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The postsynaptic effects of antidepressant drugs in the rat anococcygeus muscle

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The effects of antidepressants of tricyclic (amitriptyline, nortriptyline, protriptyline, doxepin, imipramine, and desipramine) and atypical (maprotiline, nomifensine, tandamine, viloxazine, CGP 6085A, and YM-08054-1) structures on contractile responses to exogenously applied acetylcholine and (−)-noradrenaline were studied in rat isolated anococcygeus muscle previously incubated with 6-hydroxydopamine. Atropine, amitriptyline, protriptyline, doxepin, imipramine, maprotiline and nortriptyline inhibited contractile responses to acetylcholine whereas desipramine, nomifensine, tandamine, viloxazine, CGP 6085A and YM 08054–1 did not. The contractile responses to (−)-noradrenaline were inhibited by low concentrations of tricyclic antidepressants and by higher concentrations of the atypical agents. These results illustrate that, in the preparation, the order of potency of antidepressants as muscarinic and as postsynaptic α-adrenoceptor antagonists is similar. The ability of tricyclic, but not atypical agents, to increase the concentration of noradrenaline bound to postsynaptic α-adrenoceptors may be severely limited by the antagonistic effect these agents have at this receptor.

The therapeutic effects of tricyclic antidepressants in depression is commonly attributed to long term adaptive changes resulting from their ability to inhibit the neuronal uptake of (−)-noradrenaline and/or 5-hydroxytryptamine (Sulser et al 1978) while the untoward and/or toxic effects are generally attributed to antagonistic effects, particularly at muscarinic receptors. As any α-adrenoceptor blocking activity possessed by antidepressants will decrease the concentration of noradrenaline bound to the α-adrenoceptor, the effects of a series of drugs of tricyclic or atypical structure as postsynaptic α-adrenoceptor antagonists have been assessed on contractile responses to exogenously applied (−)-noradrenaline in the rat isolated anococcygeus muscle. The antimuscarinic activity of the agents on responses to acetylcholine was also determined as it seems likely that agents devoid of this property (and of the unwanted effects associated with it), but with an antidepressant effect, will be the preferred clinical agents. The effects were compared with those of atropine and phentolamine. A preliminary account of some of these findings has been presented to the Australian Physiological and Pharmacological Society (Doggrell 1980a).

METHODS

Mature male Wistar rats were killed by a blow at the base of the skull and exsanguinated. Anococcygeus muscles were dissected as described by Gillespie (1972). All experiments were in the presence of a modified Krebs solution [Composition (mM): NaCl, 116, KCl, 5·4; $CaCl_2$, 2·5; $MgCl_2$, 1·2; NaH_2PO_4, 1·2; $NaHCO_3$, 22·0; D-glucose, 11·2; Na_2EDTA, 0·04], equilibrated with 5% CO_2 in oxygen, at 37 °C. Paired individual muscles were mounted separately under 0·5 g tension in 5 ml organ baths containing Krebs solution. The methods were those of Doggrell (1980b).

Muscles were incubated in 6-hydroxydopamine, 10^{-3} M for 3 h (see Doggrell & Woodruff 1978), so that any effects of drugs on responses to exogenously applied noradrenaline must occur predominantly postsynaptically. The tissues were then washed by overflow for 30 min in Krebs solution. One concentration of the drug under investigation was present in the Krebs solution of a single anococcygeus muscle from the beginning of the wash period. The other tissue of the pair remained in Krebs solution throughout. Concentration-response curves for agonists were determined non-cumulatively. On each tissue, concentration-response curves to increasing concentrations of both acetylcholine and (−)-noradrenaline were obtained; their initial concentrations were 10^{-6} and 10^{-8} M, respectively. Exposure to agonist was continued for 30 s or until a maximum response was obtained. The tissues were then allowed to recover, with a minimum period of 5 min, before further addition of agonist occurred. 30 min was allowed to elapse between each response curve. Contractile responses were recorded

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isometrically with force displacement transducers (Grass model FT03.C) connected to a polygraph (Grass model 79B).

