THE PHYSIOLOGICAL AND PHARMACOLOGICAL ACTIONS AND FATE OF SEVERAL SYMPATHOMIMETIC AMINES IN VASCULAR BEDS

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GENERAL SUMMARY

An historical survey of the fate of amines in the mammalian body has been presented in the introduction of this thesis and the role of monoamine oxidase in particular, has been considered in detail. Monoamine oxidase is a collective term embracing a number of isoenzymes which vary in their relative proportions in different mammalian tissues and certain metabolic effects in such tissues may possibly be related to involvement of specific and separate forms of monoamine oxidase.

Recent advances in the knowledge of noradrenaline uptake mechanisms and the pharmacology of monoamine oxidase and catechol-O-methyl transferase have been incorporated into the review of the physiological action and fate of noradrenaline at the noradrenergic nerve terminal. The concept of noradrenaline storage pools has provided a logical means of interpreting some of the dynamic changes which occur within the nerve terminal, although there is much that requires further clarification.

The rabbit ear artery has been used extensively in studies on the control of vascular sensitivity and one of the main purposes of this thesis has been to further explore the role which monoamine oxidase plays in the mechanisms involved. In order to do this, it was first necessary to investigate the distribution

of the enzyme in the ear artery and this aspect was studied in some detail by histochemical methods. Extraneuronal monoamine oxidase was demonstrated mainly throughout the media and chronic sympathetic denervation did not perceptibly alter its concentration in this region.

There was a surprising inability to observe any accumulation of the enzyme at the medial-adventitial border of the vessel, as the sympathetic nerve terminals, which are known to contain monoamine oxidase, are concentrated at this site. It may be, however, that the small size of the nerve terminals precludes their detection by the sensitivity of the histochemical method employed and by the resolution of the light microscope. An alternative explanation may be that the affinity of intraneuronal monoamine oxidase for the tryptamine hydrochloride substrate in the histochemical procedure, is less than that of extraneuronal monoamine oxidase.

In order to further assess the role of monoamine oxidase in the ear artery and also the effect on vascular sensitivity of its distribution in the artery wall, the physiological effects of several sympathomimetic agents and monoamine oxidase inhibitors were studied in detail. The sympathomimetic amine, tyramine, has been shown by others to have largely an indirect action on smooth muscle and the indirect component is mediated by release of

noradrenaline from the noradrenergic storage structures in the sympathetic nerve terminal. The experiments performed previously in this laboratory have shown that the ear artery is much more sensitive to extraluminally-applied tyramine than to intraluminally-applied tyramine.

Three possible explanations for the lower sensitivity of the artery to intraluminal tyramine had been previously postulated, namely: (1) a permeability barrier located somewhere between the intima and the medial-adventitial border of the artery; (2) a major site of loss (possibly represented by monoamine oxidase in the media) located between the intima and the medial-adventitial border and (3) dilution of the intraluminally-applied tyramine in the vicinity of the medial-adventitial border by the tyramine-free solution bathing the adventitia externally. Accordingly, the effects of monoamine oxidase inhibition on the sensitivity of the ear artery to tyramine were investigated by means of the inhibitors, iproniazid and nialamide.

Monoamine oxidase inhibition was found to enhance the sensitivity of the ear artery to both intraluminal and extraluminal tyramine. The gain in sensitivity to intraluminal tyramine, however, was much greater than that to extraluminal tyramine and the difference between the intraluminal and extraluminal potencies of the drug was much less marked after monoamine oxidase

inhibition. In view of the extraneuronal monoamine oxidase present throughout the media of the artery wall, it is suggested that the high ratio of activity of extraluminal tyramine to intraluminal tyramine which normally prevails, is due to enzymatic destruction (by monoamine oxidase) of the intraluminally-applied tyramine as it diffuses from the intima to the sympathetic nerve terminals situated at the medial-adventitial border of the artery.

Experiments were also performed in an attempt to elucidate the possible role of monoamine oxidase in the response of the rabbit ear artery to noradrenaline. It was thought possible that intraneuronal monoamine oxidase may influence the response of the ear artery to intraluminal noradrenaline in a different fashion than that to extraluminal noradrenaline. Results from other centres have been conflicting, in that intraneuronal monoamine oxidase has been assigned an important role in the response of the guinea-pig atrium to noradrenaline, whereas extraneuronal catechol-O-methyl transferase and monoamine oxidase were considered to be of greater importance in the response of the rabbit aortic strip to noradrenaline.

The effect of monoamine oxidase inhibition on the noradrenaline response in the rabbit ear artery was examined with particular reference to the kinetics of the response and procedures which modified the effects of enzyme inhibition by disrupting or

inhibiting uptake and binding of noradrenaline by the sympathetic nerves and nerve terminals, were also applied. Such techniques included destruction of the nerves by chronic sympathetic denervation, inhibition of noradrenaline uptake by cocaine and depletion of the noradrenaline stores by reserpine.

The phenomenon of secondary sensitization, reported by others to occur following the application of noradrenaline to the monoamine oxidase-inhibited guinea-pig atrium, was also noted with extraluminal application of noradrenaline to the monoamine oxidase-inhibited rabbit ear artery. Secondary sensitization was manifested by a secondary response (reported also in the cat nictitating membrane) and by delayed recovery following washout of noradrenaline. Both the secondary response and delayed recovery were abolished by chronic sympathetic denervation or by cocaine applied concurrently with the extraluminal noradrenaline. Uptake of noradrenaline by the sympathetic nerve terminals thus appeared to be essential for the occurrence of secondary sensitization.

