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The contractile effect of the ghrelin receptor antagonist, D-Lys^3-GHRP-6, in rat fundic strips is mediated through 5-HT receptors

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Abstract

Ghrelin is an orexigenic peptide present in the stomach with gastroprokinetic properties. Previous in vivo studies have shown that the ghrelin receptor antagonist, D-Lys^3-GHRP-6, reduced food intake and delayed gastric emptying in rodents but these effects are at variance with the normal phenotype of the ghrelin knockout mice. To verify the specificity of the effects observed with D-Lys^3-GHRP-6 this study aimed to investigate the pharmacology of D-Lys^3-GHRP-6 in vitro.

Rat fundic strips were suspended in a tissue bath and the contraction of strips to 10^{-5} M of ghrelin, GHRP-6 or D-Lys^3-GHRP-6 was measured isometrically in the absence and presence of blockers.

Neither ghrelin, nor GHRP-6, induced significant contractions in the absence of electrical field stimulation thereby excluding the presence of ghrelin receptors on smooth muscle cells. In contrast D-Lys^3-GHRP-6, induced a pronounced biphasic contraction of 13.9±1.8% and 40.5±3.2% relative to the response to 60 mM KCl. The contraction was blocked by the 5-HT$_{1,2}$ receptor antagonist methysergide and was markedly reduced by the 5-HT$_{2B}$ receptor antagonist, yohimbine, which also profoundly affected 5-HT induced contractions in fundic strips. The existence of 5-HT$_{2B}$ receptors in the fundus was confirmed by use of the 5-HT$_{2B}$ receptor agonist, BW 723C86.

In contrast to ghrelin and GHRP-6, the ghrelin receptor antagonist, D-Lys^3-GHRP-6, induced pronounced smooth muscle contractions in the rat fundus by interacting with 5-HT$_{2B}$ receptors. This may question the role of endogenous ghrelin in the effects observed with D-Lys^3-GHRP-6 on food intake and gastric emptying in vivo.

Keywords: Ghrelin receptor antagonist; 5-HT receptor; Contraction study

1. Introduction

The hunger hormone ghrelin was isolated from rat stomach extracts by Kojima et al. (1999). The receptor for ghrelin, the growth hormone secretagogue receptor, was already identified by Howard et al. (1996) prior to the endogenous ligand by the use of synthetic growth hormone secretagogues (e.g. growth hormone releasing peptide, GHRP-6). The latter were initially discovered by Bowers et al. (1977) as enkephalin analogues that affected the release of growth hormone (GH). Although ghrelin is a potent GH secretagogue in man and rodents (Date et al., 2000; Takaya et al., 2000), the activity of this peptide is not restricted to the hypothalamic–pituitary axis. Ghrelin is now considered as the first systemically active orexigenic hormone in man and rodents that induces weight gain by stimulating an acute increase in food intake as well as by decreasing fat utilization under conditions of negative energy balance (Tschop et al., 2000; Nakazato et al., 2001).

The fact that the endocrine cells of the stomach are the major source of ghrelin already suggested that ghrelin may have important effects on gastric motility. Moreover, ghrelin is structurally most related to motilin, an important regulator of gastrointestinal motility. In rodents ghrelin but also the growth hormone secretagogue, GHRP-6, was found to accelerate gastric emptying, stimulate interdigestive motility, enhance small bowel transit and overcome postoperative ileus (Trudel et al., 2002; De Winter et al., 2004; Depoortere et al., 2005; Kitazawa et al.,

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2005; Fujino et al., 2003). Ghrelin also enhances gastric emptying in patients with diabetic and iodopathic gastroparesis and stimulates interdigestive motility in healthy volunteers (Murray et al., 2005; Tack et al., 2005, 2006). In vivo studies with the ghrelin receptor antagonist, D-Lys^3-GHRP-6, in mice showed that antagonism of the ghrelin receptor reduced food intake and body weight gain but also delayed gastric emptying, implying a physiological role for endogenous ghrelin in the regulation of these processes (Asakawa et al., 2003; Kitazawa et al., 2005). However, these effects of D-Lys^3-GHRP-6 are at variance with the normal gastric emptying and normal patterns of daily food observed in the ghrelin knockout mice (De Smet et al., 2006). To verify the specificity of the effects observed with the putative ghrelin receptor antagonist, D-Lys^3-GHRP-6, on gastric emptying and food intake in vivo, this study aimed to investigate the pharmacology of D-Lys^3-GHRP-6 in the rat fundus in vitro.

