ELSEVIER

European Journal of Pharmacology 312 (1996) 215–225

ejp

# Characterisation of a postjunctional 5-ht$_7$-like and a prejunctional 5-HT$_3$ receptor mediating contraction of rat isolated jejunum

Peter G. McLean, Ian M. Coupar *

*School of Pharmaceutical Biology and Pharmacology, Victorian College of Pharmacy, Monash University, Parkville, Victoria 3052, Australia*

Received 12 March 1996; revised 14 May 1996; accepted 11 June 1996

**Abstract**

The 5-HT (5-hydroxytryptamine)-induced contractile biphasic concentration-effect curve in rat isolated jejunum was investigated. The $pEC_{50}$ values for the first and second phases were 8.0 and 6.1, respectively. The responses were insensitive to atropine (0.1 μM), ketanserin (2 μM), (−)-pindolol (5 μM), yohimbine (0.1 μM) and GR 113808 ({1-[2-(methyl-sulphonylamino)ethyl]-4-piperidinyl}methyl 1-methyl-1*H*-indole-3-carboxylate, 1 μM) but susceptible to cocaine (10 μM). The low affinity phase was blocked by tetrodotoxin (1 μM), ondansetron (1 μM) and SR48968 (*S*)-*N*-methyl-*N*-[4-(4-acetylamino-4-phenyl piperidino)-2-(3,4-dichlorophenyl)butyl]benzamide, 0.1 μM). The high affinity phase was antagonised non-surmountably by fluoxetine (1 μM) methysergide (0.1 μM), spiperone (0.1 μM) and methiothepin (0.1 μM). Ritanserin (0.01–0.1 μM) and mesulergine (0.01–0.1 μM) acted as surmountable, competitive antagonists with $pA_2$ values of 8.0 and 8.1, respectively. Clozapine (0.1 μM) was a surmountable antagonist with an apparent $pA_2$ value of 8.0. The rank potency order of the 5-HT receptor agonists was 5-CT (5-carboxyamidotryptamine) ≥ 5-HT = 5-methoxytryptamine ≥ α-methyl-5-HT ≫ 8-OH-DPAT ((±)-2-dipropyl-amino-8-hydroxy-1,2,3,4-tetrahydronaphthalene) > dipropyl-5-CT > renzapride = sumatriptan. The responses to 5-HT and 5-CT were not potentiated by pargyline (10 and 100 μM). It is suggested that rat jejunum contains a neuronal 5-HT$_3$ receptor facilitating neurokinin release and a contractile smooth muscle 5-HT receptor with a pharmacological operational profile similar to the cloned 5-ht$_7$ receptor.

*Keywords:* 5-HT (5-hydroxytryptamine, serotonin); Jejunum, rat; Smooth muscle contraction; 5-ht$_7$ receptor; 5-HT$_3$ receptor; Neurokinin, tachykinin NK$_2$ receptor

## 1. Introduction

At least 14 distinct subtypes of mammalian 5-HT receptors have been identified via molecular and pharmacological approaches which can be classified into seven classes of receptor each with unique structural, transductional and operational characteristics (Hoyer et al., 1994; Martin and Humphrey, 1994). The four major classes are the 5-HT$_1$, 5-HT$_2$, 5-HT$_3$, and 5-HT$_4$ receptors for which endogenous functional equivalents have been identified. In addition the 5-ht$_5$, 5-ht$_6$, and 5-ht$_7$ (lower case indicates no functional correlate) receptor genes have been cloned recently, but these receptors have yet to be fully characterised on the basis of functional information in intact tissues.

It has been observed that 5-HT produces a contraction of smooth muscle in a number of isolated intestinal tissues (Drakontides and Gershon, 1968; Costa and Furness, 1979a,b; Furukawa, 1978). The majority of published data with regard to the characterisation of the receptors mediating these responses have been performed using guinea-pig intestinal tissues. The receptors mediating contraction of the guinea-pig ileum have been shown to be neuronally located 5-HT$_3$ and 5-HT$_4$ receptors (Craig and Clarke, 1990; Craig et al., 1990) and neuronal 5-HT$_4$ receptors mediate contraction of the guinea-pig colon (Elswood et al., 1991; Wardle and Sanger, 1992). In contrast, only very limited and preliminary data are available with regard to the effect of 5-HT on rat intestinal tissue. It has been reported that 5-HT produces a relaxation mediated by a 5-HT$_4$ receptor (Tuladhar et al., 1991; McLean et al., 1995), and a contraction (Bubenik, 1986; Fox-Robichaud and Collins, 1986) of the rat small intestine. To date, the 5-HT receptor type mediating this contraction has not been fully characterised. The contraction was, however, shown to be greater in the jejunum than the ileum (Tuladhar et al., 1992), is dependent on calcium (Suer et al., 1992), sensitive to indomethacin (Tuladhar et al., 1992), undergoes

* Corresponding author. Tel.: 61 3 9903 9567; fax: 61 3 9903 9638.


*PII* S0014-2999(96)00456-6

desensitisation readily by prolonged exposure to this amine (Gillian and Pollock, 1980) and consists of a direct muscle action and a larger indirect neuronal action (Fox et al., 1986) which is insensitive to cholinoceptor blockade (Vermillion and Collins, 1988). There is, however, a conflicting report in which neuronal blockade failed to inhibit the 5-HT-induced contraction (Vermillion and Collins, 1988). The contractile action of 5-HT in the rat intestine has been used to investigate tissue responsiveness to 5-HT (Farmer and Laniyonu, 1984; Vermillion and Collins, 1988) and as an assay tissue for 5-HT (Bashir et al., 1992).

To date there is only preliminary characterisation of the receptor mediating the 5-HT-induced contraction of the rat small intestine. Methysergide, a non-selective 5-$HT_1$/5-$HT_2$ receptor antagonist, was shown to behave as a non-competitive antagonist in this preparation (Bubenik, 1986; Furukawa, 1978). The 5-HT-induced contraction of the rat jejunum was shown to be insensitive to the 5-$HT_2$ receptor antagonist ketanserin, but paradoxically sensitive to another non-selective 5-$HT_2$ receptor antagonist, cyproheptadine (Vermillion and Collins, 1988). This profile is not consistent with any of the 'classical' 5-HT receptors (Bradley et al., 1986) but may suggest an involvement of the putative 5-$ht_7$ receptor (Hoyer et al., 1994) at which cyproheptadine and methysergide have high affinities relative to ketanserin (Lovenberg et al., 1993; Plassat et al., 1993; Shen et al., 1993). At present, no selective 5-$ht_7$ receptor agonists or antagonists are known, however rank orders of agonist and antagonist potencies have been determined at central endogenous 5-$ht_7$ receptors and at receptors expressed from the 5-$ht_7$ receptor genes, both positively linked to adenylyl cyclase. As such, a unique pharmacological profile for 5-$ht_7$ receptors has emerged which to date is consistent across species (Boess and Martin, 1994; Eglen et al., 1994). 5-$ht_7$ receptors exhibit high affinity ($pK_i$ 8.1–9.9) for 5-carboxamidotryptamine (5-CT), 5-HT and 5-methoxytryptamine, moderate affinity ($pK_i$ 6.4–7.8) for (±)-2-dipropyl-amino-8-hydroxy-1,2,3,4,-tetrahydronaphthalene (8-OH-DPAT), mesulergine, ritanserin and spiperone and low affinity ($pK_i$ < 6.0) for (−)-pindolol, sumatriptan and ketanserin (Lovenberg et al., 1993; Plassat et al., 1993; Shen et al., 1993; To et al., 1995). In the rat, the 5-$ht_7$ receptor appears to be predominantly expressed centrally, with no evidence of expression in any of the peripheral tissues tested (intestinal tissues not tested, Lovenberg et al., 1993). Plassat et al. (1993), however, detected 5-$ht_7$ receptor mRNA in mouse heart and intestine.

There have been limited reports of functional assays which show 5-$ht_7$-like receptor profiles such as the guinea-pig hippocampus (Shenker et al., 1987; Tsou et al., 1994) and a number of peripheral tissues including porcine vena cava (Sumner et al., 1989), canine coronary artery (Cushing and Cohen, 1992), marmoset aorta (Dyer et al., 1994) guinea-pig ileum (Feniuk et al., 1983; Carter et al., 1995) and rabbit vascular rings (Martin and Wilson, 1995).

The aim of the present study was to functionally characterise further the receptor type(s) mediating the 5-HT-induced contraction of rat jejunum by use of selective agonists and antagonists and also to investigate the mechanisms involved in the contraction.

