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COMPARISON OF THE EFFECTS OF METHOXAMINE WITH THOSE OF NORADRENALINE AND PHENYLEPHRINE ON SINGLE CEREBRAL CORTICAL NEURONES

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1 The technique of microelectrophoresis was used to compare the actions of methoxamine, noradrenaline and phenylephrine on single neurones in the somatosensory cerebral cortex of the rat.

2 Methoxamine evoked only excitatory responses on cortical neurones. The methoxamine-sensitive cells were also excited by phenylephrine; cells excited by methoxamine could either be excited or depressed by noradrenaline.

3 Methoxamine appeared to be less potent than either noradrenaline or phenylephrine in evoking excitatory responses.

4 Responses to methoxamine had a slower time course than responses to either noradrenaline or phenylephrine, both the latencies to onset and the recovery times being longer for responses to methoxamine than for responses to noradrenaline or phenylephrine.

5 When the absolute mobilities of methoxamine, noradrenaline and phenylephrine were compared using an *in vitro* method, no significant differences were found between the mobilities of the three ionic species, suggesting that the three drugs have similar transport numbers. Thus the differences in potency between methoxamine and the other two drugs, and the difference between the time courses of responses to methoxamine and the other two drugs, are presumably of biological origin.

6 The α-adrenoceptor antagonist, phenoxybenzamine, antagonized equally excitatory responses to methoxamine and noradrenaline, and responses to methoxamine and phenylephrine, without affecting responses to acetylcholine.

7 When responses to methoxamine and noradrenaline and responses to methoxamine and acetylcholine were summated on the same cells, the net responses were smaller than those expected on the basis of additive effects; the deviation from additivity was greater in the case of the summation of responses to methoxamine and noradrenaline than in the case of summation of responses to methoxamine and acetylcholine. This observation is consistent with the hypothesis that the interaction between methoxamine and noradrenaline follows the model of competitive dualism, whereas the interaction between methoxamine and acetylcholine follows the model of functional synergism.

8 The results suggest that methoxamine may act as a partial agonist at excitatory α-adrenoceptors on cerebral cortical neurones.

Introduction

In a previous paper we showed that the α-adrenoceptor stimulating agent, methoxamine (see Trendelenburg, 1972), when applied by microelectrophoresis, had an excitatory effect on cortical neurones; in contrast, noradrenaline evoked either excitatory or depressant responses (Bevan, Bradshaw & Szabadi, 1977). This observation is in agreement with the sugestion that cerebral cortical neurones may contain both α-adrenoceptors and β-adrenoceptors, the α-adrenoceptors mediating excitatory, and the β-adrenoceptors mediating depressant responses (Bevan *et al.*, 1977; Szabadi, 1979). Our previous observations also showed that methoxamine was less potent than phenylephrine in evoking excitatory responses on cortical neurones (Bevan *et al.*, 1977).

In the present paper we describe some further observations concerning the action of methoxamine on cortical neurones. Firstly, we compared the agonistic effect (potency and time course of responses) of methoxamine with those of noradrenaline and phenylephrine. Secondly, in an attempt to provide further evidence for the activation of α-adrenoceptors by methoxamine on cortical neurones, we compared the effect of the α-adrenoceptor blocking agent, phenoxybenzamine (Nickerson, 1967), on responses to methoxamine and noradrenaline and on responses to methoxamine and

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phenylephrine. Thirdly, we examined the hypothesis that methoxamine acts as a partial agonist at α-adrenoceptors on cortical neurones, by comparing the summation of responses to methoxamine and noradrenaline ('competitive dualism', see Methods), and the summation of responses to methoxamine and acetylcholine ('functional synergism', see Methods).

Some of the results presented here have been communicated to the British Pharmacological Society (Bradshaw, Pun, Slater & Szabadi, 1980a).

Methods

Pharmacological experiments

Male albino Wistar rats (250 to 350 g) were anaesthized with halothane (0.8 to 1.0%). Our methods for the surgical preparation of the animals, for the manufacture of six-barrelled micropipettes (of tip diameter 3.0 to 5.0 μm), for the extracellular recording of action potentials and for the microelectrophoretic application of drugs have been described elsewhere (Bradshaw, Szabadi & Roberts, 1973b; Bradshaw, Roberts & Szabadi, 1974). Spontaneously active neurones were studied in the cerebral cortex (stereotaxic coordinates, according to König & Klippel (1963): A 4.8–6.5, L 0.9–2.4).

