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SHORT COMMUNICATIONS

A comparative study on the pre- and post-synaptic alpha blocking activity of a series of benzodioxanes

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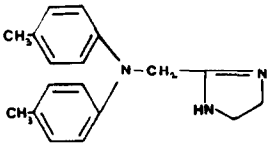
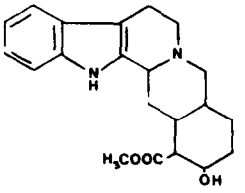
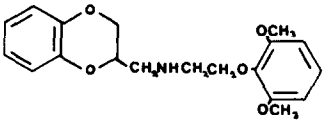
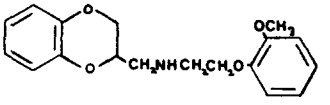
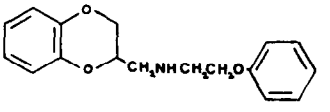
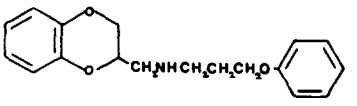
There is now increasing evidence for the existence of presynaptic α-adrenoceptors located at noradrenergic varicosities both in the peripheral and central nervous system[1, 2]. Activation of these presynaptic α-adrenoceptors by α-adrenoceptor agonists results in a decreased release of noradrenaline by nerve impulses[3]. Recent observations indicate differences between presynaptic and classical postsynaptic α-adrenoceptors in their sensitivity to both agonists and antagonists[4, 5]. The present study was undertaken to investigate a series of benzodioxanes, which have previously been evaluated for α-adrenoceptor blocking activity on postsynaptic α-adrenoceptors, for their activity and relative potencies on presynaptic α-adrenoceptors, in the rat vas deferens.

Table 1. Comparison of the antagonistic potency of α-adrenoceptor blocking agents on pre- and post-synaptic α-adrenoceptors. The results are expressed as mean pA_2 values ±S.E.M. and the number of experiments undertaken to determine the mean are shown in parentheses.

pA_2 PRESYNAPTIC		COMPOUND	STRUCTURE	★ pA_2 POSTSYNAPTIC	
8·30 ± 0·01	(6)	PHENTOLAMINE		8·7 ± 0·03	(4)
8·32 ± 0·16	(6)	YOHIMBINE		8·1 ± 0·06	(4)
6·24 ± 0·03	(8)	WB 4101		9·6 ± 0·04	(6)
6·15 ± 0·05	(8)	WB 4085		8·62 ± 0·09	(6)
6·31 ± 0·06	(6)	WB 4082		4·88 ± 0·02	(5)
6·58 ± 0·02	(6)	WB 4093		<3·3	(4)

★ DATA PREVIOUSLY REPORTED[10, 11]

The experimental procedure used to quantitate the effects of the antagonists at presynaptic α-adrenoceptors was essentially similar to that employed by Drew[6]. Vasa deferentia from male Charles River rats (200–250 g) were set up in organ baths and bathed in a 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 and glucose 11.1, which was maintained at 37° and aerated with a mixture of 5% CO_2 in O_2. Silver electrodes were placed near the top and bottom of the tissue and the intramural nerves of the vas deferens were stimulated by square wave pulses of 3 ms duration, 20–25V, a submaximal voltage, at a frequency of 0.1 Hz. Isometric contractions were recorded using Devices transducers and two-channel recorders.

Cumulative dose–response curves to clonidine, a selective presynaptic α-adrenoceptor agonist[7], were obtained in 10 preparations. In other preparations varying concentrations of the antagonists were allowed a contact time of 15 min before initial challenge with clonidine. pA_2 values were calculated according to the method of Arunlakshana *et al.*[8].

Low frequency (0.1 Hz) electrical stimulation of the rat isolated vas deferens produces regular contractions or 'twitches'[9] that were inhibited by guanethidine and clonidine. The inhibition of the 'twitch' response by clonidine was competitively antagonised by α-adrenoceptor blocking agents whilst the inhibition produced by guanethidine was unaffected by α-adrenoceptor blocking agents confirming that the twitch responses resulted from nerve stimulation rather than direct stimulation of the muscle and that clonidine inhibits the twitch response by a presynaptic mechanism involving α-adrenoceptors. Results showed that all the blockers studied exhibited a competitive type of blockade in that the antagonists all caused a parallel shift of the clonidine—dose response curve to the right. The pA_2 values for these compounds on presynaptic α-adrenoceptors are shown in Table 1; also shown are the pA_2 values against noradrenaline on postsynaptic α-adrenoceptors previously obtained on isolated rat vas deferens. The results confirm previous reports that Yohimbine and Phentolamine are potent presynaptic α-adrenoceptor blocking agents[12, 13]. Results on the benzodioxane series of drugs show that though their postsynaptic antagonistic potencies vary over a wide range, their presynaptic potencies exhibit a very close range of activity.

The benzodioxane WB 4101 has been shown to be a very potent antagonist at both peripheral and central postsynaptic α-adrenoceptors[10, 14]. Recently it has been suggested[11] that the potent postsynaptic α-adrenoceptor antagonism exhibited by WB 4101 depends not only on the benzodioxan moiety of the compound but that incorporated into the receptor is at least one other subsite for aromatic interaction at a specific distance from the subsite for nitrogen interaction and that the 2,6-dimethoxy substituents play an important role in the drug-receptor interaction.

The structural requirements for WB 4101 necessary for potent α-adrenoceptor blocking activity on postsynaptic α-adrenoceptors are not paralleled on its presynaptic α-adrenoceptor blocking activity. Neither alteration of the methoxy substituent groups nor an increase in chain length between the nitrogen and benzene ring significantly affected presynaptic α-adrenoceptor blocking activity, whilst such structural modification had previously been shown to produce a marked reduction in postsynaptic α-adrenoceptor blocking activity.

It has been suggested[15, 16] that antagonists of the α-adrenoceptor (postsynaptic) which exhibit widely differing chemical structure, may bind to the central, nucleophilic group within the receptor, as do agonists, but that the rest of the molecule may bind over a wide area around this group. It is possible that this situation also exists on presynaptic α-adrenoceptors. However, it has been shown[5, 13] that the relative degree of activity for both agonists and antagonists differs markedly between pre and postsynaptic receptors, and the results of this present study lend weight to these observations in that a series of α-adrenoceptor blocking agents with closely related chemical structures exhibit totally different degrees of relative activity on the two receptor populations. Results therefore suggest that pre- and postsynaptic α-adrenoceptors are not identical but that the benzodioxan moiety may be important for the interaction at both sites.

It is possible that the benzodioxanes interact with a different type of presynaptic α-adrenoceptor with perhaps different recognition sites to that occupied by phentolamine and yohimbine. Recently Bryant *et al.*[17] found that both phenoxybenzamine and phentolamine in contrast to piperoxan, a benzodioxane, had no effect in enhancing the stimulation—evoked overflow of noradrenaline in guinea-pig hypothalamic slice; a result which is suggestive of differences in α-adrenoceptor organization at the presynaptic level.

Experiments are in progress to determine the presynaptic activity of these benzodioxanes in other tissues with a view that they will reveal whether pre- and postsynaptic α-adrenoceptors can indeed be considered as two major subclasses with respect to drug sensitivity or whether the classification by Langer[1] of postsynaptic receptors as α_1 and presynaptic receptors as α_2 should be extended.

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