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The influence of amine metabolizing enzymes on the pharmacology of tyramine in the isolated perfused mesenteric arterial bed of the rat

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1 The pressor response to the infusion of tyramine (Tyr) into the isolated perfused mesenteric arterial bed of the rat has been studied at both a low and a high dose (0.2 and 2.0 μmol) and the effect of monoamine oxidase-A (MAO-A) and semicarbazide-sensitive amine oxidase (SSAO) inhibition was examined. Very little MAO-B activity is found in homogenates of this tissue when Tyr is used as substrate.

2 Inhibition of SSAO by treating rats with 1 mg kg^{-1} (E)-2-(3',4'-dimethoxyphenyl)-3-fluoroally lamine (MDL 72145) 1 h before dissection, had no significant effect on the maximum pressure attained or the area under the curve (AUC) of the response to both low and high doses of Tyr. Inhibition of MAO-A, by inclusion of 10 μM clorgyline in the perfusing fluid, resulted in no significant potentiation at both low or high doses of Tyr. The inhibition of both these enzymes together substantially increased the AUC of the pressor response.

3 Cocaine (3 μM) significantly potentiated the responses to adrenaline (Ad). At this dose, cocaine significantly reduced the peak height and the AUC of the responses to both doses of Tyr.

4 Inhibition of extraneuronal uptake mechanisms with corticosterone (29 μM) did not potentiate the response to Ad and did not significantly alter the response to Tyr (low dose).

5 The effects of MDL 72145 and clorgyline on the directly acting amine, Ad, were studied. MDL 72145 caused a small but significant increase in the EC_{50} and in the maximum response to Ad, whilst clorgyline (10 μM) increased the EC_{50} value slightly and decreased the maximum response. When the two inhibitors were used in combination, a significant increase in the maximum response but with no change in the EC_{50} was seen.

6 These data indicate that the action of Tyr in this vascular bed is, at least, partly indirect. Inactivation of Tyr is effected by both MAO-A and SSAO in the blood vessel wall. Inhibition of both enzymes seems to be necessary to achieve a significant potentiation of the pressor response. The effects of these enzyme inhibitors on directly acting amines may mask any potentiation of the response when MAO-A or SSAO alone are inhibited.

Introduction

Tyramine (Tyr) metabolism in tissue homogenates of rat mesenteric blood vessels is catalysed by monoamine oxidase type A (MAO-A; EC 1.4.3.4) and by an amine oxidase that is resistant to inhibition by the acetylenic inhibitors of MAO, such as clorgyline (Coquil *et al.*, 1973). This enzyme activity has been called semicarbazide-sensitive amine oxidase (SSAO, EC 1.4.3.6) and its ability to metabolize Tyr added to the fluid perfusing isolated mesenteric blood vessels of the rat has been demonstrated (Elliott *et al.*, 1989). Unlike MAO, which is a mitochondrial enzyme (Greenawalt & Schnaitman, 1970), SSAO resides in the plasma membrane of smooth muscle cells (Wibo *et al.*, 1980; Lyles & Singh, 1985). This cellular location has led to speculation that this enzyme might have access to circulating amines before they have entered cells, (Callingham *et al.*, 1983) but the physiological function of SSAO remains unknown. SSAO inhibition has been shown to potentiate the contractile response of the rat aorta to tryptamaine, but only after MAO had been inhibited (Lyles & Taneja, 1987). Although Tyr is a substrate for MAO-B there is little of this enzyme present in rat mesenteric blood vessels and therefore it does not

contribute significantly to the metabolism of this amine in homogenates of this tissue (Coquil *et al.*, 1973; Elliott *et al.*, 1989).

Tyr has been shown to release [^{3}H]-noradrenaline from labelled stores in the isolated perfused mesenteric arterial bed of the rat (George & Leach, 1975). In order to act in this way, Tyr must pass from the lumen of the vessel, across the medial layer, to the adventitial-medial junction, where the nerve endings are located (Hodge & Robinson, 1972). Tyr also causes pressor responses in the perfused mesenteric vascular bed, which can be prevented by blockade of neuronal uptake with cocaine (George & Leach, 1973). Tyr has also been shown to have some direct action on vascular smooth muscle (Toda *et al.*, 1978; Iriarte *et al.*, 1985) which may involve stimulation of α-adrenoceptors (Krishnamurty & Grollman, 1972; Miyahara & Suzuki, 1985). However, much of the evidence indicating that inhibition of MAO leads to potentiation of the actions of Tyr has been complicated by the fact that many of the agents used also inhibit SSAO (see Lyles, 1984).

