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The influence of monoamine oxidase activity on the release of noradrenaline by tyramine

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Abstract—The influence of monoamine oxidase (MAO) activity on the kinetic characteristics of noradrenaline (NA) release evoked by tyramine has been examined. Dog splenic artery strips were incubated with [^{3}H]NA after inhibition of catechol-*O*-methyltransferase (COMT) and of extraneuronal uptake. In some experiments MAO was also inhibited. The strips were then perifused for 200 min. Some strips were exposed to tyramine (1·5, 40 and 3240 μmol L^{-1}) from the 100th to the 200th min of perifusion. In control experiments (i.e. in the absence of tyramine) most of the [^{3}H]NA accumulated in the strips (83% of tissue activity) and did not contribute to the efflux. The value of this "bound fraction" (the NA located at a site(s) from which it could not be displaced by a simple concentration gradient) was the same whether or not MAO was inhibited. At all concentrations, tyramine mobilized only one NA compartment. Increasing the concentration of tyramine resulted in a decrease of the "bound fraction", which became negligible for the highest concentration of tyramine used (3240 μmol L^{-1}), regardless of MAO being inhibited or not. However, for the lower concentrations of tyramine, MAO inhibition resulted in an increase in the amine's releasing effect. The formation of 3,4-dihydroxy-phenylglycol (DOPEG) increased with increase of tyramine from the 1·5 to 40 μmol L^{-1} concentration, but not beyond. The ratio NA/DOPEG increased for all concentrations of tyramine. Thus, it was not possible to exclude an inhibitory effect of tyramine on MAO activity with the highest concentration used.

When the releasing effect of tyramine (0·49–3240 μmol L^{-1}) was studied by Brandão et al (1981), it was shown that the amine, in a concentration-dependent manner, mobilized [^{3}H]noradrenaline accumulated in tissue, the releasable pool becoming almost equal to total noradrenaline (NA) when the highest concentration of tyramine was used. Since in those experiments monoamine oxidase (MAO) was inhibited, it was not possible to know the influence of deamination on the releasing effect of tyramine. This may be of relevance, as tyramine is a good substrate of MAO and intraneuronal MAO activity is high (Cassis et al 1986; Grohmann 1987). Furthermore, Leitz & Stefano (1971) proposed that the releasing effect of tyramine was dependent on its inhibitory effect of MAO.

We have continued this investigation to clarify the influence of MAO activity on the releasing effect of tyramine on NA stores and to determine if it has an inhibitory effect on MAO activity.

Material and methods

Mongrel dogs (10–20 kg) were anaesthetized with sodium pentobarbitone (30 mg kg^{-1} i.v.). Segments of proximal splenic artery were removed, and helically cut strips were prepared. Each strip, 4 cm long and weighing about 120 mg, was preincubated for 30 min in Krebs solution at 37°C, gassed with 95% O_2 and 5% CO_2, in the presence of 0·1 mmol L^{-1} 3,4-dihydroxy-2-methyl-propiophenone (U-0521) and 28 μmol L^{-1} cortisol, to block catechol-*O*-methyltransferase (COMT) and extraneuronal uptake, respectively (UC strips). Some strips were also preincubated with 0·1 mmol L^{-1} pargyline to block the MAO (PUC strips). The strips were then incubated for 60 min in 3 mL of the same medium (i.e. with U-0521 + cortisol or U-0521 + cortisol + pargyline), to which (−)-7-[^{3}H]NA (with 70% of total tritium in the 7 position and 30% in the 8 position, 23·1 Ci $mmol^{-1}$ specific activity) was added; the final concentration of [^{3}H]NA was 1·4 μmol L^{-1}. After incubation, the strips were continuously perifused for 200 min in a 1 mL glass organ bath; Krebs solution (with U-0521 and cortisol as above) was pumped through the bath at a constant rate of 0·8 mL min^{-1}, and the overflow was collected. In PUC strips, pargyline was present only during the preincubation and loading. Details of the perifusion method and of determination of total radioactivity, NA and its metabolites, have been previously described (Brandão 1977). In some experiments, tyramine was present in the perifusion fluid from 100–200 min. The composition of Krebs solution was the same as that used by Brandão et al (1985). Efflux curves (based on rates of efflux in nmol g^{-1} min^{-1}) were analysed by "backward peeling" as described by Henseling et al (1976). Tissue activity at 100 min (^{3}H 100) was obtained by adding the ^{3}H existing in the tissue at the end of the experiment to the ^{3}H collected in the overflow from 100–200 min of perifusion. The "bound fraction", i.e. radioactivity not contributing to the efflux of the identified compartments (presumably, [^{3}H]NA trapped at a site(s) from where it could not be released by simple concentration gradients), was also determined as described by Henseling et al (1976). "Bound fraction" % is the ratio between the bound fraction and ^{3}H 100 multiplied by 100 (Brandão et al 1981).

