J. Pharm. Pharmacol. 1984, 36: 668–672
Received February 1, 1984

The action and interaction of β-phenethylamines and imidazolines on prejunctional α_2-adrenoceptors of guinea-pig ileum in the presence of the non-competitive antagonist benextramine

D. R. MOTTRAM* AND Y. THAKAR

School of Pharmacy, Liverpool Polytechnic, Liverpool L3 3AF, UK

The effects of benextramine, a selective non-competitive, irreversible antagonist of α-adrenoceptors, against α_2-adrenoceptor agonists has been investigated in isolated field-stimualted guinea-pig ileum. Benextramine was equipotent against the imidazoline, clonidine and the thiazoloazepine B-HT920, however, higher concentrations of benextramine were required for an equivalent non-competitive antagonism of the β-phenethylamine, α-methylnoradrenaline. Using benextramine to differentially block the agonist effects of clonidine but not α-methylnoradrenaline it was shown that clonidine can competitively antagonize the effects of α-methylnoradrenaline. From these and previous results on the differential effects of imidazoline-like and β-phenethylamine-like drugs on α_2-adrenoceptors, it is proposed that two distinct populations of these receptors exist.

The first observation that differences existed between the interaction of imidazolines and β-phenethylamines with α-adrenoceptors was made by Ruffolo et al (1977), reporting a lack of cross-desensitization between the two classes of agonists. Since then, a number of other reports have indicated that imidazolines and β-phenethylamines interact differentially with α-adrenoceptors (Ruffolo et al 1979, 1983; Pelayo et al 1980; Langer & Dubocovich 1981; Mottram 1982, 1983a, b; de Jonge et al 1983). Such results have led to speculation that two subsites may exist on α-adrenoceptors through which imidazoline-like and β-phenethylamine-like agonists can interact.

It has been shown (Mottram 1983a) that competitive antagonists of α-adrenoceptors that block the pre-junctioned effect of clonidine, produce only a partial antagonism of the pre-junctional α_2-adrenoceptor effects of α-methylnoradrenaline and adrenaline. The present study investigates the differential responses of these chemically distinct agonists to antagonism by a non-competitive α-adrenoreceptor antagonist benextramine. Benextramine (*NN'*-bis(6-(o-methoxy-benzylamino)-n-hexyl)-cystamine) blocks post-junctional α-adrenoceptors in a non-competitive and irreverible manner (Melchiorre et al 1978). Pre-junctionally, benextramine has been shown to block clonidine non-competitively though this blockade appeared to be slowly reversible (Belleau et al 1982). Unlike other non-competitive antagonists of α-adrenoceptor it is suggested that benextramine is selective in its reactivity with the α-adrenoceptor, through a buried thiol group (Belleau 1982). Therefore covalent binding by benextramine should ensure equivalent antagonism of all agonists on the α_2-adrenoceptor at each concentration of benextramine used.

* Correspondence.

MATERIALS AND METHODS

Pre-junctional α-adrenoceptor activity was measured in isolated field-stimulated guinea-pig ileum. Tissues were set up according to the method of Drew (1978) and bathed in Krebs solution containing (μM) cocaine 3, propranolol 1, prazosin 0·3, to inhibit neuronal uptake, and to block β- and α_1-adrenoceptors respectively. The bathing solution was maintained at 37 °C and aerated with 5% CO_2 and 95% O_2. Field stimulation of tissues was produced by square wave pulses of 1 ms duration at a frequency of 0·1 Hz using submaximal voltages generated by Grass stimulators. Isometric contractions were recorded with Lectromed 2 oz strain gauges and two-channel recorders.

Cumulative dose-response curves to clonidine, B-HT920 (6-allyl-2-amino-5,6,7,8-tetrahydro-4H-thiazolo[4,5-d]-azepine) and α-methyl noradrenaline were obtained before and after exposure to benextramine, in concentrations between 0·1 and 10 μM. Tissues were exposed to benextramine for 30 min

before washout with fresh Krebs solutions and 10 min later cumulative dose-response activities for agonists repeated.

In a further set of experiments, tissues were pre-dosed with benextramine (1 μM) which was left in contact with tissues for 30 min. Tissues were then washed with fresh Krebs solution and 5 min later varying concentrations of clonidine (0·3 to 3 μM) added to the bath and left for 5 min. Cumulative doses of α-methylnoradrenaline were then added and its inhibitory effect on the twitch response of the ileum recorded.

