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Differential blockade of pre- and postsynaptic α-adrenoceptors by the 2-*R* and 2-*S* enantiomers of WB4101

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Agonists and antagonists of the α-adrenoceptor have been shown to possess different degrees of potency at the pre- and postsynaptic levels. The present investigation was undertaken to see whether the two optical isomers of WB4101 likewise exhibit differential blockade at the two receptor sites. This was achieved by measuring the potencies of 2-*R* and 2-*S* WB4101 against noradrenaline-induced contractions of the rat isolated vas deferens and against clonidine-induced inhibition of the twitch response in field stimulated rat vas deferens. Results showed that though the enantiomers exhibited widely differing potency, as indicated by calculations of pA_2 values, at the postsynaptic level, they possessed almost identical potency presynaptically. In addition, 2-aminomethyl-1, 4-benzodioxan itself both exhibited reasonable presynaptic α-adrenoceptor blocking activity, whilst being devoid of activity postsynaptically. The values for slope on the Schild plots for 2-*R* and 2-*S* WB4101 indicated that they produced classical competitive antagonism at the postsynaptic receptor but at the presynaptic level the values for slope were indicative of a non-competitive type of blockade. Results are discussed in terms of recent suggestions of that agonists and antagonists of the α-adrenoceptor may not share a common mechanism or site of action at the presynaptic level.

In recent years the concept of presynaptic α-adrenoceptors has been introduced with the suggestion that these receptors may have a physiological role to play in the regulation of transmitter release (Langer 1977; Starke 1977). Recently, Kalsner (1980) has cast some doubt over this presynaptic adrenoceptor theory having shown that phenoxybenzamine and noradrenaline do not appear to have a common mechanism or site of action presynaptically.

WB4101 (2-(*N*-[2,6-dimethoxyphenyl-oxyethyl])-aminomethyl-1,4-benzodioxane) , a potent competitive antagonist of postsynaptic α-adrenoceptors (Mottram & Kapur 1975) has a significantly lower antagonistic potency at presynaptic α-adrenoceptors (Kapur & Mottram 1978; Butler & Jenkinson 1978). This type of differential blockade is also seen with a number of structurally related analogues of WB4101, widely differing potencies of which at postsynaptic α-adrenoceptors are contrasted with their remarkably consistent degree of antagonistic potency at the presynaptic level. This observation is in accord with the widely held view that the pre- and postsynaptic α-adrenoceptors differ in their response to sympathomimetic amines or their antagonists (Barowski et al 1977; Doxey et al 1977).

Recently, Nelson et al (1979) have synthesized the 2-*R* and 2-*S* enantiomers of WB4101 and found the 2-*S* enantiomer to be some 40–50 times more potent than the 2-*R* enantiomer in blocking the postsynaptic α-adrenoceptor-mediated response of methoxamine in rabbit aortic strip.

The present investigation was initially undertaken to compare the pre- and postsynaptic antagonistic activities of 2-*R* and 2-*S* WB4101 to see whether differential blockade between the two receptor populations was evident with isomers as well as structural analogues of WB4101 and to determine the degree of selectivity exhibited by the presynaptic α-adrenoceptor. Preliminary results were presented to the British Pharmaceutical Conference (Mottram 1980).

MATERIALS AND METHODS

For the determination of postysynpatic α-adrenoceptor antagonism, male rats, 175–250 g, were killed by a blow to the head. The vasa differentia were removed, stripped of extraneous material and suspended in 10 ml organ baths, containing Tyrode solution (composition (mM): NaCl 90·4, KCl 2·7, $MgCl_2$ 2·1, $CaCl_2$ 1·8, NaH_2PO_4 0·4, $NaHCO_3$ 12 and glucose 5·6 maintained at 37 °C and aerated with a mixture of 95% O_2 and 5% Co_2.

