I declare that this thesis is of my own composition and that it is a record of original work conducted in the Department of Human Physiology and Pharmacology at the University of Adelaide during the years 1964, 1965, 1966 and 1967.

The experimental work described herein has not been submitted for any other degree, award or diploma.

I would like to record my gratitude to Professor R. F. Whelan for his guidance and encouragement throughout the duration of my studies in his department.

I would like to thank my colleagues Dr. C. J. Cooper, Dr. J. D. Fewings, Dr. R. L. Hodge, Dr. S. L. Skinner and Dr. J. A. Walsh with whom I have had the privilege and pleasure of working during various stages of these investigations and Mrs. C. Contos, Miss F. Katic and Mrs. B. Starr for providing excellent technical assistance. I am particularly grateful to those colleagues, students and patients who willingly acted as subjects for these investigations.

I am also indebted to the various drug companies for

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Finally, I am grateful to the University of Adelaide for employing me in the capacity of Temporary Lecturer in 1964 and then as a Lecturer in 1965, 1966 and 1967.

INTRODUCTION

Richard Bright (1836) was one of the first to suggest that a humoral factor may be released from the diseased kidney and involved in the pathogenesis of hypertension. In his analysis of a group of patients with renal disease and albuminous urine he observed ... "that the hypertrophy of the heart seems, in some degree, to have kept pace with the advance of the disease in the kidneys: for in by far the majority of cases, where the muscular power of the heart was increased, the hardness and contraction of the kidney bespoke the probability of a long continuance of the disease." Furthermore, he noted that ... "out of fifty-two cases of hypertrophy, no valvular disease whatsoever could be detected in thirty-four", and that therefore ... "This naturally leads us to look for some less local cause, for the unusual efforts to which the heart has been impelled: and the two most ready solutions appear to be, either that the altered quality of the blood (as a result of renal disease) affords irregular and unwonted stimulus to the organ immediately; or, that it so affects the minute and capillary circulation, as to render greater action necessary to force the blood through the distant subdivisions of the vascular system."

From a remarkable series of experiments in man and animals,

extending over a period of many years, Brown-Sequard and his co-workers had concluded that a number of organs in the body produced an internal secretion which was vital for life (Brown-Sequard, 1889,1893 ; Brown-Sequard and d'Arsonval,1892). He extended these observations to the kidney (...."le rein possede une secretion interne d'une tres grande importance"....) and suggested that the internal and external secretions were independent of each other (Brown-Sequard,1893).

However, Oliver, in 1897, observing the effects of renal extracts on the blood vessels of the frog mesentery, was unable to demonstrate the hypothetical vasoconstrictor substance suggested by Bright.

Then, in 1898, Tigerstedt and Bergman performed a remarkably detailed series of experiments, from which they were able to conclude that an extract from the cortex of rabbit kidneys possessed marked pressor activity. They named this pressor substance renin and were able to study its properties and explore its mechanism of action. They characterized its chemistry and concluded, among other things, that it was present only in the cortex of the kidney and "is delivered to the blood flowing through the kidney". They further demonstrated that the pressor effect was long-lasting, that nephrectomized rabbits were

hypersensitive, and that tachyphylaxis occurred. They observed that the pressor action of renin was unmodified by such procedures as cardiac denervation, cervical cord transection or even destruction of the entire spinal cord. They concluded (for rather confusing reasons) that the most likely site of action was on the 'peripheral centres of vascular nerves' (referring probably to the sympathetic ganglia) and not on the vascular smooth muscle directly. They tentatively suggested that renin may be important in maintaining normal vascular tone and that either increased production or decreased destruction of renin may explain the association of heart hypertrophy and certain diseases of the kidney, by virtue of an increase in vascular resistance.

The collective observations of Bright, Brown-Sequard and Tigerstedt and Bergman had laid the foundation of a hypothesis to explain the association of renal disease and hypertension but attempts to develop a reproducible experimental model in animals were largely unsuccessful (references cited by Goldblatt, 1937 b). Although a number of workers were able to find pressor activity in renal extracts (Shaw, 1906; Bingel and Strauss, 1909; Pearce, 1909) and even increased pressor activity in extracts from 'ischaemic' kidneys (Shaw, 1906), this was also true for a variety of other organs (Vincent and Sheen, 1903; Pearce, 1909; Collip, 1928) and furthermore, many of these extracts had quite prominent depressor

activity (Pearce, 1909; Miller and Miller, 1911) suggesting that the effects were due to the products of autolysis or bacterial action rather than to any specific substances (Hessel and Hartwich, 1932).

Then, from the results of experiments begun in 1928, Goldblatt, Lynch, Hanzal and Summerville (1934) reported that sustained hypertension could be produced in dogs by partially obstructing the renal artery of one or both kidneys. This was subsequently confirmed in a variety of animals (Goldblatt, 1937 a; Wilson and Pickering, 1938; Wilson and Byrom, 1939; Goldblatt, Kahn and Lewis, 1943). With a more reliable experimental model now available, renewed interest was shown in the aetiology of hypertension and particularly the role of renin in this situation, and the next ten years saw an increasing amount of activity in this particular field.

A neurogenic mechanism in the aetiology of renal hypertension seemed unlikely since the response to constriction of the renal artery was not altered by either sympathectomy (Goldblatt, Gross and Hanzal, 1936, 1937; Blalock and Levy, 1937; Heymans, Bouckaert, Elaut, Bayless and Samaan, 1937; Freeman and Page, 1937; Alpert, Alving and Grimson, 1937; Glenn, Child and Page, 1938; Verney and Vogt, 1938) or by renal denervation (Page, 1935; Collins,

1936; Glenn, Child and Heuer, 1937; Blalock and Levy, 1937; Verney and Vogt, 1938).

A humoral mechanism had been sought repeatedly since the initial observations of Bright, Brown-Sequard and Tigerstedt, but pressor material could not be demonstrated with any consistency in the plasma of hypertensive man or experimental animals (Page, 1936; Friedman and Prinzmetal, 1936; Prinzmetal and Friedman, 1936; Prinzmetal, Friedman and Rosenthal, 1936; Katz, Friedman, Rodbard and Weinstein, 1939). However, this seemed the most likely explanation since removal of the affected kidney or relief of the arterial compression restored the blood pressure to normal (Goldblatt, Lynch, Hanzal and Summerville, 1934; Dicker, 1937 a; Houssay and Fasciolo, 1937 a), and there was some evidence that extracts of the affected kidneys from animals with renovascular hypertension had increased pressor activity (Shaw, 1906; Harrison, Blalock and Mason, 1936; Harrison, Blalock, Mason and Williams, 1937).

More definite evidence of a humoral mechanism came from the experiments of Houssay and Fasciolo (1937 b) and Dicker (1937 b), both of whom observed a pressor effect when the affected kidneys from dogs with Goldblatt hypertension were grafted into the necks of normal and nephrectomized dogs. Similarly, Glenn, Child and Heuer (1937) were able to produce hypertension in dogs by constricting

the artery of a single kidney transplanted to the groin. These results were supported by those of Fasciolo, Houssay and Taquini (1938), and others, demonstrating increased vasoconstrictor activity in the renal venous blood of 'ischaemic' kidneys.

Renin was suggested as the most probable humoral agent in the pathogenesis of experimental renal hypertension, whether due to renal artery constriction or cellophane perinephritis (Page, 1940; Page and Helmer, 1940), and further it was suggested that the juxtaglomerular cells, described by Ruyter (1925), may be the site of its formation in the kidney (Goormaghtigh, 1939; Dunlhue and Candon, 1940). This concept was supported by the subsequent observation of Dunlhue (1941) that rabbits with experimental hypertension showed an initial increase in juxtaglomerular cell granularity, and finally, in 1956, Peart, Gordon, Cook and Pickering confirmed that renin in the rabbit kidney was virtually confined to those areas containing glomeruli.

Then the observations that renin itself had no vasoconstrictor activity (Friedman, Abramson and Marx, 1938) and that blood colloids were required for its activation (Kohlstaedt, Helmer and Page, 1938) led two independent groups of investigators (Page and Helmer, 1939, 1940; Braun-Menendez, Fasciolo, Leloir and Munoz, 1939, 1940 a; Munoz, Braun-Menendez, Fasciolo, and Leloir, 1939) to the realization that although renin was the probable humoral agent

released from the kidney, it functioned as an enzyme to form the active vasoconstrictor principle, angiotonin (Page and co-workers) or hypertensin (Braun-Menendez and co-workers), from the pseudo-globulin fraction of the plasma proteins. After much discussion (Page, Helmer, Plenti, Kohlstaedt and Corcoran, 1943; Braun-Menendez, Fasciolo, Houssay, Leloir, Munoz and Taquini, 1943), the unified name of angiotensin was eventually chosen for this active principle in 1958 (Braun-Menendez and Page).

Skeggs, Marsh, Kahn and Shumway (1954 a) suggested that angiotensin was probably a polypeptide and that it existed in two forms, I and II, and they subsequently purified them both (Skeggs, Marsh, Kahn and Shumway, 1954 b; Skeggs, Kahn and Shumway, 1956 a) although a relatively impure preparation had been produced by Edman in 1946.

With purer preparations of angiotensin available, the amino-acid composition and sequence could be investigated. This was attempted by Bumpus and Page (1954), who found fourteen amino-acids in their preparation, and eventually accomplished by Lentz, Skeggs, Woods, Kahn and Shumway (1956), who determined both the amino-acid composition of angiotensin II and its relationship to angiotensin I, and the amino-acid sequence of angiotensin I and II (Skeggs, Marsh, Kahn and Shumway, 1955; Skeggs, Lentz, Kahn, Shumway and Woods, 1956). Other authors at the same time had determined the amino-acid

sequence and composition of the decapeptide (Peart, 1955; Peart, 1956 a, b; Elliott and Peart, 1956), but the existence of two forms of angiotensin (Skeggs, Marsh, Kahn and Shumway, 1954 a; Helmer, 1957), and the need for converting enzyme (Skeggs, Kahn and Shumway, 1956 b) apparently were not recognized.

The determination of the amino-acid composition and sequence of angiotensin II enabled a synthetic form of the hormone to be prepared and this was accomplished simultaneously by Bumpus, Schwarz and Page (1957) and Rittel, Iselin, Kappeler, Rinniker and Schwyzer (1957). The availability of a pure preparation of synthetic angiotensin, which was biologically identical to the natural preparation (Bumpus, Schwarz and Page, 1957) enabled the pharmacology to be determined more precisely than was possible prior to this time.

The pharmacological actions of angiotensin had, in effect, been documented in regard to the earlier investigations into the mechanism of action of renin (Tigerstedt and Bergman,1898 ; Bingel and Strauss,1909; Hessel,1938; Merrill, Williams, J.R. and Harrison, 1938; Merrill, Williams, R.H. and Harrison, 1938; Friedman, Abramson and Marx, 1938; Katz and Friedberg,1938; Helmer and Page, 1939; Page, 1939; Houssay and Dexter, 1942) and in general, there was agreement that the pressor action of renin was not significantly modified by such procedures as sympathectomy, destruction of the spinal cord,

adrenalectomy, hypophysectomy or treatment with ergotamine, piperoxane or cocaine. It is interesting that a number of workers had been able to modify the response to renal artery constriction by adrenalectomy and hypophysectomy (Goldblatt, 1937 a; Page and Sweet, 1937) even though the humoral agent responsible for renovascular hypertension was thought to be renin (Kohlstaedt and Page, 1940; Braun-Menendez, Fasciolo, Leloir and Munoz, 1940 a; Leo, Prinzmetal and Lewis, 1940; Page, 1940). It is now apparent that the acute response to renal artery constriction is renin dependent, as suggested by the work of Collins and Hamilton (1940), Dunlhue (1941) and Pickering (1945), and that in the chronic situation a secondary mechanism is involved (Pickering, 1945; Floyer, 1954; Govaerts, 1954; Grollman, 1954), possibly through the sympathetic nervous system (Dunlhue, 1941; Reed, Saplrstein, Southard and Ogden, 1944; Taquini, Blaquier and Bohr, 1961; Dickinson and Lawrence, 1963; McCubbin and Page, 1963 a, b; Yu and Dickinson, 1965).

Investigations into the mechanism of action of angiotensin itself began with the discovery of its role as the active vasoconstrictor principle in the pressor action of renin (Page and Helmer, 1940; Braun-Menendez, Fasciolo, Leloir and Munoz, 1940 a). The findings were essentially the same as for renin, in that the pressor response was unaffected by such procedures as adrenalectomy, pithing, ergotamine, cocaine and atropine (Houssay and Dexter, 1942). Tachy-

phylaxis occurred but was less easily demonstrated than with renin and the pressor response to acute injections was of shorter duration than with renin although similar curves could be obtained by continuous infusion. With regard to the peripheral circulation, Abell and Page (1942, 1946) demonstrated that intravenous angiotensin produced a marked constriction of the arterioles of the rabbit ear and mesentery whereas the response was less marked in the venules and absent in the capillaries. Hill and Andrus (1940) and Lorber (1942) found that angiotensin reduced coronary blood flow and increased the force of contraction without any significant effect on heart rate in the isolated perfused heart of the cat.

Some of the earliest investigations into the cardiovascular actions of angiotensin in man were performed by Battro, Braun-Menendez, Lanari and Leloir (1940) and Battro, Gonzalez-Segura and Lanari (1941) who demonstrated a pressor action with a rise in both systolic and diastolic blood pressure and a bradycardia mediated by the vagus. Corcoran, Kohlstaedt and Page (1941) demonstrated a rise in blood pressure and a fall in renal blood flow, the latter resulting from an action on the efferent arteriole. A more detailed study was performed by Wilkins and Duncan (1941) who found an increase in mean arterial pressure and pulse pressure with an overall fall in limb blood flow and a fall in skin temperature following the intravenous administration of angiotensin. There was a rise in central

venous pressure and a slight fall in cardiac output. The bradycardia that appeared during the pressor response was not only abolished by atropine but was often reversed to a slight tachycardia. Similar results, with regard to the changes in cardiac output and blood pressure during intravenous injections or infusions of angiotensin, were obtained by other workers (Bradley and Parker, 1941; Taylor and Page, 1943; Middleton and Wiggers, 1944). There was general agreement that the intravenous administration of angiotensin resulted in an elevation in mean arterial pressure, an effect that was not modified by spinal anaesthesia in man or in animals (Gregory, Levine and Lindley, 1944) or dibenamine pre-treatment in dogs (Youmans and Rankin, 1947) and was therefore thought to be the result of an overall increase in peripheral vascular resistance mediated by a direct vasoconstrictor action on specific, non-adrenergic receptors. The stroke volume was slightly reduced and the degree to which the cardiac output fell was determined by the degree of bradycardia, which in itself was an inconstant finding (Tigerstedt and Bergman, 1898).

The orientation of most studies at this time, and indeed afterwards, was towards a correlation of the cardiovascular actions of angiotensin with the observed haemodynamic changes in essential and renovascular hypertension. It was not until synthetic angiotensin became readily available in 1957 that the more detailed investiga-

tions of its cardiovascular effects and mechanism of action were begun. In the meantime, most of the activity in the renin-angiotensin field was concentrated on such aspects as the assay and purification of the reactants in the system, determination of their structure, and the role and mechanism of action of renin in renovascular hypertension.

A concurrent development at this time was a growing interest in the role of the adrenal cortex in the pathogenesis of renovascular hypertension. In their initial report on experimental hypertension Goldblatt, Lynch, Hanzal and Summerville (1934) had suggested that such a relationship might exist. In 1936, Page and Sweet noted that in dogs, hypophysectomy almost completely abolished the response to renal artery constriction, and that in the established case of Goldblatt hypertension it resulted in a return of blood pressure to near normal levels. They concluded that this effect was probably an indirect one due to the absence of the normal secretions of the adrenal cortex or possibly the thyroid (Page and Sweet, 1937).

It was subsequently confirmed that removal of the adrenal cortex prevented the rise in pressure following renal artery constriction and abolished the hypertension in the established case (Goldblatt, 1937 a, b; Blalock and Levy, 1937; Page, 1938) whereas

removal of the adrenal medulla alone did not alter the response in either case (Goldblatt, 1937a,b).

A relationship between the juxtaglomerular cells, which were thought to contain renin (Goormaghtigh, 1939; Dunlhue and Candon, 1940; Dunlhue, 1941) and the adrenal cortex was suggested by Dunlhue in 1946. He observed hypertrophy and an increase in granularity of these cells in rabbits, dogs and monkeys following bilateral adrenalectomy. Subsequently Hartroft and Hartroft (1953, 1955) described a secretory cycle in these cells, with degranulation during salt loading and increased granularity during salt deprivation.

More conclusive evidence in support of a role of renin in adrenocortical function came from the work of Bessinger and Wakerlin (1948). These authors measured the renin content of canine kidneys following unilateral renal artery constriction and found that the contralateral, nonconstricted kidney contained no renin in this situation, but that bilateral adrenalectomy favoured its return. Furthermore, the administration of desoxycorticosterone acetate reduced the renin content of the constricted kidney without affecting the already zero levels in the contralateral unconstricted kidney. Further confirmation of this work came from Deane and Masson (1951) who observed an increase in width of the zona glomerulosa of the adrenal cortex of rats following the subcutaneous injec-

tion of hog renin or the development of membrane hypertension. Tobian, Janecek and Tomboulian (1959) repeated the work of Bessinger and Wakerlin (1948) and were able to show a direct correlation between renin content of the kidney and the degree of juxtaglomerular cell granulation. Similar evidence was reported by Pitcock, Hartroft and Newmark (1959) who found an increase in renal pressor activity and juxtaglomerular cell granularity in the kidneys of sodium deficient rats.

Gross and his co-workers had arrived at similar conclusions (Gross and Sulser, 1957) and suggested that the renin-angiotensin system controlled sodium metabolism by the trophic action of angiotensin on the adrenal cortex to release aldosterone (Gross, 1958, 1960). Confirmation came from Genest, Nowaczynski, Kolw, Sandor and Biron (1960) who found a two to tenfold increase in urinary aldosterone levels during and immediately following prolonged intravenous infusion of angiotensin in man. This work was subsequently confirmed in a number of different animals by several authors (Laragh, Angers, Kelly and Lieberman, 1960; Mulrow and Ganong, 1961; Carpenter, Davis, Ayers and Casper, 1961; Bartter, Casper, Delea and Slater, 1961; Blair-West, Coghlan, Denton, Goding, Munro, Peterson and Wintour, 1962). The long-lasting effect of angiotensin in promoting aldosterone secretion from the adrenal cortex, together with the action of this latter hormone on sodium metabolism, suggest

that these mechanisms may be important in the pressor action of angiotensin, at least in the chronic situation (Davis, 1962, 1963; Laragh, Cannon, Ames, Sicinski and Borkowski, 1963).

A number of other pharmacological actions and interactions of renin and angiotensin have been suggested (reviewed by Page and Bumpus, 1961; Brown,Davies, Lever and Robertson, 1966). Of particular interest with regard to the material presented in this thesis have been suggestions from animal studies that the pressor response to angiotensin may be a complex of direct constrictor action at the arteriolar level, and a number of indirect actions such as release of adrenal medullary hormones, interaction with the autonomic nervous system and interaction with other circulating hormones and vasoactive substances.

The direct vasoconstrictor action of angiotensin, which is its most prominent cardiovascular effect, appears to be mediated through specific, non-adrenergic receptors. The requirements for biological activity of angiotensin, with regard to structural configuration, have been reviewed by Page and Bumpus (1961), Gross (1963) and Khairallah and Page (1963).

The possibility that angiotensin might release adrenal medullary hormones was first suggested by the experiments of Williams

and Grossman (1938). They noticed that the injection of a perfusate from hog kidneys into anaesthetized rats resulted in a pressor response which had two components. The initial sharp increase in blood pressure that occurred was adrenaline-like in that it was enhanced by cocaine and diminished by ergotamine. The second component was a less marked but more prolonged pressor effect, resembling that of renin. The pressor effect of an extract from the cortex of hog kidneys (renin) was similarly modified by cocaine and ergotamine (Williams, 1938). It was this evidence that led Williams (1938) to suggest that part of the pressor response to renin may be due to release of adrenaline from the adrenal gland. However, the boiled perfusate was still active in the isolated limb (Williams and Grossman, 1938) and adrenal medullectomy did not alter the response to renin (Williams, Diaz, Burch and Harrison, 1939), suggesting that the adrenaline-like substance ("perfusin") was either derived from renin or from the kidney tissue itself rather than from the adrenal gland.

Then in 1940, Braun-Menendez, Fasciolo, Leloir and Munoz (1940 b) demonstrated that angiotensin released adrenaline from both the normal and denervated adrenal gland. Sevy and Ohler (1953) observed a reduction in the adrenal ascorbic acid concentration in rats following administration of hog renin, an effect that was prevented by adrenergic blockade with dibenzyline. In

1959, Renson, Barac and Bacq reported that angiotensin caused a contraction of the nictitating membrane in cats pre-treated with cocaine and that this effect was abolished by phenoxybenzamine. Kaneko, McCubbin and Page (1961) demonstrated adrenal medullary discharge by angiotensin in the dog, following sensitization with ganglion-stimulating agents.

More recent evidence of adrenal medullary stimulation by angiotensin has come from the work of Cession and Cession-Fossion (1963) and Feldberg and Lewis (1963, 1964). Brody (1966) reported a reduced pressor response to angiotensin in rats following adrenal demedullation, and White and Ross (1966) and Ross and White (1966) observed a similar effect in the cat following ligation of the adrenal blood vessels. A dose-dependent effect of angiotensin in releasing adrenaline from the adrenal medulla of dogs was demonstrated by Peach, Cline and Watts (1966). However, Vincent, Kashemsant, Cuddy, Fried, Smulyan and Eich (1965) found no change in urinary vanillylmandelic acid levels during pressor infusions of angiotensin in man.

The earlier work on angiotensin had led most investigators to conclude that the pressor effect of the hormone relied upon its direct vasoconstrictor action and was independent of the central nervous system (Braun-Menendez, 1956; Page and Bumpus, 1961).

isolated observations,notably those of Dock and his co-workers, that destruction of the vasomotor centres in a variety of animals with established renal hypertension, restored the blood pressure to normal, were largely ignored (Dock and Rytand, 1934; Dock, 1940; Dock, Shidler and Moy, 1942). Robertson and Rubin (1958, 1962) demonstrated that both angiotensin I and angiotensin II stimulated cholinergic neurones of the guinea-pig ileum and Khairallah and Page (1961), who arrived at a similar conclusion, proposed a ganglionic site of action.

Then in 1961, Bickerton and Buckley, using the synthetic octapeptide, described a central sympathetic stimulating action of angiotensin in the dog, which made a significant contribution to the pressor effect. However, Laurence and Nagle (1963) were unable to greatly modify the pressor response to angiotensin in man with either bretylium or guanethidine. Nevertheless, the work of Bickerton and Buckley and their co-workers (Halliday and Buckley, 1962; Buckley, Bickerton, Halliday and Kato, 1963) stimulated interest in the possibility of this and other indirect mechanisms in the pressor action of angiotensin. Laverty (1963) observed a nervously mediated vasoconstriction (or vasodilatation with lower doses) in the hind-limb of the anaesthetized rat during intravenous infusions of angiotensin, which he concluded was due to central sympathetic stimulation. A post-ganglionic site of action has been suggested by the

work of Benelli, Della Bella and Gandini (1964). They found that angiotensin greatly potentiated the height of the contractions of the isolated vas deferens of the guineapig during electrical stimulation of the hypogastric nerve and that the pressor response in anaesthetized and spinal cats was greatly reduced by nicotine or tetramethylammonium. Liebau, Distler and Wolff (1966) from observations of the rat aortic strip preparation have described a tyramine-like action of angiotensin. Zimmerman (1962) and Zimmerman and Gomez (1965) suggest that angiotensin enhances the effect of normally functioning sympathetic nerves by either facilitating release of the neurotransmitter or preventing its re-uptake. A ganglionic site of action has been suggested by the work of Lewis and Reit (1965) in the cat. With regard to the heart, a number of workers have observed positive inotropic and chronotropic effects of angiotensin mediated via the sympathetic nervous system (Nishith, Davis and Youmans, 1962; Berry, Austen and Clark, 1964; Vogin and Buckley, 1964; Krasney, Paudler, Smith, Davis and Youmans, 1965; Krasney, Paudler, Hogan, Lowe and Youmans, 1966). McGiff and Fasy (1964, 1965) have been able to abolish the renal vasoconstriction during intravenous infusions of angiotensin by various forms of sympathetic blockade and denervation.

Despite this evidence from animal studies of a widespread participation of the sympathetic nervous system in the cardiovascular

response to angiotensin, the contribution of this indirect mechanism to the total pressor response is uncertain. Confusion has arisen from attempts to compare the mechanism of the pressor response to acute infusions of angiotensin with the mechanism of chronic renal hypertension. In renal hypertension the initial mechanism is a humoral one, namely renin (Page, 1939), and is unmodified by various forms of sympathectomy. However, in chronic renal hypertension a major part of the elevation in blood pressure appears to be through a neurogenic mechanism (Reed, Sapirstein, Southard and Ogden, 1944; Taggart and Drury, 1940; Pickering, 1945; Taquini, Blaquier and Bohr, 1961; McCubbin and Page, 1963 a, b). In a similar fashion, the pressor response to acute intravenous infusions of angiotensin does not appear to receive a significant contribution from any sympathetic stimulating action (Braun-Menendez, 1956; Page and Bumpus, 1961) but such is not the case in the chronic situation. From the results of experiments where chronic infusions of angiotensin and renin were administered to normal animals, in an attempt to mimic the situation in chronic renal hypertension, the suggestion has been made that a secondary neurogenic mechanism appears after a time and is responsible for maintaining the elevation in arterial pressure independently of the direct vasoconstrictor action of the drug (Hill and Pickering, 1939; Blacket, Depoorter, Pickering, Sellers and Wilson, 1950; Dickinson and Lawrence, 1963; Brown, Chapuis and Robertson, 1963, 1964; Yu and Dickinson, 1965;

McCubbin, De Moura, Page and Olmsted, 1965), which may at this time be diminished on the basis of tachyphylaxis (Gross, Bock and Turrian, 1961). This suggestion is well supported by the experiments of McCubbin, De Moura, Page and Olmsted (1965) in particular, where a hypertensive effect of initially subpressor amounts of angiotensin was observed after several days of continuous infusion.

Although there is a growing body of evidence to suggest participation of the sympathetic nervous system in the cardiovascular response to acute and chronic infusions of angiotensin the role of this mechanism in the aetiology of either renal or essential hypertension remains to be elucidated.

The suggestion that the vasoconstrictor action of angiotensin may be enhanced by an interaction with a variety of vasoactive substances (Braun-Menendez, 1956) has added a further indirect mechanism of interest. Verney and Vogt (1938) observed an increase in the pressor response to intravenous tyramine in renal hypertensive dogs and an occasional enhancement of the response to adrenaline. Braun-Menendez, Fasciolo, Leloir and Munoz (1939, 1940 b) and Braun-Menendez, Fasciolo, Leloir, Munoz and Taquini (1946) reported a similar potentiation of the pressor action of angiotensin by tyramine. McCubbin and Page (1963 b) in repeating the work of Verney and Vogt (1938) observed a potentiation of endogenously

released noradrenaline (by tyramine) when administered in combination with angiotensin in the dog whereas the response to exogenous noradrenaline was virtually unchanged. More recently Sakurai and Hashimoto (1965) reported an enhanced response of the perfused rabbit ear vessels to noradrenaline and tyramine when administered with subthreshold amounts of angiotensin. The pressor response to tyramine in the intact rabbit was also enhanced by subthreshold amounts of angiotensin. Striking potentiation by serotonin of the constrictor response to angiotensin in the isolated perfused central ear artery of the rabbit was observed by de la Lande, Cannell and Waterson (1966) although Hurwitz, Campbell, Gordon and Haddy (1961) were unable to demonstrate a similar interaction in the denervated dog forelimb.

However, the importance of interactions between administered angiotensin and the normal levels of endogenous noradrenaline and serotonin and the contribution of these interactions to the total pressor response to angiotensin have not been determined.

There is, then, considerable evidence from animal studies to suggest that apart from direct receptor stimulation a number of indirect mechanisms may contribute to the vasoconstrictor and possibly the pressor action of angiotensin. However, apart from the work of de Pasquale and Burch, (1963) who demonstrated a sympathetic

component in the action of angiotensin on forearm veins, there is little corroborative evidence from studies in man (Laurence and Nagle, 1963; Vincent, Kashemsant, Cuddy, Fried, Smulyan and Eich, 1965; Braun-Menendez, 1956; Page and Bumpus, 1961). Part of the work to be described in this thesis is an attempt to define some of these mechanisms in man.

SUMMARY

In summary, the association between renal disease and hypertension was recognized as early as 1836 and the suggestion was made at that time that the diseased kidney may produce a circulating pressor substance (Bright, 1836).

The discovery of renin by Tigerstedt and Bergman in 1898 lent support to this hypothesis but subsequent workers in the early part of this century were unable to consistently demonstrate this substance in the kidney or to find elevated levels of pressor material in plasma and tissues of patients with hypertension and furthermore a reliable and reproducible experimental model could not be produced by various forms of renal damage. This and other evidence raised considerable doubt as to whether renin existed as a specific renal pressor substance and whether it was important in the aetiology of hypertension.

Then in 1934, the experimental techniques of Goldblatt and his co-workers produced a reliable experimental model and in a remarkably active period from 1934 to 1940 the existence of renin was recognized, its properties categorized and the discovery made of its enzymatic function in the formation of the active constrictor principle, angiotensin. However attempts to correlate the

activity of the renin-angiotensin system and the properties of the reactants with all forms of hypertension together with the use of impure preparations resulted in some confusion.

With the development of reliable assay procedures and the availability of synthetic preparations of angiotensin, the more subtle actions and interactions of the system are becoming manifest. Much interest now centres around the many indirect actions of angiotensin and their possible role in normal physiology as well as in the genesis of hypertension.

The work to be described in this thesis was therefore undertaken with three main purposes in mind:-

(1) To categorize more fully the cardiovascular actions of angiotensin in man, with particular emphasis on the peripheral vascular responses.

(2) To explore the possibility of indirect mechanisms of action of angiotensin in man.

(3) To investigate the role of the renin-angiotensin system in renal and essential hypertension.

In most of the experiments the responses to noradrenaline were used as a comparison. The normal responses were obtained from volunteer male medical students who were subjected to a variety of

procedures. Various patients were also studied, including patients with hypertension (primary, renovascular and malignant), cervical and lumbar sympathectomy, and autonomic nervous system degeneration.

As an appendix to the investigations of the indirect mechanisms of action of angiotensin in man a limited study was made in dogs using similar experimental designs.

SECTION I

METHODS

VENOUS OCCLUSION PLETHYSMOGRAPHY

Francis Glisson, in 1677, was one of the first to use the technique of plethysmography. He constructed a large glass cylinder into which the whole arm could be placed, the open end sealed to the upper arm and the whole instrument filled with water. An open tube at one end, into which the water had risen, enabled him to observe the changes in limb volume during muscular activity.

The technique was modified by Bulson in 1862, who designed an instrument capable of recording the volume changes graphically, which is embodied in the name of the technique ("plethysmos" ... increase , "graphein" ... to write).

By application of intermittent venous occlusion, as suggested by Brodie and Russell in 1905, the rate of blood flow in an organ could be determined quantitatively. This modification was adapted for use in the human limb by Hewlett and van Zwaluwenberg in 1909, and the upper limb partitioned to enable hand (Lewis and Grant, 1925) or forearm blood flow (Freeman, 1935) to be determined independently. Grant and Pearson (1938) suggested that the hand be excluded from forearm blood flow measurements by arterial occlusion at the wrist.

With the use of the water-filled plethysmograph for measuring blood flow in human limbs (Lewis and Grant, 1925) a method was devised for sealing the instrument using a rubber sleeve or glove attached to the plethysmograph (Krogh, Landis and Turner, 1932) and the correct water temperature determined for both the hand and forearm segments to allow an accurate measurement of resting blood flow (Barcroft and Edholm, 1946).

The technique has been critically examined on several occasions and shown to give an accurate measurement of blood flow (Landowne and Katz, 1942; Formel and Doyle, 1957; Greenfield, 1960).

The method used in the present studies was basically the same as that described by Barcroft and Swan (1953), the only modifications being in design of equipment.

Fig. 1-1 shows the equipment in operation. Water-filled plethysmographs, similar to the type described by Greenfield (1954) and Greenfield, Whitney and Mowbray in 1963 (Fig. 1-2), were used in most studies, the exception being a few experiments in Section 4 where a capacitance plethysmograph was used (Willoughby, 1965; Fewings and Whelan, 1966). The capacitance plethysmograph is shown in Fig. 1-3.

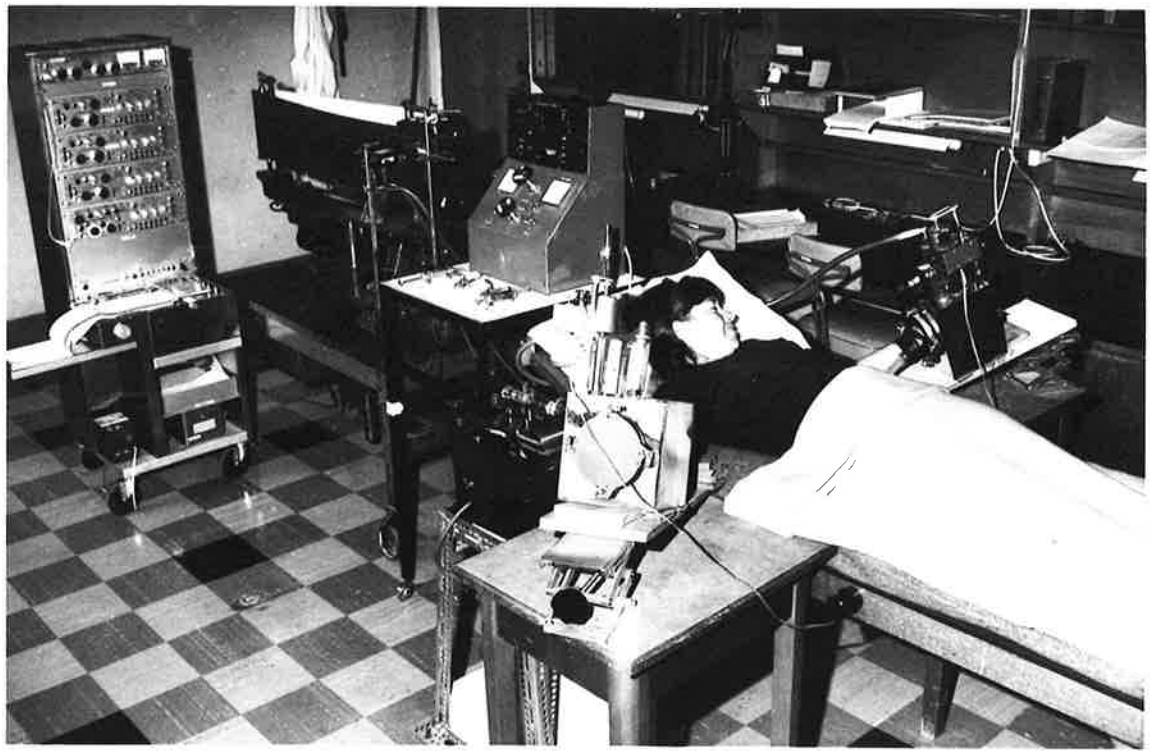

Fig. I-1 The general laboratory set-up showing a Grass polygraph, Brodie-Starling kymograph with smoked drum attached, sequence timer and collecting and wrist occlusion cuff pressure monitors. The subject is shown lying on the couch with bilateral hand plethysmography in progress.

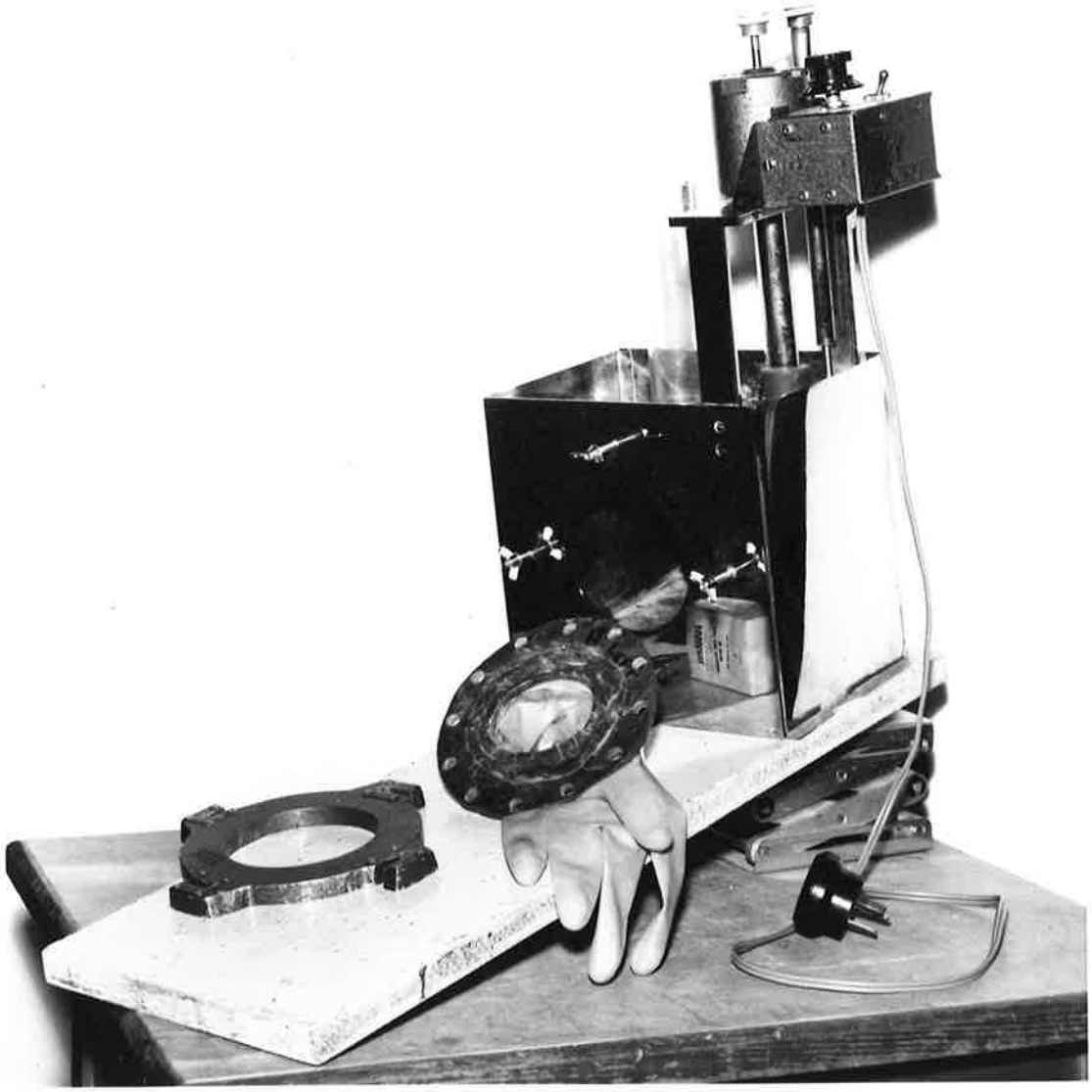

Fig. I-2 The water-filled plethysmograph showing the rubber glove and sealing plate used for hand plethysmography.

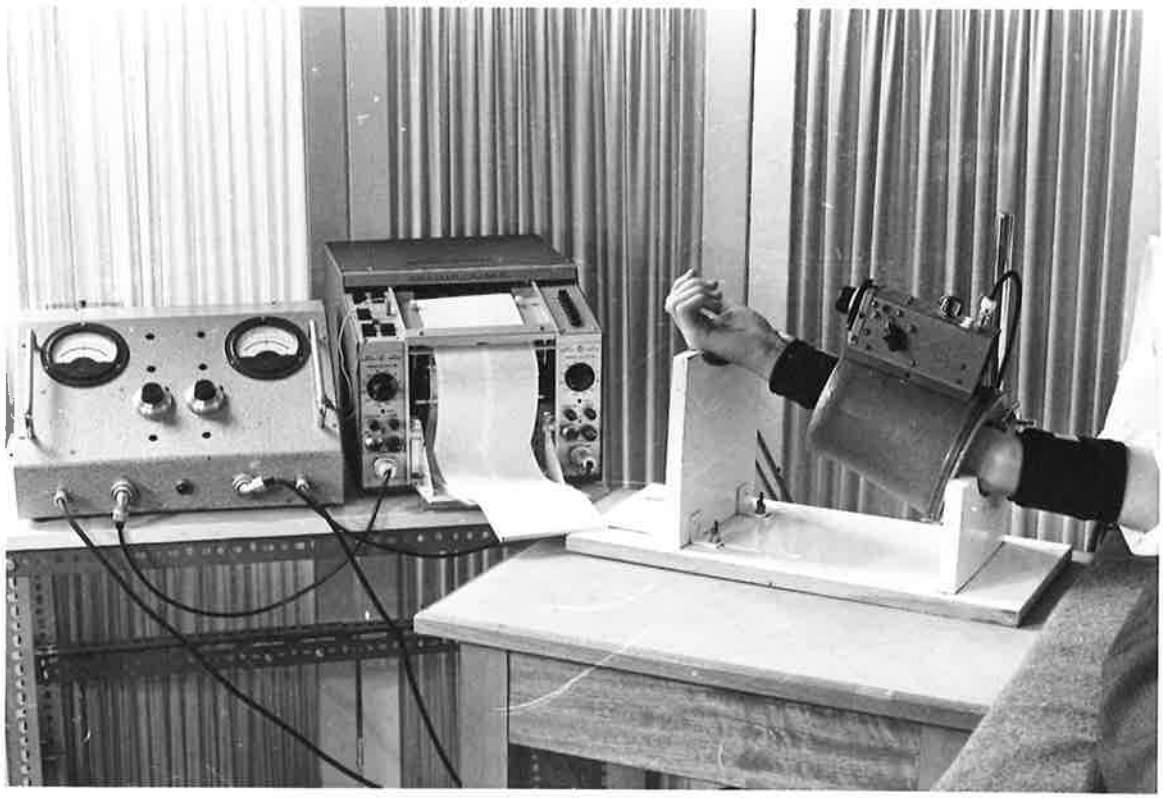

Fig. I-3 The laboratory set-up for capacitance plethysmography showing the control unit, recorder and the plethysmograph applied to the forearm, with wrist occlusion and forearm collecting cuffs in place.

Collecting cuff inflation and deflation were made automatic by means of a sequence timer (Paton Industries, Adelaide) and electrically operated solenoid valves releasing air from reservoirs at constant pressure, the reservoirs being filled from compressed air cylinders using constant pressure valves (Fig. 1-1).

With the exception of a few experiments where capacitance plethysmography was used (Fig. 1-3), hand and forearm blood flow were recorded on a smoked drum (Fig. 1-1) and the actual flow calculated by the method of Barcroft and Swan (1953). The method of reading the flow values from the kymograph tracing is shown in Fig. 1-4.

