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Inhibitory Effect of 5-Hydroxytryptamine on Rat Stomach Fundus: Mediated Indirectly by Activation of Noradrenaline Release

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Abstract

Biphasic cumulative concentration-response curves to 5-hydroxytryptamine (5-HT) and α-methyl-5-hydroxytryptamine (α-Me-5-HT) using rat stomach fundus in the presence of 50 μM pargyline suggested two sites of interaction (high and low affinity).

The order of agonist potencies of 5-HT agonists for the high-affinity (contractile) response confirmed reports of a strong correlation of the 5-HT_{2B} (contractile) receptor on fundus with reported rat brain (radioligand binding) pK_d values at the 5-HT_{2C} receptor ($r = 0{\cdot}94$, $b = 0{\cdot}93$ $P < 0{\cdot}01$). 1(1-Naphthyl)-piperazine and ketanserin antagonized the high-affinity responses. The order of potencies for the low affinity (inhibitory) response was: α-Me-5-HT > 5-HT. Preincubation with mianserin or *S*-(−)propranolol, pretreatment with reserpine and removal of the mucosa layer resulted in amplification of the contractile effects and disappearance or reversal of the inhibitory effects of 5-HT.

Activation of a second receptor site inducing catecholamine release may explain this effect of 5-HT on rat stomach fundus.

The rat stomach fundus preparation continues to be used extensively to bioassay 5-hydroxytryptamine-like activity (Vane 1957; Van Nueten et al 1983; Wrigglesworth 1983; Cohen 1989). Pharmacological characterization, sequencing and cloning of the 5-HT_{2B} receptor responsible for the contractile effect of 5-hydroxtryptamine (5-HT) on rat fundus has been attained (Foguet et al 1992; Kursar et al 1992; Humphrey et al 1993). Interestingly, 5-HT induces not only a pronounced contractile response in gastrointestinal smooth muscle but can also evoke a relaxant effect at high concentrations. Offermeier & Äriens (1966) reported two components in the effect of 5-HT: a fast contraction at low concentrations and an autoinhibitory effect at concentrations above 10^{-5} M. These authors indicated that the inhibition was more apparent using the cumulative-concentration method and that this could be associated with activation of α-adrenoceptors. Buchheit et al (1986) corroborated the biphasic effect elicited by several 5-HT agonists on rat fundus and suggested that activation of two different receptors could explain this phenomenon. The contribution of the inhibitory response in the overall effects of 5-HT agonists on rat fundus has not been explored recently. In the present study, both contractile and inhibitory effects were observed using the cumulative method in the presence of a monoamine oxidase inhibitor (MAOI) to reduce variability of the responses by catabolism. Intrinsic activity and affinity constants (pD_2) for 5-HT and selective 5-HT agonists were determined and antagonist studies were extended using some selective 5-HT antagonists not previously reported in the literature for this tissue.

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Materials and Methods

Test compounds

5-Carboxyamidotryptamine HCl (5-CT), carbachol Cl, pargyline HCl, prazosin HCl, *S*-(−)-propranolol HCl, reserpine, 5-hydroxytryptamine creatinine sulphate complex monohydrate and tryptamine HCl were purchased from Sigma Chemical Co. (St Louis, MO). Ketanserin (+)-tartrate, α-methyl 5-hydroxytryptamine maleate (α-Me-5-HT), 2-methyl 5-hydroxytryptamine maleate (2-Me-5-HT), mianserin HCl, 1(1-naphthyl)piperazine HCl (1-NP), quipazine maleate, (±)-8-hydroxydipropyl-aminotetralin HBr (8-OH-DPAT), 1-(3-chlorophenyl)piperazine HCl (m-CPP) and 3-tropanyl-3,5-dichlorobenzoate (MDL-72222) were obtained from Research Biochemicals Inc. (Natick, MA). Atropine sulphate was from Nutritional Biochemicals Inc. (Cleveland, OH).

