Br. J. Pharmac. (1978), **63**, 671–675

THE EFFECTS OF α-ADRENOCEPTOR ANTAGONISTS ON CONTRACTILE RESPONSES TO 5-HYDROXYTRYPTAMINE IN DOG SAPHENOUS VEIN

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1 5-Hydroxytryptamine (5-HT) contracted isolated saphenous vein strips of the dog, producing a biphasic concentration-effect curve. The first phase occurred with low concentrations of 5-HT (1.0×10^{-8} to 5.0×10^{-6} mol/l) with a plateau between 1.0×10^{-6} mol/l and 1.0×10^{-5} mol/l. The second phase occurred with high concentrations of 5-HT (greater than 1.0×10^{-5} mol/l).

2 The α-adrenoceptor antagonists, phentolamine (5.0×10^{-8} to 5.0×10^{-7} mol/l), labetalol (1.0×10^{-6} to 1.0×10^{-5} mol/l) and thymoxamine (1.0×10^{-6} to 1.0×10^{-5} mol/l), antagonized responses to high concentrations of 5-HT but responses to low concentrations of 5-HT were not antagonized.

3 The effects of high concentrations of 5-HT were antagonized by cocaine (1.0×10^{-6} to 1.0×10^{-5} mol/l) and were not evident in veins removed from dogs pretreated with syrosingopine.

4 It is concluded that in the saphenous vein, low concentrations of 5-HT act directly on specific 5-HT receptors and that high concentrations of 5-HT also act indirectly on α-adrenoceptors by displacing noradrenaline from neuronal stores.

Introduction

As a part of a study on the interactions of antagonists with receptors for 5-hydroxytryptamine (5-HT) and noradrenaline in vascular smooth muscle, experiments were carried out on the isolated saphenous vein of the dog. In this preparation the concentration-effect curve for 5-HT was found to consist of two distinct phases. Since it is known that 5-HT can interact with α-adrenoceptors in addition to a specific action on 5-HT receptors (Apperley, Humphrey & Levy, 1976), the effects of α-adrenoceptor antagonists on these contractile responses to 5-HT were investigated in order to characterize the receptors involved.

Methods

Dog saphenous vein strip

Dog saphenous vein strips were isolated and prepared as described previously (Humphrey, 1978) so that isometric contractions could be recorded.

Agonists and antagonists

The potency of agonists and antagonists was quantified in a similar manner to that described previously (Apperley *et al.*, 1976). In order to investigate the effect of antagonists on both phases of the 5-HT concentration-effect curve the first curve was obtained with concentrations up to only 5×10^{-6} mol/l. The second curve, either in the absence (control) or presence of varying concentrations of antagonist, was obtained with 5-HT concentrations up to 2.0×10^{-4} mol/l. In this way no desensitization occurred such that the dose-ratios of the first phase of the 5-HT control curves were close to one. However, only a rough estimate of antagonist potency against the second phase of the 5-HT concentration-effect curve could be made, by comparing the second phase in the strips exposed to the antagonists with that in the control strip for each experiment. Since complete curves for the second phase could not be obtained, an approximate pA_2 was obtained from the dose-ratio produced by a single antagonist concentration using the Gaddum equation (Gaddum, 1957).

Syrosingopine pretreatment

Dogs were pretreated with syrosingopine (0.5 mg/kg intravenously) 18 to 24 h before induction of anaesthesia. The syrosingopine base (Ciba) was dissolved in the manner described by Orlans, Finger & Brodie (1960).

