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Characterization of presynaptic 5-HT receptors on adrenergic nerves supplying the bovine ovarian follicle

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1 The effects of 5-hydroxytryptamine (5-HT) on contraction and release of [^{3}H]-noradrenaline were investigated *in vitro* in bovine ovarian follicle strips. Using available selective agonists and antagonists, an effort was made to characterize the type of receptor mediating the inhibitory effect of 5-HT on neurogenic contraction and release of [^{3}H]-noradrenaline by electrical field stimulation.

2 5-Hydroxytryptamine inhibited the neurogenic contraction and release of [^{3}H]-noradrenaline evoked by electrical field stimulation in a concentration-dependent manner. Like 5-HT, 5-carboxamidotryptamine (5-CT) and methysergide reduced the transmitter release as well as the neurogenic contraction, whereas 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) failed to inhibit both responses in concentrations up to 0.1 μM.

3 The 5-HT (1 μM)-induced inhibition of contractile responses was more evident during stimulation at low frequencies (4 and 8 Hz) than during high frequency electrical stimulation (16 and 32 Hz).

4 Methiothepin (1 μM) and methysergide (10 μM) significantly antagonized the inhibitory effect of 5-HT on the electrically evoked release of tritium, whereas cyanopindolol, MDL 72222 and ketanserin (all 0.1 μM) were without effect. In addition, ketanserin, MDL 72222, cimetidine, pyrilamine, atropine, propranolol and indomethacin were without effect on the 5-HT-induced inhibition of the neurogenic contraction.

5 It is suggested that 5-HT inhibits the electrically evoked transmitter release from adrenergic nerves in the bovine ovarian follicle wall via prejunctional 5-HT$_1$-like receptors. This was based on the findings that 5-CT was a potent agonist, methiothepin an antagonist and the lack of effect of MDL 72222, cyanopindolol and ketanserin.

Introduction

Evidence has been presented for more than one type of neuronal receptor for 5-hydroxytryptamine (5-HT) in various peripheral tissues. Both excitatory and inhibitory effects of 5-HT have been demonstrated in postganglionic sympathetic neurones, where inhibition is suggested to be mediated by the 5-HT$_1$ type of receptor (Fozard, 1984a). Radioligand binding studies in the central nervous system have shown that 5-HT$_1$ receptors can be divided into at least three different subclasses, namely 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1C}$ receptors (Pedigo *et al.*, 1981; Middlemiss & Fozard, 1983; Pazos *et al.*, 1984). In certain peripheral organs the inhibitory effect of 5-HT on the sympathetic transmission seems to be mediated by receptors that cannot be classified as any of these subtypes. These 'atypical' 5-HT receptors, often called 5-HT$_1$-like receptors have so far been demonstrated in saphenous vein (McGrath, 1977; Feniuk *et al.*, 1979), vas deferens (Kapur & Mottram, 1979), myocardium (Martinez & Lokhandwala, 1980), femoral vasculature (Feniuk *et al.*, 1981b) and kidney (Charlton *et al.*, 1986).

Preovulatory follicles of the mammalian ovary receive a dense autonomic innervation supplying contractile cells in the theca externa layer of the follicle (Owman *et al.*, 1979). Although it has previously been shown, *in vitro* and *in vivo*, that 5-HT can both contract and relax the follicle wall, the receptors involved have not been characterized (O'Shea & Phillips, 1974; Gimeno *et al.*, 1976; Talbot & Schroeder, 1982). Neither have any previous studies dealt with interactions between 5-HT and adrenergic nerves in the ovary.

The aim of the present investigation was to provide a basis for understanding the role of 5-HT in ovarian function by elucidating whether 5-HT has prejunctional effects on the transmitter release from local adrenergic nerves. The bovine follicle provides a suitable model because the nerve-induced contraction

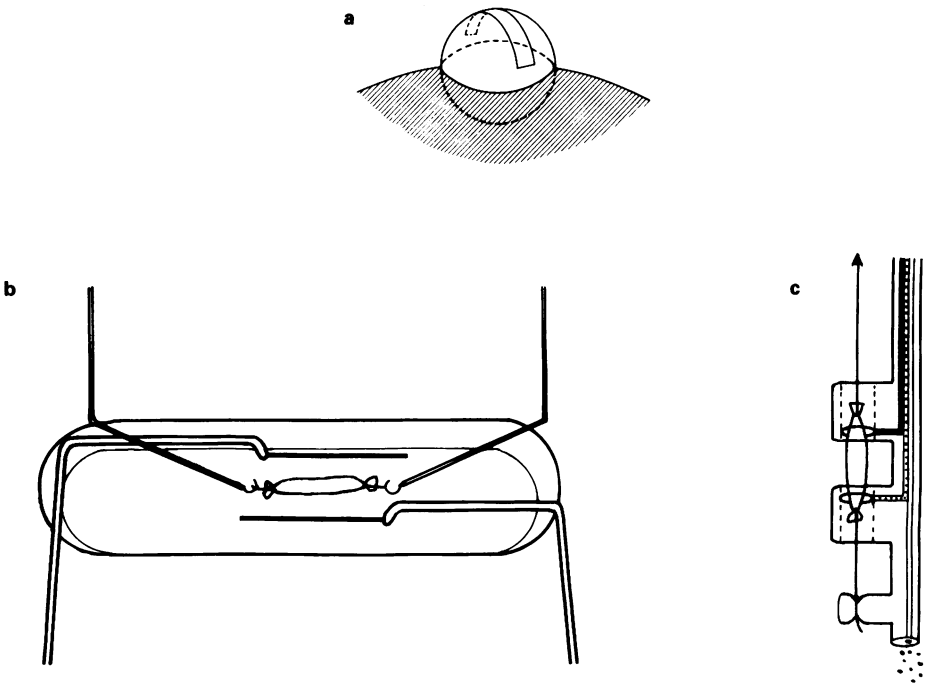

Figure 1 Schematic illustration of follicle strip preparation. The 2 × 10 mm strip was cut from the protruding part of the Graafian follicle (a). The strip was mounted in a 7.5 ml organ bath between holders, one adjustable and one connected to a force-displacement transducer, for recording of motor activity during electrical field stimulation between two parallel platinum-wire electrodes (b). Following incubation of the follicle strip in the presence of [^{3}H]-noradrenaline the tritium efflux was determined during electrical field stimulation of the strip mounted inside two ring-form platinum electrodes (c).

involves mainly the adrenergic nerves (Walles *et al.*, 1982) and the corresponding postjunctional receptors in the neuromuscular complex are well characterized (Walles *et al.*, 1975).