## 2. Materials and methods

### 2.1. Preparation and experimental design

The jejunum (0–25 cm distal from the ligament of Trietz) was removed from male Hooded Wistar rats (200–250 g) and transferred to warm oxygenated Krebs-Henseleit solution of the following composition (mM): NaCl, 118; KCl, 4.7; $NaHCO_3$, 25; $KH_2PO_4$, 1.2; $CaCl_2$, 2.5; $MgSO_4$, 1.2; D-(+)-glucose, 11. The mesentery was cut away and the lumen flushed carefully. From each rat, five segments of jejunum between 4–5 cm in length were suspended in the longitudinal plane and bathed with Krebs-Henseleit solution oxygenated with 95% $O_2$ and 5% $CO_2$ and maintained at 37°C. The tissues were equilibrated for 45 min with washes every 15 min until a stable baseline and a consistent response to acetylcholine (1 μM) was observed. No tension was applied initially but was gradually applied to a maximum tension of 1 g over a period of 15 min. Ugo Basile isotonic transducers connected to a Grass model 79D polygraph or a MacLab/4e (ADInstruments) were used to measure isotonic changes in length of the tissues.

### 2.2. Effects of agonists and antagonist

Non-cumulative concentration-effect curves for the 5-HT-induced contractions were constructed as preliminary experiments showed that the maximum response obtained using a cumulative concentration-effect curve was markedly reduced. The curves were established by adding increasing concentrations of agonist to the bathing solution at intervals of at least 10 min. Each concentration of agonist was in contact with the tissue until the maximum contractile response to that concentration was obtained (usually < 1 min). Earlier experiments showed that the intervals were sufficient to avoid tachyphylaxis. Each strip was only used to record one concentration-effect curve as previous experiments also showed that multiple concentration-effect curves to 5-HT were not reproducible in any of the tissues studied. Subsequently, paired tissues were used, one serving as a control, and the others to compare the responses of other agonists or in the presence of different concentrations of an antagonist. The antagonists were incubated for 30 min before determination of the agonist concentration-effect curve and were re-applied after each concentration of agonist. The relative effect of the agonists in the presence or absence of antagonists were compared as a percentage of the maximum contraction obtained with acetylcholine (1 μM).

In separate experiments the effect of cocaine (10 μM), fluoxetine (1 μM) and pargyline (10 and 100 μM, 30 min pre-incubation) on the 5-HT-induced contractions were investigated. Each compound, separately, reduced the contractions relative to control responses and hence were not used in subsequent experiments.

### 2.3. Selectivity of antagonists

Each of the antagonists tested against the 5-HT-induced contractions were also tested against acetylcholine-induced contractions. A control contraction to acetylcholine (1 μM) was established and this concentration of acetylcholine was repeated in the presence of the antagonist.

### 2.4. Description of concentration-effect curves

The concentration-effect curves were described as per Buchheit et al. (1985). Contractions were expressed as either a percentage of the maximal response to the agonist or as a percentage of the contraction to acetylcholine (1 μM) and were plotted as mean values in order to obtain log concentration-effect curves. The agonist-induced effects were expressed in terms of their potency as $EC_{50}$ values relative to individual maxima. $pEC_{50}$ values were calculated using non-linear regression analysis for each preparation from the 50% response level and expressed as arithmetic means ± S.E.M. The equipotent concentration-ratio (ECR) for the test agonist relative to 5-HT (5-HT = 1) was calculated by dividing the $EC_{50}$ value for the agonist by the $EC_{50}$ value for 5-HT. The $pEC_{50}$ value is the $-\log_{10}$ $EC_{50}$ value. Bi-phasic curves were described as follows: for each part the fractional response contributing to the total response, the maximum was calculated and indicated as $E_{max\ 1}$ and $E_{max\ 2}$. The 5-HT concentrations which produced $E_{max\ 1}/2$ and $E_{max\ 2}/2$ are denoted $EC_{50\text{-}1}$ and $EC_{50\text{-}2}$, respectively. The maximum effect ($E_{max}$) value is expressed as the percentage contraction relative to the acetylcholine (1 μM)-induced contraction. $EC_{50}$ and $E_{max}$ values were obtained by using a non-linear curve fitting analysis.

Monophasic concentration-effect curves were analysed by fitting a four parameter logistic equation to the data to obtain location and slope parameters. The equation is: $y = a + b/\{1 + 10^{-d(c+\log[A])}\}$. Biphasic curves were analysed by fitting a modified four parameter logistic equation: $y = a + b[\{f/(1 + 10^{c1+\log[A]})\} + \{1 - f/(1 + 10^{c2+\log[A]})\}]$ where $A$ is the agonist concentration, $a$ is the basal value, $b$ is the vertical range, $c$ is the $pEC_{50}$ ($c1$ is $pEC_{50\text{-}1}$ and $c2$ is $pEC_{50\text{-}2}$), $d$ is the mid-point slope (Lew, 1995) and $f$ is the fraction of the receptors that are activated with a potency described by $c1$. The remainder of the receptors are activated with a potency described by $c2$. The data points were fitted to the equations using a non-linear curve fitting analysis program (Graph Pad Prism, Graph Pad Software, San Diego, CA, USA).

### 2.5. Data analysis

The potencies of the antagonists were expressed as either a full $pA_2$ value or an apparent $pA_2$ value. The full $pA_2$ value was calculated using the method of Arunlakshana and Schild (1959) and expressed with 95% confidence limits which was computed using the 3 point method of Tallarida and Murray (1987). The assumption of simple competition (i.e. slope of unity) between antagonist and agonist at the receptor was checked with a Schild plot (Arunlakshana and Schild, 1959). An apparent $pA_2$ estimate was calculated from the Furchgott (1972) relationship: $pA_2 = \log(CR - 1) - \log[B]$, where CR is the concentration ratio of agonist used in the presence and absence of antagonist ($B$) and is expressed as $pA_2 \pm$ S.E.M. The concentration ratios required for the above analyses were determined using $EC_{50}$ values in the presence and absence of antagonist. The number of observations is indicated by $n$, which represents the number of animals.

The significance of differences between the values was determined by use of Student's paired or unpaired $t$-test. Concentration-effect curves were compared using two-way ANOVA (analysis of variance, Graph Pad Prism, Graph Pad Software, San Diego, CA, USA). The criterion for statistical significance was set at $P < 0.05$.

### 2.6. Drugs

5-Carboxyamidotryptamine (5-CT), dipropyl-5-CT, clozapine, 5-methoxytryptamine, α-methyl-5-HT, (±)-2-dipropyl-amino-8-hydroxy-1,2,3,4,-tetrahydronaphthalene (8-OH-DPAT), mesulergine, (−)-pindolol and spiperone were purchased from Research Biochemicals International (Natick, MA, USA). Acetylcholine chloride, atropine sulphate, and 5-hydroxytryptamine creatinine sulphate were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Tetrodotoxin was purchased from Calbiochem (La Jolla, CA, USA). Yohimbine hydrochloride was purchased form ICN Biomedicals (Aurora, OH, USA). Cocaine hydrochloride (Glaxo, Melbourne, Australia), fluoxetine (Eli Lilly, Indianapolis, IN, USA), GR 113808 ({1-[2-(methylsulphonylamino)ethyl]-4-piperidinyl}methyl-1-methyl-1*H*-indole-3-carboxylate, Glaxo, Melbourne, Australia), ketanserin (Janssen-Cilag, Sydney, Australia), methysergide hydrogen maleate (Sandoz, Basle, Switzerland), methiothepin (Roche, Basle, Switzerland), ondansetron hydrochloride (Glaxo, Melbourne, Australia), renzapride (Smith Kline Beecham Pharmaceuticals, Surrey, UK), ritanserin (Janssen, Beerse, Belgium), SR48968 (*S*)-*N*-methyl-*N*-[4-(4-acetylamino-4-phenyl piperidino)-2-(3,4-dichlorophenyl)butyl]benzamide, Sanofi Research, Montpellier, France) and sumatriptan (Glaxo, Melbourne, Australia) were donated. Methysergide hydrogen maleate was dissolved in ethanol 90% and diluted with (+)-tartaric acid 0.1% in distilled water. Clozapine was dissolved in dimethylsulphoxide and subsequently diluted in distilled

water. All other compounds were dissolved in distilled water.

## 3. Results

### 3.1. Description of the concentration-effect curve to 5-HT

The concentration-effect curve to 5-HT in the absence of antagonists or tetrodotoxin was analysed using a non-linear curve fitting program and the model for the interaction of 5-HT with two independent receptors was found to provide a fit which converged giving an $R^2$ value of 0.87, providing a better fit than the single receptor model ($R^2$ = 0.83).

