Effects of nomifensine on noradrenaline accumulation and contractile responses in the rat anococcygeus muscle

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Nomifensine is a potent inhibitor of noradrenaline and dopamine uptake into rat brain synaptosomes but only a weak inhibitor of 5-hydroxytryptamine uptake (Tuomisto, 1977). The potency of nomifensine in inhibiting noradrenaline uptake into synaptosomes from rat hypothalamus is similar to that of the tricyclic antidepressant, nortriptyline (Schacht & Heptner, 1974). There is a correlation between the ability of tricyclic antidepressants, such as nortriptyline, to inhibit noradrenaline accumulation and to potentiate the contractile responses to noradrenaline in the rat anococcygeus muscle (Doggrell & Woodruff, 1977). In the present study, we have examined the effects of nomifensine on the accumulation of noradrenaline and on the contractile responses to exogenously applied noradrenaline in the rat anococcygeus muscle.

Male rats (350 g) were killed and the anococcygeus muscles dissected out as described by Gillespie (1972). The accumulation of noradrenaline and the contractile responses were measured as described by Doggrell & Woodruff (1977). Thus, for the accumulation experiments, muscles were mounted on wire frames under 0·2–0·5 g tension in Krebs solution, gassed with 5% CO_2 in oxygen. Tritiated noradrenaline (final concentration of 61 pmol ml^{-1}) was added to a final volume of 5 ml. The mixture was incubated for 20 min at 37°, the tissue was then blotted, washed for 10 min in 5 ml of Krebs solution, again blotted dry and weighed. The radioactivity in the tissue and incubation medium was determined and the tissue:medium ratios calculated.

Parallel experiments were performed in which the effects of nomifensine and cocaine on noradrenaline accumulation were studied. In these experiments the incubations were carried out in Krebs solution containing different concentrations of these drugs. Inhibition of noradrenaline accumulation was expressed as a percentage of control values. Dose-response curves were plotted as % inhibition of noradrenaline accumulation against log M concentration of uptake inhibitor and the IC50 values (concentration causing 50% inhibition of noradrenaline accumulation) were determined by regression line analysis.

For the contractile response experiments, individual muscles were mounted under 0·2–0·5 g tension in 10 ml organ baths containing Krebs solution at 37°, gassed with 5% CO_2 in oxygen. Contractile responses were recorded using an isometric transducer and a Servoscribe pen recorder. When the effects of nomifensine or cocaine on responses were studied, these drugs were present in the Krebs solution throughout.

Responses were calculated as a percentage of the maximum response. A regression line was calculated for each dose-response curve over the range 20 to 80% of the maximum response and the pD2 value (negative logarithm of molar concentration of agonist producing 50% of the maximum response) was determined. The pD_2 values, under different conditions, were compared by Student's *t*-test and considered significantly different whenever $P < 0.05$. The ability of drugs to potentiate or inhibit responses is expressed as the dose-ratios, which were obtained by taking the antilogarithm of the difference between the mean pD_2 values obtained in the presence and absence of drugs.

(−)-[7-^{3}H]Noradrenaline hydrochloride (specific activity 10·3 Ci $mmol^{-1}$) was obtained from Amersham and diluted as described by Doggrell & Woodruff (1977). The other drugs used were carbachol, cocaine hydrochloride (BDH); methoxamine hydrochloride (Burroughs-Wellcome); nomifensine hydrogen maleate (Hoechst); and (−)-adrenaline bitartrate, (−)-noradrenaline bitartrate, tyramine hydrochloride, and acetylcholine chloride (Koch-Light).

In the study of (−)-noradrenaline accumulation in the muscle, the control tissue:medium ratios were 5·5 ± 0·5 (s.e. mean; n = 4). Nomifensine (3×10^{-10}–10^{-3} M) and cocaine (10^{-7}–10^{-3} M) inhibited noradrenaline accumulation. The IC50 values for nomifensine and cocaine were $1{\cdot}80 \times 10^{-8}$ M and $9{\cdot}16 \times 10^{-7}$ M, respectively. Thus, nomifensine was a very potent inhibitor of noradrenaline accumulation.