Differences between means were compared by Student's *t*-test and those with a *P* value of 0·05 or less were considered significant.

Drugs used were cortisol phosphate (Vitória, Lisboa, Portugal), pargyline hydrochloride (Sigma, St. Louis, Mo, USA), tyramine hydrochloride (Sigma, St. Louis, MO, USA),U-0521 (The Upjohn Co., Kalamazoo, MI, USA).

Results

Accumulation of NA. Strips in the presence of cortisol and U-0521 (UC strips) showed a ^{3}H 100 which was about 2·3 times higher than that of strips which were also treated with pargyline (PUC strips) (see below, Fig. 2).

Spontaneous efflux. The rate of efflux between 100–200 min for UC strips (Fig. 1) declined slowly, with a half time of 108·0 min (k = 0·00642 min^{-1}), the "bound fraction" representing 83 ± 3% of ^{3}H 100 (Fig. 2). In PUC strips the rate of efflux was lower than in UC strips (Fig. 1), but neither the half-life efflux nor the "bound fraction" were significantly different from those obtained in UC strips.

Overflow induced by tyramine. Exposure of the UC strips to tyramine increased the rate of ^{3}H-efflux for each concentration used. The rate of efflux reached its maximum at about 120 min of perifusion, and subsequently showed a monophasic exponential decline with time (Fig. 1). The half-life ^{3}H-efflux induced by each concentration of tyramine tended to decrease when the amine concentration was increased, but the differences were not significant (Fig. 2). The use of higher concentrations of tyramine decreased the "bound fraction", which became negligible at the highest concentration (Fig. 2). The ^{3}H-efflux induced by tyramine was represented mainly by NA and 3,4-dihydroxy-phenylglycol (DOPEG). The increase in tyramine concentration induced only change in the rate of efflux of DOPEG, but there was a marked increase in the efflux of NA (Fig. 3A), the ratio

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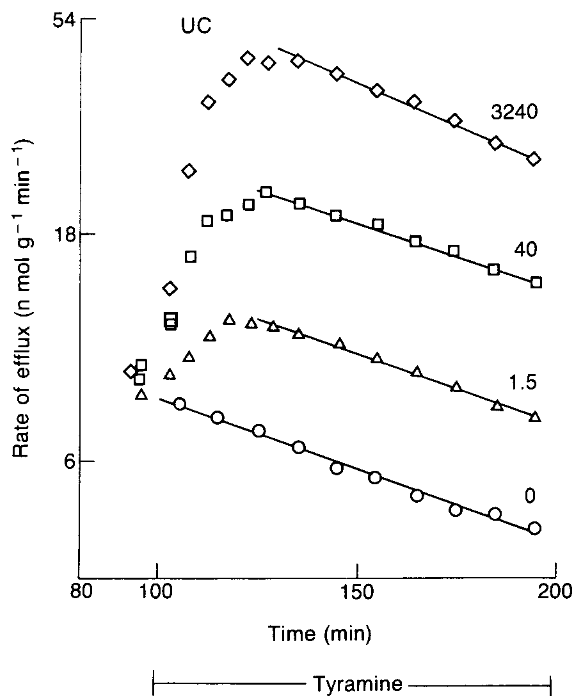