Some of the results have been presented at the Federation meeting of American Societies for Experimental Biology, March 1987.

Methods

Tissue preparation

Bovine ovaries were obtained from a local slaughter-house about 20 min after animal death. Only ovaries with well-developed Graafian follicles (10–20 mm in diameter) were removed. They were immediately put in ice-cold (0–5°C) buffered Krebs-Ringer solution. All follicles used protruded above the ovarian surface and their apical region was thin and transparent. No further estimation of the cyclic stage was carried out. Strips, 10 × 2 mm, were cut from the protruding part of the follicle (Figure 1), and a 5–0 silk ligature was tied around each end of the strip.

Recording of motor activity

The strip was mounted longitudinally in a 7.5 ml mantled organ bath (Figure 1), with one end of the preparation attached to a force-displacement transducer (Grass FT03C) and the other to an adjustable device to obtain passive load of the preparation. The bath contained a Krebs-Ringer solution of the following composition (mM): NaCl 118, KCl 4.69, $CaCl_2$ 2.52, $MgSO_4$ 1.16, $NaHCO_3$ 24.90, KH_2PO_4 1.18, glucose 5.56, Ca-EDTA 0.03. The buffer solution was continuously bubbled with a gas mixture of 88.5% O_2 and 11.5% CO_2 to give a mean pH of approximately 7.4. All experiments were performed at 37°C. Isometric contractions were electrically amplified and recorded on a Grass Model 7D Polygraph. The follicle strip was given a load of 4 mN immediately after being set-up and was allowed to relax to a steady level of tension (about 2 mN) for a period of 45 min, during which time the preparation was washed repeatedly with buffer solution.

For electrical field stimulation of the nerves in the preparation, platinum electrodes were placed on each side parallel to the strip (Figure 1) at a distance of a few mm from the preparation. The electrodes were connected to a stimulator where trains of monophasic square-wave pulses (9 V between the electrodes) were provided at a pulse duration of 1 ms and a frequency of 8 Hz in most experiments, giving approximately 70% of maximum contraction (Walles *et al.*, 1982). Each train of pulses lasted for 5 s and was followed by a 99 s pause.

In the quantitative evaluations of drug actions on the electrically-induced contractions, the mean effect of 5 consecutive control trains was calculated and set as 100%. This was followed by a new stimulation period in the presence of the drug, where the mean % effect was calculated from another set of 5 stable consecutive trains. The maximum inhibition (I_{max}) was noted together with the concentration giving half-maximum effect, expressed as the negative logarithm (pD_2 value). In order to avoid tachyphylaxis, a new strip was tested at each dose level to achieve the total number of experiments based on 4–11 strips.

To examine the effect of 5-HT on contraction induced by exogenous noradrenaline, two cumulative concentration-response curves for noradrenaline were made in each strip. The maximum contractile force (mN) was determined from the first curve. The second concentration-response curve was performed in the absence (control) and presence (test strip) of 5-HT (3 μM). The EC_{50} values for the second curves were calculated and compared. The E_{max} values were determined from the second contraction and expressed as a percentage of the maximum contractile force elicited in the first curve. 5-HT was administered 15 min before the initial addition of noradrenaline.

The drugs were administered into the organ chamber in volumes of 30 or 90 μl. The blocking agents were added 10–15 min before 5-HT except for indomethacin, which was added 30 min before 5-HT. The effects of methiothepin and ketanserin on the neurogenic contraction were tested after 10–15 min and after 25 min. A control strip which was not exposed to the drug was run simultaneously for both experiments.

Recording of tritium release

The technique used in this study has been described previously in detail (Wikberg & Axelsson, 1980). The strips were preincubated for 10 min in Krebs-Ringer buffer solution (for composition, see above) followed by incubation in the presence of [^{3}H]-noradrenaline (0.1 μM) together with 10 μM normetanephrine and 0.2 mg ml^{-1} ascorbic acid for 40 min, all at 37°C. The preparation was subsequently washed 5 times for 10 min, each time with 250 ml buffer solution containing normetanephrine, ascorbic acid and 0.6 μM desipramine (all present throughout the experiment). It was mounted in perspex holders and surrounded by two platinum ring electrodes (Figure 1) and put in vials containing 4 ml of Krebs-Ringer solution at 37°C. The tissue preparations were transferred every 2 min (for each stimulation and resting period) to new vials containing buffer solution. At the end of the experiment the radioactivity released in the buffer solution of each vial was determined in a scintillation counter (Nuclear Chicago) after addition of 10 ml Picofluor 30 (Packard). The counting efficiency, determined by an internal standard, was 25%. The tissue preparation was dissolved in 1 ml Soluene (Packard) and 10 ml of Instafluor (Packard) was added for scintillation counting of remaining radioactivity. This allowed for the determination of fractional tritium release throughout an individual experiment, expressed as % of total radioactivity (c.p.m.) measured in each individual 2 min fraction.