Rat stomach fundus (modified preparation)

Fundal portions of rat stomach were obtained from fasted Sprague-Dawley rats, 150–250 g (Simonsen Labs, Gilroy, CA) and mounted using a combination of the methodologies reported by Buchheit et al (1986) and Cohen & Fludzinski (1987), with slight modifications from our laboratory. Briefly, four fundal strips were obtained from one stomach and mounted in individual 5-mL double-jacketed chambers containing modified-Krebs solution, composition (mM): NaCl, 118·2; KCl, 4·6; $CaCl_2$, 1·6; KH_2PO_4, 1·2; $MgSO_4$, 1·2; $NaHCO_3$, 24·8; dextrose, 10 and pargyline HCl, 0·05) kept at 37°C and aerated with a mixture of 95% O_2–5% CO_2. Resting tension was adjusted to 4 g during a period of 10 min or until the baseline became stable. Recordings were obtained using a Grass polygraph

system (DC preamplifier Model 7P1B; amplifier 70AE; ink writer oscillograph Model 7WC16PA; calibration 1 cm = 1 g = −50 mV). Strips were allowed to incubate for 1 h before any drug was added. Fundal contractility after 5-HT addition was used as a reference control for maximum response in every tissue and to check for the integrity of the preparation before the bioassay. Aqueous solutions of the 5-HT agonists were prepared immediately before each experiment. Dilutions of all the compounds were made following the procedure of Van Rossum (1963). In the case of 5-HT and other drugs of suspected instability, stock solutions were kept on ice during the experiment. Due to the relatively low solubility of MDL 72222 in water, solutions prepared with this compound were heated at 37°C, sonicated for 5 min and then stirred mechanically at 37°C until use. Each experiment was initiated by challenging the fundal strips with two consecutive additions of 5-HT (10^{-6} M) followed in each instance by washing the tissue five times. After recovery, a cumulative concentration–response to 5-HT was carried out following the procedure of Van Rossum & Van den Brink (1963) and Van Rossum (1963). In preliminary experiments, the average maximum response to 5-HT observed on the rat fundus consistently occurred at the cumulated concentration of 10^{-6} M; therefore, the response elicited by 10^{-6} M 5-HT (cumulative concentration) was considered to be the maximum effect for each tissue and was used to convert the raw data to effect/maximum effect values. Cumulative concentration–response (CCR) curves for the test agonists were carried out in the same manner after tissue washing and recovery.

In experiments using reserpine-pretreated rat fundus, stomach fundus was obtained from Sprague Dawley rats injected intraperitoneally with 5 mg kg^{-1} reserpine, 18 h before the experiment. Mucosa-denuded fundus strips were prepared by rubbing off the mucosal layer of stomach fundus with a cotton applicator. Preparations and experiments with agonists and antagonists on reserpinized and mucosa-denuded fundi were carried out following the same protocol described for untreated fundus.

Statistical analysis of the data involved converting the raw data to percentage data by dividing each measured effect by the maximum effect seen with 5-HT. The concentration–response curves for 5-HT and the corresponding agonist were graphed and those points that appeared to be part of the asymptotic high or low parts of the concentration–response sigmoidal curve were discarded before linear regression analysis. Regression analysis was used to determine slope (b) and ED50 values and their respective 95% confidence limits (Tallarida & Murray 1987; Malone 1990). A correlation coefficient (r) and its level of significance (P) were calculated for each curve (Malone 1990). The affinity constants (pD_2) and their 95% confidence limits values were determined for all pure compounds studied. Intrinsic activity was calculated as the average maximum effect observed for each agonist studied (Malone 1990).

Antagonist studies

CCR curves for 5-HT were obtained and, after repeated tissue washing, the fundal strips were incubated with a fixed concentration of the antagonist under study allowing equilibration for 30–45 min. Responses to 5-HT were then obtained again in the presence of various concentrations of the antagonist and CCR curves plotted. Three different concentrations of each antagonist were used to complete a family of curves for each tissue strip.

In the case of 5-HT agonists, each study started with two individual challenges with 5-HT (10^{-6} M) followed by a CCR curve for 5-HT. After repeated washing, the corresponding 5-HT agonist CCR curve was obtained. Following the control response of the agonist under study, fundal strips were incubated in a fixed concentration of the antagonist drug for 30–45 min. Responses to the 5-HT agonist were then obtained in the presence of the antagonist drug by increasing stepwise the cumulative concentration of the compound under study. The experiment was repeated using at least three different dose levels of the antagonist and one agonist for each tissue strip.