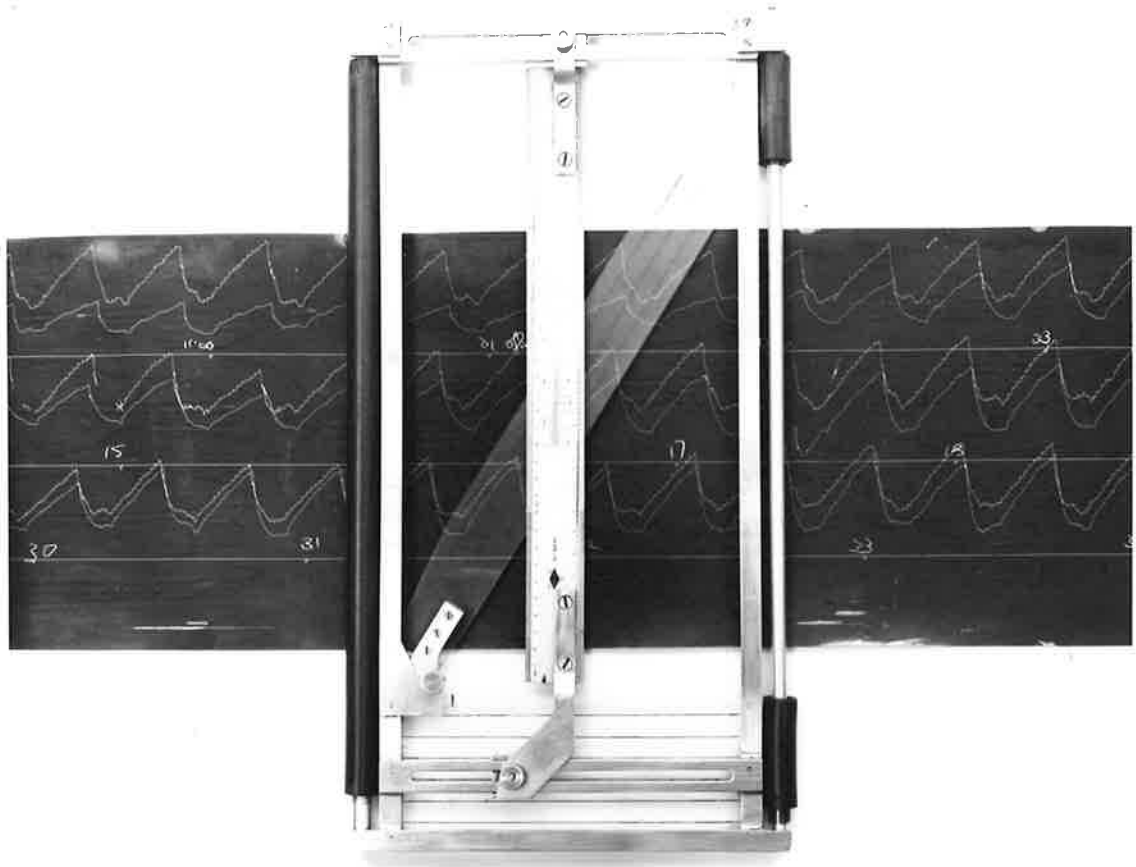

Fig. I-4 Flow-measuring apparatus shown superimposed on a typical venous occlusion plethysmographic kymograph tracing.

INTRAVASCULAR INFUSIONS

General Considerations

Drug effects in the cardiovascular system are a combination of the direct or local action, usually mediated through specific receptors, and the indirect or general effects such as baroreceptor stimulation, interaction with the autonomic nervous system and release of various hormones.

The relative contributions of these direct and indirect actions to the total cardiovascular response to a particular drug are largely dependent upon the dose administered and upon the route of administration. A combined response will best be seen on intravenous infusion but certain of the nonspecific responses to a pressor or depressor drug may confuse the general appreciation of the cardiovascular response. However, it is possible to examine the direct action alone without complicating systemic effects by using the intra-arterial route of administration and choosing the doses such that effects on the general circulation or the production of subjective symptoms such as pain, are avoided. Furthermore, by using the intra-arterial route in studies of drug responses in the human forearm and hand it is possible to confine the effects to the uninfused limb. This enables the opposite uninfused limb to act as a control during the infusions and detect spontaneous variations in

flow unrelated to drug action. This is particularly important with regard to flow measurements in the hand where the sympathetic innervation is particularly dense and the resting level of flow quite variable.

Naturally both the intravenous and intra-arterial routes of administration expose different aspects of drug action but the ability to separate the local and systemic effects gives a more complete picture.

Intra-arterial Infusions

These were administered through a 21-gauge, short-bevel, 3.2 cm. needle or 6.0 cm. polythene catheter (Intra-medic PE 90, internal diameter 0.76 mm.) inserted into the brachial artery in the cubital fossa.

The needles were inserted percutaneously under local anaesthesia (2% Lignocaine) and threaded centripetally up the lumen of the artery. They were then taped in place and remained there for the duration of the experiment (Fig. 1-5).

In certain experiments monitoring of systemic arterial pressure with transducer systems was also required at some stage, in addition to intra-arterial infusion, and in these cases a

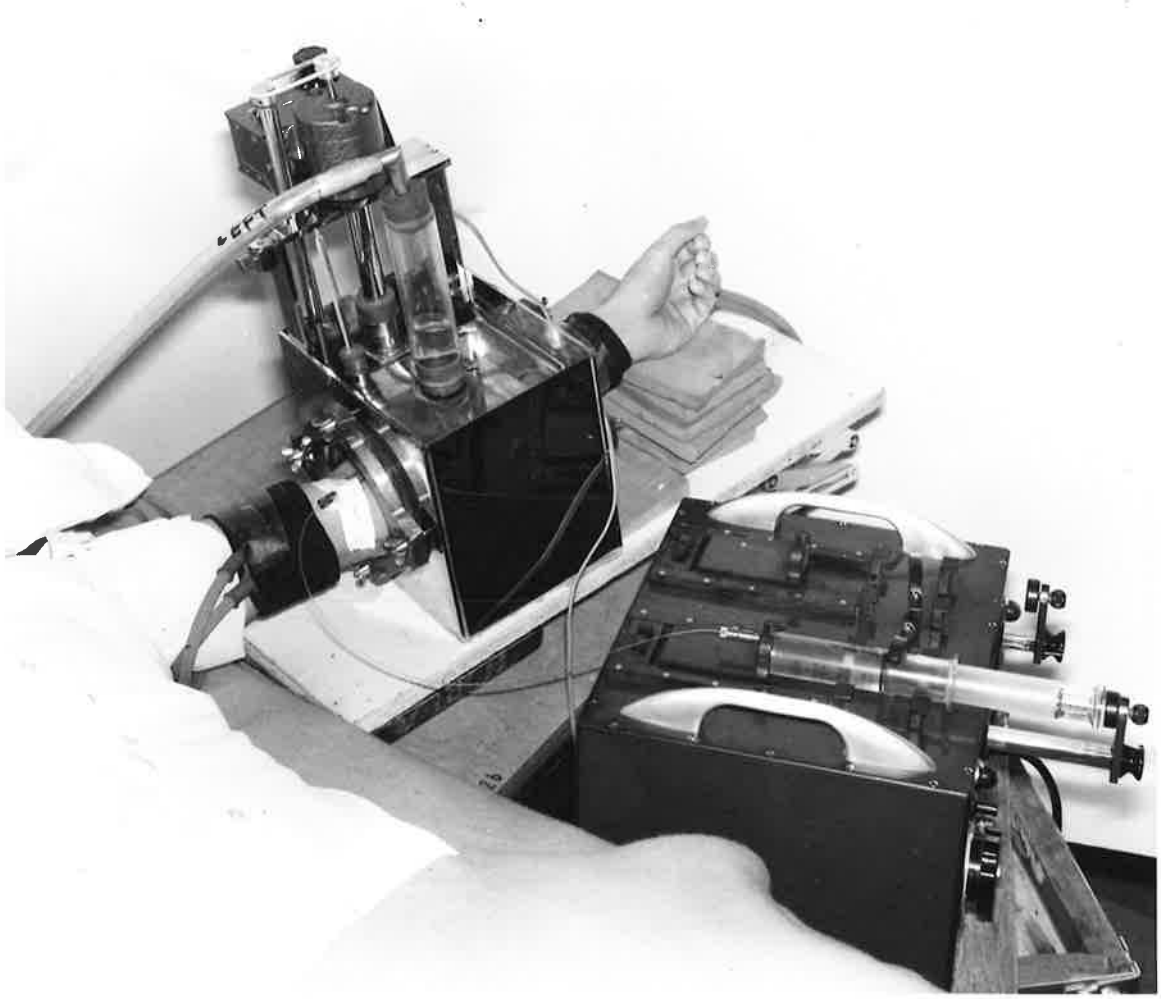

Fig. I-5 Measurement of forearm blood flow showing the intra-arterial needle attached by a polythene connection to the controlled infusion apparatus.

polythene catheter was used instead of a needle. The catheters were inserted using a modified Seldinger technique (Seldinger, 1953). The artery was entered in the usual fashion, using a 19-gauge, short-bevel, 3.2 cm. needle. A short measured section of nylon fishing line (Perlon, 17 lb. breaking strain with a diameter of less than 0.76 mm.) was then inserted through the needle and the needle withdrawn. The nylon line was then used as a guide for insertion of the catheter and subsequently withdrawn. The technique is shown diagramatically in Fig. 1-6.

The catheter or needle, having been inserted, was then attached via a 30 cm. section of polythene tubing (Portex 49A, internal diameter 0.86 mm.) to a mechanically driven 50 ml., all glass syringe in a controlled infusion apparatus (Fig. 1-7).

Most infusions were given at a rate of 2.0 ml. per min. but this could be varied from 0.5 ml. to 4.0 ml. per min. when required. The total dead space in the system between the syringe and the artery was approximately 0.17 ml. and represented a delay of 5 to 6 sec. when infusions were given at the rate of 2.0 ml. per min. Saline (0.9% weight/volume, Boots) was infused during control periods and was also used as a vehicle for the drugs.

The arrangement of recording equipment and infusion

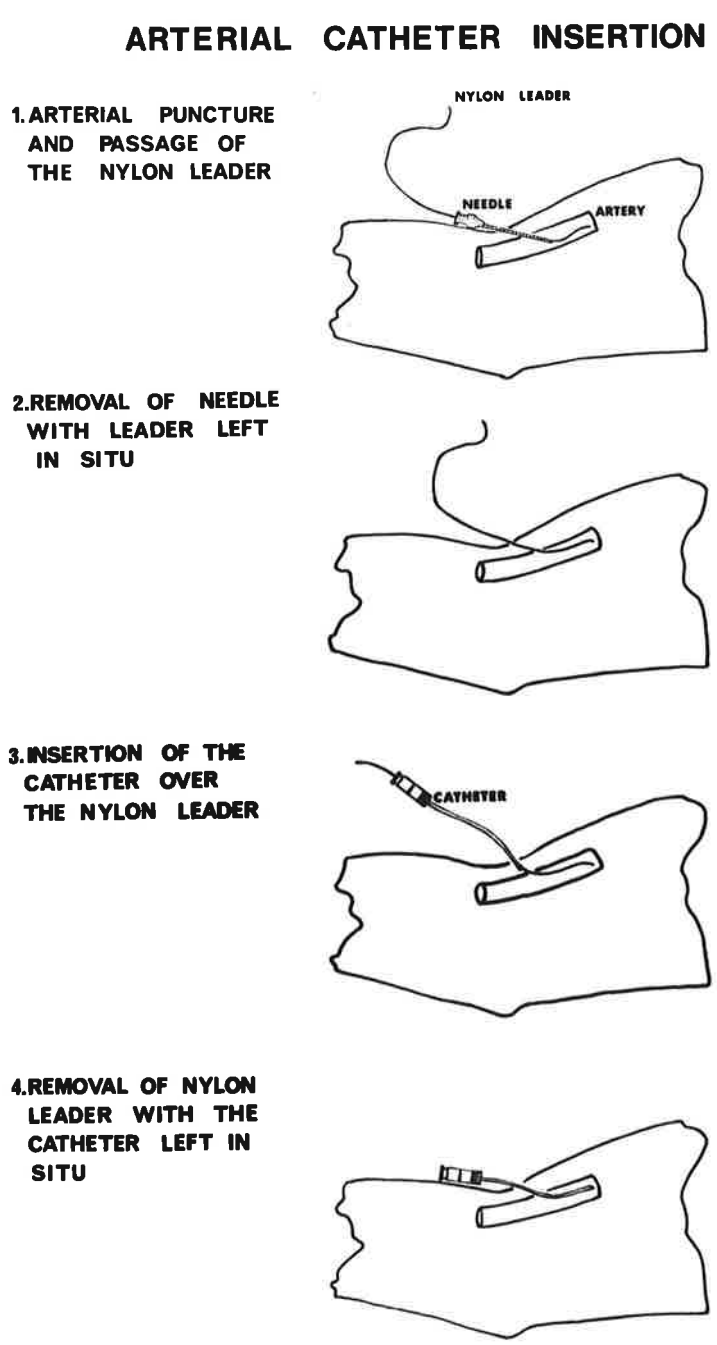

Fig. I-6 Diagramatic sequence of events in arterial catheter insertion.

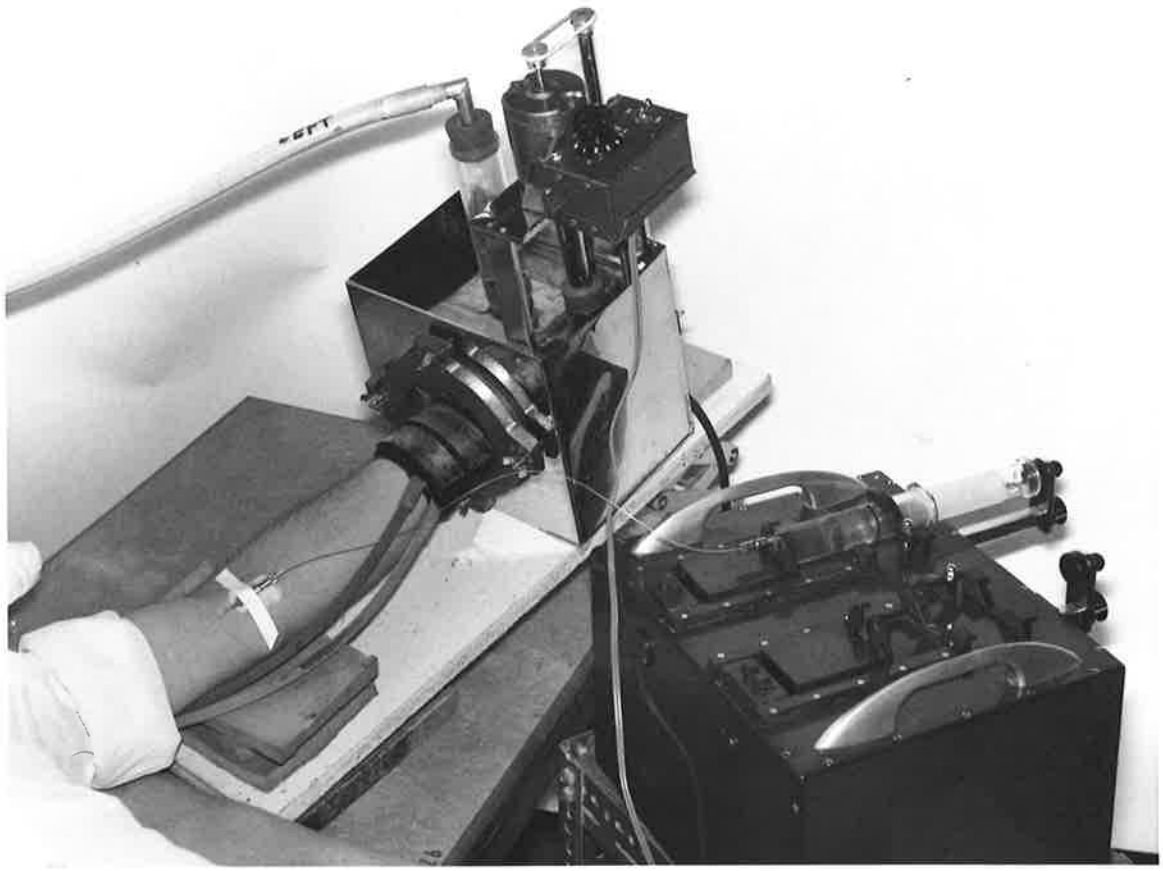

Fig. I-7 Measurement of hand blood flow showing the intra-arterial needle attached by a polythene connection to the controlled infusion apparatus.

apparatus is shown in Fig. 1-1.

Intravenous Infusions

These were administered through a polythene catheter (Intracath No. 17, Bardic) inserted centripetally into an ante-cubital vein under local anaesthesia (Lignocaine 2%). Care was taken to ensure that the tip of the catheter was unobstructed by clothing or by the inflatable venous collecting cuff during forearm flow measurements. The infusion apparatus and connecting tubing were identical to that used for intra-arterial infusions. Infusions were usually given at the rate of 2.0 ml. per min. and the total dead space in the system was approximately 0.17 ml. resulting in a delay of 5 to 6 sec.

PRESSURE MEASUREMENTS

Arterial Pressure Measurement

In most experiments arterial pressure was recorded from a polythene catheter inserted into the brachial artery in the cubital fossa of one arm as described earlier. The catheter was then connected by a short segment of polythene tubing to a Statham P23 Dc pressure transducer and the output recorded on a Grass polygraph (Model 5D). The catheter-transducer system contained a static column of heparinized saline (50 units per ml.) which was flushed from time to time through the side arm of a three-way tap (Fig. 1-8).

In a few experiments, where insertion of a catheter was not possible, usually for anatomical reasons, the arterial pressures were recorded from a 21-gauge needle inserted as for intra-arterial infusions. The needles were connected via a short length of polythene tubing to one side of a differential electrocapacitance manometer (N.E.P. London) and the signal from this passed to a Bevelec carrier amplifier and recorded on an ultraviolet oscillograph (N.E.P. type 1050). To avoid clotting in the needle, and therefore interference with the pressure record, a continuous infusion of heparinized saline (50 units per ml.) was infused through the whole system at the rate of 0.1 ml. to 0.5 ml. per min. and this

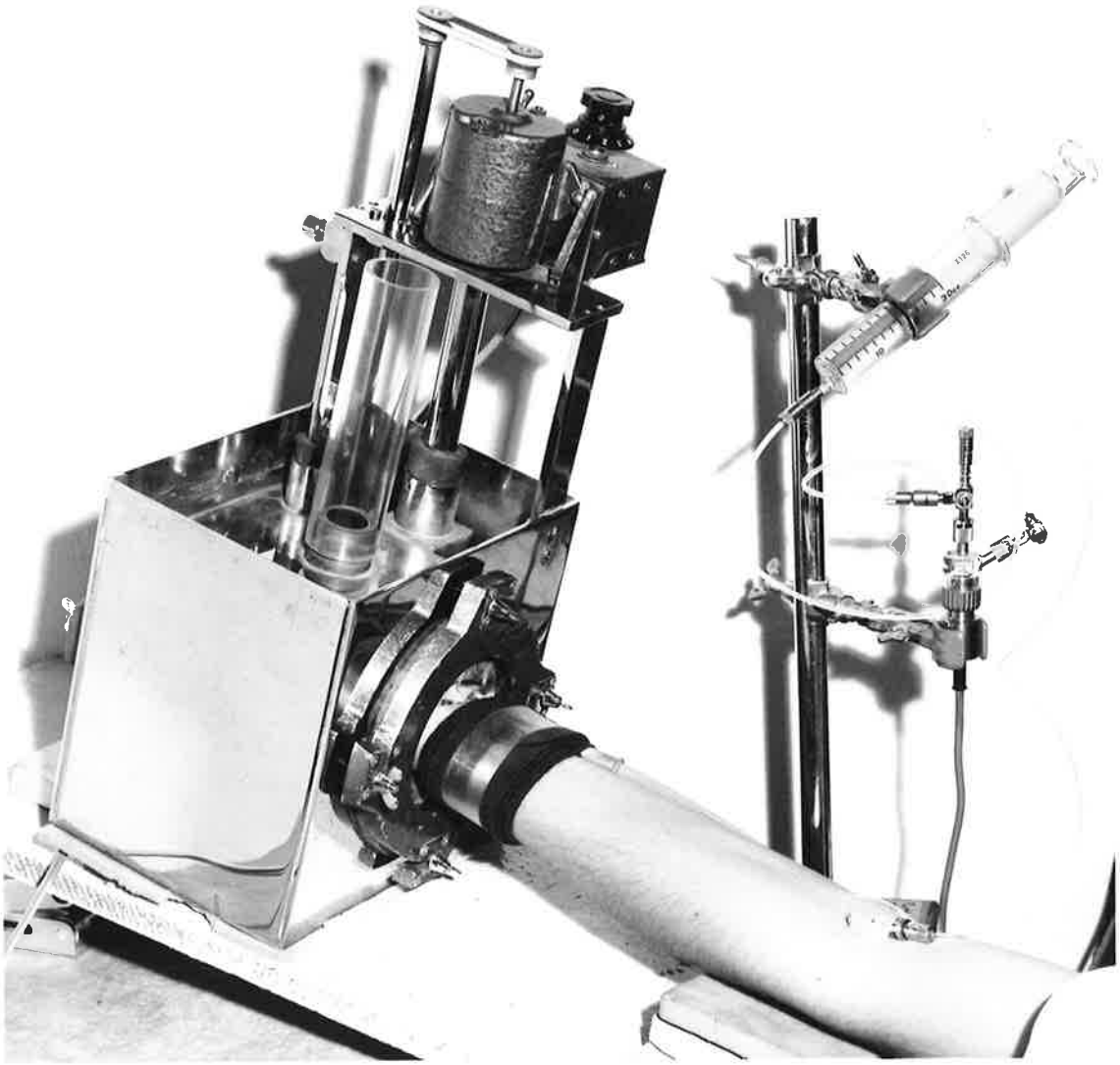

Fig. I-8 Measurement of systemic arterial blood pressure with a Statham transducer attached to an intra-arterial catheter.

did not distort the pressure record. The equipment is shown in Fig. I-9.

An electrical calibration was built into both systems but was checked with a mercury manometer at the end of each experiment.

Central Venous Pressure

Central venous pressure was recorded from a polythene catheter (Intramedic PE 90) inserted into an ante-cubital vein by the modified Seldinger technique described earlier.

The catheter length required was determined in each subject prior to insertion to ensure that the tip was in the great veins in the neck at the level of the mid-clavicle and a true record of central venous pressure was obtained.

Recordings were made using a differential electrocapacitance manometer (N.E.P. London) and an ultraviolet oscillograph (N.E.P. type 1050) as described earlier. The catheter-manometer system contained a static column of heparinized saline (50 units per ml.) which was flushed from time to time through the side-arm of a three-way tap. The arrangement of equipment was similar to that described for arterial pressure measurement.

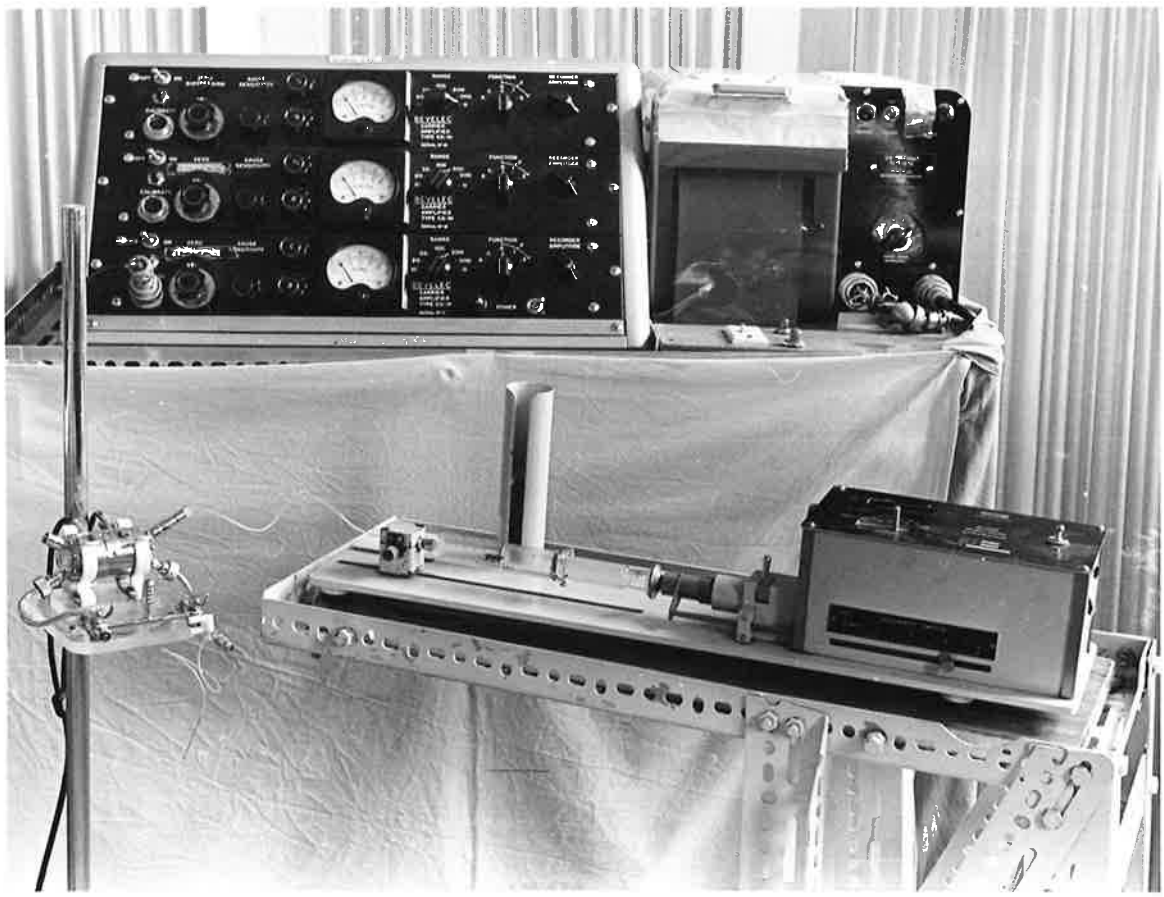

Fig. I-9 Measurement of systemic arterial pressure with a differential electrocapacitance manometer, slow-infusion pump and ultraviolet oscilloscope.

The system was calibrated against a simple water manometer and the readings expressed in terms of cm. of water.

EXPRESSION OF RESULTS

Arterial pressure was expressed in the conventional terms of systolic, diastolic and mean pressures. However, most of the comparisons of pressor responses to different drugs were expressed in terms of mm. Hg. mean pressure, calculated from the formula ...

Mean pressure = Diastolic + 1/3 (Systolic - Diastolic).

The values for mean pressure were also used in the calculation of resistance changes.

Blood flow was expressed in terms of ml. blood flow per 100 ml. hand or forearm tissue per min. and plotted either as instantaneous flow values or minute averages.

When the dose-response relationship was required for comparisons between different drugs or different subjects the percentage changes in blood flow or blood pressure were determined. With all intravascular infusions this was obtained from differences between the average flow or pressure value for the 2 min. prior to drug infusion and for the last 2 min. of the infusion period, by which time the response to the drug had become stable.

However, with most intra-arterial infusions, an additional correction was made with regard to the flow changes. Since the

small doses given did not cause systemic effects the blood flow on the uninfused side was regarded as a control. In this way, during calculation of the percentage changes in flow, allowance could be made for spontaneous variations in flow by assuming that in the absence of the infusion the two sides would maintain the same relationship as in the preinfusion period (Duff, 1952). Naturally this correction could not be used for those experiments where the sympathetic nervous transmission to the infused side had been interrupted in any way.

Resistance changes were calculated from the ratio ...

$$\frac{\text{Mean blood pressure (mm. Hg.)}}{\text{Blood flow (ml. per 100 ml. of hand or forearm per min.)}}$$

and percentage changes in resistance in response to drug infusion were calculated in the same fashion as for blood flow.

SECTION 2

A COMPARISON OF THE ACTIONS OF ANGIOTENSIN AND NORADRENALINE ON THE CIRCULATION IN MAN.

INTRODUCTION

Angiotensin is the most powerful pressor substance known to man. When compared to noradrenaline in this regard it is six to ten times more active on a weight basis or fifty to sixty times on a molar basis (Finnerty, Massaro, Chupkovich and Tuckman, 1961; McQueen and Morrison, 1961; De Bono, Lee, Mottram, Pickering, Brown, Keen, Peart and Sanderson, 1963). Both angiotensin and noradrenaline are potent vasoconstrictor agents and on intravenous infusion will elevate total peripheral resistance. It is to this property that they appear to owe their pressor action rather than to any significant effect on cardiac output (Finnerty, Massaro, Chupkovich and Tuckman, 1961; Cumming, 1963).

Although a number of vascular beds have been studied in man (De Bono, Lee, Mottram, Pickering, Brown, Keen, Peart and Sanderson, 1963; Wilkins and Duncan, 1941; Bock, Krecke and Kuhn, 1958) and in animals (Barer, 1961; Mandel and Saplrstein, 1962; Haddy, Molnar, Borden and Texter, 1962) the site of the major increase in peripheral resistance during systemic infusions of angiotensin remains uncertain.

The first section of this thesis is devoted to a study of the cardiovascular actions of angiotensin II in man during both

intra-arterial and intravenous infusions. Systemic arterial and central venous pressures, heart rate, and hand and forearm blood flow were measured. The responses to noradrenaline have been used as a comparison since the cardiovascular effects of this drug, in most cases, are due to direct receptor stimulation. This is almost certainly true of the hand vascular responses during both local and systemic infusions of noradrenaline and a comparison of the angiotensin responses during both methods of administration might expose the suggested indirect constrictor mechanisms of this drug.

METHODS

The subjects for the experiments were colleagues and volunteer medical students. The laboratory temperature was maintained at 23°-24°C. and the subjects rested on a couch for at least 30 min. before observations began.

Intra-arterial infusions were administered into the brachial artery of one arm. Saline (0.9% w/v) was given throughout the control periods and was also used as a vehicle for the drugs. Blood flow in the hand or forearm was recorded three to five times every minute in both limbs by venous occlusion plethysmography using water-filled, temperature-controlled plethysmographs. The temperature of the water was 32° and 34°C. for the hand and forearm respectively. The blood flow measured in the uninfused limb was regarded as a control.

Intravenous infusions were administered through a polythene catheter inserted into an antecubital vein. Blood pressure was recorded from a needle or catheter in the brachial artery of one arm. Heart rate was read from the blood pressure tracing. During intravenous infusions, hand and forearm blood flow were recorded simultaneously in opposite limbs (Fig. 2-1). Vascular resistance changes were calculated and in some experiments central

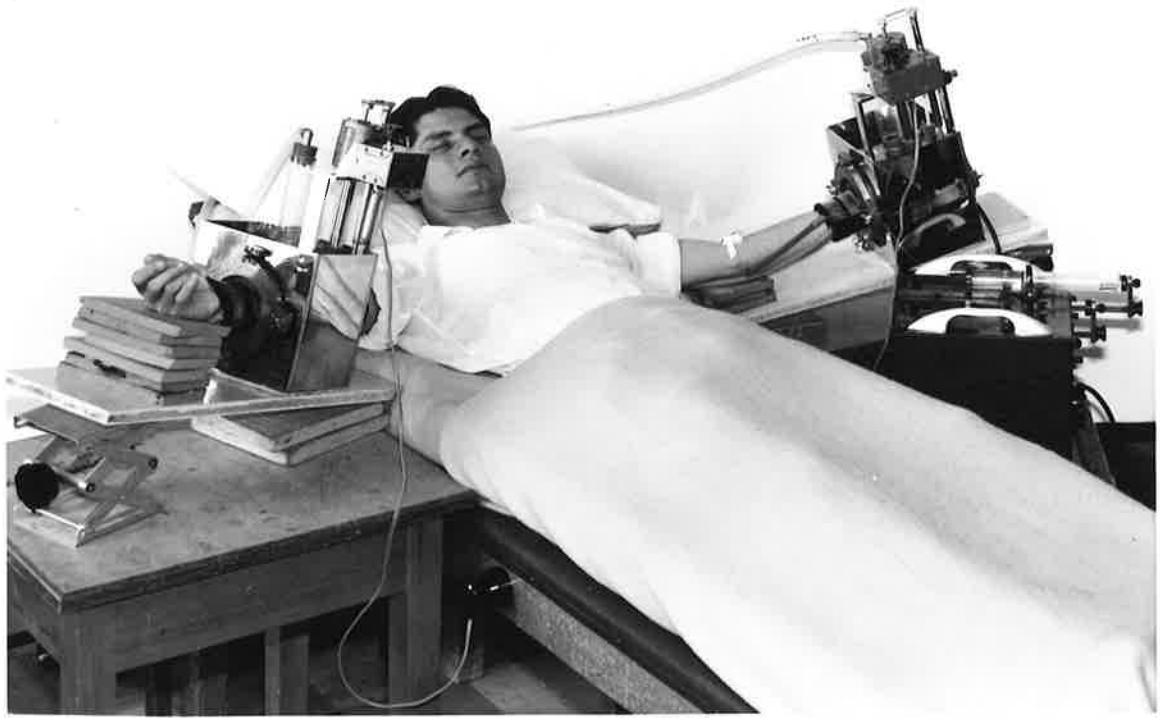

Fig. 2-1 The arrangement of equipment for the simultaneous measurement of hand and forearm blood flow and the intravenous infusion of drugs.

venous pressure was measured.

The drugs used were noradrenaline bitartrate monohydrate (Levophed, Winthrop) and angiotensin II (val^5-hypertensin II-asp-β-amide, Hypertensin, Ciba). Doses of noradrenaline are expressed as weights of the base and of angiotensin as weights of the amide. Ascorbic acid (1 : 50,000) was added to the noradrenaline solutions.

The calculation of percentage changes in the various parameters during intra-arterial and intravenous infusions has been outlined in the Methods section.

RESULTS

Hand Vessel Responses:-

a. Intra-arterial infusions. Both angiotensin and noradrenaline reduced hand blood flow, the response to angiotensin being slightly slower in onset than that to noradrenaline and taking longer to wear off, this latter effect being more noticeable with higher doses of angiotensin (Fig. 2-2).

Fig. 2-3 (left-hand frame) illustrates the percentage falls in hand blood flow resulting from the intra-arterial infusion of 25, 50 and 100 ng. per min. for 4 min. of both drugs. The plotted values are the averaged responses from five subjects, most of whom received two infusions at each dose level (see legend, Fig. 2-3). A comparison of the dose-response curves obtained with the two drugs indicates that, with regard to their local vasoconstrictor action on the hand vessels, noradrenaline is two to three times more potent than angiotensin.

b. Intravenous infusions. Both angiotensin and noradrenaline reduced hand blood flow, the response to angiotensin being slightly slower in onset and offset (Fig. 2-4).

Fig. 2-5 illustrates the percentage falls in hand blood

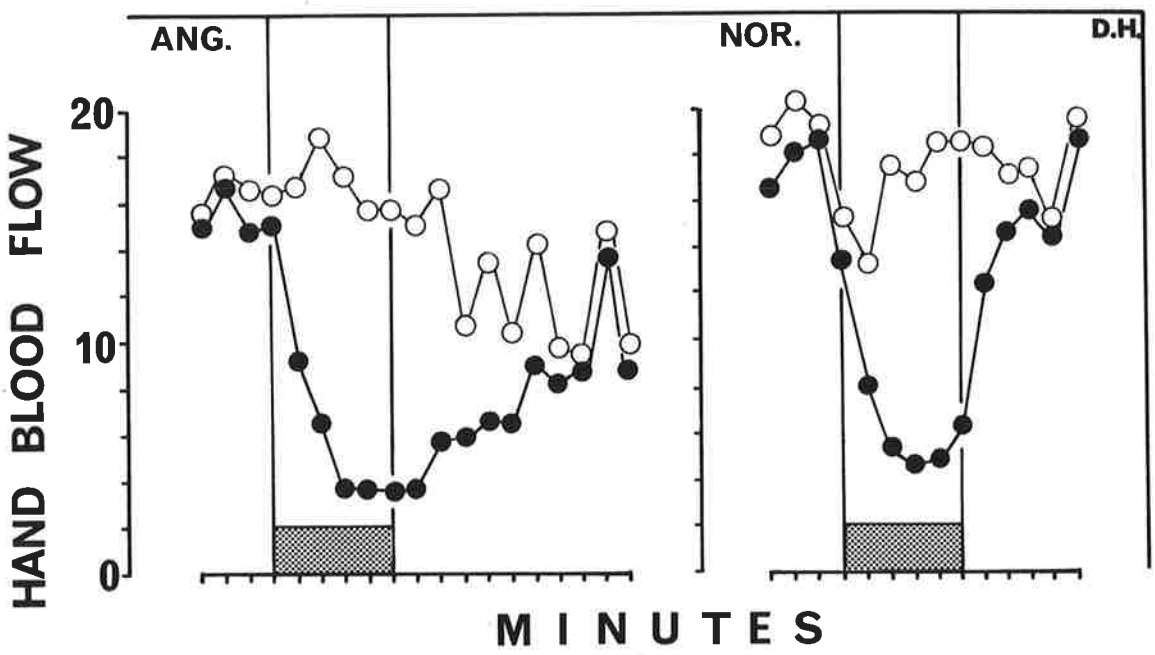

Fig. 2-2 The response of the hand vessels (● , infused hand; O , control hand) during intra-arterial infusion of angiotensin (900 ng./min. for 5 min., left of figure) and noradrenaline (300 ng./min. for 5 min., right of figure).

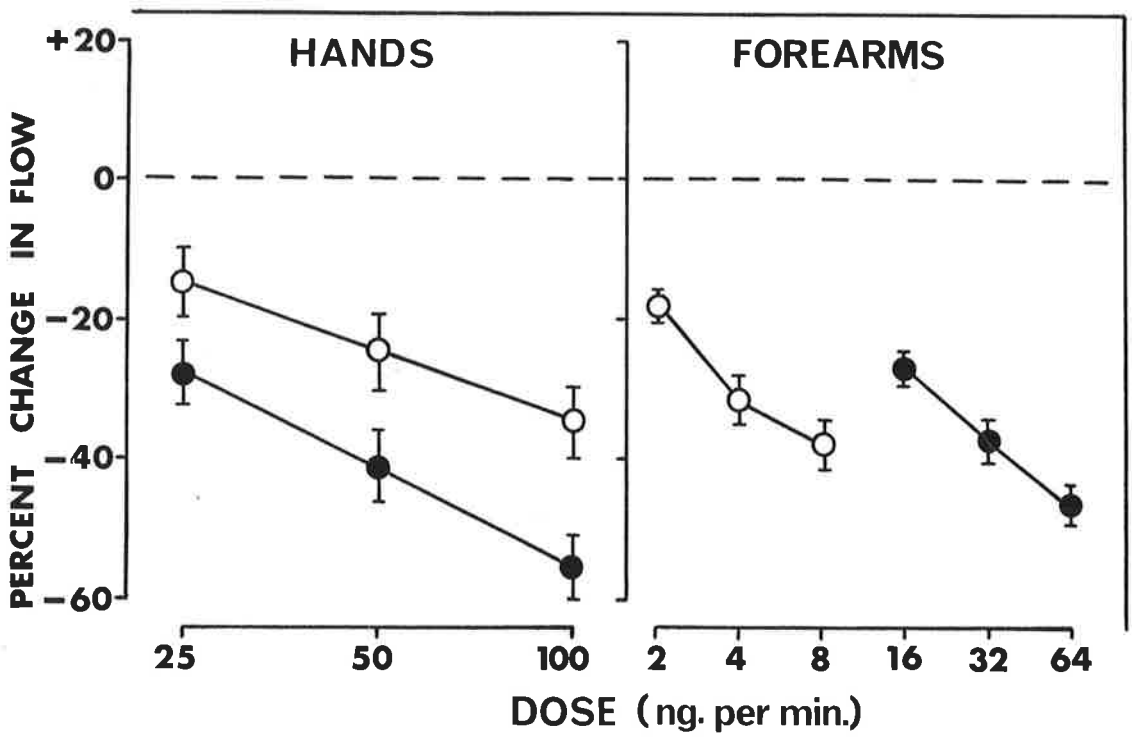

Fig. 2-3 The mean percentage changes in hand blood flow (left-hand frame) and forearm blood flow (right-hand frame) during intra-arterial infusions of angiotensin (O) and noradrenaline (●) in the doses indicated.

The hand flow values were obtained from five subjects, each point representing the mean of eight values in the case of angiotensin and nine values in the case of noradrenaline. The forearm flow values were obtained from five subjects, two infusions of each drug being given at each dose level in each subject. The vertical lines through the flow values represent one standard error on either side of the mean.

Fig. 2-4 The changes in arterial blood pressure, heart rate, hand blood flow and resistance and forearm blood flow and resistance during intravenous infusions of angiotensin (O , 2.0 µg./min. for 5 min.) and noradrenaline (● , 16.0 µg./min. for 5 min.), being the averaged data of paired infusions from seven subjects. For each parameter the mean value for the appropriate minute has been calculated and plotted as an individual point. The blood pressure responses (top record) have been superimposed, that to angiotensin being indicated by the dotted lines and that to noradrenaline by the solid lines. The period of infusion (5 min. in each case) is indicated by the hatched area.