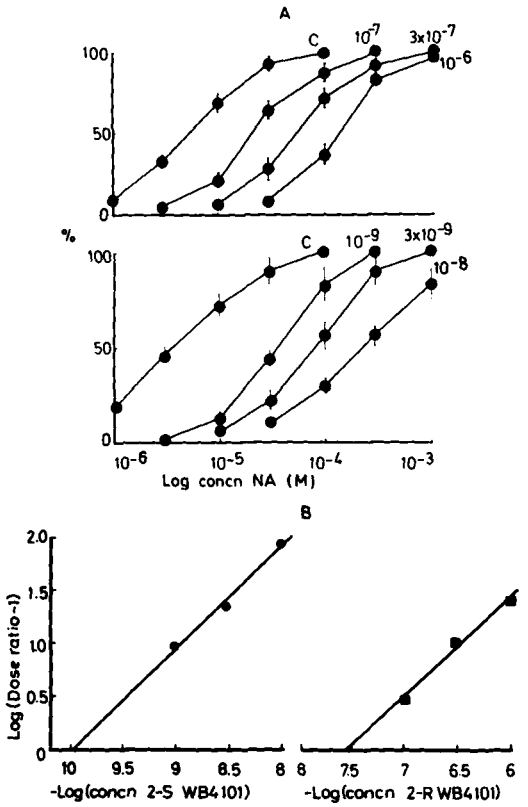

FIG. 1A. Effect of increasing concentration of 2-*R* (upper graph) and 2-*S* (lower graph) WB4101 on the cumulative dose-response curve to noradrenaline. Each point is the mean ± s.e. from 6 experiments. B. Also shown are the Schild plots for the 2-*R* and 2-*S* enantiomers of WB4101 derived from these graphs.

Isometric contractions were recorded with Devices 2 oz strain gauge transducers and two-channel recorders. Cumulative dose-response curves to noradrenaline were recorded before and after exposure to the antagonist. One hour after recording the first dose-response curve, the antagonist was administered and, 2 min later, the second dose-response curve recorded. Control dose-response curves to noradrenaline were recorded 1 h after the first recording without prior addition of the antagonist.

Presynaptic α-adrenoceptor antagonistic activity was measured on vasa diferentia, from rats 175–250 g, bathed in Mg-free Krebs solution of the following composition (mM) NaCl, 119, $CaCl_2$ 2·6 $NaHCO_3$ 25, KCl 4·7, KH_2PO_4 1·2, Glucose 11·1. The tissues were maintained at 37 °C and aerated with 95% O_2 and 5% CO_2. Field stimulation of the tissues was obtained by silver electrodes placed near the top and bottom of the vasa through which square wave pulses of 3 ms duration, 0·1 Hz frequency and submaximal voltage, were passed. Isometric 'twitch' contractions were measured by Devices transducers and recorders.

Cumulative dose-response curves for the clonidine-induced inhibition of the twitch response were recorded before and after exposure to antagonists.

Mean values for dose-response relationships were calculated and plotted graphically. From these results dose ratios were calculated and pA_2 values obtained using the method of Arunlakshana & Schild (1959).

RESULTS

Postsynaptic antagonistic activity

Both the 2-*R* and 2-*S* enantiomers of WB4101 produced parallel shifts of the noradrenaline dose-response curve. The shift in the curve was dose dependent and increasing concentrations of both 2-*S* WB4101 (10^{-9} to 10^{-8} M) and 2-*R* WB4101 (10^{-7} to 10^{-6} M) produced similar degrees of blockade (Fig. 1).

From these curves dose ratios were measured at the 50% response level and from these results plots were made according to the method of Arunlakshana and Schild (1959) (Fig. 1). The slopes and

Table 1. Results of Schild analyses on postsynaptic (A) and presynaptic (B) α-adrenoreceptor antagonistic potency of the enantiomers of WB4101 and related compounds.

		pA_2				Correlation
A. Compound	(n)	value	s.d.	Slope	s.d.	coefficient
WB4101 (racemic mixture)	(7)	9·80	0·09	0·97	0·13	0·990
2-*R* WB4101	(6)	7·55	0·20	0·92	0·16	0·984
2-*S* WB4101	(7)	9·98	0·15	0·96	0·09	0·995
2-Aminomethyl 1,4-benzodioxan	(4)	Inactive up to a concn of 7×10^{-4} M				
1,4-Benzodioxan	(4)	Inactive up to a concn of 10^{-3} M				
B. WB4101 (racemic mixture)	(7)	6·24	0·07	1·33	0·14	0·992
2-*R* WB4101	(7)	6·42	0·08	1·56	0·13	0·996
2-*S* WB4101	(6)	6·37	0·06	1·22	0·08	0·998
2-Aminomethyl 1,4-benzodioxan	(6)	5·73	0·16	1·16	0·23	0·981
1,4-Benzodioxan	(6)	4·78	0·09	1·05	0·12	0·992

n = The number of experiments.

intercepts for these plots were calculated by regression analysis and from these results the potencies of the two enantiomers of WB4101 were ascertained. These are indicated by the pA_2 values shown in Table 1.

