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An analysis of the mechanism of 5-hydroxytryptamine-induced vasopressor responses in ganglion-blocked anaesthetized dogs

W. FENIUK, J. HARE AND P. P. A. HUMPHREY*

Department of Pharmacology and Department of Biochemistry, Glaxo Group Research Limited, Ware, Hertfordshire, SG12 0DJ, U.K.

5-Hydroxytryptamine (5-HT) administered intravenously (i.v., 1–30 $\mu g\ kg^{-1}$) to ganglion-blocked anaesthetized dogs produced dose-related increases in diastolic blood pressure and we have analysed the mechanism involved. Cyproheptadine and methysergide (10–100 $\mu g\ kg^{-1}$ i.v.) were potent and specific antagonists of the 5-HT induced rise in blood pressure, while the α-adrenoceptor blocking agent phentolamine (0·3–3 $mg\ kg^{-1}$ i.v.) also caused dose-related inhibition. Syrosingopine pretreatment converted the vasopressor action of 5-HT to a vasodepressor action and acute bilateral adrenalectomy caused a marked reduction in the 5-HT-induced rise in blood pressure. In two dogs, 5-HT (30 $\mu g\ kg^{-1}$ i.v.) markedly increased the venous plasma concentrations of noradrenaline and adrenaline. We concluded that the 5-HT-induced rise in diastolic pressure in the ganglion blocked anaesthetized dog is due largely to the release of catecholamines of which a substantial component is from the adrenal gland. The rise in diastolic blood pressure is specifically blocked by low doses of cyproheptadine and methysergide suggesting that the release of catecholamines is mediated by specific 5-HT receptors located mainly within the adrenal medulla.

The intravenous administration of 5-hydroxytryptamine (5-HT) to a variety of laboratory animals produces complex and variable effects on arterial blood pressure (Page & McCubbin 1953). However, in ganglion-blocked anaesthetized dogs 5-HT produces dose-related vasopressor responses and this preparation has therefore been used to quantitate the activity of 5-HT receptor blocking drugs in vivo (Fanchamps et al 1960; Stone et al 1961; Turner 1965; Saxena et al 1971).

Earlier studies have shown that 5-HT can induce catecholamine release from the adrenal gland of the cat (Reid & Rand 1952; Reid 1952) and more recently Eble et al (1972) have shown that intravenously administered 5-HT causes increases in plasma adrenaline concentrations which are sufficient to account for the concomitant vasopressor responses. These findings strongly suggest that the vasopressor effects of 5-HT are indirect and involve catecholamine release, although this has been disputed (Page & McCubbin 1953; Stone et al 1961). Why, therefore, are these effects also antagonized by 5-HT receptor-blocking drugs?

This question prompted us to re-investigate the mechanism involved in the 5-HT mediated vasopressor response in ganglion-blocked anaesthetized dogs. A preliminary account of some of these results has been presented to the British Pharmacological Society (Feniuk & Humphrey 1979).

* Correspondence.

METHODS

Beagle dogs (7–11 kg) of either sex were anaesthetized with barbitone sodium (300 $mg\ kg^{-1}$ i.p.) following induction with thiopentone sodium (25 $mg\ kg^{-1}$ i.v.). Animals were artifically respired with room air using a Palmer ventilation pump (100–150 ml) at 20 cycles min^{-1}. Body temperature was maintained at 38–39 °C. Aortic blood pressure was recorded via the right femoral artery using a Bell and Howell pressure transducer (type 4-422-0001) and heart rate derived from the blood pressure signal using a Devices ratemeter (type 2750). Drugs were administered into the left femoral vein and all dogs except one (see Fig. 1) were pretreated with mecamylamine 5 $mg\ kg^{-1}$ i.v. 30 min before the start of the experiment to produce long lasting ganglion-blockade (Edge et al 1960).

Blood gas analysis

In most experiments aortic blood obtained via the femoral artery was sampled at the start and end of each experiment. The samples were analysed at 37 °C using a Radiometer ABL1 blood gas analyser.

The initial values (uncorrected for temperature) were: pH 7·42 ± 0·01; P_{CO_2} 35·4 ± 1·3 mm Hg; P_{O_2} 97·2 ± 3·9 mm Hg. At the end of each experiment they were pH 7·38 ± 0·01; P_{CO_2} 37·4 ± 1·3 mm Hg and P_{O_2} 94·4 ± 5·0 mm Hg. Each value is the mean value (± s.e. mean) from 24 dogs.