Two barrels of each micropipette contained 4.0 M NaCl, one for recording action potentials, the other for current balancing. The remaining barrels contained drug solutions. The following drug solutions were used: (−)-noradrenaline bitartrate (0.05 M, pH 3.0 to 4.0); (−)-phenylephrine hydrochloride (0.05 M, pH 5.0 to 5.5); methoxamine hydrochloride (0.05 M, pH 3.5 to 5.5); acetylcholine chloride (0.05 M, pH 3.5 to 5.5); phenoxybenzamine hydrochloride (0.01 M, pH 2.5 to 3.0).

Two measures for the relative potencies of the agonists were used: (a) the equipotent current ratio was defined as the ratio of ejecting currents needed to evoke responses of approximately equal magnitude (maximum change in firing rate not differing by more than 20%) to the two agonists compared; (b) the equicurrent magnitude ratio was defined as the ratio of the sizes of responses to two agonists (measured as the maximum change in firing rate), evoked by identical ejecting currents.

The effect of the antagonist phenoxybenzamine was evaluated by the procedures described previously (Bevan *et al.*, 1977; Bevan, Bradshaw, Pun, Slater & Szabadi, 1978a). The response to an agonist was regarded as antagonized if its size ('total spike number', see Bradshaw *et al.*, 1973b) was reduced by at least 50%.

The interaction between agonists was examined by comparing the conformity of the interaction to two theoretical models of drug interaction: competitive dualism and functional synergism. The net response in both cases is expected to be smaller than that calculated on the basis of simple additive effects; the discrepancy between net response and additivity, however, is predicted to be greater in the case of competitive dualism than in the case of functional synergism (see Ariëns, Simonis & van Rossum, 1964a; Ariëns, Simonis & van Rossum, 1964b). On each cell, the maximum evokable response to each agonist (methoxamine, noradrenaline, acetylcholine) was first established. Then, an attempt was made to establish submaximal and approximately equivalent responses (in terms of the equilibrium change in firing rate) to noradrenaline and acetylcholine. After the standard responses to the individual agonists had been established, the net responses to the combined application of methoxamine and noradrenaline, and to the combined application of methoxamine and acetylcholine were evoked. In the case of each combined response, the percentage deviation from additivity was calculated.

Measurement of the release of noradrenaline from micropipettes (in vitro)

The absolute mobilities of methoxamine and noradrenaline were compared by the method of Bradshaw, Pun, Slater & Szabadi (1980b). Six-barrelled micropipettes were used in these experiments. Two types of experiments were conducted. In Experiment 1, three barrels of each micropipette were filled with 0.05 M [methylene-^{14}C]-noradrenaline bitartrate plus 0.05 M noradrenaline bitartrate; the remaining three barrels contained 0.05 M [methylene-^{14}C]-noradrenaline bitartrate plus 0.5 M noradrenaline hydrochloride. In Experiment 2, three barrels of each micropipette were filled with 0.05 M [methylene-^{14}C-noradrenaline bitartrate plus 0.5 M noradrenaline bitartrate; the remaining three barrels contained 0.05 M [methylene-^{14}C]-noradrenaline bitartrate plus 0.05 M methoxamine hydrochloride. D,L-[Methylene-^{14}C]-noradrenaline bitartrate was obtained from the Radiochemical Centre, Amersham; the specific activity of the 0.05 M solution was 1.0 mCi/mmol. The pH of the combined solutions were: 3.30 (0.05 M noradrenaline bitartrate plus 0.5 M noradrenaline bitartrate); 3.24 (0.05 M noradrenaline bitartrate plus 0.05 M noradrenaline hydrochloride); and 3.30 (0.05 M noradrenaline bitartrate plus 0.05 M methoxamine hydrochloride).