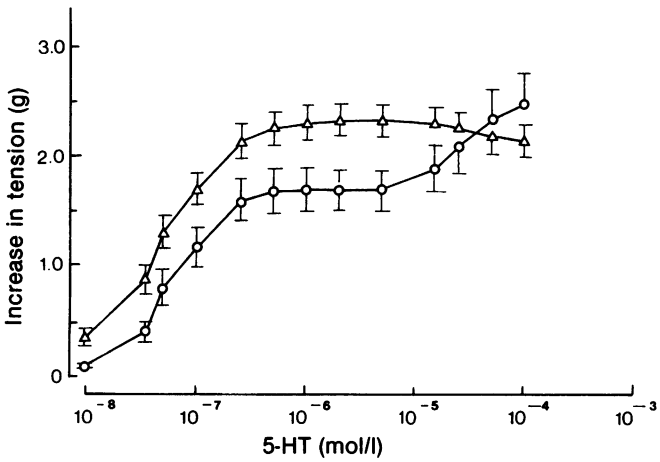

Figure 1 Dog isolated saphenous vein. Concentration-effect curves for contractions produced by 5-hydroxytryptamine (5-HT) in veins removed from untreated dogs (○) and dogs pretreated with syrosingopine (△), 0.5 mg/kg intravenously 18 to 24 h previously. Each point is the mean of 20 determinations and vertical lines show s.e. means. Note the absence of a second phase of the concentration-effect curve in veins from syrosingopine pretreated dogs.

Drugs

The following drugs were used: 5-hydroxytryptamine (serotonin) creatinine sulphate (5-HT), mol. wt. 405.4 (Koch-Light); phentolamine methane sulphonate, mol. wt. 377.5 (Ciba); labetalol hydrochloride, mol. wt. 364.9 (AH 5158, Allen & Hanburys); thymoxamine hydrochloride, mol. wt. 315.8 (Warner) and cocaine hydrochloride, mol. wt. 339.8 (May & Baker).

Stock solutions of 5-HT ($>1.0 \times 10^{-2}$ mol/l) were made up in distilled water and all subsequent dilutions were dissolved in isotonic saline (0.9% w/v NaCl solution). Phentolamine, labetalol, thymoxamine and cocaine were dissolved in isotonic saline.

Results

Agonists

5-Hydroxytryptamine (1.0×10^{-8} to 1.0×10^{-4} mol/l) contracted dog saphenous vein strips producing a biphasic concentration-effect curve. The first phase occurred with low concentrations of 5-HT such that a plateau of 1.70 ± 0.20 g (mean ± s.e. mean, $n = 20$) was reached at 5.0×10^{-6} mol/l (Figure 1). The second phase occurred with higher concentrations of 5-HT such that a total contraction of 2.48 ± 0.30 g was recorded at 1.0×10^{-4} mol/l.

In contrast, noradrenaline (1.0×10^{-7} to 1.0×10^{-4} mol/l) and methoxamine (1.0×10^{-7} to 1.0×10^{-4} mol/l) produced single phase concentration-effect curves with maximal contractions of 3.56 ± 0.24 g and 3.94 ± 0.33 g respectively at 1.0×10^{-4} mol/l (Humphrey, 1978).

Table 1 The interaction of 5-hydroxytryptamine (5-HT), noradrenaline and methoxamine with α-adrenoceptor antagonists in dog saphenous vein

		5-HT (a)	*5-HT (b)*	*Noradrenaline*†	*Methoxamine*†
Phentolamine	pA_2	6.11 (5.92–6.30)	7.74* (7.55–7.93)	7.00 (6.69–7.31)	7.90 (7.66–8.14)
	Slope	0.95 (0.54–1.36)	—	1.01 (0.70–1.32)	1.00 (0.80–1.20)
Labetalol	pA_2	<5.0	5.95* (5.19–6.71)	6.25 (5.22–7.28)	7.06 (6.87–7.25)
	Slope	—	—	0.54 (0.13–0.95)	0.95 (0.77–1.13)
Thymoxamine	pA_2	<5.0	6.07* (5.78–6.36)	5.37 (4.07–6.67)	7.11 (6.80–7.42)
	Slope	—	—	0.50 (0.05–0.95)	0.83 (0.66–1.00)

Each value is the mean of 4–8 observations (95% confidence limits).
(a) = 1.0×10^{-8} to 5.0×10^{-6} mol/l; (b) = 1.0×10^{-5} to 2.0×10^{-4} mol/l. † Results from Humphrey (1978); * approximate pA_2 values (see Methods).