Four 2 min periods of transmural electrical stimulation (5 Hz, 1 ms duration of the monophasic pulses, 10 V over the electrodes) were applied to each strip at 10 min (S1), 24 min (S2), 36 min (S3) and 48 min (S4) after washing and mounting the tissue. S1 and S2 served as controls in each experiment, whereas S3 was performed in the presence of test substances, followed by a washout period of the agonist and another control stimulation (S4).

The agonists were added immediately after S2, so that the preparation was exposed to each compound for a total of 12 min, including S3. In experiments with 5-HT, the antagonists were introduced in the first vial and were subsequently present during the remaining period of the experiment. The effect of methiothepin and ketanserin on electrically-evoked tritium release was investigated after 10 min (S3) and after 22 min (S4). The S3/S2 ratio and the S4/S2 ratio were compared with those of control experiments. All test substances used were added to the vials in volumes of 10–20 μl. Basal tritium efflux was expressed as the mean concentration in three previous vials before each individual stimulation and was set as 100%. Stimulation-evoked release was then related to the basal tritium efflux value. The effect of 5-HT and related agonists and antagonists was assessed by comparing the release of tritium during S3 with that of the expected efflux, which was calculated as an average of the amounts released during S2 and S4. In control experiments the amount released during S3 did not differ significantly from the calculated average of the amounts during S2 and S4.

Analysis of data

Student's t test for unpaired observations was used to compare mean values ± s.e.means in all experiments. P values less than 0.05 were regarded as significant.

Regression lines were obtained by the method of least squares.

Drugs

The following drugs were used: 5-hydroxytryptamine creatinine sulphate, tetrodotoxin, normetanephrine HCl, pyrilamine maleate, (−)-arterenol HCl, ethylenediamine tetraacetic acid disodium-calcium salt (Ca-EDTA) (all from Sigma, St Louis, MO, U.S.A.), phentolamine mesylate (Regitin; Ciba-Geigy, Basel, Switzerland), propranolol HCl (Inderal; ICI, Macclesfield, U.K.), atropine sulphate (ACO, Solna, Sweden), cimetidine (Tagamet; SK & F, Welwyn Garden City, Hertfordshire, U,K.), ketanserin tartrate (R49945; Janssen, Beerse, Belgium), indomethacin (Confortid; Dumex, Copenhagen, Denmark), methiothepin maleate (Roche, Basel, Switzerland), bufotenin (N,N-dimethyl-5-HT binoxalate), (±)-cyanopindolol, mesulergine HCl and methysergide hydrogen maleinate (gifts from Dr G. Engel, Sandoz, Basel, Switzerland), 5-carboxamidotryptamine HCl (5-CT, gift from Dr P.P.A. Humphrey, Glaxo, Ware, U.K.), 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT, gift from Dr B. Andersson, Department of Pharmacology, Göteborg, Sweden), 1'H,3',5'H-tropan-3-yl 3,5-dichlorobenzoate methane sulphonate (MLD 72222, gift from Dr J. Fozard, CRMI, Strasbourg, France), desipramine HCl (Pertofrin; Ciba-Geigy, Basel, Switzerland) and (−)-ring-2,5,6-[^{3}H]-noradrenaline (52.9 Ci mmol^{-1}; New England Nuclear, Dreieich, GFR).

Results

The follicle strip preparations showed no spontaneous contractility. In each individual strip the electrical field stimulation induced reproducible contractile responses, which were totally inhibited by tetrodotoxin (0.1 μM), indicating that only nerves in the preparation were activated without direct stimulation of the muscle cells (Moore & Narahashi, 1967). The α-adrenoceptor antagonist, phentolamine (1 μM) inhibited the neurogenic contractions by 87%, whereas controls or preparations treated with 1 μM atropine were only slightly reduced (by 15%) or not affected at all. Thus, the main part of the contractile response appears to be mediated by the extensive system of adrenergic nerves present in the follicle wall (Stefenson *et al.*, 1981).

The concentration-response curve for noradrenaline was unaltered in the presence of 3 μM 5-HT. ($EC_{50} = 3.03 \pm 0.24 \times 10^{-6}$ M, $n = 4$ for the controls and for the test group it was $3.16 \pm 0.58 \times 10^{-6}$ M, $n = 5$.) Also the E_{max} values were not significantly different (64 ± 3.6% for the controls and 61 ± 4.8% for the test group).

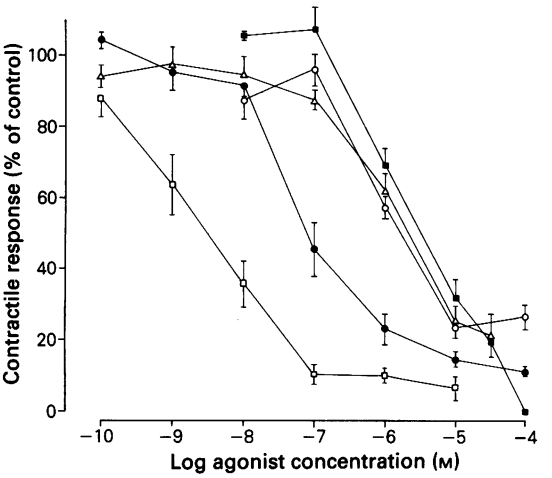

Figure 2 Contractile response to electrical field stimulation of follicle strips is reduced in the presence of 5-hydroxytryptamine (●), bufotenin (○), 8-hydroxy-2-(di-n-propylamino)tetralin (△), methysergide (■) and 5-carboxamidotryptamine (□). Values are means of 4–13 experiments at each concentration; vertical lines indicate s.e.mean.