Our methods for the collection of samples have been described in detail elsewhere (Bradshaw *et al.*, 1973a). Sample collection periods of 10 min were used. Initially the rate of spontaneous release of radioactive noradrenaline was measured in the absence of any electrophoretic current (2 samples). Then the rate of release of radioactive noradrenaline was measured in the presence of ejecting currents of

+25 nA, +50 nA, +75 nA and +100 nA (2 samples each) passed through each of the three barrels containing one of the drug combinations. After a further 2 samples of spontaneously released radioactivity had been collected, the process was repeated for the three barrels containing the other drug combination. Finally, a further two samples of spontaneously released radioactivity were collected. The mean distintegrations per minute (d/min) obtained from the 6 samples of spontaneously released noradrenaline were subtracted from the d/min obtained from each sample collected in the presence of an ejecting current; the remaining d/min were used for calculation of the rate of electrophoretic release. The apparent transport number of noradrenaline, in the presence of each kind of 'foreign' ion, was calculated from the following formula:

$$n = R_e zF/3i,$$

where R_e is the rate of electrophoretic release (mol/s), z is the valency (z = 1 for noradrenaline), F is Faraday's constant, and i is the intensity of the ejecting current (A) passed through each of the three barrels containing the drug combination in question.

Results

Comparison of responses to the agonists

Comparison of direction of responses Out of 199 cells responding to methoxamine, each was excited by the drug. Of the 109 cells which responded to both methoxamine and noradrenaline, methoxamine excited all the cells, whereas noradrenaline excited 95 cells and depressed 14 cells. Of the 90 cells which responded to both methoxamine and phenylephrine, all were excited by both drugs.

Comparison of apparent potencies of methoxamine and noradrenaline The equipotent current ratio (methoxamine/noradrenaline) was calculated for 25 cells yielding equivalent excitatory responses to the two agonists. On each cell methoxamine appeared to be less potent than noradrenaline. The mean equipotent current ratio (2.59 ± 0.24) was significantly greater than 1.0 (t test, $P < 0.001$), indicating the lower apparent potency of methoxamine.

The equicurrent magnitude ratio (methoxamine/noradrenaline) was calculated for 11 cells to which the two agonists were applied with identical ejecting currents. The mean equicurrent magnitude ratio (0.76 ± 0.05) was significantly less than 1.0 (t test, $P < 0.001$), indicating the lower apparent potency of methoxamine.

Comparison of apparent potencies of methoxamine and phenylephrine The mean equipotent current ratio (methoxamine/phenylephrine) (3.73 ± 0.90) was significantly greater than 1.0 (t test, $P < 0.001$), and the mean equicurrent magnitude ratio (methoxamine/phenylephrine) (0.52 ± 0.11) was significantly smaller than 1.0 (t test, $P < 0.02$), indicating the lower apparent potency of methoxamine than of phenylephrine. Current-response curves for the two agonists obtained on one cell are shown in Figure 1a: methoxamine was less potent than phenylephrine, the current-response curve for methoxamine having a lower maximum than that for phenylephrine.

Comparison of the time course of responses to methoxamine and noradrenaline Two time course parameters were measured: latency to onset and recovery time (see Bradshaw *et al.*, 1973b). The ratios (methoxamine/noradrenaline) of these time course parameters were calculated on 25 cells giving equivalent responses (in terms of the maximum changes in firing rate; see Bevan *et al.*, 1978b) to the two amines. Both the latency ratio and the recovery time ratio were significantly greater than 1.0 (see Table 1).

Comparison of the time courses of responses to methoxamine and phenylephrine Both the latency ratio and the recovery time ratio (methoxamine/phenylephrine) were significantly greater than 1.0 (see Table 1).

Time course parameters of responses to the agonists: between-cell comparison The mean values of the time course parameters for all the cells studied are shown in Table 2. Responses to methoxamine had significantly longer latencies and recovery times than responses to either noradrenaline or phenylephrine. The response to noradrenaline had a significantly shorter recovery time than the response to phenylephrine (t test, $P < 0.01$); there was no significant difference between the latencies of responses to noradrenaline and phenylephrine (t test, $P > 0.1$).