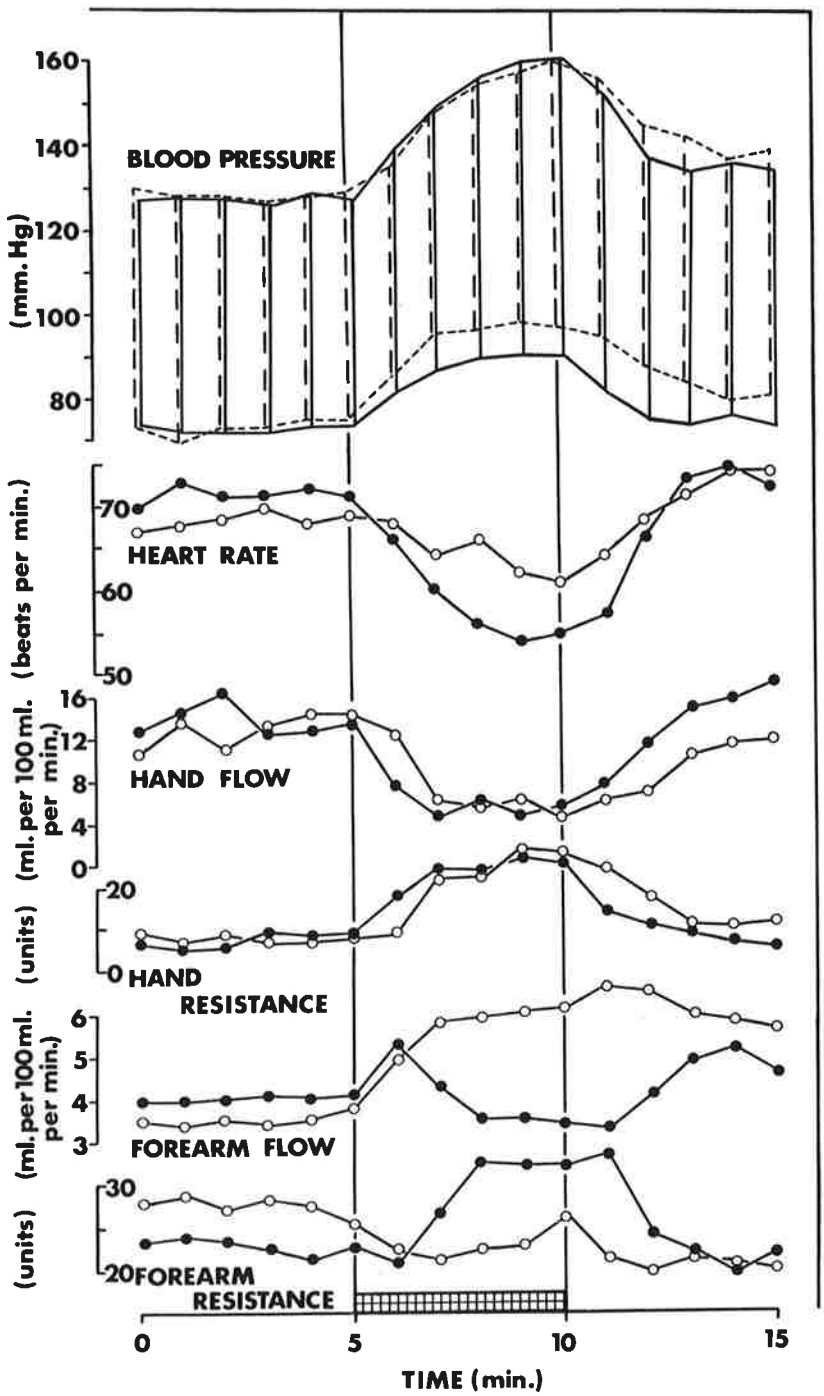
160
140
120
100
80
(mm. Hg)
BLOOD PRESSURE
70
60
50
(beats per min.)
HEART RATE
16
12
8
4
0
(ml. per 100 ml. per min.)
HAND FLOW
20
0
(units)
HAND RESISTANCE
6
5
4
3
(ml. per 100 ml. per min.)
FOREARM FLOW
30
20
(units)
FOREARM RESISTANCE
0
5
10
15
TIME (min.)

Fig. 2-4

flow (upper left-hand frame) and corresponding increases in calculated hand vascular resistance (lower left-hand frame) in response to the intravenous infusion of angiotensin (0.5, 1.0 and 2.0 µg. per min. for 5 min.) and of noradrenaline (4.0, 8.0 and 16.0 µg. per min. for 5 min.). Each value plotted is the averaged response obtained from seven subjects.

A comparison of the dose-response curves obtained (Fig. 2-5, upper left-hand frame) indicates that on intravenous infusion angiotensin is between eight and ten times more constrictor in the hand vessels than is noradrenaline. This ratio of potencies is in marked contrast to that found during intra-arterial administration where angiotensin was only one half to one third as constrictor as noradrenaline.

Fig. 2-6 (upper frames) illustrates the relationship between percentage change in hand vascular resistance and percentage increase in mean blood pressure for all doses of the two drugs and contains results from ten subjects, including the seven mentioned above and three others in whom for various reasons the full set of six infusions was not given. Each point in the figure is an individual value from one of the ten subjects. With both angiotensin and noradrenaline a positive correlation was found (angiotensin, $r = +0.529$, $n = 29$, $p < 0.01$; noradrenaline, $r = +0.452$,

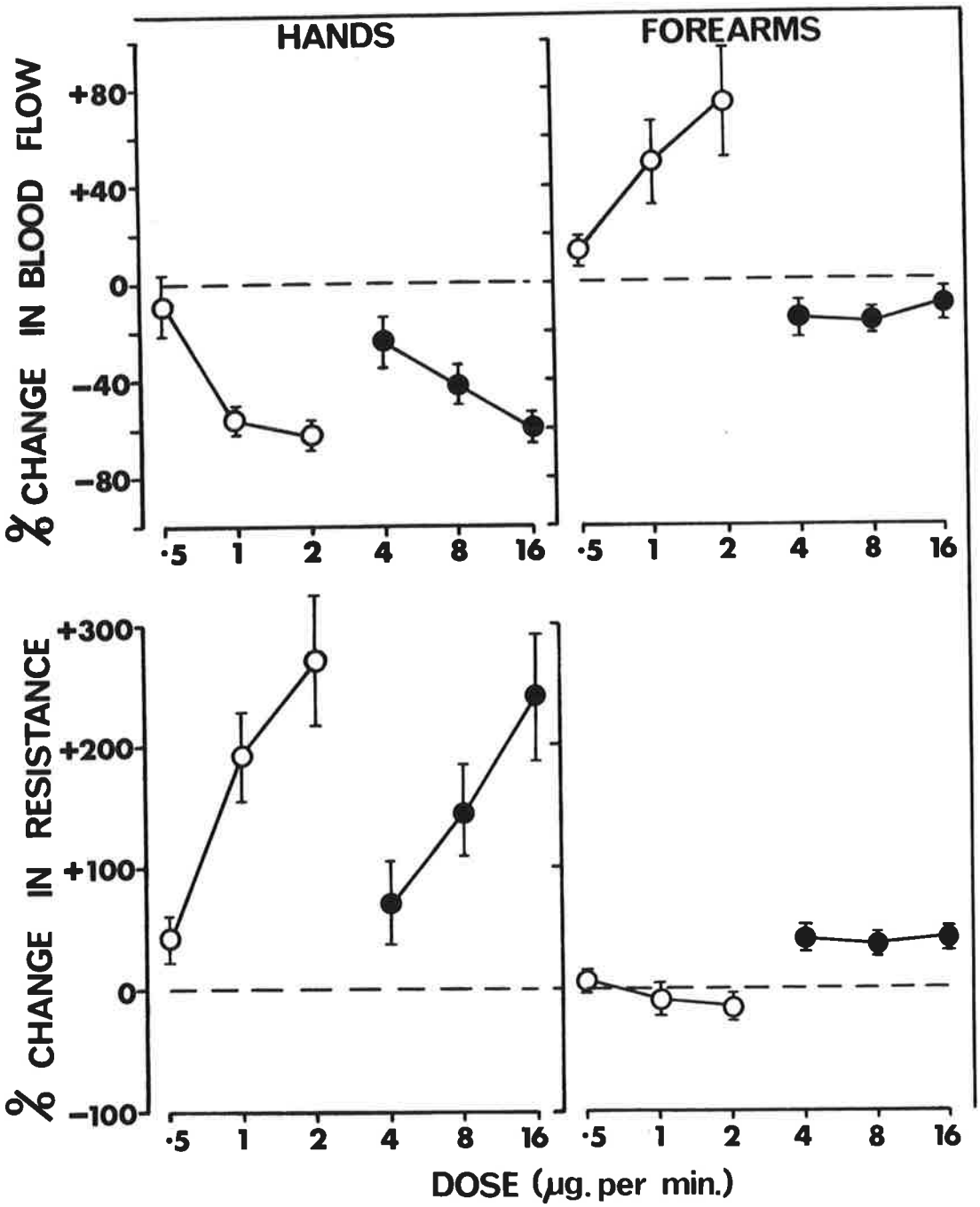

Fig. 2-5 The mean percentage changes in hand blood flow and vascular resistance (left-hand frames) and forearm blood flow and vascular resistance (right-hand frames) during intravenous infusions of three doses of angiotensin (O) and three doses of noradrenaline (●) in seven subjects. The vertical lines through each point represent one standard error on either side of the mean.

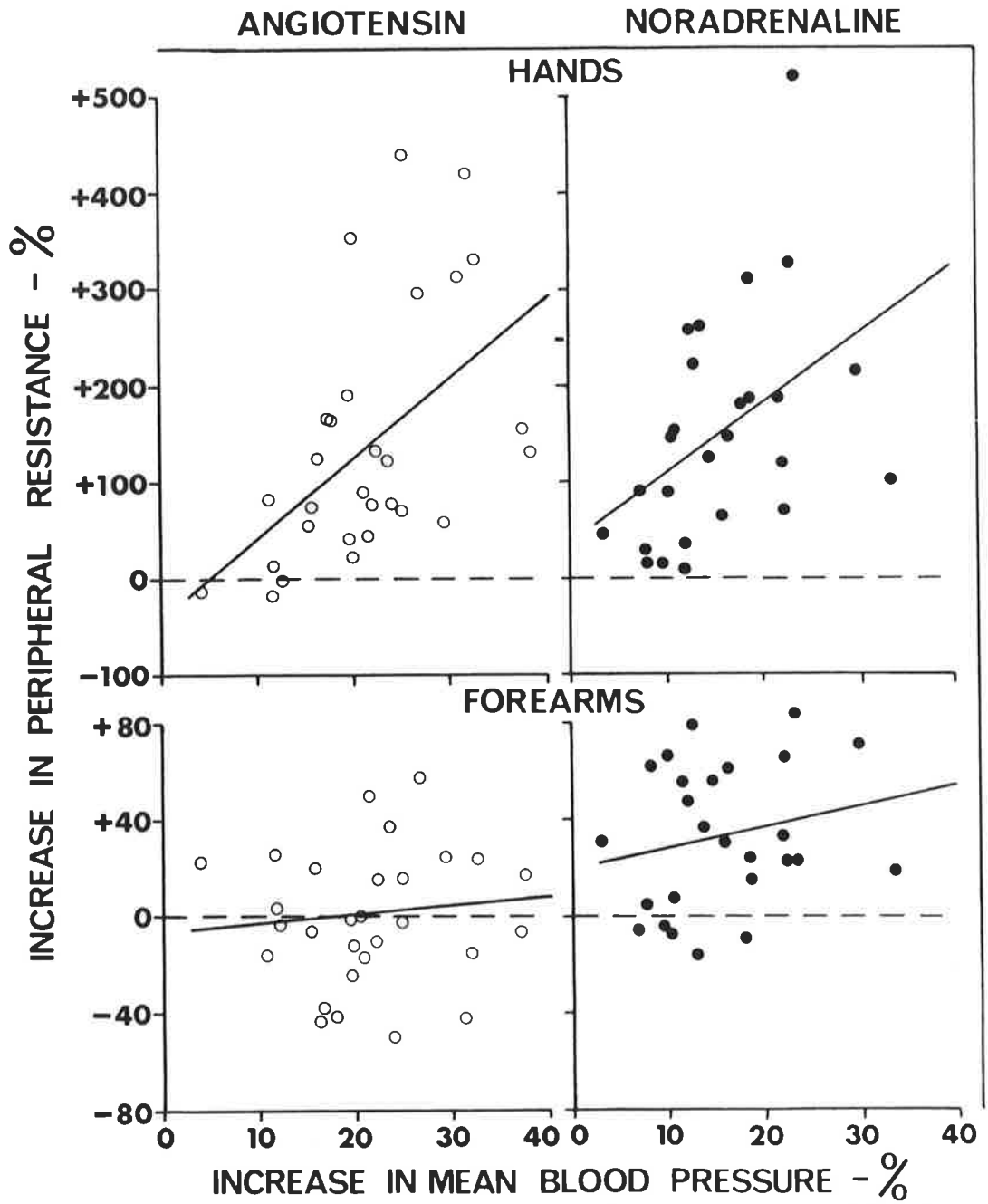

Fig. 2-6 Relationship between percentage increase in mean blood pressure and percentage change in vascular resistance in the hand (upper frames) and the forearm (lower frames) during intravenous infusions in ten subjects of angiotensin (O) in doses ranging from 0.5 to 2.0 µg./min., and noradrenaline (●) in doses ranging from 4.0 to 16.0 µg./min. The regression lines for angiotensin (upper frame, y = 8.6x - 45.01; lower frame, y = 0.28x + 35.25; lower frame, y = 0.805x + 19.34) are shown.

$n = 26$, $p < 0.05$) and there was no significant difference between their regression coefficients ("t" test, $0.7 < p < 0.8$).

Forearm Vessel Responses:-

a. Intra-arterial infusions. Both angiotensin and noradrenaline exhibited a dose-dependent vasoconstrictor action. As in the hand the constrictor effect of angiotensin was slower to develop and persisted for a longer period after the infusion, especially with larger doses.

Fig. 2-3 (right-hand frame) illustrates the percentage falls in forearm blood flow in response to intra-arterial infusion of angiotensin (2.0, 4.0 and 8.0 ng. per min. for 4 min.) and of noradrenaline (16.0, 32.0 and 64.0 ng. per min. for 4 min.). The plotted values are the averaged responses from 5 subjects, in whom each dose of angiotensin and noradrenaline was given twice.

A comparison of the dose-response curves obtained with the two drugs indicates that, with regard to their local vasoconstrictor action on the forearm vessels, angiotensin is approximately four times more potent than noradrenaline.

b. Intravenous infusions. During noradrenaline infusions the forearm flow initially increased then fell. After the infusion

ceased the flow rose to exceed the control value before returning to the pre-infusion level (Fig. 2-4). Forearm vascular resistance rose and had recovered within 3-5 min. after the end of the infusion. With angiotensin the forearm blood flow usually rose to about double the resting level and the effect lasted for up to 10 min. after the infusion had ceased. Calculated forearm resistance fell slightly, but the change was not statistically significant (Fig. 2-4).

Fig. 2-5 illustrates the percentage changes in forearm blood flow (upper right-hand frame) and calculated forearm vascular resistance (lower right-hand frame) in response to intravenous infusion of angiotensin (0.5, 1.0 and 2.0 µg. per min. for 5 min.) and of noradrenaline (4.0, 8.0 and 16.0 µg. per min. for 5 min.). Each value plotted is the averaged response obtained from seven subjects. During angiotensin infusions the forearm blood flow increased, the effect increasing with increasing doses. The calculated forearm resistance, however, did not exhibit any significant change. The increase in forearm blood flow was therefore probably the result of the increase in perfusion pressure, but an active dilator effect of angiotensin on muscle vessels, offset by skin vessel constriction, cannot be excluded. Noradrenaline caused a slight fall in forearm blood flow and approximately a 40% increase in vascular resistance, neither of which showed a relation-

ship to dose. Noradrenaline intravenously often causes an increase in muscle blood flow which may offset the constriction in skin and result in little change in total forearm flow (Cooper, Fewings, Hodge, Scroop and Whelan, 1964) and since the muscle dilator action increases with increasing doses it could account for the constancy of the forearm flow and resistance changes at all dose levels.

A comparison of the dose-response curves obtained indicates that on intravenous infusion angiotensin is a dilator of forearm vessels and noradrenaline an overall constrictor. This is in contrast to the intra-arterial results where angiotensin was approximately four times more constrictor than noradrenaline. This shift in the relative potency of the two drugs on the forearm vessels, with angiotensin becoming less constrictor on intravenous administration, is in direct contrast to the hand vessel results where the shift in relative potency was such that angiotensin became more constrictor on intravenous infusion.

To facilitate this comparison the blood flow changes in the hand and forearm during both intra-arterial and intravenous infusions of angiotensin and noradrenaline have been grouped together in Fig. 2-7.

Fig. 2-6 (lower frames) illustrates the relationship be-

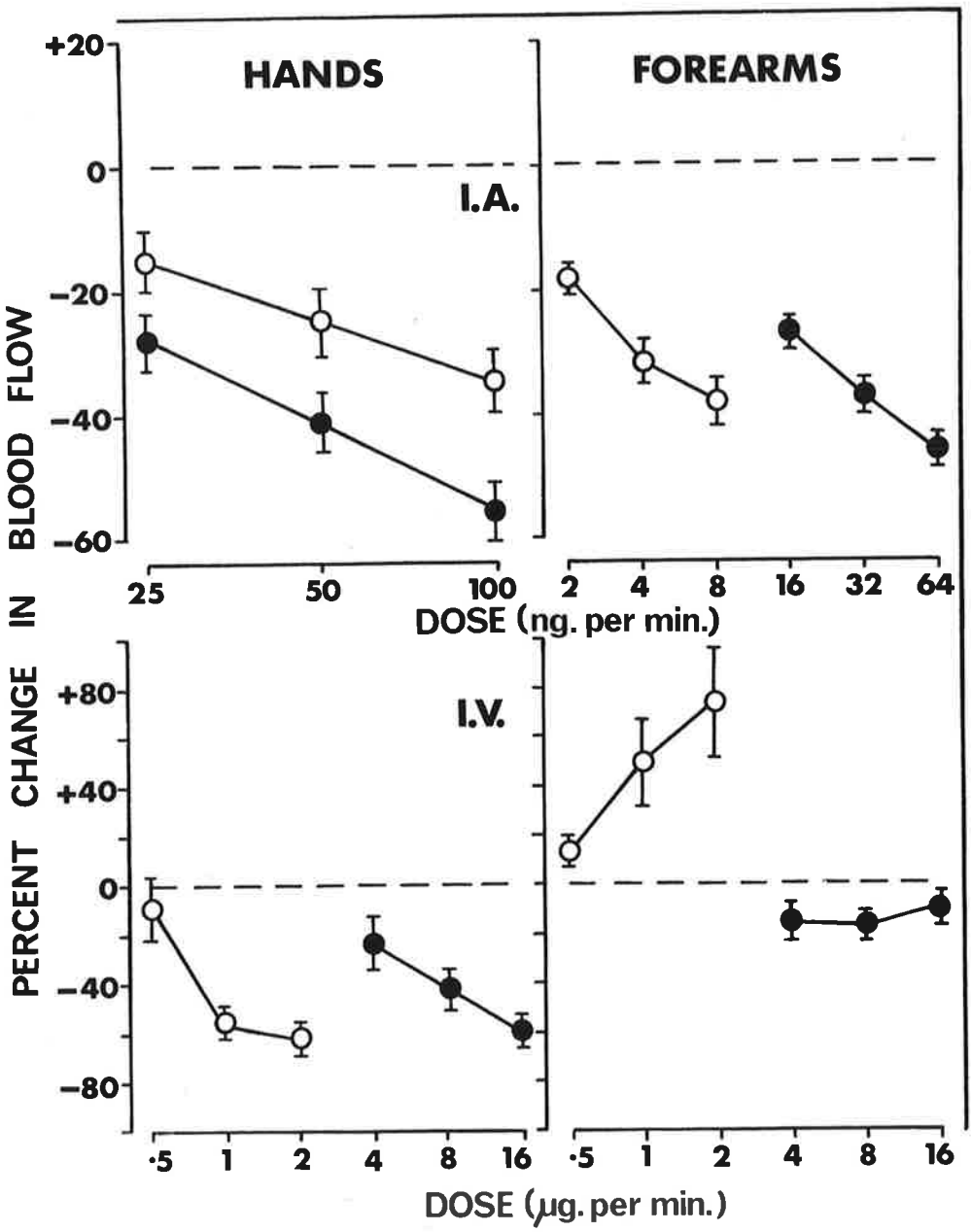

Fig. 2-7 The mean percentage changes in hand blood flow (left-hand frames) and forearm blood flow (right-hand frames) during intra-arterial (upper frames) and intravenous (lower frames) infusions of angiotensin (O) and noradrenaline (●) in the doses indicated. The intra-arterial results were obtained from five subjects in each case (see legend Fig. 2-3). The intravenous results were obtained from the same seven subjects. The vertical lines through each point represent one standard error on either side of the mean.

tween percentage change in forearm vascular resistance and percentage increase in mean blood pressure for all doses of the two drugs and contains results from ten subjects, including the seven mentioned above and three others in whom for various reasons the full set of six infusions was not given. Each point in the figure is an individual value from one of the ten subjects. No significant correlation was found with either angiotensin ($r = + 0.069$, $n = 29$, $p > 0.1$) or noradrenaline ($r = + 0.198$, $n = 26$, $p > 0.1$).

Blood Pressure:-

The intravenous infusion of both angiotensin and noradrenaline elevated mean blood pressure. When doses of the two drugs were chosen which produced similar elevations in systolic blood pressure (Fig. 2-4) the diastolic blood pressure always rose more quickly and to a higher level with angiotensin and the rise in mean blood pressure was correspondingly greater. After noradrenaline infusion both the systolic and diastolic pressures returned to pre-infusion levels more rapidly than after angiotensin.

Fig. 2-8 shows the percentage increases in mean blood pressure in response to intravenous infusion of angiotensin (0.5, 1.0 and 2.0 µg. per min. for 5 min.) and of noradrenaline (4.0, 8.0 and 16 µg. per min. for 5 min.), each plotted value being the averaged

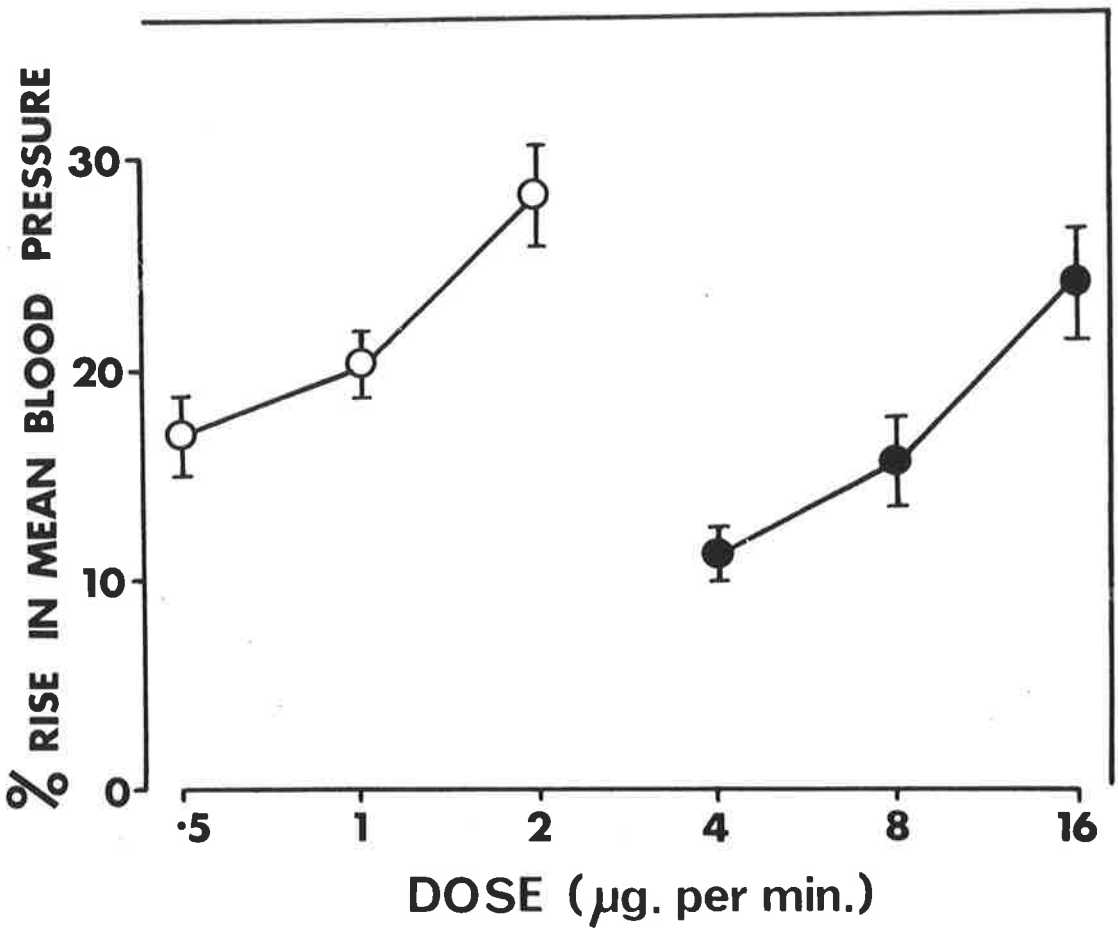

Fig. 2-8 The percentage rise in mean blood pressure following intravenous infusions of three doses of angiotensin (O) and three doses of noradrenaline (●). Each point represents the mean of seven subjects and the vertical line through each point represents one standard error on either side of the mean.

response from seven subjects. On a weight basis angiotensin was approximately fifteen times more effective in elevating mean blood pressure than was noradrenaline.

Heart Rate:-

The heart rate was reduced during most intravenous infusions of angiotensin and in all cases with noradrenaline but for similar increases in mean blood pressure the effect was greater with noradrenaline (Fig. 2-4).

Fig. 2-9 shows the relationship between the increase in mean blood pressure and the bradycardia for all doses of the two drugs. The figure includes the results from the seven subjects mentioned above and a further ten, who, for various reasons, did not receive the full set of six drug infusions. In the case of noradrenaline there is a close relationship ($r = -0.57$, $n = 35$, $p < 0.001$) but no such relationship was found with angiotensin ($r = +0.955$, $n = 39$, $p > 0.1$).

Central Venous Pressure:-

The central venous pressure and brachial arterial blood pressure were simultaneously recorded in six subjects and intravenous infusions of various doses of angiotensin and noradrenaline given. The central venous pressure rose with both drugs but this

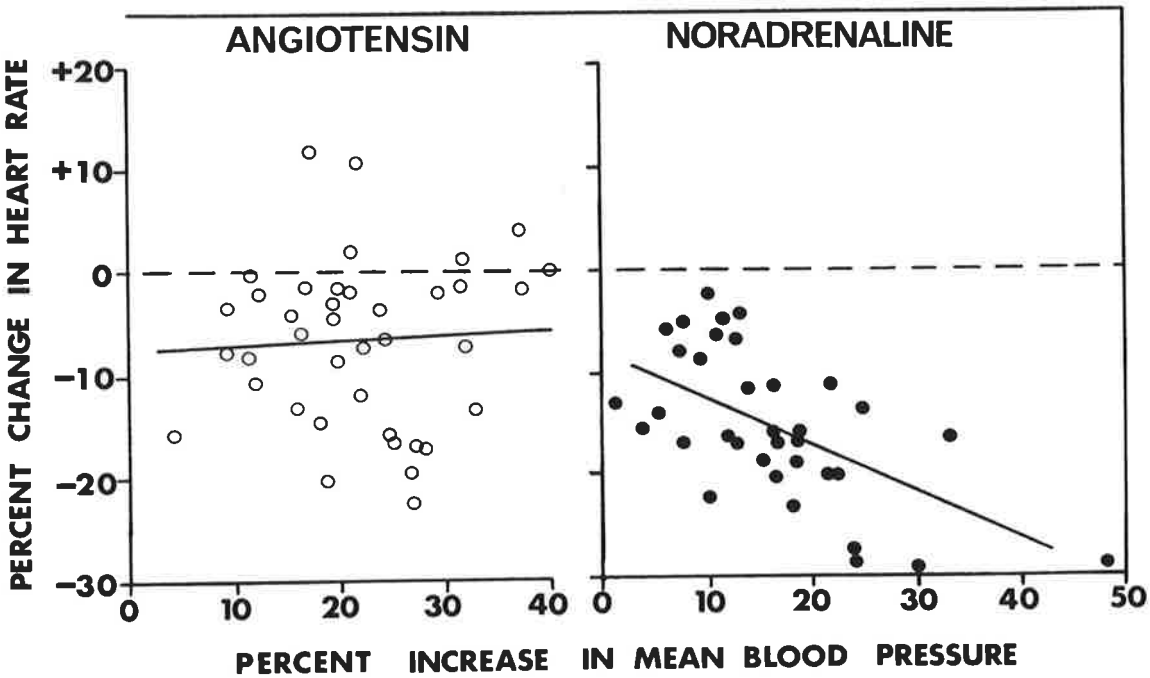

Fig. 2-9 The relationship between percentage change in heart rate and percentage increase in mean blood pressure in seventeen subjects during intravenous infusions of angiotensin (O) in doses ranging from 0.5 to 2.0 µg./min., and noradrenaline (●) in doses ranging from 2.5 to 16.0 µg./min. The regression lines for angiotensin ($y = 0.052x - 7.621$) and noradrenaline ($y = -0.449x - 8.02$) are shown.

was much more marked with noradrenaline for a given rise in mean blood pressure. In Fig. 2-10 the increase in central venous pressure in cm. of water is plotted against increase in mean arterial pressure for both drugs. A positive correlation was found for noradrenaline ($r = + 0.736$, $n = 9$, $p < 0.05$) but no significant relationship appeared with angiotensin ($r = + 0.318$, $n = 10$, $p > 0.1$) and there was a significant difference between their regression coefficients ("t" test, $p < 0.001$).

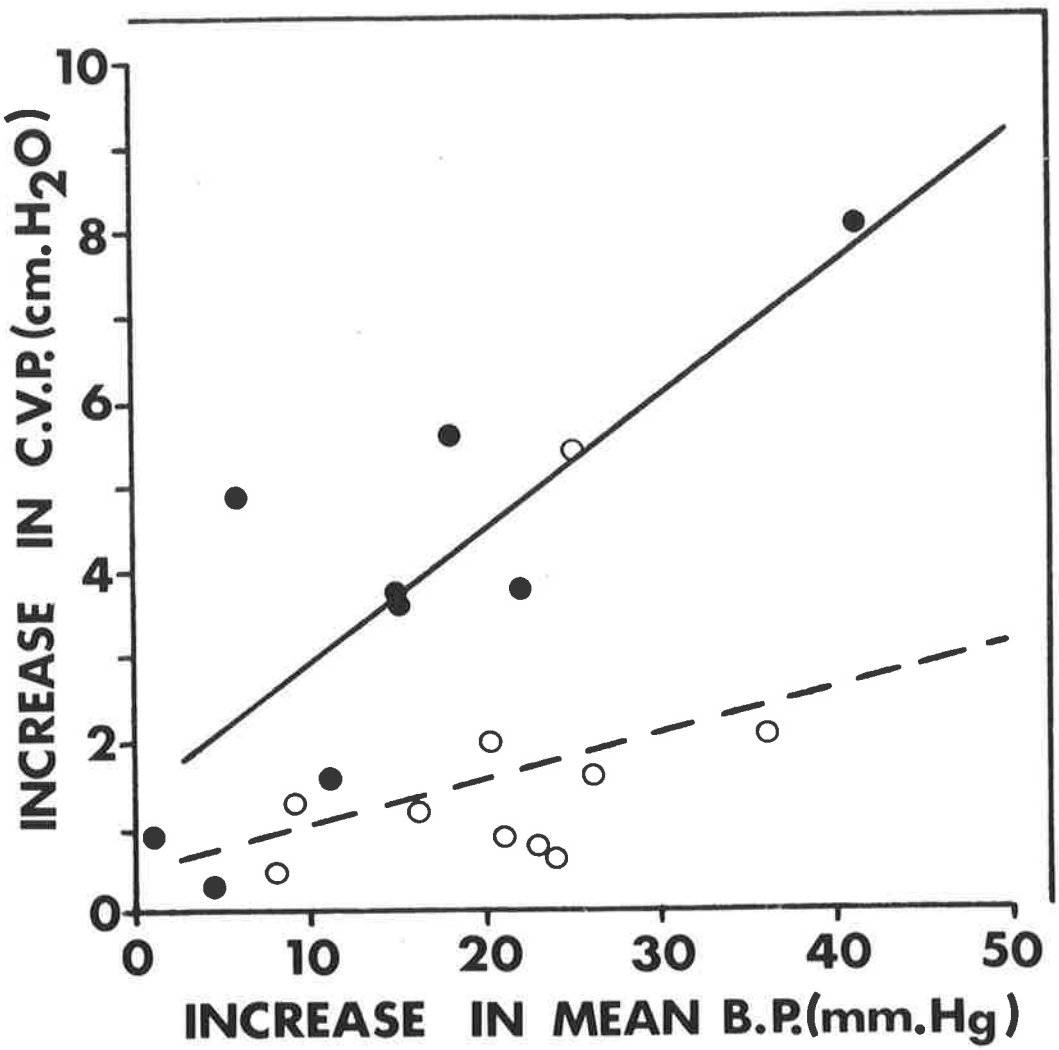

Fig. 2-10 Relationship between the rise in central venous pressure and the rise in mean blood pressure in seven subjects during intravenous infusion of angiotensin (O) in doses ranging from 0.5 to 2.0 μg./min., and noradrenaline (●) in doses ranging from 2.5 to 10.0 μg./min. The regression lines for noradrenaline (solid line, $y = 0.126x + 1.53$) and angiotensin (dotted line, $y = 0.0372x + 0.78$) are shown.

DISCUSSION

The most striking feature to emerge from this study, when the results from intra-arterial and intravenous administration were compared, was the change in the ratios of the relative constrictor activities of the two drugs in the peripheral vessels (Fig. 2-7.).

With regard to the hand vessels both angiotensin and noradrenaline reduced hand blood flow, whether administered intra-arterially or intravenously. However, intra-arterial angiotensin was about one half as effective as noradrenaline, whereas on intravenous infusion it became approximately eight times more potent.

These experiments provide no explanation for this difference in potency with the two routes of administration, although a number of possibilities exist. Since the rates of inactivation of angiotensin and noradrenaline are very similar, the relative potencies of their direct actions should be unchanged by the route of administration. This would suggest that the observed enhancement of the constrictor action of angiotensin on intravenous infusion is due to the activation of some indirect mechanism, either adding to the direct constrictor action of angiotensin or opposing that of noradrenaline.

There are a number of factors which might oppose the direct constrictor action of noradrenaline. With equipressor doses of the two drugs the eight-fold discrepancy remains so that a high pressure baroreceptor reflex is unlikely to be involved. However, with such doses, noradrenaline raises central venous pressure more than does angiotensin and this could result in a reflex from the low-pressure baroreceptors with release of sympathetic tone in the hand vessels as has been shown to occur in the forearm (Roddie, Shepherd and Whelan, 1957). Noradrenaline has also been shown to have a central effect in inhibiting sympathetic tone (Taylor and Page, 1951) and this may oppose the direct constrictor action of the drug. Furthermore, Lever, Mowbray and Peart (1961) postulate the release of a vasodilator substance by intravenous noradrenaline, so that both a neural and a humoral mechanism could be activated on intravenous infusion and oppose the direct constrictor action of the drug.

The discrepancy between the constrictor potencies of the two drugs on the hand vessels when given by the two routes could also be accounted for if intravenous angiotensin enhanced its direct constrictor action by activating some indirect constrictor mechanism. This indirect effect might be a central one on the vasomotor centres since Bickerton and Buckley (1961) have found that a large part of the pressor action of angiotensin in the dog

is due to a central action mediated peripherally through the sympathetic nervous system. Laverty (1963) has found similar evidence for a sympathetically-mediated vasoconstriction in the hind-limb of the anaesthetized rat during intravenous infusions of angiotensin which he concludes is due to a central action of the drug. De Pasquale and Burch (1963) have found evidence for a sympathetically-mediated venoconstrictor action of angiotensin in man. The work of Benelli, Della Bella and Gandini (1964) in the guinea-pig and Zimmerman (1962) and Zimmerman and Gomez (1965) in the dog suggests that the interaction between angiotensin and the sympathetic nervous system is a peripheral postganglionic one. However, such an action would contribute equally to the responses on intra-arterial and intravenous infusion and would not explain the present results in man. The possibility of the release of a second vasoconstrictor substance also needs to be considered since a number of workers have demonstrated the release of adrenal-medullary hormones by angiotensin in animals (Braun-Menendez, Fasciolo, Leloir and Munoz, 1940 b; Cession and Cession-Fossion, 1963; Feldberg and Lewis, 1963, 1964). However, it is not known whether angiotensin releases adrenal catecholamines in man although the available evidence would suggest that it does not, at least in the doses used in the present experiments (Vincent, Kashemsant, Cuddy, Fried, Smulyan and Eich, 1965).

A difference between intra-arterial and intravenous

potencies was also observed in the case of the forearm vessels. However, it was the reverse of the hand vessel results, in that intra-arterially angiotensin had four times the constrictor action of noradrenaline whereas intravenously noradrenaline was more constrictor (Fig. 2-7). If an indirect mechanism is involved here, with either angiotensin or noradrenaline, any hypothesis offered to explain it would be contrary to that offered with regard to the findings in the hand vessels, with the possible exception of the adrenal-medullary stimulating action of angiotensin (Feldberg and Lewis, 1963, 1964). If the indirect mechanism were the release of adrenaline then this hormone, with its constrictor effects on skin vessels and dilator effects on muscle vessels could explain both the hand and forearm findings.

A further point of interest emerged from the intra-arterial studies. When the effects of intra-arterial infusions of angiotensin and noradrenaline on the forearm and hand vessels are compared it is notable that the degree of constriction produced with equal doses of noradrenaline is the same for the two vascular beds (Fig. 2-3) whereas with angiotensin similar responses in the forearm and hand were only obtained if twenty-five times the dose was given to the hand vessels. This could imply a genuine difference in forearm and hand vascular sensitivities to angiotensin or a different mechanism of action in each vascular bed. It might also mean that

angiotensin is rapidly inactivated in transit from the point of infusion at the elbow, to the hand vessels, as occurs with bradykinin (Saamell and Eskes, 1962) and acetylcholine (Duff, Greenfield, Shepherd and Thompson, 1953).

The contribution of the limb vessel responses to the overall increase in peripheral vascular resistance during angiotensin infusions is probably a minor one. On intravenous infusion the hand blood flow was reduced with both drugs and there was a parallel increase in vascular resistance. In the case of the forearms, however, intravenous angiotensin resulted in an increase in blood flow which was probably due to dilatation of muscle vessels (Bock, Krecke and Kuhn, 1958) and forearm vascular resistance did not alter significantly. Thus, while constriction of the hand vessels, which are mainly skin, may contribute to the pressor effect of angiotensin, any constriction of skin vessels in the forearm is offset by the dilatation, either active or passive, in the underlying muscle. It is probable, therefore, that the major rise in vascular resistance during intravenous infusions of angiotensin lies in vascular beds other than those of the limbs.

The observations of the relative effects of angiotensin and noradrenaline on the arterial pressure are similar to those of other authors although the difference in relative potencies found

in the present experiments is larger (Finnerty, Massaro, Chupkovich and Tuckman, 1961; De Bono, Lee, Mottram, Pickering, Brown, Keen, Peart and Sanderson, 1963). Angiotensin is approximately fifteen times more potent when the doses of the drugs are expressed by weights. If the comparison is made on a molar basis, angiotensin is seen to be approximately eight times more pressor than noradrenaline, but since the "molecule-to-receptor" relationship is unknown the weight basis for comparison is probably more appropriate.

The bradycardia seen with noradrenaline infusions can be attributed to a reflex vagal effect (Goldenberg, Pines, Baldwin, Green and Roh, 1948) initiated by the rise in arterial pressure with which it shows a highly significant correlation. The bradycardia with angiotensin is probably also vagal in origin (Wilkins and Duncan, 1941) but it is less marked than with noradrenaline when given in approximately equipressor doses and shows no correlation with the pressor response, suggesting that angiotensin may have a stimulating action on the heart which opposes a reflex slowing. Such a stimulating action is unlikely to be a direct effect of the drug since Koch-Weser (1964), Hill and Andrus (1940) and Lorber (1942) have observed no positive chronotropic effect on the heart of the kitten, frog, dog or cat. Release of adrenal medullary hormones by angiotensin (Feldberg and Lewis, 1964) could play a part in countering the reflex bradycardia. A central action of

angiotensin is indicated by the work of Bickerton and Buckley (1961), among others, and this supports the suggestion of Nishith, Davis and Youmans (1962) that angiotensin may have a central cardio-accelerator action, exerted peripherally through the sympathetic nerves to the heart. If such a stimulating effect increased with increasing doses of angiotensin it could account for the observation that the bradycardia shows no correlation with the degree of hypertension. An inconsistent bradycardia was reported by Tigerstedt and Bergman (1898) in their initial observations on renin and has since been reported by other authors with angiotensin itself (Sannerstedt, Bojs and Varnauskas, 1963). The lesser degree of bradycardia with angiotensin possibly accounts for the greater rise in diastolic pressure with this drug than with noradrenaline when the increases in systolic pressure are equal (Fig. 2-4).

The difference between angiotensin and noradrenaline in the degree to which they increase central venous pressure is unexplained. The increase with both drugs is probably due to the bradycardia since it is reduced or abolished by atropine (Bock and Gross, 1961) and the greater rise in venous pressure with noradrenaline may be related to the more marked bradycardia produced by this drug. It may also be relevant that angiotensin is not as marked a constrictor of capacitance vessels as is noradrenaline

(Folkow, Johansson and Mellander, 1961; Haddy, Molnar, Borden and Texter, 1962; De Pasquale and Burch, 1963).

This evidence of an indirect mechanism in the vasoconstrictor action of angiotensin prompted further exploration and this will be discussed in the next section of the thesis.

SUMMARY

1. Intravenous and intra-arterial infusions of noradrenaline and angiotensin were given in normal human subjects and the changes in brachial arterial blood pressure, central venous pressure, heart rate and hand and forearm blood flow were recorded.

2. In the hand vessels, intra-arterial angiotensin was less constrictor than noradrenaline (angiotensin:noradrenaline = 1:2-3) whereas on intravenous infusion angiotensin was much more constrictor than noradrenaline (angiotensin:noradrenaline = 8-10:1).

3. In the forearm vessels, intra-arterial angiotensin was more constrictor than noradrenaline (angiotensin:noradrenaline = 4:1) whereas on intravenous infusion angiotensin induced a passive increase in forearm blood flow whilst noradrenaline caused an overall reduction in forearm blood flow and an increase in vascular resistance.

4. When doses were expressed on a weight basis angiotensin was found to be fifteen times more effective in raising the arterial blood pressure than was noradrenaline. The diastolic and mean pressures were elevated to a lesser degree by noradrenaline

than by angiotensin with doses which had the same effects on systolic pressure.

5. The bradycardia which accompanied noradrenaline infusions showed a correlation with the rise in mean arterial pressure while that with angiotensin was less marked and did not correlate with the pressure rise.

6. Central venous pressure was increased with both drugs, the rise being more marked with noradrenaline for a given rise in mean arterial pressure. A positive correlation between venous and arterial pressure rise was found with noradrenaline but not with angiotensin infusions.

7. The possibility of the involvement of central vasomotor effects and release of adrenal medullary hormones in the cardiovascular actions of angiotensin was discussed.

SECTION 3

INDIRECT CONSTRICTOR MECHANISM OF ANGIOTENSIN IN MAN

PART A

EVIDENCE FOR PREGANGLIONIC SYMPATHETIC STIMULATION DURING INTRAVENOUS INFUSIONS OF ANGIOTENSIN IN MAN.

INTRODUCTION

In the preceding section evidence was presented in support of an indirect mechanism of action of angiotensin, activated by intravenous infusion and contributing to the hand vessel constriction. Most of the indirect mechanisms that have been suggested from animal experimentation rely upon alpha-adrenergic receptor stimulation, whether this be by transmitter released from the sympathetic nerve terminals as a result of pre- or postganglionic stimulation (Bickerton and Buckley, 1961; Halliday and Buckley, 1962; Zimmerman, 1962; Benelli, Della Bella and Gandini, 1964; Laverty, 1963; Nishith, Davis and Youmans, 1962; McGiff and Fasy, 1964, 1965; Lewis and Reit, 1965) or by catecholamine released by angiotensin from the adrenal medulla (Braun-Menendez, Fasciolo, Leloir and Munoz, 1940b; Renson, Barac and Bacq, 1959; Cession and Cession-Fossion, 1963; Feldberg and Lewis, 1964).