Determination of pA_2 values (± s.e.) for competitive antagonists was obtained from Schild plots using values obtained from the individual experiments (Tallarida & Murray 1987). pD'_2 values (± s.e.) of noncompetitive antagonists were obtained by the method described by Van Rossum (1963) using the individual data. In this case, the individual pD'_2 values for antagonists were determined for each concentration of antagonist according to the following equation:

$$PD'_2 = PD'_x + \log(x + 1) \quad (1)$$

where pD'_x is equal to the log concentration of the antagonist and x represents the percentage of maximum inhibition of the intrinsic activity of the agonist tested in the presence of a fixed concentration of the antagonist.

Correlation analysis was used to compare published affinity constants of 5-HT compounds for the different 5-HT receptor subtypes in the brain (pK_D) and the affinity values for agonists (pD_2) and for competitive antagonists (pA_2) in rat fundus as calculated in the present study. The affinity values (pK_D) of the 5-HT drugs on rat brain membranes were those reported in the literature as determined by radioligand binding assays on different preparations (Glennon 1987; Hoyer 1989).

Results

Characterization of the high affinity (contractile) response using 5-HT agonists and antagonists

Selective and nonselective 5-HT agonists for the 5-HT_1-, 5-HT_2-, 5-HT_3- and 5-HT_{1A}- and 5-HT_{1B}- receptor subtypes were tested on the rat fundus preparation. The order of potencies of the high affinity (contractile) response for the 5-HT agonists was: 5-HT > α-Me-5-HT > quipazine > tryptamine > 5-CT > 8-OH-DPAT > m-CPP > 2-Me-5-HT and the order of intrinsic activity was: m-CPP > 5-CT > 5-HT > 8-OH-DPAT > tryptamine > quipazine > α-Me-5-HT > 2-Me-5-HT. A summary of pharmacological constants for the various 5-HT and cholinergic agonists tested is shown in Table 1. At concentrations higher than 10^{-6} M, clear relaxant effects were observed only with α-Me-5-HT and 5-HT (Fig. 1).

The nonselective 5-HT_1/5-HT_2 antagonist ℸ-NP was the only antagonist that demonstrated competitive antagonism towards 5-HT on the rat fundus preparation (pA_2 = 8·14 ±

Table 1. Relative intrinsic activities, affinities and other pharmacological constants of cholinergic and 5-HT agonists.

	Regression				Affinity		
	Slope ± s.e. (b)	r	*P*	Intrinsic activity (%)	pD_2	(95% confidence limits)	n*
5-HT (High)	0·28 ± 0·01	0·92	< 0·001	100·0	7·96	(7·92–8·00)	427
5-HT (Low)	−0·52 ± 0·05	0·67	< 0·001	18·0	4·48	(4·43–4·53)	58
Furtrethonium	0·87 ± 0·09	0·87	< 0·001	246·8	6·53	(6·30–6·76)	35
Carbachol	2·98 ± 0·28	0·86	< 0·001	272·1	7·84	(7·73–7·95)	35
5-CT	0·23 ± 0·02	0·82	< 0·001	108·3	6·72	(6·45–6·99)	56
α-Me-5-HT (High)	0·17 ± 0·01	0·90	< 0·001	49·6	7·71	(7·61–7·81)	40
α-Me-5-HT (Low)	−0·38 ± 0·07	0·78	< 0·001	11·2	5·36	(5·25–5·47)	24
2-Me-5-HT	0·05 ± 0·01	0·20	> 0·20	18·1	(8·93)†	–	30
8-OH-DPAT	0·37 ± 0·02	0·93	< 0·001	92·5	5·14	(5·05–5·23)	35
m-CPP	0·64 ± 0·08	0·83	< 0·001	120·2	4·74	(4·63–4·85)	32
Tryptamine	0·23 ± 0·01	0·96	< 0·001	80·5	6·79	(6·71–6·87)	49
Quipazine	0·13 ± 0·01	0·77	< 0·001	65·8	6·96	(6·75–7·17)	63

* n represents the total number of points used to calculate the regression line constants.
† This curve was not significant by regression analysis so the estimated pD_2 value has little validity.