In order to examine whether or not SSAO is involved in the termination of the actions of circulating Tyr, it was decided to determine the relative contributions of SSAO and MAO to Tyr inactivation in a bed of resistance blood vessels. The isolated perfused mesenteric arterial bed of the rat was chosen for this purpose. Preliminary results of this study have been described elsewhere (Elliott & Callingham, 1988).

Methods

The method of isolation and perfusion at constant flow (2 ml min^{-1}) of the mesenteric arterial bed of the rat was followed as previously described (Elliott *et al.*, 1989) and the pharmacological responses to the infusion of 20 ml of Krebs solution containing 100 μM Tyr were recorded at the same time as the metabolites appearing in the perfusate were measured. The 5 groups of preparations examined for their ability to metabolize Tyr were also evaluated for the pressor response to Tyr. These results are described here for comparison with another series of experiments where the responses to a lower dose of Tyr were also studied.

The experimental protocol for the examination of the low dose of Tyr differed slightly from that previously described (Elliott *et al.*, 1989). Once again, preparations from both control rats and rats injected with 1 mg kg^{-1} MDL 72145 1 h before dissection were examined. In some preparations clorgyline (1 or 10 μM) was added to the Krebs solution for the first 30 min of the equilibration period, after which time, perfusion with clorgyline-free Krebs solution was resumed. Following the 45 min equilibration period, a dose-response curve to adrenaline (Ad) was constructed. Doses of Ad (0.6 to 20 nmol) were administered (in 200 μl of Krebs solution) through an injection port into the perfusing fluid. Delay between injection and the beginning of the response was less than 5 s. The maximum increase in pressure was recorded. When the effects of cocaine (3 μM) and corticosterone (29 μM) were studied, these drugs were added to the Krebs solution 20 min before the infusion of Tyr. Two further doses of Ad were injected and the responses compared with those obtained in the absence of these drugs. Corticosterone was dissolved in ethanol before dilution in Krebs solution. Addition of this vehicle alone had no effect on the responses to Ad.

Three parameters of the Tyr pressor response were measured, the maximum rise in pressure, the area under the curve (AUC) of the response and the time for the response to return to within 1 mmHg of the resting perfusion pressure. The AUC of the response was measured by cutting out the chart recording and weighing the paper. A value of 1 mmHg above the resting pressure was used as the end point of the response as some preparations settled at a new resting pressure 1 mmHg or less above the original resting pressure. None of the preparations failed to return to within 1 mmHg of the baseline perfusion pressure.

The effect of corticosterone on the responses of this preparation to Ad was investigated further in some control preparations. Responses to Ad at doses around the EC_{50} value (1 and 2 nmol) were obtained in the absence of any drug. Following this, corticosterone (29 μM) or cocaine (3 μM) was added to the Krebs solution and 15 min later the doses were repeated. Finally, the second drug (cocaine or corticosterone) was added and the responses repeated in the presence of both drugs. Repetition of these doses of Ad without the addition of either cocaine or corticosterone to the Krebs solution did not result in a significant alteration in the response observed. In a small number of experiments, corticosterone was replaced with metanephrine.