Despite this and other evidence from animal studies there is little from studies in man to support the suggestion of an indirect mechanism contributing to the vasoconstrictor action of angiotensin, with the exception of aldosterone release which is unlikely to be important in short term infusions. Vincent, Kashemsant, Cuddy, Fried, Smulyan and Eich (1965) found no change in urinary vanillylmandelic acid levels during pressor infusions of angiotensin in man

using doses similar to those described in the previous section of this thesis. Furthermore, the pressor response to intravenous infusions of angiotensin is not obviously modified in adrenalectomized patients (Statius van Eps, Smorenberg-Schoorl, Zurcher-Mulder, de Vries and Borst, 1962; Biron, 1964). The only evidence in support of a sympathetic stimulating action of angiotensin in man is that of De Pasquale and Burch (1963). They found that the constriction of forearm venous segments during systemic infusions of angiotensin was entirely abolished by sympathetic blockade. However, Laurence and Nagle (1963) were unable to significantly modify the pressor response to intravenous angiotensin in man by prior administration of bretylium or guanethidine.

In the present study the responses of the hand vessels during intravenous infusions of angiotensin before and after treatment of the hand with sympathetic and adrenergic blocking drugs and in sympathectomized, denervated and nerve-blocked limbs demonstrate that angiotensin has a central stimulant action on the sympathetic nervous system in man. The effect of lumbar sympathectomy on the foot vessel responses to intravenous angiotensin was also determined in one subject. To exclude the possibility of a peripheral sympathetic stimulating action in the hand vessels the response to intra-arterial angiotensin was determined before and after alpha-adrenergic receptor-blockade and sympathetic denervation.

METHODS

General:-

The subjects for all experiments rested recumbent on a couch in a temperature-controlled laboratory for at least one hour before observations began, during which time recording apparatus was applied and infusion needles inserted.

Intra-arterial drug infusions were administered into the brachial artery at the elbow and intravenous infusions through a polythene catheter inserted into an ante-cubital vein. Saline (0.9%, w/v) was given throughout the control periods and was also used as a vehicle for the drugs. Blood flow in both hands or feet (Fig. 3-1) was recorded three to five times every min. by venous occlusion plethysmography using water-filled, temperature-controlled plethysmographs.

Arterial blood pressure was recorded with a Statham P 23Dc transducer attached to an arterial catheter as described in the methods and hand and foot vascular resistance calculated from the ratio:-

$$\frac{\text{mean blood pressure (mm. Hg.)}}{\text{blood flow (ml./100ml. hand or foot/min.)}}$$

Anaesthetic block of the nerves to the forearm and hand

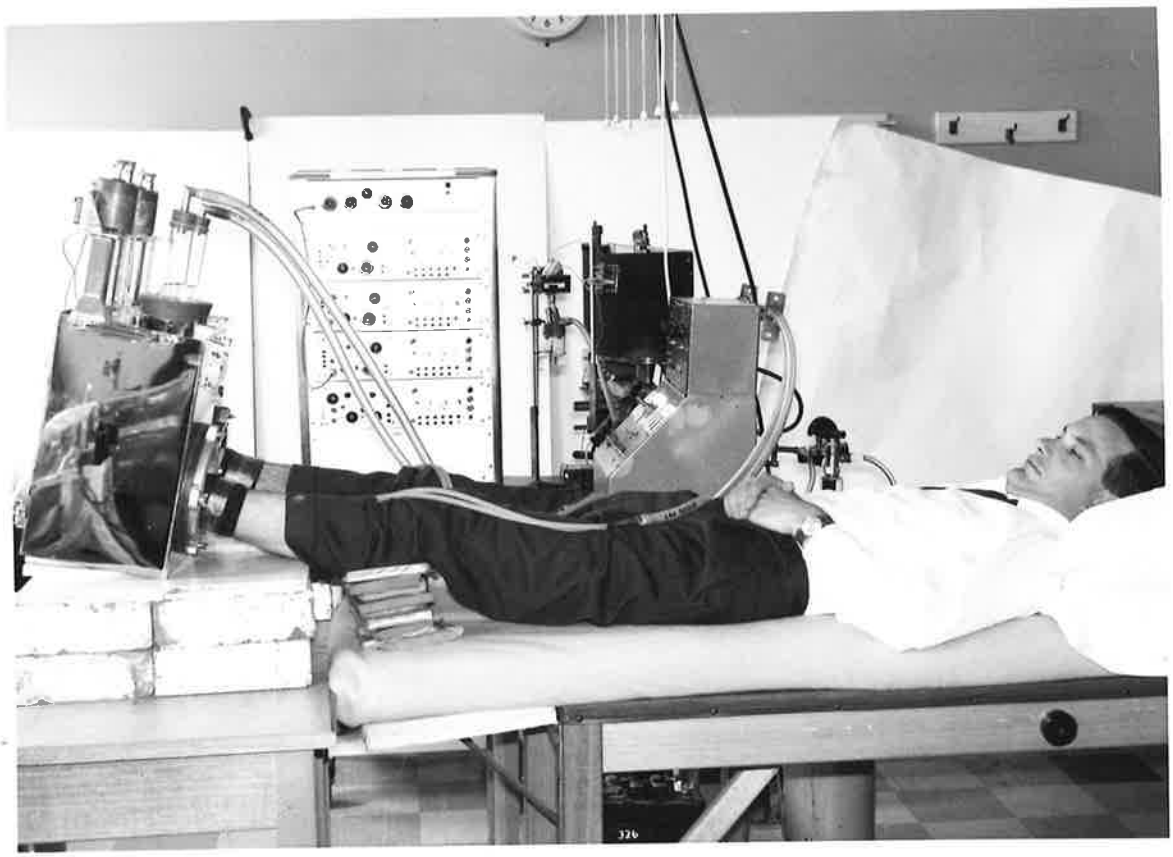

Fig. 3-1 The arrangement of equipment for the measurement of bilateral foot blood flow.

was carried out using the technique described by Webling (1960). Lignocaine (20-25 ml. of 1.0%) containing adrenaline (1:200,000) was infiltrated on either side of and behind the brachial artery just below the level of the anterior axillary fold. The block became complete in 15-30 min. and its effectiveness was determined by loss of sensation in the skin of the hand and forearm, loss of motor power, and the absence of a constrictor response of the hand vessels following application of ice to the neck (Fewings, Hodge, Scroop and Whelan, 1964).

The drugs used were angiotensin II (val^5-hypertensin II-asp-β-amide, Hypertensin, Ciba) noradrenaline bitartrate monohydrate (Levophed, Winthrop), adrenaline hydrochloride (D.H.A.), phenoxybenzamine hydrochloride (Dibenyline, S.K.F.) and bretylium tosylate (Darenthin, B.W.). Doses of noradrenaline and adrenaline are expressed as weights of their bases and of angiotensin, phenoxybenzamine and bretylium as weights of their salts. Ascorbic acid (1:50,000) was added to the noradrenaline and adrenaline solutions.

Subjects:-

Normal students and colleagues acted as subjects in those experiments where the effect of acute alpha-receptor blockade and sympathetic denervation on the responses of the hand vessels to angiotensin was being examined. The subjects were kept warm and

the room temperature high (25-30°C.) in order to reduce the resting vasomotor tone and minimize the increase in flow above the control side which followed administration of blocking agents to one hand.

Eight patients were also studied:-

(a) Surgical sympathectomy (three patients). Two patients (W.W. and R.T.) had undergone bilateral cervical sympathectomy for mild Raynaud's phenomenon and one of these (R. Taylor) was studied before and after sympathectomy. A further patient (D.B.) had undergone unilateral lumbar sympathectomy for mild Raynaud's phenomenon in the feet and was also studied before and after operation.

(b) Traumatic sympathectomy (two patients). One of the patients (H.v.d.S.) had sustained a unilateral brachial plexus avulsion with complete denervation nine months previously and the other (R. Tree) a complete cervical cord transection at C6-7 four years previously constituting a preganglionic sympathectomy.

(c) Idiopathic sympathectomy (three patients). All three patients (A.H., E.H. and P.B.) in this group suffered from idiopathic autonomic nervous system degeneration.

The completeness of sympathetic denervation was determined by one or more of the following tests:-

(1) The absence of a constrictor response in the hand ves-

sels during intra-arterial infusions of tyramine and ephedrine and following the application of ice to the neck. These stimuli (with the exception of tyramine and ephedrine in the preganglionic sympathectomy) normally produce a fall in hand blood flow which is dependent upon an intact sympathetic nerve supply (Parks, Skinner and Whelan, 1961; Whelan, 1967).

(2) The response of systemic arterial pressure, heart rate and forearm vascular resistance during postural changes and Valsalva's manouvre (Sharpey-Schafer, 1953; Roddie, Shepherd and Whelan, 1958; Mason, Kopin and Braunwald, 1966).

(3) The absence of sweating on indirect body-heating (Guttman, 1947).

In most experiments the constrictor response to noradrenaline was used as a monitor, since the unchanged response following the various forms of sympathetic denervation indicated that the vessels were capable of responding to a direct acting drug.

RESULTS

Intravenous Infusions:-

In the normal subject intravenous infusions of angiotensin caused an elevation in blood pressure and a reduction in hand blood flow, the degree and time course of which were symmetrical in the two hands (Fig. 3-2). The responses of one hand could thus be used as a control for the other when this had been sympathectomized, nerve-blocked, or subjected to the influence of autonomic blocking agents given by infusion into the brachial artery. With regard to the latter their action was confined to the limb into which they were infused and they did not enter the circulation in sufficient amounts to have any generalized effects.

(a) Phenoxybenzamine; Fig. 3-2 illustrates the results obtained from one of four subjects who received the alpha-receptor-blocking agent phenoxybenzamine (0.5 mg./min. for 3 min.) into the brachial artery of one arm. The fall in hand blood flow during intravenous angiotensin (1.0 µg./min. for 5 min.) was almost abolished on the treated side, while that of the control side was unimpaired. In every case the constrictor responses of the hand vessels to ice on the neck or to intravenous noradrenaline (10.0 µg./min. for 5 min.) were abolished or very much reduced on the blocked side, demonstrating the effectiveness of alpha-receptor blockade. The blood flow and resistance changes in the control and treated hands

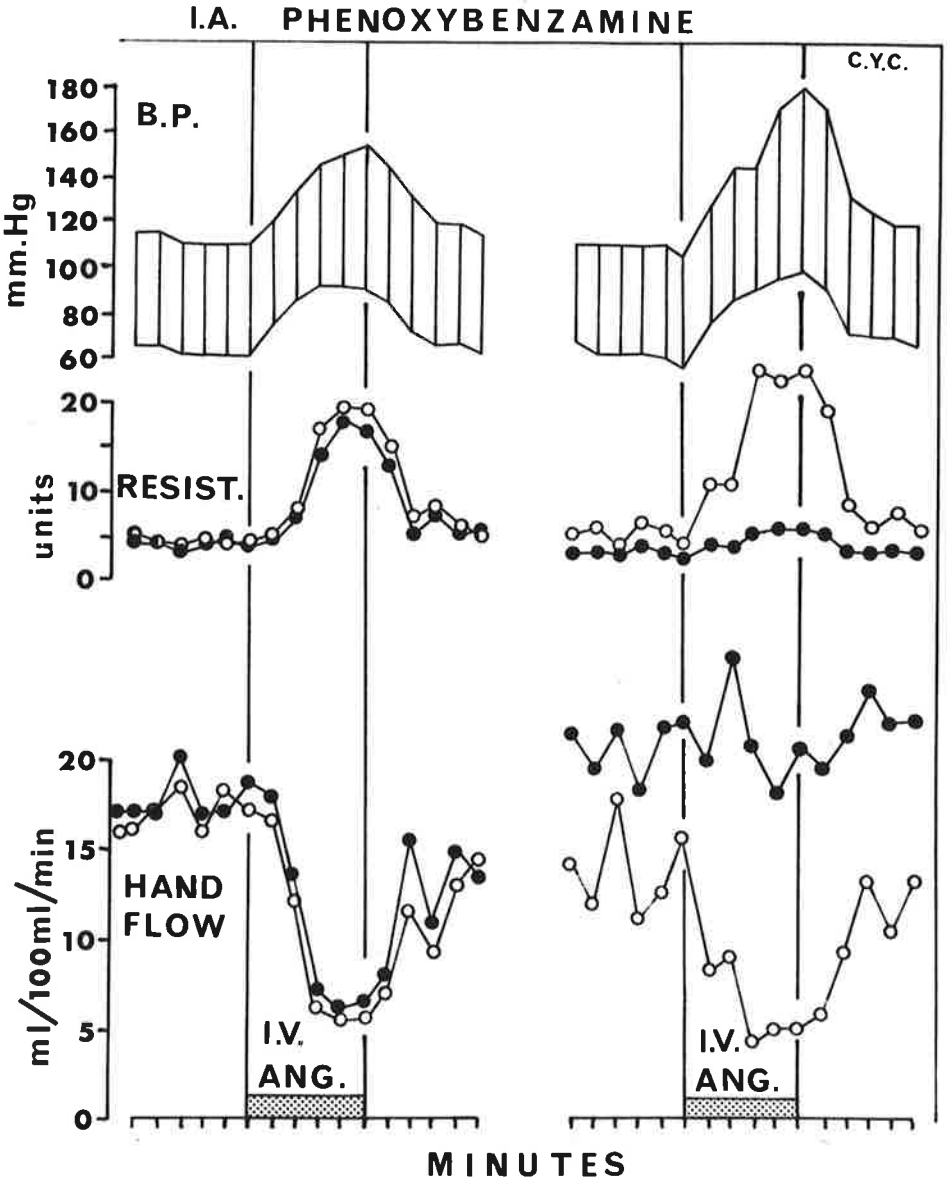

Fig. 3-2 The changes in arterial blood pressure, vascular resistance and blood flow in the left (●) and right (O) hands of subject C.Y.C. during intravenous infusions of angiotensin (1.0 μg./min. for 5 min.) before (left of figure) and after (right of figure) introduction of phenoxybenzamine (0.5 mg./min. for 3 min.) into the brachial artery of the left arm.

during angiotensin infusion in each subject are summarized in Table I.

(b) Bretylium tosylate. Fig. 3-3 illustrates the results of one experiment in which bretylium tosylate (4.0 mg./min. for 5 min.) was infused intra-arterially into one hand 60 min. prior to intravenous drug administration. The reflex constrictor response of the hand vessels of the treated side to ice applied to the neck was almost completely abolished (Cooper, Fewings, Hodge and Whelan, 1963). The responses of the vessels to intravenous adrenaline and noradrenaline were unaffected by the administration of bretylium, illustrating the ability of both hands to respond equally and symmetrically to a directly acting constrictor agent. Intravenous infusions of angiotensin (1.0 µg./min. for 5 min), caused the usual reduction in blood flow in the control hand but only slightly reduced the flow in the bretylium-treated hand. The flow and hand vascular resistance changes are summarized in Table I.

(c) Sympathectomy. Fig. 3-4 shows the effects of intravenous infusions of angiotensin and noradrenaline in one of the patients (W.W.) who had undergone bilateral cervical sympathectomy. Angiotensin (0.5 µg./min. for 5 min.) had no constrictor effect on the hand vessels. A slight increase in hand flow occurred which was presumably a passive response to the rise in blood pressure since hand vascular resistance showed little change (Table I).

Table I. The mean level of blood flow (ml./100 ml./min.) and vascular resistance (mm. Hg./ml./100 ml./min.) in the control and treated limbs in the last two minutes before intravenous infusion of angiotensin (B) and during the last two minutes of the five minute infusion period (D), together with the respective percentage changes in these parameters (+, increase; -, decrease). In the case of treatment with phenoxybenzamine, bretylium and nerve-block, the control values refer to the opposite hand measured simultaneously, both limbs having been shown to respond symmetrically to angiotensin before the various forms of treatment. The control values in the patient with brachial plexus avulsion (H.v.d.S.) and in the patient with unilateral lumbar sympathectomy (D.B.) refer to the flow measured simultaneously in the opposite normally innervated hand and foot respectively. In the case of the patient with cervical sympathectomy (W.W.), the patient with autonomic nervous system degeneration (A.R.V.H.) and the patient with cervical cord transection (R.T.), both hands were sympathetically denervated and no control was available.

TABLE I

SUBJECT	TREATMENT	CHANGE IN FLOW AND RESISTANCE											
		CONTROL						TREATED					
		Flow			Resistance			Flow			Resistance		
		B	D	%	B	D	%	B	D	%	B	D	%
C.Y.C.	PHENOXYBENZAMINE	12.3	4.8	-60	6.2	23.0	+270	20.7	20.1	- 3	3.7	5.7	+ 54
R.W.R.	PHENOXYBENZAMINE	15.4	13.1	-15	5.4	8.5	+ 57	14.8	15.1	+ 2	5.6	7.4	+ 32
C.M.S.	PHENOXYBENZAMINE	16.3	11.8	-28	4.8	8.6	+ 79	26.7	26.9	+0.7	2.9	3.8	+ 31
R.J.G.	PHENOXYBENZAMINE	31.6	11.2	-65	2.6	9.9	+280	31.6	30.8	-2.5	2.6	3.6	+ 38
M.G.H.	BRETYLIUM	17.3	8.0	-53	5.0	15.3	+206	19.5	16.6	- 15	4.4	7.3	+ 66
W.W.	CERVICAL SYMPATHECTOMY	-	-	-	-	-	-	9.8	12.4	+ 27	8.2	9.3	+ 13
D.B.	LUMBAR SYMPATHECTOMY	2.6	1.5	-42	41.2	87.3	+112	8.1	7.5	- 7	13.2	17.5	+ 33
A.R.V.H.	AUTONOMIC DEGENERATION	-	-	-	-	-	-	5.9	7.1	+ 20	13.7	16.0	+ 17
J.A.W.	NERVE BLOCK	16.4	9.0	-45	4.8	13.4	+179	30.6	37.1	+ 21	2.5	3.3	+ 32
H.v.d.S.	BRACHIAL AVULSION	9.2	2.4	-74	7.6	40.0	+426	7.3	7.9	+ 8	9.5	12.1	+ 27
R.T.	CERVICAL CORD TRANSECTION	-	-	-	-	-	-	8.4	17.7	+111	7.4	4.6	- 38

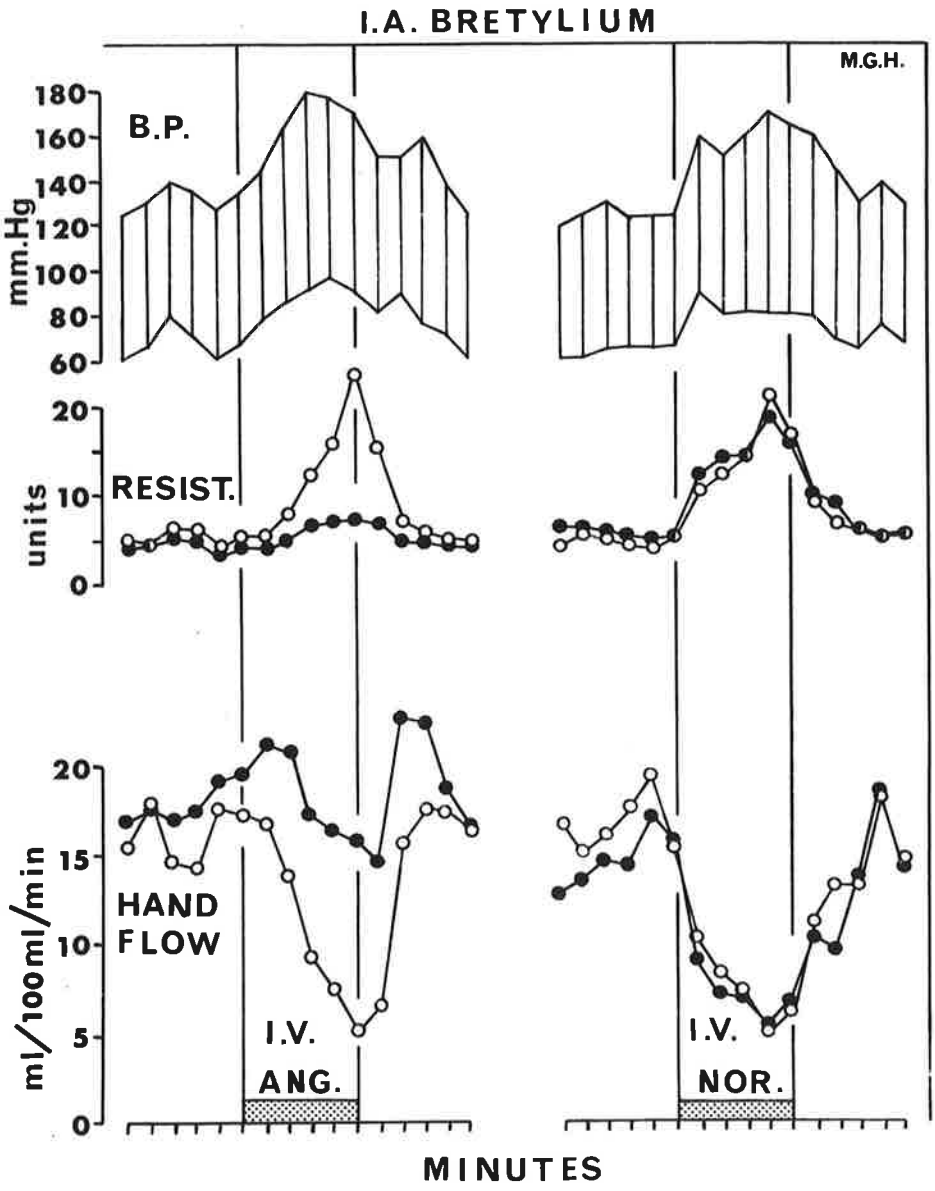

Fig. 3-3 The changes in arterial blood pressure, vascular resistance and blood flow through the left (●) and right (O) hands of subject M.G.H. during intravenous infusions of angiotensin (1.0 µg./min.; left of figure) and noradrenaline (10.0 µg./min.; right of figure)) 60 min. after the introduction of bretylium tosylate (4.0 mg./min. for 5 min.) into the brachial artery of the left arm.

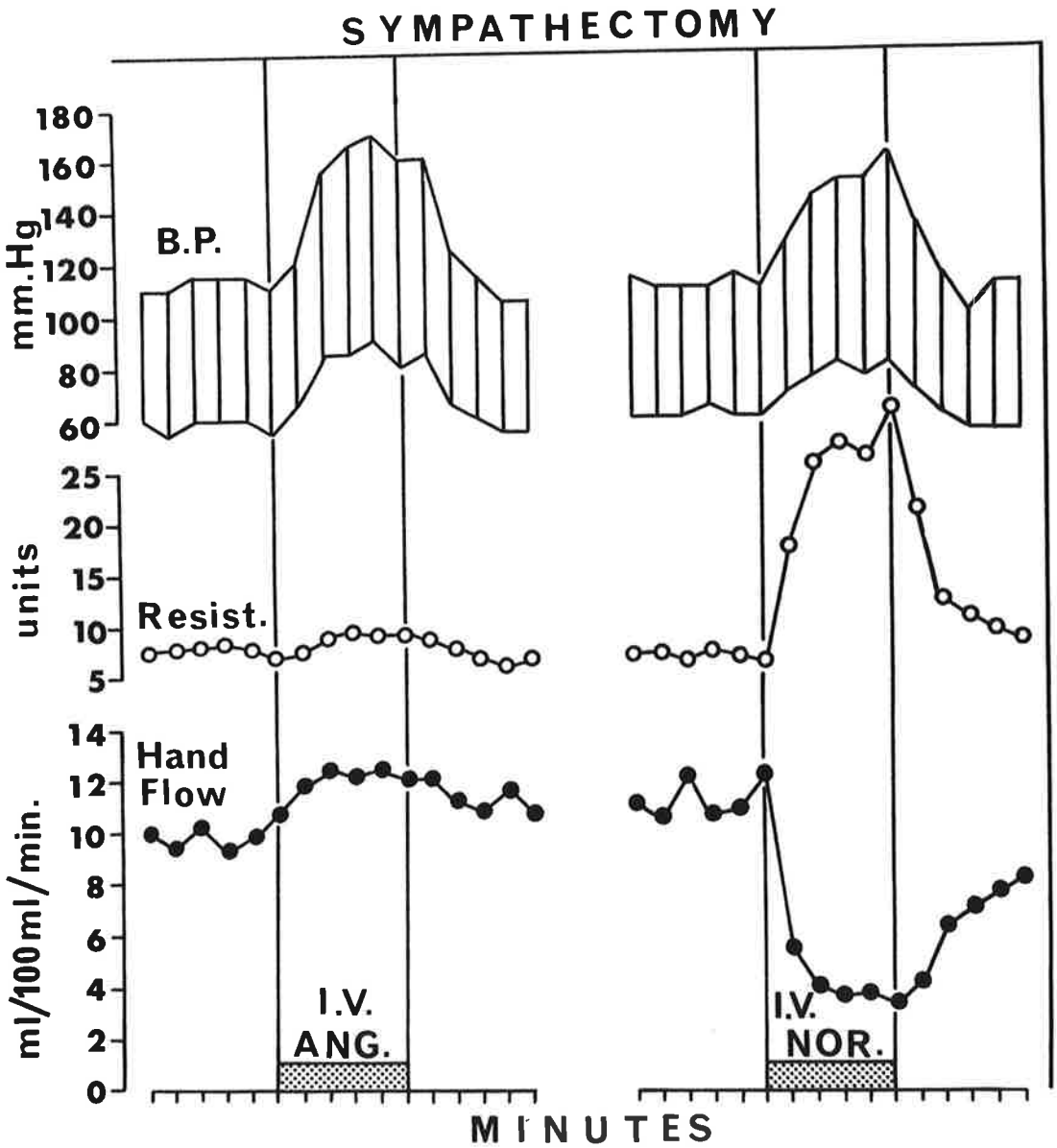

Fig. 3-4 The response of arterial blood pressure, hand blood flow (●) and hand vascular resistance (O) in the sympathectomized patient, W.W., during intravenous infusions of angiotensin (0.5 µg./min., left of figure) and noradrenaline (5.0 µg./min., right of figure).

Marked constrictor responses to adrenaline and noradrenaline (5.0 µg./min. for 5 min.) were obtained with associated changes in vascular resistance.

Similar findings were obtained in the patient (A.H.) with autonomic degeneration in whom all sympathetic activity to the upper limbs had been shown to be completely absent. Intravenous infusion of angiotensin (0.25 µg./min. for 5 min.) caused a marked elevation of blood pressure and an increase in hand blood flow (Fig. 3-5). The hand vascular resistance was only slightly increased (Table I). The pressor response to noradrenaline was also enhanced but the hand vessel response was the usual vasoconstriction.

Fig. 3-6 shows the responses of the foot vessels to intravenous infusion of angiotensin in a patient (D.B.) who had undergone unilateral lumbar sympathectomy three months previously. The control foot responded normally with a constriction but the response was abolished on the sympathectomized side. Prior to operation both feet had responded equally to angiotensin. The flow and foot vascular resistance changes are summarized in Table I.

(d) Nerve block:- Complete anaesthetic block of the sympathetic nerves to the hand resulted in abolition of the constrictor response of the vessels to intravenous angiotensin. Fig. 3-7 shows a comparison of the responses of the nerve-blocked left

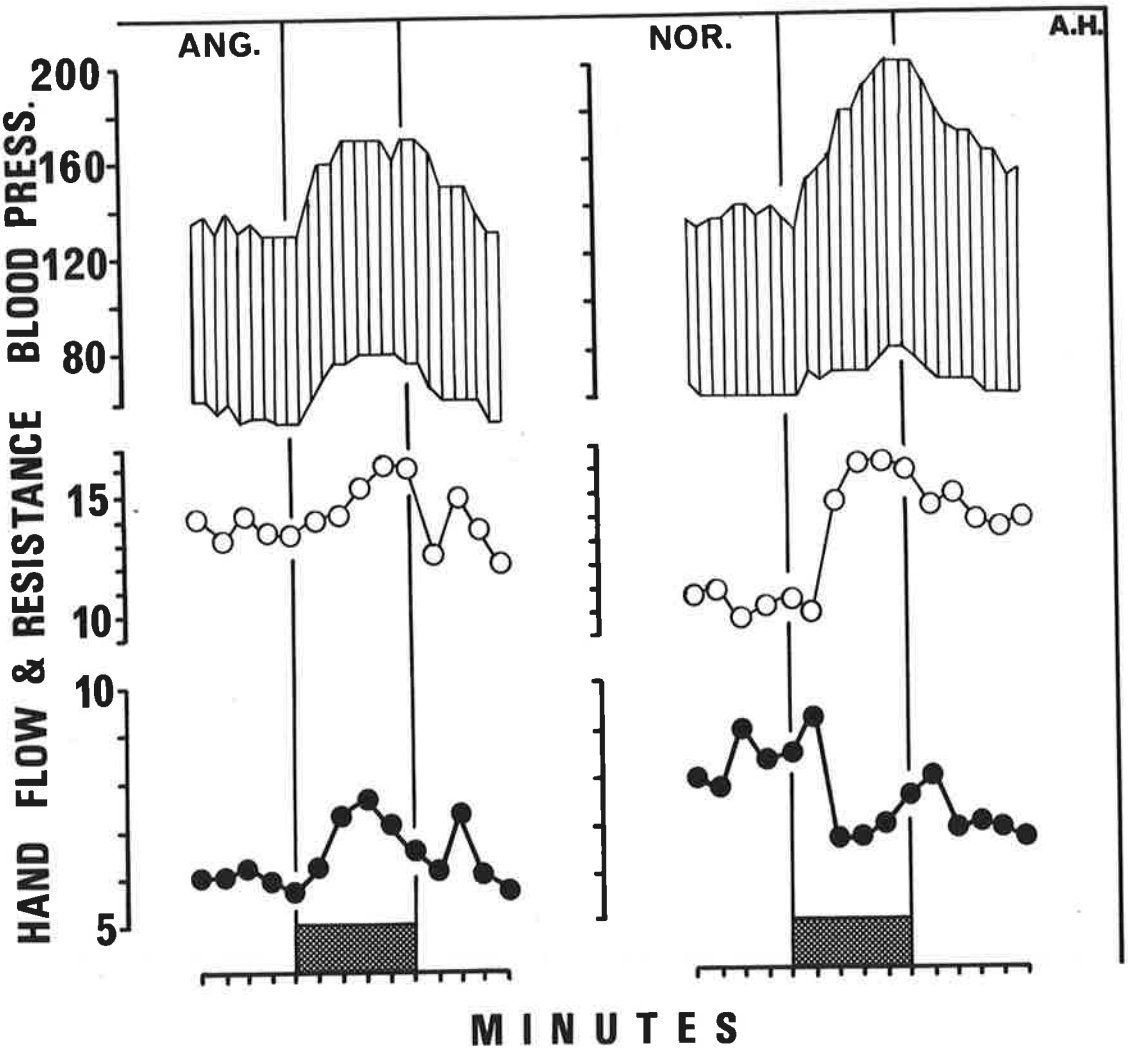

Fig. 3-5 The response of arterial blood pressure, hand vascular resistance (O) and hand blood flow (●) in patient A.H. during intravenous infusions of angiotensin (0.25 µg./min. for 5 min., left of figure) and noradrenaline (2.0 µg./min. for 5 min., right of figure).

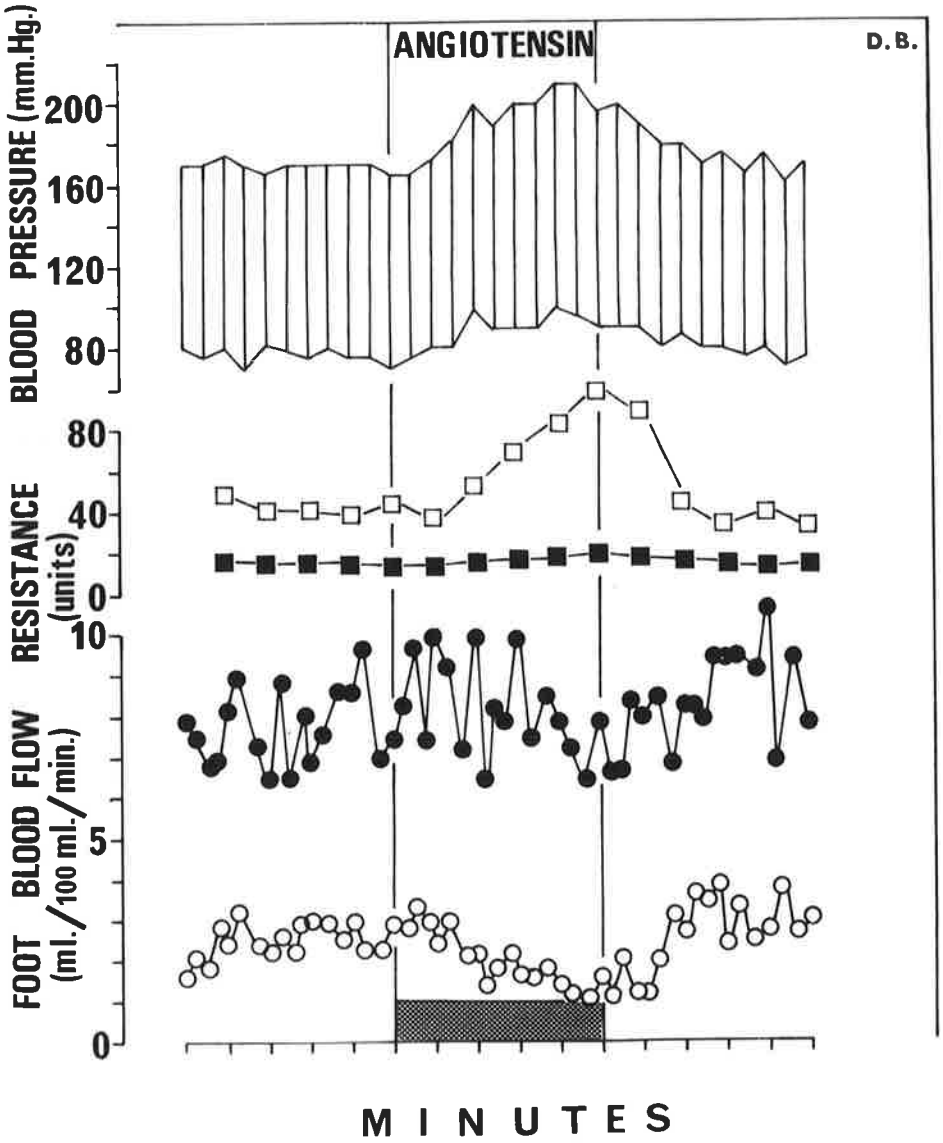

Fig. 3-6 The changes in arterial blood pressure, foot blood flow and foot vascular resistance in the sympathectomized (● , ■) and normally innervated (O , □) feet of patient D.B. in response to the intravenous infusion of angiotensin (1.0 μg./min. for 5 min.)

hand and the control right hand in one subject (J.A.W.) to angiotensin and to noradrenaline given intravenously in doses which produced approximately equal elevations in the blood pressure. Angiotensin (2.0 µg./min. for 5 min., left of figure) caused the usual reduction in flow in the control hand, but no vasoconstriction was seen on the blocked side. The flow through this hand increased passively due to the rise in perfusion pressure and the hand vascular resistance increased by less than one-fifth of that seen in the control side (Table I). Similar responses to the above were obtained with two other such infusions of angiotensin (1.5 µg./min. for 7 min. and 2.0 µg./min. for 5 min.) in the 2 hr. following the block, during which time no constriction of the hand vessels on the blocked side occurred following the application of ice to the neck although the control hand responded normally throughout.

Before the block of the nerves to the left hand was carried out the vessels of both hands had been shown to give similar constrictor responses to intravenous angiotensin. Both the blocked and the control hands showed comparable constrictor responses to intravenous noradrenaline (15.0 µg./min. for 5 min., right of figure) which demonstrated that the vessels of the blocked hand were capable of a normal response to a directly-acting vasoconstrictor agent.

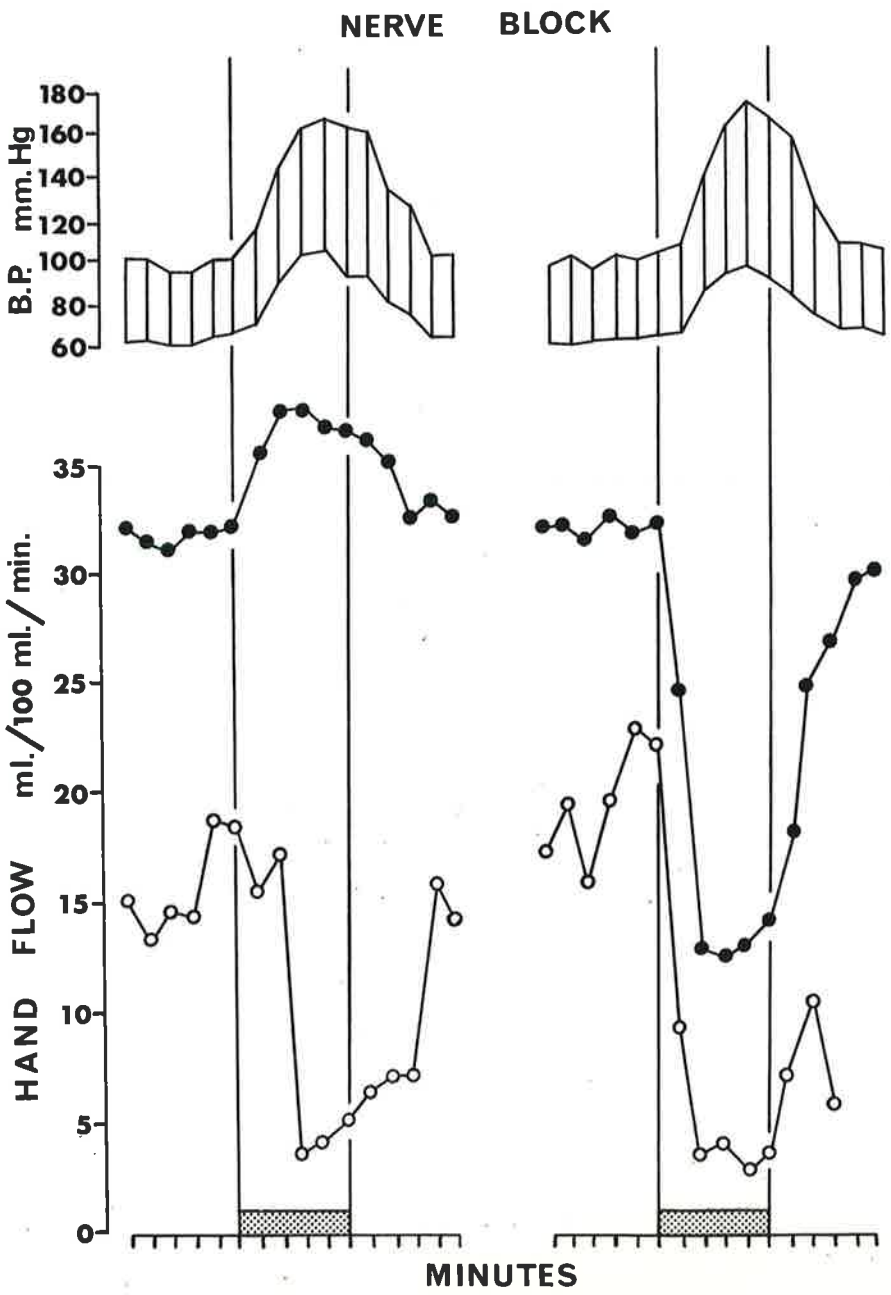

Fig. 3-7 The response of the arterial blood pressure and blood flow through the nerve-blocked hand (●) and through the control hand (O) of the subject J.A.W. during intravenous infusions of angiotensin (2.0 µg./min. for 5 min., left of figure) and noradrenaline (15.0 µg./min. for 5 min., right of figure).

(e) Denervated limbs. The responses of the hand vessels to intravenous infusions of angiotensin and noradrenaline in a patient (H.v.d.S.) who had sustained avulsion of the brachial plexus 9 months previously are illustrated in Fig. 3-8. The opposite arm was normal and used as a control. Angiotensin produced the usual reduction in hand blood flow on the control side but not on the denervated side and the increase in the vascular resistance through this hand was very small compared with the control (Table I). However, both hands showed a vasoconstriction with intravenous noradrenaline although the response was very much more marked and persisted for a longer time on the denervated side than on the control side. This would seem to indicate a striking increase in the sensitivity of the denervated vessels to catecholamines. A similar hyper-sensitivity of the hand vessels to intravenous noradrenaline was seen in another patient of this series (W.W.) following sympathectomy and has been reported elsewhere (Parks, Skinner and Whelan, 1961).

Fig. 3-9 shows the responses of blood pressure and blood flow in one hand (both were similarly denervated and gave identical responses) together with calculated resistance changes during the intravenous infusion of angiotensin (0.25 µg./min. for 5 min.) and noradrenaline (4.0 µg./min. for 5 min.) in the patient (R. Tree) with complete C6-7 spinal cord transection. Angiotensin caused a

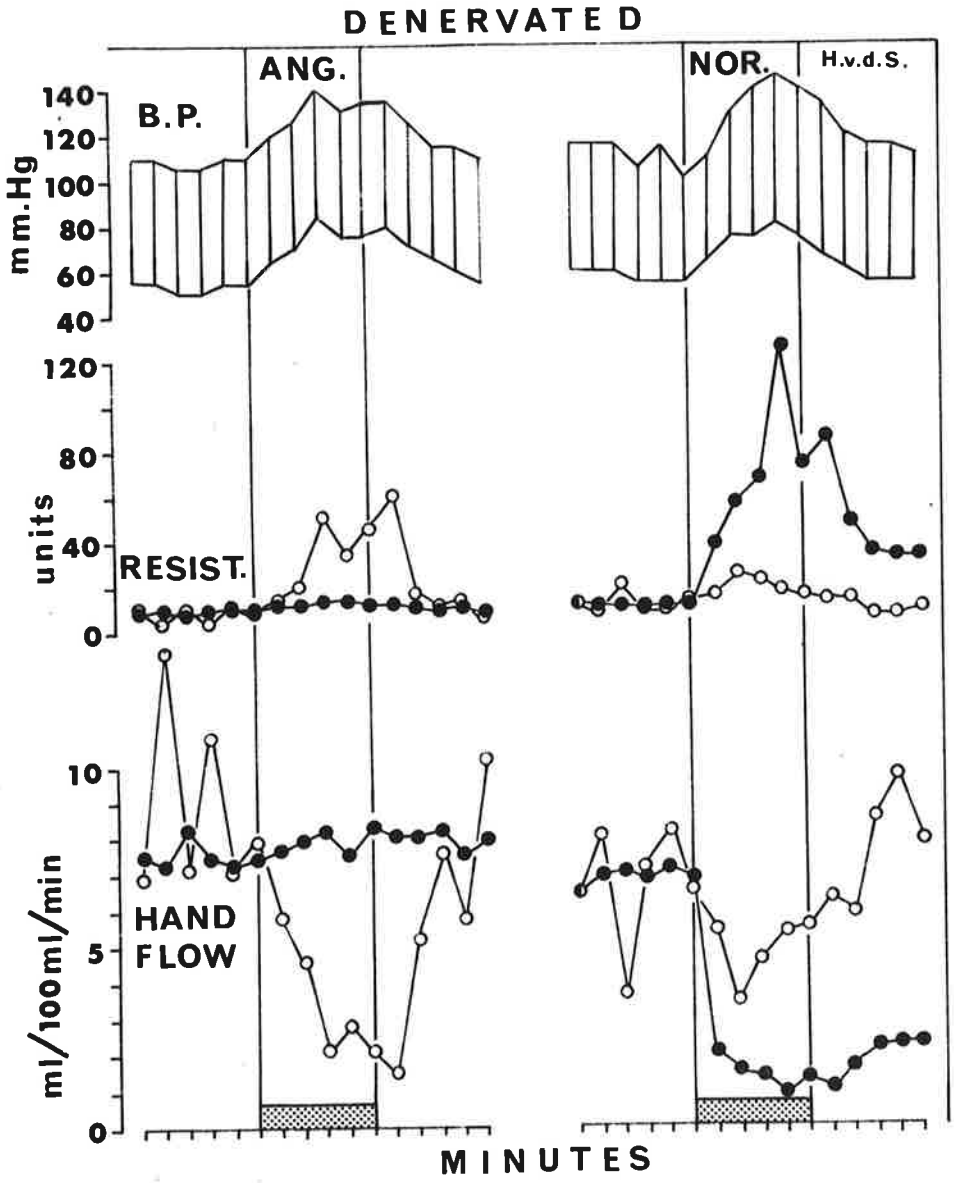

Fig. 3-8 The response of the arterial blood pressure, hand blood flow and hand vascular resistance in the denervated (●) and control (O) hands of patient H. v.d. S. during intravenous infusions of angiotensin (1.5 µg./min. for 5 min., left of figure) and noradrenaline (10.0 µg./min. for 5 min., right of figure).