0·37; b = −0·92). *S*-(−)-Propranolol did not antagonize the spasmogenic effects of 5-HT at any of the concentrations used. However, at concentrations above 10^{-7} M, this compound appeared to amplify the contractile responses of 5-HT on fundus. The intrinsic activity value of 5-HT was increased up to 1·5 (150%) (Fig. 2). Ketanserin exhibited noncompetitive inhibition of the contractile responses of 5-HT and α-Me-5-HT [pD'_2 values = 5·60 (5·27–5·93) for 5-HT and = 6·68 (6·16–7·20) for α-Me-5-HT]. Ketanserin also partly reversed the low affinity (inhibitory) effects of 5-HT at lower concentrations. MDL 72222 exhibited only a slight noncompetitive antagonism to 5-HT. The calculated pD'_2 value was 6·46 (6·02–6·90).

A highly significant correlation ($P < 0·01$, r = 0·94, b = 0·93) between the contractile 5-HT_{2B} receptor and the 5-HT_{2C} receptor on rat brain membranes (previously denominated 5-HT_{1C}) was confirmed when pD_2 values for the six agonists (5-HT, tryptamine, α-Me-5-HT, quipazine, 5-CT and 8-OH-DPAT) and the pA_2 for 1-NP all tested here, were related to pK_d values reported in the literature (Fozard 1984; Glennon 1987; Hoyer 1989). Lack of correlation was observed for 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_2 and 5-HT_3 receptors.

Effect of mianserin on responses elicited by 5-HT and α-Me-5-HT

The relatively selective 5-HT_{2C} antagonist mianserin did not antagonize the contractile effect of 5-HT in a range of concentrations from 10^{-8} to 10^{-6} M. However, this compound reversed the low affinity (inhibitory) response of 5-HT. The maximum reversal was observed at a concentration of 10^{-7} M. Higher concentrations of mianserin resulted in decreased amplification. The calculated intrinsic activity of 5-HT in the presence of 1×10^{-7} M mianserin was 1·70 (170% that of the control response) (Fig. 3). The high affinity (contractile) response of the rat fundus to α-Me-5-HT was also slightly amplified by the presence of mianserin in the range of concentrations used. On the other hand, the low affinity (inhibitory) response was completely reversed by mianserin. Again a concentration-response shift was only observed at 10^{-8} and 10^{-7} M with higher concentrations of mianserin not producing a greater effect (Fig. 4).

Effect of 5-HT on carbachol-precontracted fundus

Untreated control and mucosa-denuded fundal strips were precontracted maximally with a single concentration of 10^{-6} M carbachol. After maximal contraction had been attained, 5-HT was cumulatively added until a maximum concentration of 10^{-4} M had been obtained. Neither spasmogenic nor inhibitory effects of 5-HT were observed in these two preparations at the concentrations of 5-HT used (Fig. 5).

Low affinity response and adrenergic blockade

To study the effect of 5-HT on catecholamine release, three different approaches were taken: the use of a preparation where catecholamine content had been depleted by exposure to the alkaloid reserpine; the use of a preparation where the mucosa layer had been partly removed by dissecting out the myomucosal plexus neurons; and the use of a mucosa-denuded preparation to which a combination of prazosin (α_1-blocker) and *S*-(−)-propranolol (β-blocker) was added. The effects of 5-HT in these preparations, as compared with untreated intact fundus, can be observed in Fig. 6. A full reversal of the inhibitory phase of the 5-HT curve was seen

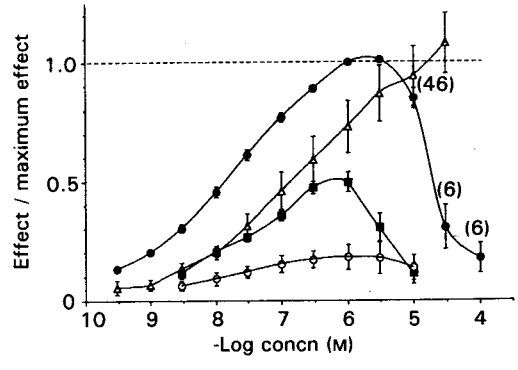

FIG. 1. Effect of selective 5-HT agonists on rat isolated stomach fundus; 5-HT (●, n = 61), 5-CT (△, n = 6), α-Me-5-HT (■, n = 8) and 2-Me-5-HT (○, n = 6). Points equal mean values and bars indicate s.e.m. Where n differs from that specified, it is noted in parentheses in the figure.

