Br. J. Pharmac. (1970), **40**, 249–256.

The role of monoamine oxidase in the response of the isolated central artery of the rabbit ear to tyramine

I. S. DE LA LANDE, B. D. HILL, L. B. JELLETT AND J. M. McNEIL

Department of Human Physiology and Pharmacology, University of Adelaide, and the Institute of Medical and Veterinary Science, Adelaide, Australia

Summary

1. Monoamine oxidase activity was demonstrated histochemically throughout the media of the rabbit ear artery but not in the adventitia at its border with the media where the sympathetic nerve terminals are concentrated. Neither intensity nor distribution of enzyme activity was perceptibly altered up to 60 days after sympathetic denervation.

2. Iproniazid and nialamide increased the sensitivity of the artery to intraluminal tyramine much more than that to extraluminal tyramine, so that the difference between the potencies for the two routes of administration became less marked.

3. The results indicate that inactivation by monoamine oxidase in the media is a factor contributing to the relatively low potency of intraluminal tyramine on the artery.

Introduction

Evidence was presented in an earlier study (de la Lande & Waterson, 1968) that the action of extraluminal tyramine on the rabbit ear artery was largely indirect, that is, mediated by the sympathetic nerves. Intraluminal tyramine relative to extraluminal tyramine was less potent, and the indirect component of its action was much less prominent. A possible explanation of these differences is that intraluminal tyramine is inactivated by monoamine oxidase in the media of the artery, thus limiting its access to the nerve terminals. The latter are located in the adventitia adjacent to the media (de la Lande & Waterson, 1967; Waterson & Smale, 1967). In the present study, the effects of monoamine oxidase inhibition on the responses to intraluminal and extraluminal tyramine have been investigated. We have also made observations on the distribution of monoamine oxidase in the rabbit ear artery, which supplement the brief account on it by Koelle & Valk (1954). These workers detected enzyme activity in the media but not in the adventitia of rabbit ear arteries.

Methods

Perfusion

The method of isolating the central artery of the rabbit ear and conditions for perfusing the vessel employed in the present study were identical with those described in detail by de la Lande & Waterson (1968). The principle of the method

is that the artery segment (approximately 2 cm in length) is suspended in an organ bath so that its adventitial surface is bathed in Krebs bicarbonate solution (extraluminal solution) and perfused through its lumen with Krebs bicarbonate solution (intraluminal solution) in such a way that the intraluminal and extraluminal solutions do not mix. The intraluminal perfusion is maintained at a constant rate so that constriction of the artery increases perfusion pressure. The response to tyramine was measured by the magnitude of the sustained increase in perfusion pressure which occurs during the period of contact of the artery with tyramine. Tyramine was added either to the reservoir containing the intraluminal fluid, or to the extraluminal fluid in the organ bath. The nature of the responses to tyramine were discussed in an earlier paper (de la Lande & Waterson, 1968).

Estimation of sensitivity

The effects of monoamine oxidase inhibitors were measured by determining the ratio of the equi-potent concentrations of tyramine in the control and monoamine oxidase inhibited arteries. In early experiments, the responses to individual doses of tyramine were recorded, the interval between doses being 10–20 min. Concentration-response curves to intraluminal and to extraluminal tyramine were obtained, and the ratio of the equi-potent concentrations (sensitivity ratio) determined at an arbitrarily-chosen level of response (60 mmHg increase in perfusion pressure, 1 mmHg$\equiv$1·333 mbar). In later studies (see **Results**) threshold responses were employed. These were estimated by commencing with a sub-threshold concentration of tyramine, and raising it in multiples of 2 or 2·5, at intervals of 5 min, until a response of 2 mm or greater was obtained. Threshold responses were chosen as a basis for comparison in order to minimize exposure of the artery to high concentrations of tyramine. To avoid bias in the estimates of relative threshold concentrations due to changes in sensitivity to tyramine occurring during the course of an experiment (2–4 h), the order in which intraluminal and extraluminal tyramine were applied to the artery was alternated between experiments.

