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Pre-junctional α_2-adrenoceptor activity of B-HT920

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An in-vitro study has been carried out on the pre-junctional α_2-adrenoceptor activity of the thiazoloazepine derivative B-HT 920 (6-allyl-2-amino-5,6,7,8-tetrahydro-4H-thiazolo-[4,5-d]-azepine) using isolated, field-stimulated rat vas deferens and guinea-pig ileum. The α_2-selective agonists clonidine (an imidazoline derivative) and α-methyl noradrenaline (a β-phenethylamine derivative) were compared. Results show that B-HT 920 is a potent agonist on pre-junctional α_2-adrenoceptors and is competitively antagonized by the selective α_2-adrenoceptor antagonist yohimbine. The characteristics of the pharmacological responses obtained with B-HT920 indicate that it interacts with the receptor in an imidazoline-like, rather than a β-phenethylamine-like, manner.

The sub-division of α-adrenoceptors is now accepted as being based on the relative activity of agonists and antagonists on the receptor subtypes (Berthelson & Pettinger 1977; Starke & Langer 1979) independent of their anatomical location. Thus α_1-adrenoceptors are selectively activated by phenylephrine and methoxamine and antagonized by prazosin; whilst α_2-adrenoceptors are selectively activated by clonidine and α-methyl noradrenaline and antagonized by yohimbine (Melchiorre 1980).

There is, however, increasing evidence that the sub-classification of α-adrenoceptors must be extended. Multiple binding sites on α-adrenoceptors in the c.n.s. have been described (Weinreich et al 1981; Weinreich & Seeman 1981) and more specifically, it has been postulated that the α_2-adrenoceptor may be sub-divided into high- and low-affinity binding sites (Hoffman et al 1980; Rouot et al 1980; Jarrott et al 1982), as indicated by the differential pharmacological responses observed between agonists of different chemical structure *viz*: the phenethylamine-like drugs (adrenaline and α-methyl noradrenaline) and the imidazoline-like drugs (clonidine and tramazoline) (Ruffolo et al 1977; Finch et al 1978; Mottram 1982).

Kobinger & Pichler (1980) and Van Meel et al (1981) have recently reported that B-HT 920 (6-allyl-2-amino-5,6,7,8-tetrahydro-4H-thiazolo-[4,5-d]-azepine) is a highly selective agonist on the α_2-adrenoceptor in-vivo, and a thiazolazepine derivative, which is not structurally related to phenethylamine or the imidazoline type of adrenoreceptor agonists. This study was therefore undertaken to investigate the activity of B-HT920 as an α_2-adrenoceptor agonist in-vitro and to determine whether it exhibits clonidine-like, or phenethylamine-like activity using field-stimulated rat vas deferens and guinea-pig ileum.

MATERIALS AND METHODS

Stripped vasa deferentia from male Wistar rats, (175–225 g) were suspended in organ baths in a solution consisting of (mM) NaCl 119, $CaCl_2$ 2·6, $NaHCO_3$ 25, KCl 4·7, KH_2PO_4 1·2 and glucose 11·1, maintained at 37 °C and aerated with a mixture of 5% CO_2 in 0_2. 3μM cocaine was incorporated into the bathing solution to inhibit the neuronal uptake process (this concentration was chosen since Pelayo et al (1980) and Langer & Dubocovich (1981) have shown that higher concentrations decrease imidazoline-induced inhibition of transmitter release). Isometric contractions were recorded by means of force-displacement transducers coupled to polygraph recorders (Devices). Guinea-pig ileum preparations were set up according to the method of Drew (1978). Field stimulation of the tissues was produced by square wave pulses of 1 ms (guinea-pig) or 3 ms (rat vas deferens) duration at a frequency of 0·1 Hz using sub-maximal voltages generated by Grass Stimulators.