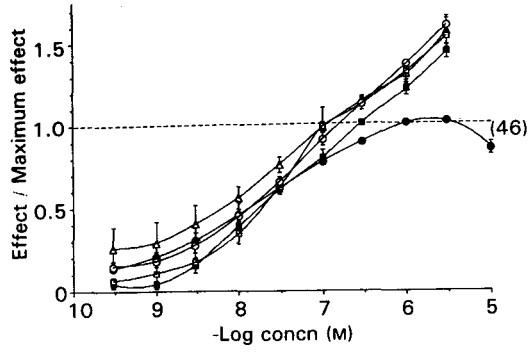

FIG. 2. Effect of *S*-(−)-propranolol on the response elicited by 5-HT on rat fundus in-vitro (incubation time = 45 min); 5-HT alone (●, n = 61), 5-HT/propranolol 10^{-7} M (△, n = 4), 5-HT/propranolol 3×10^{-7} M (■, n = 4), 5-HT/propranolol 10^{-6} M (□, n = 4), 5-HT/propranolol 3×10^{-6} M (○, n = 4). Points equal mean values and bars indicate s.e.m. Where n differs from that specified, it is noted in parentheses in the figure.

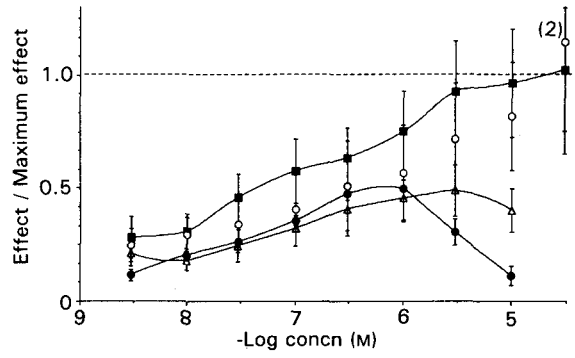

FIG. 4. Cumulative concentration-response curves for α-Me-5-HT on rat isolated fundus in the presence of various concentrations of mianserin (incubation time = 30 min); α-Me-5-HT alone (●, n = 8), α-Me-5-HT/mianserin 10^{-8} M (△, n = 4), α-Me-5-HT/mianserin 10^{-7} M (■, n = 4), α-Me-5-HT/mianserin 10^{-6} M (○, n = 4). Points equal mean values and bars indicate s.e.m. Where n differs from that specified, it is noted in parentheses in the figure.

in reserpinized tissue; the maximum intrinsic activity was 1·85 (185%) and achieved at a concentration of 3×10^{-5} M 5-HT. Mucosa-denuded fundus in the presence and absence of adrenergic blockers exhibited much the same behaviour; the intrinsic activity values were 1·40 (140%) and 1·12 (112%), respectively, at a concentration of 3×10^{-5} M. In the absence of adrenergic blockers, the mucosa-denuded fundus clearly did not exhibit a biphasic CCR curve, the inhibitory phase was absent and the intrinsic activity was only slightly amplified. The 5-HT curve became asymptotic at concentrations greater than 10^{-6} M 5-HT.

Discussion

This study confirms previous findings that the spasmogenic receptor of fundus is not a 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2A}, 5-HT_{2C} or 5-HT_3 receptor. Although this receptor has been very significantly correlated with the 5-HT_{2C} receptor subtype by us ($r = 0{\cdot}94$, $P < 0{\cdot}01$) and by others, mRNA for the 5-HT_{2C} receptor is absent in rat stomach fundus (Baez et al 1990). Furthermore, biochemical information on the transduction mechanism for the 5-HT_{2C} receptor on choroid plexus has been associated with an increase in phosphoinositide (PI) turnover (Conn et al 1986) and 5-HT does not increase PI hydrolysis in concentrations up to 10^4 M on this tissue. The stomach fundus 5-HT receptor has been classified as a member of the 5-HT_2 family, but is clearly a different receptor subtype. Foguet et al (1992) and Kursar et al (1992) cloned, sequenced and pharmacologically characterized the stomach fundus receptor and classified it as a 5-HT_{2F} receptor. In the most current 5-HT receptor classification this receptor is denominated 5-HT_{2B} by Humphrey et al (1993).