Nomifensine (10^{-5}–10^{-6} M) alone did not cause contractile responses of the muscle. The contractile responses to (−)-noradrenaline were potentiated ×28·8, ×309·0, ×19·1 and ×13·2 by 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} M nomifensine, respectively. The responses to (−)-adrenaline were potentiated ×7·8, ×50·1 and ×14·1 by 10^{-5}, 10^{-6} and 10^{-7} M nomifensine, respectively. Thus, 10^{-6} M nomifensine caused a large potentiation of responses to (−)-noradrenaline (Fig. 1A) and to (−)-adrenaline. The responses to tyramine were inhibited (×36·2) by 10^{-5} M nomifensine. Lower concentrations of nomifensine (10^{-6}–10^{-8} M) had no effect on responses to tyramine. 10^{-6} M nomifensine inhibited responses to methoxamine (×11) but potentiated responses to acetylcholine (×14) and carbachol (×8). The potentiation of responses to acetylcholine by 10^{-6} M nomifensine was more pronounced at low concentrations than high concentrations of acetylcholine (Fig. 1B).

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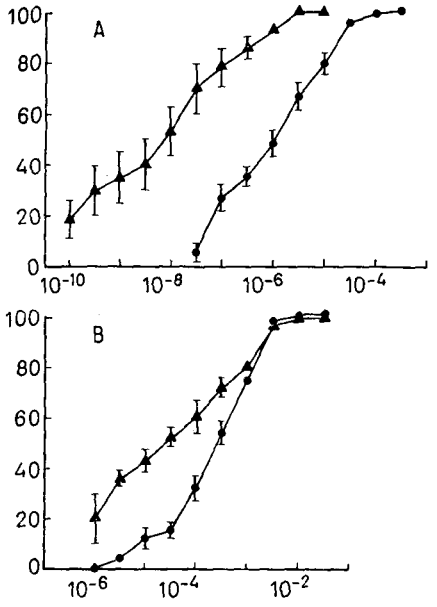

FIG. 1. Effect of nomifensine on responses to A (−)-noradrenaline (M) and B acetylcholine (M) in the rat anococcygeus muscle. Control responses (●—●) and in the presence of 10^{-6}M nomifensine (▲—▲). All responses are expressed as a percentage of the maximum response (ordinate). Each value is the mean ±s.e.m. of 4–6 observations.

In the presence of cocaine (3×10^{-6} M), the responses to (−)-noradrenaline and (−)-adrenaline were potentiated ×5·3 and ×8·7, respectively. Cocaine (3×10^{-6} M) had no effect on responses to tyramine, methoxamine and acetylcholine.

The accumulation of low concentrations of noradrenaline by the rat anococcygeus muscle is predominantly neuronal (Nash, Gillespie & Robertson, 1974). Furthermore, following incubation in the presence of low concentrations of noradrenaline, all the radioactivity in the muscle behaves as authentic noradrenaline (Doggrell & Woodruff, 1977). In the present paper, we used a low concentration of noradrenaline in order to study neuronal accumulation.

Nomifensine was a potent inhibitor of noradrenaline accumulation in the rat anococcygeus muscle, having a similar potency to nortriptyline (Doggrell & Woodruff, 1977). This is in agreement with the findings of Schacht & Heptner (1974), who used synaptosomes from rat hypothalamus.

Doggrell & Woodruff (1977) found a correlation between the ability of tricyclic antidepressants, such as nortriptyline, to inhibit noradrenaline accumulation and potentiate contractile responses to noradrenaline in the rat anococcygeus muscle. However, in the present study, there was not a good correlation between the ability of nomifensine to inhibit noradrenaline accumulation and potentiate contractile responses to noradrenaline; 10^{-6} M nomifensine caused an unusually large potentiation of responses to noradrenaline.

Nomifensine (10^{-6}–10^{-8} M) and cocaine (3×10^{-6} M) did not inhibit contractile responses to tyramine. When noradrenaline uptake is not fully inhibited, as with these drug concentrations, the effect on responses to tyramine is twofold. First, there is a partial inhibition of tyramine uptake, thus less noradrenaline than normal is released. Secondly, there is a partial inhibition of the uptake of noradrenaline released by tyramine and thus a higher proportion of noradrenaline will reach the receptors than normal. These two effects oppose each other and consequently there may be no effect on responses to tyramine.

Methoxamine is not a substrate for uptake into noradrenergic neurons (Trendelenburg, Maxwell & Pluchino, 1970). Methoxamine is, however, more susceptible to α-adrenoceptor blockade than noradrenaline (Doggrell & Woodruff, 1977). The responses to methoxamine were inhibited by 10^{-6} M nomifensine, this inhibition must reflect some α-adrenoceptor blocking activity. The unusually large potentiation of responses to noradrenaline by nomifensine (10^{-6} M) is even more surprising when the α-adrenoceptor blocking activity of this drug is taken into account.

