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Postjunctional effects of mianserin and its metabolites on the rat isolated anococcygeus muscle

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After rat isolated anococcygeus muscle had been treated with 6-hydroxydopamine to abolish noradrenergic transmission, the effects of mianserin and its metabolites alone on tone and on the contractile responses to methacholine and phenylephrine were studied. Mianserin ($\geq 3 \times 10^{-5}$ M) and 8-hydroxymianserin ($\geq 3 \times 10^{-6}$ M), but not desmethylmianserin and mianserin-*N*-oxide, induced contractions. The contractions to mianserin were not altered by phentolamine, atropine, cyproheptadine, ouabain and verapamil but were reduced in tissues treated with indomethacin. Thus the responses to mianserin may, in part, be mediated by the synthesis of prostaglandins. Mianserin (10^{-5} M) and 8-hydroxymianserin (10^{-7}–10^{-5} M) potentiated, mianserin-*N*-oxide had no effect, and desmethylmianserin (10^{-7}–10^{-5} M) inhibited methacholine-induced contractions. The inhibition of responses to phenylephrine was concentration-related with desmethylmianserin (10^{-7}–10^{-5} M) and mianserin-*N*-oxide (10^{-6}–10^{-5} M) but not with mianserin or 8-hydroxymianserin (10^{-7}–10^{-5} M). The effects of mianserin and its metabolites on responses to methacholine and phenylephrine were similar in the absence and presence of indomethacin. These results suggest that mianserin and 8-hydroxymianserin increase the tissue's sensitivity to methacholine by an action at the level, or distal to, the muscarine receptor. In addition to blocking α_1-adrenoceptors, mianserin and 8-hydroxymianserin may have another postjunctional action at, or beyond, the α_1-adrenoceptor.

Mianserin is an effective antidepressant in man (e.g. Freighner et al 1983). However there is no consistent relationship between the plasma concentrations of mianserin and its therapeutic response (van Riezen et al 1981) probably because the metabolites of mianserin (i.e. desmethylmianserin, 8-hydroxymianserin and mianserin-*N*-oxide; see Pinder & van Delft 1983) contribute to the overall therapeutic effect. Although the pharmacological effects of mianserin have been extensively studied (see reviews by Peet & Behagel 1978; Marshall 1983) those of the metabolites have not. I have examined the effects of mianserin and its metabolites on the tone of the rat isolated anococcygeus muscle and on the contractile responses to methacholine and phenylephrine, an α_1-adrenoceptor selective agonist. A preliminary account of these findings have been presented to the Australasian Society for Clinical and Experimental Pharmacologists (Doggrell 1984).

METHODS AND MATERIALS

Mature male Wistar rats were stunned and exsanguinated. Anococcygeus muscles were removed and dissected free of surrounding connective tissue. All experiments were performed in the presence of a modified Krebs solution of the following 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 and Na_2EDTA 0·04, equilibrated with 5% CO_2 in O_2 at 37 °C. Contractile responses were recorded isometrically with force displacement transducers (Grass model FTO3.C) and displayed on a polygraph (Grass model 79B). In each series of experiments, the individual values obtained were compared by Student's paired *t*-test and were considered to be significant when $P < 0{\cdot}05$. mean values + s.e. mean were also obtained.

Individual anococcygeus muscles were mounted under 0·5 g tension in 5 ml organ baths containing Krebs solution. 6-Hydroxydopamine (10^{-3} M) was added to the Krebs solution of each tissue for 60 min. Tissues were then washed by overflow in drug-free Krebs solution for 120 min. This procedure gives a selective abolition of noradrenergic transmission in the rat isolated anococcygeus muscle (discussed by Doggrell & Waldron 1982). After denervation, the resting tension on each tissue was reset at 0·5 g and the tissues were treated as follows.

When the effect of mianserin and its metabolites alone were studied on tone, tissues were further washed in drug-free Krebs solution for 90 min before being exposed to mianserin or one of its metabolites non-cumulatively. Tissues were allowed to recover for 10 min between each challenge. Because of its

poor solubility in distilled water, the maximal final concentration tested was 5×10^{-4} M for mianserin and 10^{-5} M for desmethylmianserin, mianserin-*N*-oxide and 8-hydroxymianserin. In some experiments, a sustained contraction to mianserin at 10^{-4} M was obtained and the effects of the addition of drugs on this contraction studied. Three tissues were used for each drug tested. In other experiments, a contraction to mianserin at 10^{-4} M was obtained and then tissues were allowed to recover during washing by overflow with Krebs solution. One tissue was then treated with indomethacin while the other muscle of the pair remained untreated. After 60 min each tissue was exposed to mianserin again.