Unlike most of the 5-HT agonists tested here, α-Me-5-HT had a pD_2 value approaching that of 5-HT; however, α-Me-5-HT was only a partial agonist on rat fundus, with an

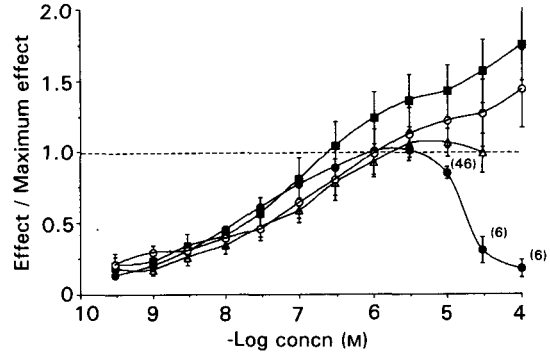

FIG. 3. Effect of mianserin on the response elicited by 5-HT on rat isolated stomach fundus (incubation time = 30 min); 5-HT alone (●, n = 61), 5-HT/mianserin 10^{-8} M (△, n = 4), 5-HT/mianserin 10^{-7} M (■, n = 4), 5-HT/mianserin 10^{-6} M (○, n = 4). Points equal mean values and bars indicate s.e.m. Where n differs from that specified, it is noted in parentheses in the figure.

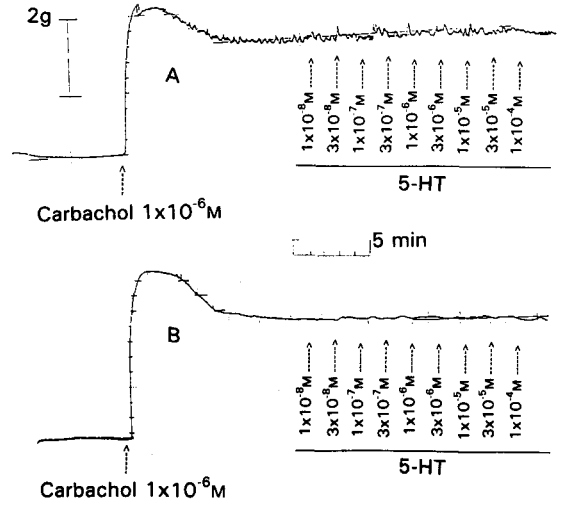

FIG. 5. Effects of 5-HT on carbachol-(10^{-6} M) precontracted fundal strips. A. Control strips in Krebs solution; B. mucosa-denuded fundal strips incubated with both prazosin and *S*-(−)-propranolol, 10^{-7} M each. Arrows indicate addition of carbachol and 5-HT to the organ chambers.

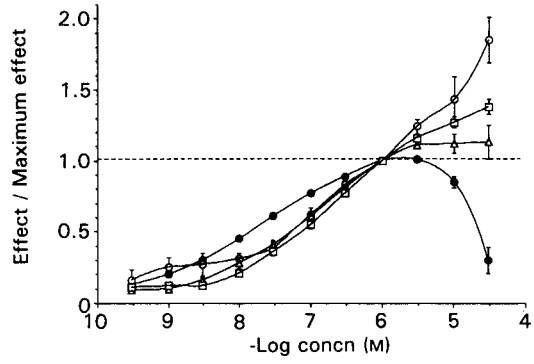

FIG. 6. Effects of 5-HT on intact (●, n = 61), reserpine-treated (○, n = 4) and mucosa-denuded fundus in the absence (△, n = 4) and in the presence of the adrenergic blockers prazosin and *S*-(−)-propranolol (□, n = 4, 10^{-7} M each). Points equal mean values and bars indicate s.e.m.

intrinsic activity of 0·50 (50% of the maximum effect of 5-HT, Fig. 1). The CCR curve was clearly biphasic and resembled that seen for 5-HT, suggesting similar receptor activation by 5-HT and α-Me-5-HT.

The nonselective 5-HT_1/5-HT_2 antagonist 1-NP was the only compound tested which competitively antagonized the contractile effects of 5-HT in the preparation at the three different concentrations used. The results confirmed the work of Cohen & Fludzinski (1987) using a single concentration (10^{-7} M) of this antagonist to demonstrate the competitive antagonism of the relatively selective 5-HT_1 agonists 5-CT, TR3369 (indorenate) and MK212.

Ketanserin and MDL 72222 (antagonists for the 5-HT_2 and 5-HT_3 receptors, respectively) did not show competitive antagonism in the present study. Interestingly, some non-competitive antagonism was exhibited by ketanserin and MDL 72222 to the responses of 5-HT and α-Me-5-HT on fundus.