Monoamine oxidase inhibition

(*a*) *In vivo.* Iproniazid, 200 mg/kg (1·1 mmol/kg), was administered intraperitoneally to rabbits 24 h before removal of the arteries.

(*b*) *In vitro.* Two arteries, one from each ear of the same rabbit, were excised. Nialamide, 0·4 mM, or iproniazid, 0·55 mM, were added to both the extraluminal and the intraluminal solution used for one of the arteries ; one hour later both solutions were changed to drug free Krebs solution. The second artery was perfused with Krebs solution only for 1 h and served as a control. Before commencing applications of tyramine both arteries were perfused for 10 min after the initial 1 h perfusion.

(*c*) At the end of most of these experiments, the arteries were examined histochemically for monoamine oxidase activity.

Histochemistry

The distribution of monoamine oxidase was examined histochemically by a method based on that described by Glenner, Burtner & Brown (1957). The principle of the method is that tryptamine is oxidized by monoamine oxidase in the tissue

section to indolyl-3-acetaldehyde, which in turn reduces nitro-blue tetrazolium to a blue-staining formazan precipitate. The blue precipitate indicates the position of monoamine oxidase. The tissues examined were either the excised central ear artery or the whole ear containing the vessels and nerves *in situ*. Fresh, frozen cryostat sections (10–20 μm in thickness) were cut at a temperature of −25° C, mounted on glass slides and air-dried for 10–15 min. The sections were then incubated at 37° C for 45 min to 2 h in a medium consisting of tryptamine hydrochloride 25 mg, sodium sulphate 4 mg, nitro-blue tetrazolium 5 mg, 5 ml of 0·1 M phosphate buffer (pH 7·6) and 15 ml of distilled water. Following incubation, the sections were washed in running water for 2 min, fixed in 10% neutral formalin for 24 h and then mounted in glycerin jelly.

The method used differs from the method of Glenner *et al.* (1957) in the following respects. Incubation periods longer than 45 min were used in attempts to delineate structures such as nerve bundles, and because staining in arteries which had been perfused for long periods was less intense than in non-perfused arteries. Incubation periods longer than 45 min have also been employed by Härkönen (1964) and Fujiwara, Tanaka, Hikosaka & Okegawa (1966). Second, to avoid removal of certain purple and red colours which may also indicate monoamine oxidase activity, sections were mounted in glycerin jelly instead of being dehydrated, cleaned and mounted in Permount, as suggested by Pearse (1960).

Another modification was that sections were sometimes incubated in media containing tetranitro-blue tetrazolium instead of nitro-blue tetrazolium since the former is more easily reduced and in theory should provide more accurate localization of the enzyme (Pearse, 1963).

To assist in distinguishing between enzymic and artefactual staining, the following controls were regularly employed: (1) monoamine oxidase inhibited arteries; (2) omission of substrate (tryptamine).

Sympathetic denervation

The central artery of one ear was denervated in each of ten rabbits by removing the respective superior cervical ganglion as described by de la Lande & Rand (1965). The arteries of both denervated and control ears were removed for histochemistry after intervals of 12 to 21 days (eight rabbits), 30 days (one rabbit) and 60 days (one rabbit) after operation. The effectiveness of denervation was judged by the appearance on the denervated side of pupillary constriction and vasodilatation of the ear. Another test used was the inability of the denervated ear artery, when subsequently perfused *in vitro*, to respond to electrical field stimulation under conditions which caused marked constriction of the contralateral innervated ear artery (de la Lande, Frewin & Waterson, 1967).

Drugs used

Iproniazid (Roche); nialamide (Pfizer); cocaine hydrochloride (Macfarlane-Smith); (−)-noradrenaline bitartrate (Koch-Light); tyramine hydrochloride (Koch-Light).

Results

Sensitivities to tyramine of arteries from seven iproniazid-pretreated rabbits are compared with the sensitivities of arteries from ten untreated rabbits in Table 1.