5-HT produced two distinct phases of contraction. The contractions at concentrations of 5-HT below 1 μM (first phase) developed slowly and were sustained. Higher concentrations (second phase) produced both a rapid contraction and a subsequent fading returning to a contraction similar to that produced by the maximum concentration of the first phase (Fig. 1).

The contribution of the interaction of 5-HT with the high potency receptors responsible for the first phase of the concentration-effect curve ($E_{max\,1}$) was calculated to be 54.6 ± 12.12% of the total response and the $pEC_{50\text{-}1}$ value was 8.0 ± 0.62. The values for the second phase of the curve were: $E_{max\,2}$ = 66.2 ± 8.40% of the contraction caused by acetylcholine (1 μM, Table 1); $pEC_{50\text{-}2}$ = 6.1 ± 0.46. ($n$ = 6).

The maximum response to 5-HT caused by the cumulative addition of 5-HT was significantly less than that using a non-cumulative concentration-effect curve ($E_{max}$ = 42.2 ± 0.92, $n$ = 4, $P < 0.05$). In other experiments designed to investigate whether tachyphylaxis developed within the non-cumulative concentration-effect curve to 5-HT, repeated addition of 5-HT (1 μM) produced consistent and reproducible responses for at least the amount of time required to construct a full concentration-effect curve to 5-HT. Tachyphylaxis did, however develop when a second non-cumulative concentration-effect curve was repeated on the same tissue.

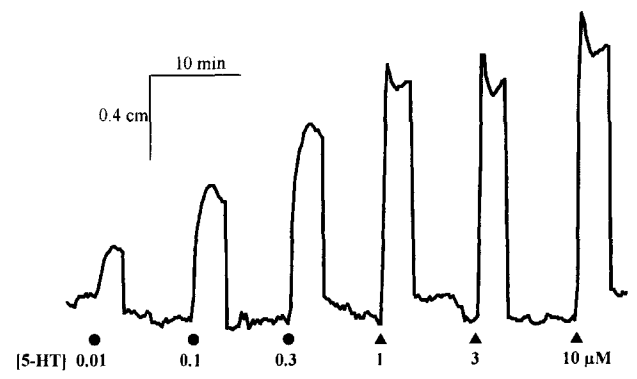


Fig. 1. Representative trace showing the contractile effect of 5-HT on the longitudinal muscle of rat jejunum. ● and ▲ represent the response from the first and second phase, respectively of the bi-phasic concentration-effect curve to 5-HT.

Table 1
Summary of agonist potency ($pEC_{50}$), equipotent concentration ratio (ECR) and maximum effect values from the 5-HT receptor agonist-induced tetrodotoxin-insensitive contraction of rat isolated jejunum

| Agonist | $pEC_{50}$ | ECR [a] | $E_{max}$ (%ACh) | $n$ |
|---|---|---|---|---|
| 5-HT | 6.94 ± 0.19 | 1.00 | 73.4 ± 3.5 | 4 |
| 5-CT | 7.56 ± 0.47 | 0.23 | 72.3 ± 7.2 | 4 |
| 5-MeOT | 6.95 ± 0.21 | 0.97 | 83.2 ± 4.7 | 4 |
| α-Methyl-5-HT | 6.84 ± 0.44 | 1.24 | 64.1 ± 10.1 | 7 |
| 8-OH-DPAT | 4.56 ± 0.21 | 239.4 | 42.6 ± 6.8 | 4 |
| Renzapride | < 4 | > 300 | < 20 | 4 |
| Sumatriptan | < 4 | > 300 | < 20 | 4 |
| Dipropyl-5-CT | < 4 | > 300 | < 20 | 3 |

[a] Equipotent concentration ratio, 5-MeOT = 5-methoxytryptamine.

### 3.2. Influence of atropine on 5-HT-induced contractions

Atropine (0.1 μM) completely abolished the response to acetylcholine (1 μM) in all tissues tested ($n$ = 4), however the 5-HT-induced contractions were not affected in the presence of atropine. The concentration-effect curves to 5-HT in the absence and presence of atropine (0.1 μM) were superimposable ($P > 0.05$, two-way ANOVA, $n$ = 4).

### 3.3. Influence of methysergide on 5-HT-induced contractions

Without affecting the acetylcholine (1 μM)-induced contraction, methysergide (0.1 μM) abolished the first phase of the response to 5-HT transforming it to a monophasic curve. The second phase was unaffected by methysergide, the $pEC_{50\text{-}2}$ value (5.7 ± 0.10) in the absence being not significantly different from the $pEC_{50}$ value (5.6 ± 0.09) in the presence of methysergide. Methysergide also did not effect the maximal response to 5-HT (Fig. 2, $n$ = 4).

### 3.4. Influence of tetrodotoxin on 5-HT-induced contractions

Tetrodotoxin (1 μM) did not affect the response to acetylcholine (1 μM). Fig. 3 shows the 5-HT concentration-effect curve in the presence of tetrodotoxin (1 μM). The curve was transformed from bi- to mono-phasicity, the second phase of the curve being almost completely abolished. Tetrodotoxin significantly reduced the maximum response of the concentration-effect curve to 5-HT by 42.6 ± 1.63% ($P < 0.05$, $n$ = 5). Tetrodotoxin also caused a small rightward shift of the first phase of the concentration-effect curve, but this shift was not statistically signifi-

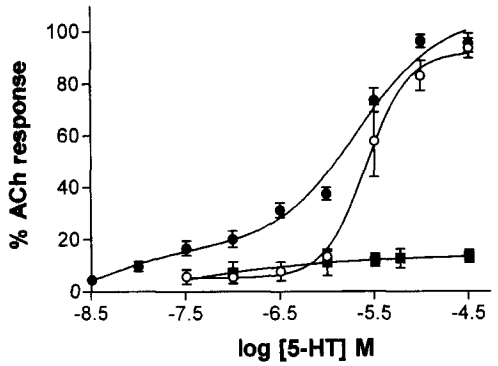


Fig. 2. The effect of methysergide and tetrodotoxin on the concentration-effect curve to 5-HT in the isolated longitudinal muscle preparation from rat jejunum. Concentration-effect curves were constructed to 5-HT (●, $n = 5$), and 5-HT in the presence of methysergide (○, 0.1 μM, $n = 4$), and in the presence of methysergide (0.01 μM) + tetrodotoxin (1 μM, ■, $n = 4$). The theoretical curves were fitted using non-linear regression analysis for a two and one site model in the absence (●) and presence (○) of methysergide respectively. Each point represents individual mean responses with S.E.M. represented by the vertical bars.

cant ($P > 0.05$, 2-way ANOVA). The $pEC_{50}$ value (7.2 ± 0.20, $n = 5$) obtained from the monophasic concentration-effect curve in the presence of tetrodotoxin did not differ significantly ($P > 0.05$) from the $pEC_{50\text{-}1}$ (8.0 ± 0.62, $n = 5$) value obtained without tetrodotoxin. The residual response to 5-HT in the presence of tetrodotoxin (1 μM) was blocked by methysergide in a non-surmountable fashion (Fig. 2).

### 3.5. Influence of ondansetron on 5-HT-induced contractions

In the presence of ondansetron (1 μM) a similar effect was observed to that with tetrodotoxin. The first phase of the concentration-effect curve to 5-HT was left unaffected, the $pEC_{50}$ values in the presence (8.1 ± 0.76) and absence (8.0 ± 0.88) of ondansetron being not significantly different ($P > 0.05$, $n = 5$). The response to 5-HT obtained in the second phase was antagonised in a non-surmountable fashion, the maximum response being reduced by 52.1 ± 3.90% ($P < 0.05$, $n = 5$). Ondansetron did not affect the acetylcholine-induced contraction.

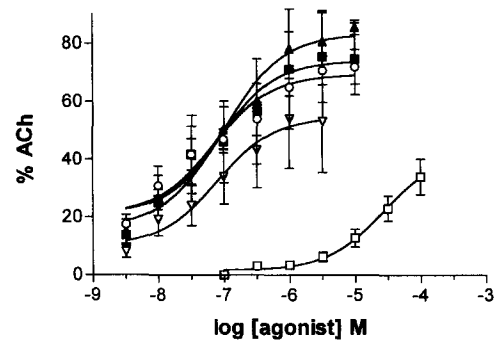


Fig. 3. Concentration-contraction effect curves to 5-HT and a series of 5-HT receptor agonists on the longitudinal muscle of the rat jejunum. Non-cumulative concentration-effect curves were constructed to 5-HT (○, $n = 4$), 5-methoxytryptamine (▲, $n = 4$), 5-CT (■, $n = 4$), α-methyl-5-HT (▽, $n = 7$) and 8-OH-DPAT (□, $n = 4$) in the presence of tetrodotoxin (1 μM). Data are mean ± s.e.mean from $n$ tissues.