Summation of responses to the agonists

In order to test the hypothesis that methoxamine acts as a partial agonist at α-adrenoceptors on cortical neurones, net responses obtained by the summation of responses to methoxamine and noradrenaline were compared with those obtained by the summation of responses to methoxamine and acetylcholine (see Methods). Such comparisons were made on 12 cells. An example is shown in Figure 1b. It is apparent that, while the net response to methoxamine and acetylcholine was approximately equal to the sum of the individual responses, the net response to methoxamine and noradrenaline was smaller than the response to noradrenaline alone. On each of the 12

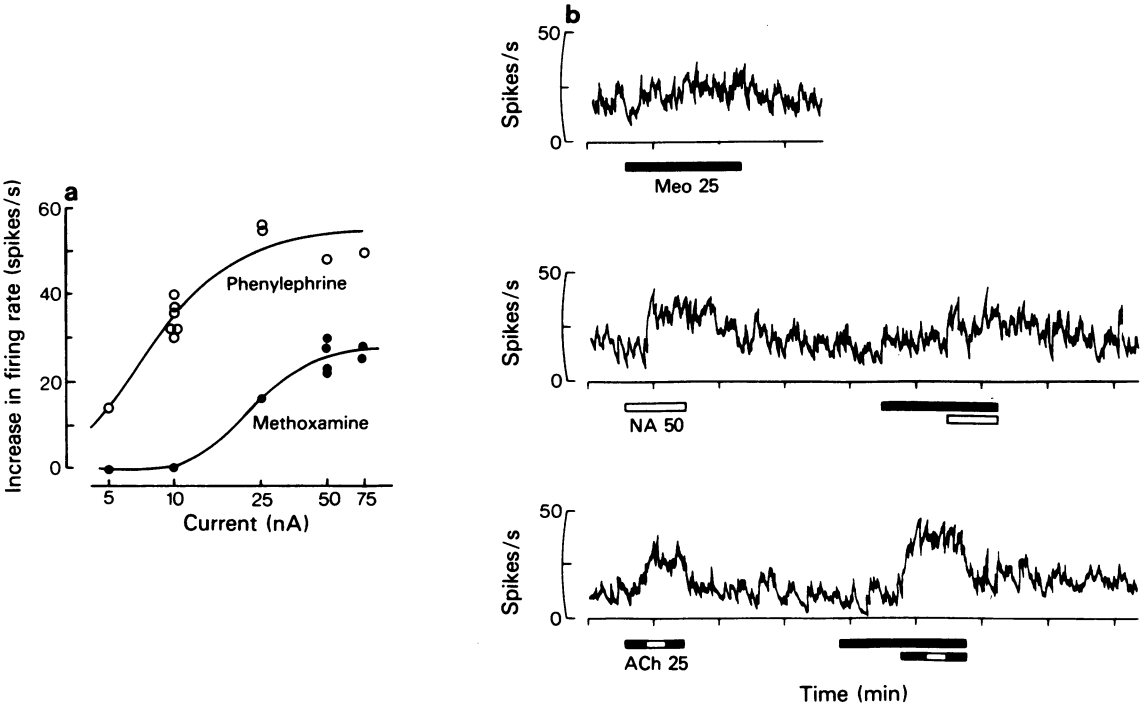

Figure 1 Evidence for the partial agonistic action of methoxamine at α-adrenoceptors on cortical neurones. (a) Comparison of the responses of a single cortical neurone to phenylephrine and methoxamine: current-response curves. Each response was measured as the maximum equilibrium increase in firing rate in the presence of a given ejecting current. Ordinate scale: increase in firing rate (spikes/s); abscissa scale: intensity of ejecting current (nA) on a log scale. Note that the current-response curve for methoxamine had a lower maximum than that for phenylephrine. (b) Summation of responses to methoxamine (Meo) and acetylcholine, and of responses to methoxamine and noradrenaline (NA). Excerpts from the rate meter recording of the firing rate of a single cortical neurone; ordinate scale: firing rate (spikes/s), abscissa scale: running time (min). Horizontal bars below the traces indicate microelectrophoretic drug applications; numbers refer to intensities of ejecting current. Upper trace: excitatory response to methoxamine (25 nA). Middle trace: excitatory response to acetylcholine (25 nA) and the net excitatory response evoked by the simultaneous application of methoxamine and acetylcholine. Lower trace: excitatory response to noradrenaline (50 nA) and the net response evoked by the simultaneous application of methoxamine and noradrenaline. Note that while the summation of responses to methoxamine and acetylcholine resulted in a net response which was larger that the response to acetycholine alone, the summation of responses to methoxamine and noradrenaline resulted in a net response which was smaller than the response to noradrenaline alone.