The drugs used were: benextramine (*NN'*-bis(6-(o-methoxy-benzylamino)-n-hexyl) cystamine) tetrachloride monohydrate, (Aldrich), B-HT920 (Dr Karl Thomae), clonidine hydrochloride (Boehringer Ingelheim), cocaine hydrochloride (Merck), (−)-erythro α-methylnoradrenaline hydrochloride (Hoechst), prazosin hydrochloride (Pfizer) and proproanolol hydrochloride (Sigma). All drug solutions were made up freshly for each experiment.

RESULTS

The potentiation of the twitch response of field stimulated ileum, normally seen with antagonists of α_2-adrenoceptors, was absent with benextramine. On the contrary, a dose-dependent inhibition of the twitch response was observed. An immediate reduction in the magnitude of the twitch response was obtained after the addition of benextramine to the Krebs solution (Fig. 1). Two to three minutes after addition, the maximal inhibitory effect was achieved, then a slight recovery was sometimes observed. Dose-dependent inhibition occurred at concentrations of benextramine above 0·1 μM and Fig. 1 shows a plot of dose against percentage inhibition of the twitch.

Benextramine, 10 μM did not inhibit the twitch response significantly more than benextramine, 3 μM.

The α_2-adrenoceptor antagonist activity of benextramine was determined against the selective α_2-agonists α-methylnoradrenaline (a β-phenethylamine), clonidine (an imidazoline) and B-HT920 (a thiazoloazepine, Kobinger & Pichler 1980; Van Meel et al 1981). The relative activities of the three agonists are shown in Table 1, in which their intrinsic activities and affinities, in terms of −log EC50 values, are listed.

Benextramine produced a non-competitive antagonism against all three agonists. Interestingly, benextramine was equipotent against clonidine and B-HT920 (Fig. 2. A, B) but it required a seven-fold

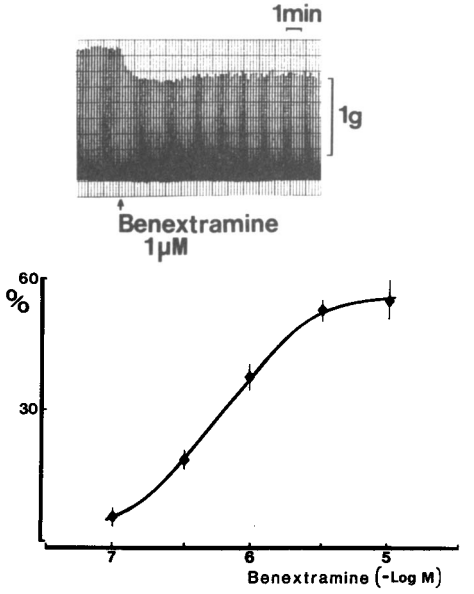

FIG. 1. Trace showing the inhibitory effect of benextramine 1 μM, on the twitch response of field-stimulated guinea-pig ileum. The magnitude of the contraction is indicated by the vertical 1 g calibration. The graph shows the negative log dose-response relationship for the benextramine-induced inhibition of the twitch response of field-stimualted guinea-pig ileum. Each point is the mean ± s.e.m. from at least 11 experiments.

increase in the concentration of benextramine to produce an equivalent degree of blockade of α-methylnoradrenaline (Fig. 2, C) under identical experimental conditions.

The cumulative addition of clonidine to field-stimulated guinea-pig ileum invariably failed to produce complete inhibition of the twitch response in accord with its partial agonist activity on α-adrenoceptors. On the other hand, cumulative addition of α-methylnoradrenaline invariably induced maximal inhibition of the twitch response.

It was observed, however, that clonidine 10 μM was able to partially reverse the inhibitory effect of α-methylnoradrenaline on the twitch response (Tracing on Fig. 3) to the level equivalent to that to

Table 1. Relative activities of α-methylnoradrenaline, clonidine and B-HT920 on pre-junctional α-adrenoceptors in field-stimulated guinea-pig ileum.

Compound	Intrinsic activity	−log ED50	n
α-Methylnoradrenaline	1.00	7·38 ± 0·08	11
Clonidine	0·83 ± 0·02	8·46 ± 0·09	15
B-HT920	0·92 ± 0·02	6·86 ± 0·05	16