Drugs and solutions

The modified Krebs-Henseleit solution had the following composition (mM):- NaCl 118, KCl 4.57, $CaCl_2$ 1.27, KH_2PO_4 1.19, $MgSO_4$ 1.19, $NaHCO_3$ 25 and glucose 5.55. Tyramine hydrochloride, adrenaline bitartrate, metanephrine hydrochloride and corticosterone were all obtained from Sigma Chemical Co., Poole, Dorset. MDL 72145 was a gift from Merrell Dow Research Institute, Strasbourg, France and clorgyline hydrochloride was a gift from May and Baker Ltd., (now Rhône-Poulenc), Eccles,

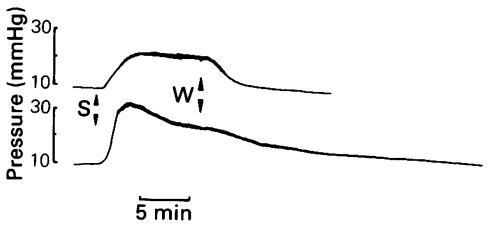

Figure 1 Typical responses of the isolated perfused mesenteric arterial bed of the rat to infusion of modified Krebs-Henseleit solution containing 10 μM tyramine (Tyr). The infusion started at 'S' and a total of 20 ml was perfused at a flow rate of 2 ml min^{-1}. At 'W' perfusion with Tyr-free Krebs solution was resumed and the Tyr was washed out of the preparations. The upper trace shows the response of a preparation from a control rat. The lower trace is a preparation from a rat that was treated with MDL 72145 1 h before use and the preparation was perfused with Krebs solution containing 1 μM clorgyline for 30 min at the start of the experiment. The baseline pressure was 9.8 mmHg for both preparations and the maximum pressure attained, the AUC of the response and the time for the response to return to within 1 mmHg of the original baseline pressure, were measured.

Manchester. Cocaine hydrochloride BP was purchased from Macarthys Medical Ltd., Romford, Essex. All other reagents were of analytical grade where possible.

Statistical analysis of the results

Statistical significance of the Tyr responses was evaluated by Student's t test (unpaired) comparing each group with the control. The significance of changes in response to Ad in the presence of cocaine or corticosterone were analysed by a paired Student's t test. The dose-response curves were fitted to the observations using a non-linear regression programme (Harwell Library non-linear programme VBO1A) which calculated the Hill slope, the EC_{50} and the maximum pressure response in each case. The equation employed was:

$$\text{Increase in pressure} = Resp_{max} \times D^n/(D^n + EC_{50}{}^n)$$

where D is the dose of Ad added, n is the Hill coefficient, EC_{50} is the dose giving the half maximal response and $Resp_{max}$ is the maximum response. Each point was weighted according to the reciprocal of its variance. The curves were also compared with the control curve using the method of De Lean *et al.* (1978), fitting the two curves simultaneously with none of the parameters constrained and then constraining each in turn (NAG library routine EO4 FDF). The F-test was applied to the sum of the squares of residuals in each situation and a change in the parameter was taken to be significant when $P < 0.05$.

Results

Responses of the preparations to infusion of Tyr (10 μM) are exemplified in Figure 1. The pressure rose within 1 min of the start of the infusion and rapidly reached its maximum. In some preparations, this level was maintained throughout the Tyr infusion but, in others, it fell gradually. This was seen in all

Table 1 Responses of isolated perfused mesenteric arteries to the infusion of tyramine (10 μM)

Group	(n)	*Max. pressure* (mmHg)	*AUC of response* (arbitrary units)	*Time to washout* (min)
Control	(8)	18.7 ± 3.7	108.0 ± 20.5	6.9 ± 0.5
Clorgyline (1 μM)	(8)	18.8 ± 2.7	118.6 ± 17.5	9.3 ± 0.7*
Clorgyline (10 μM)	(6)	16.5 ± 1.8	106.0 ± 11.8	7.9 ± 0.4
MDL	(8)	17.4 ± 3.6	109.0 ± 21.2	11.2 ± 0.7**
MDL + clorgyline	(8)	28.0 ± 5.8	204.0 ± 28.9*	21.4 ± 1.1***
Cocaine	(6)	7.6 ± 1.4*	47.8 ± 8.0*	5.0 ± 0.5*
CCS	(6)	14.5 ± 1.4	90.2 ± 8.0	7.0 ± 0.3