The values obtained were compared using Student's paired *t*-test and were considered to be significantly different when $P < 0.05$. When the maximum responses (g), in the presence and absence of drugs, were not significantly different, responses were calculated as a percentage of the maximum of the individual response curve (i.e. normalized). The slope (difference in percentage maximum of the response/unit of log M concentration of agonist) of each concentration-response curve was determined by regression line analysis (over the range 20–80% of the maximum response). When the maximum responses were not significantly different, a pD_2 value was also determined from the regression line analysis. For each pair of tissues, the ability of a drug to inhibit responses is expressed as the dose-ratio (the antilog of the difference between the pD_2 values in the presence and absence of drugs). On the assumption of competitive antagonism, pA_2 values were calculated for each pair of tissues. In addition, mean slopes, mean pD_2 values, and mean pA_2 values were determined. When the maximum responses (g), in the presence and absence of drugs, were significantly different, responses were calculated as a percentage of the maximum response of the control concentration-response curve.

Drugs used were: tandamine hydrochloride* (Ayerst Research Laboratories), CGP 6085A [4-(5,6-dimethyl-2-benzofuranyl)piperidine hydrochloride]*, despiramine hydrochloride*, imipramine hydrochloride*, maprotiline hydrochloride*, and phentolamine mesylate* (Ciba-Geigy Ltd), nortriptyline hydrochloride* (Eli Lilly and Co. Ltd.), nomifensine maleate* (Hoechst N.Z. Ltd), viloxazine hydrochloride* (ICI N.Z. Ltd.), amitriptyline hydrochloride* and protriptyline hydrochloride* (Merck Sharpe & Dohme N.Z. Ltd.), doxepin hydrochloride* (Pfizer Laboratories Ltd.), acetylcholine chloride, atropine sulphate and (−)-noradrenaline bitartrate (Sigma Chemicals Ltd.), and YM-08054-1 [2-(7-indenyloxymethyl)morpholine hydrochloride]* (Yamanouchi Pharmaceuticals Co. Ltd.). Compounds indicated with an asterisk were generously donated by the companies shown.

RESULTS

Effect of atropine and phentolamine on responses to acetylcholine and (−)-noradrenaline

Atropine (10^{-9}–10^{-6} M) had no effect on the magnitude of maximal responses to acetylcholine. The slopes of the concentration-response curves to acetylcholine were increased and the submaximal responses, at the level of the 50% response, were inhibited in a manner not consistent with competitive antagonism (Table 1). Thus the apparent pA_2 values for atropine ranged from 7·46 to 9·30 (Table 1). The effect of atropine, 10^{-6} M, on responses to acetylcholine is shown in Fig. 1.

Table 1. The effect of atropine on the contractile responses to acetylcholine in the rat anococcygeus muscle.

	Slope	Dose-ratio (inhibition) (Mean ± s.e.m.)	pA_2
Control	34·11 ± 1·69 (6)		
10^{-9} M Atropine	58·28 ± 1·69 (6)*	4·0 ± 1·0 (6)	9·30 ± 0·20 (6)
Control	35·59 ± 4·29 (4)		
10^{-8} M Atropine	80·24 ± 9·55 (4)*	5·0 ± 0·8 (4)	8·58 ± 0·08 (4)
Control	45·14 ± 6·13 (4)		
10^{-7} M Atropine	87·45 ± 4·91 (4)*	16·3 ± 6·2 (4)	8·08 ± 0·18 (4)
Control	39·95 ± 7·79 (4)		
10^{-6} M Atropine	101·88 ± 18·07 (4)*	49·0 ± 29·8 (4)	7·46 ± 0·24 (4)

* $P < 0.01$, paired *t*-test.
(n) = number of observations.

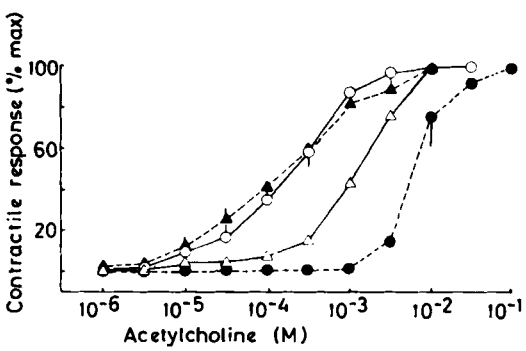

FIG. 1. Effect of atropine and amitriptyline on responses to acetylcholine. Responses to acetylcholine in the absence (○—○) and presence (●—●) of 10^{-6} M atropine, and in the absence (▲—▲) and presence (△—△) of 10^{-6} M amitriptyline. All responses are expressed as a percentage of the maximum response. Each value is the mean ± s.e.m. from 4 preparations.