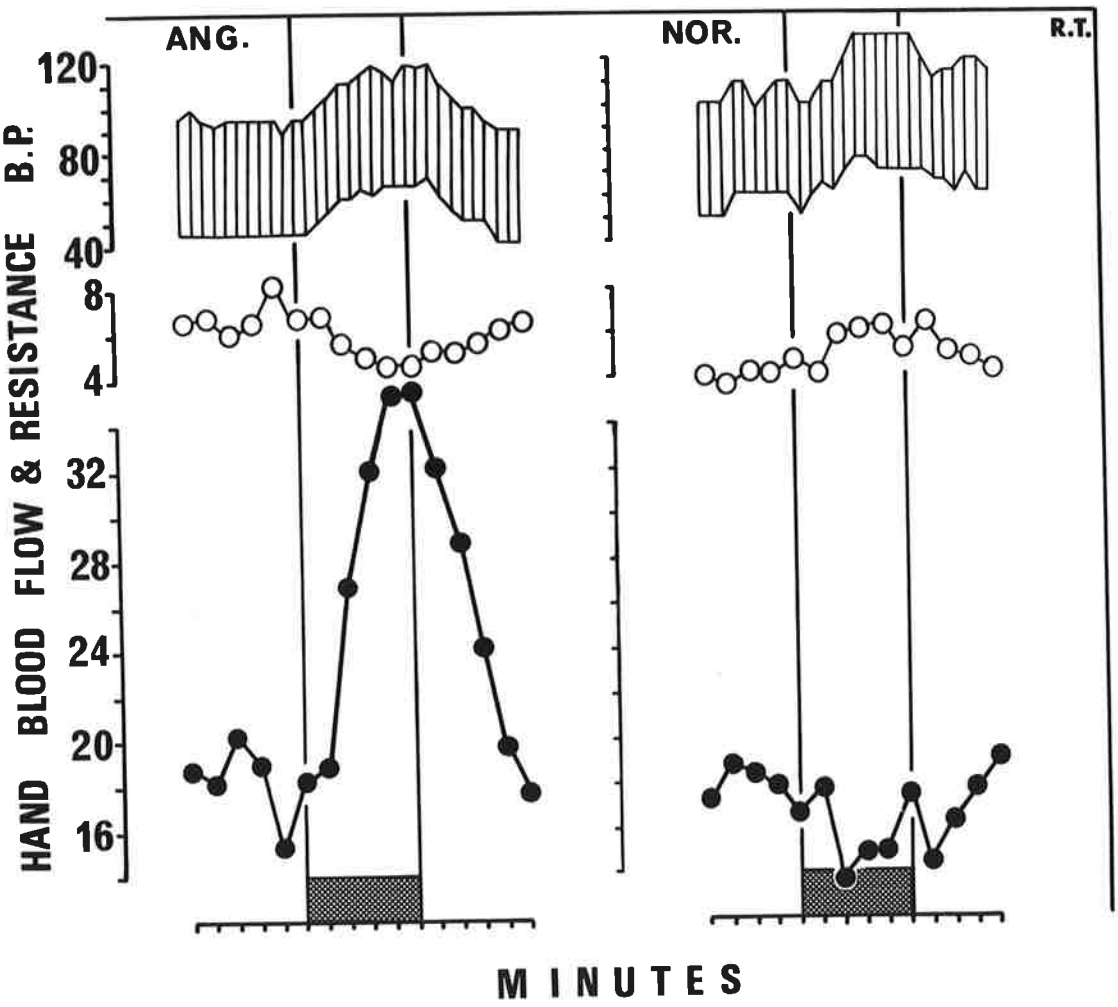

Fig. 3-9 The response of the arterial blood pressure, hand blood flow (●) and hand vascular resistance (O) in patient R. Tree during the intravenous infusion of angiotensin (0.25 µg./min. for 5 min., left of figure) and noradrenaline (4.0 µg./min. for 5 min., right of figure).

marked increase in arterial blood pressure and a striking increase in hand blood flow, calculated hand vascular resistance falling slightly. Since the post-ganglionic pathway is intact in this patient the absence of the normal constrictor response of the hand vessels indicates that the site of the sympathetic stimulating action of angiotensin is preganglionic. The small fall in calculated vascular resistance that occurred was unexpected and suggests a dilator mechanism in addition to the expected passive increase in blood flow in response to the increase in mean arterial pressure.

Intravenous noradrenaline increased arterial blood pressure and although hand blood flow fell and calculated resistance rose the response in this vascular bed was less than normally seen with this dose.

Intra-arterial infusions:

(a) Phenoxybenzamine. During these experiments the hand blood flows were deliberately kept high to avoid any marked change in the resting levels throughout the experiment that might otherwise occur following alpha-receptor blockade and release of sympathetic tone. Therefore, any difference in the constrictor action of each drug before and after alpha-receptor blockade was unlikely to be due to a dilutional effect or an altered flow distribution pattern. It is interesting, nevertheless, that when the flows were

high and in the absence of any other treatment, the constrictor action of angiotensin was reduced to a greater degree than was that of noradrenaline. Since the dilutional effect would have been the same with each drug it is possible that the destruction of angiotensin was accelerated by the high flow rate. These, then, are the reasons for the relatively high doses of both drugs, angiotensin in particular, which were used, but despite this there were no systemic effects as evidenced by the absence of a constrictor response on the control side.

Fig. 3-10 illustrates the response of the hand vessels in one subject to infusion into the brachial artery of angiotensin in a dose of 0.9 µg./min. for 5 min. The reduction in blood flow that occurred was similar to that produced by noradrenaline in a dose of 0.3 µg./min. for 5 min. The constrictor response to angiotensin was unaffected by the prior infusion of phenoxybenzamine intra-arterially in a dose of 0.5 mg./min. for 3 min., which was sufficient to abolish, almost completely, the constrictor action of noradrenaline. Six other experiments of this kind, each on a different subject, gave similar results with angiotensin in doses of 1.0 to 3.0 µg./min. for 5 min. The results of all these experiments are summarized in Fig. 3-11.

(b) Sympathectomy. Fig. 3-12 shows the responses of the

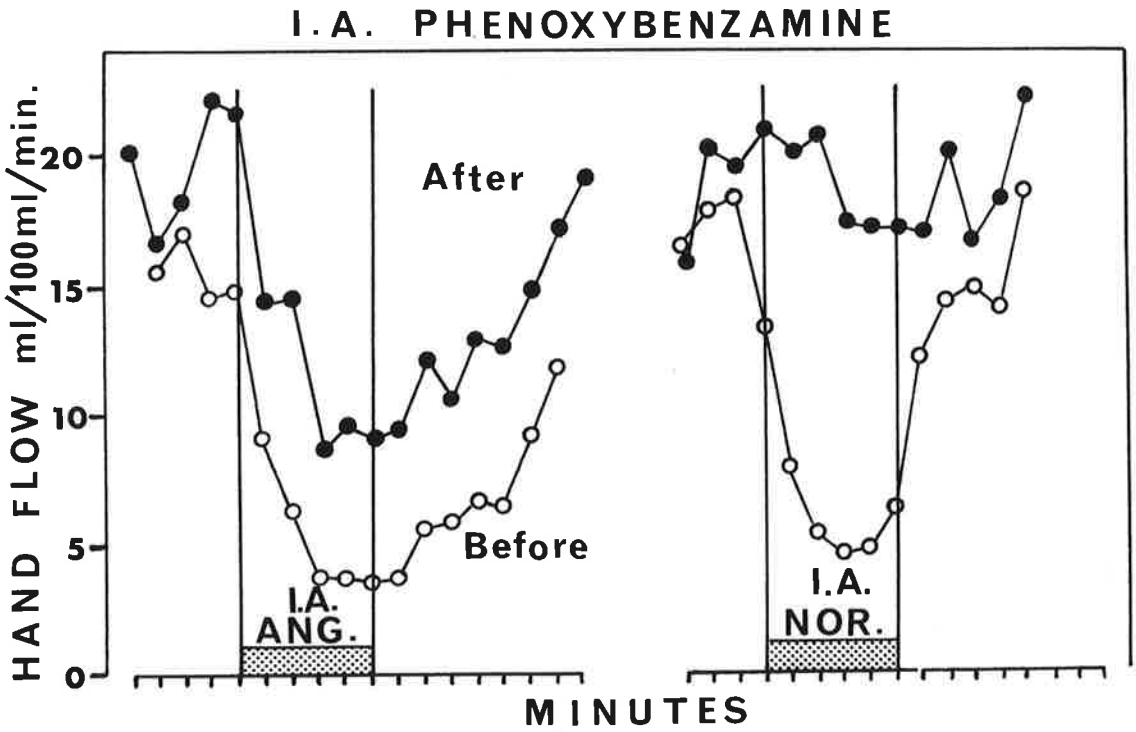

Fig. 3-10 The responses of the blood flow through the left hand of subject D.H. during intra-arterial infusions of angiotensin (0.9 µg./min., left of figure) and noradrenaline (0.3 µg./min., right of figure) before (O) and after (●) introduction of phenoxybenzamine (0.5 mg./min. for 3 min.) into the brachial artery of the same arm.

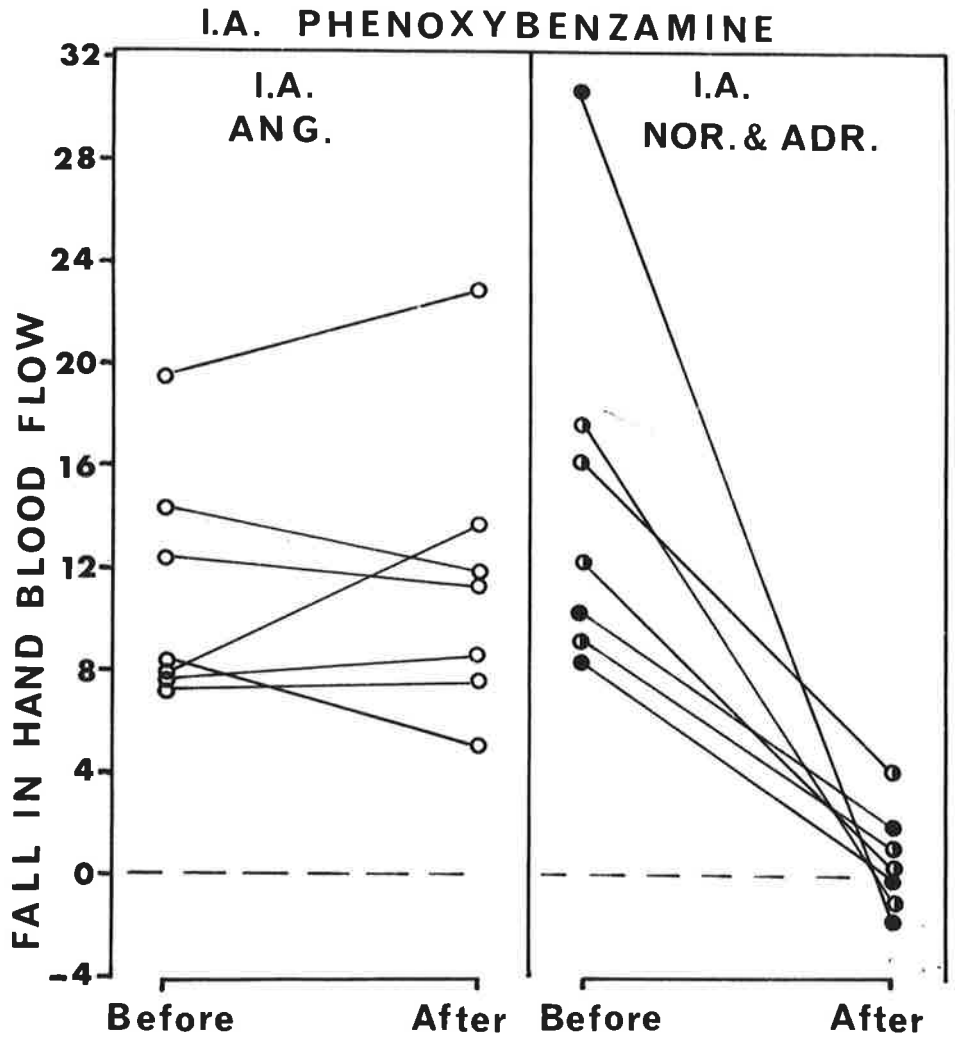

Fig. 3-11 The fall in hand blood flow in seven subjects produced by intra-arterial infusion of angiotensin (O) and noradrenaline (●) or adrenaline (◑) before and after intra-arterial infusion of phenoxybenzamine (0.5 mg./min. for 3 min.).

hand vessels during intra-arterial infusion of several different doses of angiotensin and noradrenaline in the two patients with cervical sympathectomy, one of whom (R. Taylor) was studied before (R.T.1) and after (R.T.2) operation and the other only post-operatively (W.W.), and in the three patients with autonomic degeneration (A.H., E.H. and P.B.). The dose-response curves obtained have been superimposed on the hatched area in the figure, which includes one standard deviation about the mean responses for each of the three doses of angiotensin and noradrenaline when administered to five normal subjects.

With the exception of patient W.W., in whom the responses to both angiotensin and noradrenaline were greater than normal, sympathetic denervation had no appreciable effect on the constrictor response of the hand vessels to intra-arterial angiotensin. The responses to noradrenaline, however, showed an increase above normal in most patients, at all dose levels. The hand vessel responses to angiotensin in patient R.T. were not appreciably altered by sympathectomy although the noradrenaline responses were slightly enhanced following sympathectomy.

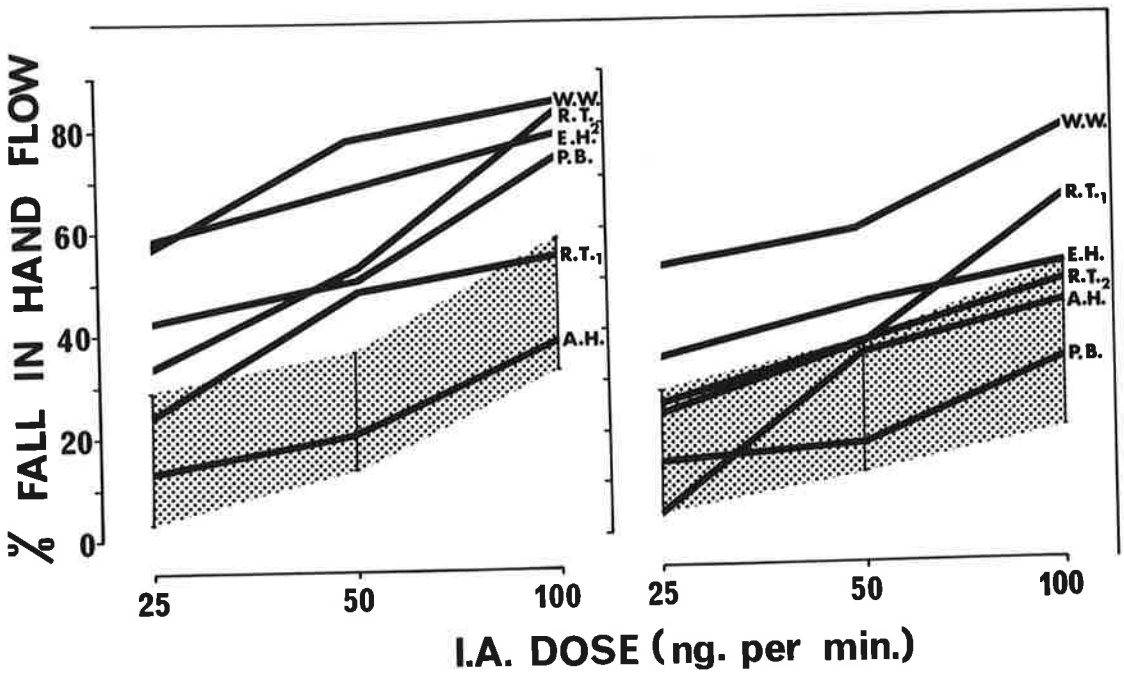

Fig. 3-12 The hatched areas in both frames include one standard deviation about the mean constrictor responses of the hand vessels for each of the three doses of noradrenaline (left hand frame) and angiotensin (right hand frame), when administered to five normal subjects.

The superimposed dose-response curves (▬▬) were obtained from three patients with autonomic nervous system degeneration (A.H., E.H. & P.B.) and two patients with cervical sympathectomy (W.W. & R.T.). Patient R.T. was studied before ($R.T._1$) and after ($R.T._2$) sympathectomy. These curves were constructed from the values for the mean percentage falls in hand blood flow in response to intra-arterial infusion of noradrenaline (left hand frame) and angiotensin (right hand frame) in the doses shown.

DISCUSSION

The abolition of the constrictor responses to intravenous angiotensin in the hand treated with phenoxybenzamine, while the response in the control hand was unaffected, demonstrated the adrenergic nature of the response, but since phenoxybenzamine blocks the action of both sympathetically released and circulating catecholamines this evidence did not distinguish between sympathetic nerve activity and the arrival of an adrenal hormone. However, the marked reduction in the constrictor response of the hand vessels by treatment with bretylium demonstrated that the response was largely mediated by way of the sympathetic nerves, and this conclusion was confirmed by the absence of constriction of the vessels in the sympathectomized, nerve-blocked and denervated hands.

In the bretylium-treated hand the constrictor response to intravenous angiotensin was not completely abolished and a slight fall in hand blood flow persisted. Since the dose of bretylium used did not block sympathetic activity completely, and a small reflex response to ice applied to the neck persisted after 60 min. (Cooper, Fewings, Hodge and Whelan, 1963), the slight degree of vasoconstriction might have been due to residual sympathetic nerve activity.

In the case of the sympathectomized,nerve-blocked and

denervated hands sympathetic denervation was judged to be almost complete. In these hands an increase in blood flow occurred during the angiotensin infusions which was probably due to the raised perfusion pressure since the calculated hand vascular resistance showed only a small increase in each case.

These small increases in resistance indicated that the hand vessels were not behaving in a completely passive manner and it is probable that the increases were due to a local myogenic response to the increased distending pressure (Bayliss, 1902). The possibility cannot be excluded, however, that small amounts of a circulating hormone, such as adrenaline, may have been responsible. Angiotensin has been shown to release adrenal medullary catecholamines in the cat (Feldberg and Lewis, 1964) but the evidence so far available does not suggest a similar action in man (Vincent, Kashemsant, Cuddy, Fried, Smulyan and Eich, 1965; Biron, 1964). Furthermore, the almost complete abolition of the constrictor response to intravenous angiotensin in the denervated hand vessels of the patient with brachial plexus avulsion, despite the observed enhancement of the constrictor response in these vessels to infused noradrenaline, suggests that angiotensin, in the doses used in these experiments, did not significantly increase the circulating levels of noradrenaline.

A direct action of angiotensin is unlikely to be responsible for the small residual increase in resistance in the denervated vessels in view of the likely effectiveness of the concentrations of angiotensin calculated to be arriving in the hand during intravenous infusions. At the level of hand blood flow existing during these experiments doses of 1.0 µg./min. or more had to be given intra-arterially in order to produce a constrictor response comparable to that seen during intravenous infusions of 1.0 to 2.0 µg./min. An intra-arterial dose of 1.0 µg./min. can be calculated to result in an arriving blood concentration in the hand of approximately 10 ng./ml. whereas during intravenous infusions of 1.0 µg./min. the maximum concentration which would be arriving in the hand is approximately 0.2 ng./ml. at the end of the first min. and rising to 1.0 ng./ml. at the end of the fifth min. The actual blood concentration arriving in the hand is certain to be very much less than this maximum value, during intravenous infusions, because of the rapid destruction of angiotensin in the circulation and although these calculations are only a rough approximation, such concentrations are unlikely to have any appreciable direct constrictor effect on the hand vessels. This was apparent from the results in the previous section of this thesis.

While the above considerations make it improbable that the direct local constrictor action of angiotensin plays any part

in the responses of the hand vessels during intravenous infusions, nevertheless, a direct effect, alone or in combination with a sympathetically-mediated constriction, could occur in other vascular beds, if these were more sensitive to the direct action of angiotensin than are the vessels of the hand. The forearm vessels have been shown, in the previous section of this thesis, to be twenty-five times more responsive than the hand vessels to intra-arterial angiotensin. This suggests, at first sight, that the direct action of angiotensin could play an important or even dominant role in the responses of this vascular bed during intravenous infusions. However, it was demonstrated in the previous section of this thesis, that intravenous infusions of angiotensin produced no change in overall forearm vascular resistance (Fig. 2-5) suggesting that the increase in total peripheral resistance during pressor infusions of angiotensin occurs in vascular beds other than those of the limbs. Although only one patient was available for study, the foot vasoconstriction during intravenous infusions of angiotensin appears to be largely sympathetically-mediated, and, like the hand and forearm, its contribution to the overall rise in peripheral resistance is probably small. McGiff and Fasy (1964) studied the renal circulation in the dog and found that treatment with bretylium and guanethidine, acute denervation of the kidney, spinal anaesthesia and section of the spinal cord at C 1 abolished the renal vasoconstrictor effect of intravenous infusions of angiotensin. The relative contrib-

utions of direct and indirect actions of angiotensin in other vascular beds in man and in animals have yet to be explored.

The direct effect of angiotensin on the hand vessels, seen on intra-arterial infusion, was unaffected by alpha-receptor blockade or sympathetic denervation, indicating that the site of the sympathetic stimulating action of angiotensin, which is seen during intravenous infusions in man, is not in the more peripheral part of the postganglionic fibre as has been suggested by the work of Zimmerman (1962), Zimmerman and Gomez (1965) and Benelli, Della Bella and Gandini (1964) in animals. Although a ganglionic site of action of angiotensin has been suggested by the work of Lewis and Reit (1965) in the cat this is unlikely to be true in man since the constrictor response in the hand vessels was abolished in the patient with complete cervical cord transection, in whom the sympathetic ganglia and postganglionic pathways were intact, as judged by a normal constrictor response of the hand vessels to intra-arterial tyramine. This result further indicates that the site of the sympathetic stimulating action of angiotensin in man is preganglionic and is most probably in the medullary vasomotor centres. Its action here is probably a highly specific one since apart from the possible role of the sympathetic nerves in the changes in heart rate during angiotensin infusions, that was suggested from the results in the previous section of this thesis

and from animal studies (Nishith, Davis and Youmans, 1962; Krasney, Paudler,Smith, Davis and Youmans, 1965; Krasney, Paudler, Hogan, Lowe and Youmans, 1966), there was no evidence of widespread autonomic stimulation with the doses used in the present experiments. Other studies in man have demonstrated that the vasomotor centres can be influenced in a very specific manner by different stimuli. For example, a rise in blood temperature results in release of sympathetic tone to skin vessels of the limbs without affecting the underlying muscle vessels (Roddie, Shepherd and Whelan, 1956) and conversely, muscle vessel tone is modified by postural baroreceptor stimulation which is without effect on the skin vessels (Roddie, Shepherd and Whelan, 1957). Thus the nature of the functional organization of the sympathetic nervous system in man makes it possible for angiotensin to have a selective action on some of the medullary centres and modify tone in certain vascular beds while others remain unaffected.

In conclusion, these experiments have demonstrated a preganglionic sympathetic stimulating action of angiotensin in man. The contribution made by this mechanism to the pressor response during intravenous infusions forms the subject matter of the next section of this thesis.

SUMMARY

1. Intravenous infusions of angiotensin in man caused a vasoconstriction in the hand which was almost abolished by prior administration of phenoxybenzamine and bretylium tosylate. The response was absent in the surgically sympathectomized hand, in the nerve-blocked hand, in the hand of a patient who had sustained brachial plexus avulsion and in the hands of one patient with a C6-7 cord transection.

   In one patient the vasoconstriction in the foot vessels was abolished by lumbar sympathectomy.

2. It is concluded that angiotensin administered intravenously has a preganglionic stimulating action on the sympathetic vasomotor system and that in the case of the hands, and probably the feet, this is the sole cause of the vasoconstriction.

3. Whether this preganglionic stimulation of the sympathetic system occurs in the vasomotor centres of the medulla or elsewhere in the preganglionic pathways could not be determined from these experiments.

4. Angiotensin, when given intra-arterially, exhibits a constrictor action which is unaffected by phenoxybenzamine or various forms

of sympathetic denervation and is therefore considered to be a direct action of the drug on the vascular smooth muscle. This result almost certainly excludes a post-ganglionic site of action of angiotensin in the limb vessels of man.

5. While the increase in peripheral resistance in the hand and foot during intravenous infusions of angiotensin is almost entirely nervously mediated, the relative contribution made by the direct and indirect actions of angiotensin to the responses of other vascular beds was not determined in these experiments.

PART B

THE EFFECTS OF TOTAL ALPHA-ADRENERGIC RECEPTOR BLOCKADE AND SYMPATHETIC DENERVATION ON THE PRESSOR ACTION OF ANGIOTENSIN IN MAN.

INTRODUCTION

The results presented in the previous section demonstrated that preganglionic stimulation of the sympathetic nervous system is responsible for all the vasoconstriction in the hand and the foot during intravenous infusions of angiotensin in man. It is possible that this action of angiotensin occurs in a number of vascular beds and if so could contribute significantly to the total increase in peripheral resistance which occurs. However, the assessment of drug-induced flow changes in the individual vascular beds in man, other than those of the extremities, is difficult, and it becomes even more so if one attempts to examine the effects on these responses of various forms of treatment such as sympathectomy and adrenergic-receptor blockade. For this reason the effects of most drugs on the peripheral circulation in man are deduced from measurements of changes in such parameters as blood pressure and cardiac output.

In the present study, the effect of intravenously administered angiotensin on systemic arterial pressure, heart rate and hand blood flow was determined in normal subjects before and after systemic alpha-adrenergic receptor blockade with phentolamine, in patients with autonomic nervous system degeneration, and in one patient with traumatic section of the spinal cord at C6-7. The

pressor response to angiotensin in man is almost entirely dependent upon its vaso-constrictor action. Therefore, if sympathetic stimulation makes an important contribution to the vasoconstrictor action of angiotensin in other vascular beds in man apart from the hand and foot then these various procedures mentioned above should significantly modify the pressor response.

METHODS

General:-

The subjects for all experiments rested recumbent on a couch in a temperature-controlled laboratory (23-25°C.) for at least one hour before observations began, during which time recording apparatus was applied and infusion needles and catheters inserted.

The infusions were all intravenous and were administered into an antecubital vein. Arterial blood pressure was recorded from a catheter inserted into the brachial artery and heart rate read directly from the blood pressure tracing. Blood flow was measured in both hands by venous occlusion plethysmography.

The drugs used were angiotensin II (val^5-hypertensin II-asp-β-amide, Hypertensin, Ciba); phentolamine methanesulphonate (Regitine, Ciba); noradrenaline bitartrate monohydrate (Levophed, Winthrop); tyramine hydrochloride (Sigma); ephedrine hydrochloride (D.H.A.). Doses of angiotensin, phentolamine, tyramine and ephedrine are expressed as weights of their salts and of noradrenaline as weights of the base.

Subjects:-

(a) Normal students. Six healthy male medical students whose ages ranged from 19-21 years acted as subjects in the experiments designed to determine the effect of the alpha-adrenergic receptor-blocking drug, phentolamine, on the responses of the hand blood vessels and systemic arterial pressure during intravenous infusions of angiotensin and noradrenaline. Phentolamine was chosen as the alpha-receptor blocking drug because of its short duration of action which is an important property for short-term experiments on normal subjects. Abolition of the hand vasoconstriction during intravenous angiotensin and noradrenaline, and of the blood pressure response during infusion of the latter, were used as monitors of the effectiveness of alpha-receptor blockade.

(b) Patients. Three patients with idiopathic degeneration of the autonomic nervous system (A.H., E.H. and P.B.) and one patient with complete cervical cord transection at C6-7 (R. Tree) were studied. These patients also acted as subjects for some of the experiments in the preceding section and the completeness of sympathetic denervation was determined by one or more of the various tests outlined in the methods of that section.

RESULTS

(a) Phentolamine - normal subjects

Fig. 3-13 shows the responses of the hand vessels and arterial pressure in one of the six normal subjects during the intravenous infusion of phentolamine alone and then phentolamine in combination with a pressor dose of angiotensin. In this subject phentolamine alone (1.0 mg./min. for 5 min.) had almost no effect on either blood pressure or hand blood flow.

Approximately 40 min. later, when the effects of phentolamine had disappeared, an infusion of angiotensin was begun (1.0 µg./min.) through a second venous catheter inserted in the contralateral arm, and this resulted in the characteristic elevation in arterial pressure and fall in hand blood flow. Within 10 min. these responses had become stable and the 5 min. phentolamine infusion (1.0 mg./min.) was then repeated, the angiotensin infusion continuing. This resulted in a return of hand blood flow to resting values but no significant change in the pressor response. After termination of the phentolamine infusion the angiotensin infusion continued for a further 15 min., during which time the hand vessel constriction gradually reappeared. The angiotensin infusion was then terminated and both arterial pressure and hand blood flow returned to preinfusion levels.

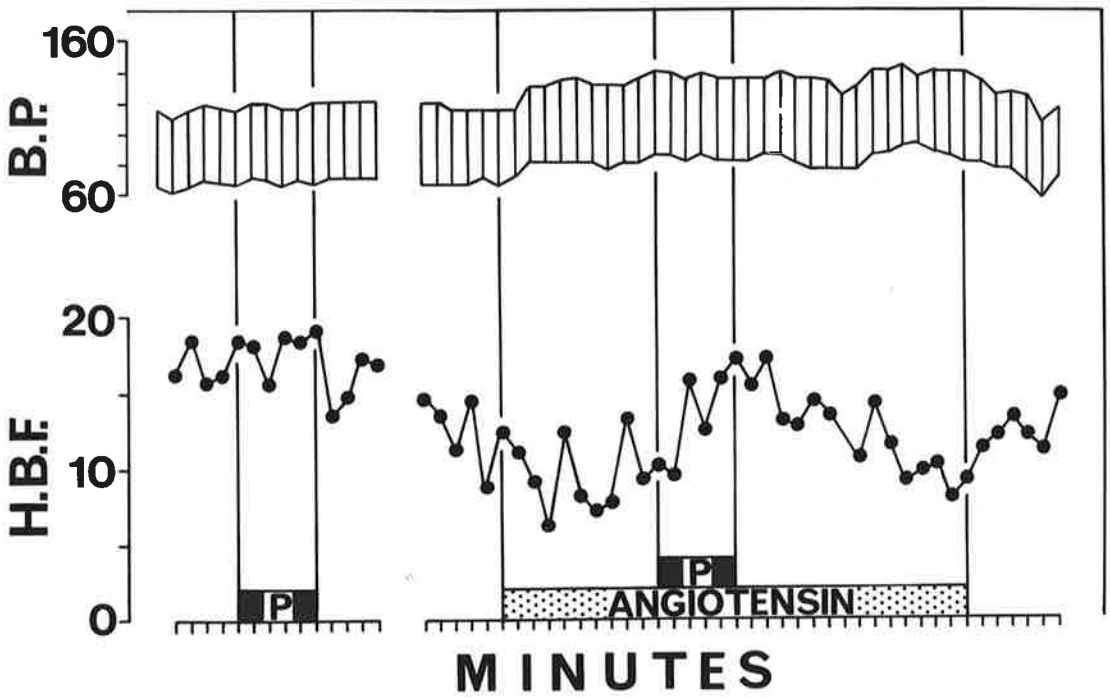

Fig. 3-13 The response of arterial blood pressure and hand blood flow during the intravenous infusion of phentolamine (P, 1.0 mg./min. for 5 min.) when given alone (left of figure) and when given during an intravenous infusion of angiotensin (1.0 µg./min. for 30 min., right of figure).

In contrast to this failure of phentolamine to affect the pressor response to angiotensin, it greatly reduced the blood pressure rise produced by intravenous noradrenaline (15.0 µg./min., Fig. 3-14).

Similar results to those in Fig. 3-13 were obtained in each of the remaining five subjects and the results of all six have been grouped in Fig. 3-15. When the blood pressure was elevated during the angiotensin infusions the small reductions in blood pressure produced by phentolamine were not significantly different from those in the normotensive situation ($p > 0.9$) whereas the increases in hand blood flow produced by phentolamine were significantly greater whether expressed on an absolute or percentage basis ($0.001 < p < 0.01$).

These results indicate that phentolamine was effective in reversing the sympathetically-mediated hand vasoconstriction produced by angiotensin but did not significantly modify the pressor response.

Idiopathic Autonomic Degeneration

Two male patients (A.H. and E.H.) both aged 67 years, and one female (P.B.) aged 45 years were studied and judged to have widespread autonomic nervous system degeneration.

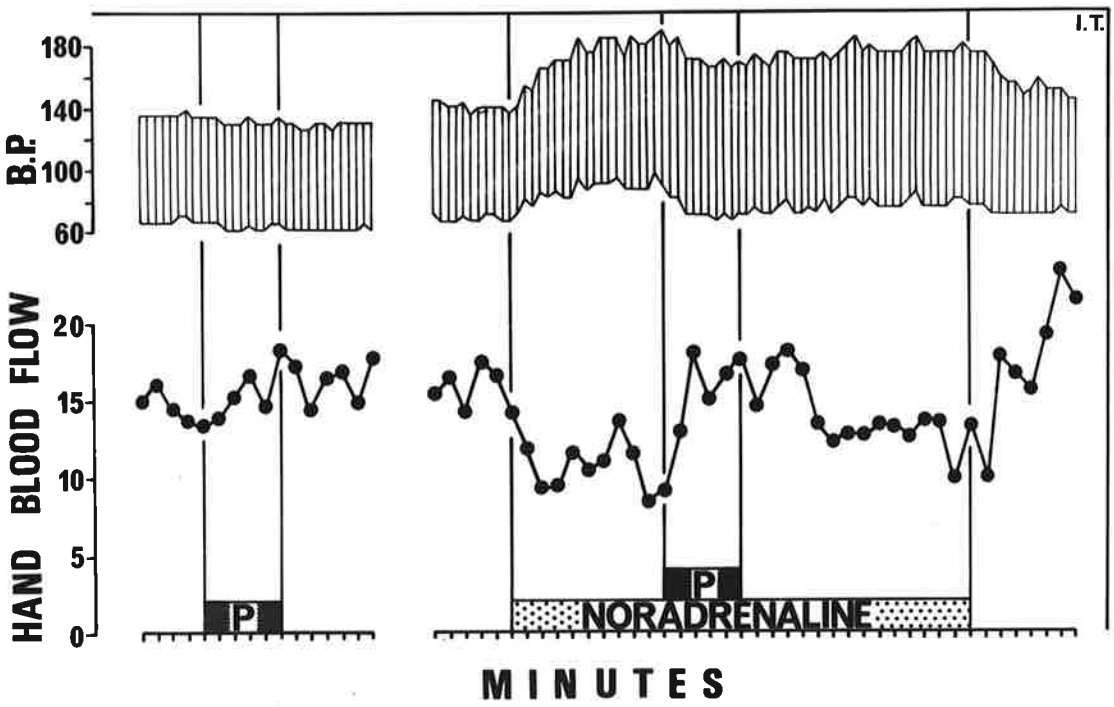

Fig. 3-14 The response of arterial blood pressure and hand blood flow during the intravenous infusion of phentolamine (P, 1.0 mg./min. for 5 min.) when given alone (left of figure) and when given during an intravenous infusion of noradrenaline (15.0 µg./min. for 30 min., right of figure).

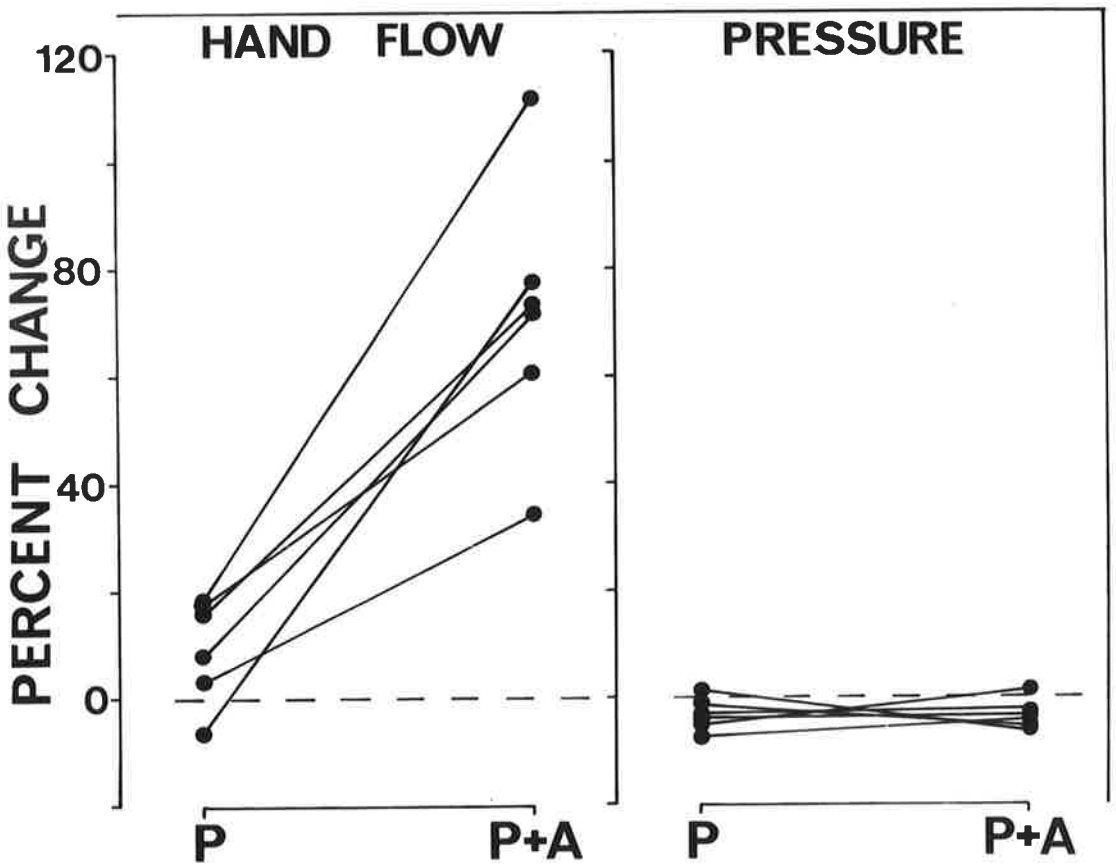

Fig. 3-15 The percent change in hand blood flow (left of figure) and mean arterial blood pressure (right of figure) in each of six subjects during the intravenous infusions of phentolamine when given alone (P) and when given in the same dose during an intravenous infusion of angiotensin (P+A).

Fig. 3-16 shows the changes in blood pressure and hand blood flow and resistance during intravenous infusions of angiotensin (0.25 µg./min. for 5 min.) and noradrenaline (2.0 µg./min. for 5 min.) in one of these patients (A.H.), each of whom showed a similar response.

Angiotensin caused a 27% increase in mean arterial pressure which is almost three times greater than that seen in normal subjects (Scroop, Walsh and Whelan, 1965). Due to autonomic denervation the heart rate in this patient was extremely stable and was unchanged by angiotensin infusion. The hand blood flow rose during the infusion period, presumably as a passive response to the increase in arterial pressure since calculated resistance was almost unchanged.

The pressor response to noradrenaline was enhanced but the hand vessel response was the usual vasoconstriction and increase in vascular resistance. The reflex bradycardia normally seen with pressor infusions of noradrenaline was replaced by a small tachycardia, presumably reflecting the unopposed positive chronotropic action of the drug.

Although the degree of autonomic denervation in these

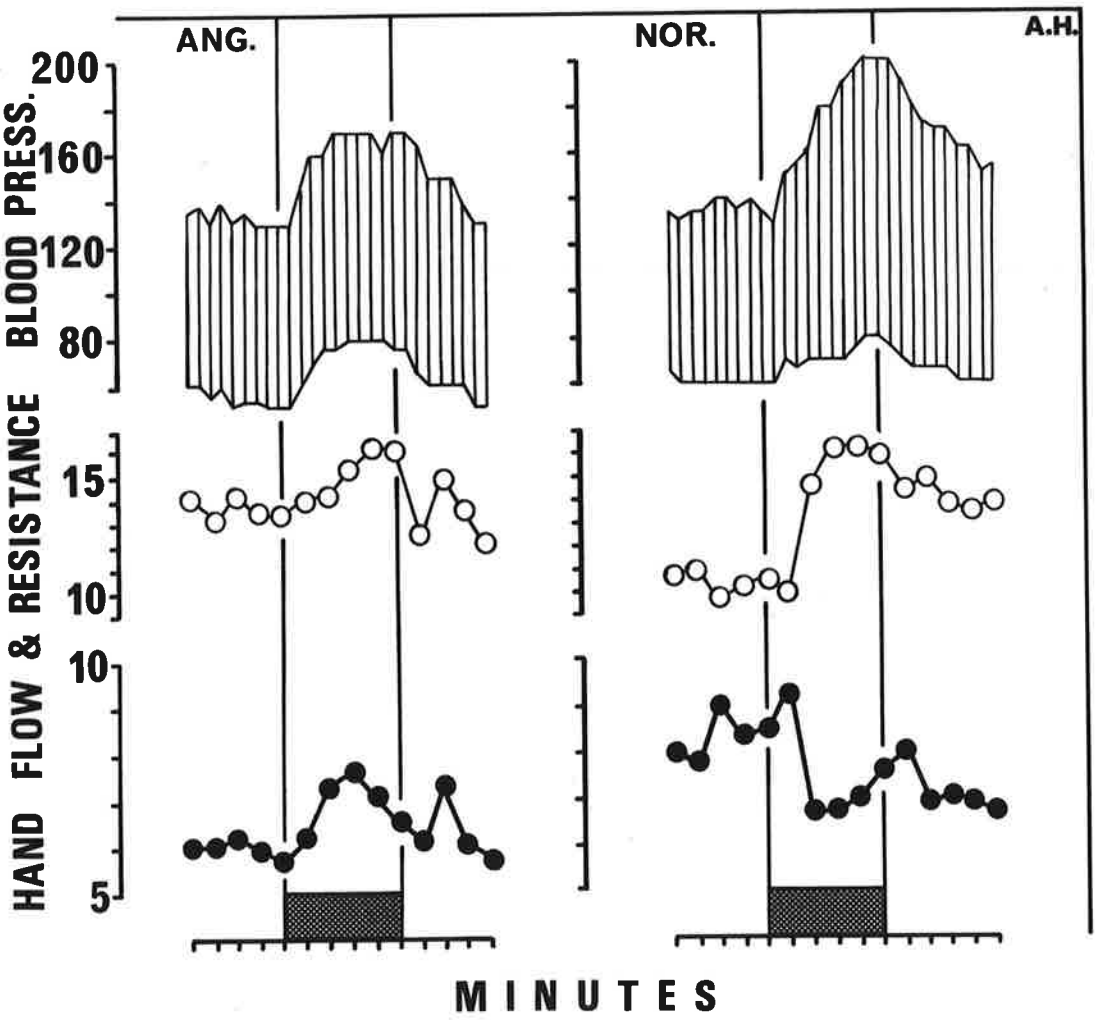

Fig. 3-16 The response of arterial blood pressure, hand vascular resistance (O) and hand blood flow (●) in patient A.H. during the intravenous infusion of angiotensin (0.25 µg./min. for 5 min., left of figure) and noradrenaline (2.0 µg./min. for 5 min., right of figure).

patients is not known with certainty the areas of involvement are certainly widespread and one might anticipate from the abnormal hand and forearm responses to the various test procedures mentioned in the methods, that there is a considerable reduction in the sympathetic innervation to the entire peripheral circulation. If this is so it appears that activation of the sympathetic nervous system does not contribute significantly to the pressor action of angiotensin.