### 3.6. Influence of 5-HT uptake and monoamine oxidase inhibitors and other 5-HT receptor antagonists on 5-HT-induced contractions

The serotonin-selective uptake inhibitor, fluoxetine (1 μM) non-surmountably abolished the first phase of the response to 5-HT and caused small but statististically non-significant shift (dose ratio = 2.0 ± 0.80, $P > 0.05$, $n = 5$) of the second phase. The maximum response to 5-HT was not affected in the presence of fluoxetine. Cocaine (10 μM) also abolished the 5-HT-induced responses in the first phase, but also reduced the responses in the second phase, in a non-surmountable fashion, causing a reduction of the maximum response value by 49.1 ± 1.20% ($P < 0.05$, $n \geq 4$).

Pargyline (100 μM, $n = 3$ and 10 μM, $n = 4$), without affecting the resting baseline, did not increase the potency of the 5-HT- or 5-CT-induced, tetrodotoxin-insensitive responses. At 100 μM pargyline pre-incubation caused a significant ($P < 0.05$) 41.0% reduction in the maximum response to 5-HT and a rightward shift with a concentration ratio of 9.4. Pre-incubation with pargyline (10 μM) also caused a significant 26.1% (5-HT) and 38.8% (5-CT) reduction in maximum response and a rightward shift of 4.1 (5-HT) and 4.7 (5-CT).

Ketanserin (2 μM, $n = 6$) and GR 113808 (1 μM, $n = 5$) did not affect the potency or $E_{max}$ values of either phase of the concentration-effect curve to 5-HT.

### 3.7. Influence of a tachykinin antagonist on 5-HT-induced contractions

The tachykinin $NK_2$ receptor antagonist, SR48968 (0.1 μM), produced a tetrodotoxin-like effect. The first phase

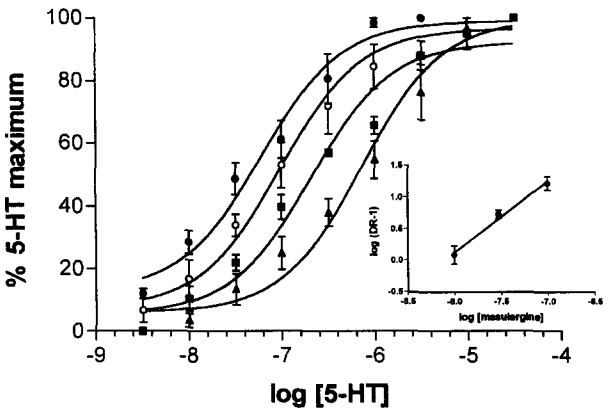


Fig. 4. Antagonism of the tetrodotoxin-insensitive responses to 5-HT by mesulergine in the rat jejunum. Data obtained in the absence (●), or presence of mesulergine 0.01 μM (○), 0.03 μM (■), and 0.1 μM (▲). The slope of Schild regression (inset) was 1.13 ± 0.15. The $pA_2$ estimate was 8.10 (95% C.L. 7.88–8.32, $n = 4$).

Table 2
Comparison of antagonist affinities in rat isolated jejunum, guinea-pig isolated ileum and recombinant rat and mouse expressed receptor systems

| Antagonist | Rat jejunum ($pA_2$) | Guinea-pig ileum ($pK_B$) Carter et al., 1995 | Rat ($pK_i$) Lovenberg et al., 1993 | Mouse ($pK_i$) Plassat et al., 1993 | Rat ($pK_i$) Shen et al., 1993 |
|---|---|---|---|---|---|
| Mesulergine | 8.1 | 7.8 | 7.9 | 7.6 | 8.1 |
| Ritanserin | 8.0 | | 7.6 | | 7.8 |
| Clozapine | 8.0 | 7.3 | > 7.6 | 7.7 | 7.9 |
| Fluoxetine | (1 μM)[a] | | | | |
| Methysergide | (0.1 μM)[a] | 7.6 | 7.8 | 8.2 | 7.9 |
| Spiperone | (0.1 μM)[a] | 7.6 | | 7.2 | |
| Methiothepin | (0.1 μM)[a] | | 9.1 | 7.7 | 9.0 |
| Pindolol | < 6 | < 6 | < 6 | < 5 | |
| Ketanserin | < 6 | < 6 | | 6.4 | 6.7 |
| Ondansetron | < 6 | < 6 | | | |
| Yohimbine | < 7 | | | | |
| GR 113808 | < 6 | < 6 | | | |

[a]Non-surmountable antagonism (concentration tested).

of the concentration-effect curves to 5-HT in the absence and presence of SR48968 were superimposable ($P > 0.05$). The application of SR48968, abolished the second phase causing a significant reduction in the response to 5-HT reducing the maximum response value by 49.7 ± 1.46% ($P < 0.05$, $n = 4$).

### 3.8. Characterisation of the 5-HT receptor mediating the tetrodotoxin-insensitive, 5-HT-induced contractions

#### 3.8.1. 5-HT receptor agonists

Fig. 3 and Table 1 show the effects of the 5-HT receptor agonists in the presence of tetrodotoxin. All agonists used produced a contraction which was characteristic of the first phase of the control bi-phasic concentration-effect curve to 5-HT and there was no evidence of a relaxant response with any of the agonists tested. Each of the agonists shown in Fig. 3 produced a concentration-related contraction leading to a mono-phasic concentration-effect curve over a range of 0.003–100 μM. 5-HT produced its effect with a mean $pEC_{50}$ value of 6.9 ± 0.19 and a maximum effect value of 73.4 ± 3.50% of the acetylcholine-induced contraction ($n = 4$).

The rank order of potency of the agonists tested was 5-CT ≥ 5-HT = 5-methoxytryptamine ≥ α-methyl-5-HT ≫ 8-OH-DPAT > dipropyl-5-CT > renzapride = sumatriptan (Fig. 3, Table 1). Maximum effect values were similar except for 8-OH-DPAT which was only 42.6 ± 6.85% at the maximum concentration tested. Dipropyl-5-CT, renzapride and sumatriptan produced relatively much smaller responses, the maximum effect values all being less than 20% of the contraction to acetylcholine. $pEC_{50}$ values, equipotent concentration ratios and maximum effect values for each agonist are shown in Table 1.

#### 3.8.2. 5-HT receptor antagonists

Contractile responses to 5-HT and 5-CT were non-surmountably antagonised by methysergide (0.1 μM, $n = 4$). Spiperone (1 μM, $n = 6$) and methiothepin (0.1 μM, $n = 4$) also caused a non-surmountable antagonism of the tetrodotoxin-insensitive, 5-HT-induced contraction. Ritanserin (0.01–0.1 μM) and mesulergine (0.01–0.1 μM) acted as surmountable, competitive (Schild slope not different from unity) antagonists against 5-HT with constrained $pA_2$ values of 8.0 (6.9–9.2, Schild slope = 1.2 ± 0.83, $n = 5$) and 8.1 (7.9–8.3, Schild slope = 1.1 ± 0.15, $n = 4$, Fig. 4), respectively. Clozapine (0.1 μM) was also a surmountable antagonist yielding an apparent $pA_2$ value of 8.0 ± 0.15 ($n = 4$). (−)-Pindolol (5 μM, $n = 5$), ketanserin (2 μM, $n = 6$), ondansetron (1 μM, $n = 5$), yohimbine (0.1 μM, $n = 4$) and GR 113808 (1 μM, $n = 5$) were without antagonist effect on the concentration-effect curve to 5-HT (see Table 2).

## 4. Discussion

The results from the present study suggest the involvement of two different 5-HT receptor types, $5\text{-ht}_7$ and $5\text{-HT}_3$, in the 5-HT-induced contraction of rat jejunum. The $5\text{-ht}_7$ receptor appears to be located on the smooth muscle of the rat jejunum, as it is tetrodotoxin-insensitive, and is responsible for the first, high potency phase of the response to 5-HT. It seems that a neurokinin, possibly neurokinin A, is involved in the non-cholinergic, tetrodotoxin-sensitive response to $5\text{-HT}_3$ receptor activation which mediates the second lower potency phase of the response.