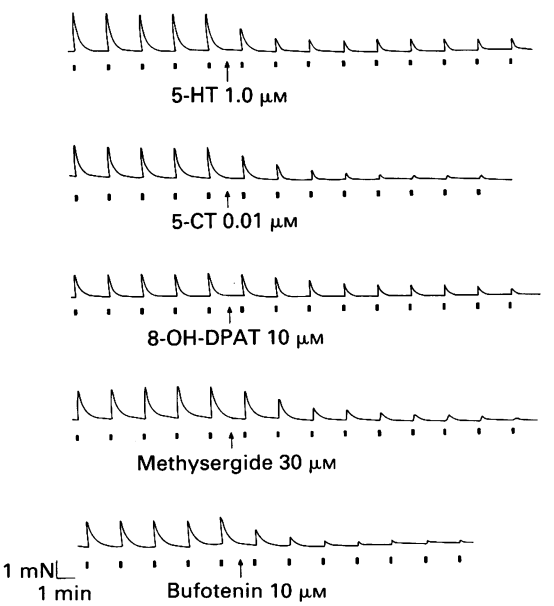

Figure 3 Single experiments on follicle strips showing effect of 5-hydroxytryptamine receptor agonists on contractions induced by nerve stimulation as indicated (9 V, 8 Hz, 1 ms pulse duration). 5-HT = 5-hydroxytryptamine, 5-CT = 5-carboxamidotryptamine, 8-OH-DPAT = 8-hydroxy-2-(di-n-propylamino)tetralin.

The neurogenic contraction was inhibited by 5-HT in a concentration-dependent manner (Figures 2 and 3). The effect was rapid in onset, being evident within 2–3 min after addition of the amine (Figure 3), and it lasted for at least the 30 min test period. The inhibition of the contractile response by 1 μM 5-HT was in general more pronounced at lower frequencies of electrical field stimulation (4 and 8 Hz), when over 75% of the response was abolished, compared to high frequency stimulation (16 and 32 Hz) (as seen in Figure 4).

Also 5-CT, bufotenin, 8-OH-DPAT and methysergide inhibited the contractile response to electrical field stimulation (Figures 2 and 3), 5-CT being the most potent. Bufotenin, 8-OH-DPAT, and methysergide were weaker at inhibiting the neurogenic contraction than 5-HT. The effect of 8-OH-DPAT was slow and gradual in onset (Figure 3) and a maximum response was only obtained after 20 min. Methysergide caused a total blockade of the response, whereas the other agonists produced incomplete inhibition of the neurogenic contraction (Figure 2 and Table 1). From the pD_2 values of the agonists tested, as presented in Table 1, the relative potencies were in the order 5-CT > 5-HT > 8-OH-DPAT > bufotenin > methysergide. Of the agonists tested 5-CT was about 40 times more active than 5-HT itself. This indicated that the 5-hydroxytryptaminergic inhibition of the nerve-induced contraction involved a 5-HT_1-like receptor; this was studied further by examining the electrically-evoked release of tritium from follicular nerves preloaded with [^{3}H]-noradrenaline.

The pattern of fractional release in two sets of experiments is shown in Figure 5. Radioactivity collected during S1 of the electrical field stimulation increased by 260 ± 62% compared to the basal efflux. The amount of tritium released was reduced progres-

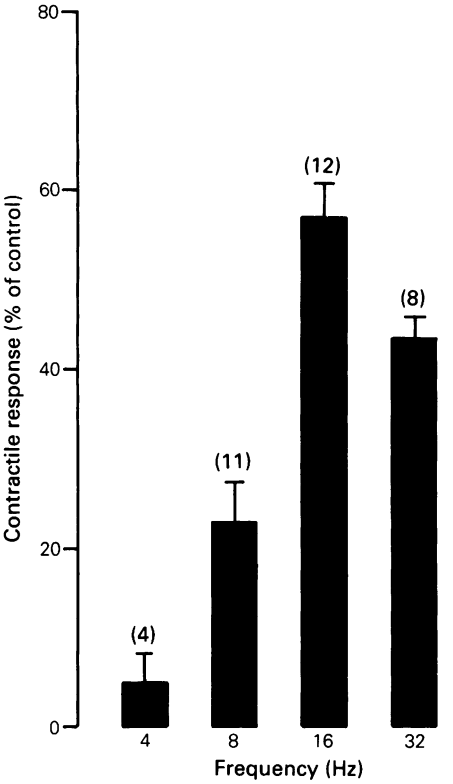

Figure 4 Effect of 5-hydroxytryptamine (1 μM) on contractile response induced by electrical field stimulation of nerves. The inhibitory effect is more prominent at lower levels of stimulation frequencies (4 and 8 Hz) compared to high frequency stimulation (16 and 32 Hz). Values are means and vertical lines show s.e.means. Number of experiments is indicated in parentheses.

Table 1 Inhibition of the nerve-induced contraction by five 5-HT receptor agonists in bovine isolated follicle strips, expressed as maximum inhibition (I_{max}) and the negative log of drug concentration producing half-maximum effect (pD_2)

Agonist	I_{max} (%)	pD_2	n	$\frac{EC_{50}\ value\ for\ 5\text{-}HT}{EC_{50}\ value}$
5-CT	93.40 (87.05–99.75)	8.70 (8.35–9.05)	6	44.0
5-HT	88.80 (85.86–91.74)	6.99 (6.74–7.24)	5	1.0
8-OH-DPAT	78.75 (66.72–90.78)	6.20 (6.00–6.40)	4	0.18
Bufotenin	73.50 (66.64–80.36)	6.13 (6.07–6.19)	4	0.16
Methysergide	100	5.40 (5.15–5.65)	4	0.027

Data are expressed as mean values (95% confidence limits). *n* indicates number of experiments.
5-CT = 5-carboxamidotryptamine, 5-HT = 5-hydroxytryptamine, 8-OH-DPAT = 8-hydroxy-2-(di-n-propylamino) tetralin.