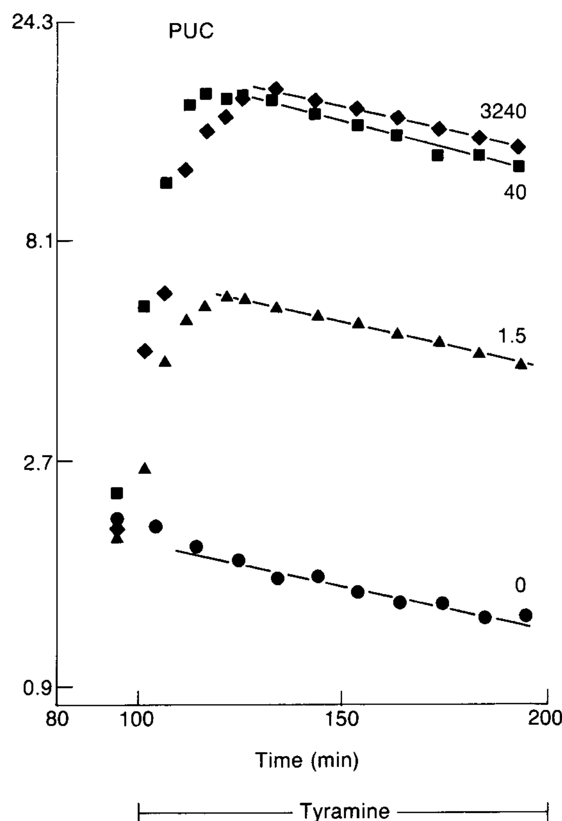

FIG. 1. [^{3}H] efflux from dog splenic artery strips from 90 to 200 min of perifusion. Tyramine was present from 100 to 200 min. Shown are the average efflux curves from UC and PUC strips. Ordinates—rate of efflux (in nmol g^{-1} min^{-1}; log scale); abscissae—time (in min) after the onset of washout. Compartmental analysis (see Methods) was carried out by retrograde regression analysis (solid line).

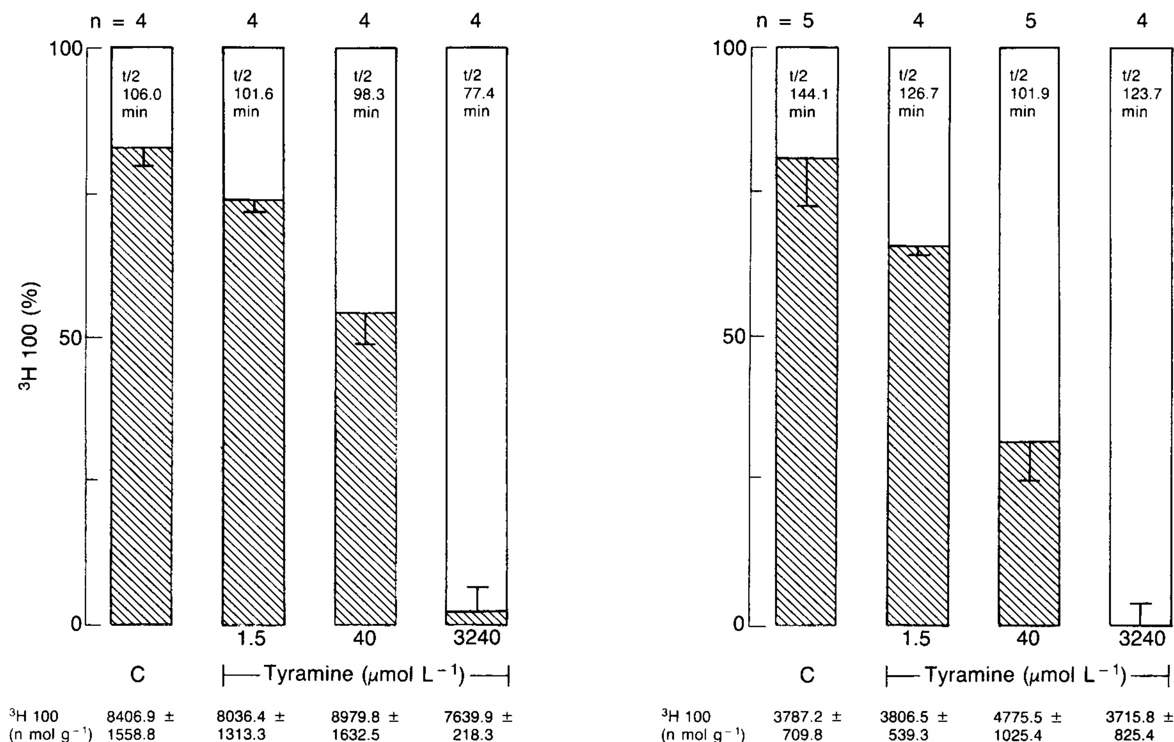

FIG. 2. Influence of tyramine on [^{3}H] efflux (from 100 to 200 min of perifusion) in dog splenic artery strips, in UC and PUC conditions (see Methods). Shown are arithmetic means ± s.e.m. for ^{3}H 100 and bound fraction % (shaded area), and geometric means for half time ($t_{\frac{1}{2}}$) of efflux. BF % = "bound fraction" % = BF × 100/^{3}H 100; ^{3}H 100 = total activity in tissue at t = 100 min of perifusion; C-control conditions. n = no. of experiments.

NA/DOPEG increasing with increase of tyramine concentration (Fig. 3B). However, for each tyramine concentration the ratio NA/DOPEG was constant after the steady-state had been attained. The ^{3}H-activity accumulated in the strips at the end of the experiments was almost totally represented by NA (about 95%, n = 8).