The role of 5-HT in the control of gastrointestinal smooth muscle is not clear partly due to the high degree of heterogeneity in 5-HT receptor type, function and distribution. Substantial diversity of action is also evident in the different regions of the gastrointestinal tract and between species. As more 5-HT receptor types are identified the role of 5-HT becomes clearer and this is evident with the recent identification of the $5\text{-HT}_4$ receptor which has been

characterised in the alimentary tract of a number of species, including man (see Hoyer et al., 1994; McLean et al., 1995; McLean and Coupar, 1995, 1996a,b). Further clarification of this situation may occur following the identification of a novel 5-HT receptor type with the current appellation, 5-$ht_7$, having at present no definitive endogenous correlate.

To date, only a very preliminary description is available of the mechanism and the receptor type mediating the contractile action of 5-HT in the rat jejunum. The contraction is mediated by a direct muscle and an indirect neuronal action. The response is sensitive to antagonism by methysergide and cyproheptadine but is insensitive to ketanserin and atropine (see Introduction for references). The results of the present study are consistent with these data and show, in addition, that 5-HT produced a bi-phasic concentration-effect curve, the second phase of which is mediated via a non-cholinergic neuronal pathway as the response was abolished by tetrodotoxin and not affected by atropine. The residual response produced by the lower concentrations of 5-HT, which accounted for approximately half the total response, was abolished by methysergide but not ketanserin. In fact, neither phase of the concentration-effect curve was affected by ketanserin. Atropine also had no effect on the direct muscle action of 5-HT. Additionally, the response was susceptible to desensitisation as was shown by the reduced responses elicited by cumulative compared to non-cumulative addition of 5-HT and also by the observation that two consecutive concentration-effect curves were not superimposable, confirming the report by Gillian and Pollock (1980).

Subsequent experiments were designed to further investigate the receptor subtypes responsible for each component of the contractile response and also to identify the neurotransmitter involved in bringing about the neuronally mediated component of the contraction. Since the release of tachykinins has been implicated in the contractile action of 5-HT in the guinea-pig ileum (Buchheit et al., 1985; Ramirez et al., 1994) and trachea (Buckner et al., 1993) we examined the effect of a tachykinin receptor antagonist on the 5-HT-induced contractions. The rat small intestine contains mainly tachykinin $NK_2$ receptors (Boyle et al., 1993; Croci et al., 1994). Subsequently, SR48968, a tachykinin $NK_2$ receptor selective antagonist with a $pA_2$ at tachykinin $NK_2$ receptors of between 7.5–10.5 (Advenier et al., 1992; Croci et al., 1994; Edmonts-Alt et al., 1992; Maggi et al., 1993; Zeng et al., 1995) was chosen to investigate the role of tachykinins in the 5-HT-induced contraction. SR48968 abolished the second phase of the concentration-effect curve without affecting the first phase. The $5\text{-}HT_3$ receptor antagonist, ondansetron produced a similar effect to SR48968 in that the second phase was antagonised, without affecting the first phase. These data, together with the observations that $5\text{-}HT_1$, $5\text{-}HT_2$, and $5\text{-}HT_4$ receptor antagonism with methysergide, ketanserin and GR 113808 did not prevent the second phase suggest that the neuronal component is mediated via the activation of prejunctional $5\text{-}HT_3$ receptors. $5\text{-}HT_{1P}$ receptors, which are located on enteric nerves (Branchek et al., 1988; Gershon et al., 1991), can be excluded as 5-methoxytryptamine and 5-CT which were full agonists in the present study are inactive at the $5\text{-}HT_{1P}$ receptor (see Hoyer et al., 1994). These $5\text{-}HT_3$ receptors are possibly located on excitatory motorneurons which release tachykinins. Indeed, the excitatory motor neurons which innervate the longitudinal muscle of the guinea-pig small intestine have been shown to utilise acetylcholine and neurokinins (Furness et al., 1994). The tachykinin released is possibly neurokinin A as it is the preferred endogenous ligand for the tachykinin $NK_2$ receptor and neurokinin A contracts the smooth muscle of the rat duodenum (Regoli and Nantel, 1991; Croci et al., 1994). These findings are also supported by the observation by Fox et al. (1986) who reported a postjunctional action of substance P in rat jejunum. Other studies have also reported a link between $5\text{-}HT_3$ receptors and tachykinin release. In guinea-pig ileum tachykinins are involved in the response to $5\text{-}HT_3$ receptor stimulation (Ramirez et al., 1994). Endogenous 5-HT was shown to modulate the release of tachykinins from the rat spinal cord via $5\text{-}HT_3$ receptors (Saria et al., 1991). 5-HT was also shown to facilitate the evoked release of neurokinins from central terminals of primary sensory neurons via $5\text{-}HT_3$ receptors in the rat spinal cord (Saria et al., 1990).

Interestingly, the use of the serotonin uptake blockers cocaine and fluoxetine and the monoamine oxidase inhibitor pargyline did not potentiate the 5-HT-induced contraction but caused a reduction in the response. Further studies with uptake and metabolism inhibitors are required to fully establish whether uptake and/or metabolism modify responses to 5-HT in the rat jejunum. Fluoxetine inhibited the first phase of the curve to 5-HT in a non-surmountable fashion and caused a small but statistically non-significant shift of the second phase without affecting the maximum response. Cocaine inhibited the 5-HT-induced responses in both phases causing a significant reduction of the maximum response. A possible explanation for these effects may be due to the 5-HT receptor blocking actions of cocaine and fluoxetine which have been reported in a number of tissues (Fozard et al., 1979; Cortes et al., 1978; Lucchelli et al., 1995). The $5\text{-}HT_3$ receptor-ion channel complex is a site for the inhibitory action of cocaine (Fan et al., 1994) whereas fluoxetine has a moderate affinity for $5\text{-}HT_2$ receptors (Wood et al., 1993) and negligible affinity for $5\text{-}HT_3$ receptors (Angel et al., 1993). These data provide further evidence in favour of a $5\text{-}HT_3$ receptor mechanism mediating the second phase of the concentration-effect curve. As with methysergide, spiperone and methiothepin, it was not possible to obtain an estimate for the affinity of cocaine and fluoxetine versus 5-HT in the first phase of the concentration-effect curve as they too caused a non-surmountable antagonism. The in-

hibitory action of pargyline is difficult to explain but is possibly due to an antagonist action of pargyline, however, no study, to our knowledge, has reported such an action of pargyline. Another possible explanation is that pre-incubation with pargyline may cause a desensitisation effect via elevation of endogenous 5-hydroxytryptamine. A similar observation was reported by Fan (1994) who showed that monoamine oxidase inhibition caused a desensitisation of the 5-$HT_3$ receptor-mediated response in rat nodose ganglion neurones.

The receptor type involved in the tetrodotoxin-insensitive response was investigated using a series of selective 5-HT receptor agonists and antagonists. Tetrodotoxin was used at an appropriate neurotoxic concentration (1 μM) to enable the direct muscle effects to be studied in isolation. However, it has been noted that tetrodotoxin does not inhibit all neuronal function and as such it is possible that the receptor is located neuronally. The data obtained from this series of experiments correlate well with the pharmacological profile of the recently described 5-$ht_7$ receptor (see Hoyer et al., 1994). Specifically, the contractile action of 5-HT in the rat jejunum in the presence of tetrodotoxin was mimicked by a number of compounds for which the rank order of potency was 5-CT ≥ 5-HT = 5-methoxytryptamine ≥ α-methyl-5-HT ≫ 8-OH-DPAT > dipropyl-5-CT > renzapride = sumatriptan. This is consistent with data from 5-$ht_7$ receptors which stimulate adenylyl cyclase expressed in HeLa cells (Lovenberg et al., 1993) or monkey kidney cells (COS-7) (Plassat et al., 1993). The very weak agonist action of sumatriptan, dipropyl-5-CT and renzapride in the present study is in agreement with the 5-$ht_7$ receptor profile and the partial agonist action of 8-OH-DPAT is in accordance with its activity at 5-$ht_7$ receptors (Lovenberg et al., 1993; Carter et al., 1995).