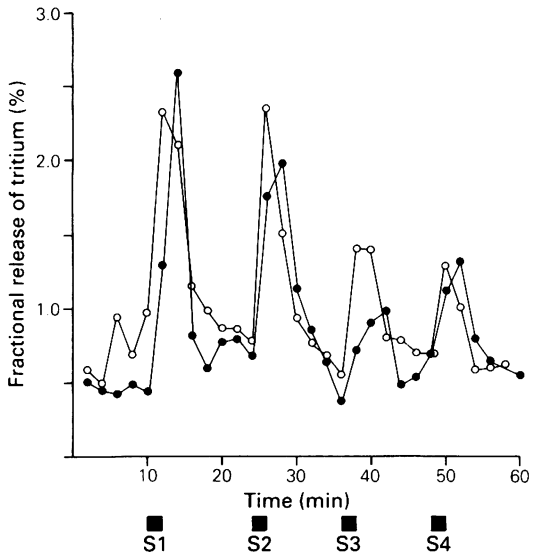

Figure 5 Fractional release of tritium expressed as % of total radioactivity (ordinate scale) including effects of electrical field stimulation (5 Hz, 1 ms, 10 V) at S1–S4. (●) 5-Hydroxytryptamine (5-HT) was present 10 min before and during S3. (○) Represents corresponding results from control experiment in the absence of 5-HT. The abscissa scale shows the time after washing and mounting of the preparation.

sively during each period (S1–S4) of electrical stimulation, and when the decline was expressed in logarithmic form it represented a line with a correlation coefficient, $r = 0.98$. Thus, the release during S3, in the absence of test substance, was not significantly different from the mean release of S2 + S4. The tritium efflux induced by field stimulation was totally blocked by 0.1 μM tetrodotoxin.

The basal release of tritium in preparations not subjected to electrical field stimulation was unaffected by 5-HT (tested up to 1 μM). As shown in Figure 6 the tritium release during electrical field stimulation (S3) was reduced in a concentration-dependent manner by 5-HT (0.01, 0.03, 0.1 and 1 μM). Following washout of 5-HT between S3 and S4 the tritium efflux during S4 returned to values not significantly different from the corresponding controls not exposed to 5-HT.

Of the other agonists tested 5-CT (0.1 μM) was most potent, reducing the stimulation-evoked tritium efflux by 80% whereas methysergide only caused a minor reduction of the tritium efflux (about 30%). 8-OH-DPAT (0.1 μM) had little or no effect on the electrically-evoked tritium release. However, in high concentrations (10 μM) it evoked a persistent and marked reduction which did not return to the control level following washout.

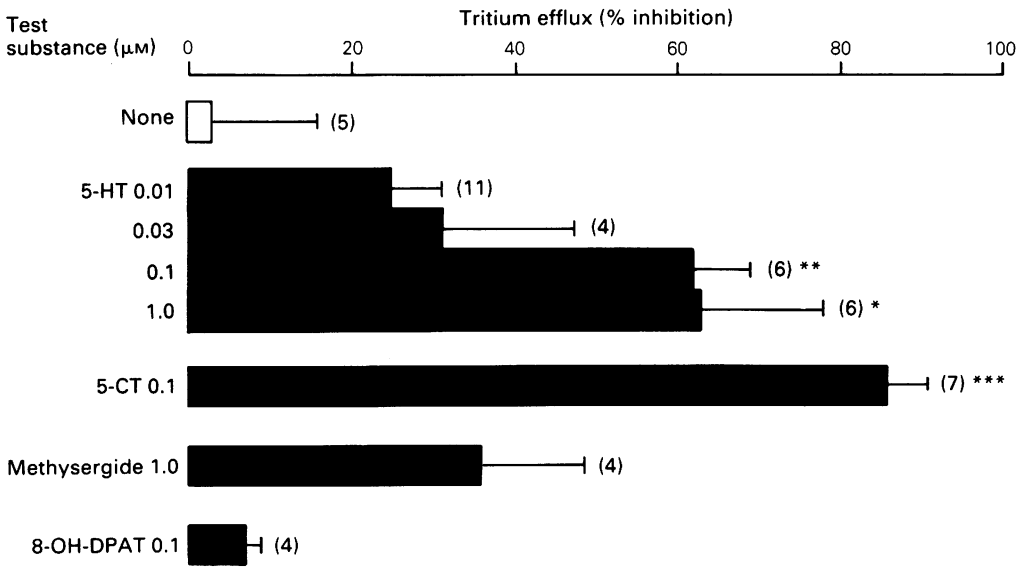

Figure 6 The electrically evoked (5 Hz, 1 ms, 10 V) efflux of tritium following incubation in [^{3}H]-noradrenaline was markedly inhibited by various 5-hydroxytryptamine (5-HT) receptor agonists. Each column represents mean of number of experiments indicated in parentheses; horizontal lines indicate s.e.mean. Differences in comparison with control (in absence of test substance, open column) according to Student's t test: $^*P < 0.05$, $^{**}0.001\ P < 0.01$, $^{***}P < 0.001$. 5-CT and 8-OH-DPAT represent 5-carboxamidotryptamine and 8-hydroxy-2-(di-n-propylamino)tetralin, respectively.

