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β-ADRENOCEPTOR BLOCKING AGENTS AND RESPONSES TO ADRENALINE AND 5-HYDROXYTRYPTAMINE IN RAT ISOLATED STOMACH AND UTERUS

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1 Four β-adrenoceptor blocking agents, (±)- and (+)-propranolol, practolol and oxprenolol, were found to antagonize, apparently competitively, the responses of both the rat isolated stomach and uterus to 5-hydroxytryptamine (5-HT).

2 The pA_2 values for each of these agents as antagonists of the contractile action of 5-HT on the rat stomach were found to be: (±)-propranolol, 6.08; (+)-propranolol, 4.94; practolol, 3.43; and oxprenolol, 5.99. These values were very similar to the corresponding figures for antagonism of 5-HT-induced contractions of the uterus.

3 pA_2 values for antagonism of adrenaline-induced relaxations by the four blocking agents on the rat stomach and uterus did not differ from the values for 5-HT blockade.

4 To antagonize contractile responses to acetylcholine of the rat stomach it was necessary to give 100 times more (±)-propranolol than was needed to antagonize responses to 5-HT.

Introduction

The α-adrenoceptor blocking agent, phenoxybenzamine, can antagonize the contractile responses to both adrenaline and 5-hydroxytryptamine (5-HT) in various types of smooth muscle (Gyermek, 1961; Innes, 1962; Gay, Rand & Wilson, 1967). Innes (1962) showed that contractile responses to both 5-HT and adrenaline were not blocked by phenoxybenzamine in strips of cat spleen which were protected by a high concentration of either 5-HT or adrenaline throughout exposure to the blocking agent. This indicated that adrenaline and 5-HT produced their effects by combining with the same receptors.

In other types of smooth muscle, such as the rat uterus and stomach, and guinea-pig trachea, where catecholamines cause relaxation by stimulating adrenoceptors, 5-HT causes contractions. Here too the effects of 5-HT, but not catecholamines, are antagonized by relatively low concentrations of phenoxybenzamine (Vane, 1957; Erspamer, 1953).

Catecholamine-induced relaxation, on the other hand, can be effectively blocked by low concentrations of propranolol (Furchgott, 1972). These findings suggested that the receptors through which the contractile effects of 5-HT are mediated resembled the α-adrenoceptors for catecholamines, but differed from β-adrenoceptors. We were, therefore, surprised to find while studying the effects of propranolol on various types of smooth muscle, that this agent appeared to antagonize competitively the contractions elicited by 5-HT at the same concentrations at which it blocked the relaxations produced by adrenaline.

The purpose of this investigation was to study in more detail the effect of propranolol on responses to 5-HT in preparations relaxed by catecholamines. Attempts were made to differentiate the specific action of propranolol on 5-HT receptors from a general depressant effect of this agent on smooth muscle, by use of another agonist and by comparing its effects with those of three other β-receptor blocking agents.

Methods

Rat isolated stomach preparation

Male Wistar albino rats weighing 120-150 g were killed, the stomach removed, and a strip of the fundus prepared as described by Vane (1957). The preparation was set up under a tension of 1 to 2 g in a 10 ml isolated organ bath containing Tyrode solution, (NaCl, 8.0; KCl, 0.2; $CaCl_2$, 0.2; $MgSO_4$, 0.2; $NaHCO_3$, 1.0; $NaHPO_4$, 0.05; glucose

1.0 g/l), at 37°C, bubbled with 95% O_2 and 5% CO_2.

Movements were recorded by means of a Grass force displacement transducer on a multichannel Grass polygraph.

Doses of 5-HT were injected into the bath every 3 min and left in contact for 30 seconds. Dose-response curves were determined with at least four dose levels of the amine, each dose being given at least twice.

The response of the tissue to 5-HT was constant after 30-45 min after which the blocking agents could be introduced.

To obtain graded relaxations to adrenaline on the stomach preparation we found it necessary to increase the tone of the tissue by maintaining a concentration of 2.6×10^{-6} M 5-HT in the Tyrode solution (Armitage & Vane, 1964). Adrenaline was administered at 4 min intervals and left in contact with the tissues for 30 seconds. Four dose levels were used, each twice, to establish control dose-response curves.