Further evidence for the existence of 5-$ht_7$ receptors in the rat jejunum has been obtained by the use of 5-HT receptor antagonists. Prior to the present study only preliminary data existed with regard to the action of 5-HT receptor antagonists on the 5-HT-induced contraction of rat jejunum. Methysergide acted as a non-competitive antagonist in the present study and in the studies by Bubenik (1986) and Furukawa (1978). The present study also confirmed the observation by Vermillion and Collins (1988) that ketanserin did not affect the contraction. These workers also reported a blocking action of cyproheptadine. The present study, using a number of 5-HT receptor antagonists, extended these findings whereby non-surmountable antagonism of the tetrodotoxin-insensitive response to 5-HT was observed with methysergide (also against 5-CT), spiperone and methiothepin. Competitive antagonism was observed with ritanserin ($pA_2 = 8.0$) and mesulergine ($pA_2 = 8.1$), and clozapine acted as a surmountable antagonist (apparent $pA_2 = 8.0$). These affinity values correlate well with those from other studies of the 5-$ht_7$ receptor (Roth et al., 1994; Bard et al., 1993; Lovenberg et al., 1993; Plassat et al., 1993; Ruat et al., 1993; Shen et al., 1993; see also Table 2). The receptor is unlikely to be a 5-$HT_3$ receptor as these are found exclusively associated with neurones (Hoyer et al., 1994) which is supported by the lack of antagonist action of ondansetron. An important difference between 5-$HT_{1A}$ and 5-$ht_7$ receptor pharmacology is in the relative affinity for (−)-pindolol (see Eglen et al., 1994). Consistent with a lack of affinity for the 5-$ht_7$ receptor, (−)-pindolol was without antagonist effect in this study. Also ketanserin (Van Nueten et al., 1981), and GR 113808 (Gale et al., 1994; Grossman et al., 1993) were without antagonist effect indicating that the receptor is not of the 5-$HT_{2A}$ or 5-$HT_4$ receptor type. However, ketanserin has been shown to have a moderate affinity for the 5-$ht_7$ receptor ($pK_D = 6.4–6.6$, Plassat et al., 1993; Shen et al., 1993) and at the concentration used in the present study (2 μM) it may be expected to antagonise the response. However, other groups have shown ketanserin to have much lower affinity values at 5-$ht_7$ receptors (< 5.1, Ruat et al. (1993); < 6.0 Carter et al. (1995)) providing an explanation for its lack of effect in the present study. Yohimbine, having a high affinity for 5-$HT_{2B}$ receptors was used to discriminate between the 5-$HT_{2B}$ and 5-$ht_7$ receptor. At a concentration consistent with its $pA_2$ at the cloned rat 5-$HT_{2B}$ receptor (Wainscott et al., 1993) and the 5-$HT_{2B}$ receptors in rat stomach fundus ($pA_2 = 7.9$, Baxter et al., 1994), yohimbine did not affect the 5-HT-induced response, excluding the possibility that the response is mediated by a 5-$HT_{2B}$ receptor. Finally, the 5-$HT_5$ and 5-$HT_6$ receptors can be ruled out as the response was antagonised by mesulergine with an affinity value not consistent with either of these receptors (8.1, this study; < 6.0 5-$HT_5$, Plassat et al., 1992; Erlander et al., 1993; 5.8, 5-$HT_6$, Monsma et al., 1993). Further evidence against a 5-$HT_6$ receptor mechanism is that 5-CT has submicromolar affinities at the 5-$HT_6$ receptor ($pK_i = 6.1$, Monsma et al., 1993) whereas in the present study it was the most potent agonist tested ($pEC_{50} = 7.6$). In addition, fluoxetine was an insurmountable antagonist in the present study at a concentration almost half that of its $K_i$ at 5-$HT_6$ receptors (Monsma et al., 1993).

There is some discrepancy between the agonist $EC_{50}$ values measured in this study and their corresponding binding affinity values in other studies (Plassat et al., 1993; Shen et al., 1993). However, the agonist potency values in the present study are in agreement with the study by Carter et al. (1995); 5-HT (5.5), 5-CT (7.6) and 5-methoxytryptamine (5.7) which is the only other study to date that characterises a gastrointestinal functional 5-$ht_7$ receptor. It is conceivable that effective uptake and metabolism inhibition may yield agonist potency values which are more in line with their higher binding affinities. However, the current inhibitory effects observed with fluoxetine, cocaine and pargyline do not allow such an investigation. In addition, the study by Carter et al. (1995) used uptake and metabolism inhibitors yielding potency values

which are in line with the present study. This indicates that uptake and metabolism may not be an important factor in the determination of agonist potency values in the rat jejunum. Although there is some difference between the functional potency values and binding affinity values, the rank order of the values are consistent with other reports.

The guinea-pig ileum is the only gastrointestinal preparation, to date, in which a 5-ht$_7$ receptor has been identified using functional pharmacological methods (Carter et al., 1995). Other endogenous correlates of the 5-ht$_7$ receptor have been functionally characterised in transiently expressed cells (Bard et al., 1993; Tsou et al., 1994) and on smooth muscle (Carter et al., 1995; Cushing and Cohen, 1992; Dyer et al., 1994; Martin and Wilson, 1995; Sumner et al., 1989). In each of the above functional preparations the 5-ht$_7$ receptor-mediated response was a relaxation. This response is consistent with the coupling mechanism of the 5-ht$_7$ receptor, which in all reports, to date, is activation of adenylyl cyclase. In the present study the response is a contraction which is not consistent with a positive coupling to adenylyl cyclase. The operational characteristics of the currently described system best fit that of a 5-ht$_7$ receptor, however it is difficult to explain the apparent discrepancy in the nature of the response and possibly indicates a neuronal action or a different coupling mechanism. Further experiments are required to fully elucidate the signal transduction mechanism of this 5-ht$_7$-like receptor in rat jejunum.

In conclusion, this study has demonstrated that rat jejunum contains a 5-HT receptor with an operational profile similar to the cloned 5-ht$_7$ receptor which activates smooth muscle contraction, and a neuronal 5-HT$_3$ receptor which stimulates a tachykinin-releasing nerve also causing a contraction. The 5-ht$_7$-like receptor described in this study is an example of a possible endogenous correlate of the cloned 5-ht$_7$ receptor and awaits further transductional and molecular characterisation and identification in this preparation.

## References


Advenier, C., N. Rouissi, Q.T. Nguyen, X. Emonds-Alt, J.C. Breliere, G. Neliat, E. Naline and D. Regoli, 1992, Neurokinin A ($NK_2$) receptor revisited with SR 48968, a potent non-peptide antagonist, Biochem. Biophys. Res. Commun. 184, 1418.

Angel, I., H. Schoemaker, M. Prouteau, M. Garreau and S.Z. Langer, 1993, Litoxetine: a selective 5-HT uptake inhibitor with concomitant 5-HT$_3$ receptor antagonist and antiemetic properties, Eur. J. Pharmacol. 232, 139.

Arunlakshana, O. and H.O. Schild, 1959, Some quantitative uses of drug antagonists, Br. J. Pharmacol. Chemother. 14, 48.

Bard, J.A., J. Zgombick, N. Adham, P. Vaysse, T.A. Branchek and R.L. Weinshank, 1993, Cloning of a novel serotonin receptor (5-HT$_7$) positively linked to adenylate cyclase, J. Biol. Chem. 31, 23422.

Bashir, A.A., K. Vasudevan, V. Vasanthi and P.S.R.K. Haranath, 1992, Use of rat jejunum for assay of acetylcholine, Indian J. Med. Res. 96, 258.

Baxter, G.S., O.E. Murphy and T.P. Blackburn, 1994, Further characterization of 5-hydroxytryptamine receptors (putative 5-HT$_{2B}$) in rat stomach fundus longitudinal muscle, Br. J. Pharmacol. 112, 323.

Boess, F.G. and I.L. Martin, 1994, Review: molecular biology of 5-HT receptors, Neuropharmacology 33, 275.

Boyle, S.J., S. Manley, K.-W. Tang, K. Meecham, S. Guard, K.J. Watling, G.N. Woodruff and A.T. McKnight, 1993, Affinity of the $NK_2$ antagonist SR48968 at $NK_3$-tachykinin and μ-opioid receptors, Br. J. Pharmacol. 108, 24P.

Bradley, P.B., G. Engel, W. Feniuk, J.R. Fozard, P.P.A. Humphrey, D.N. Middlemiss, E.J. Mylecharane, B.P. Richardson and P.R. Saxena, 1986, Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine Neuropharmacology 25, 563.

Branchek T.A., G.M. Mawe and M.D. Gershon, 1988, Characterization and localization of a peripheral neural 5-hydroxytryptamine receptor subtype (5-HT$_{1P}$) with a selective agonist, $^3$H-5-hydroxyindalpine, J. Neurosci. 8, 2582.

Bubenik, G.A., 1986, The effect of serotonin, *N*-acetylserotonin, and melatonin on spontaneous contractions of isolated rat intestine, J. Pineal. Res. 3, 41.