Cumulative dose-response curves for the clonidine-, B-HT920- and α-methylnoradrenaline-induced inhibition of the twitch response were recorded before and after exposure to varying concentrations of the antagonists yohimbine, WB 4101 (2-*N*(2,6-dimethoxy-phenoxyethyl)-aminomethyl)-1,4-benzodioxan HCl), WB 4085 (2-*N*(2′-methoxyphenoxyethyl)aminomethyl)-1,4-benzodioxan HCl) and WB 4093 (2-*N*(3′-phenoxy-propyl)aminomethyl-4^1,4-benzodioxan HCl). From the dose-response curves dose ratios were calculated and pA_2 values obtained using the method of Arunlakshana & Schild (1959). In experiments where α-methylnoradrenaline was used as the pre-junctional α_2-adrenoceptor agonist, prazosin (50 nM) was added to the bathing solution to prevent post-junctionally mediated contractions of the vasa (Brown et al 1980).

RESULTS

B-HT920, like clonidine and α-methyl noradrenaline, produced an effective inhibition of the electrically-evoked twitch response of the rat vas deferens and guinea-pig ileum (Table 1). The IC50 values for clonidine were similar in both rat vas deferens and guinea-pig ileum and closely paralleled the values previously reported by Drew (1977, 1978). B-HT920 exhibited a 2-fold difference in IC50 values between rat vas deferens and guinea-pig ileum (Fig. 1). Responses to B-HT920 begin at 10 nM in both tissues but the two curves exhibit differing slopes, which may reflect a different affinity for B-HT920 on the two receptor populations or indicate a difference in the ability of the drug to reach the receptor site in the two tissues. There was no statistically significant difference between the IC50 values for α-methylnoradrenaline on rat vas deferens and guinea-pig ileum.

Table 1. Inhibitory activity (measured as IC50 values $\pm$ s.e.m.) of agonists on field stimulated rat vas deferens and guinea-pig ileum.

	Rat vas deferens	Guinea-pig ileum
Clonidine	3·5 ± 0·2 nM	2·5 ± 0·5 nM
B-HT920	76 ± 18 nM	134 ± 15 nM
α-Methylnoradrenaline	98 ± 21 nM	59 ± 22 nM

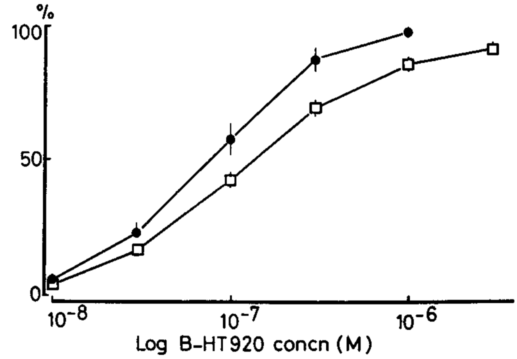

FIG. 1. Cumulative dose-response curves for the B-HT920 inhibition of field-stimulated rat vas deferens (●——●) and guinea-pig ileum (□——□). Each point is the mean ± s.e. from 11 (rat vas deferens) and 15 (guinea-pig ileum) experiments.

The α_2-selective antagonist, yohimbine, produced a competitive antagonism against the clonidine and B-HT920-induced inhibition of the twitch response in rat vas deferens (Fig. 2 A, B) from which pA_2 values of 8·21 ± 0·15 and 7·76 ± 0·07 respectively, were calculated (see Table 2). Against α-methylnoradrenaline, however, yohimbine did not produce the same degree of competitive antagonism. Below 1 μM it does not antagonize α-methylnoradrenaline. At 2 μM yohimbine shifts the dose-response curve for α-methylnoradrenaline to the right but 10 and 50 μM failed to shift the curve futher (Fig. 2C). Similarly the potent α-adrenoceptor antagonist WB4101 (Mottram & Kapur 1975) failed to produce parallel shifts in the dose-response curves for α-methylnoradrenaline,