Phentolamine, 10^{-7} and 10^{-6} M, had no effect on the magnitude of maximal responses or slopes of concentration-response curves to (−)-noradrenaline. Submaximal response to (−)-noradrenaline were inhibited ×19·0 ± 1·9(4) [mean dose-ratio ± s.e.m., n = 4] and ×148·4 ± 44·4(4) by 10^{-7} and 10^{-6} M phentolamine, respectively. The pA_2 values for phentolamine were independent of concentration and were approximately 8·15. The effect of phentol-

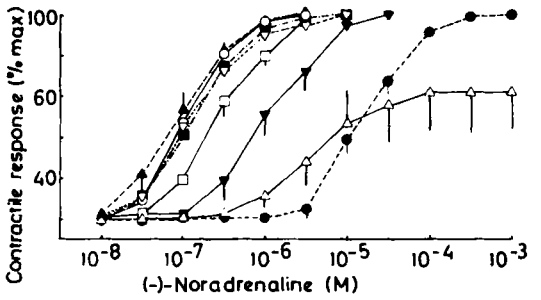

FIG. 2. Effect of phentolamine and amitriptyline on responses to (—)-noradrenaline. Responses to (—)-noradrenaline in the absence (○—○) and presence (●—●) of 10^{-6} M phentolamine, absence (▲—▲) and presence (△—△) of 10^{-6} M amitriptyline, absence (▽—▽) and presence (▼—▼) of 10^{-7} M amitriptyline, and absence (■—■) and presence (□—□) of 10^{-6} M amitriptyline. All responses are expressed as a percentage of the maximum response (see Methods section for further details). Each value is the mean ± s.e.m. from 4 preparations.

amine, 10^{-6} M, on responses to (−)-noradrenaline is shown in Fig. 2.

Atropine (10^{-9}–10^{-6} M) had no effect on responses to (−)-noradrenaline and the responses to acetylcholine were unaltered by phentolamine (10^{-7} and 10^{-6} M).

Effect of antidepressants on responses to acetylcholine and (−)-noradrenaline

None of the antidepressants had an effect on the resting tone of the muscles.

(a) *Drugs of tricyclic structure*

Amitriptyline (10^{-8}, 10^{-7}, 10^{-6} M), nortriptyline, desipramine (10^{-7}, 10^{-6} M) and imipramine, protriptyline, and doxepin (5×10^{-8}, 10^{-7}, 10^{-6} M) had no effect on the magnitude of maximal responses to acetylcholine. The slopes of the concentration-response curves to acetylcholine were increased and the submaximal responses inhibited by high concentrations of drug (with the exception of desipramine) (Table 2). The effect of amitriptyline, 10^{-6} M, on responses to acetylcholine is shown in Fig. 1.

With the exception of 10^{-6} M amitriptyline, the drugs had no effect on the magnitude of maximal responses or slopes of concentration-response curves to (−)-noradrenaline. Amitriptyline, 10^{-6} M, reduced the magnitude of maximal responses to (−)-noradrenaline: control 5·75 ± 0·79 (4) (mean g ± s.e.m., n = 4) and in the presence of amitriptyline, 10^{-6} M, 3·60 ± 1·26(4). Submaximal responses to (−)-noradrenaline were reduced by the drugs at all concentrations (Table 2). Amitriptyline was the most potent inhibitor of responses to (−)-noradrenaline. Its effects in comparison with phentolamine are illustrated in Fig. 2. The tertiary compounds, amitriptyline and imipramine, were more potent than nortriptyline and desipramine, their respective secondary derivatives (Table 2).

Table 2. The effect of tricyclic antidepressants on the contractile responses to acetylcholine and to (—)-noradrenaline in the rat anococcygeus muscle.