Dosing procedure

5-Hydroxytryptamine or phenylephrine was administered intravenously in increasing doses (1–100 $\mu g\ kg^{-1}$) at 15 min intervals in order to obtain a control dose-effect curve for the increase in diastolic blood pressure. This curve was then repeated three times with a 30 min interval between each curve. Antagonists were administered in increasing doses 15 min before the start of the second, third and fourth dose-effect curve. In some experiments 0·9% w/v sodium chloride (saline) was administered in volumes equivalent to those used for the antagonists so that spontaneous changes in agonist sensitivity could be assessed.

Syrosingopine pretreatment

Dogs were pretreated with syrosingopine (0·5 mg kg^{-1} i.v. 48 h and 1 mg kg^{-1} i.v. 24 h) before each experiment. The syrosingopine base was dissolved according to Orlans et al (1960).

Bilateral adrenalectomy

Dose-effect curves to 5-HT were obtained in dogs before and after acute bilateral adrenalectomy or sham operation. Bilateral adrenalectomy was carried out by occlusion of the major blood vessels and removal of the glands by electro-cautery.

Catecholamine assay

The technique used was essentially that of Callingham & Barrand (1976). Plasma catecholamines were *O*-methylated using [^{3}H-methyl]-s-adenosylmethionine and catechol-*O*-methyl transferase to form [^{3}H]normetanephrine and [^{3}H]metanephrine from noradrenaline and adrenaline respectively. These were then extracted, acetylated and separated by descending paper chromatography. The radioactivity associated with each metabolite was determined and a comparison made with standard amounts (200 pg) of catecholamine following addition to plasma (250 μl).

Before assay, venous blood samples (10 ml) were collected in plastic syringes before and after administration of 5-HT and centrifuged at 4 °C at 650 *g* for 10 min. The plasma supernatant was removed and stored at 4 °C until analysed for catecholamine concentrations. All assays were performed in duplicate within 36 h of blood collection.

Statistics. If not stated, values given are the mean ± s.e. mean of n observations (dogs).

Drugs used. Cyproheptadine hydrochloride (Merck, Sharp and Dohme); 5-hydroxytryptamine creatinine sulphate (Koch-Light); mecamylamine hydrochloride (Merck, Sharp and Dohme); methysergide bimaleate (Sandoz); phentolamine mesylate (Ciba); phenylephrine hydrochloride (Koch-Light); propranolol hydrochloride (ICI); syrosingopine (Ciba). All drugs except syrosingopine (see syrosingopine pretreatment) were initially dissolved in distilled water and all subsequent dilutions were made with saline. All doses in the text refer to the weight of free base.

RESULTS

In the anaesthetized dog 5-HT (1–100 $\mu g\ kg^{-1}$ i.v.) produced variable effects on arterial blood pressure which were often complex and consisted of both pressor and depressor responses (see Fig. 1a). After the administration of mecamylamine (5 mg kg^{-1} i.v.), these same doses of 5-HT produced only dose-related increases in blood pressure (see Fig. 1b). In the remainder of this study all dogs were treated with mecamylamine (see methods) and for simplicity only the effects of drugs on the rise in diastolic pressure are described.

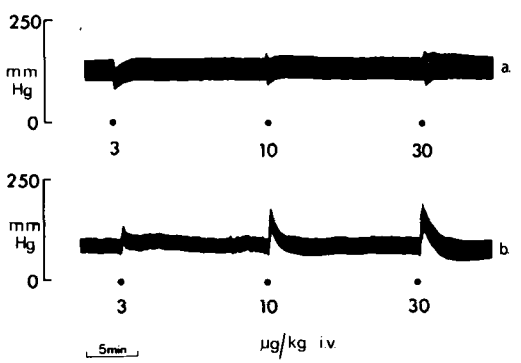

FIG. 1. The effects of 5-hydroxytryptamine (●) 3, 10 and 30 $\mu g\ kg^{-1}$ i.v. on aortic blood pressure in an anaesthetized dog (a) before and (b) after mecamylamine 5 mg kg^{-1} i.v.

Blood pressure and heart rate were reduced by mecamylamine after 30 min to 93/59 ± 3/2 mm Hg and 110 ± 3 beats min^{-1} (mean ± s.e. mean, n = 37) respectively and remained close to these levels throughout the experimental period regardless of subsequent procedures, dosing with antagonists, etc. Higher doses of mecamylamine (10 mg kg^{-1} i.v.) did not reduce blood pressure further.