S-(−)-Propranolol, a relatively selective 5-HT_1 antagonist with adrenergic β-blocker effects, did not antagonize 5-HT even at concentrations as high as 3×10^{-6} M. In addition, this compound was found to amplify the high-affinity (contractile) response to 5-HT (Fig. 2).

The biphasic effects obtained with α-Me-5-HT and 5-HT in the present work would seem to support the suggestion of Buchheit et al (1986) that 5-HT could indeed have two different sites of action on rat stomach fundus and the contribution of a second (low-affinity) interaction was sufficiently interesting to require further investigation.

Strong correlation between the 5-HT_{2C} receptor and the high-affinity response of 5-HT on rat fundus indicated that testing the effect of mianserin as an antagonist for 5-HT and α-Me-5-HT in our preparation was necessary. Some differences were found between the results of the present study and those obtained by Cohen (1989). We were unable to demonstrate any type of antagonism by mianserin for the high-affinity responses of both 5-HT and α-Me-5-HT. Moreover, this compound amplified contractile responses for both compounds in the tissue. Mianserin reversed low-affinity inhibition even at concentrations as low as 10^{-8} M, at which concentration the compound exhibits cross affinity for the 5-HT_2 and α-adrenergic receptors (Figs 3, 4). The reversing effects of mianserin were concentration-related but somewhat diminished at the highest concentration.

In an attempt to observe a clearer inhibitory response by 5-HT on rat stomach fundus, carbachol-precontracted tissue was utilized. However, neither inhibition nor contraction by 5-HT on untreated control and mucosa-denuded tissues was seen, as would be expected if inhibitory effects by 5-HT were mediated through a direct activation of a second (low affinity) receptor. The stronger carbachol effects on gastric smooth muscle masked completely any 5-hydroxytryptamine relaxant effects on the tissue indicating cholinergic activation appears not to be affected by this inhibition.

Reversal of the low affinity response of 5-HT by *S*-(−)-propranolol and mianserin, but not by other 5-HT_1 antagonists lacking adrenergic blocking effect, suggested that this inhibition could be related to intramural catecholamine release. To demonstrate if blockade of catecholamines or reduction of catecholamine content would make a difference in the 5-HT curve, three different approaches were utilized: the use of a reserpinized preparation where catecholamine storage had been depleted; the use of a preparation where the mucosa layer had been partly removed taking out myomucosal plexus neurons; and the use of a mucosa-denuded preparation incubated with a combination of prazosin and *S*-(−)-propranolol. The effects of 5-HT in these preparations, as compared with intact fundus, suggested an activation of catecholamine release by 5-HT on rat fundus and that both depletion of catecholamines and the blockade of adrenergic receptors show only a monophasic 5-HT curve (contractile effects). The maximum effect for 5-HT in reserpinized tissue was greatly amplified (185%) and the maximum effect was not seen at the maximum concentration used (3×10^{-5} M). It would appear that the true maximum occurs at even greater concentrations (Fig. 6). This finding certainly can affect the calculation of valid pD_2 and intrinsic activity values for 5-HT, α-Me-5-HT and other 5-HT agonists as obtained in the first part of the study, although it is conceivable that the order of potencies could remain unchanged.

Clineschmidt et al (1985) tested the effects of α_1- and α_2-adrenergic antagonists on the response elicited by 5-HT on fundus and demonstrated that these compounds were either weak antagonists of 5-HT or ineffective in this preparation. Results of the present research, however, indicate that these compounds do amplify the response of 5-HT and it is possible that the previously reported lack of response of these compounds may be due to the low 5-HT concentrations used.

It would appear that the inhibitory phase observed with 5-HT and α-Me-5-HT on rat fundus may be due to catecholamine release by either activation of a second receptor site present in intramural neurons located near or within the mucosa layer or by the intake of 5-HT into the neurons causing catecholamine release. The contribution of the low-affinity inhibition needs to be explored as a factor when calculating intrinsic activity and affinity constants in the future.