Spinal cord transection at C 6-7

The patient (R. Tree) was a 25 year-old male and the cord lesion was post-traumatic, having occurred 4 years previously. The transection was complete at C 6-7, constituting a complete preganglionic sympathectomy.

Fig. 3-17 shows the hand blood flow and blood pressure responses together with the calculated resistance changes during the intravenous infusion of angiotensin (0.25 µg./min. for 5 min.) and noradrenaline (4.0 µg./min. for 5 min.).

Angiotensin caused a 32% increase in mean arterial pressure which is more than three times the normal response to this dose. There was a striking increase in hand blood flow and calcu-

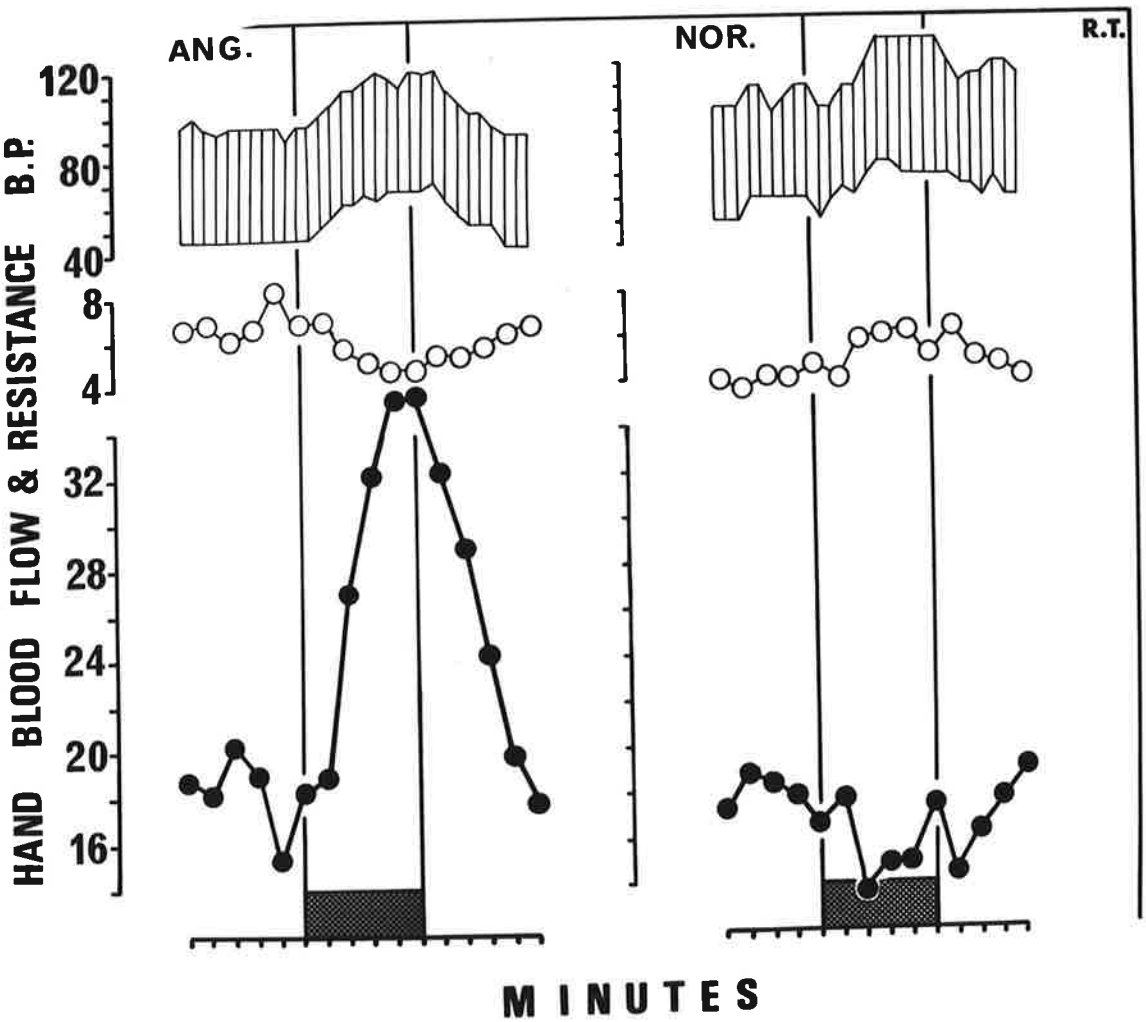

Fig. 3-17 The response of arterial blood pressure, hand vascular resistance (O) and hand blood flow (●) in patient R.T. during the intravenous infusion of angiotensin (0.25 µg./min. for 5 min., left of figure) and noradrenaline (4.0 µg./min. for 5 min., right of figure).

lated hand vascular resistance actually fell slightly. This small fall in calculated vascular resistance was unexpected and suggests a dilator mechanism in addition to the expected passive increase in blood flow in response to the increase in mean arterial pressure.

Intravenous noradrenaline resulted in a 38% increase in mean arterial pressure which is a three-fold increase over that seen in normal subjects. Although hand blood flow fell and calculated resistance rose the response was less than normally seen with this dose.

DISCUSSION

Laurence and Nagle (1963) were unable to modify significantly the pressor response to angiotensin in man with either bretylium or guanethidine. McCubbin and Page (1963,b) and Gross, Montague, Rosas and Bohr (1965), from studies in animals, also concluded that the sympathetic nervous system did not make a major contribution to the angiotensin pressor response. However, in the original experiments of Bickerton and Buckley (1961) the adrenergic-blocking drug, piperoxane, greatly reduced the pressor response.

In the present study, the cardiovascular sympathetic innervation of all the patients and students who acted as subjects had been interrupted as completely as possible and in a variety of ways. In the normal subjects where phentolamine was used to interfere with sympathetic nerve activity by virtue of its alpha-receptor blocking properties, the hand vasoconstriction during intravenous infusions of angiotensin was abolished in every case, yet the pressor responses were not significantly modified. Although it is unlikely that the alpha-receptor blockade was absolutely complete, nevertheless, one would have expected some modification of the angiotensin pressor response if sympathetic nerve stimulation made a significant contribution to the total pressor response.

Despite the evidence of considerable cardiovascular denervation in the two patients with autonomic nervous system degeneration, and in the quadriplegic patient, the pressor responses to both angiotensin and noradrenaline were approximately three times greater than normally seen with the doses given. The fact that both drugs showed a similar degree of enhancement suggests a common mechanism and the most likely one is abolition of compensatory baroreceptor reflex mechanisms.

These observations suggest that the contribution of sympathetic nerve stimulation to the vasoconstrictor action of angiotensin in vascular beds other than the hand is not very great and that the increase in total peripheral resistance is due to a predominance of the direct, non-adrenergic vasoconstrictor action of the drug.

The failure of phentolamine to modify the pressor response to angiotensin indicates that adrenal medullary release of catecholamines by angiotensin does not make an important contribution to the pressor response in man. This suggestion is supported by Vincent, Kashemsant, Cuddy, Fried, Smulyan and Eich (1965) who found no change in the urinary vanillylmandelic acid levels following prolonged intravenous infusions of angiotensin in man, using doses similar to those in the present study. Furthermore, the

pressor response to intravenous infusions of angiotensin is not obviously modified in adrenalectomized patients (Statius van Eps, Smorenberg-Schoorl, Zurcher-Mulder, de Vries and Borst, 1962; Biron, 1964).

It seems then that the contribution made by catecholamines released from the adrenal medulla and by sympathetic nerve stimulation to the total angiotensin pressor response in man is a small one. This is contrary to the findings in animals but may reflect species differences or differences in experimental design. The magnitude of the dose of angiotensin administered and the duration of exposure to it may also determine the extent to which the above indirect mechanisms contribute to the pressor response. For example, the degree of adrenal medullary stimulation in both the dog and cat appears to be dose-dependent (Peach, Cline and Watts, 1966; Lewis, 1964) and logically is more apparent following close intra-arterial (coeliac) injection than on intravenous administration (Lewis, 1964). The dose of angiotensin administered will also influence the degree of sympathetic stimulation as will the density of sympathetic innervation in individual vascular beds. Furthermore, there is evidence to suggest that with prolonged intravenous infusions of angiotensin, the initial pressure rise is due to direct action on the blood vessels but this is subsequently replaced by an indirect sympathetic mechanism which maintains the elevation of arterial

pressure largely by itself (Hill and Pickering, 1939; Blacket, Depoorter, Pickering, Sellers and Wilson, 1950; Dickinson and Lawrence, 1963; Brown, Chapuis and Robertson, 1964; Yu and Dickinson, 1965). This evidence is well supported by the experiments of McCubbin, de Moura, Page and Olmsted (1965) where a hypertensive effect of angiotensin, in doses which were initially subpressor, appeared after several days of continuous infusion. In renal hypertension the initial mechanism is also thought to be a humoral one (renin-angiotensin-aldosterone) and unmodified by various forms of sympathectomy, whereas in the chronic situation a major part of the elevation in pressure appears to be through a neurogenic mechanism (Reed, Sapirstein, Southard and Ogden, 1944; Pickering, 1945; Taggart and Drury, 1940; Taquini, Blaquier and Bohr, 1961; McCubbin and Page, 1963 a, b).

It appears that the observed sympathetic stimulating action of angiotensin in the hand and foot vessels, where it is responsible for almost all the vasoconstriction, is not sufficiently widespread or is not of sufficient magnitude to contribute significantly to the pressor action of the hormone. However, this finding does not exclude the possibility that larger doses of angiotensin than those which can safely be given acutely in normal subjects, or a longer duration of exposure, might produce a significant degree of sympatho-adrenal stimulation.

This suggestion is supported by results obtained in a patient with renovascular hypertension (presented in Section 5). This patient had grossly elevated plasma renin activity (Skinner, 1967), and presumably, therefore, elevated levels of angiotensin in plasma since the reactivity of her hand vessels to local infusions of angiotensin was greatly reduced (Fig. 5-3). There was marginal elevation on two occasions of the 24 hour urinary levels of 3-methoxy 4-hydroxy mandelic acid and the intravenous infusion of phentolamine (1.0 mg./min. for 5 min.) resulted in a fall in blood pressure which was significantly greater than normal (Fig. 3-18). This test was positive on two separate occasions. There was no evidence of phaeochromocytoma from the other investigations performed and following removal of the affected kidney (post-traumatic renal artery stenosis with a small kidney) her blood pressure, phentolamine response and hand vascular reactivity to angiotensin together with plasma renin activity all returned to, and have remained at, normal levels. Although only one patient is involved it is tempting to suggest that the positive phentolamine test, the marginally elevated levels of urinary 3-methoxy 4-hydroxy mandelic acid and the observed tachyphylaxis to infused angiotensin imply that the direct action of angiotensin has become less important in maintaining her hypertension and has been replaced by an indirect sympatho-adrenal mechanism. Further studies in such patients, where the hypertension is entirely the result of elevated plasma

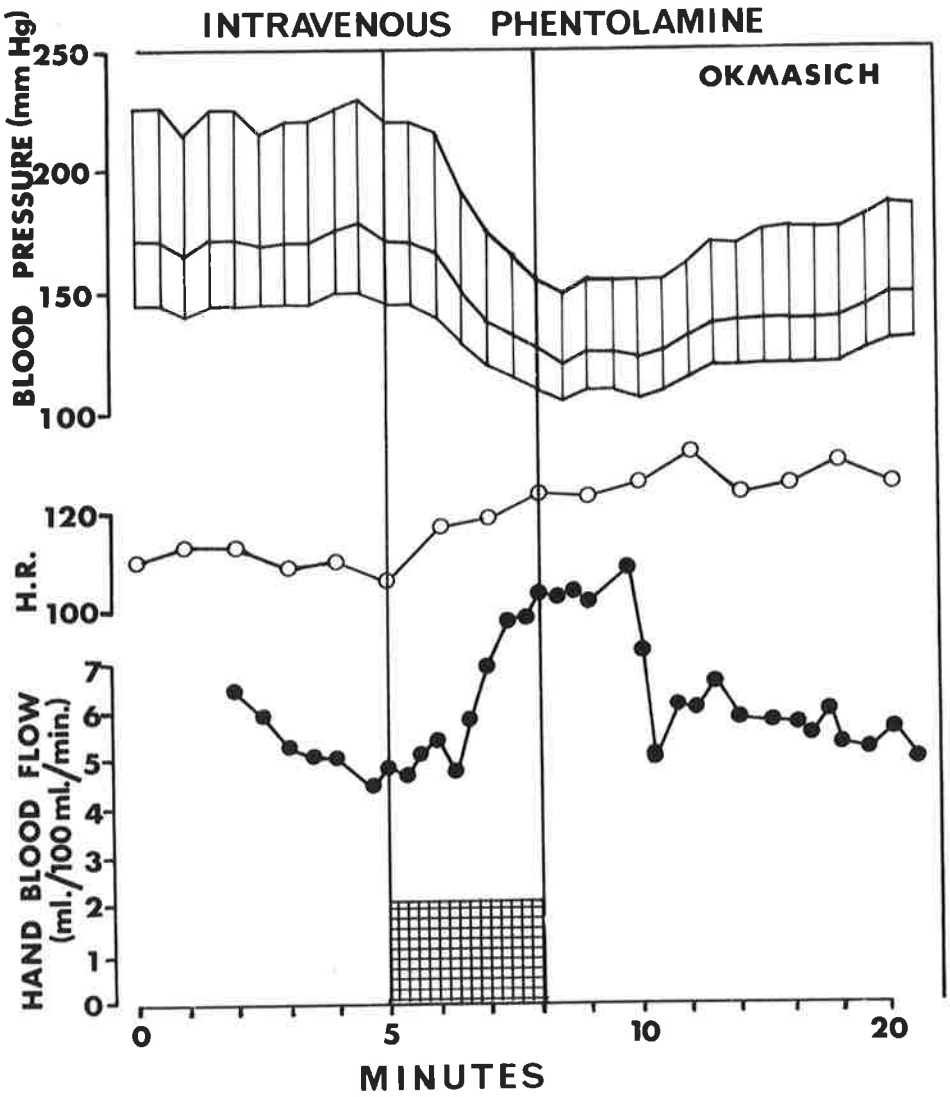

Fig. 3-18 The response of arterial blood pressure, heart rate (O) and hand blood flow (●) pre-operatively in patient M.O. during the intravenous infusion of phentolamine (1.0 mg./min. for 3 min.).

angiotensin levels (at least initially), are required before any definite conclusions can be drawn.

SUMMARY

1. Intravenous phentolamine abolished the sympathetically-mediated hand vasoconstriction appearing during intravenous infusions of angiotensin in normal subjects. However, the blood pressure response was not significantly modified indicating that the sympathetic stimulating action of angiotensin in the doses and duration of exposure used, did not make a major contribution to the overall increase in peripheral resistance.

2. This conclusion is supported by the observation of enhanced pressor responses to angiotensin in three patients with autonomic nervous system degeneration and in one patient with complete cervical cord transection.

3. The failure of phentolamine to modify the pressor response to angiotensin indicates that with the doses used adrenal medullary release of catecholamines, which is known to occur in animals during angiotensin infusions, does not make a significant contribution to the pressor response in man.

SECTION 4

INTERACTIONS BETWEEN ANGIOTENSIN, NORADRENALINE AND SEROTONIN IN THE PERIPHERAL BLOOD VESSELS IN MAN.

INTRODUCTION

The first report of an interaction between angiotensin and other vasoactive substances was that of Braun-Menendez, Fasciolo, Leloir and Munoz (1939, 1940 a) and Braun-Menendez, Fasciolo, Leloir, Munoz and Taquini (1946) who observed that both ephedrine and tyramine potentiated the pressor action of angiotensin in the dog. Prior to this Verney and Vogt (1938) had reported an increase in the pressor response to intravenous tyramine and occasionally that to adrenaline in renal hypertensive dogs. McCubbin and Page (1963 b) also observed an enhancement of the tyramine response in the presence of angiotensin but found that the response to exogenous noradrenaline was not similarly potentiated.

More recently, Sakurai and Hashimoto (1965) reported an enhanced response of the vessels of the perfused rabbit ear to noradrenaline and tyramine when administered in combination with subthreshold amounts of angiotensin. The pressor response to tyramine in the intact rabbit was also enhanced by subpressor amounts of angiotensin.

Striking potentiation by serotonin of the constrictor response to angiotensin and noradrenaline in the isolated perfused central artery of the rabbit ear and human digital artery (obtained

at post-mortem) was observed by de la Lande, Cannell and Waterson (1966). Hurwitz, Campbell, Gordon and Haddy (1961) were unable to demonstrate potentiation by serotonin of the constrictor responses to angiotensin and noradrenaline in the denervated dog forelimb as a whole, although the venous constrictor actions of both drugs were enhanced by serotonin.

These results of animal studies indicated that a similar mechanism might also exist in man. In order to examine this possibility the responses of the hand and forearm vessels have been observed during intra-arterial infusions of angiotensin, noradrenaline and serotonin in various combinations.

METHODS

The subjects were volunteer medical students who lay supine on a couch in a temperature-controlled laboratory for at least one hour before observations began, during which time the infusion needle was inserted and recording apparatus applied.

The infusions were all intra-arterial and were administered to the hand and forearm through the brachial artery at the elbow. Water-filled plethysmographs were used to record blood flow through the hands and forearms on most occasions but in some experiments forearm flow was measured with an electrocapacitance plethysmograph (Willoughby, 1965; Fewings and Whelan, 1966).

In each subject an intra-arterial infusion of one of the three drugs was administered in a dose sufficient to cause an obvious and reproducible effect on hand or forearm blood flow. Then a threshold dose (i.e. one which had little or no effect) of one of the other two drugs was determined. When these responses had been established the threshold infusion was recommenced and after four to five min. both drugs were given simultaneously from the same syringe, having been mixed in such concentrations that the dose rate of each was the same as that when administered singly.

Percentage changes in hand or forearm blood flow during

infusions of each of the three drugs, whether alone or in combination with one of the other two, were determined from the averaged flow values for the two min. prior to drug infusion and for the last two min. of the infusion period, by which time the responses to the drugs had become stable. The doses used did not cause systemic effects and hence the blood flow in the opposite arm was regarded as a control. Allowance could then be made for spontaneous variations in flow unrelated to drug action by assuming that in the absence of the drug infusion the two sides would have maintained the same relationship to each other as in the preinfusion period (Duff, 1952). Allowance was also made for any small effect of the "threshold" drug alone by appropriate correction of the theoretical flow value obtained as above.

The drugs used were angiotensin II (val^5-hypertensin II-asp-β-amide, Hypertensin, Ciba), noradrenaline bitartrate monohydrate (Levophed, Winthrop) and serotonin (5-hydroxytryptamine creatinine sulphate, SR 134, Sandoz). Doses of noradrenaline are expressed as weights of the base and of angiotensin and serotonin as weights of their salts. Ascorbic acid (1 : 50,000) was added to the noradrenaline solutions.

All statistical analyses were done using the Student's "t" test on paired data within individuals.

RESULTS

Hand Vessel Responses

(a) Angiotensin-noradrenaline interaction. Fig. 4-1 shows the hand vessel responses in one subject during intra-arterial infusion of noradrenaline alone (400 ng./min. for 4 min.) and then in combination with intra-arterial angiotensin (400 ng./min.) during the last 4 min. of an 8 min. infusion of this drug. The first 4 min. of this 8 min. infusion period demonstrate that this dose of angiotensin alone in this particular subject had a negligible effect, but when the noradrenaline infusion, which by itself produced a 57% fall in hand blood flow, was repeated in combination with this threshold dose of angiotensin during the last 4 min. the resultant fall in hand blood flow was 96%.

Similar results were obtained in three of four other subjects and the results from all five are summarized in Fig. 4-2 (upper left-hand frame). In some subjects the combined infusions were repeated with almost identical results. When allowance was made for an occasional small constrictor effect of angiotensin by determining the effect of an 8 min. infusion of this drug, it was found that angiotensin significantly enhanced the constrictor action of noradrenaline in the hand blood vessels ($0.02 < p < 0.05$).

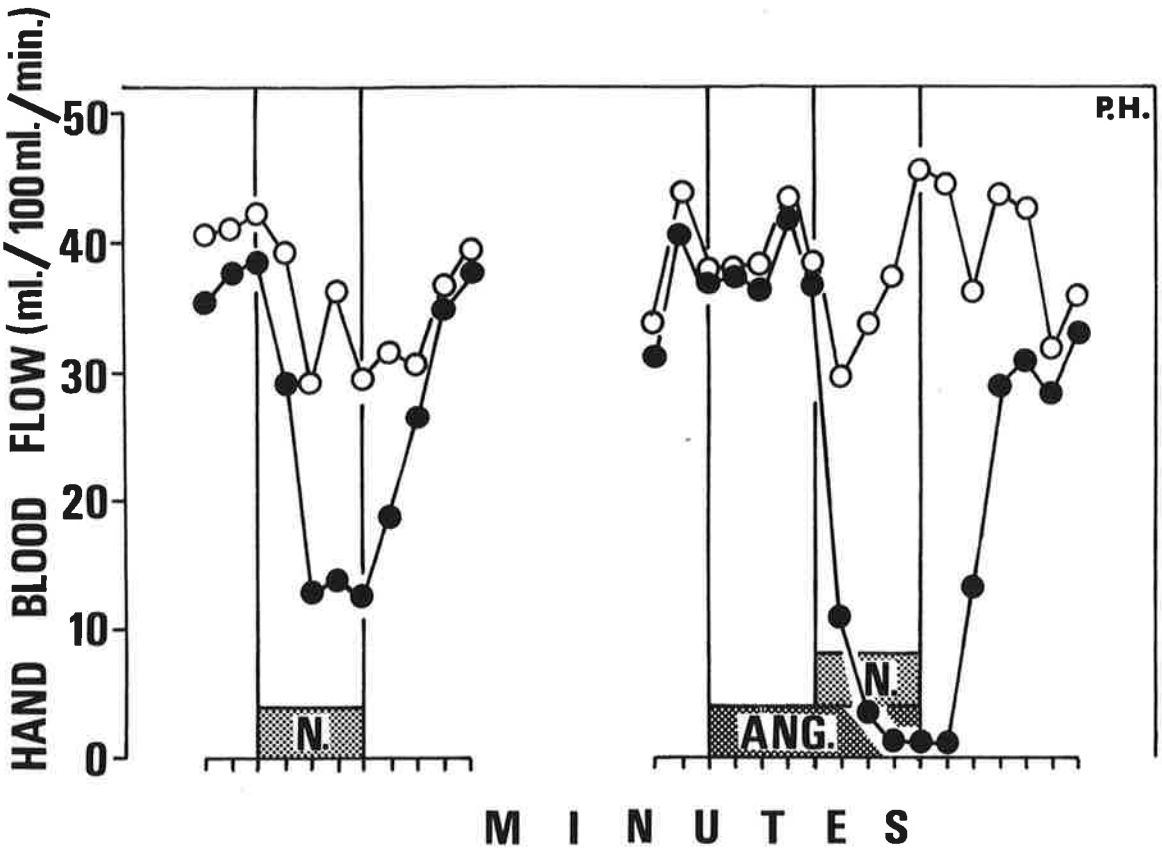

Fig. 4-1 The effect of intra-arterial noradrenaline (400 ng./min. for 4 min.) on hand blood flow (● , infused hand; ○ , control hand) when given alone (left of figure) and then during the last 4 min. of an 8 min. intra-arterial infusion of angiotensin (400 ng./min., right of figure).

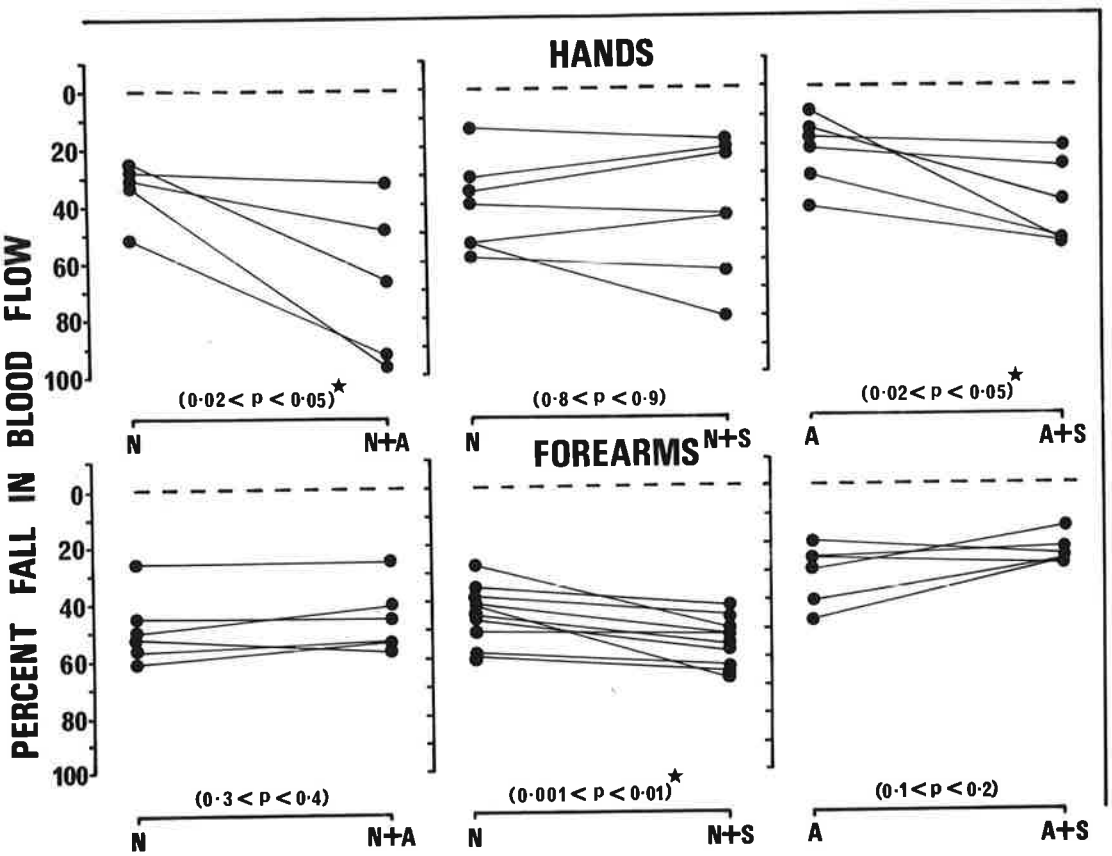

Fig. 4-2 The percentage falls in hand blood flow (upper three frames) and forearm blood flow (lower three frames) in response to intra-arterial infusions of noradrenaline alone (N) and then in combination with intra-arterial angiotensin (N+A, left-hand frames; 5 hand subjects and 6 forearm subjects) and serotonin (N+S, middle frames; 7 hand subjects and 10 forearm subjects), and to angiotensin alone (A) and then in combination with serotonin (A+S, right-hand frames; 6 hand subjects and 6 forearm subjects). Allowance has been made for the constrictor action of the threshold drug in each experiment. The results from the paired "t" tests are shown in brackets, those combinations resulting in significant potentiation being indicated thus * .

(b) Noradrenaline-serotonin interaction. Fig. 4-2 (upper middle frame) shows the mean constrictor responses of the hand vessels of seven subjects to noradrenaline alone and then the mean responses to the same dose when administered in combination with subthreshold doses of serotonin. As in the previous experiments each subject received several infusions of noradrenaline alone at the same dose level (usually 100 ng./min. for 4 min.) to obtain a mean dose-response value and then this dose was repeated during the last 4 min. of an 8 min. infusion of serotonin in a "threshold" dose (usually 250-500 ng./min. for 8 min.). Some of the combined infusions were repeated and a mean value obtained. In calculating the constrictor responses to noradrenaline in the presence of serotonin allowance was made for any small serotonin effect. The constrictor action of intra-arterial noradrenaline in the hand vessels was not significantly altered in the presence of serotonin ($0.80 < p < 0.90$) and in many cases the response was actually reduced.

(c) Angiotensin-serotonin interaction. Fig. 4-3 shows the hand vessel responses in one subject during intra-arterial infusion of angiotensin alone (200 ng./min. for 5 min.) and then in combination with intra-arterial serotonin (500 ng./min.) during the middle 5 min. of a 15 min. infusion period of this drug. In this subject this dose of serotonin alone had a negligible effect, but when the infusion of angiotensin, which alone produced a 14% fall in hand

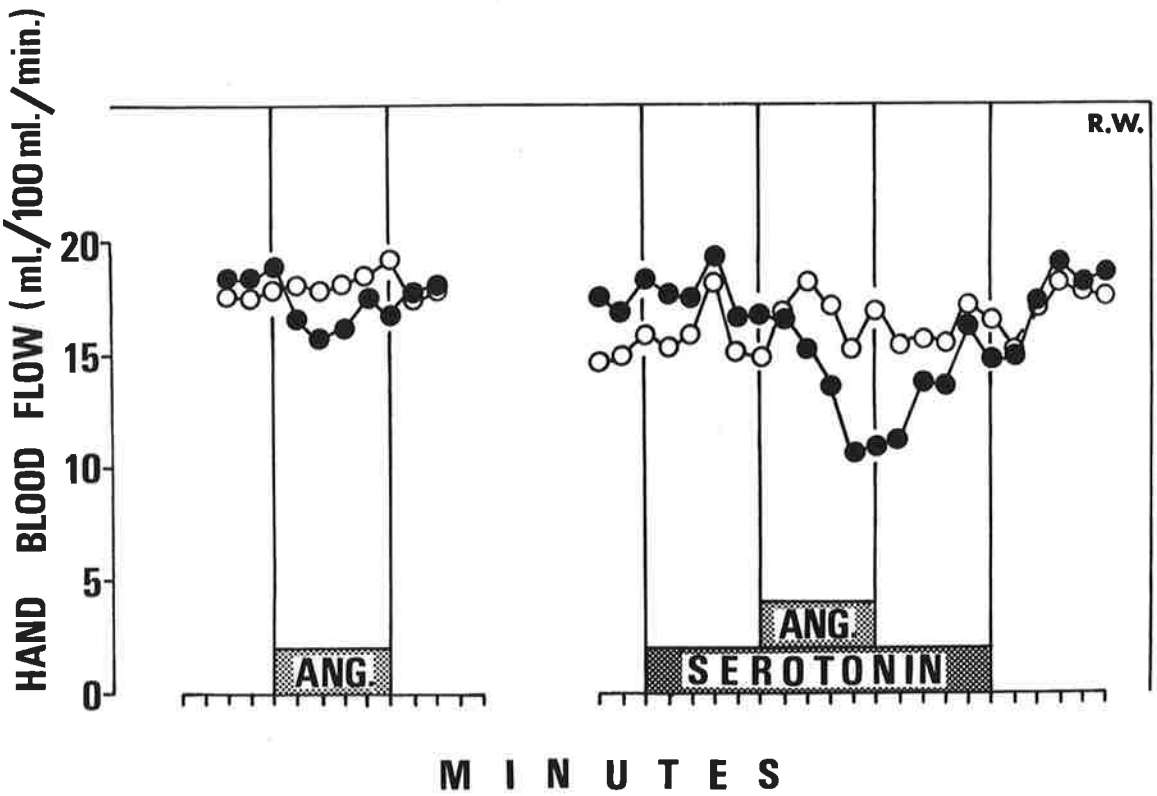

Fig. 4-3 The effect of intra-arterial angiotensin (200 ng./min. for 5 min.) on hand blood flow (● , infused hand; O , control hand) when given alone (left of figure) and then during the middle 5 min. of a 15 min. intra-arterial infusion of serotonin (500 ng./min., right of figure).

blood flow, was repeated against this background the enhancement of constrictor effect to produce a 38% fall in flow was greater than could be explained by summation alone. When the angiotensin infusion was terminated, the serotonin infusion continuing, the blood flow took much longer to return to pre-angiotensin infusion levels than it did following angiotensin alone.

A similar enhancement of the constrictor effect of intra-arterial angiotensin on the hand blood vessels in the presence of serotonin was seen in five other subjects and the enhancement was significant when allowance was made for any serotonin effect ($0.02 < p < 0.05$). The results from all six subjects are summarized in Fig. 4-2 (upper right-hand frame).

Forearm Vessel Responses

(a) Angiotensin-noradrenaline interaction. Fig. 4-2 (lower left-hand frame) shows the mean constrictor responses of the forearm vessels of six subjects to noradrenaline alone (in most experiments 25-50 ng./min. for 4 min.) and then the mean responses to the same dose when administered during the last 4 min. of 8 min. infusion periods of "threshold" doses of angiotensin (in most cases 0.25-0.5 ng./min. for 8 min.). To obtain mean responses most infusions were given more than once and in calculating the constrictor responses to noradrenaline in the presence of angiotensin allowance

was made for any small angiotensin effect. The constrictor action of intra-arterial noradrenaline in the forearm vessels was not significantly altered in the presence of angiotensin ($0.3 < p < 0.4$). This is contrary to the findings in the hand vessels with the same combination and experimental design.

(b) Noradrenaline-serotonin interaction. Fig. 4-4 shows the forearm vascular responses in one subject during intra-arterial infusion of noradrenaline alone (50 ng./min. for 4 min.) and then together with a "threshold" dose of serotonin (500 ng./min.) during the last 4 min. of a 9 min. infusion of this drug. Noradrenaline alone in this subject produced a 25% fall in forearm blood flow but when administered in combination with serotonin the resultant fall in forearm blood flow when corrected for the serotonin effect was 50%. Similar results were obtained in nine other subjects and the results from all ten have been summarized in Fig. 4-2 (lower middle frame). In all subjects the constrictor responses of the forearm vessels to intra-arterial noradrenaline were significantly enhanced in the presence of serotonin ($0.001 < p < 0.01$).

(c) Angiotensin-serotonin interaction. Fig. 4-2 (lower right-hand frame) illustrates the percentage falls in forearm blood flow in each of six subjects in response to intra-arterial angiotensin alone and then in combination with a "threshold" intra-arterial

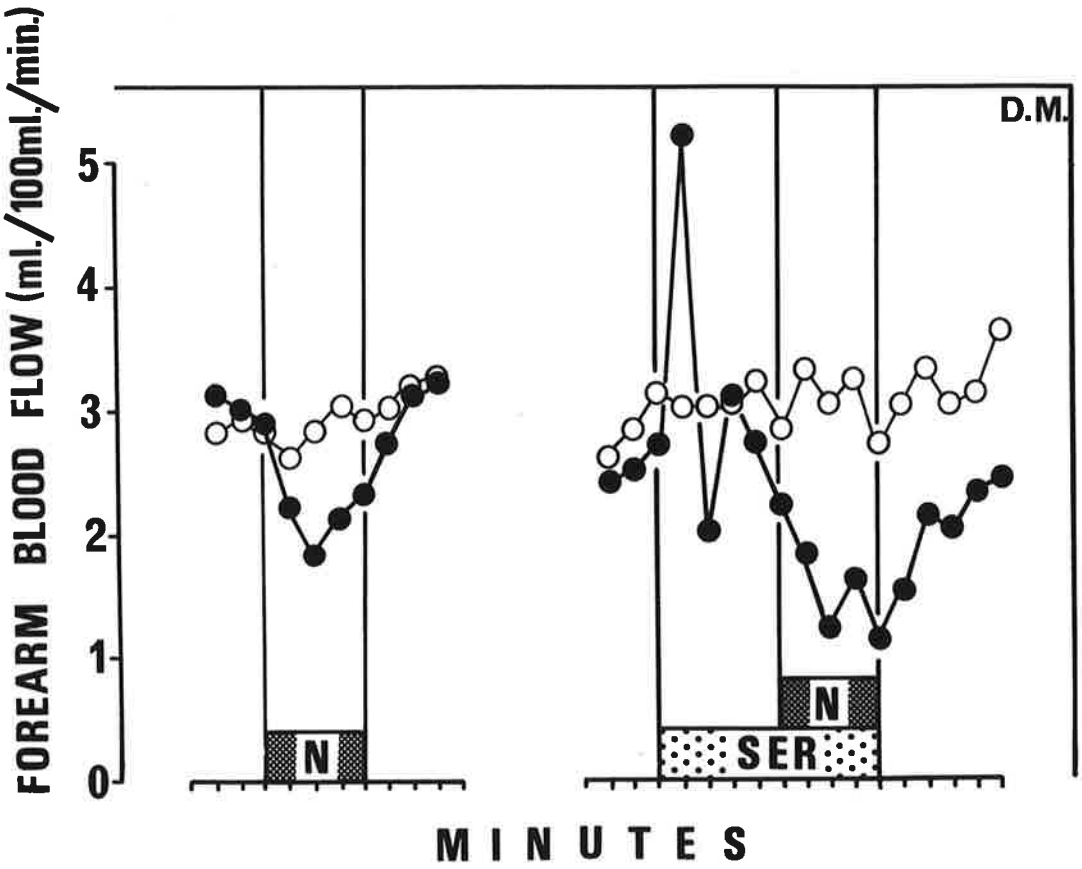

Fig. 4-4 The effect of intra-arterial noradrenaline (50 ng./min. for 4 min.) on forearm blood flow (● , infused forearm; O , control forearm) when given alone (left of figure) and then during the last 4 min. of a 9 min. intra-arterial infusion of serotonin (500 ng./min., right of figure).

infusion of serotonin. In three of the six subjects shown serotonin caused a marked reduction in the constrictor response to angiotensin whereas in the other three there was a small enhancement of the response. Collectively the constrictor action of angiotensin was not significantly altered in the presence of "threshold" amounts of serotonin ($0.1 < p < 0.2$). In contrast, significant potentiation was seen in the hand vessels. As in the other experiments the doses of serotonin used had very little effect on forearm blood flow but allowance was made for this in the calculations.

DISCUSSION

de la Lande, Cannell and Waterson (1966), using the isolated, perfused central artery of the rabbit ear and the digital artery of man (obtained at post-mortem), were able to show potentiation of the constrictor responses to angiotensin and noradrenaline in the presence of subthreshold concentrations of serotonin and concluded that serotonin was the sensitizing agent in these experiments. They also observed an augmented response to noradrenaline in the presence of subthreshold concentrations of angiotensin in the digital artery (personal communication).

In the present study a complicated pattern of interactions between angiotensin, noradrenaline and serotonin was observed and whether or not potentiation occurred seemed to depend not only on the two drugs chosen but also on the vascular bed to which they were administered. Statistically significant potentiation was seen with combinations of angiotensin and noradrenaline and angiotensin and serotonin in the hand but not in the forearm. Conversely, statistically significant potentiation was seen with combinations of noradrenaline and serotonin in the forearm but not in the hand. Furthermore, even with those combinations where collectively there was significant potentiation there were some experiments in which the combined response was very little different

from the individual response. The degree of potentiation seen in isolated animal and human arterial preparations is often quite large, being of the order of a two to twenty-fold increase in constrictor effect (de la Lande, Cannell and Waterson, 1966). Such potentiation would be of considerable importance if it occurred in an intact vascular bed. However, the present results suggest that while statistically significant degrees of potentiation can be demonstrated with some combinations of angiotensin, serotonin and noradrenaline the enhancement is usually very small and is unlikely to be of biological importance.

In the case of the isolated vessel studies the preparation has very little if any tone and is perfused at a low pressure with Krebs or other similar solution. It is therefore not exposed to the normal plasma levels of the hormones under study and may readily exhibit major degrees of potentiation when these are added. In the case of the vessels of the intact limb, however, maximal interaction may already be established between the normal circulating levels of angiotensin, noradrenaline and serotonin and little further potentiation can be achieved by increasing the levels in the perfusing blood.

The mechanism of the potentiation is not clear from these experiments, nor is it possible to decide which of the drugs was

the more important sensitizing factor. This would have required the construction of dose-response curves and the use of receptor-blocking drugs which were not possible to include in the experimental design. de la Lande, Cannell and Waterson (1966) suggest that the mechanism of sensitization may be through changes in the resting membrane potential. For example, serotonin may shift the resting potential of the smooth muscle cell towards the critical firing level, thereby increasing excitability and enhancing the response to some additional constrictor agent. Competitive interactions between the various drugs for receptor sites or catabolic enzymes are other possible mechanisms (Furchgott, 1954; Zeller, Barsky and Berman, 1955; Axelrod and Tomchick, 1960).

In conclusion, interactions between angiotensin and other circulating vaso-active substances, such as noradrenaline and serotonin, are unlikely to make a major contribution to the vasoconstrictor action of the hormone.

SUMMARY

1. The constrictor action of noradrenaline in the hand vessels was significantly enhanced in the presence of threshold amounts of angiotensin as was that of angiotensin in the presence of threshold amounts of serotonin. No such potentiation was seen with the same combinations in the forearm.

2. In the forearm significant potentiation was only seen with constrictor doses of noradrenaline in the presence of threshold amounts of serotonin. No potentiation was seen with this combination in the hand vessels.

3. Although statistically significant potentiation was seen with all three possible combinations of the drugs it was not common to both vascular beds studied. Furthermore it was often of a minor degree and inconsistent within the one combination. These considerations make it unlikely that these interactions are of biological importance in man.