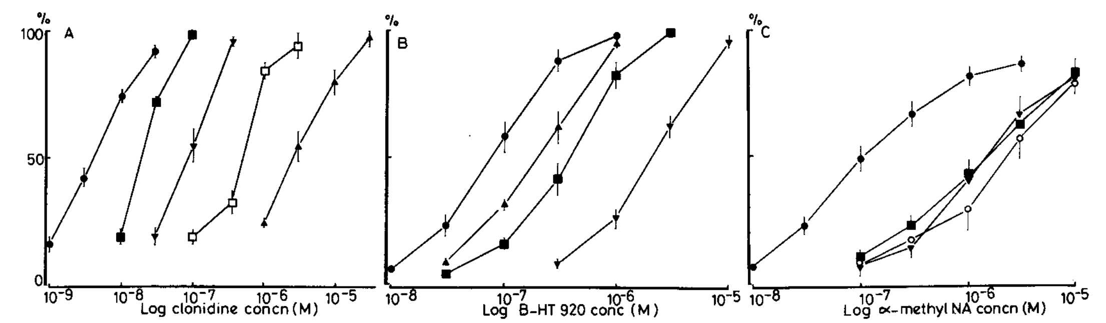

FIG. 2. The effect of increasing concentrations of yohimbine on the cumulative dose-response curves for the clonidine-, B-HT920 and α-methylnoradrenaline-induced inhibition of field-stimulated rat vas deferens. A. (●——●) control dose-response curve for clonidine. Dose-response curves for clonidine in the presence of 20 nM (■——■), 200 nM (▼——▼), 2 μM (□——□) and 20 μM (▲——▲) yohimbine. Each point is the mean ± s.e. from 7 experiments. B. (●——●) control dose-response curve for B-HT920. Dose-response curves for B-HT920 in the presence of 30 nM (▲——▲), 100 nM (■——■) and 1 μM (▼——▼) yohimbine. Each point is the mean ± s.e. from 6 experiments. C. (●——●) control dose-response curve for α-methyl noradrenaline. Dose-response curves for α-methylnoradrenaline in the presence of 2 μM (▼——▼), 10 μM (■——■) and 50 μM (●——●) yohimbine. Each point is the mean s.e. from 5 experiments.

whilst competitively antagonizing both clonidine and B-HT920, (see Table 2). Unlike yohimbine, WB4101 produced similar antagonistic activity against both the agonists (pA_2: 6·24 and 6·44), as did WB4085 and WB4093.

Table 2. Antagonist activity, expressed as pA_2 values (Arunlakeshana & Schild 1959), for a series of antagonists against the inhibitory activity of clonidine and B-HT920 on the field-stimulated vas deferens.

	Clonidine			B-HT920		
	$pA_2 \pm$ s.e.	Slope	n	$pA_2 \pm$ s.e.	Slope	n
Yohimbine	8·21 ± 0·15	0·79	5	7·76 ± 0·07	0·81	6
WB4101	*6·24 ± 0·03	1·33	8	6·44 ± 0·11	1·07	5
WB4085	*6·15 ± 0·05	0·91	6	6·40 ± 0·12	1·05	5
WB4093	*6·58 ± 0·02	1·12	6	6·42 ± 0·02	1·15	5

* Data previously reported (Kapur & Mottram 1978)

The antagonistic activity of yohimbine and WB4101 against the clonidine- and B-HT920-induced inhibition of the twitch response in guinea pig ileum is shown in Table 3. Results exhibit a similar profile of activity to that seen in the rat vas deferens. α-Methylnoradrenaline again proved resistant to further competitive blockade at concentrations of the antagonists above 3 μM.

Table 3. Antagonistic activity (expressed as pA_2 values) for yohimbine and WB4101 against the inhibitory activity of clonidine and B-HT920 on field-stimulated guinea-pig ileum.

	Clonidine			B-HT920		
	$pA_2 \pm$ s.e.	Slope	n	$pA_2 \pm$ s.e.	Slope	n
Yohimbine	7·74 ± 0·24	1·16	7	7·52 ± 0·50	0·78	6
WB4101	6·62 ± 0·49	1·01	7	6·52 ± 0·48	0·96	7

During experiments in which tissues were allowed to recover following cumulative dosing with the three agonists under investigation, recovery was extremely rapid after α-methylnoradrenaline, whereas after clonidine or B-HT920 it was prolonged even after repeated washes with fresh Krebs solution. These observations applied equally in both the rat vas deferens and the guinea-pig ileum.