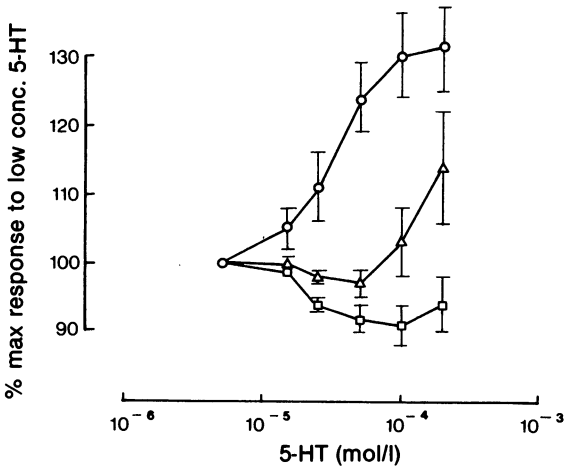

Figure 2 Dog isolated saphenous vein. Concentration-effect curves for contractions produced by high concentrations of 5-hydroxytryptamine (5-HT). The ordinate scale represents tension changes relative to the plateau response produced by low concentrations of 5-HT (see text). Each point is the mean of 5 determinations and vertical lines show s.e. means. The contractions evident in the control curve (○) were reduced or abolished by cocaine at 1.0×10^{-6} mol/l (△) and 1.0×10^{-5} mol/l (□). The apparent relaxation seen in the presence of cocaine represents the spontaneous fade of the plateau response produced by low concentrations of 5-HT

Antagonists

The effects of the α-adrenoceptor antagonists phentolamine, labetalol and thymoxamine on responses to 5-HT are summarized in Table 1.

Effect on low concentrations of 5-hydroxytryptamine (1.0×10^{-8} to 5.0×10^{-6} mol/l). Labetalol and thymoxamine did not antagonize responses to low concentrations of 5-HT even at concentrations up to 1.0×10^{-5} mol/l. Only high concentrations of phentolamine ($> 7.5 \times 10^{-7}$ mol/l) antagonized these responses to 5-HT, producing parallel displacement of the concentration-effect curves (Table 1).

Effect on high concentrations of 5-hydroxytryptamine (1.0×10^{-5} to 2.0×10^{-4} mol/l). Labetalol (1.0×10^{-6} to 1.0×10^{-5} mol/l), thymoxamine (1.0×10^{-6} to 1.0×10^{-5} mol/l) and phentolamine (5.0×10^{-8} to 5.0×10^{-7} mol/l) antagonized responses to high concentrations of 5-HT. These concentrations of antagonists were similar to those required to antagonize responses to noradrenaline or methoxamine (Table 1).

Effect of syrosingopine pretreatment

5-Hydroxytryptamine concentration-effect curves were obtained in veins removed from dogs pretreated with syrosingopine. A maximal contraction of 2.34 ± 0.15 g (mean ± s.e. mean, $n = 20$) occurred at 2.0×10^{-6} mol/l with no further contraction even when concentrations of 5-HT up to 1.0×10^{-4} mol/l were used (Figure 1).

Effects of cocaine

Cocaine (1.0×10^{-6} to 1.0×10^{-5} mol/l) had no effect on responses to low concentrations of 5-HT but responses to high concentrations of 5-HT were reduced or abolished (Figure 2).