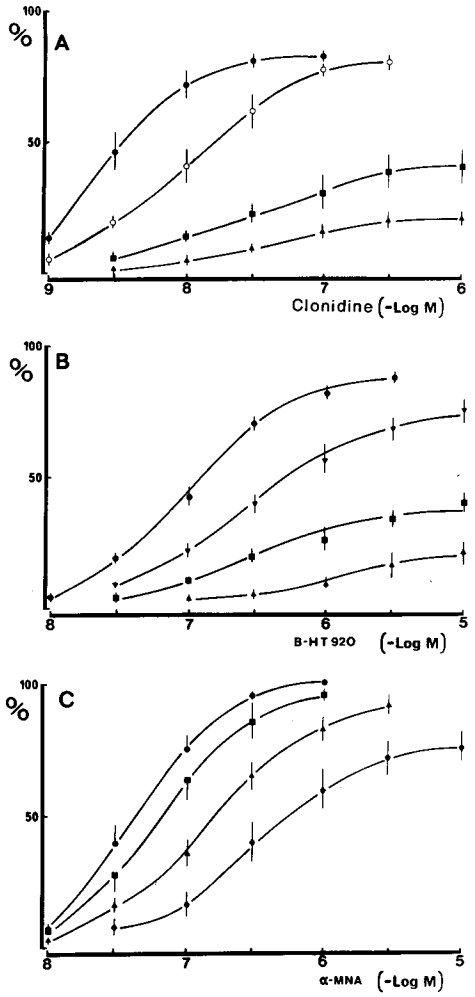

FIG. 2. A. Cumulative dose-response curves for clonidine-induced inhibition of field-stimulated guinea-pig ileum, alone (●——●) and in the presence of 0·3 μM (○——○), 1 μM (■——■) or 3 μM (▲——▲) benextramine. Each point is the mean ± s.e.m. from 8–10 experiments. B. Cumulative dose-response curves for B-HT920-induced inhibition of field-stimulated guinea-pig ileum, alone (●——●) and in the presence of 0·3 μM (▼——▼), 1 μM (■——■) or 3 μM (▲——▲) benextramine. Each point is the mean ± s.e.m. from 9–11 experiments. C. Cumulative dose-response curves for α-methylnoradrenaline-induced inhibition of field-stimulated guinea-pig ileum alone (●——●) and in the presence of 0·3 μM (■——■) 1 μM (▲——▲) or 3 μM (◆——◆) benextramine. Each point is the mean ± s.e.m. from 9–12 experiments.

which clonidine alone inhibited the response (histograph on Fig. 3). This result indicated an antagonistic effect of clonidine to α-methylnoradrenaline. To investigate this further, increasing concentrations of clonidine (1–10 μM) were used to antagonize the cumulative dose-response effect of α-methylnoradrenaline in field-stimulated guinea-pig ileum pretreated with 1 μM benextramine for 30 min. Pretreatment with benextramine in this manner ensures a differential non-competitive blockade of clonidine whilst leaving the effects of α-methylnoradrenaline virtually intact (see Fig. 2A, C). Results show that clonidine acts as a competitive antagonist to α-methylnoradrenaline-induced inhibition of the twitch response (Fig. 4), with a $-\log K_B$ value of 6·30 ± 0·23 (K_B = [antagonist]/(dose-ratio − 1), Furchgott 1972), at the 1 μM concentration of clonidine.

DISCUSSION

Benextramine is a non-competitive and irreversible antagonist of postjunctional α-adrenoceptors (Melchiorre et al 1978). Prejunctionally however, benextramine blockade is reported to be slowly reversible (Belleau et al 1982) with a partial recovery of response after 40 min of repeated changes of bathing fluid. Benextramine did not potentiate the twitch response of field-stimualted guinea-pig ileum as normally seen following the addition of an α_2-adrenoceptor antagonist (Drew 1978). The reason for this may lie in the fact that the potentiation was masked by the dose-dependent depression of the twitch response observed in this tissue (Fig. 1). This depression is attributable to the muscarinic receptor-blocking activity of benextramine (Benfey et al 1979), since the twitch response in guinea-pig ileum is mediated via the release of neuronal acetylcholine. Our results indicated a maximal muscarinic inhibi-

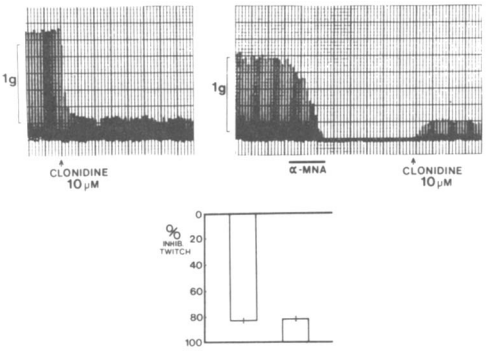

FIG. 3. Traces showing the twitch response to field-stimulated guinea-pig ileum. Left hand trace shows the inhibitory effect of a maximal dose (10 μM) of clonidine. Right hand trace shows the inhibitory effect of α-methylnoradrenaline, producing complete inhibition of the twitch, followed by partial recovery of the twitch response following the addition of 10 μM clonidine. The histogram shows the mean percentage inhibition of the twitch response to clonidine, 10 μM (83·4 ± 1·7, n = 8) (the maximum inhibition obtainable with clonidine) and the mean percentage inhibition by α-methylnoradrenaline followed by clonidine, 10 μM (81·5 ± 1·7, n = 10).