In PUC strips the rate of ^{3}H efflux induced by tyramine was less than in UC strips (Fig. 1). In UC strips the ^{3}H-efflux increased with each concentration of tyramine, whereas in PUC strips it increased only when the amine concentration rose from 1·5 to 40 μmol L^{-1}. The half-life of efflux was similar for the three concentrations of tyramine used (Fig. 2). All the half-life efflux values obtained in PUC strips were longer than those obtained in UC strips with similar concentrations of tyramine (144 vs 108, 127 vs 102, 102 vs 98, 124 vs 77 for PUC vs UC strips, respectively, in control conditions, or 1·5, 40 and 3240 μmol L^{-1} tyramine); however the differences were only significant at the 3240 μmol L^{-1} concentration ($P < 0·05$). The "bound fraction" values were significantly smaller in PUC strips than in UC strips, both at 1·5 ($P < 0·02$) and 40 μmol L^{-1} ($P < 0·05$), but not for the highest concentration (Fig. 2).

Discussion

When we compared the ^{3}H 100 values for UC and PUC strips we observed that the presence of pargyline (100 μmol L^{-1}) during pre-incubation and loading (see Methods) markedly decreased the [^{3}H]NA accumulated in the strips. This can be explained by an inhibition of neuronal uptake by pargyline (Hendley & Snyder 1968; Azzaro & Demarest 1982).

The present results, obtained in the splenic artery under UC conditions (see Methods) showed that the spontaneous ^{3}H-efflux after the 100 th min of perifusion had a long half-life (about 108 min) and most of the radioactivity (about 83%) accumulated did not contribute to the efflux, i.e. remained as a "bound fraction" which corresponded almost completely to [^{3}H]NA. These kinetic characteristics are in good agreement with those observed for [^{3}H]NA of vesicular origin (Henseling et al 1976; Eckert et al 1976; Brandão et al 1981). With our experimental conditions the results showed that the size of the [^{3}H]NA pool mobilized by tyramine was dependent on the amine's concentration and increased with it, the "bound fraction" becoming almost nil for the highest concentration. Furthermore, it was shown that tyramine evoked a monoexponential decline of [^{3}H]NA efflux in this preparation; which is in good agreement with results obtained in the heart by Neff et al (1965). The [^{3}H] efflux induced by tyramine the final result of a number of processes, such as accumulation of tyramine by the uptake$_1$ mechanism, metabolism in the cytoplasm, vesicular uptake, conversion to octopamine and displacement of NA, outward transport of NA, diffusion through the adventitia and media, and extraneuronal uptake. Thus, to make the study more feasible, the model was simplified by the inhibition of one or more of these mechanisms, which, however, are seldom complete, especially when high concentrations of tyramine are used.

In experiments with dog saphenous vein (Brandão et al 1980) it was shown that DOPEG was almost the only intraneuronal metabolite of Na released by tyramine under these conditions, and a good indicator of MAO activity. This is more evident if it is considered that the ^{3}H-labelling of NA used results in a decreased rate of deamination and in the formation of tritiated water, due to the presence of tritium in position 8 (Grohmann et al 1987). Accordingly, in the present experiments the only metabolite studied was DOPEG. When the efflux of DOPEG was compared with that of [^{3}H]NA, the increase of tyramine concentration slightly increased the rate of efflux of DOPEG