The maximum rise in diastolic pressure produced by 5-HT was normally about 70 mm Hg and was achieved with a dose of 30 μg kg^{-1}. Higher doses always caused a smaller rise in pressure (e.g. see Fig. 2). The dose-effect curves to 5-HT were reproducible and there was little or no difference between four such curves when saline was administered between each. Doses of 5-HT greater than 3 μg kg^{-1} also caused a tachycardia; the increase in rate produced by a dose of 100 μg kg^{-1} was 37 $\pm$ 4 beats min^{-1} (mean $\pm$ s.e. mean from 18 dogs).

The intravenous administration of the α-adrenoceptor agonist, phenylephrine, also caused a dose-related increase in diastolic blood pressure but little or no change in heart rate.

Effect of cyproheptadine and methysergide

The 5-HT antagonists, cyproheptadine and methysergide (10–100 μg kg^{-1} i.v.) caused a dose-related antagonism of the 5-HT-induced rise in diastolic pressure with a small rightward displacement of the control dose-effect curve and a marked suppression of the maximum (Figs 2 and 3). Neither cyproheptadine nor methysergide antagonized the rise in diastolic pressure produced by the α-adrenoceptor agonist phenylephrine (Figs 2 and 3).

Cyproheptadine and methysergide also inhibited the 5-HT-induced tachycardia in a dose related manner. At 100 μg kg^{-1} i.v., cyproheptadine and methysergide reduced the tachycardia produced by 5-HT (100 μg kg^{-1} i.v.) by a mean value of 37 $\pm$ 15% (n = 5) and 71 $\pm$ 7% (n = 4) respectively.

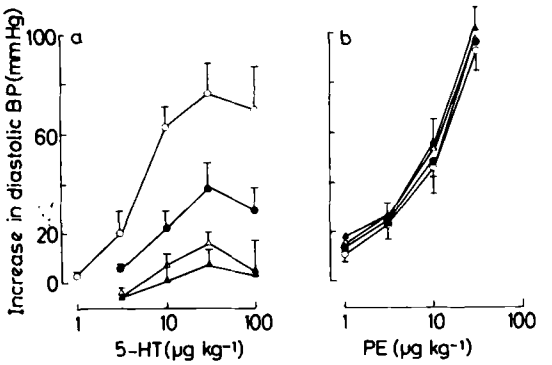

FIG. 3. Anaesthetized dog dosed with mecamylamine (5 mg kg^{-1} i.v.) The increase in diastolic blood pressure produced by (a) 5-HT and (b) phenylephrine before (○) and after methysergide [10 μg kg^{-1} i.v. (●), 30 μg kg^{-1} i.v. (△), and then 100 μg kg^{-1} i.v. (▲)]. Each point is the mean value ($\pm$ s.e. mean) obtained from 4 dogs.

Effect of phentolamine

The α-adrenoceptor blocking agent phentolamine (0·3–3 mg kg^{-1} i.v.) caused a dose-related inhibition of the 5-HT-induced rise in diastolic blood pressure As with cyproheptadine and methysergide, there was a small rightward displacement of the control dose-effect curve with a marked suppression of the maximum response (Fig. 4). In contrast, phentolamine produced a parallel displacement to the right of the vasopressor dose-effect curve to the α-adrenoceptor agonist phenylephrine (Fig. 4).

Phentolamine had no effect on the tachycardia produced by 5-HT.

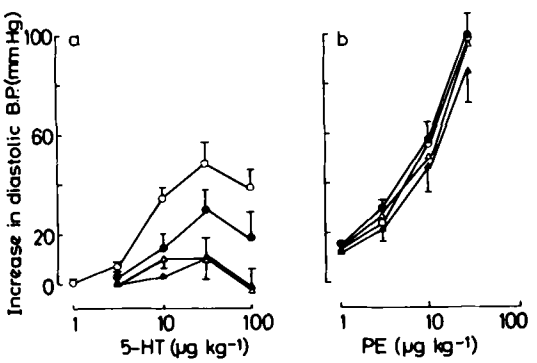

FIG. 2. Anaesthetized dog dosed with mecamylamine (5 mg kg^{-1} i.v.). The increase in diastolic blood pressure produced by (a) 5-hydroxytryptamine and (b) phenylephrine before (○) and after cyproheptadine [10 μg kg^{-1} i.v. (●), 30 μg kg^{-1} i.v. (△) and then 100 μg kg^{-1} i.v. (▲)]. Each point is the mean value ($\pm$ s.e. mean) obtained from 5 dogs (a) and 4 dogs (b).