SECTION 5

VASCULAR REACTIVITY STUDIES IN HYPERTENSIVE PATIENTS

INTRODUCTION

A number of studies have suggested that in primary hypertension there is an increase in the vascular reactivity to certain of the naturally occurring hormones (Goldenberg, Pines, Baldwin, Green and Roh, 1948; Greisman, 1954; Doyle and Black, 1955; Duff, 1956; Doyle, Fraser and Marshall, 1959). However, this may be more apparent than real in that an alteration in arterial wall histology could offer an explanation on simple physical principles (Folkow, 1956; Conway, 1958; Hinke, 1965). If this is the case then there should be a non-specific increase in vascular reactivity to all vasoactive agents, and while this has indeed been described (Doyle, Fraser and Marshall, 1959) there have also been reports to the contrary (Duff, 1956; Hinke, 1965).

In most previous studies angiotensin and noradrenaline have been given by intravenous infusion and changes in vascular reactivity deduced from the pressor responses or by calculating changes in total peripheral resistance. While observations of changes in reactivity in this situation are important they are difficult to interpret because systemic vascular responses provoke compensatory mechanisms and these may be modified by treatment. Furthermore, there are difficulties in achieving the necessary accuracy of dosage rate and blood pressure recording using conventional intra-

venous drip and sphygmomanometry and these difficulties may account for the diverse results from tests of this type (Kaplan and Silah, 1964; Morgan, 1965; Breckenridge, 1965; Guedon, Godard, Gregoire and Chapman, 1965). In addition there may be potential harm resulting from raising the blood pressure further in a patient who is already hypertensive (Lancet, 1964 a, b).

For these reasons, in the present study, drugs were infused into the brachial artery to avoid systemic effects. The reactivity of the hand vessels to angiotensin and noradrenaline was determined plethysmographically in patients with primary, renovascular and malignant hypertension and correlated with plasma renin activity. Normotensive subjects were used as controls.

METHODS

General :

The laboratory temperature was maintained at 23-24°C. and both the normotensive and hypertensive subjects rested recumbent on a couch for at least 30 min. before observations began, during which time the recording apparatus was applied and the infusion needle inserted.

Intra-arterial infusions were administered through a 21-gauge needle inserted centripetally under local anaesthesia into the brachial artery at the elbow. Drugs were infused over 4 min. periods from a constant infusion apparatus delivering 2 ml. per min. Saline (0.9%, w/v) was administered throughout the control periods and was also used as a vehicle for the drugs.

Blood flow in both hands was recorded 3-5 times every minute by venous occlusion plethysmography.

The drugs used were noradrenaline bitartrate monohydrate (Levophed, Winthrop) and angiotensin II (val^5-hypertensin II-asp-β-amide, Hypertensin, Ciba). Doses of noradrenaline are expressed as weights of the base and of angiotensin as weights of the amide. Ascorbic acid (1:50,000) was added to the noradrenaline solutions.

Subjects :

Normotensive subjects --- 5 normal male medical students were studied to obtain the normal responses to angiotensin and noradrenaline. Their ages ranged from 19 to 27 years.

Primary hypertension --- 19 patients were included in this group (5 women and 14 men) whose ages ranged from 17 to 48 years and to whom various doses of angiotensin and noradrenaline were administered. All these patients had been subjected to intensive investigation, including renal arteriography, intravenous pyelography, phentolamine test, estimation of the 24 hour urinary excretion levels of 3-methoxy 4-hydroxy mandelic acid (M.H.M.A.) and assay of plasma renin activity. It was concluded from the results of these and other tests that the patients in this group had primary hypertension. Most of the patients were on treatment at the time of the study.

Renovascular hypertension --- 3 patients were included in this group on the basis of selective renal arteriography and assay of plasma renin activity.

1. M.O. --- a 25 year old woman with unilateral renal artery stenosis (probably post-traumatic) and hypertension of approximately one month duration. Her plasma renin activity was

195 ng./ml./hr. (normal 1.7±0.5 ng./ml./hr.). She was studied one week before and one week after operative removal of the affected kidney.

2. R.F. --- a 51 year old man with three renal arteries on the left, one of which was markedly stenosed, and two on the right, both of which were similarly stenosed. He had been hypertensive for at least one year and in addition had polycythaemia, probably of renal origin. He was studied on two occasions, on and off treatment with the same result. He has not been submitted to surgery because of the multiple stenoses and bilateral involvement. His plasma renin activity was measured on the two occasions he was studied with values of 3.8 and 3.5 ng./ml./hr.

3. F.M. --- a 58 year old man with a ten year history of hypertension which had recently increased in severity. Serial 24 hour urinary M.H.M.A. estimations were at or slightly above the upper limit of the normal range. Both kidneys appeared small on intravenous pyelography. Plasma renin activity measured at the time of the sensitivity studies was 7.5 ng./ml./hr.

Malignant hypertension --- 2 patients, one woman (M.L.) aged 33 years and one man (M.S.) aged 42 years, were studied. They were classified as malignant from their clinical course, urinalysis and fundoscopic examination. Both had normal renal arteriograms but

elevated plasma renin activity (6.0 and 8.0 ng./ml./hr. respectively). One has since died and the other has been controlled and is no longer in the malignant phase.

Plasma renin activity :

Plasma renin activity was assayed in all subjects by Dr. S.L. Skinner using a method developed by him in this laboratory (Skinner, 1967).

RESULTS

Primary hypertension

All 19 patients in this group received 200 ng./min. for 4 min. of both angiotensin and noradrenaline and the mean percentage falls in hand blood flow in response to these particular doses of the two drugs, together with their appropriate standard deviations, are shown in Fig. 5-1. The responses of the hypertensive patients were not significantly different from normal.

Nine of the patients (2 women and 7 men) received the same three doses of angiotensin (200, 400 and 800 ng./min. for 4 min.) and of noradrenaline (100, 200 and 400 ng./min. for 4 min.) and the mean percentage fall in hand blood flow ar each dose level, together with the appropriate standard deviation, has been plotted against log-dose of each drug and the resultant dose-response curves are shown in Fig. 5-2. The hatched areas in the figure include one standard deviation about the mean responses obtained from the 5 normotensive subjects for the appropriate dose of each drug. As in Fig. 5-1, the mean responses to both drugs were less in the hypertensive patients but were not significantly different from normal.

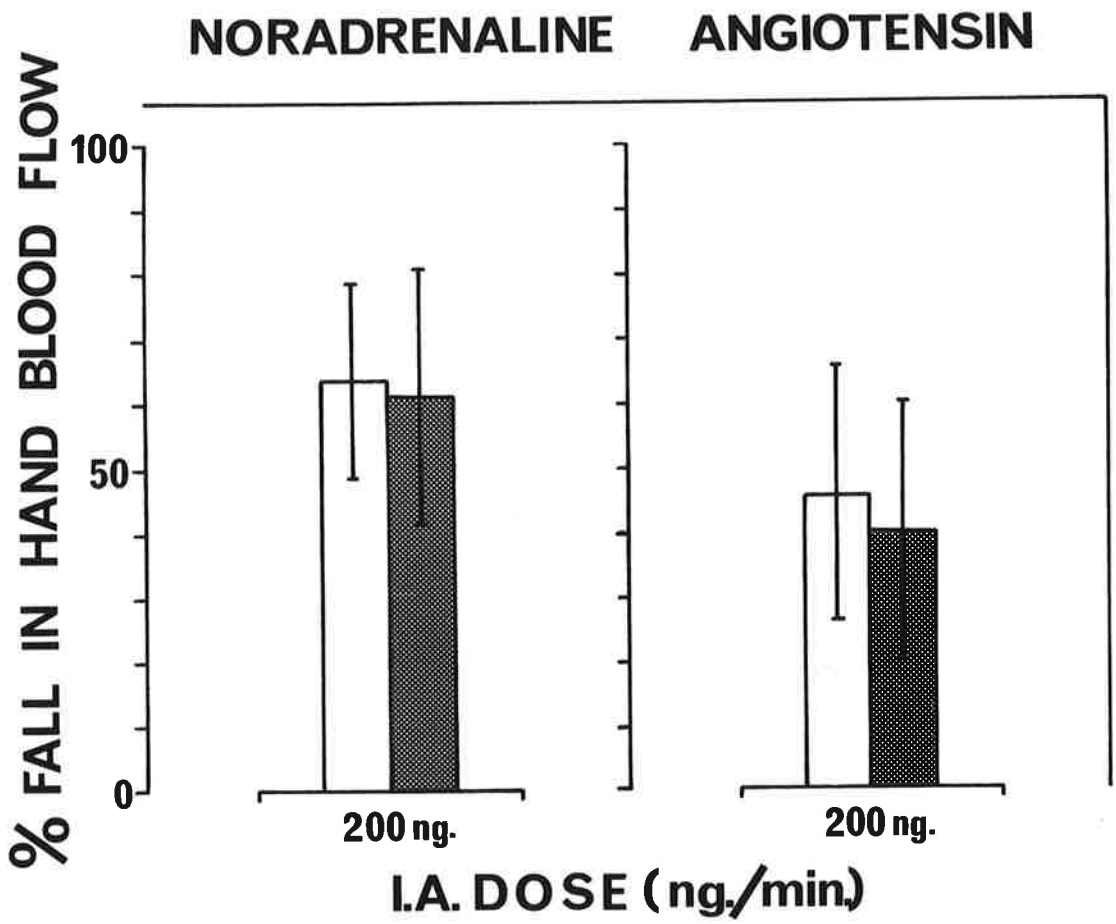

Fig. 5-1 The vertical columns represent the mean percentage falls in hand blood flow in response to intra-arterial infusions of noradrenaline (left-hand frame) and angiotensin (right-hand frame) at a rate of 200 ng./min. for 4 min. in 5 normotensive (open columns) and 19 primary hypertensive (hatched columns) individuals. The vertical lines through the top of each column represent one standard deviation on either side of the mean.

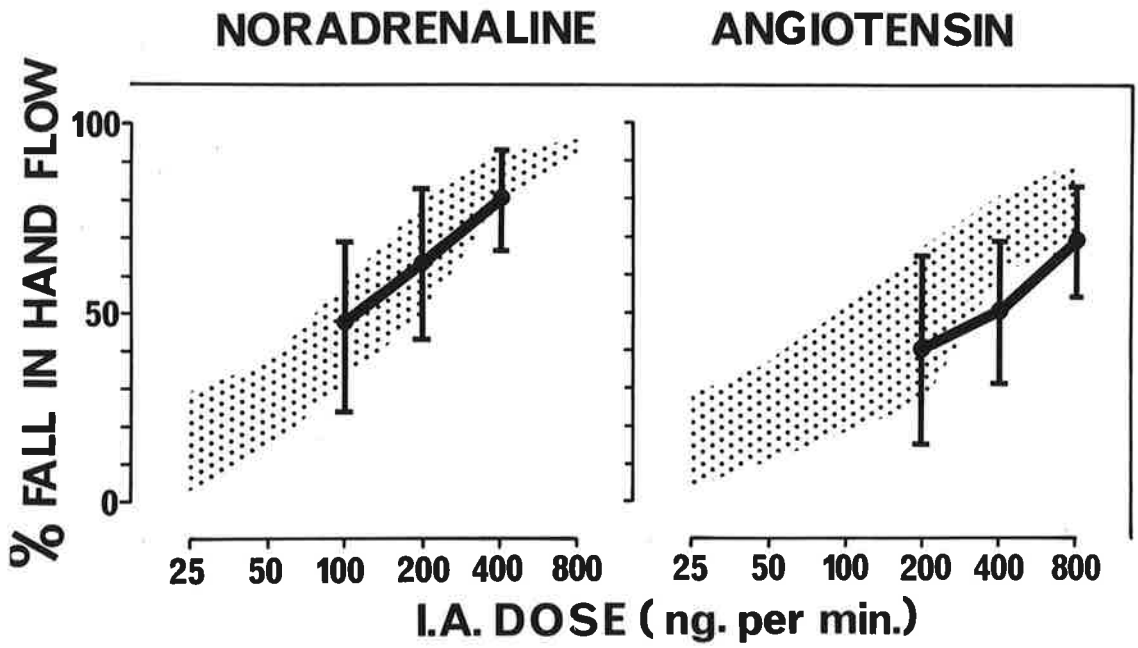

Fig. 5-2 The hatched areas in both frames include one standard deviation about the mean responses for each of the 6 doses of noradrenaline and angiotensin administered to 5 normotensive subjects.

The 2 superimposed dose-response curves (●——●) were constructed from values for the mean percentage fall in hand blood flow in 9 patients with primary hypertension in response to intra-arterial infusion of noradrenaline (left-hand frame) and angiotensin (right-hand frame) in the doses shown. The vertical lines through each point represent one standard deviation on either side of the mean.

Renovascular hypertension

The percentage falls in hand blood flow in patient M.O. have been plotted against log-dose of angiotensin and noradrenaline at several dose levels, before and after operative removal of her affected kidney and the resultant curves are shown in Fig. 5-3. Before operation when she was hypertensive and the plasma renin activity was markedly elevated there was a significant reduction in hand vascular reactivity to angiotensin. One week after operation when her blood pressure and plasma renin activity had returned to normal her hand vascular responses to angiotensin were also in the normal range. The reactivity of her hand vessels to noradrenaline was unchanged by surgery, being on both occasions at the upper limit of normal.

Patient R.F. was studied both on and off treatment. The curves obtained were almost identical and in both instances the reactivity of the hand vessels to angiotensin was significantly reduced whereas that to noradrenaline was at the upper limit of the normal range. The dose-response curves obtained on one of these occasions are shown in Fig. 5-3.

Patient F.M. showed a similar reduction in hand vascular reactivity to angiotensin whereas the responses to noradrenaline were in the normal range. The curves obtained are shown in Fig. 5-3.

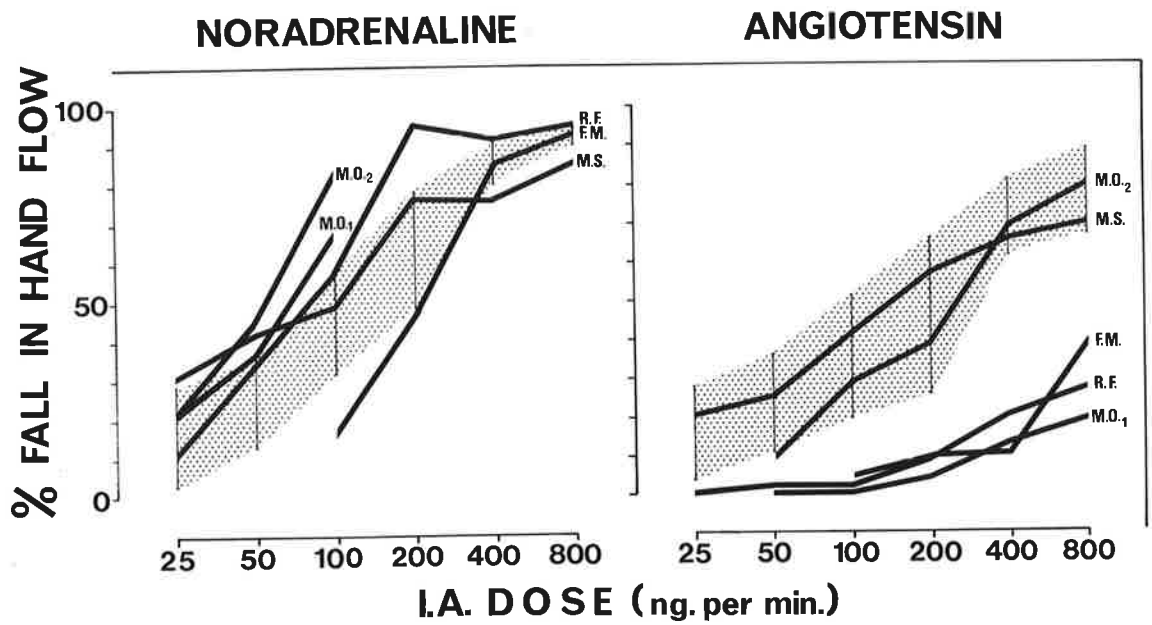

Fig. 5-3 The hatched areas in both frames include one standard deviation about the mean responses for each of the 6 doses of noradrenaline and angiotensin administered to 5 normotensive subjects.

The superimposed dose-response curves shown (▬▬) were constructed from the values for the mean percentage fall in hand blood flow in response to intra-arterial infusion of noradrenaline (left-hand frame) and angiotensin (right-hand frame) in the doses shown. The curves were obtained from one patient with malignant hypertension (M.S.) and three patients with renovascular hypertension (M.O., R.F. and F.M.). Patient M.O. was studied before ($M.O._1$) and after ($M.O._2$) removal of the affected kidney.

Malignant hypertension

The reactivity of the hand vessels to angiotensin and noradrenaline in both these patients was in the normal range and the dose-response curves obtained from one of these patients (M.S.) are shown in Fig. 5-3.

DISCUSSION

While cardiovascular hyper-reactivity to both systemic and local infusions of noradrenaline has been reported previously in primary hypertension (Greisman, 1954; Goldenberg, Pines, Baldwin, Green and Roh, 1948; Doyle, Fraser and Marshall, 1959), this was not seen in the present study. However, our results do agree with those of Duff (1956) who also found a normal response in the hand vessels to intra-arterial infusions of noradrenaline. The normotensive subjects were in a younger age group than those with primary hypertension but this was also the case in the study of Doyle, Fraser and Marshall (1959) where hyper-reactivity of the forearm vessels to both noradrenaline and angiotensin was demonstrated. This may indicate that an increase in reactivity of vascular beds other than the hand is responsible for the increased pressor response to intravenous infusions of noradrenaline observed by other authors.

The marked hypo-reactivity to angiotensin in the three patients with renovascular hypertension is in agreement with the increase in plasma renin activity and presumably plasma angiotensin levels. Although there are no similar studies for comparison Kaplan and Silah (1964) found a reduced pressor response to systemic infusions of angiotensin in such patients. The restoration of a

normal response to angiotensin on the return of normal plasma renin activity following surgery in patient M.O. suggests that the elevation in systemic arterial pressure in this patient was due to the vasoconstrictor action of large amounts of circulating angiotensin and that the hyporeactivity pre-operatively was a manifestation of angiotensin tachyphylaxis (Gross, Bock and Turrian, 1961). In pregnancy, Addison's disease and hepatic cirrhosis the plasma renin activity is also elevated, in the absence of hypertension, and these patients also show hyporeactivity to infused angiotensin (Chesley, Talledo, Bohler and Zuspan, 1965; Brown, Davies, Doak, Lever and Robertson, 1963; Kuchel, Horky, Pazourek and Gregorova, 1964; Laragh, 1962; Johnston and Jose, 1963; Segel, Bayley, Paton, Dykes and Bishop, 1963).

The two patients with malignant hypertension who were studied both had elevated plasma renin activity and the finding of normal vascular reactivity to angiotensin contrasts with the marked reduction in the blood pressure response to intravenous infusions of angiotensin reported by Kaplan and Silah (1964) in patients of this type. This discrepancy is difficult to explain and a further study of such patients is required.

In summary, the hand vessels of those patients with primary and malignant hypertension responded normally to local infusions of

angiotensin and noradrenaline. On the other hand, those patients with renovascular hypertension had a markedly reduced response to angiotensin and a normal response to noradrenaline. The correlation between plasma renin activity and vascular reactivity to angiotensin is not a good one emphasizing the complexity of the renin-angiotensin system.

SUMMARY

1. The reactivity of the hand blood vessels to local intra-arterial infusions of angiotensin and noradrenaline was determined in patients with renovascular, malignant and primary hypertension and compared with that in normotensive individuals and correlated with plasma renin activity.

2. Nineteen patients with primary hypertension and normal plasma renin activity were studied and in all of them the angiotensin and noradrenaline responses were within normal limits.

3. In three patients with renovascular hypertension, each of whom had elevated plasma renin activity, there was a marked reduction in the reactivity of the hand vessels to angiotensin, whereas that to noradrenaline was at the upper limit of the normal range. One of these patients had a unilateral nephrectomy and following this her plasma renin activity, blood pressure and vascular reactivity to angiotensin returned to normal levels.

4. In two patients with malignant hypertension plasma renin activity was elevated but the vascular reactivity to angiotensin and noradrenaline was normal.

SECTION 6

EVIDENCE OF SYMPATHETIC STIMULATION DURING INTRAVENOUS INFUSIONS OF ANGIOTENSIN IN THE DOG

INTRODUCTION

The results presented in the previous sections of this thesis provide evidence that angiotensin has a preganglionic sympathetic stimulating action in man, which, although responsible for almost all the vasoconstriction in the hand and foot, does not appear to be sufficiently widespread or of sufficient magnitude to contribute significantly to the pressor action. Furthermore, there was no evidence from these experiments of an increase in the plasma catecholamine levels during intravenous infusions of angiotensin in man which is contrary to the results from many animal studies (Feldberg and Lewis, 1964; Brody, 1966; Peach,Cline and Watts, 1966; White and Ross, 1966; Ross and White, 1966).

However, it is possible that the relatively small doses of angiotensin which are permissible in human subjects do not release a significant amount of catecholamine from either the sympathetic nerve terminals or the adrenal medulla and therefore those experiments reported in the previous sections were repeated in animals where larger doses could be given.

The changes in brachial artery blood flow, systemic arterial blood pressure and, in some experiments, heart rate and central venous pressure were examined during infusions of angiotensin and

noradrenaline in anaesthetized dogs.

The observations were made both before and after sympathetic denervation or alpha-adrenergic receptor-blockade of one forelimb only, and before and after total alpha- and beta-adrenergic receptor-blockade of the cardiovascular system. The effectiveness of alpha- and beta-receptor blockade was determined by administration of noradrenaline, adrenaline and isoprenaline.

METHODS

Twenty-eight mongrel dogs weighing 10-30 kg. were anaesthetized with sodium thiopentone (20 mg./kg.) and maintained on closed circuit respiration with halothane, nitrous oxide and oxygen.

Intravenous infusions and injections were administered through a polythene catheter (Intramedic PE 90, Clay-Adams) inserted into a femoral vein and arterial pressure was measured from a similar catheter in the femoral artery (Fig. 6-1). The method of administration of intravenous infusions and the measurement of arterial pressure was identical with that described for human experiments. Central venous pressure was recorded with a Statham P 23Dc transducer connected to a catheter which was inserted into a femoral vein in a similar fashion to that described in the human experiments.

Intra-arterial infusions were administered through a polythene catheter (Sterivac No. 1) inserted into a side-branch of one brachial artery distal to the site of blood flow measurement (Fig. 6-1). The infusion technique was the same as that used in the human experiments.

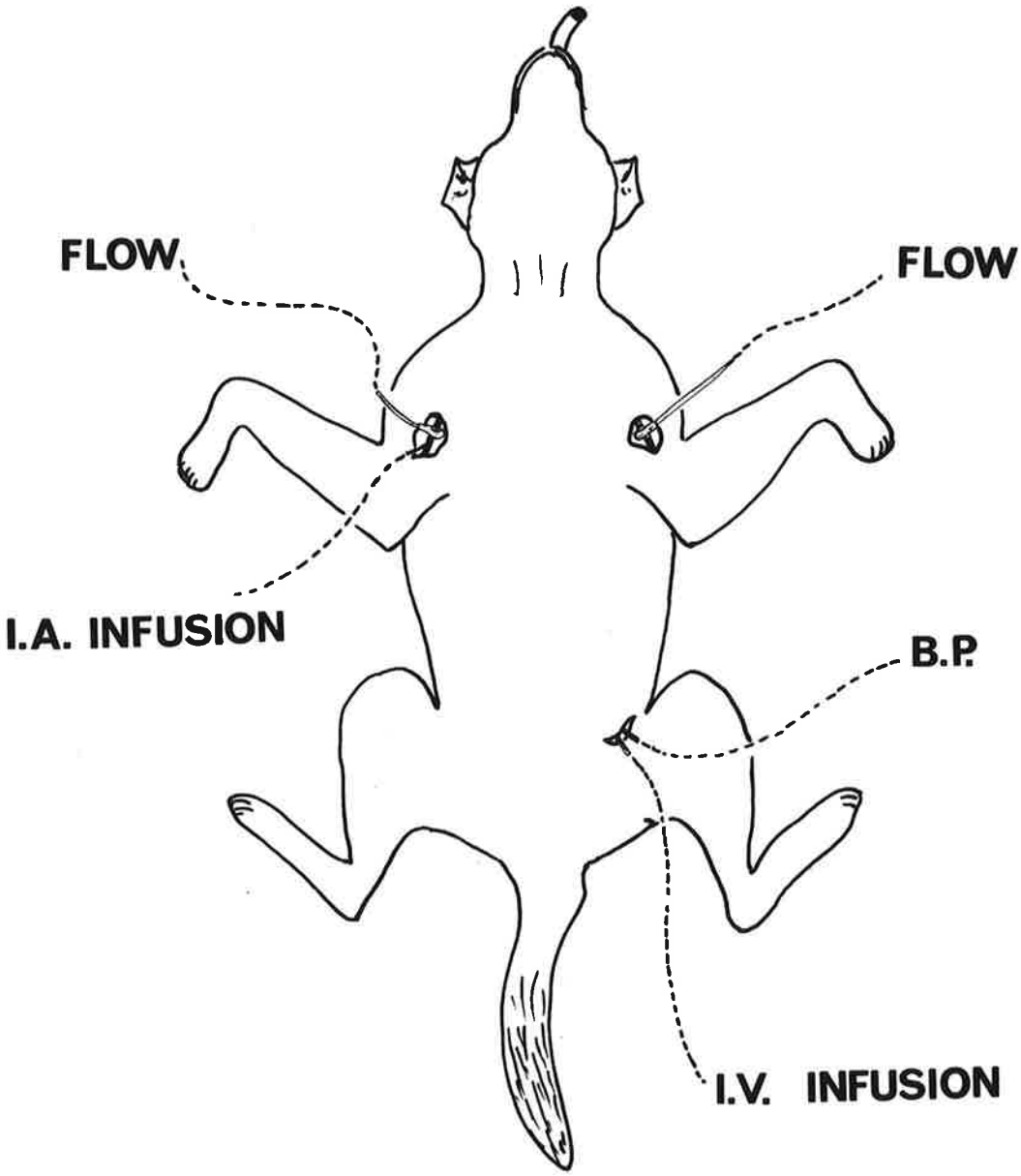

Fig. 6-1 Diagram of dog lying on its back with flow-meters in place and the sites for administering the intra-arterial (I.A.) and intra-venous (I.V.) infusions, and for recording arterial blood pressure (B.P.), indicated.

Blood flow was measured in one or both brachial arteries at the sites indicated in Fig. 6-1, using square-wave electromagnetic flowmeters (Model EMP - 4051, Carolina Medical Electronics), and Grass polygraph (model 5D). The flow probes were calibrated prior to experimentation. This enabled the appropriate probe factor to be determined from the haematocrit and absolute flow could then be measured. Probes of 5 mm. circumference were satisfactory for most brachial flow measurements.

Heart rate was recorded with a Grass Model 5P4 tachograph where the amplitude of the pen deflection is a measure of the period between successive beats, and is therefore the reciprocal of the rate.

The drugs used were angiotensin II (val^5-hypertensin II-asp-β-amide, Hypertensin, Ciba); noradrenaline bitartrate monohydrate (Levophed, Winthrop); phentolamine methanesulphonate (Regitine, Ciba); isoprenaline hydrochloride (Isuprel, Winthrop); adrenaline hydrochloride (D.H.A.); phenoxybenzamine hydrochloride (Dibenyline, S.K.F.) and propranolol (Inderal, I.C.I.). Doses of angiotensin, phentolamine, isoprenaline, phenoxybenzamine and propranolol are expressed as weights of their salts and of noradrenaline and adrenaline as weights of their bases. Ascorbic acid (1:50,000) was added to the adrenaline and noradrenaline solutions.

RESULTS

(a) Alpha-receptor blockade of one forelimb :-

Fig. 6-2 shows the changes in systemic blood pressure and right and left brachial artery blood flow following the intravenous injection (arrow) of angiotensin (2.5 µg. in 1.0 ml. injected in a 10 sec. period) before (A) and after (B) treatment of the right forelimb with phentolamine (125 µg./min. given by continuous infusion throughout the recording period.

Before treatment (Fig. 6-2, A) the blood flow in both limbs showed an initial transient increase in flow which rapidly gave way to a marked fall in flow of approximately equal magnitude in each limb. The blood pressure showed a similar initial transient increase which was followed by a more sustained rise. Both the flow and pressure changes had returned to pre-injection levels within 6-7 min.

An intra-arterial infusion of phentolamine was then begun through a side-branch of the right brachial artery distal to the flowmeter (Fig. 6-1) in a dose of 125 µg./min., which produced very little change in resting flow on the infused side and was without significant systemic effect. The angiotensin injection

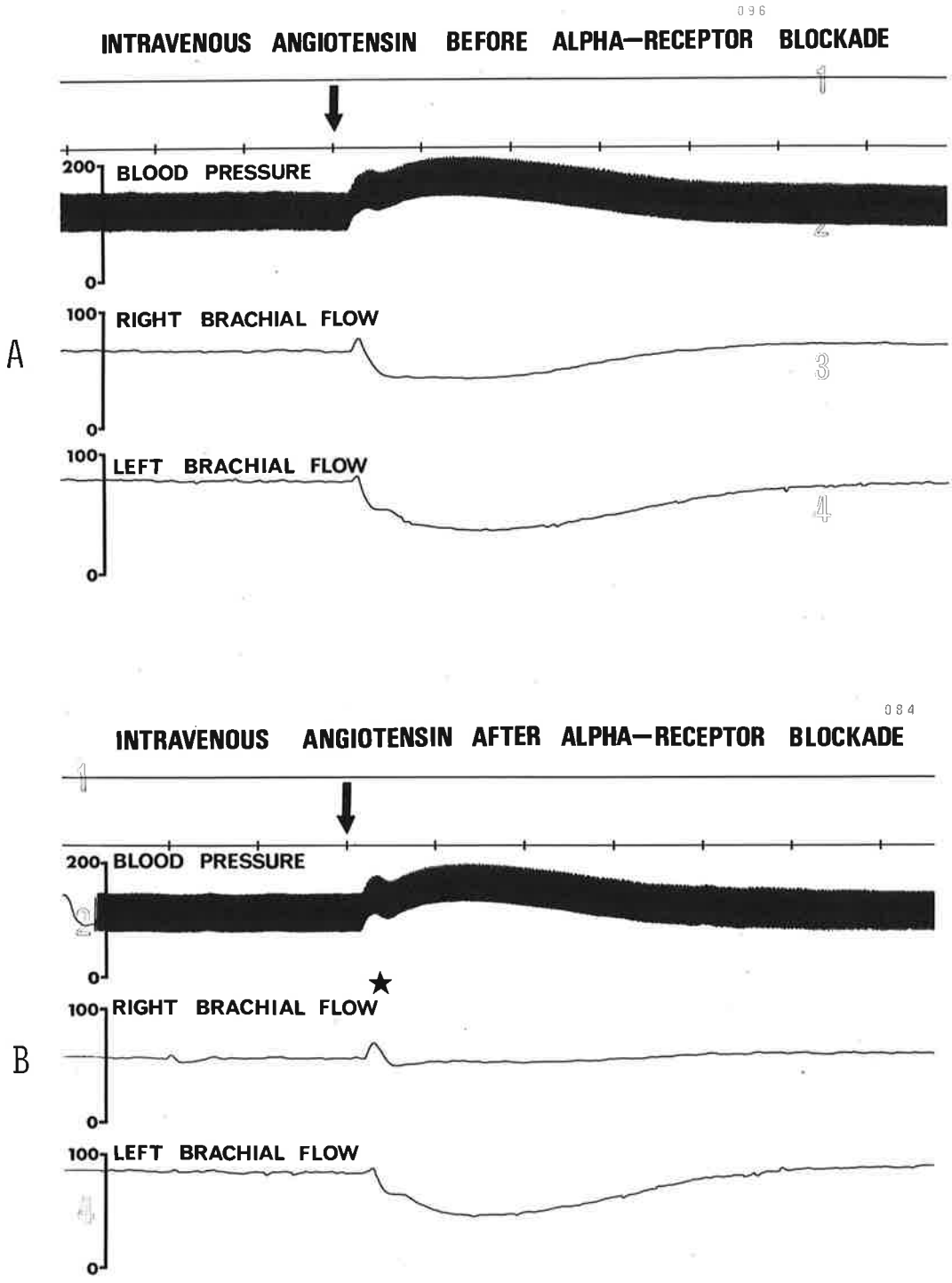

Fig. 6-2 The response of arterial blood pressure and right and left brachial artery flow following the intravenous injection (arrow) of angiotensin (2.5 µg. in 1.0 ml.) before (A) and after (B) treatment of the right forelimb with phentolamine (125 µg./min. throughout the entire recording period).

(2.5 µg. in 1.0 ml.) was then repeated, the phentolamine infusion continuing throughout (Fig. 6-2,B). The blood pressure response and the initial transient increases in limb flow were little changed. However, on this occasion no fall in blood flow was seen in the phentolamine-treated limb whereas the untreated limb showed essentially the same response as previously.

The effectiveness of this dose of phentolamine in producing local alpha-receptor blockade is demonstrated in Fig. 6-3. Following the intravenous injection of noradrenaline (5.0 µg. in 1.0 ml. injected over 10 sec.) a fall in blood flow occurred in the untreated left limb whereas the blood flow in the opposite right limb, which was receiving an intra-arterial of phentolamine throughout (125 µg./min.), showed a small increase, possibly as a passive response to the increase in systemic blood pressure.

Blockade of the constrictor action of intravenous angiotensin in the right forelimb of this dog was also seen when the angiotensin was administered by continuous intravenous infusion rather than as a single injection. Before phentolamine treatment (Fig. 6-4,A) angiotensin (2.5 µg./min. for 5 min.) produced a sustained decrease in blood flow throughout the infusion period which was of approximately equal magnitude in both limbs. There was a rise in both systolic and diastolic blood pressure and an

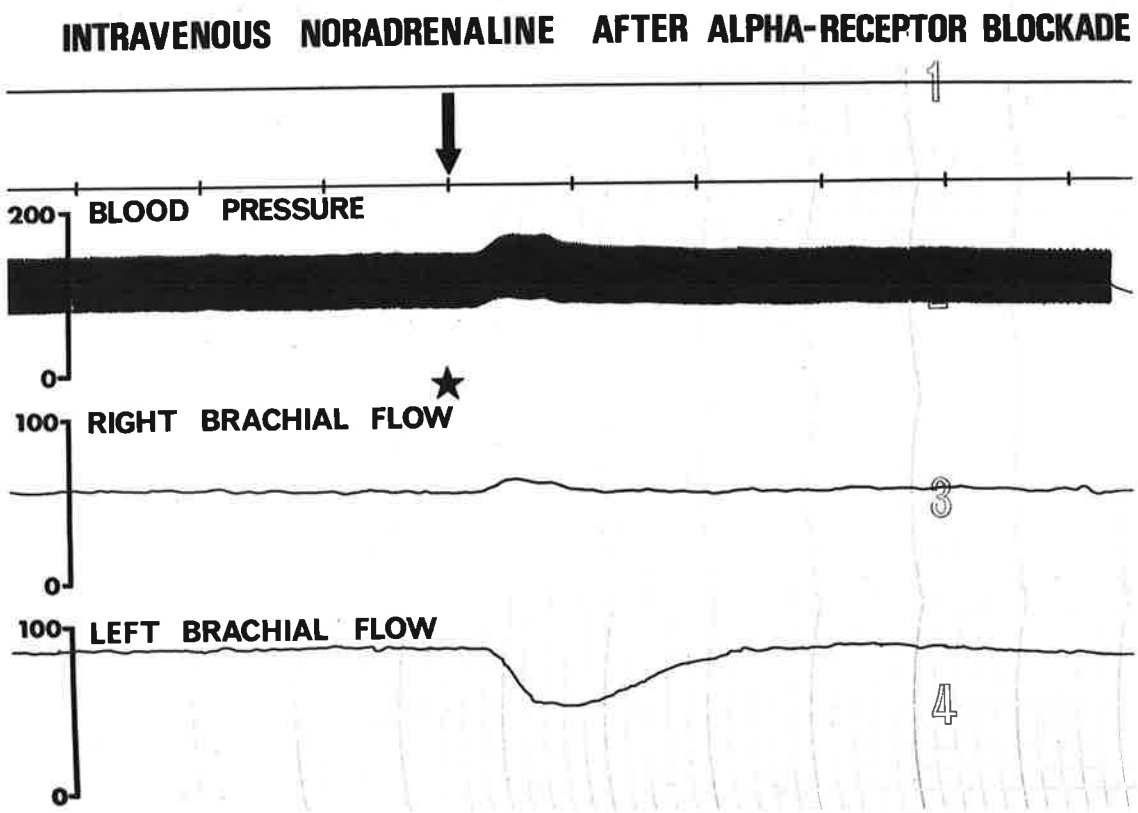

Fig. 6-3 The response of arterial blood pressure and right and left brachial artery flow following the intravenous injection (arrow) of noradrenaline (5.0 µg. in 1.0 ml.) after treatment of the right forelimb with phentolamine (125 µg./min. throughout the entire recording period).

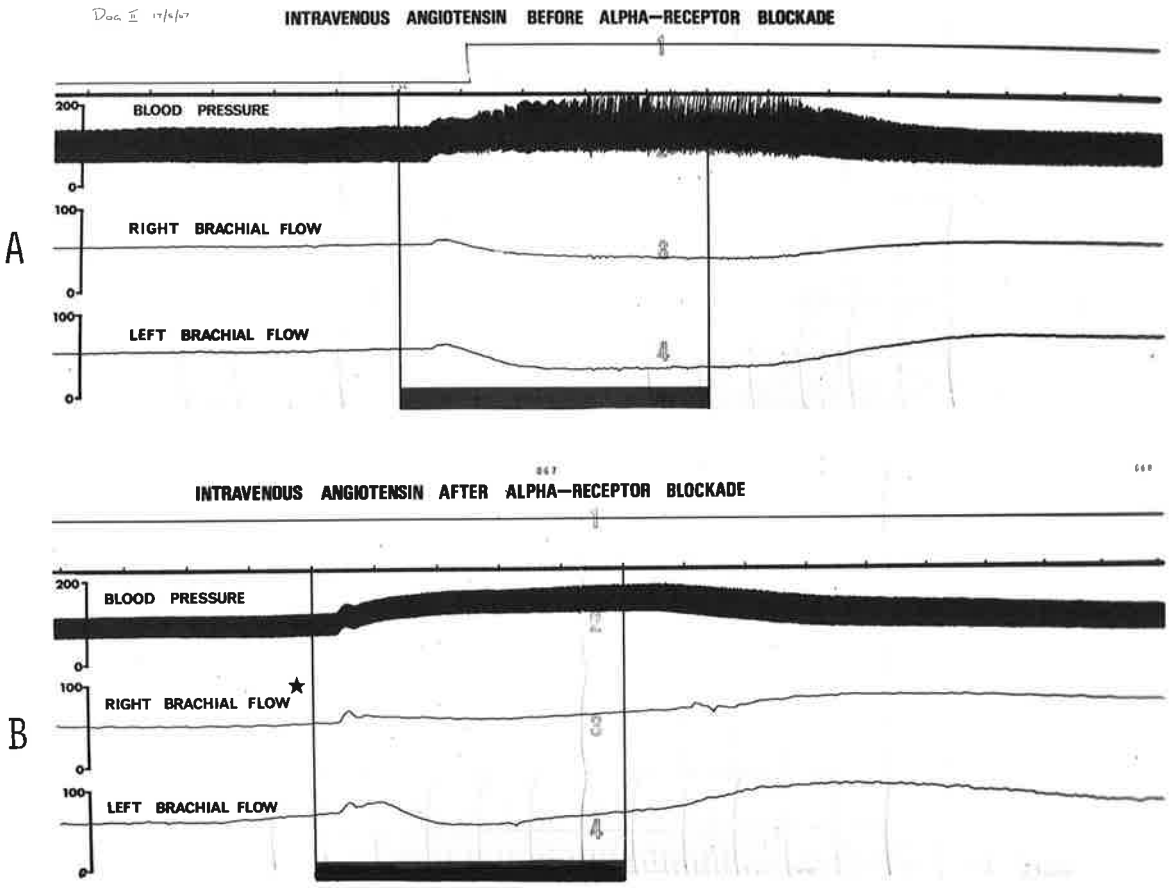

Fig. 6-4 The response of arterial blood pressure and right and left brachial artery flow during the intravenous infusion of angiotensin (2.5 µg./min. for 5 min.) before (A) and after (B) treatment of the right forelimb with phentolamine (125 µg./min. throughout the recording period.

abnormal cardiac rhythm appeared. After treatment of the right forelimb with phentolamine (125 µg./min. throughout the observation period) the constrictor response to the same dose of angiotensin was abolished whereas the untreated limb showed essentially the same response as before (Fig. 6-4,B). Although the magnitude of the pressor response was almost unchanged the rhythm disturbance did not appear on this occasion.

(b) Acute denervation of one forelimb:-

Fig. 6-5 shows the changes in systemic blood pressure and right brachial artery blood flow during the intravenous infusion of angiotensin (2.0 µg./min. for 5 min.) before (A) and after (B) acute surgical denervation of the right forelimb.

Before denervation angiotensin produced a fall in brachial artery flow and a rise in systemic blood pressure, a minor rhythm change occurring towards the end of the infusion period.

The brachial plexus of nerves, which embraces the brachial artery at this level, was then severed and the brachial artery adventitia stripped to ensure maximum sympathetic denervation of the forelimb vascular bed. The angiotensin infusion was then repeated and on this occasion (Fig. 6-5,B) brachial artery flow showed a marked increase. The pressure rise was almost unchanged

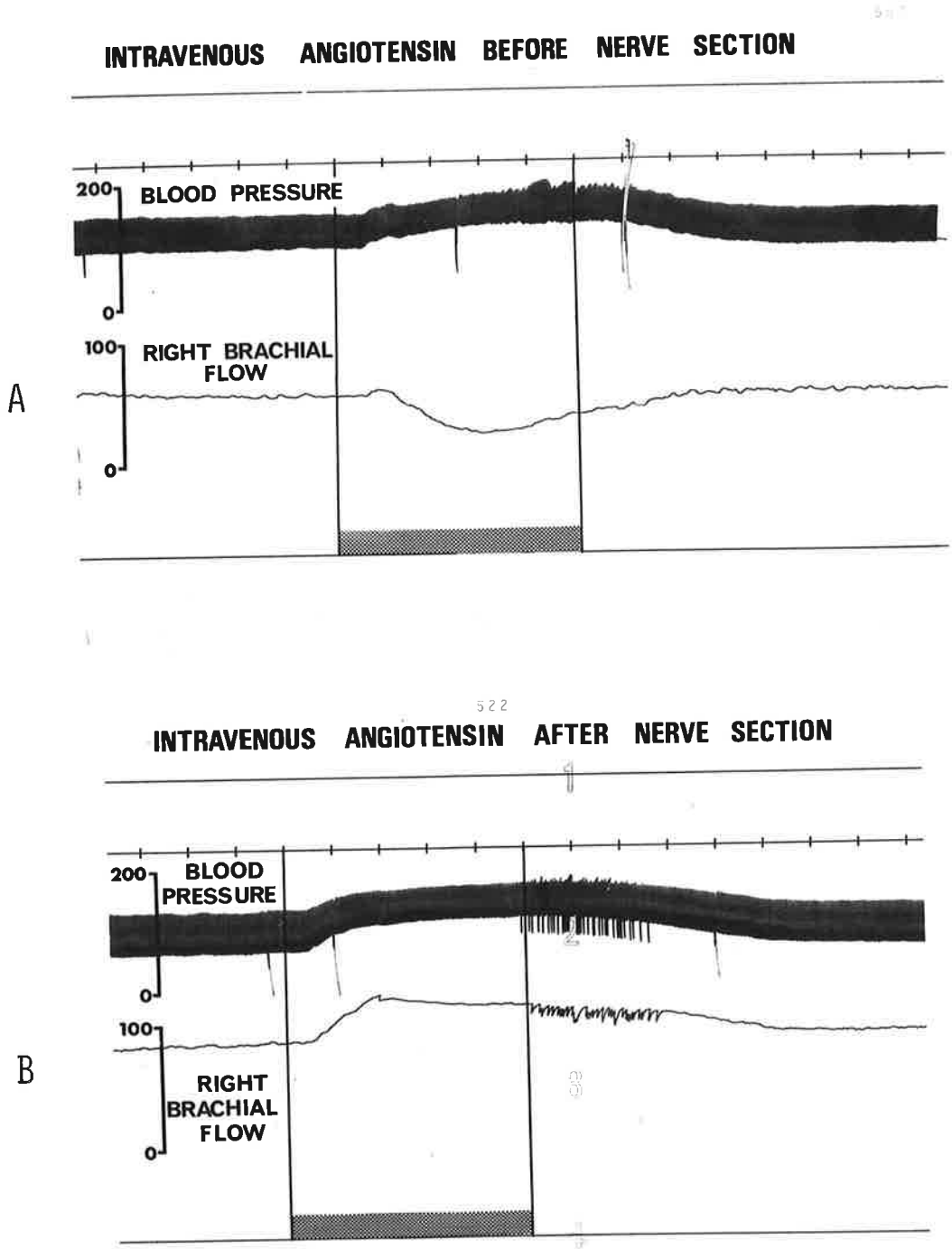

Fig. 6-5 The response of arterial blood pressure and right brachial artery flow during the intravenous infusion of angiotensin (2.0 µg./min. for 5 min.) before (A) and after (B) section of the nerves to the right forelimb.