Tyr was infused into each preparation in modified Krebs-Henseleit solution at a flow rate of 2 ml min^{-1} and a total of 20 ml of Krebs solution containing 10 μM Tyr was infused before perfusion with Tyr-free Krebs solution was resumed. The maximum pressure attained, the AUC of the Tyr response and the time for the response to return to within 1 mmHg of the baseline following the return to perfusion with Tyr-free Krebs solution, have been measured. The groups were composed of preparations from control rats (control), from control rats perfused with clorgyline for 30 min at the start of the experiment (1 μM or 10 μM), from rats treated with 1 mg kg^{-1} MDL 72145 1 h before dissection (MDL), from MDL 72145-treated rats that have also been perfused with Krebs solution containing 1 μM clorgyline, and from control rats with 3 μM cocaine (Cocaine) or 29 μM corticosterone (CCS) added to the Krebs solution 20 min before the Tyr infusion. In each case the mean response ± s.e.mean has been given for 6 to 8 preparations in each group. The values obtained have been compared with the control values by an unpaired Student's t test (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

Table 2 Responses of the isolated perfused mesenteric arteries to the infusion of tyramine (100 μM)

Group	(n)	*Max. pressure* (mmHg)	*AUC of response* (arbitrary units)	*Time to washout* (min)
Control	(8)	29.3 ± 4.1	150.1 ± 13.8	13.6 ± 0.9
Clorgyline (10 μM)	(6)	39.9 ± 7.4	191.2 ± 24.2	16.6 ± 1.7
MDL	(6)	19.9 ± 5.5	169.1 ± 38.7	22.8 ± 3.5*
MDL + clorgyline	(4)	33.0 ± 4.4	302.5 ± 23.2**	44.0 ± 2.4***
Cocaine	(6)	16.5 ± 1.3*	88.4 ± 12.3**	5.8 ± 0.5***

Twenty ml of Krebs solution containing 100 μM Tyr was infused and the same response parameters were measured as described in the legend to Table 1. The 5 groups of preparations examined at this higher dose of Tyr were the same as those described in Table 1 (the same abbreviations have been used) except that no group treated with 1 μM clorgyline was examined, nor were the effects of corticosterone on the response to this dose, tested.

groups of preparations but generally the higher the peak pressure the more likely there was to be a gradual fall in the perfusion pressure during the Tyr infusion. When the perfusing fluid was changed to Tyr-free Krebs solution the pressure began to fall within 1 min. In Figure 1 the upper trace is from a control preparation and the lower trace from a preparation treated with both MDL 72145 and clorgyline (1 μM). The time for the pressure to return to the resting level was considerably longer in the second preparation than in the first. The pattern of responses to the higher dose of Tyr was very similar. All three parameters were substantially increased following the high dose of Tyr when compared with the low dose. The data for the responses to both low and high doses of Tyr are given in Tables 1 and 2 respectively. In each case the AUC of the Tyr response was only significantly increased when preparations from MDL 72145-treated rats were also treated with clorgyline. MDL 72145 treatment alone significantly increased the time for the response to return to within 1 mmHg of the resting level following wash out of both high and low doses of Tyr. Any increase in the peak response or the AUC of the response caused by clorgyline or MDL 72145 treatment alone did not reach statistical significance. Cocaine (3 μM) depressed all three parameters significantly at both high and low doses of Tyr, whereas corticosterone (tested on the low dose only), had no effect on any of these parameters.

The dose-response curves to Ad in the five groups of preparations where the low dose of Tyr was tested are shown in Figure 2 and represent the computer-generated best fit curves. The three parameters generated for each curve are shown in Table 3. When the curves were compared with the control curve by a simultaneous curve fitting method, the small increase in EC_{50}, seen in the MDL 72145-treated and clorgyline-treated (both 1 and 10 μM) groups, reached statistical significance, as did the increase in the maximum response for the MDL 72145 group and group treated with both MDL 72145 and clorgyline. There was also a significant decrease in the maximum response in the group of preparations treated with clorgyline (10 μM). No significant alteration occurred in the Hill slope in any of the groups tested.