The effects of nialamide, 0·4 mM, perfused *in vitro* for 1 h in each of eight arteries are summarized in Table 2. The data in Table 2 are based on threshold responses to tyramine since it was apparent during the experiments with iproniazid that the sensitivity of the arteries tended to decline during the course of repeated applications of intraluminal and extraluminal tyramine and responses to larger doses tended to be erratic. Nevertheless, the trends exhibited in both series of experiments were identical. The effect of the monoamine oxidase inhibitors was to increase the sensitivity to both intraluminal and extraluminal tyramine, but the magnitude of the increase was very much greater in the case of intraluminal tyramine (approximately 60 fold compared with 10 fold). It will be noted that the net effect of inhibition of monoamine oxidase was to reduce greatly the difference between the potencies of intraluminal and extraluminal tyramine which prevails in the normal artery. To determine whether the indirect or direct components of tyramine's action on the ear artery had been enhanced by monoamine oxidase inhibition, the effect of cocaine on the sensitivity to tyramine was examined. In three arteries which had been previously perfused with nialamide for 1 h, the presence of cocaine, 3 μM, in both the intraluminal and extraluminal solutions, resulted in a decrease in sensitivity to intraluminal tyramine of approximately 20 fold, and to extraluminal tyramine of approximately 12 fold.

Histochemistry

The distribution of monoamine oxidase activity in the ear artery is illustrated in Fig. 1a, which shows a result typical of more than sixty arteries examined. The main feature is that the blue staining, characteristic of monoamine oxidase activity, is confined entirely to the media. Enzyme activity was either not present in the

TABLE 1. *Effect of iproniazid on sensitivity to tyramine*

	Tyramine	Control	Iproniazid treated	Gain in sensitivity
Equipotent concentrations (×0·01 mM)	Intraluminal	17 ± 17	$0{\cdot}28 \pm 0{\cdot}19$	×60
	Extraluminal	$1{\cdot}4 \pm 1$	$0{\cdot}11 \pm 0{\cdot}08$	×12
Ratio of equipotent concentrations	$\frac{\text{Intraluminal}}{\text{Extraluminal}}$	$9{\cdot}5\ ^{+13{\cdot}2}_{-\ 5{\cdot}5}$	$2{\cdot}4^{+3{\cdot}8}_{-1{\cdot}5}$	

Arithmetic means ± S.D. are shown for concentrations and the gain in sensitivity represents the ratio of the mean of the values for control and treated. The ratios for $\frac{\text{Intraluminal}}{\text{Extraluminal}}$ potency are the geometric means ± S.D. of the ratios determined in separate experiments. For experimental details see text.

TABLE 2. *Effect of nialamide on sensitivity to tyramine*

	Tyramine	Control	Nialamide treated	Gain in sensitivity
Equipotent concentrations (×0·01 mM)	Intraluminal	$5{\cdot}3 \pm 3{\cdot}6$	$0{\cdot}09 \pm 0{\cdot}09$	$\times 67^{+177}_{-\ 49}$
	Extraluminal	$0{\cdot}44 \pm 0{\cdot}16$	$0{\cdot}06 \pm 0{\cdot}03$	$\times 7{\cdot}7\ ^{+\ 8{\cdot}4}_{-\ 4{\cdot}0}$
Ratio of equipotent concentrations	$\frac{\text{Intraluminal}}{\text{Extraluminal}}$	$9{\cdot}5\ ^{+11{\cdot}8}_{-\ 5{\cdot}3}$	$1{\cdot}1^{+1{\cdot}6}_{-0{\cdot}6}$	

Arithmetic means ± S.D. are shown for concentrations. The gain in sensitivity ± S.D. was calculated from geometric means of responses of control and treated arteries and the ratio ± S.D. for the $\frac{\text{Intraluminal}}{\text{Extraluminal}}$ potency also represent ratios based on geometric means of individual experiments. For experimental details see text.

adventitia and endothelium or was so slight that it was not distinguishable from an artefact. Staining of the media did not occur (*a*) in the absence of substrate: (*b*) in arteries from rabbits which had been pretreated with iproniazid, 200 mg/kg; or (*c*) in arteries which had been perfused with iproniazid, 0·55 mM, or nialamide, 0·4 mM, *in vitro* (Fig. 2). The staining was both diffuse and granular, and was uniformly distributed throughout the media. There was no evidence of more intense staining at the medial-adventitial border where the sympathetic nerve terminals are