When the effects of indomethacin, mianserin and its metabolites were examined on contractile responses to agonists, one of the pair of anococcygeus muscles was treated with one of these drugs while the other was not. Only one concentration of a drug was used for each pair of muscles. Tissues were allowed to equilibrate for 60 min before concentration-response curves for methacholine and then phenylephrine were determined non-cumulatively for each tissue with 30 min being allowed to elapse between responses. Exposure to an agonist was continued for 30 s or until a maximum response was obtained and tissues were allowed to recover for a minimum of 5 min before further agonist was added. When indomethacin was present, it was continuously in the Krebs solution from 60 min before concentration-response curves were initiated.

When the maximum responses (g), to agonists with or without added drug, were not significantly different, responses were calculated as a percentage of the maximum response of the individual response curve (i.e. normalized). Then the slope (difference in percentage maximum of the response/unit of logarithm molar concentration of agonist) and the pD_2 value (negative logarithm of the molar concentration of agonist producing 50% of the maximum response) for each concentration-response curve was computed by regression line analysis (over the range 20–80% of the maximum response). For each pair of tissues, the ability of a drug to potentiate or to inhibit responses was expressed as the concentration-ratio (the antilogarithm of the difference between the pD_2 value with and without the drug). When the maximum responses (g), with or without drug were significantly different, responses were calculated as a percentage of the maximum response of the response curve from the untreated muscle.

The drugs used were mianserin hydrochloride*, 8-hydroxymianserin*, desmethylmianserin* and mianserin-*N*-oxide* (Organon), phentolamine mesylate* (Ciba-Geigy), verapamil hydrochloride* (Knoll) and atropine sulphate, cyproheptadine hydrochloride, 6-hydroxydopamine hydrochloride, indomethacin, methacholine chloride, ouabain octahydrate and (−)-phenylephrine hydrochloride (Sigma Chemical Co.). Compounds indicated with an asterisk were donated. All drugs (except 6-hydroxydopamine and indomethacin) were dissolved in distilled water. 6-Hydroxydopamine was dissolved in Krebs solution and indomethacin was made up as a stock solution of 8×10^{-3} M in sodium carbonate (3×10^{-3} M) just before use. In the study of the effect of indomethacin on responses to agonists, sodium carbonate was added to the tissues untreated with indomethacin.

RESULTS

Mianserin, desmethylmianserin and mianserin-*N*-oxide (each at 10^{-8}–10^{-5} M) and 8-hydroxymianserin at 10^{-8}–10^{-6} M had no effect on the resting tone of the muscle. Mianserin ($\geq 3 \times 10^{-5}$ M) caused sustained contractions whereas 8-hydroxymianserin ($\geq 3 \times 10^{-6}$ M) produced contractions that were not maintained (Fig. 1). 8-Hydroxymianserin was more potent in contracting the tissue than mianserin. The contraction to mianserin at 10^{-4} M was not altered by phentolamine (10^{-6} M), atropine (10^{-6} M), cyproheptadine (10^{-8} M) ouabain (10^{-5} M), or verapamil (10^{-5} M), but was reduced by prior treatment with indomethacin, 8×10^{-6} M) for 60 min (data not shown).

Indomethacin (8×10^{-6} M) had no effect on the resting tone or on contractile responses to methacholine and phenylephrine. The contractions induced by 8-hydroxymianserin (10^{-5} M) in 11 of 19 tissues lasted >2½ h and these tissues were not challenged with other agonists. The 8-hydroxymianserin-induced tone disappeared in the 8 other tissues challenged in <2½ h. To ensure that no

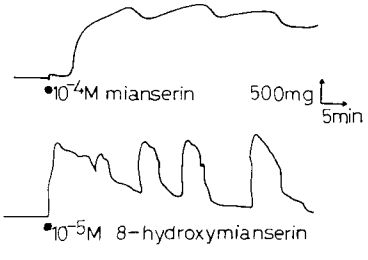

FIG. 1. Contractile responses to mianserin at 10^{-4} M (●) and to 8-hydroxymianserin at 10^{-5} M (■) in the rat anococcygeus muscle.

effects of 10^{-5} M 8-hydroxymianserin on tone were involved in effects on contractile responses to methacholine or phenylephrine, the 8 tissues were continually incubated in the presence of 8-hydroxymianserin until 30 min had elapsed after any induced tone had disappeared and for a minimum period of 60 min. The tissues were then exposed to methacholine and/or phenylephrine.