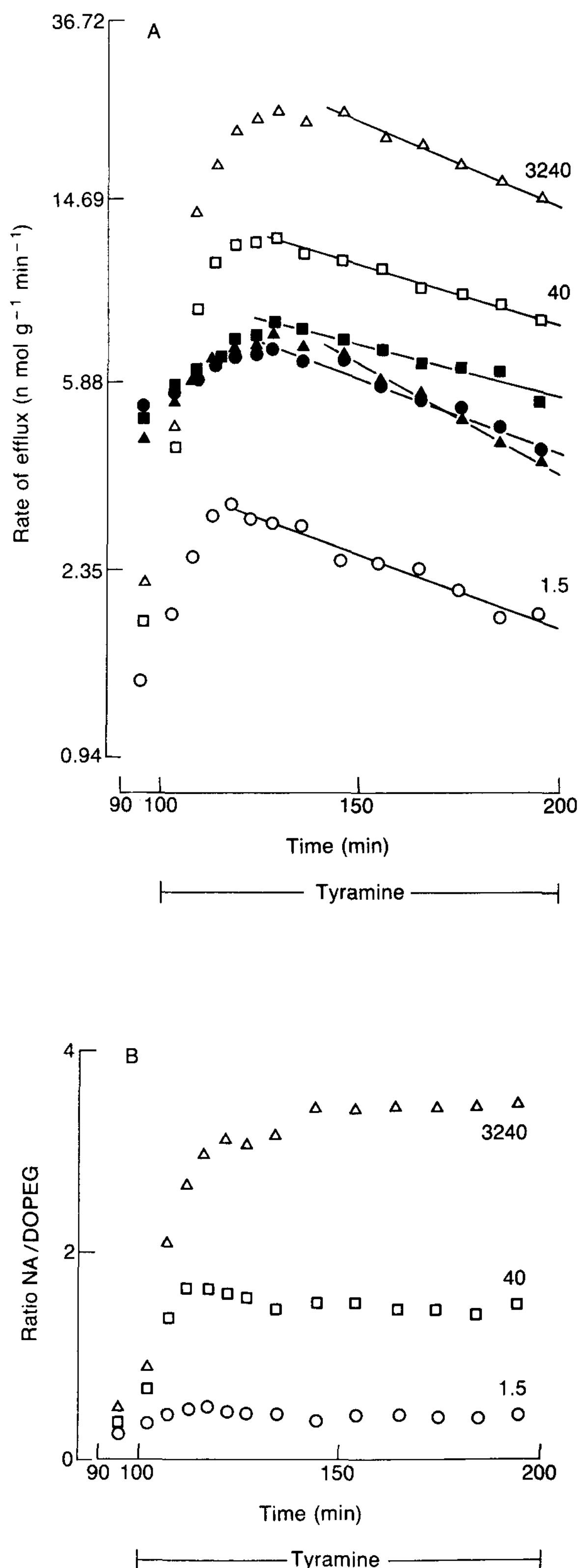

FIG. 3. A. Efflux of [^{3}H]NA (open symbols) and [^{3}H]DOPEG (closed symbols) in the presence of tyramine, under UC conditions. Shown are the average efflux curves. Ordinates—rate of efflux (in nmol g^{-1} min^{-1}; log scale); abscissae—time (in min) after the onset of washout. Compartmental analysis was carried out from retrograde regression analysis (solid line). B. Influence of tyramine on the ratio NA/DOPEG for each concentration. n = no. of experiments.

only at the 1·5 and 40 μmol L^{-1} concentrations, whereas the rate of efflux of [^{3}H]NA was always markedly increased. As a consequence, under steady-state conditions, the ratio of [^{3}H]NA/DOPEG increased with tyramine concentration. This, in addition to its well-known releasing effect of vesicular NA (responsible for the decrease of "bound fraction"), could be due to other small effects that tended to cancel the increase of DOPEG. Recent data (Langeloh 1986) have shown that the inward transport of tyramine makes the carrier available for the outward transport of NA by a facilitating exchange diffusion. In addition, the co-transport of sodium (together with tyramine) appears to increase the affinity of NA to the carrier inside the axoplasmic membrane (Bönisch 1986). As a consequence of these two effects, the efflux of NA is increased without simultaneous increase of DOPEG. However, a further possibility cannot be excluded, namely, substrate saturation by tyramine (with the increase of unchanged NA efflux); this may occur for the highest concentration of tyramine, as suggested by Brandão et al (1980). More recently, Langeloh & Trendelenburg (1987), studying the releasing effect of three groups of indirectly-acting amines (substrates, inhibitors, and neither substrates nor inhibitors of MAO) proved that for concentrations between 1–10 mmol L^{-1}, tyramine did not increase the release of [^{3}H]NA while increasing the NA/DOPEG ratio. These results confirm that high concentrations of tyramine inhibit MAO, having a releasing effect similar to the one shown by indirectly-acting amines that are inhibitors of MAO. The inhibition of MAO (PUC strips) did not modify significantly the kinetic characteristics of spontaneous efflux. However, MAO inhibition increased the releasable pool of [^{3}H]NA mobilized by the lower concentrations of tyramine used (1·5, 40 μmol L^{-1}). These results indicate that when MAO is intact, the access of tyramine to the vesicles is reduced by MAO activity; this suggests that inhibition of MAO by pargyline permits a higher degree of accumulation of tyramine in the axoplasm. Furthermore, as shown in Fig. 1, under UC conditions the maximum efflux rate increases with the increase of Ty concentration (from 40 to 3240 μmol L^{-1}), while under PUC conditions no such increase was observed. This could suggest that after inhibition of deamination intraneuronal tyramine competes with NA for the efflux mechanism.

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