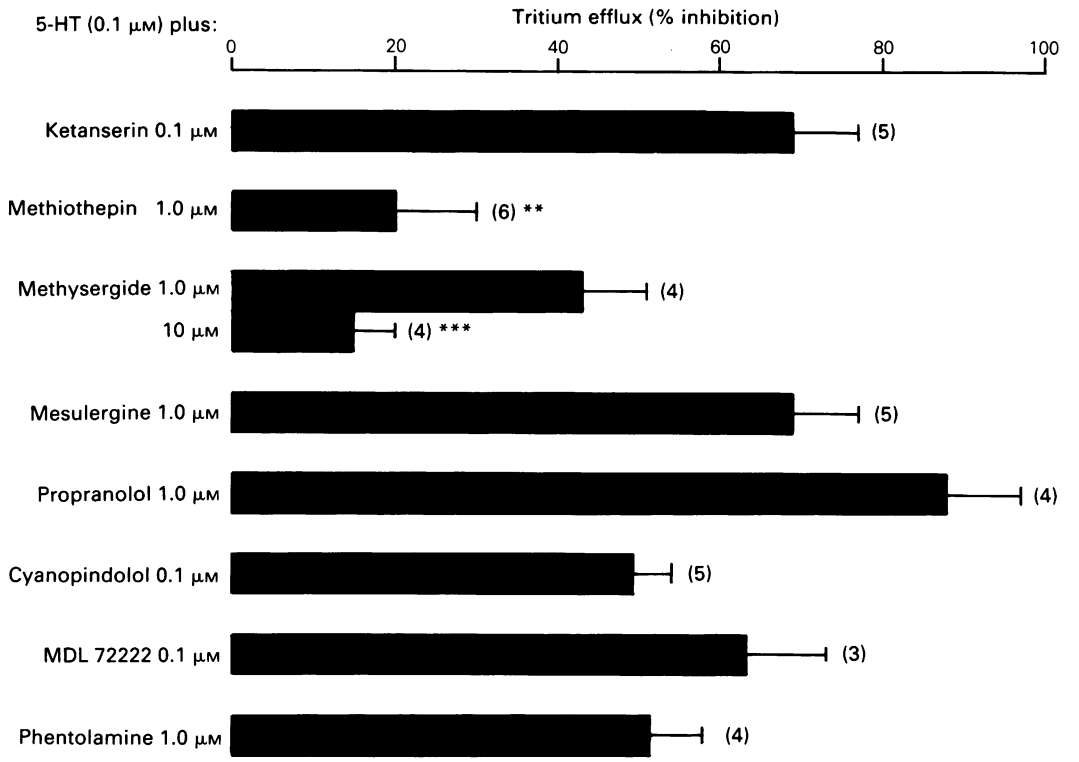

Figure 7 Effect of various antagonists on the 5-hydroxytryptamine (0.1 μM)-induced inhibition of tritium release evoked by nerve stimulation in follicle strips previously incubated with [^{3}H]-noradrenaline. Values are means of number of experiments indicated in parentheses; horizontal lines show s.e.mean. Stimulation parameters as indicated in Figure 6. **$0.001 < P < 0.01$, ***$P < 0.001$, compared to effect of 5-HT (0.1 μM) alone (Student's t test).

The effects of antagonists on the inhibition of tritium efflux induced by 0.1 μM 5-HT are presented in Figure 7. Methysergide (10 μM) and methiothepin (1.0 μM) markedly reduced (by more than 65%) the 5-HT induced inhibition of the electrically-evoked tritium release. The inhibition by 1 μM methysergide was not statistically significant. Neither mesulergine (1 μM) nor ketanserin (0.1 μM) antagonized the 5-HT response. Propranolol (1 μM) and cyanopindolol (0.1 μM) were also without effect or slightly enhanced the 5-HT-induced inhibition of stimulation-evoked tritium release. Also the 5-HT$_3$ receptor antagonist MDL 72222 was without effect on the 5-HT-induced reduction in tritium efflux. Further, the α-adrenoceptor antagonist phentolamine (1 μM) failed to antagonize the inhibitory effect of 5-HT.

The lack of antagonism of the 5-hydroxytryptaminergic inhibition was also confirmed for the electrically-evoked motor response, as tested with ketanserin (0.1 μM) (see Figure 8), MDL 72222 (0.1 μM) and propranolol (1 μM). In addition cimetidine (10 μM), pyrilamine (10 μM), atropine (10 μM), and indomethacin (100 μM) were without effect on the motor response. As expected, ketanserin did not affect the inhibition induced by 5-CT (Figure 8).

Effects of the antagonists alone on the stimulation-evoked tritium release are presented in Figure 9. Ketanserin (0.1 μM) and methiothepin (1 μM) had no significant influence on the tritium release when investigated 10 and 25 min after the drug administration. MDL 72222 (0.1 μM) was also without effect on the electrically-evoked tritium release, whereas cyanopindolol (0.1 μM) and propranolol (1 μM) reduced the tritium release by about 20%. Mesulergine (1 μM) increased the efflux by 30%.

Discussion

This study was undertaken to investigate the effect of 5-HT on the contraction of the ovarian follicle induced by electrical field stimulation of nerves innervating the