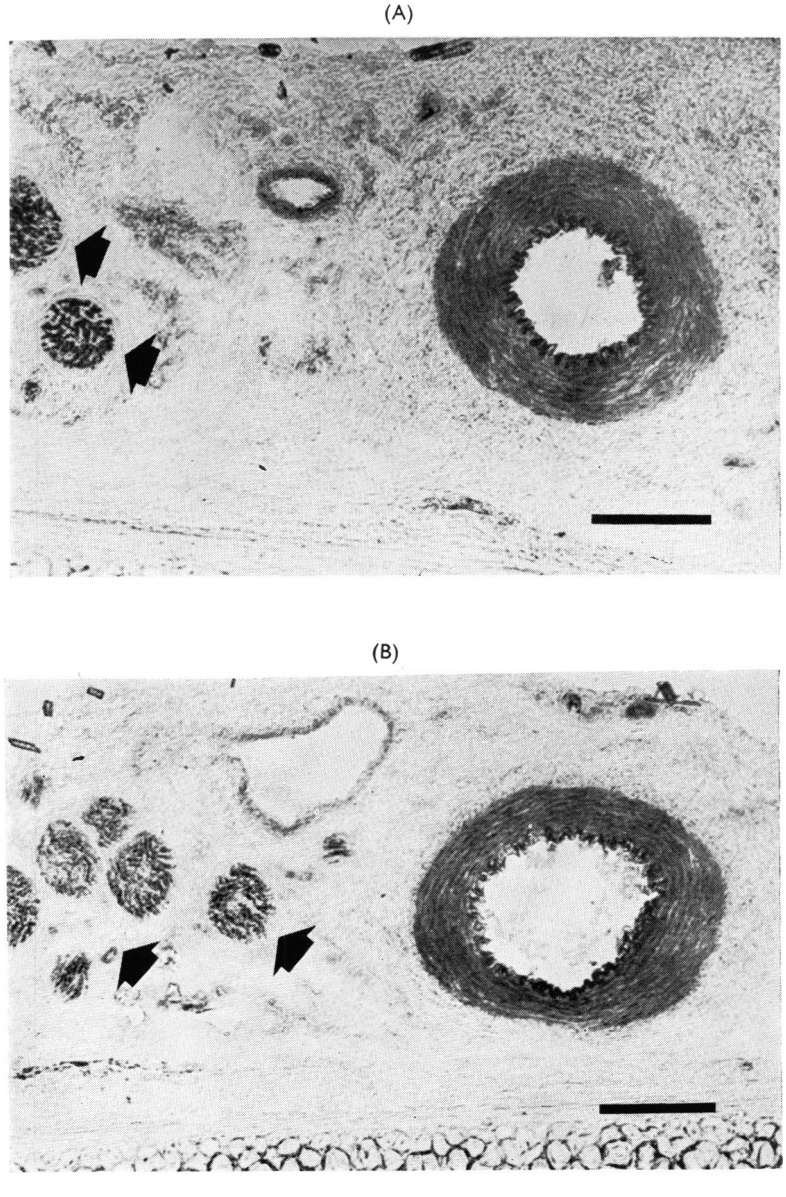

FIG. 1. Histochemical analysis for monoamine oxidase in transverse sections of a control artery (A), and the artery from the opposite ear which had been denervated by removal of the superior cervical ganglion 24 days previously (B). Staining is present in the media of each artery, and also in adjacent mixed nerve bundles (shown by arrows). Section thickness, 20 μm; scale, 200 μm.

known to be highly localized (de la Lande & Waterson, 1967 ; Waterson & Smale, 1967), nor could the distribution and intensity of staining in chronically denervated arteries be distinguished from that in normal innervated arteries (compare Figs. 1a and 1b). These findings suggested that the distribution of the enzyme in the ear artery was largely extraneuronal. Fig. 1 also shows bundles of one of the major afferent nerves (ventral auricular) in the ear. These nerves displayed monoamine oxidase activity in both control and chronically denervated arteries. Staining of myelinated nerves has been described by Yasuda & Montagna (1960), Shanthaveerappa & Bourne (1964) and Härkönen (1964).