Buchheit, K.H., E. Gunter, E. Mutschler and B. Richardson, 1985, Study of the contractile effect of 5-hydroxytryptamine (5-HT) in the isolated longitudinal muscle strip from guinea-pig ileum: evidence for two distinct mechanisms, Naunyn-Schmiedeberg's Arch. Pharmacol. 329, 36.

Buckner, C.K., N. Liberati, D. Dea, D. Lengel, C. Stinson-Fisher, J. Campbell, S. Miller, A. Shenvi and R.D. Krell, 1993, Differential blockade by tachykinin NK1 and NK2 receptor antagonists of bronchoconstriction induced by direct-acting agonists and the indirect-acting mimetics capsaicin, serotonin and 2-methyl-serotonin in the anesthetized guinea pig, J. Pharmacol. Exp. Ther. 267, 1168.

Carter, D., M. Champney, B. Hwang and R.M. Eglen, 1995, Characterization of post-junctional 5-hydroxytryptamine (5-HT) receptor mediating relaxation of guinea-pig isolated ileum, Eur. J. Pharmacol. 280, 243.

Cortes, A. G. Merino and E. Contreras, 1978, Effect of fluoxetine (Lilly II0I40) on serotonin, noradrenaline and acetylcholine in isolated organs, Cell. Mol. Biol. 23, 179.

Costa, M. and J.B. Furness, 1979a, The sites of action of 5-hydroxytryptamine in nerve-muscle preparations from guinea-pig small intestine and colon, Br. J. Pharmacol. 65, 237.

Costa, M. and J.B. Furness, 1979b, On the possibility that an indoleamine is a neurotransmitter in the gastrointestinal tract, Biochem. Pharmacol. 28, 565.

Craig, D.A. and D.E. Clarke, 1990, Pharmacological characterisation for a neuronal receptor for 5-hydroxytryptamine in guinea-pig ileum with properties similar to the 5-HT4 receptor, J. Pharmacol. Exp. Ther. 252, 1378.

Craig, D.A., R.M. Eglen, L.K.M. Walsh, L.A. Perkins, R.L. Whiting and D.E. Clarke, 1990, 5-Methoxytryptamine and 2-methyl-5-hydroxytryptamine-induced desensitisation as a discriminative tool for the 5-HT$_3$ and putative 5-HT$_4$ receptors in the guinea-pig ileum, Naunyn-Schmiedeberg's Arch. Pharmacol. 345, 264.

Croci, T., X. Emonds-Alt, G. Le Fur and L. Manara, 1994, In vitro characterization of the non-peptide tachykinin NK1- and NK2-receptor antagonists, SR140333 and SR48968 in different rat and guinea-pig intestinal segments, Life Sci. 56, 267.

Cushing, D.J. and M.L. Cohen, 1992, Sertonin-induced relaxation in canine coronary artery smooth muscle, J. Pharmacol. Exp. Ther. 263, 123.

Drakontides, A.B. and M.D. Gershon, 1968, 5-Hydroxytryptamine receptors in the mouse duodenum, Br. J. Pharmacol. 33, 480.

Dyer, S.M., S. Bexis, M.T. Mane, I.S. De La Lande, D.B. Frewin and R.J. Head, 1994, Interactions between 5-hydroxytryptamine, noradrenaline and the thromboxane-A(2) mimetic U44069 in the marmoset isolated aorta, Clin. Exp. Pharmacol. Physiol. 21, 201.

Edmonts-Alt, X., P. Villain, P. Goulaouic, V. Proietto, D. Van Broeck, C. Advenier, E. Naline, G. Neliat, G. Le Fur and J.C. Breliere, 1992, A

potent and selective non-peptide antagonist of the neurokinin A ($NK_2$) receptor, Life Sci. 50, PL101.

Eglen, R.M., L. Jakeman and R.A. Alvarez, 1994, The 5-hydroxytryptamine$_7$ receptor, Expert Opin. Invest. Drugs, 3, 175.

Elswood, C.J., K.T. Bunce and P.P.A. Humphrey, 1991, Identification of putative 5-$HT_4$ receptors in guinea-pig ascending colon, Eur. J. Pharmacol. 196, 149.

Erlander M.G., T.W. Lovenberg, B.M. Baron, L. DeLecea, P.E. Danielson, M. Racke, A.L. Slone, B.W. Siegel, P.E. Foye, K. Cannon, J.E. Bruns and J.G. Sutcliffe, 1993, Two members of a distinct subfamily of 5-hydroxytryptamine receptors differentially expressed in rat brain, Proc. Natl. Acad. Sci. USA 90, 3452.

Fan, P., 1994, Effects of antidepressants on the inward current mediated by 5-$HT_3$ receptors in rat nodose ganglion neurones, Br. J. Pharmacol. 112, 741.

Fan, P., S. Visentin and F.F. Weight, 1994, Effects of cocaine on the serotonin-induced inward current in rat nodose ganglion neurons, J. Pharmacol. Exp. Ther. 271, 262.

Farmer, S.G.. and A.A. Laniyonu, 1984, Effects of *p*-chlorophenylalanine on the sensitivity of rat intestine to agonists and on intestinal 5-hydroxytryptamine levels during *Nippostrongylus brasiliensis* infection, Br. J. Pharmacol. 82, 883.

Feniuk, W., P.P.A. Humphrey and A.D. Watts, 1983, 5-Hydroxytryptamine-induced relaxation of isolated mammalian smooth muscle, Eur. J. Pharmacol. 96, 71.

Fox, D.A., J.R. Herman and P. Bass, 1986, Differentiation between myenteric plexus and longitudinal muscle of the rat jejunum as the site of putative enteric neurotransmitters, Eur. J. Pharmacol. 131, 39.

Fox-Robichaud, A.E. and S.M. Collins, 1986, Altered calcium handling properties of jejunal smooth muscle from the nematode-infected rat, Gastroenterology 91, 1462.

Fozard, J.R., A.T.M.M. Ali and G. Newgrosh, 1979, Blockade of serotonin receptors on autonomic neurones by (−)-cocaine and some related compounds, Eur. J. Pharmacol. 59, 195.

Furchgott, R.F., 1972, The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory, in: Catecholamines, Hand. Exp. Pharmacol., Vol. 33, eds. H.R. Blaschko and E. Muscholl (Spinger-Verlag: Berlin, Heidelberg, New York) p. 283.

Furness, J.B., J.C. Bornstein, S. Pompolo, H.M. Young, W.A.A. Kunze and H. Kelly, 1994, The circuitry of the enteric nervous system, Neurogastroenterol. Mot. 6, 241.

Furukawa, K., 1978, Mechanism of contraction and relaxation in the response to 5-hydroxytryptamine of isolated rat ileum, Nippon Yakurigaku Zasshi 74, 525.

Gale, J.D., C.J. Grossman, J.W.F. Whitehead, A.W. Oxford, K.T. Bunce and P.P.A. Humphrey, 1994, GR 113808: a novel, selective antagonist with high affinity at the 5-$HT_4$ receptor, Br. J. Pharmacol. 111, 332.

Gershon, M.D., P.R. Wade, E. Fiorica-Howells, A.L. Kirchgessner and H. Tamir, 1991, 5-$HT_{1P}$ receptors in the bowel: G-protein coupling, localistion and function, in: Serotonin: Biology, Receptors and Functional Effects, eds. J.R. Fozard and P.R. Saxena (Birkhauser Verlag, Basel, Switzerland) p. 133.

Gillian, M.G.C. and D. Pollock, 1980, Acute effects of morphine and opioid peptides on the motility and responses of rat colon to electrical stimulation, Br. J. Pharmacol. 68, 381.

Grossman, C.J., J.D. Gale, K.T. Bunce, G.J. Kilpatrick, J.W.F. Whitehead, A.W. Oxford and P.P.A. Humphrey, 1993, Development of a radioligand binding assay for the 5-$HT_4$ receptor: use of a novel antagonist, Br. J. Pharmacol. 108, 106P.

Hoyer, D., D.E. Clarke, J.R. Fozard, P.R. Hartig, G.R. Martin, E.J. Mylecharane, P.R. Saxena and P.P.A. Humphrey, 1994, International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin), Pharmacol. Rev. 46, 157.

Lew, M.J., 1995, Extended concentration-response curves used to reflect full agonist efficacies and receptor occupancy-response coupling ranges, Br. J. Pharmacol. 115, 745.