DISCUSSION

B-HT920 has previously been shown to be a highly selective α_2-adrenoceptor agonist, in-vivo, exhibiting a higher α_2/α_1 agonist activity that clonidine (Kobinger & Pichler 1980). In the present in-vitro study B-HT920 has been shown to inhibit the electrically-evoked twitch response of rat vas deferens and guinea-pig ileum. B-HT920 was less potent than clonidine having a log potency ratio of 1·34 in rat vas deferens and 1·73 in the guinea-pig ileum. Kobinger & Pichler (1980) have reported a like difference in potency between B-HT920 and clonidine in increasing blood pressure in pithed rats, and Van Meel et al (1981) have shown B-HT920 to be less potent than clonidine on presynaptic cardiac α_2-adrenoceptors also in pithed rats.

IC50 values for B-HT920 were found to be significantly different in the rat vas deferens and the guinea-pig ileum (Table 1), as shown by the difference in the slopes of the two curves (Fig. 1). It is possible that this difference in effect on the two tissues may be due to the α_2-adrenoceptors having slightly different properties in various tissues, as suggested by Reichenbacher et al (1982). Alternatively, the increase in the slope of the dose-response curve to B-HT920 in the rat vas deferens compared with the guinea-pig ileum, may be explained by the fact that B-HT920 has potent stimulatory effects on dopamine autoreceptors (Andén et al 1982). It has been established by Tayo (1979) that, although pre-junctional α_2-adrenoceptors of both tissues are the same, the pre-junctional dopamine receptors differ in the two tissues. Additionally Gyorgy et al (1981) have shown that dopaminergic agonists increase the steepness of the slope of the frequency-response curves in field-stimulated rat vas deferens.

Yohimbine is one of the most selective blocking agents of α_2-adrenoceptors (Starke et al 1975; Borowski et al 1977; Weitzell et al 1979) and was chosen to antagonize the clonidine-, α-methylnoradrenaline- and B-HT920-induced inhibition of field-stimulated rat vas deferens and guinea-pig ileum, tissues reported to have the same type of pre-junctional α_2-adrenoceptor (Drew 1978). Results in Fig. 2, Table 2 and Table 3 indicate that B-HT920 is competitively blocked by yohimbine in a manner similar to that observed with clonidine, in marked contrast to the type of blockade that yohimbine exerts on α-methylnoradrenaline against which competitive antagonism only extends from 1 to 2 μM yohimbine. This has previously been reported by Mottram (1982) and concurs with other reports of striking differences between the α-adrenoceptor agonist activities of β-phenethylamines and imidazolines (Ruffolo et al 1977, 1979; Kobinger et al 1980; Miller et al 1980). B-HT920 would, therefore, appear to fall into the 'imidazoline-type' of α_2-adrenoceptor agonists.

A notable feature of the antagonism by 1,4 benzodioxanes, of clonidine at pre-junctional α_2-

adrenoceptors is the similarity in the potency as measured by their pA_2 values. This contrasts with their disparate potencies against the post-junctionally, α_1-adrenoceptor-mediated responses of noradrenaline (Kapur & Mottram 1978; Mottram 1981). We investigated whether such a consistency of antagonism existed towards B-HT920, a non-imidazoline derivative, on pre-junctional α_2-adrenoceptors. That such a consistency does exist is shown in Table 2 and confirms that B-HT920 exerts its α_2-adrenoceptor activity through an imidazoline-like interaction with the receptor.

Further support for an imidazoline-, rather than a β-phenethylamine-, type interaction of B-HT920 with α_2-adrenoceptors comes from the observed rate of recovery following agonist inhibition of the twitch response. In both tissues used, α-methyl noradrenaline exhibited a rapid recovery of the twitch response after a single change of Krebs solution, as has been previously reported by Gyorgy et al (1981) and Mottram (1982). This rapid reversal of the α-methylnoradrenaline response may reflect a low affinity between the agonist and the pre-junctional α-adrenoceptor and may account for the failure of α-methylnoradrenaline to induce a 100% inhibition of the twitch response in the rat vas deferens (Fig. 2C). The recovery following inhibition by B-HT920 was prolonged even with repeated changes of the Krebs solution, and was therefore in accord with the recovery pattern of clonidine.

Thus I conclude that B-HT920 is a potent agonist on prejunctional α_2-adrenoceptors in-vitro. Results add further support to the concept that β-phenethylamines and imidazolines interact through different sites on α-adrenoceptors and it appears from this study that derivatives of thiazoloazepine, such as B-HT920, interact through the imidazoline site.

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