Discussion

The histochemical studies have confirmed, by a different technique, the original observations of Koelle & Valk (1954) that monoamine oxidase activity is located in the media of the artery. There was no evidence of enzymic activity in the region of the medial-adventitial border or of a change in distribution or intensity of activity following chronic denervation. This latter finding is in agreement with that of Armin, Grant, Thompson & Tickner (1953), who showed that monoamine oxidase activity in ear arteries, when measured by the rate of oxidation of tyramine, was unaffected by chronic sympathetic denervation. It may be concluded that the contribution of intraneuronal monoamine oxidase to the total enzyme activity, when tryptamine or tyramine are substrates, is small compared with that of the enzyme in the media, and that the latter enzyme is largely extraneuronal in distribution.

The selective enhancement of sensitivity to intraluminal tyramine caused by monoamine oxidase inhibition implies that the enzyme plays a more important role in the response to intraluminal tyramine than in the response to extraluminal tyramine, while the inhibitory action of cocaine indicates that the gain in sensitivity

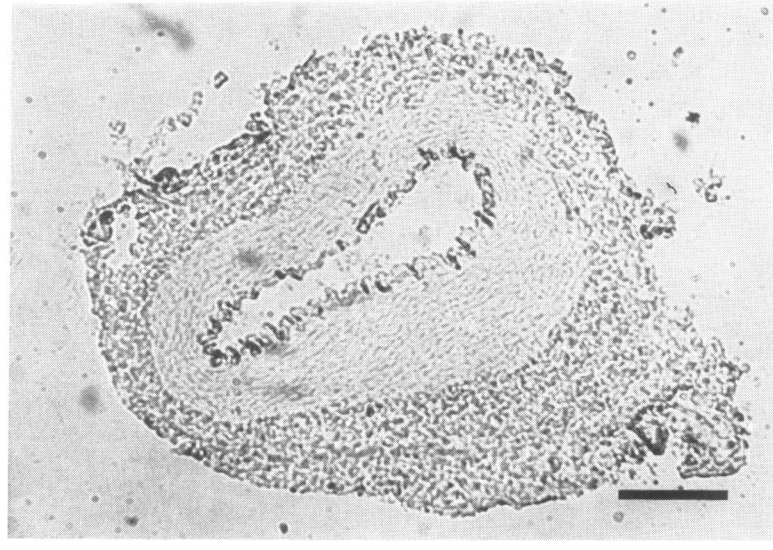

FIG. 2. Ear artery which had been perfused with 0·4 mM nialamide for 1 h, showing absence of staining reaction for monoamine oxidase in the media. Section thickness: 20 μm ; scale: 200 μm.

is due mainly to an increase in the indirect component of tyramine's action. Such an increase could be explained in two ways, either by an increase in the amount of tyramine reaching the nerve terminals from the intima or by an enhancement of the action of tyramine on the nerve terminals. Since the latter effect would be common to both intraluminal and extraluminal tyramine, it may account for the increase in sensitivity to extraluminal tyramine but cannot account for the approximately six-fold greater increase in sensitivity to intraluminal tyramine. Hence our data support the suggestion (de la Lande & Waterson, 1968) that intraluminal tyramine is inactivated by monoamine oxidase in the media as it diffuses towards the nerve terminals, thus accounting for its low constrictor potency compared with that of extraluminal tyramine.

It is conceivable that monoamine oxidase is not the only factor contributing to loss of tyramine as the latter diffuses across the media. Monoamine oxidase has been shown to be associated with mitochondria (Cotzias & Dole, 1951 ; Hawkins, 1952 ; Blaschko, Hagen & Hagen, 1957 ; Schnaitman, Erwin & Greenawalt, 1967), so that presumably tyramine must enter the smooth muscle cells of the media in order to be metabolized. Entry of tyramine may still occur, despite monoamine oxidase inhibition. Nevertheless, loss of intraluminal tyramine by this mechanism must be small compared with loss due to inactivation by monoamine oxidase, as the difference between the intraluminal and extraluminal sensitivities to tyramine in the monoamine oxidase inhibited arteries was less than twofold in most experiments.