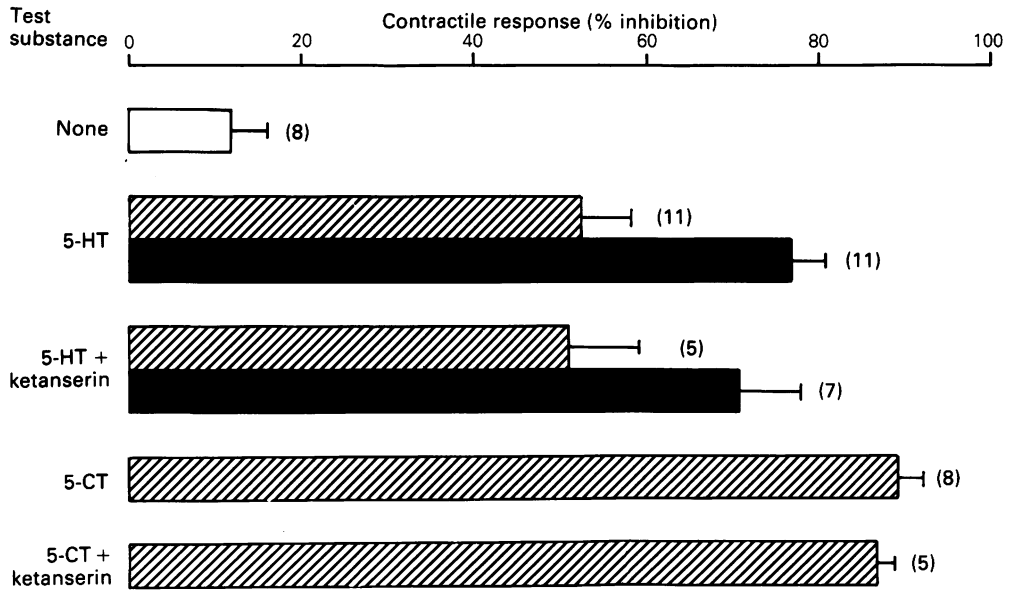

Figure 8 The inhibition of the nerve-induced contraction induced by 5-hydroxytryptamine (5-HT, 0.1 μM hatched column; 1 μM closed columns) and 5-carboxamidotryptamine (5-CT, 0.1 μM) are not antagonized by ketanserin (0.1 μM). The control level (open column reflects individual variability of the follicle strip preparation. Each column represents mean of number of experiments indicated in parentheses; horizontal lines show s.e.mean.

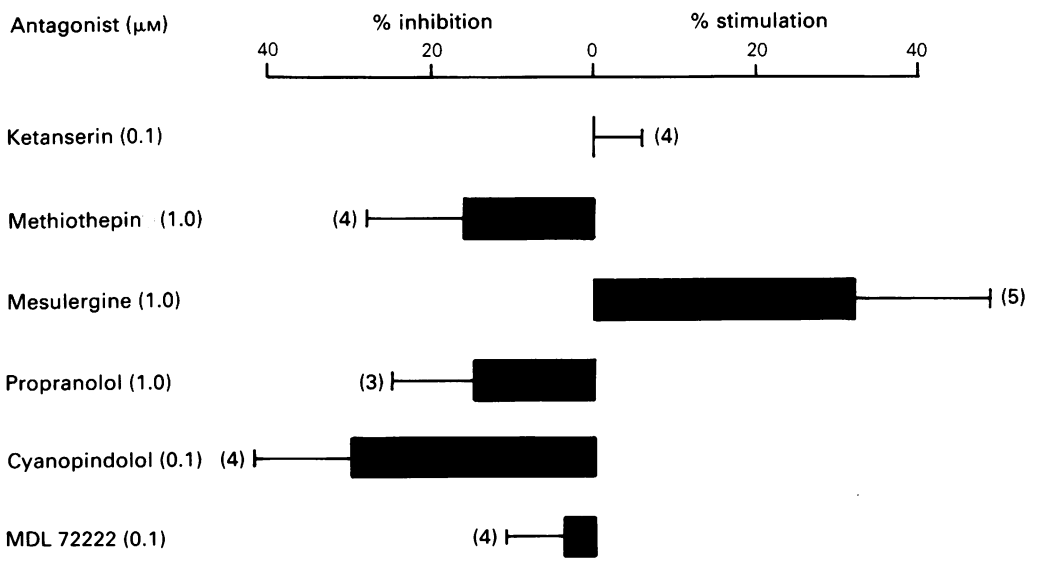

Figure 9 The antagonists tested had no or a modest effect by themselves on the tritium release evoked by electrical field stimulation of follicle strips previously incubated with [^{3}H]-noradrenaline. Each column represents mean of number of experiments indicated in parentheses; horizontal lines show s.e.mean. The ordinate scale indicates % change (inhibition or stimulation) in electrically evoked tritium efflux induced by the antagonists themselves. Stimulation parameters as indicated in Figure 6.

contractile cells in follicle theca externa. Attempts were made to characterize further the type of prejunctional receptor involved in this mechanism by measurement of [^{3}H]-noradrenaline release from the follicular nerves.

The initial experiments suggested that the effect of 5-HT was primarily mediated by a presynaptic site rather than by postjunctional actions, since 5-HT did not affect the noradrenaline-induced contractions but strongly inhibited the contractile response to electrical field stimulation. Moreover, the inhibitory effect of 5-HT was more pronounced at low stimulation frequencies, which has been reported to be characteristic for drugs affecting calcium-dependent, stimulation-evoked release of transmitter (Westfall, 1977; Feniuk *et al.*, 1979).

The electrically-evoked efflux of tritium was used as a convenient index for the release of transmitter from adrenergic nerves following incubation in [^{3}H]-noradrenaline. The method applied has been discussed in detail elsewhere (Wikberg & Axelsson, 1980). The release of tritium evoked by electrical field stimulation was markedly reduced in the presence of 5-HT. This is consistent with the results from contraction experiments and further supports the view that 5-HT inhibits release of noradrenaline through a presynaptic receptor. Presynaptic inhibitory 5-HT receptors on adrenergic nerves have been demonstrated in other sympathetically innervated peripheral organs (McGrath, 1977; Kapur & Mottram, 1979; Martinez & Lokhandwala, 1980; Feniuk *et al.*, 1981b; Charlton *et al.*, 1986).