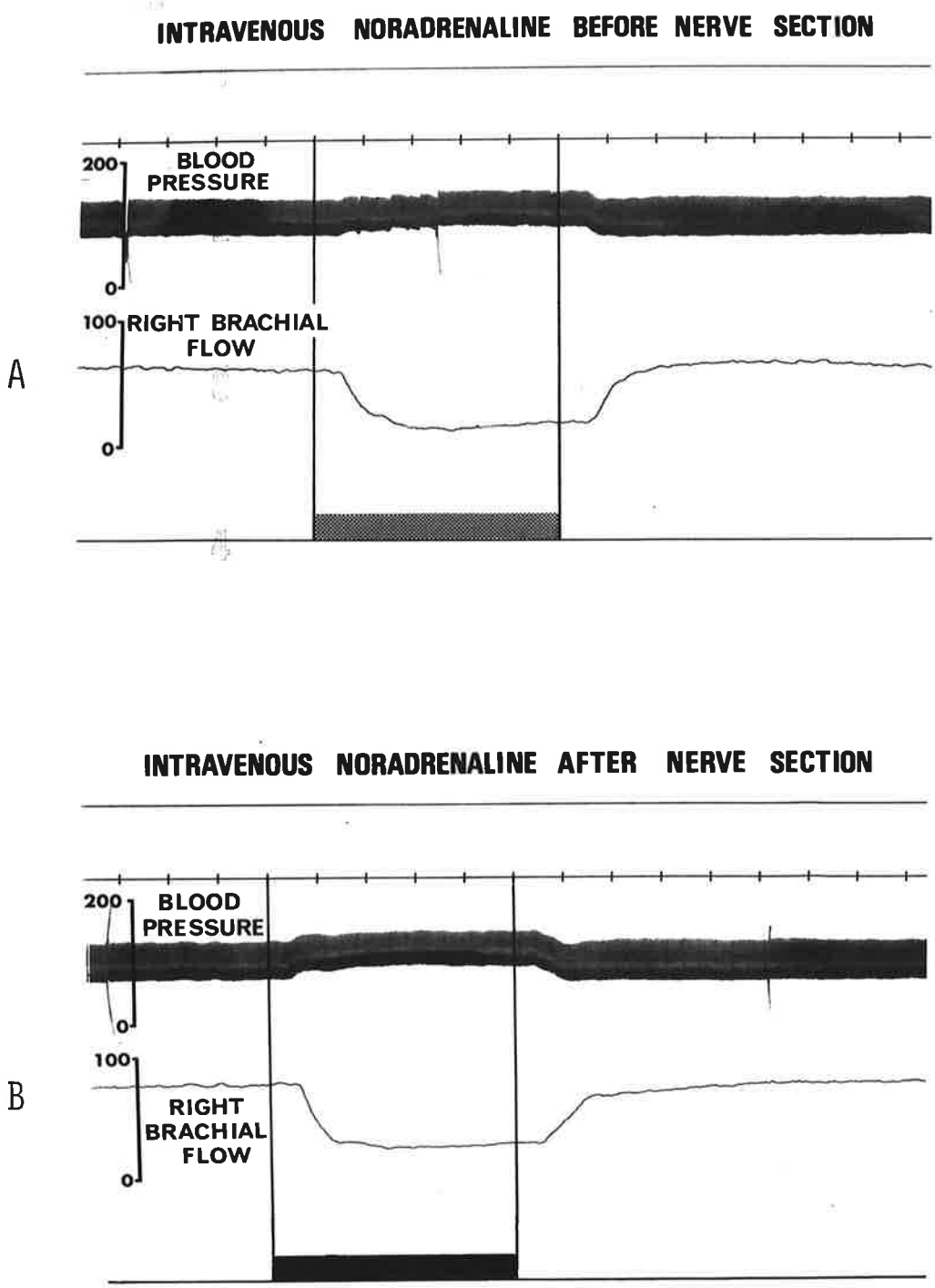

Fig. 6-6 The response of arterial blood pressure and right brachial artery blood flow during the intravenous infusion of noradrenaline (5.0 μg./min. for 5 min.) before (A) and after (B) section of the nerves to the right forelimb.

although the cardiac rhythm disturbance was more noticeable and affected the post-infusional flow level.

Interruption of the nerves had no effect on the constrictor action of intravenous noradrenaline (5.0 µg./min. for 5 min.). Neither the fall in brachial blood flow nor the rise in arterial pressure induced by noradrenaline were altered by nerve section (Fig. 6-6, A & B).

(c) Systemic alpha- and beta-receptor blockade :-

Fig. 6-7 shows the changes in central venous pressure, systemic blood pressure, right brachial artery blood flow and heart rate during the intravenous infusion of angiotensin (2.0 µg./min. for 10 min.) on three separate occasions in the same dog.

The upper tracings (Fig. 6-7, A) show the responses to angiotensin alone. The same dose of angiotensin was then repeated, firstly following total beta-receptor blockade with propranolol (250 µg./min. for 2o min. , Fig. 6-7, B) and then following combined alpha- and beta-receptor blockade with phenoxybenzamine (1.o mg./min. for 20 min.) and propranolol (Fig. 6-7, C).

In the absence of any other treatment the intravenous infusion of angiotensin (2.0 µg./min. for 10 min.) resulted in a

Fig. 6-7 The response of central venous pressure (C.V.P.), arterial blood pressure (B.P.), right brachial artery blood flow (R.B.F.) and heart rate (H.R.) during the intravenous infusion of angiotensin (2.0 µg./min. for 10 min.), administered throughout the hatched period. The time scale between the C.V.P. and B.P. records is in minutes.

A - in the absence of any other treatment.

B - after total beta-receptor blockade with propranolol.

C - after combined alpha- and beta-receptor blockade with phenoxybenzamine and propranolol.

An increase in heart rate is indicated by a decrease in width of the record and vice versa. An increase in central venous pressure is indicated by an upward movement of the tracing. These parameters were of secondary interest and no calibration is shown.

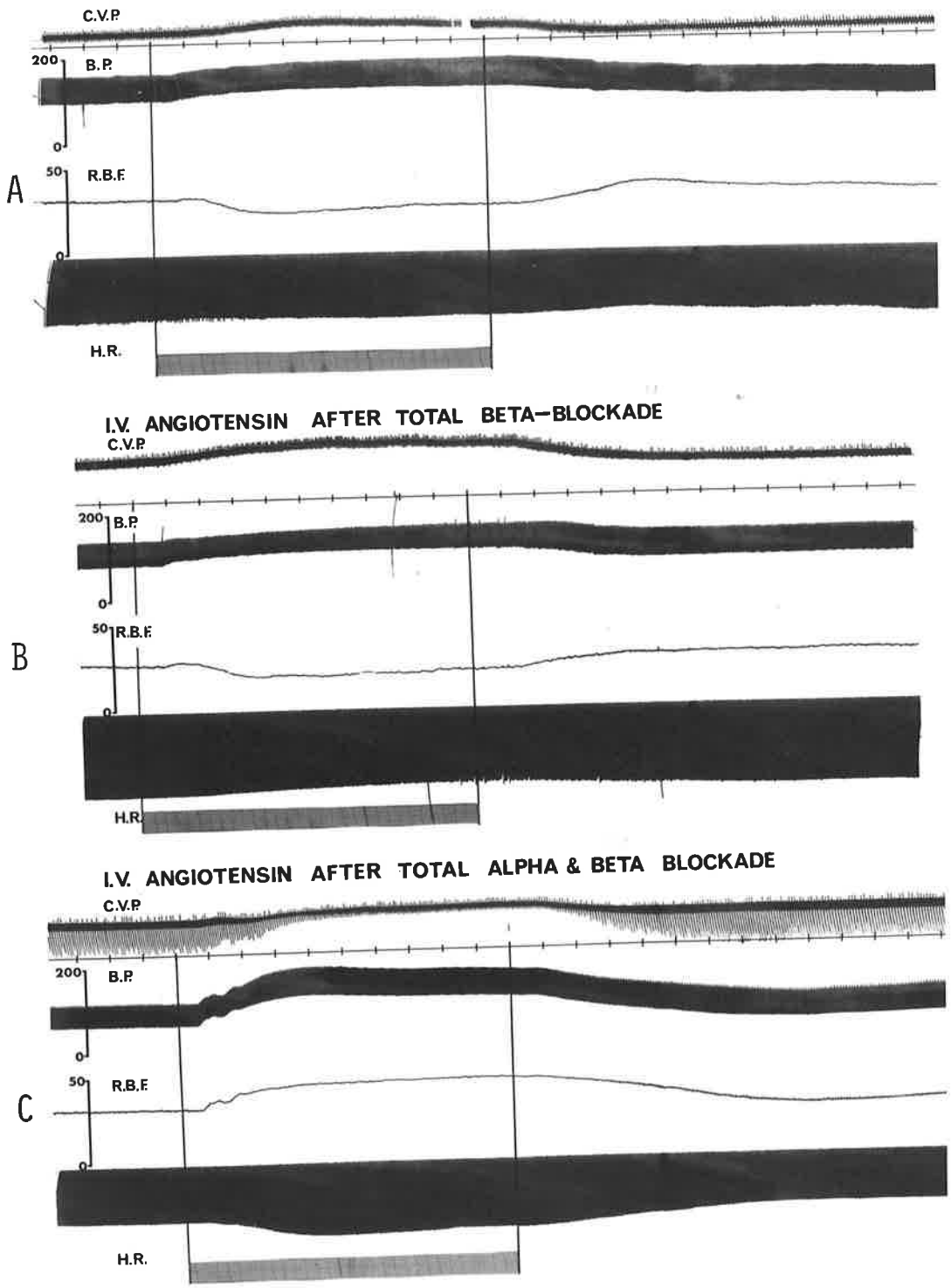
I.V. ANGIOTENSIN BEFORE TOTAL BLOCKADE
C.V.P.
200
B.P.
0
A
50
R.B.F.
0
H.R.
I.V. ANGIOTENSIN AFTER TOTAL BETA-BLOCKADE
C.V.P.
200
B.P.
0
50
R.B.F.
B
0
H.R.
I.V. ANGIOTENSIN AFTER TOTAL ALPHA & BETA BLOCKADE
C.V.P.
200
B.P.
0
50
R.B.F.
C
0
H.R.

Fig. 6-7

rise in central venous pressure and systemic blood pressure without any marked change in heart rate. Limb blood flow showed a sustained fall throughout the infusion period (Fig. 6-7, A).

Beta-receptor blockade itself resulted in a decrease in the resting heart rate and blood pressure and an increase in central venous pressure (Fig. 6-7, B). However, apart from a small increase in heart rate during the infusion period the responses to intravenous angiotensin were not appreciably altered.

Alpha-receptor blockade produced a further fall in resting blood pressure and an increase in resting heart rate (Fig. 6-7,C). The respiratory fluctuations in central venous pressure were more marked but the mean pressure was little different. However, following combined alpha- and beta-receptor blockade the rise in systemic blood pressure produced by the same dose of angiotensin as above was of much greater magnitude and was accompanied by a bradycardia. The fall in limb flow was replaced by a striking increase which paralleled the blood pressure rise and was sustained throughout the infusion period.

A similar pattern of responses was seen in the same dog with a dose of 4.0 µg./min. for 10 min. of angiotensin (Fig. 6-8, A,B & C).

Fig. 6-8 The response of central venous pressure (C.V.P.), arterial blood pressure (B.P.), right brachial artery blood flow (R.B.F.) and heart rate (H.R.) during the intravenous infusion of angiotensin (4.0 µg./min. for 10 min.), administered throughout the hatched period. The time scale between the C.V.P. and B.P. records is in minutes.

A - In the absence of any other treatment.

B - after total beta-receptor blockade with propranolol.

C - after combined alpha- and beta-receptor blockade with phenoxybenzamine and propranolol.

An increase in heart rate is indicated by a decrease in width of the record and vice versa. An increase in central venous pressure is indicated by an upward movement of the tracing. These parameters were of secondary interest and no calibration is shown.

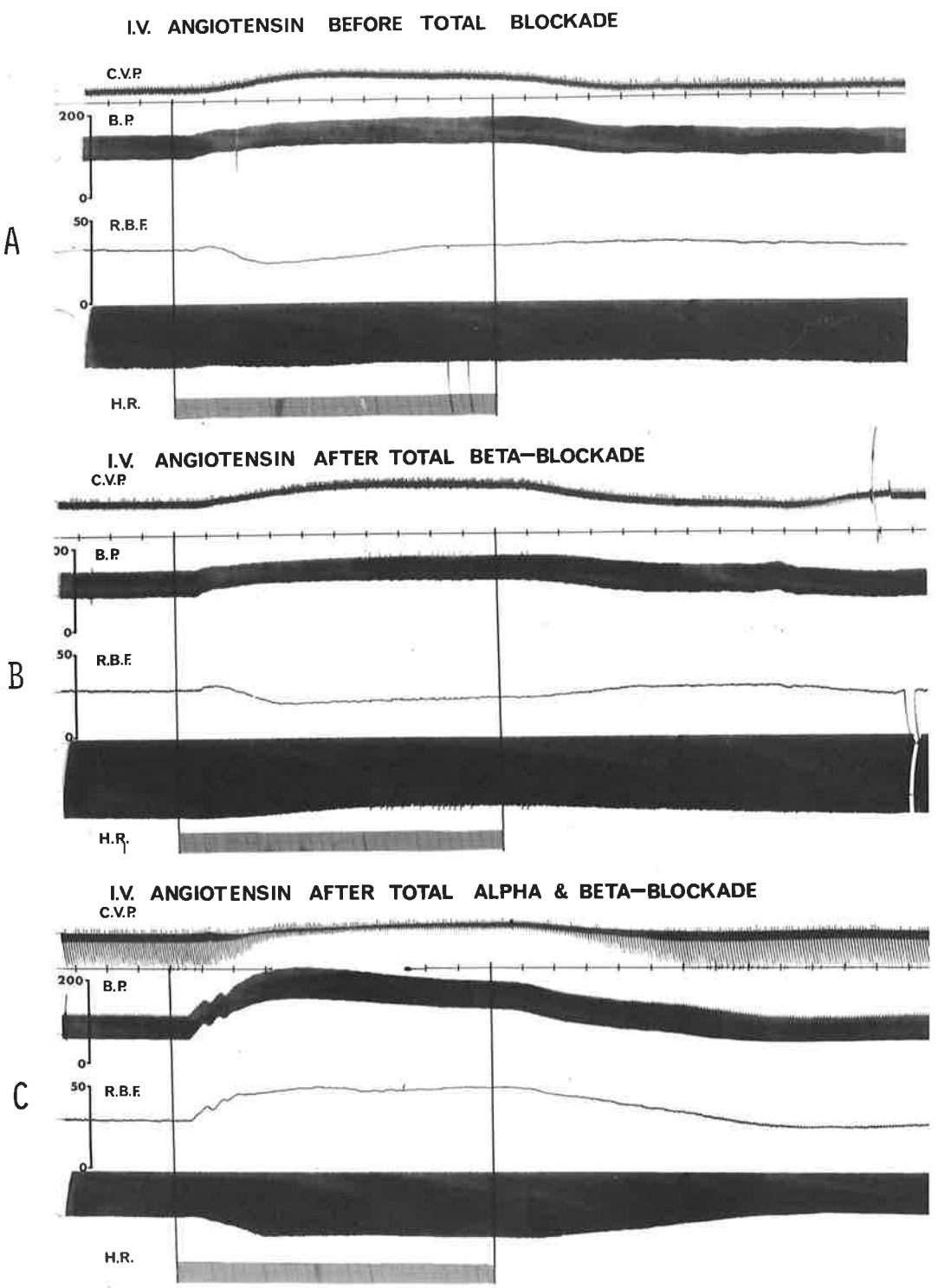
I.V. ANGIOTENSIN BEFORE TOTAL BLOCKADE
C.V.P.
200
B.P.
0
50
R.B.F.
0
H.R.
A
I.V. ANGIOTENSIN AFTER TOTAL BETA-BLOCKADE
C.V.P.
B.P.
0
50
R.B.F.
0
H.R.
B
I.V. ANGIOTENSIN AFTER TOTAL ALPHA & BETA-BLOCKADE
C.V.P.
200
B.P.
0
50
R.B.F.
0
H.R.
C

Fig. 6-8

The effectiveness of both the alpha- and beta-receptor blockade was tested by infusion of noradrenaline, isoprenaline and adrenaline. The intravenous infusion of noradrenaline (7.5 µg./min. for 10 min.) produced an increase in systemic blood pressure and central venous pressure, a fall in heart rate and a fall in brachial artery blood flow (Fig. 6-9, A). Although the bradycardia produced by noradrenaline was much reduced by beta-receptor blockade the other cardiovascular responses were little modified (Fig. 6-9, B). However, all responses were almost completely abolished by phneoxybenzamine (Fig. 6-9, C).

Intravenous isoprenaline (3.0 µg./min. for 10 min.) produced a rise in central venous pressure, brachial artery blood flow and heart rate and a fall in systemic blood pressure (Fig. 6-10, A). All these effects were abolished following treatment with propranolol (Fig. 6-10, B) and no further alteration in these responses appeared after treatment with phenoxybenzamine (Fig. 6-10, C).

Intravenous infusion of adrenaline (7.5 µg./min. for 10 min.) produced a rise in central venous pressure and heart rate and a fall in systemic blood pressure and brachial artery blood flow (Fig. 6-11, A). Beta-receptor blockade enhanced the constrictor effect of adrenaline such that there was a more marked fall in brachial artery blood flow and a rise in systemic blood pressure (Fig. 6-11, B).

Fig. 6-9 The response of central venous pressure (C.V.P.), arterial blood pressure (B.P.), right brachial artery blood flow (R.B.F.) and heart rate (H.R.) during the intravenous infusion of noradrenaline (7.5 µg./min. for 10 min.) administered throughout the hatched period. The time scale between the C.V.P. and B.P. records is in minutes.

A - in the absence of any other treatment.

B - after total beta-receptor blockade with propranolol.

C - after combined alpha- and beta-receptor blockade with phenoxybenzamine and propranolol.

An increase in heart rate is indicated by a decrease in width of the record and vice versa. An increase in central venous pressure is indicated by an upward movement of the tracing. These parameters were of secondary interest and no calibration is shown.

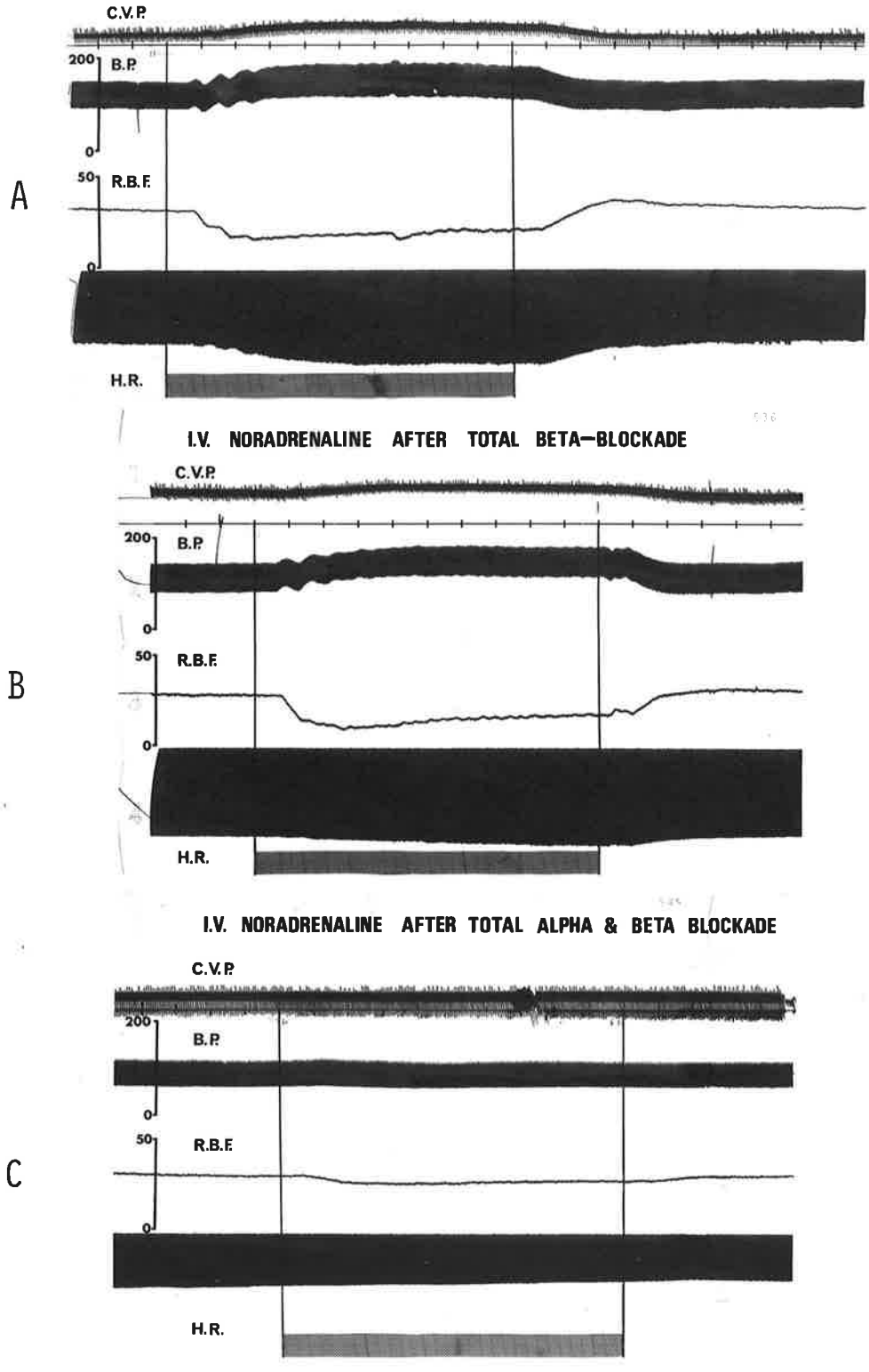
I.V. NORADRENALINE BEFORE TOTAL BLOCKADE
C.V.P.
200
B.P.
0
50
R.B.F.
0
H.R.
A
I.V. NORADRENALINE AFTER TOTAL BETA—BLOCKADE
C.V.P.
200
B.P.
0
50
R.B.F.
0
H.R.
B
I.V. NORADRENALINE AFTER TOTAL ALPHA & BETA BLOCKADE
C.V.P.
200
B.P.
0
50
R.B.F.
0
H.R.
C

Fig. 6-9

Fig. 6-10 The response of central venous pressure (C.V.P.), arterial blood pressure (B.P.), right brachial artery blood flow (R.B.F.) and heart rate (H.R.) during the intravenous infusion of isoprenaline (3.0 µg./min. for 10 min.). administered throughout the hatched period. The time scale between the C.V.P. and B.P. records is in minutes.

A - In the absence of any other treatment.

B - after total beta-receptor blockade with propranolol.

C - after combined alpha- and beta-receptor blockade with phenoxybenzamine and propranolol.

An increase in heart rate is indicated by a decrease in width of the record and vice versa. An increase in central venous pressure is indicated by an upward movement of the tracing. These parameters were of secondary interest and no calibration is shown.

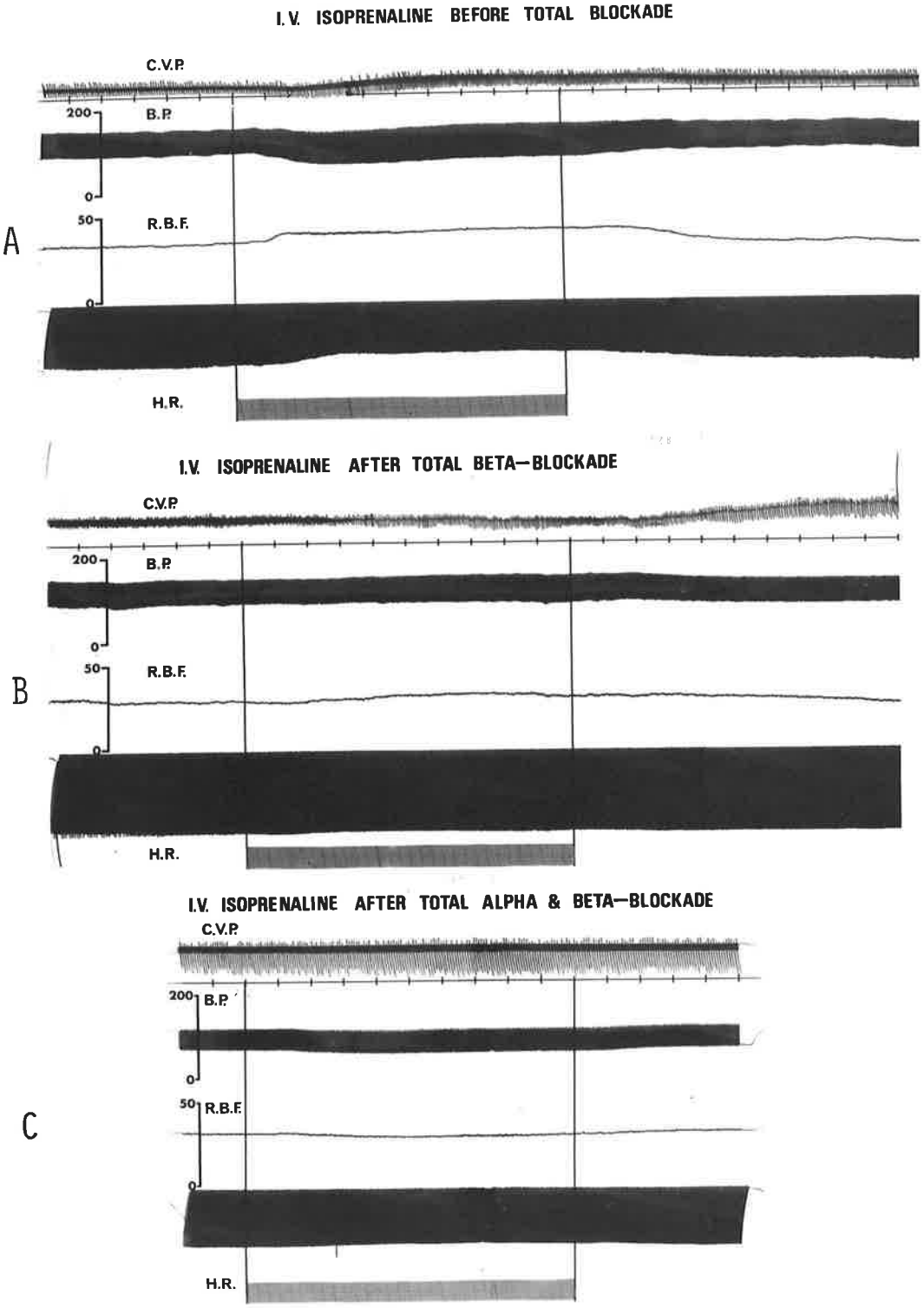
I.V. ISOPRENALINE BEFORE TOTAL BLOCKADE
C.V.P.
200
B.P.
0
50
R.B.F.
0
A
H.R.
I.V. ISOPRENALINE AFTER TOTAL BETA—BLOCKADE
C.V.P.
200
B.P.
0
50
R.B.F.
0
B
H.R.
I.V. ISOPRENALINE AFTER TOTAL ALPHA & BETA—BLOCKADE
C.V.P.
200
B.P.
0
50
R.B.F.
0
C
H.R.

Fig. 6-10

Fig. 6-11 The response of central venous pressure (C.V.P.), arterial blood pressure (B.P.), right brachial artery blood flow (R.B.F.) and heart rate (H.R.) during the intravenous infusion of adrenaline (7.5 µg./min. for 10 min.), administered throughout the hatched period. The time scale between the C.V.P. and B.P. records is in minutes.

A - in the absence of any other treatment.

B - after total beta-receptor blockade with propranolol.

C - after combined alpha- and beta-receptor blockade with phenoxybenzamine and propranolol.

An increase in heart rate is indicated by a decrease in width of the record and vice versa. An increase in central venous pressure is indicated by an upward movement of the tracing. These parameters were of secondary interest and no calibration is shown.

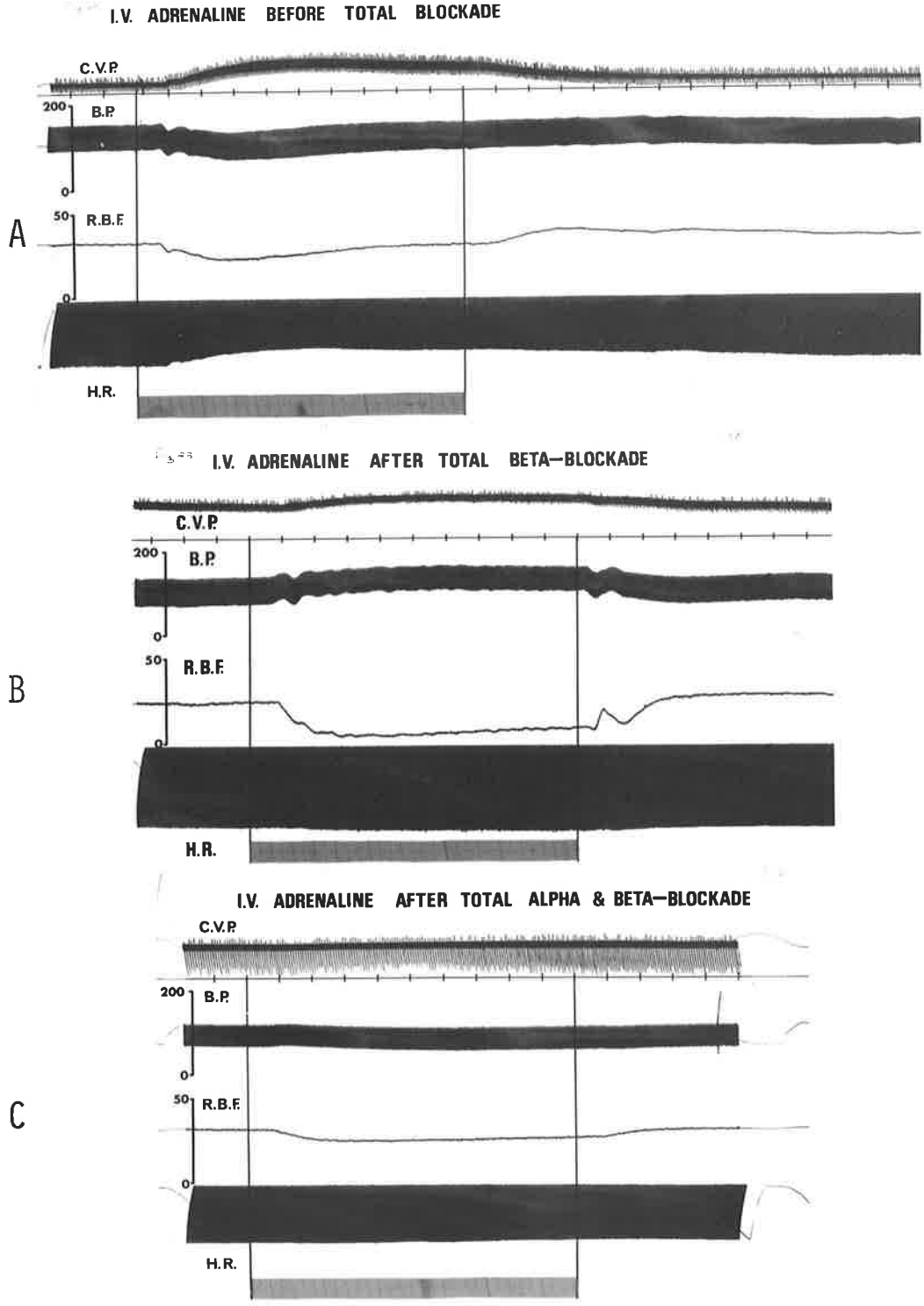
I.V. ADRENALINE BEFORE TOTAL BLOCKADE
C.V.P.
200
B.P.
0
50
R.B.F.
0
A
H.R.
I.V. ADRENALINE AFTER TOTAL BETA–BLOCKADE
C.V.P.
200
B.P.
0
50
R.B.F.
0
B
H.R.
I.V. ADRENALINE AFTER TOTAL ALPHA & BETA–BLOCKADE
C.V.P.
200
B.P.
0
50
R.B.F.
0
C
H.R.

Fig. 6-11

The bradycardia and rise in central venous pressure were less apparent. Following combined alpha- and beta-receptor blockade (Fig. 6-11, C) most of the cardiovascular response to adrenaline was abolished.

DISCUSSION

Alpha-adrenergic receptor-blockade of the forelimb vascular bed in the dog, whether produced by local or systemic infusions of the blocking drug, abolished the vasoconstriction appearing during intravenous infusions of angiotensin in much the same fashion as it did in the hand vessels of man (Scroop and Whelan, 1966).

As in man, the alpha-receptor stimulation results from release of sympathetic transmitter rather than an increase in the circulating levels of catecholamines since the constrictor response was also abolished by sympathetic denervation. This conclusion is supported by the finding that beta-receptor blockade, which had been shown to enhance the constrictor response to adrenaline in the forelimb (Fig. 6-11) had no effect on the constrictor response to angiotensin. However, other investigators have found a dose-dependent increase in the plasma catecholamine levels during angiotensin infusions (Feldberg and Lewis, 1964; Peach, Cline and Watts, 1966) and it may be that even larger doses than those used in the present experiments are required. Larger doses were used in some experiments but the development of cardiac arrythmias resulted in death or such severe cardiovascular impairment that further experimentation was impossible. Results of this type make it unlikely

that release of adrenal medullary hormones make a significant contribution to the cardiovascular actions of angiotensin.

The failure of alpha-receptor blockade to abolish the pressor response to angiotensin is in agreement with the results in man. However, the reason for the marked enhancement of the response is not clear and no such effect was seen in man with doses of alpha-blocker which greatly reduced the pressor response to infused noradrenaline. Beta-receptor blockade alone had no effect on the angiotensin pressor response but it is likely that the alpha-receptor blockade was more complete in the dog experiments such that certain of the compensatory baroreceptor reflexes were severely impaired and the pressor response was consequently enhanced.

SUMMARY

1. The reduction in brachial artery blood flow seen in the dog forelimb during intravenous infusions of angiotensin was abolished and often converted to a dilator response following either alpha-receptor blockade or sympathetic denervation.

2. This evidence, together with the failure of beta-receptor blockade alone to modify the constrictor response to angiotensin indicates that adrenal medullary release of catecholamines is unlikely to be important in the response.

3. The pressor response to intravenous angiotensin was greatly enhanced following alpha- and beta-receptor blockade of the entire cardiovascular system indicating that neither sympathetic stimulation nor adrenal medullary release of catecholamines makes an important contribution.

GENERAL SUMMARY

GENERAL SUMMARY

An historical survey of the events leading to the discovery of angiotensin was presented in the introduction to the thesis. In a review of the previous observations upon its cardiovascular actions in man and animals, reference was made to the indirect mechanisms in its cardiovascular action. These were of particular interest since the greater part of this thesis is devoted to an exploration of such mechanisms in man.

Initially a comparison was made of the cardiovascular effects of angiotensin and noradrenaline and these experiments are described in the second section. The hand vascular responses were of particular interest and provided the first evidence of an indirect vasoconstrictor mechanism in man. It was found that the local action of angiotensin on the hand vessels was only one half to one third as potent as that of noradrenaline (on a weight basis), whereas on intravenous infusion angiotensin greatly enhanced its constrictor potency in this vascular bed to become eight to ten times more active than noradrenaline. Although the mechanism of this enhanced effect of angiotensin during systemic infusions was not clear from these experiments angiotensin had been shown to release adrenal medullary catecholamines in cats and to stimulate the ~~systemic~~ sympathetic nervous system in dogs and this evidence provided the necessary clues for a further

exploration in man.

The explanation of this phenomenon was presented in the third section of the thesis where angiotensin was shown to have a stimulating action on the sympathetic vasomotor system in man. This action was only seen with systemic infusions and the site of action is preganglionic, possibly in the medullary vasomotor centres. There was no evidence of an increase in the circulating levels of catecholamines and a postganglionic sympathetic stimulating action was excluded.

The contribution of this sympathetic stimulating action to the blood pressure response during acute intravenous infusions of angiotensin in man seems to be a minor one since neither systemic alpha-adrenergic receptor blockade nor various forms of sympathetic denervation were able to reduce the response.

The suggestion that angiotensin may enhance its vasoconstrictor effect by interaction with other circulating vasoactive substances was examined in the fourth section of the thesis. When combinations of angiotensin and noradrenaline and of angiotensin and serotonin were administered to the hand vessels by intra-arterial infusion the constrictor response was greater than could be accounted

for by summation of effects alone. However, the degree of potentiation was often small and was not seen in the forearm making it unlikely that such interactions make a major contribution to the overall vasoconstrictor action of angiotensin.

To gain some idea of the importance of angiotensin in hypertension a group of such patients were studied in section five and the responses of their hand vessels to local infusions of angiotensin and noradrenaline were examined and correlated with their plasma renin activities. Only the patients with renovascular hypertension exhibited responses which were significantly different from normal. The reduction in vascular sensitivity seen in these patients is to be expected on the basis of the elevated plasma angiotensin levels, although there was no close relationship between the level of plasma renin activity and the hand vascular responses.

The possibility that the small doses of angiotensin permissible in the human experiments were insufficient to fully activate the sympathetic stimulating action of the drug or release detectable amounts of adrenal catecholamines was examined in the last section of the thesis, using anaesthetized dogs as subjects where larger doses could be given. However, the results from these experiments were basically the same as those in man in that,although angiotensin stimulated sympathetic vasoconstrictor fibres to the upper limb,

total alpha- and beta-receptor blockade did not reduce the pressor response. In fact, the pressor response was greatly enhanced following alpha-receptor blockade. There was no evidence of an increase in the circulating levels of catecholamines.

In conclusion, the vasoconstrictor action of angiotensin is a complex of direct and indirect factors. Some of these are indicated in Fig. 7-1. The work described in this thesis provides the first evidence of a sympathetic component in the vasoconstrictor action of angiotensin in man and evidence was also presented of potentiating interactions between angiotensin and other vasoactive substances. However, the direct action of angiotensin, which is presumably on specific receptors, makes the most important contribution to its overall vasoconstrictor effect, at least during short-term infusions in man.

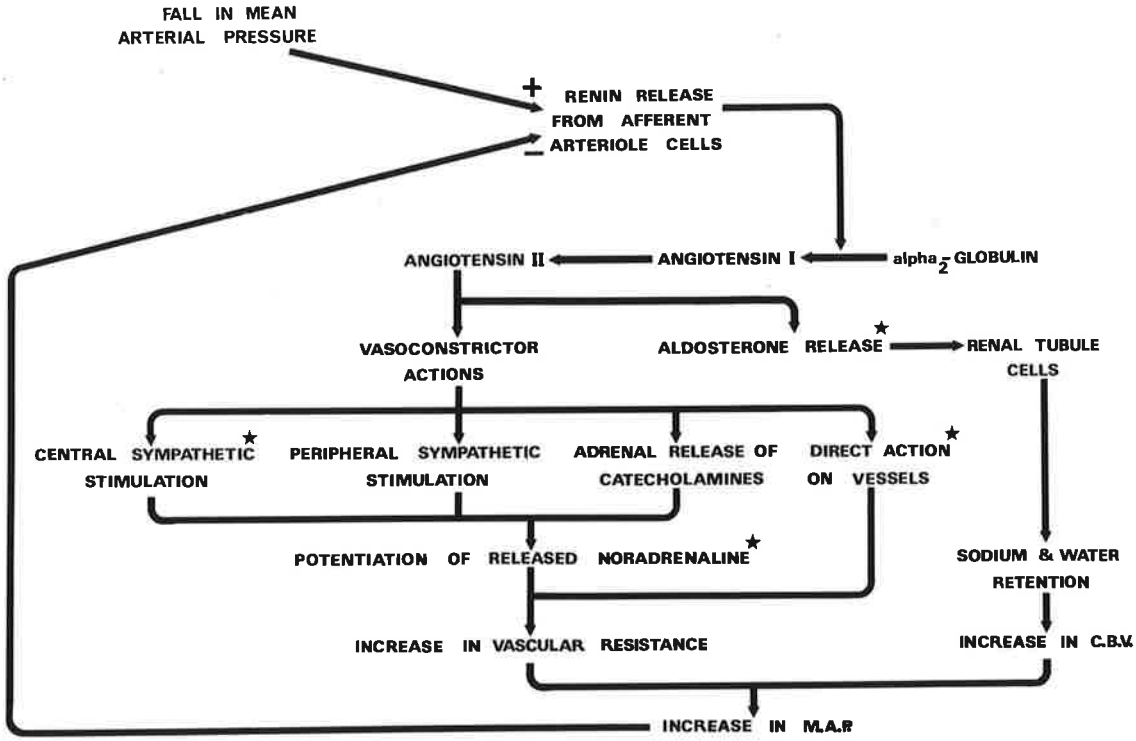

Fig. 7-1 A simplified version of the renin-angiotensin system with emphasis on the components of the vasoconstrictor action. Those indirect mechanisms of action for which there is evidence in man are indicated thus * .

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