We wish to acknowledge the skilled assistance of Miss Tiina Mannik and Miss Maria Klop. The work was assisted by a grant from the Life Insurance Medical Research Fund of Australia and New Zealand.

REFERENCES

ARMIN, J., GRANT, R. T., THOMPSON, R. H. S. & TICKNER, A. (1953). An explanation of the heightened vascular reactivity of the denervated rabbit's ear. *J. Physiol., Lond.*, **121**, 603–622.

BLASCHKO, H., HAGEN, JEAN M. & HAGEN, P. (1957). Mitochondrial enzymes and chromaffin granules. *J. Physiol., Lond.*, **139**, 316–322.

COTZIAS, G. C. & DOLE, V. P. (1951). Metabolism of amines. II. Mitochondrial localization of monoamine oxidase. *Proc. Soc. exp. Biol., N.Y.*, **78**, 157–160.

DE LA LANDE, I. S., FREWIN, D. B. & WATERSON, J. G. (1967). The influence of sympathetic innervation on vascular sensitivity to noradrenaline. *Br. J. Pharmac. Chemother.*, **31**, 82–93.

DE LA LANDE, I. S. & RAND, M. J. (1965). A simple isolated nerve-blood vessel preparation. *Aust. J. exp. Biol. med. Sci.*, **43**, 639–656.

DE LA LANDE, I. S. & WATERSON, J. G. (1967). Site of action of cocaine on the perfused artery. *Nature, Lond.*, **214**, 313–314.

DE LA LANDE, I. S. & WATERSON, J. G. (1968). The action of tyramine on the rabbit ear artery *Br. J. Pharmac.*, **34**, 8–18.

FUJIWARA, M., TANAKA, C., HIKOSAKA, H. & OKEGAWA, T. (1966). Cytological localization of noradrenaline, monoamine oxidase and acetylcholinesterase in salivary glands of dog. *J. Histochem. Cytochem.*, **14**, 483–490.

GLENNER, G. G., BURTNER, HELEN J. & BROWN, G. W. (1957). The histochemical demonstration of monoamine oxidase activity of tetrazolium salts. *J. Histochem. Cytochem.*, **5**, 591–600.

HÄRKÖNEN, M. (1964). Carboxylic esterases, oxidative enzymes and catecholamines in the superior cervical ganglion of the rat and the effect of pre- and post-ganglionic nerve division. *Acta physiol. scand.*, **63**, suppl. 237, 1–94.

HAWKINS, JOYCE (1952). The localization of amine oxidase in the liver cell. *Biochem. J.*, **50**, 577–581.

KOELLE, G. B. & VALK, A. DE T. (1954). Physiological implications of the histochemical localization of monoamine oxidase. *J. Physiol., Lond.*, **126**, 434–447.

PEARSE, A. G. E. (1960). *Histochemistry, Theoretical and Applied*, 2nd ed., p. 907. London: Churchill.

PEARSE, A. G. E. (1963). The histoenzymology of normal and diseased muscle. *Research in Muscular Dystrophy*, Proc. 2nd Symp., pp. 189–199. London: Pitman Medical Publishing Co.

SCHNAITMAN, C., ERWIN, V. G. & GREENAWALT, J. W. (1967). The submitochondrial localization of monoamine oxidase. *J. cell. Biol.*, **32**, 719–735.

SHANTHAVEERAPPA, T. R. & BOURNE, G. H. (1964). Monoamine oxidase distribution in the rabbit eye. *J. Histochem. Cytochem.*, **12**, 281–287.

WATERSON, J. G. & SMALE, D. E. (1967). Location of noradrenergic structures in the central artery of the rabbit ear. *Aust. J. exp. Biol. med. Sci.*, **45**, 301–308.

YASUDA, K. & MONTAGNA, W. (1960). Histology and cytochemistry of human skin. XX. The distribution of monoamine oxidase. *J. Histochem. Cytochem.*, **8**, 356–366.

(*Received December* 19, 1969)