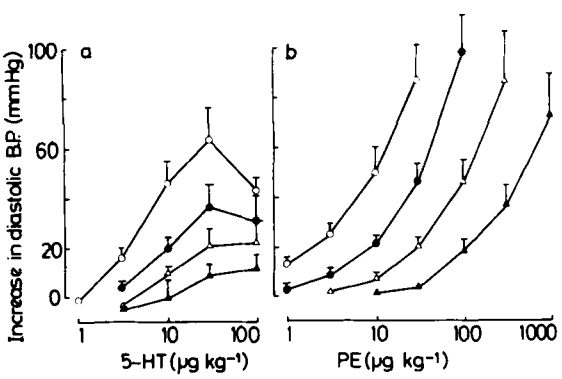

FIG. 4. Anaesthetized dog dosed with mecamylamine (5 mg kg^{-1} i.v.). The increase in diastolic blood pressure produced by (a) 5-HT and (b) phenylephrine before (○) and after phentolamine [0·3 mg kg^{-1} i.v. (●), 1·0 mg kg^{-1} i.v. (△) and then 3·0 mg kg^{-1} i.v. (▲)]. Each point is the mean value ($\pm$ s.e. mean) obtained from 4 dogs.

Effect of syrosingopine pretreatment

In dogs which had been pretreated with the catecholamine depleting agent, syrosingopine (0·5 mg kg^{-1} i.v. 48 h and 1 mg kg^{-1} i.v. 24 h previously) 5-HT caused only small falls in diastolic pressure (Fig. 5). In addition syrosingopine pretreatment reduced the tachycardia produced by 5-HT (100 μg kg^{-1} i.v.) by a mean value of 54 $\pm$ 11% (n = 4) when compared with the tachycardia produced in untreated dogs.

Catecholamine plasma concentrations

A blood sample was taken 15 min before intravenous administration of 5-HT and a second sample taken at the time of the peak of the vasopressor response to 5-HT (30 μg kg^{-1} i.v.). The results obtained in two control dogs and two dogs pretreated with syrosingopine are shown in Table 1. Plasma concentrations of noradrenaline and adrenaline were increased by 5-HT in control dogs but not in dogs pretreated with syrosingopine.

Effect of acute bilateral adrenalectomy

Acute bilateral adrenalectomy caused a significant reduction in the magnitude of 5-HT-induced vasopressor response over the whole dose range studied when compared with vasopressor responses obtained in sham-operated dogs (Fig. 6). There was also a 43 $\pm$ 14% (n = 4) reduction in the tachycardia produced by 5-HT (100 μg kg^{-1} i.v.) in the adrenalectomized dogs.

Table 1. The effect of 5-HT (30 μg kg^{-1} i.v.) on plasma concentrations of noradrenaline and adrenaline in the ganglion-blocked anaesthetized dog.

Procedure	Pretreatment	Plasma concn (pg ml^{-1}) of Noradrenaline Dog 1	Dog 2	Adrenaline Dog 1	Dog 2
Before 5-HT	None	65	75	38	33
After 5-HT		391	183	609	568
		(4·1)		(16·1)	
		Dog 3	Dog 4	Dog 3	Dog 4
Before 5-HT	Syrosingopine	147	30	110	29
After 5-HT		110	26	103	38
		(0·8)		(1·0)	

The mean × fold increase in catecholamine concentration after 5-HT is shown in brackets.

DISCUSSION

5-Hydroxytryptamine administered intravenously to ganglion-blocked anaesthetized dogs caused a dose-related increase in diastolic blood pressure confirming earlier findings in the literature (Stone et al 1961; Saxena et al 1971). Indeed this preparation is used to examine compounds for their ability to antagonize 5-HT in vivo (e.g. Turner 1965). In this study we have attempted to determine to what degree the response to 5-HT might involve catecholamine release (see introduction) and, if a 5-HT receptor is involved, what type is it? (see Apperley et al 1980).