Mianserin (10^{-8}–10^{-6} M), 8-hydroxymianserin, desmethylmianserin (both at 10^{-8} M) and mianserin-*N*-oxide (10^{-8}–10^{-5} M) had no effect on the contractile responses to methacholine. 8-Hydroxymianserin (10^{-7} M) had no effect on the magnitude of maximal responses or slopes of concentration-response curves but increased the pD_2 values (Table 1) which represented a potentiation of responses to methacholine ×2. Mianserin (10^{-5} M) and 8-hydroxymianserin (10^{-6}–10^{-5} M) potentiated responses (including maxima) to methacholine (Fig. 2). Desmethylmianserin (10^{-7}–10^{-5} M) had no effect on the magnitude of the maximal responses or slopes of concentration-response curves to methacholine but reduced the pD_2 values (Table 1). The responses to methacholine were inhibited ×1·4, ×2 and ×7 by desmethylmianserin at 10^{-7}, 10^{-6} and 10^{-5} M, respectively.

8-Hydroxymianserin, desmethylmianserin (each at 10^{-8} M) and mianserin-*N*-oxide (10^{-8}–10^{-7} M) had no effect on the contractile responses to phenylephrine. Mianserin (10^{-8}–10^{-5} M), 8-hydroxymianserin, desmethylmianserin (each at 10^{-7}–10^{-5} M) and mianserin-*N*-oxide (10^{-5} M) had no effect on the magnitude of the maximal responses to phenylephrine. Mianserin at 10^{-8} M increased, at 10^{-7} M had no effect and at 10^{-6}–10^{-5} M decreased the slopes of the concentration-response curves to phenylephrine (Table 2). The pD_2 values for phenylephrine were reduced by mianserin (Table 2) which represents inhibition ×2, ×4, ×14 and ×75 by 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} M mianserin, respectively. The inhibitory effect of 8-hydroxymianserin (10^{-7}–10^{-5} M) on responses to phenylephrine included a decrease in the slope of the concentration-response curves and in the pD_2 values (Table 2 and illustrated for 8-hydroxymianserin, 10^{-6}–10^{-5} M, in Fig. 3). The reduction in pD_2 represented inhibition ×4 and ×9 by 10^{-6} and 10^{-5} M 8-hydroxymianserin, respectively. Desmethylmianserin (10^{-7}–10^{-5} M) and mianserin-*N*-oxide (10^{-6} M) had no effect on the slopes of the

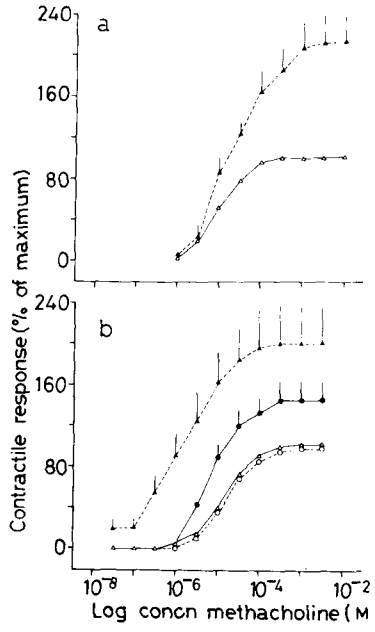

FIG. 2. Effect of mianserin (a) or 8-hydroxymianserin (b) on the contractile response to methacholine in the rat anococcygeus. Responses from: (a) 9 tissues alone in Krebs solution (△) and paired tissues in 10^{-5} M mianserin (▲); in (b) 8 tissues alone in Krebs solution (○) and paired tissues in 10^{-6} M 8-hydroxymianserin (●), and from other animals 6 tissues alone in Krebs solution (△) and paired tissues in 10^{-5} M 8-hydroxymianserin (▲). All responses are expressed as a percentage of the maximum response of the control response curve, ordinate, and plotted against the logarithm of the molar concentration of agonist (methacholine), abscissa. Each value is the mean ± s.e. mean. The potentiating effects of 10^{-5} M mianserin and 10^{-6}–10^{-5} M 8-hydroxymianserin on response to methacholine included increases in maximal response from 2·74 g ± 0·26 g (9) to 5·38 g ± 0·37 g (9), from 2·23 g ± 0·30 g (8) to 3·14 g ± 0·46 g (8) and from 2·79 g ± 0·42 g (6) to 5·00 g ± 0·57 g (6), respectively.