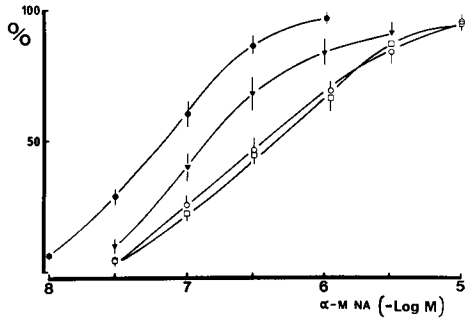

FIG. 4. Cumulative dose-response curves for α-methylnoradrenaline-induced inhibition of field-stimulated guinea-pig ileum following exposure to benextramine, 1 μM. The control dose-response curve (●——●) is shifted to the right by 1 μM (▼——▼) and 3 μM (□——□) clonidine. 10 μM clonidine (○——○) fails to shift the curve further. Each point is the mean ± s.e.m. from 8–12 exposures.

tory effect between 3 and 10 μM benextramine whilst Benfey et al had a maximum between 10 and 30 μM. This difference may be explained by their investigation of exogenous acetylcholine acting both junctionally and extra-junctionally whilst our effects are against endogenous acetylcholine confined to the junctional space.

An alternative explanation for the lack of any potentiation of the twitch response by benextramine may lie in the non-competitive nature of its antagonism. Drew (1977) suggested that there is no relationship between α-adrenoceptor blockade and potentiation of the twitch response by α-adrenoceptor antagonists and conceivably benextramine may interact at a site other than that responsible for either competitive blockade or potentiation of the twitch response.

Benextramine blocked all three agonists, clonidine, B-HT920 and α-methylnoradrenaline, non-competitively. Benextramine exhibited very similar potency against both the imidazoline, clonidine, and the thiazoloazepine, B-HT920, as expected for a selective covalently binding α-adrenoceptor antagonist (Belleau 1982). This also indicates that prejunctional inhibition of neurotransmission by B-HT920 is mediated through α_2-adrenoceptors (Mottram 1983b) rather than through dopamine receptors as suggested by Andén et al (1982), although Gorich et al (1982) reported that, in guinea-pig isolated ileum, the dopamine inhibition of cholinergic transmission is mediated through prejunctional α-adrenoceptors.

Higher concentrations of benextramine were, however, required to produce equivalent antagonism of the β-phenethylamine, α-methylnoradrenaline. Previously (Mottram, 1982, 1983a), competitive antagonists of α_2-adrenoceptors have been shown to block clonidine whilst incompletely blocking α-methylnoradrenaline, whereas in the present study the non-competitive antagonist benextramine, though requiring higher concentrations, does produce a dose-dependent antagonism of α-methylnoradrenaline. A parallel differential blockade by non-competitive antagonists against Sgd 101/75 (4[2-imidazoline-amino]-2-methylindazol-chlorhydrate), a clonidine-like compound and noradrenaline on α_1-adrenoceptors has been described by Coates et al (1982) and Coates & Weetman (1983). As a result these authors have proposed a subclassification into α_1- and α_{1S}-adrenoceptors. There may then be a subclassification of α_1-adrenoceptors, as they have proposed, but not necessarily confined to an interaction of the α_{1S} receptor with Sgd 101/75 alone since other workers have found differential α_1-adrenoceptor activity with imidazolines and non-imidazolines (Ruffolo et al 1983; Holck et al 1983).

Clonidine has been reported to be a partial agonist on α-adrenoceptors (Medgett et al 1978) an effect confirmed in the present results. In the presence of benextramine, at a concentration that differentially antagonizes clonidine but not α-methylnoradrenaline, clonidine up to a concentration of 3 μM competitively blocks α-methylnoradrenaline after which no further antagonism is achieved. This is in accord with previous observations where a dose-dependent blockade of β-phenethylamines is obtained by low concentrations of competitive α-adrenoceptor antagonists, but at higher concentrations no further antagonism is obtained. Competition between noradrenaline and clonidine on prejunctional adrenoceptors has also been decribed by Hedler et al (1983) during their investigations into the release of [^{3}H]amezinium from noradrenergic axons. They found that the increase or decrease of endogenous noradrenaline, from the nerve terminals into the neuromuscular junction, inhibited or potentiated respectively, the pre-junctional effects of clonidine.