The 5-HT antagonists cyproheptadine and methysergide were highly potent and selective antagonists of the vasopressor action of 5-HT indicating that 5-HT mediates its vasopressor effects via an action on a specific 5-HT receptor. However phentolamine also caused a dose-related antagonism of the 5-HT induced vasopressor response, in doses which caused a parallel rightward displacement of the dose-effect curves to the α-adrenoceptor agonist phenylephrine,

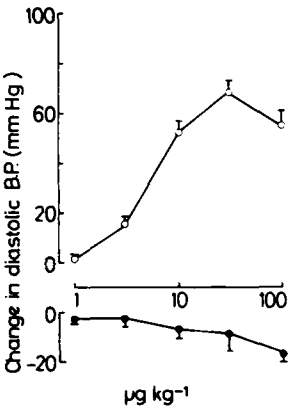

FIG. 5. Anaesthetized dog dosed with mecamylamine (5 mg kg^{-1} i.v.). The change in diastolic blood pressure produced by 5-HT in untreated dogs (○) and dogs pretreated with syrosingopine (0·5 mg kg^{-1} i.v. 48 h and 1·0 mg kg^{-1} i.v. 24 h previously) (●). Each point is the mean value (± s.e. mean) from 4 dogs.

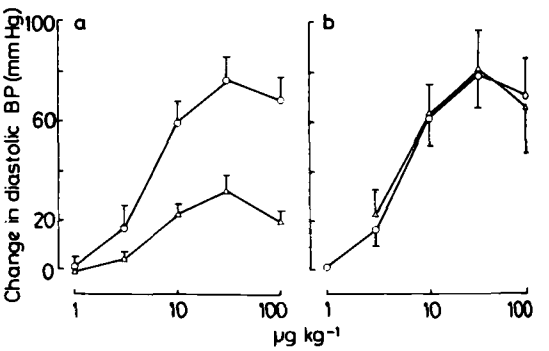

FIG. 6. Anaesthetized dog dosed with mecamylamine (5 mg kg^{-1} i.v.). The increase in diastolic blood pressure produced by 5-HT in (a) bilateral adrenalectomized dogs and (b) sham operated dogs. Dose effect-curves for 5-HT were obtained (○) before and after (△) the operative procedure. Each point is the mean (± s.e. mean) obtained from 4 dogs.

which suggests that stimulation of α-adrenoceptors is involved.

Although 5-HT can directly stimulate α-adrenoceptors in some vascular preparations (Apperley et al 1976) our results with syrosingopine suggest that an indirect action is involved. When dogs were pretreated with the catecholamine depleting agent, syrosingopine (Orlans et al 1960; Stone et al 1962), the 5-HT-induced rise in diastolic blood pressure was abolished and only small vasodepressor responses were seen. This finding suggests that the 5-HT-induced vasopressor response was mediated via catecholamine release and indeed our biochemical data (Table 1) confirms this analysis. In turn this explains the ability of phentolamine to block the rise in diastolic blood pressure produced by 5-HT. Our findings in the dog therefore appear to contrast markedly with those of Fozard & Leach (1968) in the rat in which the vasopressor response to 5-HT is not mediated indirectly and is apparently due to a direct effect of 5-HT on receptors in the vascular smooth muscle. We have now confirmed some of Fozard and Leach's observations in the rat in our own laboratories (M. Philp, personal communication) and conclude that 5-HT does not cause catecholamine release in the rat as it does in the dog.

In this study acute bilateral adrenalectomy caused a marked reduction in the magnitude of the 5-HT-induced vasopressor response. It thus appears that the adrenal gland is a major site from which 5-HT caused the release of catecholamines. Douglas et al (1967) have demonstrated that 5-HT can induce depolarization of isolated chromaffin cells from the gerbil adrenal medulla and have suggested that this is an important event leading to the release of catecholamines. We suggest that a similar situation may exist in the dog and that 5-HT causes depolarization of adrenal medullary chromaffin cells and subsequent catecholamine release by stimulation of specific 5-HT receptors. The fact that the release was potently and selectively antagonized by cyproheptadine and methysergide indicates that a stoichiometric release mechanism was not involved (see Humphrey 1978) and also rules out the possibility of stimulation of M-receptors for 5-HT (Fozard & Mobarok Ali 1978). Furthermore, in the present study methysergide did not cause any rise in diastolic blood pressure suggesting that the 5-HT receptor found in the dog saphenous vein (Apperley et al 1980) is unimportant in this release mechanism. We believe that our findings with cyproheptadine and methysergide implicate a receptor mechanism mediated via the so-called D-receptor for 5-HT (Gaddum & Picarelli 1957: see also Apperley et al 1980). Both compounds, although chemically distinct, share the property of being potent specific D-receptor blocking drugs in vitro (Fozard 1975; Apperley et al 1976) and in this study in vivo they had a similar profile of activity being potent and specific antagonists of the vasopressor action of 5-HT.