	Dose-ratio (Inhibition) Mean ± s.e.m. Against Acetylcholine	Against (−)-Noradrenaline	pA_x against (−)-noradrenaline Mean ± s.e.m.
Amitriptyline, 10^{-8} M	NI	2·2 ± 0·2 (4)	8·06 ± 0·05 (4)
Amitriptyline, 10^{-7} M	3·8 ± 1·2 (5)	10·2 ± 3·2 (4)	7·89 ± 0·12 (4)
Amitriptyline, 10^{-6} M	9·1 ± 2·1 (4)[1]	—	—
Nortriptyline, 10^{-7} M	NI	3·3 ± 0·6 (9)	7·19 ± 0·15 (9)
Nortriptyline, 10^{-6} M	9·2 ± 5·6 (6)[1]	41·2 ± 22·0 (6)	7·28 ± 0·25 (9)
Protriptyline, 5×10^{-8} M	NI	2·0 ± 1·0 (8)	7·27 ± 0·31 (8)
Protriptyline, 10^{-7} M	3·5 ± 0·7 (5)[1]	3·1 ± 1·4 (5)	6·99 ± 0·25 (5)
Protriptyline, 10^{-6} M	11·2 ± 7·0 (4)[1]	5·8 ± 2·8 (4)	6·29 ± 0·38 (4)
Doxepin, 5×10^{-8} M	NI	3·0 ± 0·6 (4)	7·72 ± 0·17 (4)
Doxepin, 10^{-7} M	4·5 ± 1·5 (6)	7·1 ± 1·5(6)	7·71 ± 0·12 (6)
Doxepin, 10^{-6} M	8·0 ± 1·0 (4)	25·1 ± 5·9 (4)	7·35 ± 0·09 (4)
Imipramine, 5×10^{-8} M	NI	3·0 ± 0·5 (8)	7·53 ± 0·10 (8)
Imipramine, 10^{-7} M	2·6 ± 0·7 (10)	10·8 ± 4·4 (10)	7·59 ± 0·21 (10)
Imipramine, 10^{-6} M	3·9 ± 0·6 (10)[1]	28·5 ± 5·1 (10)	7·36 ± 0·10 (10)
Desipramine, 10^{-7} M	NI	2·4 + 0·4 (6)	7·08 ± 013 (6)
Desipramine, 10^{-6} M	NI	12·1 ± 4·3 (6)	6·93 ± 0·13 (6)

NI = No Inhibition.
1. Inhibition included an increase in the slope of the concentration-response curve.
(n) = number of observations.

(b) *Drugs of atypical structure*

Maptrotiline (2×10^{-7}, 10^{-6} M), nomifensine, tandamine, viloxazine, CGP 6085A and YM 08054–1 (10^{-6}, 10^{-5} M) had no effect on the magnitude of maximal responses or slopes of concentration-response curves to acetylcholine or (−)-noradrenaline. Submaximal responses to acetylcholine were unaltered save by maprotiline, 2×10^{-7} and 10^{-6} M, which inhibited responses to acetylcholine ×4·1 and ×3·8, respectively. Viloxazine, 10^{-6} M, and YM 08054–1, 10^{-6} M, had no effect on submaximal responses to (−)-noradrenaline. A higher concentration of these and the other agents inhibited responses to (−)-noradrenaline (Table 3).

(c) *Postsynaptic α-adrenoceptor blocking activity*

As the antidepressants were generally more potent as postsynaptic α-adrenoceptor antagonists than in inhibiting responses to acetylcholine, we were able to assess the postsynaptic α-blocking activity (i.e. pA_2 values calculated) under two conditions: firstly, when the concentration of antidepressant had no effect on responses to acetylcholine (i.e. selective inhibition of responses to (−)-noradrenaline) and, secondly, when the concentration of drug altered responses to acetylcholine in addition to inhibiting sub-maximal responses to (−)-noradrenaline (i.e. non-selective inhibition of responses to (−)-noradrenaline). When pA_2 values under these two conditions were not significantly different (Student's unpaired *t*-test), antagonism of responses to (−)-noradrenaline was considered to be independent of effects on responses to acetylcholine. All of the inhibitory effects observed with the drugs (with the exception of maprotiline) were shown to be independent of effects on responses to acetylcholine. Maprotiline was an equipotent inhibitor of sub-maximal responses to acetylcholine and (−)-noradrenaline. The pA_2 values for antidepressants against (−)-noradrenaline (Tables 2 and 3) were all independent of concentration. From mean pA_2 values the order of potency as postsynaptic α-adrenoceptor antagonists is phentolamine ⩾amitriptyline ⩾doxepin ⩾imipramine ⩾nortriptyline ⩾ protriptyline ⩾desipramine ⩾maprotiline >nomifensine >CGP 6085A ⩾tandamine >vilaxazine > YM 08054–1.

Table 3. The effect of atypicals on the contractile responses to (—)-noradrenaline in the rat anococcygeus muscle.