The differential blocking activity of benextramine against compounds of the imidazoline-like structure and β-phenethylamines provides further clear evidence that these two types of agonists interact either through different subsites on the α_2-adrenoceptor, or through two distinct and separate populations of the α_2-adrenoceptors. On the basis of previous reports (Ruffolo et al 1979, 1983; Pelayo et al 1980; Langer & Dubocovich 1981; Mottram 1982, 1983a, b; de Jonge et al 1983) and the present results, it is proposed that two distinct adrenoceptor populations exist prejunctionally. Firstly, the imidazoline-

sensitive α_2-adrenoceptors which are activated by clonidine-like agonists, fully blocked by competitive α_2-adrenoceptor antagonists and blocked by non-selective non-competitive α-adrenoceptor antagonists such as benextramine. Secondly, the β-phenethylamine sensitive α_2-adrenoceptors which are activated by α-methylnoradrenaline-like agonists, partially blocked by competitive α_2-adrenoceptor antagonists, blocked by non-selective, non-competitive antagonists but at higher concentrations than those required to block the imidazoline sensitive α_2-adrenoceptors and partially blocked by agonists of the imidazoline type in a similar manner to the antagonism produced by competitive α_2-adrenoceptor antagonists.

Acknowledgements

The authors gratefully acknowledge the generous gifts of drugs from Boehringer Ingelheim, Hoechst, Karl Thomae and Pfizer.

REFERENCES

Andén, N. E., Golembiowska-Nikitin, K., Thornstrom, U. (1982) N-S. Arch. Pharmacol. 321: 100–104

Belleau, B. (1982) in Frontiers in cellular surface research, P.J.D. Publications Ltd, pp 37–53

Belleau, B., Benfey, B. G., Melchiorre, C. (1982) Br. J. Pharmacol. 75: 617–621

Benfey, B. G., Yong, M. S., Belleau, B., Melchiorre, C. (1979) Can. J. Physiol. Pharmacol. 57: 41–47

Coates, J., Jahn, U., Weetman, D. F. (1982) Br. J. Pharmacol. 75: 549–552

Coates, J., Weetman, D. F. (1983) Ibid. 80: 343–346

DeJonge, A., Timmermans, P. B. M. W. M., Van Zwieten, P. A. (1984) J. Pharmacol. Exp. Ther., 226: 565–571

Drew, G. M. (1977) Eur. J. Pharmacol. 42: 123–130

Drew, G. M. (1978) Br. J. Pharmacol. 64: 293–300

Furchgott, R. F. (1972) in Catecholamines, Blashchko, H., Muschol, E. (eds) Springer-Verlag, Berlin, pp 283–335

Gorich, R., Weihraugh, T. R., Kilbinger, H. (1982) Naunyn Smiedeberg's Arch. Pharmacol. 318: 308–312

Hedler, L., Starke, K., Stepeller, A. (1983) Br. J. Pharmacol. 78: 645–653

Holck, M. I., Jones, C. H. M., Haesler, G. (1983) J. Cardiovasc. Pharmacol. 5: 240–248

Kobinger, W., Pichler, L. (1980) Eur. J. Pharmacol. 65: 393–402

Langer, S. Z., Dubocovich, M. L. (1981) J. Pharmacol. Exp. Ther. 216: 162–171

Medgett, I. C., McCulloch, M. W., Rand, M. J. (1978) Naunyn Smideberg's Arch. Pharmacol. 304: 215–221

Melchiorre, C., Yong, M. S., Benfey, B. G., Belleau, B. (1978) J. Med. Chem. 21: 1126–1132

Mottram, D. R. (1982) Br. J. Pharmacol. 75: 138P

Mottram, D. R. (1983a) Arch. Int. Pharmacodyn. Ther. 264: 28–39

Mottram, D. R. (1983b) J. Pharm. Pharmacol. 35: 652–655

Pelayo, R., Dubocovich, M. L., Langer, S. Z. (1980) Eur. J. Pharmacol. 64: 143–155

Ruffolo, R. R. Jr., Dillard, R. D., Yaden, E. L., Waddell, J. E. (1979) J. Pharmacol. Exp. Ther. 211: 74–79

Ruffolo, R. R. Jr., Rice, P. J., Patil, P. N., Hamada, A., Miller, D. D. (1983) Eur. J. Pharmacol. 86. 471–475

Ruffolo, R. R. Jr., Turowski, B. S., Patil, P. N. (1977) J. Pharm. Pharmacol. 29: 378–380

Van Meel, J. C. A., DeJonge, A., Timmermans, P. B. M. W. M., Van Zwieten, P. A. (1981) J. Pharmacol. Exp. Ther. 219: 760–767