The effects of cocaine and corticosterone on the response to Ad at doses near to the EC_{50} are shown in Figure 3. Corticosterone (29 μM) alone did not alter the response to Ad (1 or 2 nmol) whereas cocaine (3 μM) significantly increased the response at

Table 3 Effect of amine oxidase inhibitors on the sensitivity of the perfused mesenteric arterial bed to adrenaline

Group	(n)	*Hill slope*	EC_{50} (nmol)	*Max. pressure* (mmHg)
Control	(8)	1.32 ± 0.08	1.56 ± 0.08	89.3 ± 1.59
Clorgyline (1 μM)	(8)	1.48 ± 0.08	1.96 ± 0.11*	90.4 ± 1.74
Clorgyline (10 μM)	(6)	1.52 ± 0.12	1.94 ± 0.16*	81.0 ± 2.24*
MDL	(8)	1.38 ± 0.03	1.96 ± 0.04**	94.0 ± 0.60*
MDL + clorgyline (1 μM)	(8)	1.46 ± 0.11	1.72 ± 0.11	103.0 ± 2.33*

The data are derived from the 5 curves shown in Figure 2. The abbreviations for the groups of preparations used are the same as those given in Table 1. A weighted non-linear regression computer programme was used to determine the line of best fit for each curve as described in Methods and the three parameters, Hill slope, EC_{50} and maximum response were generated. A method for simultaneously fitting two curves (De Lean *et al.*, 1978) with or without one of the parameters constrained was used to test for a significant difference from the control curve. (* $P < 0.05$; ** $P < 0.01$).

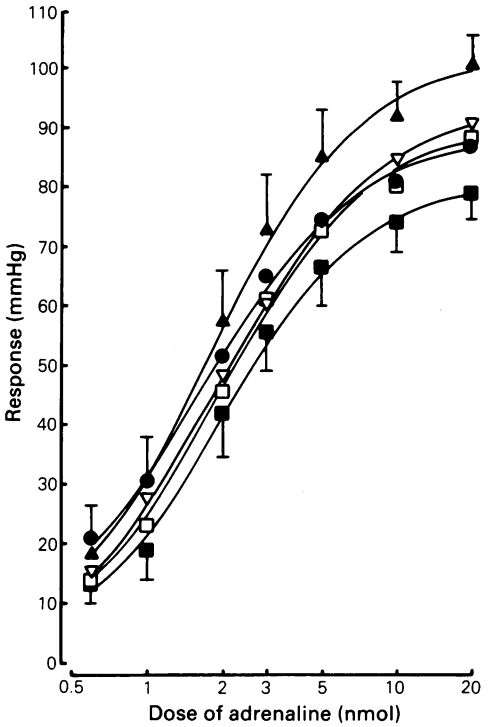

Figure 2 Dose-response curves of the isolated perfused mesenteric arterial bed of the rat to adrenaline (Ad). Doses of Ad (0.6 to 20 nmol) were injected into the perfusing fluid (in 200 μl of Krebs solution) and the maximum rise in pressure is plotted against log of the dose of Ad given. Each point represents the mean value from 6 or 8 preparations. The s.e.mean values are shown only for the two outside curves for clarity, but were of the same magnitude for the other three curves. The lines of best fit have been drawn through the points using a weighted, non-linear regression computer programme described in the methods. The values obtained for EC_{50} Hill slope and maximum response for each group of preparations are given in Table 3. The 5 curves shown are control preparations (●), preparations from rats treated with MDL 72145 (▽), preparations that have been perfused with Krebs solution containing clorgyline for 30 min (1 μM, □; 10 μM, ■) and preparations that have been perfused with Krebs solution containing 1 μM clorgyline from rats that have been treated with MDL 72145 (▲).