Table 1. The effect of metabolites of mianserin on the responses of the rat anococcygeus to methacholine and phenylephrine.

	Methacholine pD_2	Phenylephrine pD_2
Control	4·91 ± 0·06 (11)	6·33 ± 0·4 (11)
8-Hydroxy-mianserin, 10^{-7} M	5·16 ± 0·11 (11)*	6·20 ± 0·06 (11)*
Control	5·06 ± 0·05 (11)	6·56 ± 0·05 (11)*
Desmethyl-mianserin, 10^{-7} M	4·89 ± 0·09 (11)*	6·30 ± 0·05 (11)*
Control	5·14 ± 0·06 (8)	6·52 ± 0·08 (6)
Desmethyl-mianserin, 10^{-6} M	4·88 ± 0·10 (8)*	5·45 ± 0·04 (6)*
Control	4·85 ± 0·04 (8)	6·34 ± 0·03 (8)
Desmethyl-mianserin, 10^{-5} M	4·06 ± 0·07 (8)*	4·34 ± 0·07 (8)*
Control	4·87 ± 0·08 (7)	6·66 ± 0·09 (8)
Mianserin-*N*-oxide, 10^{-5} M	4·81 ± 0·10 (7)	6·46 ± 0·07 (8)*

Each value is the mean ± s.e. mean (n), where n = number of observations.
*$P < 0·05$, paired *t*-test.

Table 2. The effect of mianserin and 8-hydroxymianserin on the response of the rat anococcygeus to phenylephrine.

	Phenylephrine	
	Slopes	pD_2
Control	66·06 ± 4·08 (11)	6·61 ± 0·06 (11)
Mianserin, 10^{-8} M	81·30 ± 3·96 (11)*	6·38 ± 0·06 (11)*
Control	67·50 ± 3·81 (8)	6·63 ± 0·07 (8)
Mianserin, 10^{-7} M	66·73 ± 4·33 (8)	6·22 ± 0·09 (8)*
Control	74·42 ± 5·70 (7)	6·75 ± 0·05 (7)
Mianserin, 10^{-6} M	55·33 ± 3·35 (7)*	5·65 ± 0·07 (7)*
Control	71·79 ± 3·74 (13)	6·53 ± 0·03 (13)
Mianserin, 10^{-5} M	51·00 ± 3·40 (13)*	4·69 ± 0·09 (13)*
Control	73·40 ± 2·50 (11)*	6·33 ± 0·04 (11)
8-Hydroxy-mianserin, 10^{-7} M	66·85 ± 4·00 (11)*	6·20 ± 0·06 (11)*
Control	75·96 ± 3·71 (8)	6·55 ± 0·06 (8)
8-Hydroxy-mianserin, 10^{-6} M	58·86 ± 2·10 (8)*	5·97 ± 0·07 (8)*
Control	86·12 ± 3·69 (5)	6·28 ± 0·09 (5)
8-Hydroxy-mianserin, 10^{-5} M	52·10 ± 13·84 (5)*	5·63 ± 0·28 (5)*

Each value is the mean ± s.e. mean (n), where n = number of observations.
*$P < 0.05$, paired t-test.

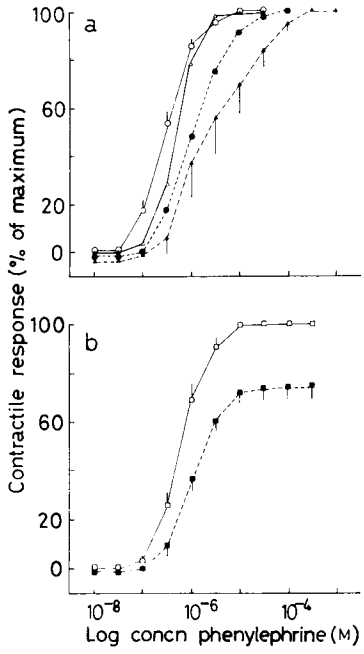

FIG. 3. Effect of 8-hydroxymianserin and mianserin-N-oxide on the contractile responses to phenylephrine in the rat anococcygeus. Response from (a) 7 tissues alone in Krebs solution (○) and paired tissues in 10^{-6} M 8-hydroxymianserin (●), and from other animals 5 tissues alone in Krebs solution (△) and paired tissues in 10^{-5} M 8-hydroxymianserin (▲): in (b) 7 tissues alone in Krebs solution (□) and paired tissues in 10^{-5} M mianserin-N-oxide (■). See legend to Fig. 2 for further details. Mianserin-N-oxide at 10^{-5} M decreased the magnitude of the maximal response to phenylephrine from 7·10 g ± 0·34 (7) to 5·20 ± 0·37 (7).