The rat anococcygeus muscle has no cholinergic innervation (Gillespie, 1972). In the present study, nomifensine (10^{-6} M) potentiated whereas cocaine (3×10^{-6} M) had no effect on responses to acetylcholine and carbachol. This potentiation must reflect a postsynaptic action of nomifensine.

These results suggest that nomifensine potentiates contractile responses to noradrenaline by two mechanisms; by inhibiting noradrenaline uptake and by a postsynaptic action.

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Persistent neuronal blockade with guanethidine in dog mesenteric arteries

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The effects of prolonged administration of guanethidine differs in several respects from those following acute dosage (Boura & Green, 1965; Laverty, 1973; Jandhyala, Clarke & Buckley, 1974). We have shown (Clarke, Jandhyala & others, 1974) that tolerance develops to the adrenergic neuronal blocking action of guanethidine to the dog heart, whereas sympathetic activity to the mesenteric arteries remains inhibited. Thus, the present study was undertaken to define further the nature and time-course of guanethidine-induced effects upon neuronal and receptor function in the mesenteric arteries of the dog. The findings show that guanethidine produces rapid but long-lasting effects which appear to be only slowly reversible with time. Additionally, persistent neuronal blockade is associated with a resistance to (+)-amphetamine-induced reversal.

Purebred beagle dogs were treated daily with oral guanethidine (2·5 mg kg^{-1}) for various times. Controls, randomly selected, received lactose. The guanethidine or lactose in a gelatin capsule was placed at the back of a dog's tongue, swallowing was initiated by neck massage. All experiments were made 18 to 22 h after the last dose. Isolated mesenteric artery preparations were obtained after the administration of sodium pentobarbitone (35 mg kg^{-1}, i.v.) as described previously (Clarke, Ertel & others, 1972). The vessels were perfused with Krebs bicarbonate solution at a constant rate (35 ml min^{-1}) and perfusion pressure recorded. At least three preparations were made from each dog and the results were pooled to give a single "n" value. The periarterial sympathetic nerves were stimulated at a supramaximal voltage, with a pulse duration of 2 ms over a range of frequencies (1–24 Hz) for 20 s at 3 to 6 min intervals. Drugs were injected into the perfusion fluid.

Fig. 1 shows the effect of treatment with guanethidine for 1 and 7 days. After only one dose of guanethidine the responses to injected noradrenaline were increased by about two-fold, but the frequency-response curve did not differ from the lactose controls. As there was increased sensitivity to injected noradrenaline some impairment of neuronal function was already present. After 7 days of treatment, neurotransmission was fully inhibited but there was no further increase in the responses to injected noradrenaline. Treatment for 2 and 8 months (Fig. 2) also failed to increase further the sensitivity to injected noradrenaline. Neuronal blockade persisted, confirming data presented previously for 6 months of treatment (Clarke, Jandhyala & others, 1974). In dogs given guanethidine for 6 months, followed by lactose administration for 2 months, the sensitivity to noradrenaline returned to within control values, but nerve mediated responses remained clearly less than those obtained in the control preparations.

It is well established that (+)-amphetamine will reverse the early sympathetic neuronal blocking action of guanethidine (Day & Rand, 1962, 1963; Boura & Green, 1965). In the current study (+)-amphetamine (4 mg, bolus dose) readily reversed the effects of guanethidine after 1 or 7 days of treatment. However,

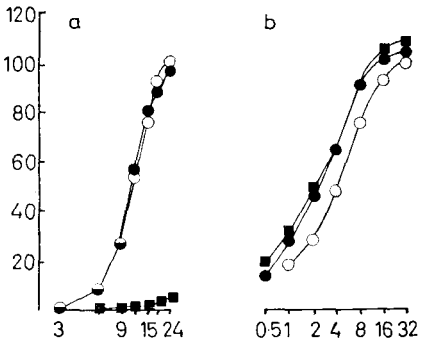

FIG. 1. Effect of guanethidine (2·5 mg kg^{-1} day^{-1}, orally) on the frequency response to (abscissae) a-periarterial nerve stimulation (Hz) and b-injected noradrenaline (μg) in isolated perfused dog mesenteric arteries. Pooled controls, lactose for 1 and 7 days (n = 6), ○—○; guanethidine for 1 day (n = 3), ●—●; guanethidine for 7 days (n = 3), ■—■. The maximum control rise in perfusion pressure (%) (ordinate) for nerve stimulation and noradrenaline was 210 ± 18 and 218 ± 12 (mm Hg, mean ± s.e.m.), respectively. Significance of differences ($P<0·05$): nerve stimulation, ■—■; noradrenaline, ■—■, ●—● (between 20 and 80% values).

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