Table 1 Comparison of the time course parameters of responses to methoxamine with those of responses to noradrenaline and phenylephrine

	Latency ratio†	*Recovery time ratio*†
Methoxamine/noradrenaline	3.09 ± 0.44** (n = 25)	2.01 ± 0.17** (n = 25)
Methoxamine/phenylephrine	4.29 ± 1.03* (n = 20)	1.49 ± 0.08** (n = 20)

† Ratios were calculated separately for each individual cell. Values are means ± s.e. mean for all cells. Significance levels (ratios significantly greater than 1.0; t test): *$P < 0.01$; **$P < 0.001$.

Table 2 Time course parameters of response to methoxamine, noradrenaline and phenylephrine

	Latency†	*Recovery time*†
Methoxamine	31.22 ± 2.84 (n = 45)	143.76 ± 5.95 (n = 45)
Noradrenaline	15.04 ± 2.86** (n = 25)	77.52 ± 5.74** (n = 25)
Phenylephrine	10.25 ± 1.18** (n = 20)	103.05 ± 8.32* (n = 20)

† Time course parameters are in seconds. Values are means ± s.e. mean for all cells. Significance levels (values significantly smaller than those for methoxamine: t test): *$P < 0.01$; **$P < 0.001$.

cells, the discrepancy between the net response observed and that expected on the basis of simple additivity was calculated, taking the sum of the individual responses as 100%. The mean discrepancy (± s.e. mean) between actual net response and that expected on the basis of additivity was: −38.35% ± 4.30% (methoxamine plus noradrenaline), and −24.55% ± 4.03% (methoxamine plus acetylcholine). The difference between these two values is statistically significant (t test; $P < 0.01$), indicating that the interaction between methoxamine and noradrenaline resulted in a greater reduction in the size of the net response than did the interaction between methoxamine and acetylcholine.