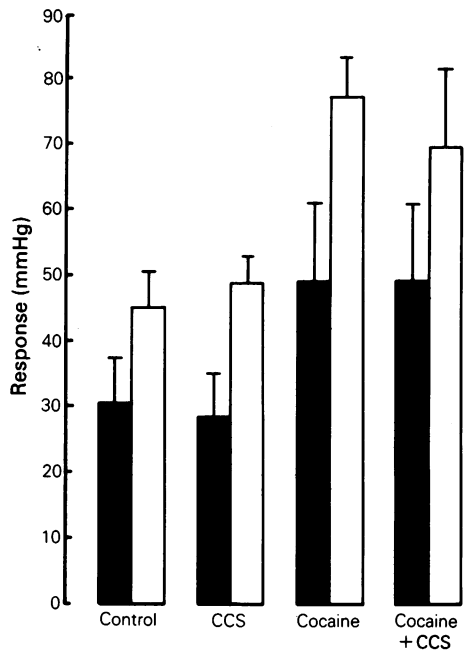

Figure 3 The effect of corticosterone and cocaine on the responses of the isolated perfused mesenteric preparation of the rat to adrenaline (Ad). The increase in pressure when 1 nmol (solid columns) or 2 nmol (open columns) of Ad was injected into the perfusing fluid (in 200 μl of Krebs solution) was recorded in the absence of either drug (Control). The responses to Ad were tested again, 15 min after the addition of either corticosterone (CCS, 29 μM) or cocaine (3 μM) to the Krebs solution. Finally the second drug was added to the Krebs solution and the responses to Ad obtained for a third time (cocaine + CCS). Each column represents the mean from at least 4 preparations with s.e.mean shown by vertical bars. The responses were compared with control by a paired t test. The addition of cocaine always resulted in a significant increase ($P < 0.05$) in the responses to both doses of Ad even when added after corticosterone (data not shown). Addition of corticosterone alone did not increase the responses significantly nor did it result in any further potentiation when added after cocaine (Cocaine + CCS).

both these doses. This increase was seen when cocaine was added first and when it was added following the addition of corticosterone. Addition of corticosterone after cocaine did not produce any further increase in the response to Ad. Metanephrine was tested in the same way as corticosterone at concentrations from 0.1 to 5 μM. No potentiation was seen at the low doses even in the presence of cocaine and at 5 μM, metanephrine produced inhibition of the responses to Ad.

Discussion

These experiments indicate, that the action of Tyr, in the perfused mesenteric arterial bed of the rat, has a substantial indirect component, confirming earlier observations (e.g., George & Leach, 1973) since pressure responses to Tyr are reduced when cocaine, at a dose which potentiates the responses to Ad, is added to the perfusing fluid. In order to act in this way, Tyr

must pass from the lumen of the vessel, across the medial layer, to the adventitial-medial junction, where the nerve endings are located (Hodge & Robinson, 1972).

When Tyr is added to the perfusing fluid, it is metabolised by SSAO and the acid metabolite, *p*-hydroxyphenylacetic acid, appears in the perfusate (Elliott *et al.*, 1989). SSAO is situated in the medial layer of the blood vessels (Lyles & Singh, 1985) and so inhibition of this enzyme might be expected to allow easier access of Tyr to the nerve endings. In the present experiments, inhibition of SSAO by MDL 72145 did not significantly increase the pressure or the AUC of the response at either of the two doses of Tyr tested. It did, however, delay the return of the pressure to the resting level. For this to occur without an increase in the AUC of the response the shape of the response must have been altered. Metabolism of Tyr by SSAO as it leaves the cells of the tissue seems to contribute to the rapid restoration of the normal resting pressure. The response may be altered because the drug treatment also reduced the postsynaptic response to α-adrenoceptor stimulation as shown by the increase in the EC_{50} value for Ad. This effect may have masked the effect of SSAO inhibition on the Tyr response. MDL 72145 when administered to rats *in vivo* at the dose used in these studies also inhibits MAO-B in tissues such as the brain and intestine (Fozard *et al.*, 1985). However, homogenates of rat mesenteric arteries possess very little MAO-B activity capable of metabolizing Tyr (Coquil *et al.*, 1973). At both the concentrations of Tyr used in these studies (100 and 10 μM) we have been unable to detect a contribution of MAO-B activity to the deamination of this amine (Elliott *et al.*, 1989, unpublished data) and so have attributed the effects seen with MDL 72145 to inhibition of SSAO in this tissue.