To determine the effect of β-receptor blocking agents on the response to both 5-HT and adrenaline, the antagonists were placed in the reservoir of Tyrode solution supplying the isolated organ bath and the tissue was washed with the drug solution for 5 min before injection of either 5-HT or adrenaline. When the responses to 5-HT or adrenaline were again constant, usually 10-15 min after contact with the antagonist, dose-response curves were redetermined.

To study the relative effect of propranolol on 5-HT and acetylcholine-induced responses, two stomach strips were set up simultaneously in adjacent baths. Dose-response curves to each agonist were obtained on one strip before and after the addition of antagonist. On the other strip only one agonist was tested at a time, so that if one agonist were to influence responses to the other, this might be detected.

Rat isolated uterus preparation

Virgin female Wistar albino rats weighing 150 to 200 g. were injected with stilboestrol (5 mg/ml in olive oil), 1 mg/kg intraperitoneally. Twenty-four hours later they were killed and the uterus removed, the horns separated and each set up in a 10 ml isolated organ bath, containing de Jalon's solution (NaCl, 9.0; KCl, 0.4; $CaCl_2$, 0.06; $NaHCO_3$, 0.15; glucose, 1.0 g/l), at 32°C, bubbled with 95% O_2 and 5% CO_2.

After allowing the tissue to rest for 30 min to reduce spontaneous contractions, dose-response curves to-5 HT were determined, by injection of at least three doses, each twice. The amine was left in contact with the tissue for 30 s and doses were given every 4 minutes.

The dose-response curves were redetermined in the presence of various concentrations of β-adrenoceptor blocking agents as described for the rat stomach preparation.

Dose-response curves to adrenaline on the uterus were obtained as described by Gaddum, Peart & Vogt (1949). Adrenaline was left in contact with the tissue 2 min before carbachol was injected. Three doses of adrenaline were used to establish a dose-response curve in the absence and presence of various concentrations of β-adrenoceptor blocking agents, as described for the rat stomach preparation.

Drugs

(−)-Adrenaline base (Boehringer Ingelheim); (±)-oxprenolol, (Ciba); acetylcholine bromide; isoprenaline hydrochloride; (±)-propranolol hydrochloride (Abic Ltd); (+)-propranolol hydrochloride (I.C.I. Ltd); practolol (Abic Ltd); 5-hydroxytryptamine creatinine sulphate; stilboestrol diproprionate.

Results

On the rat stomach preparation (±)-propranolol in concentrations of 3.8×10^{-7}M to 1.5×10^{-4}M caused a parallel shift in the dose-response curves to 5-HT without a depression of the maximum response. At these concentrations there was no significant reduction in the contractions produced by acetylcholine. Concentrations exceeding 1.5×10^{-4} M reduced the slope of the dose-response curves to 5-HT and the height of thee maximum response and also depressed responses to acetylcholine (Figures 1 and 2). The pD_2 (Ariëns & Van Rossum, 1957) of (±)-propranolol for physiological antagonism of acetylcholine was found to be 3.70.

Relaxations produced by adrenaline or isoprenaline were antagonized, apparently competitively, by virtually identical amounts of (±)-propranolol. The (+)-isomer of propranolol was a weaker antagonist than the racemic mixture, of both adrenaline and 5-HT. The ratio of antagonistic potency of (+)- to (±)- propranolol for both adrenaline and 5-HT on the rat isolated stomach was 1 to 16. Practolol also proved to be a much weaker antagonist of both amines than (±)-propranolol. The pA_2 values of these blocking agents and those of oxprenolol, against 5-HT and adrenaline effects on the rat stomach are shown in Table 1. There was no significant difference in the pA_2 values for adrenaline or 5-HT antagonism for either antagonist on this preparation.

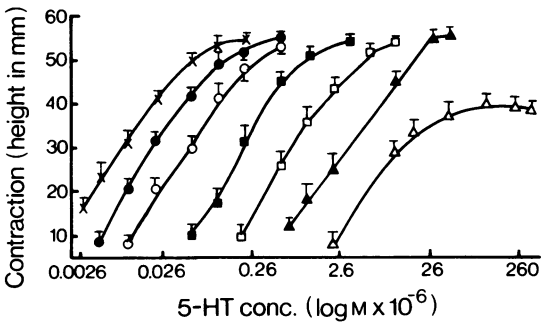

Fig. 1 Effect of increasing concentrations of (±)-propranolol on the responses of the rat isolated stomach to 5-hydroxytryptamine (5-HT). Each curve represents the mean and s.e. of 4 to 5 preparations. Ordinates: height of contraction of stomach strip in mm. (X) control; (●) 7.6×10^{-7}M; (○) 1.5×10^{-6} M; (■) 7.6×10^{-6} M; (□) 7.6×10^{-5} M; (▲) 1.5×10^{-4} M; (△) 3×10^{-4}M.