Presynaptic antagonistic activity

Antagonism of the clonidine-induced inhibition of the twitch response was produced by both the 2-*R* and 2-*S* enantiomers of WB4101. Fig. 2 shows the shift to the right of the dose-response curves for clonidine produced by increasing concentrations of

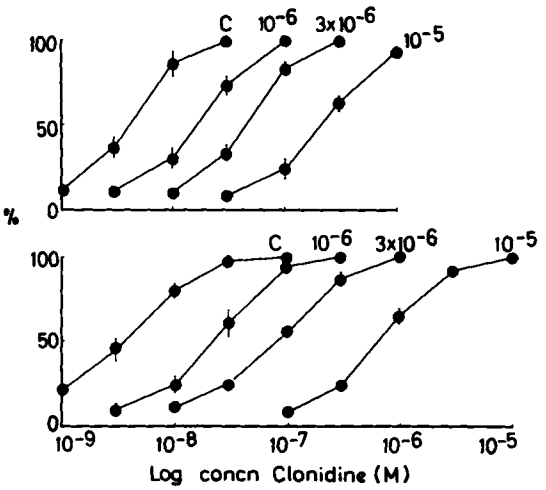

FIG. 2. Effect of increasing concentrations of 2-*R* (upper graph) and 2-*S* (lower graph) WB4101 on the dose-response curve for clonidine-induced inhibition of the twitch response to field stimulated rat vas deferens. Each point is the mean ± s.e. from 6 experiments.

the two antagonists. Unlike the postsynaptic antagonistic activity of these two enantiomers, almost identical blocking action was achieved with the same dose range (10^{-6} through to 10^{-5}M) of the drugs, at the presynaptic level. Potencies were once again measured by the method of Arunlakshana and Schild and results shown in Table 1. The pA_2 values obtained were very similar to those previously recorded (Kapur & Mottram 1978) for a number of 1,4-benzodioxan analogues of WB4101. In view of the consistency in potency, at the presynaptic level of these compounds based on 1,4-benzodioxan, it was decided to evaluate the structurally simpler analogues 2-amino methyl-1,4-benzodioxan and 1,4-benzodioxan itself, for antagonistic activity at both the postsynaptic and presynaptic level. Postsynaptic antagonistic activity against noradrenaline-induced contractions was absent with these two analogues, however, they both produced antagonistic activity against clonidine-induced inhibition of the twitch response (Fig. 3). Though obviously less potent than either the 2-*R* or 2-*S* enantiomers of WB4101 (Table 1) they were still capable of presynaptic inhibition having pA_2 values of 5·73 for 2-aminomethyl 1,4-benzodioxan and 4·78 for 1,4-benzodioxan.

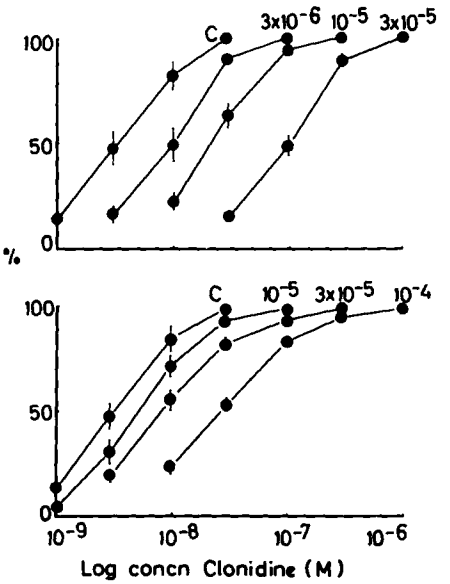

FIG. 3. Effect of increasing concentrations of 2-aminomethyl-1,4-benzodioxan (upper graph) and 1,4-benzodioxan (lower graph) on the dose-response curve for clonidine-induced inhibition of the twitch response to field stimulated rat vas deferens. Each point is the mean ± s.e. from 6 experiments.