Acknowledgements

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References

Baez, M., Yu, L., Cohen, M. L. (1990) Pharmacological and molecular evidence that the contractile response to serotonin in rat stomach fundus is not mediated by activation of the 5-hydroxytryptamine$_{1C}$ receptor. Mol. Pharmacol. 38: 31–37

Buchheit, K. H., Engel, G., Hagenbach, A., Hoyer, D., Kalkman, H. O., Seiler, M. P. (1986) The rat isolated stomach fundus strip, a model for 5-HT$_{1C}$ receptors. Br. J. Pharmacol. 88: 367P

Clineschmidt, B. V., Reiss, D. R., Pettibone, D. J., Robinson, J. L. (1985) Characterization of 5-hydroxytryptamine receptors in rat stomach fundus. J. Pharmacol. Exp. Ther. 235: 696–708

Cohen, M. L. (1989) 5-Hydroxytryptamine and nonvascular smooth muscle contraction and relaxation. In: Fozard J.R. (ed.) The Peripheral Actions of 5-Hydroxytryptamine, 1st edn. Oxford Medical Publications, Oxford, UK, pp 201–219

Cohen, M. L., Fludzinski, L. A. (1987) Contractile serotonergic receptor in rat stomach fundus. J. Pharmacol. Exp. Ther. 243: 264–269

Conn, P. J., Sanders-Busch, E., Hoffman, B. J., Hartig, P. R. (1986) A unique serotonin receptor in choroid plexus is linked to phosphatidyl inositol turnover. Proc. Natl. Acad. Sci. USA 83: 4086–4088

Foguet, M., Hoyer, D., Pardo, L. A., Parekh, A., Kluxen, F. W., Kalkman, H. O., Stuhmer, W., Lubbert, H. (1992) Cloning and functional characterization of the rat stomach fundus serotonin receptor. EMBO J. 11: 3481–3487

Fozard, J. R. (1984) MDL-72222, a potent and highly selective antagonist at neuronal 5-hydroxytryptamine receptors. Naunyn Schmiedebergs Arch. Pharmacol. 326: 36–44

Glennon, R. A. (1987) Central serotonin receptors as targets for drug research. J. Med. Chem. 30: 1–12

Hoyer, D. (1989) 5-Hydroxytryptamine receptors and effector coupling mechanisms in peripheral tissues. In: Fozard J.R. (ed.) The Peripheral Actions of 5-Hydroxytryptamine, 1st edn. Oxford Medical Publications, Oxford, UK, pp 72–99

Humphrey, P. P. A., Hartig, P., Hoyer, D. A. (1993) A proposed new nomenclature for 5-HT receptors. Trends Pharmacol. Sci. 14: 233–236

Kursar, J. D., Nelson, D. L., Wainscott, D. B., Cohen, M. L., Baez, M. (1992) Molecular cloning, functional expression, and pharmacological characterization of a novel serotonin receptor (5-hydroxytryptamine$_{2F}$) from rat stomach fundus. Mol. Pharmacol. 42: 549–557

Malone, M. H. (1990) Biometrics in the Pharmaceutical Sciences. University of the Pacific, Stockton, CA, USA

Offermeier, J., Åriens, E. J. (1966) Serotonin I: receptors involved in its action. Arch. Int. Pharmacodyn. 164: 192–215

Tallarida, R. J., Murray, R. B. (1987) Manual of Pharmacologic Calculations with Computer Programs, 2nd edn. Springer-Verlag, New York

Vane, J. R. (1957) A sensitive method for the assay of 5-hydroxytryptamine. Br. J. Pharmacol. 12: 344–349

Van Nueten, J. M., Janssen, P. A. J., Vanhoutte, P. M. (1983) Pharmacological properties of serotonergic responses in vascular, bronchial and gastrointestinal smooth muscle. In: Bevan, J.A. (ed.) Vascular Neuroeffector Mechanisms, 1st edn. Raven Press, New York, pp 237–242

Van Rossum, J. M. (1963) Cumulative dose-response curves. II: Technique for the making of dose-response curves in isolated organs and the evaluation of drug parameters. Arch. Int. Pharmacodyn. 143: 299–330

Van Rossum, J. M., Van den Brink, F. G. (1963) Cumulative dose-response curves. I: Introduction to the technique. Arch. Int. Pharmacodyn. 143: 240–246

Wrigglesworth, S. J. (1983) Heterogeneity of 5-hydroxytryptamine receptors in the rat uterus and stomach strip. Br. J. Pharmacol. 80: 691–697