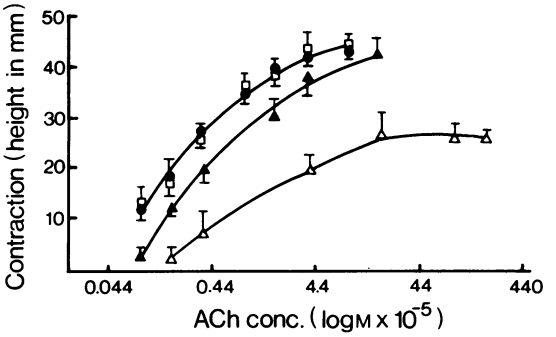

Fig. 2 Effect of increasing concentrations of (±)-propranolol on the responses of the rat isolated stomach to acetylcholine (Ach). Each curve represents the mean and s.e. of 4 preparations. Concentrations of (±)-propranolol between 7.6×10^{-7}M and 7.6×10^{-5}M, caused no significant antagonism. Uppermost curve; control (●); (±)-propranolol 7.6×10^{-5}M (□). (▲) 1.5×10^{-4}M; (△) 3×10^{-4}M.

To see whether β-adrenoceptor antagonists could block the effects of 5-HT on other preparations in which the responses of catecholamines were mediated via β-adrenoceptors, we also studied their influence on the rat isolated uterus.

On this organ also, all four blocking agents produced a parallel shift to the right in the dose-response curves to 5-HT in the same concentrations in which they antagonized the effects of adrenaline. The pA_2 values on the rat uterus for 5-HT and adrenaline antagonism are shown in Table 2.

The slopes of the graphs of the plot of log (x-1) against log M, where x is the dose-ratio and M the molar concentration of antagonist, were all close to 1 for each antagonist on both preparations (See Tables 1 and 2).

Discussion

(±)-Propranolol has been found to antagonize the effects of 5-HT on both the rat stomach and uterus. Although this agent has been shown to reduce the effects of a number of smooth muscle stimulants, including histamine and acetylcholine, on several preparations (Marmo, 1970), there are several reasons to support the contention that the 5-HT antagonism reported here is a specific effect due to competition by propranolol for 5-HT receptors, and not a general depressant effect on contractile mechanisms of the muscle.

Firstly, (±)-propranolol produced a surmountable antagonism of the responses to 5-HT at doses much lower than those which reduced the effects of acetylcholine. A local anaesthetic agent

Table 1 Effect of β-adrenoceptor antagonists on the responses of the rat stomach to 5-hydroxytryptamine (5-HT) and adrenaline.

Drug	*5-HT*			*Adrenaline*			p**
	pA_2 value Mean s.e	n	*Slope**	*pA_2 value* Mean s.e	n	*Slope**	
(±)-Propranolol	6.08 ± 0.004	5	1.20	6.35 ± 0.004	6	0.96	>0.6
(+)-Propranolol	4.94 ± 0.012	5	1.19	5.15 ± 0.012	4	1.14	>0.3
Practolol	3.93 ± 0.005	5	0.99	3.85 ± 0.006	5	1.15	>0.6
Oxprenolol	5.99 ± 0.006	5	1.16	5.76 ± 0.002	5	1.05	>0.4

*Slope refers to plot of log (x-1) against log. M (see text).
**Level of significance of the difference between the two pA_2 values.

such as procaine, with general depressant action on the muscle, would be expected to antagonize all stimulants at approximately the same concentration (Åström, 1964). The pA_2 value for 5-HT antagonism by (±)-propranolol was found to be 6.08 on the rat stomach, compared with a pD_2 value of 3.70 for physiological antagonism of acetylcholine by (±)-propranolol.