DISCUSSION

The concept of presynaptic α-adrenoceptors is now well established, and their function as part of a negative feedback system modulating transmission at the sympathetic nerve terminal is widely documented (For reviews, see Langer 1977; Starke 1977.)

Though these receptors are termed 'alpha' it has been shown repeatedly that these presynaptic α-adrenoceptors differ in their pharmacological profile from the postsynaptically located α-adrenoceptors (Dubocovich & Langer 1974; Starke et al 1975; Drew 1976; Doxey et al 1977). These studies were carried out using standard agonists and antagonists of the

α-adrenoceptor. However, even within a series of closely related analogues, a marked difference in the antagonistic potency at the presynaptic and postsynaptic level can be seen (Kapur & Mottram 1978).

The 2-*R* and 2-*S* enantiomers of WB4101 have previously been shown to possess different potencies at the postsynaptic α-adrenoceptor (Nelson et al 1979). This observation was confirmed in the present study where 2-*S* WB4101 was found to be some 270 times more potent than 2-*R* WB4101 in antagonizing the postsynaptic response to noradrenaline in rat isolated vas deferens.

The pA_2 values on rat vas deferens are somewhat higher than those obtained by Nelson et al, in rabbit aortic strips (2-*S* = 9·02 ± 0·33 and 2-*R* = 7·35 ± 0·29).

The values obtained in both studies exhibit a fair degree of consistency however, despite the difference in agonist being used and the reported variation in response to agonist and antagonists on α-adrenoceptors in different tissues and species (Barker et al 1977).

At the presynaptic level, however, the 2-*R* and 2-*S* enantiomers of WB4101 exhibit almost identical potencies, as indicated by their pA_2 values (Table 1). These figures are in accord with both the pA_2 value of 6·24 for the racemic mixture of WB4101 and with the pA_2 values (range 6·15 to 6·58) recorded for the structural analogues of WB4101 which have been published previously (Kapur & Mottram 1978). This remarkable level of consistency in potency at presynaptic α-adrenoceptors is perhaps indicative of a low degree of structural specificity required for the antagonism of presynaptic receptors.

To investigate this further 1,4-benzodioxan and 2-aminomethyl-1,4-benzodioxan were evaluated as potential antagonists of pre- and postsynaptic α-adrenoceptors. These compounds were chosen as the moiety common to all the analogues previously investigated was the 1,4-benzodioxan. It was found that neither compound possessed antagonistic activity against noradrenaline at the postsynaptic site, but both had significant activity at presynaptic α-adrenoceptors (Table 1). These results may also indicate a very low degree of structural specificity required for interaction, at least by antagonists of the benzodioxan group, at presynaptic α-adrenoceptors.

These results may be in accord with the recently published observations of Kalsner & Chan (1979) and Kalsner (1980) who have suggested that agonists and antagonists of the α-adrenoceptor may not share a common mechanism or site of action at the presynaptic level. Support for this suggestion lies in the fact that though the 2-*R* and 2-*S* enantiomers of WB4101 exhibit classical competitive antagonism at the postsynaptic site, as indicated by the values for slope on the Schild plots being to unity (Table 1), their values for slope at the presynaptic level are indicative of a non-competitive type of inhibition.

Ruffolo et al (1977) have suggested that phenethylamines and imidazolines interact at different sites on the α-adrenoceptors with a common point of overlap. It has also been suggested by Kapur et al (1978, 1979) that the α-adrenoceptor has at least two aromatic subsites and that the ability of WB4101 to bind through both these sites accounts for its exceptional potency as an α-blocking agent. In the present study, postsynaptic effects were measured using a drug of the phenethylamine type whilst presynaptic studies were with an imidazoline type compound. Therefore, presynaptically, the imidazoline site may simply be blocked by any type of compound possessing a 1,4-benzodioxan group, regardless of further reactive groups. Whereas postsynaptically the phenethylamine site may require more structurally specific interaction by antagonists through the non-benzodioxan moiety of their structure.

An alternative site of binding for 1,4-benzodioxan at the presynaptic level may be the low affinity binding site for WB4101, which Lyon & Randall (1980) have recently described and which is distinct from the clonidine or noradrenaline binding sites.

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