The other Tyr-metabolizing activity known to be present in this tissue is MAO-A, a mitochondrial enzyme present both in the nerve endings and extraneuronally (Jarrott & Iversen, 1971; Coquil *et al.*, 1973). Metabolites attributable to this activity did not appear in the perfusate, possibly because they were not readily released following their formation by the mitochondrial enzyme (Elliott *et al.*, 1989). Inhibition of this enzyme alone did not produce statistically significant increases in perfusion pressure or in the area under the response at either high or low doses of Tyr. Clorgyline has been shown to inhibit the action of directly acting sympathomimetic amines on smooth muscle preparations by blocking α-adrenoceptors (Finberg & Tenne, 1982), an effect which was thought to be reversible on wash out of the drug. In the rat mesenteric bed substantial inhibition of the response to Ad occurred in the presence of clorgyline and this effect was also reversed on washing. Nevertheless, clorgyline (10 μM) appeared to have some effect that was difficult to wash out as the EC_{50} of the dose-response curve to Ad increased and the maximum response decreased. When a lower dose of clorgyline (1 μM) was used in an attempt to overcome these problems, the EC_{50} was still increased. At the same time, the MAO-A activity was only reduced to 20% of the control value (data not shown). This shows the importance of not extrapolating from inhibition found in tissue homogenates, (where 1 μM clorgyline completely inactivates MAO-A) to the whole tissue. Potentiation of the Tyr response in other tissues was only observed following greater than 90% inhibition of MAO-A by clorgyline (Finberg & Tenne, 1982).

The evidence that MAO contributes to inactivation of Tyr can be seen from the effect of inhibiting both MAO-A and SSAO together. The AUC of the Tyr response was substantially increased at both doses due to a 3 to 4 fold increase in the time for the response to return to the baseline. It would seem, therefore, that these two enzymes work together to activate Tyr as it diffuses from the lumen of the blood vessel to its site of action. Inhibition of one of them alone has much less effect than inhibiting both together. De la Lande *et al.* (1970) found that iproniazid potentiated the action of intraluminally applied Tyr much more than extraluminal application of this amine to the isolated perfused ear artery of the rabbit. Iproniazid inhibits all the forms of amine oxidase considered here (Lyles, 1984). Similar results were obtained by Iriarte *et al.* (1985) in the rabbit aorta when both pargyline and semicarbazide were used. Rabbit blood vessels have been shown to possess SSAO as well as MAO (Rucker & Goettlich-Riemann, 1972).

The extraneuronal uptake process was considered as another possible mechanism whereby the tissue could be protected from the pressor or other effects of Tyr. Corticosterone, at a dose which gave 95% inhibition of extraneuronal uptake of noradrenaline in the rat heart (Iversen & Salt, 1970) did not alter the response to Tyr significantly. No potentiation of the tissue responses to Ad, even in the presence of cocaine to block neuronal uptake, could be demonstrated. Metanephrine, a potent and selective extraneuronal uptake blocker (Burgen & Iversen, 1965), was also tested in these present experiments, and failed to potentiate the responses to Ad. These data support those of Venning & de la Lande (1984) who concluded that rat blood vessels, unlike those of the rabbit, had a poorly developed extraneuronal uptake process. Even in rabbit blood vessels, corticosterone failed to show potentiation of Tyr applied to the intimal surface, suggesting that Tyr is not transported into cells via a corticosterone-sensitive mechanism (Iriarte *et al.*, 1985).

In conclusion, rat blood vessels contain MAO-A and SSAO, both of which appear to be important in the inactivation of Tyr in the blood vessel wall. The role of these blood vessel enzymes in protecting against ingested Tyr remains uncertain as the first enzyme activity to be encountered will be MAO in the intestinal villus. This has been shown to be responsible for a considerable, first pass metabolism of Tyr (Davis *et al.*, 1984; Hassan *et al.*, 1988) and Tyr absorbed would be exposed to MAO in the liver en route to the systemic circulation. Nevertheless, in rat *in vitro* models where MAO inhibitors are tested for their ability to potentiate the actions of Tyr, the influence of SSAO should be considered when interpreting the results. It should also be noted that, although human blood vessels possess a similar SSAO activity (Lewinsohn, 1981), it has a very low affinity for Tyr (Precious & Lyles, 1988). Thus it would be unwise to suggest, from experiments of this nature, that inhibition of SSAO in the human subject, is involved in the 'cheese reaction' (Blackwell, 1963).

J.E. is a Wellcome Veterinary Scholar.

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