Lovenberg, T.W., B. Baron, L. DeLecea, J.D. Miller, R.A. Prosser, M.A. Rea, P.E. Foye, M. Racke, A.L. Slone, B.W. Siegel, P.E. Danielson, J.G. Sutcliffe and M.G. Erlander, 1993, A novel adenylyl cyclase-activating serotonin receptor (5-$HT_7$) implicated in the regulation of mammalian circadian rhythms, Neuron 11, 449.

Lucchelli, A., M.G. Santagostino-Barbone, A. Barbieri, S.M. Candura and M. Tonini, 1995, The interaction of antidepressant drugs with central and peripheral (enteric) 5-$HT_3$ and 5-$HT_4$ receptors, Br. J. Pharmacol. 114, 1017.

Maggi, C.A., R. Patacchini, S. Giuliani and A. Giachetti, 1993, In vivo and in vitro pharmacology of SR 48,968, a non-peptide tachykinin $NK_2$ receptor antagonist, Eur. J. Pharmacol. 234, 83.

Martin, G.R. and P.P.A. Humphrey, 1994, Classification review: receptors for 5-hydroxytryptamine: current perspectives on classification and nomenclature, Neuropharmacology 33, 261.

Martin, G.R. and R.J. Wilson, 1995, Operational characteristics of a 5-HT receptor mediating direct vascular relaxtion: identity with the 5-$HT_7$ receptor?, Br. J. Pharmacol. 114, 383P.

McLean, P.G. and I.M. Coupar, 1995, 5-$HT_4$ receptor antagonist affinities of SB207710, SB205008, and SB203186 in the human colon, rat oesophagus, and guinea-pig ileum peristaltic reflex, Naunyn-Schmiedeberg's Arch. Pharmacol. 352, 132.

McLean, P.G. and I.M. Coupar, 1996a, Stimulation of cyclic AMP formation in the circular smooth muscle of human colon by activation of 5-$HT_4$-like receptors, Br. J. Pharmacol. 117, 238.

McLean, P.G. and I.M. Coupar, 1996b, Further investigation into the signal transduction mechanism of the 5-$HT_4$-like receptor in the circular smooth muscle of human colon, Br. J. Pharmacol. 118, 1058.

McLean, P.G., I.M. Coupar and P. Molenaar, 1995, A comparative study of functional 5-$HT_4$ receptors in human colon, rat oesophagus and rat ileum, Br. J. Pharmacol. 115, 47.

Monsma, F.J., Jr., Y. Shen, R.P. Ward, M.W. Hamblin and D.R. Sibley, 1993, Cloning and expression of a novel serotonin receptor with high affinity for tricyclic psychotropic drugs, Mol. Pharmacol. 43, 320.

Plassat, J.L., U. Boschert, N. Amlaiky and R. Hen, 1992, The mouse 5-$HT_5$ receptor reveals a remarkable heterogeneity within the 5-$HT_{1D}$ receptor family, EMBO J. 11, 4779.

Plassat, J.L., N. Amlaiky and R. Hen, 1993, Molecular cloning of a mammalian serotonin receptor that activates adenylate cyclase, Mol. Pharmacol. 44, 229.

Ramirez, M.J., E. Cenarruzabeitia, J. Del Rio, B. Lasheras, 1994, Involvement of neurokinins in the non-cholinergic response to activation of 5-$HT_3$ and 5-$HT_4$ receptors in guinea-pig ileum, Br. J. Pharmacol. 111, 419.

Regoli, D. and F. Nantel, 1991, Pharmacology of neurokinin receptors, Biopolymers 31, 777.

Roth, B.L., S.C. Craigo, M.S. Choudhary, A. Uluer, F.J. Monsma, Y. Shen, H.Y. Meltzer and D.R. Sibley, 1994, Binding of typical and atypical antipsychotic agents to 5-hydroxytryptamine-6 and 5-hydroxytryptamine-7 receptors, J. Pharmacol. Exp. Ther. 268, 1403.

Ruat, M., E. Traiffort, R. Leurs, J. Tardivel-Lacombe, J. Diaz, J.-M. Arrang and J.-C. Schwartz, 1993, Molecular cloning, characterization, and localization of a high affinity serotonin receptor (5-$HT_7$) activating cyclic AMP formation, Proc. Natl. Acad. Sci. USA 90, 8547.

Saria, A., F. Javorsky, C. Humpel and R. Gamse, 1990, 5-$HT_3$ receptor antagonists inhibit sensory neuropeptide release from the rat spinal cord, Neuroreport 1, 104.

Saria, A., F. Javorsky, C. Humpel and R. Gamse, 1991, Endogenous 5-hydroxytryptamine modulates the release of tachykinins and calcitonin gene-related peptide from the rat spinal cord via 5-$HT_3$ receptors, Ann. NY Acad. Sci. 632, 464.

Shen, Y., F.J. Monsma, Jr., M.A. Metcalf, P.A. Jose, M.W. Hamblin and D.R. Sibley, 1993, Molecular cloning and expression of a 5-hydroxytryptamine$_7$ serotonin receptor subtype, J. Biol. Chem. 268, 18200.

Shenker, A., S. Maayani, H. Weinstein and J.P. Green, 1987, Pharmacological characterization of two 5-hydroxytryptamine receptors coupled to adenylate cyclase in guinea-pig hippocampal membranes, Mol. Pharmacol. 31, 357.

Suer, C., A. Erenmemisoglu, S. Temocin, S. Akar, H. Beydagi and I. Taspolat, 1992, Influence of calcium removal from bath solution of isoated rat duodenum contractions, Indian J. Physiol. Pharmacol. 36, 181.

Sumner, M.J., W. Feniuk and P.P.A Humphrey, 1989, Further characterisation of the 5-HT receptor mediating vascular relaxation and elevation of cyclic AMP in porcine isolated vena cava, Br. J. Pharmacol. 97, 292.

Tallarida, R.J. and R.B. Murray, 1987, Manual of Pharmacological Calculations with Computer Programs (Springer-Verlag, Berlin).

To, Z.P., D.W. Bonhaus, R.M. Eglen and L.B Jakeman, 1995, Characterization and distribution of putative 5-ht$_7$ receptors in guinea-pig brain, Br. J. Pharmacol. 115, 107.

Tsou, A.P., A. Kosaka, C. Bach, P. Zuppan, C. Yee, L. Tom, R. Alvarez, S. Ramsey, D.W. Bonhaus, E. Stefanich, L. Jakeman, R.M. Eglen and H.W. Chan, 1994, Cloning and expression of a 5-hydroxytryptamine$_7$ receptor positively coupled to adenylyl cyclase, J. Neurochem. 63, 456.

Tuladhar, B.R., B. Costall and R.J. Naylor, 1991, Putative 5-HT$_4$ receptor involvement in the relaxations induced by 5-HT in the rat ileum, Br. J. Pharmacol. 104, 151P.

Tuladhar, B.R., B. Costall and R.J. Naylor, 1992, Variation in the relaxation response to 5-HT in different regions of the rat small intestine, Br. J. Pharmacol. 105, 278P.

Van Nueten, J.M., P.A. Janssen, J. Van Beek, R. Xhonneux, T.J. Verbeuren and P.M. Vanhoutte, 1981, Vascular effects of ketanserin (R 41 468), a novel antagonist of 5-HT$_2$ serotonergic receptors, J. Pharmacol. Exp. Ther. 218, 217.

Vermillion, D.L. and S.M. Collins, 1988, Increased responsiveness of jejunal longitudinal muscle in *Trichinella*-infected rats, Am. J. Physiol. 254, G124.

Wainscott, D.B., M.L. Cohen, K.W. Schenck, J.E. Audia, J.S. Nissen, M. Baez, J.D. Kursar, V.L. Lucaites and D.L. Nelson, 1993, Pharmacological characteristics of the newly cloned rat 5-hydroxytryptamine$_{2F}$ receptor, Mol. Pharmacol. 43, 419.

Wardle, K.A. and G.J. Sanger, 1992, Guinea-pig distal colon: a sensitive model of the putative 5-HT$_4$ receptor, Br. J. Pharmacol. 105, 279P.

Wood, M.D., A. Glen, T.P. Blackburn, J.A. Lee, J.A. Sutiphong, C. Kumar, J. Carey and J. Robinson, 1993, (−)-Fluoxetine has a high affinity for the cloned rat and human 5-HT$_{1C}$ receptor and the human 5-HT$_2$ receptor, Br. J. Pharamcol. 110, 102P.

Zeng X.P., K.H. Moore and E. Burcher, 1995, Characterization of tachykinin NK2 receptors in human urinary bladder, J. Urol. 153, 1688.