The lack of specific antagonists has made it difficult to define clearly the receptor involved in the presynaptic inhibitory action of 5-HT (Fozard, 1984a). In fact, the 5-HT receptor antagonist methysergide has been found to act as a partial agonist at this site (Watts *et al.*, 1981; Fozard, 1984a), which also appears to be the case in the present study. On the other hand it has also been shown that methysergide acts as an α-adrenoceptor antagonist (Humphrey, 1984), which could account for the complete inhibition of the neurogenic contractile response. The inhibitory action of methysergide on the tritium release is, however, difficult to explain by the blockade of prejunctional α-adrenoceptors, since this would be expected to enhance transmitter release (Starke, 1981). Indeed, the α-adrenoceptor antagonist phentolamine did induce a concentration-dependent rise in the electrically evoked tritium release (at 1 μM; a rise of 40%). Also the α_2-adrenoceptor agonist oxymetazoline inhibited the tritium release induced by neurogenic stimulation, further supporting the existence of prejunctional predominantly α_2-type adrenoceptors (unpublished observations).

The selective 5-HT$_2$ receptor antagonist ketanserin (van Nueten *et al.*, 1981) failed to block the action of 5-HT and 5-CT, suggesting that the effect of 5-HT described in this study cannot be mediated via 5-HT$_2$ receptor stimulation. Ketanserin in the concentration used (0.1 μM) probably did not act on pre- or postjunctional α-receptors, since it did not affect the tritium efflux or the contraction of the preparation induced by neurogenic stimulation. This is consistent with the findings of Göthert *et al.* (1986) for the human saphenous vein.

The possibility that the 5-HT$_3$ receptor might be involved in the effect described was excluded by experiments with the selective and potent receptor antagonist MDL 72222 (Fozard, 1984b; Bradley *et al.*, 1986), which failed to antagonize the effect of 5-HT (tested up to 0.1 μM). Furthermore, the involvement of a 5-HT$_3$ receptor did not seem plausible, since this receptor usually mediates excitatory actions of 5-HT on transmitter release (Bradley *et al.*, 1986), whereas the effect of 5-HT in the present study was constantly inhibitory.

It was unlikely that the 5-HT$_{1A}$ subtype of receptor mediated the inhibitory effect of 5-HT on the adrenergic nerves of the follicle, since 8-OH-DPAT, a selective ligand for 5-HT$_{1A}$ binding sites, was without effect in a concentration that completely displaces [^{3}H]-5-HT from these sites (Middlemiss & Fozard, 1983). Higher concentrations of 8-OH-DPAT may also stimulate α-adrenoceptors (Timmermans *et al.*, 1984), which could explain the inhibition of tritium release seen when the highest concentration of 8-OH-DPAT was used. This may also explain the results from the motor experiments, where 8-OH-DPAT inhibited the contraction induced by nerve stimulation in a concentration-dependent manner possibly via stimulation of prejunctional α-adrenoceptors.

The inhibitory 5-HT$_{1B}$ receptors have been characterized on cortical 5-hydroxytryptaminergic neurones and are blocked by certain classical β-adrenoceptor antagonists, such as propranolol and pindolol (Middlemiss, 1984; 1986; Engel *et al.*, 1986). The 5-HT response in the present study was not influenced by either propranolol or cyanopindolol, which supports the view that neither 5-HT$_{1A}$ nor 5-HT$_{1B}$ sites are involved in the 5-HT-induced inhibition of transmitter release in the follicle wall.

Mesulergine, which recently has been shown to bind to 5-HT$_{1C}$ receptors in the choroid plexus (Pazos *et al.*, 1984) was also, at a high concentration (1 μM), without effect on 5-HT-induced inhibition of noradrenaline release, indicating that this subtype of receptor probably does not mediate the prejunctional effect of 5-HT in the ovarian follicle.

The finding that the 5-HT-induced inhibition of electrically evoked tritium release was inhibited by methiothepin and methysergide, the latter in high concentrations (10 μM), suggested that the prejunctional 5-HT receptor involved might be the so-called 5-HT$_1$-like receptor (Bradley *et al.*, 1986). Methiothepin

by itself did not alter the electrically evoked tritium release, which indicates that it did not have any significant prejunctional α_2-adrenoceptor blocking effect as it had in human saphenous vein (Göthert *et al.*, 1986). In an attempt to characterize the presynaptic site of action for 5-HT, certain synthetic and putative 5-HT agonists, such as 5-CT, have been used. In our study 5-CT was about 40 times more active than 5-HT in its inhibitory effect on the neurogenic contraction. It has been suggested that this substance is rather specific for the 5-HT_1-like receptors present on adrenergic nerves (Feniuk *et al.*, 1981a; Bradley *et al.*, 1986). In the *in vitro* perfused rat kidney it is about 6 times more active than 5-HT itself (Charlton *et al.*, 1986). Bufotenin is another agonist, which in the canine saphenous vein is more potent than 5-HT but weaker than 5-CT in inhibiting stimulation-induced noradrenaline release (Engel *et al.*, 1983). In the ovarian follicle it also inhibited the adrenergic neurogenic contraction in a similar way to 5-HT, but here it was less active than 5-HT.

Definitive characterization of the 5-HT receptor involved in the inhibition of noradrenaline release from sympathetic nerves of the follicle wall is not possible until a specific and selective receptor blocking drug has been identified at this site. So far, it would be appropriate to designate this receptor as 5-HT_1-like, which is in line with the criteria for characterization of this subtype of 5-HT receptor proposed by Bradley *et al.* (1986).

The role of 5-HT in the ovary is not well understood. Besides the previously described motor effects on the follicle wall (O'Shea & Phillips, 1974; Gimeno *et al.*, 1976; Talbot & Schroeder, 1982), it is suggested that 5-HT stimulates progesterone synthesis in bovine luteal cells *in vitro* (Battista & Condon, 1986). Also the ovarian concentration of 5-HT has been shown to increase during the oestrous period in cycling rats (Clausell & Soliman, 1978). Immunocytochemical work in our laboratory with the use of currently available antisera has failed so far to reveal any 5-hydroxytryptaminergic nerves in the follicle wall. 5-HT may instead affect local receptors through the circulation or by the release from non-neural stores, sucy as enterochromaffin cells and platelets.

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