	Dose-ratio (Inhibition) Mean ± s.e.m.	pA_2 Mean ± s.e.m.
Maprotiline, 2×10^{-7} M	3·8 ± 0·9 (4)	7·03 ± 0·22 (4)
Maprotiline, 10^{-6} M	9·4 ± 1·6 (6)	6·87 ± 0·10 (6)
Nomifensine, 10^{-6} M	3·8 ± 0·8 (6)	6·34 ± 0·15 (6)
Nomifensine, 10^{-5} M	23·4 ± 3·2 (4)	6·34 ± 0·07 (4)
Tandamine, 10^{-6} M	1·5 ± 0·5 (4)	5·50 ± 0·50 (4)
Tandamine, 10^{-5} M	4·6 ± 1·7 (4)	5·35 ± 0·24 (4)
Viloxazine, 10^{-5} M	4·4 ± 1·7 (4)	5·39 ± 0·22 (4)
CGP 6085A, 10^{-6} M	1·5 ± 0·6 (5)	5·83 ± 0·26 (5)
CGP 6085A, 10^{-5} M	6·0 ± 0·4 (4)	5·69 ± 0·31 (4)
YM 08054–1, 10^{-5} M	4·9 ± 0·4 (6)	4·86 ± 0·40 (6)

(n) = number of observations.

DISCUSSION

The rat anococcygeus muscle has no acetylcholinesterase-positive nerve fibres (Gillespie 1972) and is thus assumed to have no cholinergic innervation. The tissue does contract in the presence of acetylcholine and the responses are abolished by atropine (Gillespie 1972), demonstrating the presence of muscarinic receptors. The present study shows that the inhibitory effect of atropine on responses to acetylcholine was not consistent with competitive antagonism. This effect is not due to the preincubation of the muscles with 6-hydroxydopamine because (i) this has no effect on responses to acetylcholine (Doggrell & Woodruff 1978) and (ii) a similar interaction occurs in tissues that have not been so preincubated (unpublished observation). Consequently, the interaction can be considered a genuine antimuscarinic effect.

Desipramine had the least antimuscarinic activity of the tricyclics tested, having no effect on responses to acetylcholine at 10^{-6} M (Table 2). These results are in partial agreement with those of Shein & Smith (1978) who used guinea-pig ileum. Those authors, using carbachol, also demonstrated that the tertiary amines, amitryptyline and imipramine, had more antimuscarinic activity than their secondary derivatives nortriptyline and desipramine, respectively. However, they found nortriptyline to be a more potent antimuscarinic agent than imipramine or doxepin while we found this not to be so. The difference may reflect the use of different agonists and tissues. Of the newer agents only maprotiline, which is structurally similar to the tricyclics, had appreciable antimuscarinic activity. This suggests that they may have advantages over the tricyclics as many of the side effects associated with tricyclic therapy are antimuscarinic.

There is an inverse relationship between the potency of tricyclic antidepressants as postsynaptic α-adrenoceptor antagonists and as inhibitors of the neuronal uptake of noradrenaline in the rat anococcygeus muscle (see Doggrell & Woodruff 1977). If the short term pharmacological action that correlates with antidepressant activity is the ability to increase the concentration of noradrenaline at the level of the receptor, the ability to inhibit the neuronal uptake of noradrenaline will provide a good index of antidepressant activity for agents that have little or no, but not for those agents that have, postsynaptic α-adrenoceptor antagonistic activity. A

potentiation of contractile responses to noradrenaline, in the absence of other drugs, will illustrate an effective ability to increase the concentration of noradrenaline bound to the receptor. Iprindole is 25× less potent than amitriptyline in inhibiting noradrenaline accumulation in the rat anococcygeus muscle (Doggrell & Woodruff 1977). Amitriptyline is a very potent post-synaptic α-adrenoceptor antagonist whereas under the same conditions iprindole is not ($pA_2 = 5{\cdot}70$, Doggrell 1980a). Thus it is not surprising that amitriptyline and iprindole, at 10^{-6} M, have a similar small potentiating effect on responses to noradrenaline in the rat anococcygeus muscle (Doggrell & Woodruff 1977).

In the present study the postsynaptic α-adrenoceptor blocking and antimuscarinic activities of a series of antidepressants were monitored in the same tissue under the same conditions. The order of potency of antidepressants as muscarinic and as postsynaptic α-adrenoceptor antagonists was similar.

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