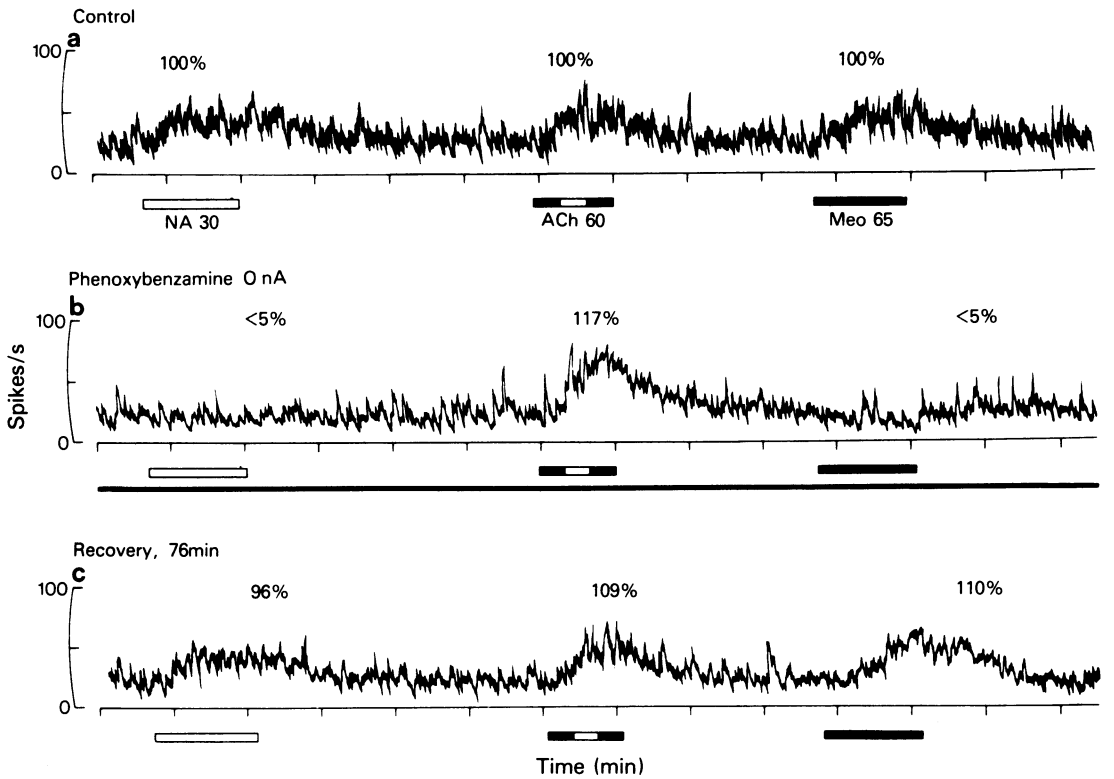

Figure 2 Effects of phenoxybenzamine on excitatory responses to noradrenaline (NA), acetylcholine (ACh) and methoxamine (Meo). Excerpts from the ratemeter recording of the firing rate of a single cortical neurone (as in Figure 1). Figures above the traces indicate total spike numbers (%), taking the size of the control response to each agonist as 100%. (a) Control responses to the agonists. (b) Responses to the agonists during the continuous application of phenoxybenzamine by diffusion (removal of the retaining current; O nA). At the start of trace (b), phenoxybenzamine had been applied continuously for 7 min. The responses to noradrenaline and methoxamine were antagonized, while the response to acetylcholine was not affected. (c) Recovery of the responses to noradrenaline and methoxamine 76 min after the application of phenoxybenzamine had been terminated.

Effect of phenoxybenzamine on responses to the agonists

Comparison of the effect of phenoxybenzamine on responses to methoxamine and noradrenaline The effect of the continuous application of phenoxybenamine was studied on 14 cells which were excited by both methoxamine and noradrenaline; all these cells were also excited by acetylcholine. On all 14, phenoxybenzamine equally and reversibly antagonised the responses to methoxamine and noradrenaline, while the responses to acetylcholine were not affected. An example is shown in Figure 2.

The effect of phenoxybenzamine was also studied on 2 cells which were excited by methoxamine and depressed by noradrenaline. On both cells, the excitatory response to methoxamine was reversibly antagonized, whereas the depressant response to noradrenaline and the excitatory response to acetylcholine were not affected.

Comparison of the effect of phenoxybenzamine on responses to methoxamine and phenylephrine The effect of the continuous application of phenoxybenzamine was studied on 26 cells excited by both methoxamine and phenylephrine. On all these cells, phenoxybenzamine antagonized equally the responses to both methoxamine and phenylephrine, while responses to acetylcholine were not affected. On 22 cells the antagonism was reversible; on 4 cells, however, no recovery of the response to methoxamine could be observed even 2 to 3 h after the application of phenoxybenzamine had been terminated. An example is shown in Figure 3.

Comparison of the absolute mobilities of methoxamine and noradrenaline

Comparison of the effects of noradrenaline bitartrate and noradrenaline hydrochloride on the release of labelled noradrenaline The effects of noradrenaline