Discussion

5-Hydroxytryptamine can act both directly (Apperley, *et al.*, 1976; Fozard, 1976; Edvinnson & Hardebo, 1976) and indirectly (Innes, 1962; Fozard & Mwaluko, 1976) on receptors for noradrenaline. In this study 5-HT has been shown to interact with α-adrenoceptors in yet another smooth muscle preparation, namely the dog saphenous vein. At low concentrations 5-HT acts on a specific 5-HT receptor (Apperley, Humphrey & Levy, 1977; Feniuk, Humphrey & Levy, 1977) and its effects are not blocked by phentolamine, labetalol or thymoxamine in concentrations that antagonize α-adrenoceptor agonists. However, high concentrations of 5-HT appear to produce contraction by activation of α-adrenoceptors, since this phase of the 5-HT concentration-effect curve was antagonized by phentolamine, labetalol and thymoxamine in concentrations that antagonized noradrenaline and methoxamine (see below). The finding that the second phase of the 5-HT concentration-effect curve was absent in veins removed from syrosingopine-pretreated animals indicates that this α-adrenoceptor activation was indirect. It is known that syrosingopine pretreatment will deplete neuronal stores (Orlans *et al.*, 1960) and therefore the absence of second phase contractile responses to high concentrations of 5-HT in preparations from pretreated animals is consistent with an indirect action as a result of noradrenaline release. It is interesting that the veins removed from syrosingopine-pretreated animals were more sensitive to 5-HT and that the maximum response was larger than the maximum of the first phase of the controls (Figure 1). This may reflect the usual super-sensitivity seen after treatment with reserpine-like compounds (Carrier & Holland, 1965).

5-Hydroxytryptamine can cause the release of noradrenaline either by uptake and stoichiometric displacement from neuronal stores, as with tyramine

(Gillis, 1964), or by depolarization of nerve endings after activation of specific 5-HT receptors (Haefely, 1974; Fozard & Mwaluko, 1976). Responses to high concentrations of 5-HT were abolished by cocaine which strongly suggests the involvement of the adrenergic uptake$_1$ mechanism (Iversen, 1967). Hence, it appears that in high concentrations 5-HT is removed by uptake$_1$, as previously shown (Thoa, Eccleston & Axelrod, 1969; Jester & Horst, 1972) and subsequently displaces noradrenaline from neuronal stores. The phenomenon is obvious in the saphenous vein because the contractile response to the released noradrenaline is additive with the smaller maximal response (compared to that of noradrenaline) which results from activation of 5-HT receptors, and because there are ample neuronal stores from which noradrenaline can be displaced (Brandao, 1976; Humphrey, 1978). This is in contrast to the rabbit aorta where the effect of the uptake$_1$ mechanism is not as marked (Brandao, 1976; Trendelenburg, 1972) and consequently, an indirect effect of high concentrations of 5-HT is small or absent (Humphrey, unpublished result).

The potency of α-adrenoceptor antagonists is markedly affected by uptake$_1$ in the saphenous vein when noradrenaline is the agonist (Humphrey, 1978) and this complicates the interpretation of the pA_2 values obtained against 5-HT. The pA_2 values obtained for labetalol and thymoxamine against high concentrations of 5-HT are similar to those obtained against noradrenaline but lower than those obtained against methoxamine (Humphrey, 1978) which has negligible affinity for uptake$_1$ (Iversen, 1965). The pA_2 value for phentolamine against high concentrations of 5-HT is closer to that obtained against methoxamine than noradrenaline. The reason for the difference between the results with phentolamine and those with labetalol and thymoxamine is not known. However, it must be emphasized that the pA_2 values against high concentrations of 5-HT are approximations (see methods) and that these differences are small compared to the differences in potency of these antagonists against methoxamine and low concentrations of 5-HT.

In the present context the important point is that the high potency of the antagonists against high concentrations of 5-HT supports the idea that 5-HT releases noradrenaline which is subsequently antagonized by α-adrenoceptor blockade.

The displacement of noradrenaline from neuronal stores by 5-HT is unlikely to be of physiological significance in view of the high concentrations of 5-HT necessary to achieve release. However, it could interfere with attempts to analyse the potency of 5-HT antagonists. In the presence of 5-HT receptor blockade, the 5-HT concentrations may have to be increased to a level that is also sufficient to release noradrenaline. The subsequent α-adrenoceptor activation would then lead to an underestimation of the potency of the 5-HT antagonist.

It is a pleasure to acknowledge the competent technical assistance of Mrs Janet Pledger.

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(*Received February 3, 1978.*
Revised March 8, 1978.)