concentration-response curves but reduced the pD_2 values to phenylephrine (Table 1) which represented an inhibition of responses ×2, ×13 and ×110 by 10^{-7}, 10^{-6} and 10^{-5} M desmethylmianserin, respectively, and ×2 by mianserin-N-oxide (10^{-6} M). The inhibitory effect of mianserin-N-oxide (10^{-5} M) on responses to phenylephrine included a decrease in the magnitude of the maximal response (Fig. 3).

In the presence of indomethacin (8×10^{-6} M), the effects of mianserin (10^{-5} M), 8-hydroxymianserin (10^{-6}–10^{-5} M), desmethylmianserin and mianserin-N-oxide (each at 10^{-5} M) on responses to methacholine and phenylephrine were similar to those observed in the absence of indomethacin (data not shown).

DISCUSSION

The rat anococcygeus muscle has an excitatory noradrenergic and an inhibitory innervation; whose transmitter is unknown (Gillespie 1980). 6-Hydroxydopamine treatment selectively depletes the noradrenaline stores and destroys the noradrenergic neurons (Doggrell & Waldron 1982). Thus the contractions to mianserin and 8-hydroxymianserin, after 6-hydroxydopamine, were not due to the release of noradrenaline. The responses to mianserin were also not due to stimulation of α_1- or α_2-adrenoceptors, muscarine, histamine or 5-hydroxytryptamine receptors as they were not altered by antagonists at these receptors (i.e. phentolamine, atropine and cyproheptadine, respectively). Other mechanisms eliminated as underlying the contractions to mianserin were effects on Na^+/K^+ ATP-ase (as the contractions were not altered by ouabain) and effects on Ca^{2+} channels (responses were not altered by verapamil). It seems likely that the contractions to mianserin are due, in part, to the stimulation of prostaglandin synthesis. Thus the contractile responses to mianserin, but not methacholine or phenylephrine, were reduced by indomethacin, a prostaglandin synthesis inhibitor.

Methacholine is slowly metabolized by acetylcholinesterase and thus it is unlikely that the ability of mianserin and 8-hydroxymianserin to potentiate contractile responses to methacholine is due to inhibition of acetylcholinesterase. The ability of mianserin and 8-hydroxymianserin to potentiate responses was also not due to the stimulation of prostaglandin synthesis as the potentiation was maintained in the presence of indomethacin. Thus,

as described for mianserin (Doggrell 1979), 8-hydroxymianserin increased the sensitivity to methacholine by an action at the level of, or distal to, the receptor. The precise mechanism underlying the potentiation remains unknown. Desmethylmianserin was the only drug tested to inhibit responses to methacholine and thus it is possibly this metabolite that is responsible for the anticholinergic effects reported by a small proportion of patients taking mianserin (cf. Freighner et al 1983).

A previous study has shown that 8-hydroxymianserin and desmethylmianserin, but not mianserin-*N*-oxide, have affinity for the α-adrenoceptors in the rat brain (Nickolson et al 1982). Thus, the inhibitory effects of desmethylmianserin on the contractile responses to methacholine and to phenylephrine probably represents antagonism at muscarine and α_1-adrenoceptors separately rather than a non-receptor mediated (direct) depressant action on contractile activity.

As mianserin, 8-hydroxymianserin and mianserin-*N*-oxide did not inhibit responses to methacholine, their inhibitory effects on responses to phenylephrine were not the result of a non-selective depressant activity and probably represent a selective action at α_1-adrenoceptors.

After the tissue had been incubated with 6-hydroxydopamine to destroy noradrenergic neurons (Doggrell & Waldron 1982), any effects of drugs on contractile responses to added noradrenaline or phenylephrine must occur postjunctionally. Under these conditions the inhibitory effect of 8-hydroxymianserin on responses to phenylephrine was neither competitive (as the slopes of curves were altered) nor concentration-related. This effect of 8-hydroxymianserin was not due to stimulation of prostaglandin synthesis as it was maintained in the presence of indomethacin. Thus, as I concluded for mianserin (Doggrell 1980), 8-hydroxymianserin may have, in addition to its ability to act as a postjunctional α_1-adrenoceptor blocker, other postjunctional actions at the level of, or distal to, the α_1-adrenoceptor.

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