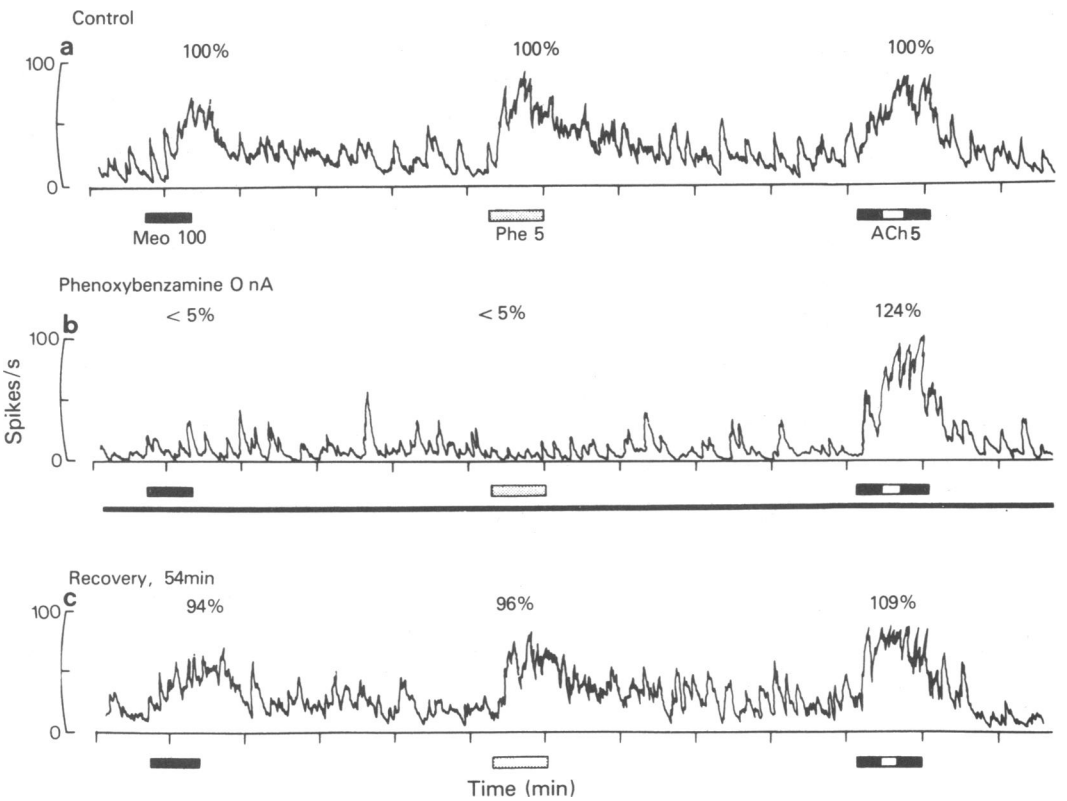

Figure 3 Effects of phenoxybenzamine on excitatory responses to methoxamine (Meo), phenylephrine (Phe) and acetylcholine (ACh). Excerpts from the ratemeter recording of the firing rate of a single cortical neurone (as in Figures 1 and 3). (a) Control responses to the agonists. (b) Responses to the agonists during the continuous application of phenoxybenzamine by diffusion (removal of the retaining current; O nA). At the start of trace (b), phenoxybenzamine had been applied continuously for 13 min. The responses to methoxamine and phenylephrine were antagonized, while the response to acetylcholine was not affected. (c) Recovery of the responses to methoxamine and phenylephrine 54 min after the application of phenoxybenzamine had been terminated.

bitartrate and noradrenaline hydrochloride on the release of labelled noradrenaline were compared using 12 micropipettes (Experiment 1, see Methods). The relationship between the intensity of the ejecting current and the rate of release of [^{14}C]-noradrenaline is shown in Figure 4a. The lines were fitted to the data by the method of least squares ($r = 0.999$, $P < 0.001$, for both lines). The linear regression equations are: $y = 0.724x + 0.026$ ([^{14}C]-noradrenaline bitartrate plus noradrenaline bitartrate), and $y = 0.726x + 0.109$ ([^{14}C]-noradrenaline bitartrate plus noradrenaline hydrochloride). In neither case does the intercept deviate significantly from zero (t test, $P > 0.05$, in both cases). The mean apparent transport number (± s.e. mean) of [^{14}C]-noradrenaline was 0.116 ± 0.005 in the presence of 0.05 M 'cold' noradrenaline bitartrate, and 0.120 ± 0.005 in the presence of 0.05 M 'cold' noradrenaline hydrochloride. The two values are not significantly different from one another (t test, $P > 0.1$), indicating that equimolar concentrations of noradrenaline bitartrate and noradrenaline hydrochloride caused similar reductions in the apparent transport number of [^{14}C]-noradrenaline. The observation would suggest that bitartrate and hydrochloride have similar absolute mobilities (see Bradshaw *et al.*, 1980b).

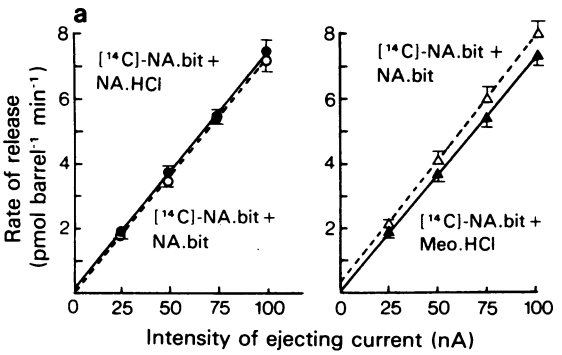

Figure 4 Relationship between intensity of ejecting current and rate of release of [^{14}C]-noradrenaline from micropipettes. (a) Comparison of the rate of release of [^{14}C]-noradrenaline from a solution of 0.05 M [^{14}C]-noradrenaline bitartrate and 0.05 M noradrenaline bitartrate (○), and from a solution of 0.05 M [^{14}C]-noradrenaline bitartrate and 0.05 M noradrenaline hydrochloride (●). Points are means ($n = 12$), vertical bars correspond to s.e. mean. (b) Comparison of the rate of release of [^{14}C]-noradrenaline from a solution of 0.05 M [^{14}C]-noradrenaline bitartrate and 0.05 M noradrenaline bitartrate (△), and from a solution of 0.05 M [^{14}C]- noradrenaline bitartrate and 0.05 M methoxamine hydrochloride (▲). Points are means ($n = 12$), vertical bars correspond to s.e. mean. In both experiments (a) and (b), the rate of release was linearly related to the intensity of the ejecting current (see text for details).