The existence of specific receptors for a naturally occurring substance like 5-HT on adrenal medullary cells is not unprecedented. Staszewska-Barczak & Vane (1965) have demonstrated that histamine can release adrenaline from the adrenal gland of the cat and this effect was selectively antagonized by mepyramine suggesting an action on histamine H_1-receptors. The adrenal glands of the anaesthetized dog secrete more adrenaline than noradrenaline in response to various stimuli (de Schaepdryver 1959) and our finding that the plasma concentrations of adrenaline increased much more than those of noradrenaline therefore suggests that the adrenal glands were primarily involved in the release of catecholamine by 5-HT. However, the adrenal glands may not be the sole site of catecholamine release involved since bilateral adrenalectomy did not cause a total inhibition of the 5-HT-induced rise in diastolic blood pressure, whilst syrosingopine pretreatment did. It may be that 5-HT can additionally induce catecholamine release by depolarization of other sites (e.g. extra-medullary chromaffin tissue or other neural elements) or alternatively the adrenalectomy may have been less than complete.

Our findings demonstrate unequivocally that the 5-HT-induced pressor response in ganglion-blocked dogs is largely mediated via catecholamine release and in particular adrenaline. Additionally the selective antagonism of this pressor response by cyproheptadine and methysergide strongly suggests the involvement of a D-receptor. Nevertheless displacement of the 5-HT dose-effect curves by increasing doses of these antagonists was always accompanied by a suppression of the maximum 5-HT-induced pressor response and requires some comment as indeed does the 'bell-shaped' nature of the control 5-HT dose-effect curve. We feel that a concomittant vasodilator action of 5-HT could account for both of these findings. Indeed the present study provides evidence that such a mechanism does occur since 5-HT caused small vasodepressor responses following syrosingopine pretreatment. An inhibitory action of 5-HT on sympathetic vascoconstrictor fibres (Feniuk et al 1979) is unlikely since the animals were ganglion-blocked and neuronal stores

of noradrenaline would have been substantially depleted by syrosingopine pretreatment. However, previous studies in vitro have shown that 5-HT has a direct vasodilator action (McKeever et al 1959; Eyre 1975) and indeed this effect is not mediated via 5-HT D-receptors (Eyre 1975).

In this study we have not attempted to analyse the haemodynamic mechanisms which contribute to the vasopressor response produced by intravenous 5-HT since this was not our intention. However, it is most likely that the predominant mechanism involved was a rise in total peripheral resistance resulting from a generalized vascoconstriction. This belief is based on the fact that in this study we measured changes in diastolic blood pressure which are generally believed to be largely indicative of changes in vascular tone (see Kennedy & Levy 1975). Furthermore we have since shown that in the anaesthetized dog, at the peak of the 5-HT-induced rise in diastolic pressure, there is a marked increase in total peripheral resistance (unpublished observations).

Although the main purpose of this study was to investigate the 5-HT-induced vasopressor response, it appeared that the tachycardia was also mainly mediated indirectly through catecholamine release. We have found that the 5-HT-induced tachycardia was markedly antagonized by propranolol (Feniuk & Humphrey, unpublished observation) and was reduced by bilateral adrenalectomy and pretreatment with syrosingopine (this study). However, since catecholamine depletion did not completely abolish the chronotropic effect of 5-HT, it may be that its action involves both a direct and an indirect component (see Trendelenburg 1960). The direct effect of 5-HT may involve a specific 5-HT receptor which appears to be present in the atria of some species (Schneider & Yonkman 1954; Trendelenburg 1960; Gonzales & Garcia 1977).

In conclusion our results confirm that the rise in diastolic blood pressure following the intravenous administration of 5-HT to ganglion-blocked dogs *is* largely mediated through catecholamine release and show that this occurs mainly from the adrenal gland. The release process appears to be susceptible to blockade by the 5-HT receptor blocking drugs cyproheptadine and methysergide and may result from D-receptor mediated depolarization of chromaffin cells.

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