The findings also implied that noradrenaline was released continuously from the nerve endings during the phase of delayed recovery, as cocaine added at this stage, caused a further increase in tone of the artery. In addition, the alpha-receptor blocking agent, phentolamine, abolished delayed recovery by

rapidly restoring the resting tone of the artery, when applied during this phase. Reserpine pretreatment in the monoamine oxidase-inhibited artery did not reduce, but tended to enhance both delayed recovery and also the constrictor response to cocaine applied during delayed recovery. It is possible, therefore, that the situation in the monoamine oxidase-inhibited rabbit ear artery resembles that described in the monoamine oxidase-inhibited guinea-pig atrium. Thus, inhibition of intraneuronal inactivation of noradrenaline causes saturation of the intraneuronal binding sites so that "free" noradrenaline accumulates within the cytoplasm of the neurone and eventually diffuses out into the region of the receptors. This explanation would therefore account for both the secondary response and delayed recovery, the latter phenomenon being attributed to the continuous diffusion of noradrenaline from the nerve terminal after noradrenaline had been washed out from the surrounding medium.

Surprisingly, these pharmacological studies indicated clearly that intraluminally-applied noradrenaline did not cause secondary sensitization in the monoamine oxidase-inhibited ear artery. These findings were directly confirmed by fluorescence histochemical experiments which showed that in monoamine oxidase-inhibited ear arteries removed from reserpine-pretreated rabbits, the concentration achieved by intraluminal noradrenaline in the

region of the sympathetic nerve terminals at the medial-adventitial border of the artery, was insignificant compared with that achieved by extraluminal noradrenaline. Several factors may contribute to the inability of intraluminal noradrenaline to achieve a significant concentration in the nerve terminals, including: (1) dilution of intraluminal noradrenaline by the noradrenaline-free extraluminal solution; (2) uptake of intraluminal noradrenaline into the smooth muscle of the media with subsequent metabolism by catechol-O-methyl transferase and (3) a possible permeability barrier to the diffusion of intraluminal noradrenaline, located somewhere between the intima and the region of the nerve terminals. However, others in this laboratory have subsequently shown, by similar fluorescence histochemical methods, that the major role is played by catechol-O-methyl transferase which metabolizes intraluminally-applied noradrenaline in the media.

The effect of monoamine oxidase inhibition on the magnitude of intraluminal and extraluminal noradrenaline responses was also studied and this indicated that the mean increases in sensitivity were only slightly above those which occurred spontaneously. This implies that metabolism of noradrenaline by monoamine oxidase in the media exerts little effect on the pharmacologically effective concentrations of noradrenaline at the noradrenergic receptors in the media. It is likely, therefore, that the very small quantity

of intraneuronal enzyme present within the nerve terminals plays a much more important role in the kinetics of the response to extraluminal noradrenaline than does the extraneuronal monoamine oxidase in the media.

Experiments investigating the action of tranylcypromine on the rabbit ear artery revealed consistent selective enhancement of extraluminal noradrenaline sensitivity. In this respect, the action of tranylcypromine closely resembled that of cocaine in inhibiting neuronal uptake of noradrenaline. Tranylcypromine failed to potentiate the extraluminal constrictor potency of the non-noradrenergic stimulant, histamine, and this also tended to support the role of the sympathetic nerve terminals and the neuronal uptake mechanism in the action of tranylcypromine on extraluminal noradrenaline sensitivity in the ear artery. Sympathetic denervation abolished the selective increase in sensitivity to extraluminal noradrenaline seen in the control arteries to which tranylcypromine had been applied, thus confirming the role of the sympathetic nerve terminals.

The effect of monoamine oxidase inhibition was also examined in man by comparing the influences of bretylium tosylate and tranylcypromine on the vasoconstrictor actions of various indirectly-acting sympathomimetic amines. The intra-arterial administration of the noradrenergic neurone blocking agent,

bretylium tosylate, was found to potentiate the vasoconstrictor effects of tyramine, methylamphetamine and ephedrine. It is suggested that this potentiating action of bretylium is due to monoamine oxidase inhibition and in the case of tyramine, which is a good substrate for monoamine oxidase, inhibition of the enzyme would increase the effective concentration of both tyramine and the noradrenaline which it releases. Methylamphetamine and ephedrine, however, are not substrates of monoamine oxidase and only enhancement of the noradrenaline concentration alone would occur, thus accounting for the lesser potentiation of their vasoconstrictor actions by bretylium.

The principal effect of bretylium is blockade of the sympathetic noradrenergic neurones. Hence, for bretylium to be effective as a monoamine oxidase inhibitor in potentiating the vasoconstrictor actions of these sympathomimetic amines on hand blood vessels at a time when reflex sympathetic activity is blocked, it is necessary to postulate that these drugs and reflex nerve activity act either on different intraneuronal noradrenergic storage compartments or by different release mechanisms. Comparison of the intra-arterial effects of bretylium and the monoamine oxidase inhibitor, tranylcypromine, on the vasoconstrictor action of the three sympathomimetic amines, showed a similar pattern of enhancement, suggesting that monoamine oxidase

inhibition may be a common factor in their potentiating actions. Tranylcypromine, however, also increased the response of the hand blood vessels to noradrenaline and this action could also contribute to its potentiation of the effects of tyramine, methylamphetamine and ephedrine.