Comparison of the effects of methoxamine hydrochloride and noradrenaline bitartrate on the release of labelled noradrenaline The effects of methoxamine hydrochloride and noradrenaline bitartrate on the release of [^{14}C]-noradrenaline were compared using 12 micropipettes (Experiment 2, see Methods). The relationship between the intensity of the ejecting current and the rate of release of [^{14}C]-noradrenaline is shown in Figure 4b. The lines were fitted to the data by the method of least squares ($r = 0.999$, $P < 0.001$, for both lines). The linear regression equations are: $y = 0.073x + 0.004$ ([^{14}C]-noradrenaline bitartrate plus methoxamine hydrochloride), and $y = 0.078x + 0.187$ ([^{14}C]-noradrenaline bitartrate plus noradrenaline bitartrate). The mean apparent transport number (± s.e. mean) of [^{14}C]-noradrenaline was 0.117 ± 0.05 in the presence of 0.05 M methoxamine hydrochloride, and 0.132 ± 0.009 in the presence of 0.05 M 'cold' noradrenaline bitartrate. The two values are not significantly different from one another (t test, $P > 0.05$), indicating that equimolar concentrations of methoxamine hydrochloride and noradrenaline bitartrate caused similar reductions in the apparent transport number of [^{14}C]-noradrenaline. Since there was no significant difference between the mobilities of the two anions (see above), this observation would suggest that methoxamine and noradrenaline have similar absolute mobilities (see Bradshaw *et al.*, 1980b).

Discussion

In the present experiment, the α-adrenoceptor stimulating agent methoxamine (see Trendelenburg, 1972) similarly to phenylephrine, had an exclusively excitatory effect on cortical neurones, confirming the hypothesis that the excitatory responses to the sympathomimetic amines are mediated by α-adrenoceptors (see Bevan *et al.*, 1977). This hypothesis is further strengthened by the action of the α-adrenoceptor blocking agent, phenoxybenzamine (see Nickerson, 1967), which antagonized equally the excitatory responses to methoxamine, noradrenaline and phenylephrine, without affecting responses to acetylcholine.

Methoxamine appeared to be less potent than either noradrenaline or phenylephrine in evoking excitatory responses. The lower potency of methoxamine is likely to reflect a genuine biological property of the drug since methoxamine did not have a lower absolute mobility than either noradrenaline (see present Results) or phenylephrine (see Bradshaw *et al.*, 1980b).

The response to methoxamine had a longer latency and a longer recovery time than responses to noradrenaline and phenylephrine. The longer latency of the response to methoxamine is likely to reflect the

lower potency of methoxamine (see Szabadi & Bradshaw, 1974), whereas the longer recovery time may reflect the fact that methoxamine has virtually no affinity for the amine-uptake mechanism (see Trendelenburg, Maxwell & Pluchino, 1970; Trendelenburg, 1972), and may thus be removed more slowly from its site of action.

The current-response curve for methoxamine had a lower maximum than that for phenylephrine (see Figure 1) suggesting that methoxamine may act as a partial agonist at α-adrenoceptors. This suggestion is further confirmed by the summation of responses to methoxamine and noradrenaline. When the summation of responses to methoxamine and noradrenaline was compared with the summation of responses to methoxamine and acetylcholine on the same cells, the combined response obtained by the summation of responses to methoxamine and noradrenaline deviated from additivity significantly more than the combined response obtained by the summation of responses to methoxamine and acetylcholine. This observation would indicate that the summation of responses to methoxamine and noradrenaline conforms to the model of competitive dualism (see Ariëns *et al.*, 1964b). Thus the α-adrenoceptors on cortical neurones in the rat appear to be similar to those in the cardiac muscle of the rabbit where methoxamine also acts as a partial agonist (Schümann & Endoh, 1976).

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