Secondly, (+)- and (±)-propranolol have been reported to differ considerably in their β-blocking potency but to be approximately equipotent in their membrane stabilizing or local anaesthetic effects (Barrett & Cullum, 1968). On the rat stomach preparation the difference in 5-HT blocking potency between these isomers was the same as the difference in their antagonistic effect on adrenaline responses. A reduction in the responses of the rat stomach to acetylcholine was produced only by much higher concentrations of both drugs. This latter depression could have resulted from their membrane stabilizing effect.

Practolol, which is said to be a relatively specific antagonist of β_1-(cardiac) receptors (Dunlop & Shanks, 1968), was found to be a much weaker antagonist than (±)-propranolol of both adrenaline and 5-HT on the rat stomach and rat uterus. Oxprenolol, a weaker membrane-stabilizing agent than propranolol (Hellenbrecht, Lemmer, Wiethold & Grobecker, 1973), but almost equipotent as an antagonist of β_1- and β_2-receptors (Brunner, Hedwall, Maier & Meier, 1970), was also equiactive against 5-HT-induced responses.

The finding that two agonists give the same pA_2 value with given antagonists in the same preparation suggests that they produce their effects by occupation of the same receptors (Arunlakshana & Schild, 1959). Thus it is conceivable that in smooth muscle where both adrenaline and 5-HT cause contraction, they each combine with the same receptors. It is, much more difficult to envisage how, in the rat stomach or uterus, occupation of the same receptor by these agonists can lead to opposing effects.

Jester & Horst (1972) suggested that 5-HT may block adrenoceptors, since it has been shown to inhibit noradrenaline's stimulation of the enzyme adenyl cyclase in erythrocyte ghosts and pineal gland. Although 5-HT and catecholamines produce the opposite effects in rat stomach, this does not appear to result from antagonism via the same receptor, for at least two reasons. If 5-HT competed with adrenaline for the same receptors in this preparation, pretreatment with high concentrations of 5-HT should completely abolish the responses to catecholamines, just as pretreatment with β-blocking agents antagonizes the responses. In our experiments, a concentration of 5-HT, 2.6×10^{-6}M was present in the bathing fluid whilst we obtained responses to adrenaline, 5.5×10^{-10}M-5.5×10^{-9} M. In the absence of 5-HT the tissue was, if anything, less sensitive to adrenaline (Armitage & Vane, 1964). Contractions of this preparation could be elicited by 5-HT, 2.6×10^{-10}M, indicating that a great excess of 5-HT was used in the experiments with adrenaline. Secondly, none of the presumably competitive adrenaline antagonists used in this study caused graded contractions of the stomach as were obtained with 5-HT, even when no 5-HT was present in the bathing fluid.

It can, therefore, be concluded that in the rat stomach and uterus preparations, 5-HT and adrenaline probably produce their effects by combining with different receptor sites which are structurally very similar. It remains to be discovered how occupation of their respective receptors results in opposite effects.

Propranolol has been found to be potent antagonist of the stimulant effect of 5-HT on guinea-pig ileum, with a pA_2 vlaue of 6.08 ± 0.029 (Schechter & Weinstock, unpublished observations). This blocking agent also abolishes the

Table 2. Effect of β-adrenoceptor antagonists on the responses of the rat uterus to 5-hydroxytryptamine (5-HT) and adrenaline.

Drug	*5-HT*			*Adrenaline*			p**
	pA_2 value Mean ± s.e.	n	*Slope**	*pA_2 value* Mean ± s.e.	n	*Slope**	
(±)-Propranolol	6.46 ± 0.015	4	1.33	6.66 ± 0.02	4	1.05	>0.3
(+)-Propranolol	5.29 ± 0.010	4	1.10	5.47 ± 0.018	4.	1.01	>0.8
Practolol	3.76 ± 0.021	4	1.20	3.93 ± 0.021	4.	1.08	>0.8
Oxprenolol	5.82 ± 0.020	4	1.25	6.25 ± 0.119	4	1.44	>0.3

*Slope refers to plot of log (x-1) against log M (see text).
**Level of significance of the difference between the two pA_2 values.

stimulant effect of 5-HT on transmission in the cat superior cervical ganglion, in doses of 2 to 10 μg Schechter & Weinstock, unpublished observations). This, taken together with the above findings suggest that propranolol and related compounds are no more specific antagonists of catecholamines than they are of 5-HT. Considerable caution should, therefore, be exercised when interpreting the actions of these β-adrenoceptor blocking agents on tissues or in the whole animal where both catecholamines and 5-HT are present.

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