2. Materials and methods

2.1. Materials

Nω-nitro-L-arginine methyl ester (L-NAME), atropine sulfate, indomethacin, methiothepin mesylate, 1-(2-Methoxyphenyl)-4-(4-phthalimidobutyl)piperazine hydrobromide (NAN-190), 5-carboxamidotryptamine maleate and yohimbine hydrochloride were purchased from Sigma (St. Louis, MO, USA). Growth hormone releasing peptide 6 (GHRP-6) and D-Lys^3-GHRP-6 were from Bachem (Bubendorf, Switzerland). Rat ghrelin, 5-hydroxytryptamine (5-HT), α-methyl-5-HT, 1-methyl-1H-indole-3-carboxylic acid, [1-[2-[(methylsulfonyl) amino]ethyl]-4-piperidinyl]methyl ester (GR113808), (2R)-1-[(3-Hydroxyphenyl)sulfonyl]-2-[2-(4-methyl-1-piperidinyl) ethyl]pyrrolidine (SB269970), ketanserin tartrate, methysergide maleate, *N*-[4-Methoxy-3-(4-methyl-1-piperazinly) phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)-1,1′-biphenyl-4-carboxamide hydrochloride (GR127935), 8-[5-(2,4-Dimethoxy-5-(4-trifluoromethylphenylsulfonamido) phenyl-5-oxopentyl]-1,3,8-triazaspiro[4,5]decane-2,4-dione hydrochloride (RS102221), *N*-(1-Methyl-1H-indo-5yl)-*N*′-(3-methyl-5-isothiazolyl)urea (SB 204741), α-Methyl-5-(2-thienylmethoxy)-1H-indole-3-ethanamine hydrochloride (BW 723C86) were obtained from Tocris Cookson (Bristol, UK). Tetrodotoxin was purchased from Roth (Karlsruhe, Germany). Ondansetron was obtained from Glaxo (Harlow, UK). {(*S*)-1,2-[3-(3,4-dichlorophenyl)-1-(3-isopropoxyphenylacetyl)piperidin-3-yl] ethyl-4-phenyl-1-azionabicyclol[2.2.2] octane, chloride} (SR140333) and [(*S*)-*N*-methyl-*N*[4-(4-acetylamino-4-phenyl piperidino)-2-(3,4-dichlorophenyl) butyl]-benzamide] (SR48968) were kindly provided by Dr. X. Emonds-Alt from Sanofi Research (Montpellier, France).

2.2. Methods

2.2.1. Tissue preparation

Male Wistar rats (350–400 g) were sacrificed by cervical dislocation, the stomach was removed and rinsed with saline. All procedures were approved by the Ethical Committee for Animal Experiments of the University of Leuven.

2.2.2. Contractility measurements

Circular strips (0.2 × 1.5 cm), freed from mucosa were cut from the fundus and suspended along their circular axis in a tissue bath filled with Krebs-buffer at 37 °C (NaCl: 120.9 mM; NaH_2PO_4: 2.0 mM; $NaHCO_3$: 15.5 mM; KCl: 5.9 mM; $CaCl_2$: 1.25 mM; $MgCl_2$: 1.2 mM; glucose: 11.5 mM) and gassed with 95% O_2/5% CO_2. Tissues were equilibrated for 2 h at optimal stretch (1.5 g), changing bath solutions every 30 min, before application of 1 or 10 μM ghrelin, GHRP-6, D-Lys^3-GHRP-6 or before establishing a concentration–response curve (1 nM–10 μM) to 5-HT, α-methyl-5-HT, 5-carboxamidotryptamine, BW 723C86 in the absence or presence of tetrodotoxin (TTX) (3 μM). The effect of blockers/receptor antagonists was tested by preincubation of the tissue for 15 min before application of 10 μM D-Lys^3-GHRP-6 or before establishing a concentration–response curve to 5-HT. Contractions were measured using an isometric force transducer/amplifier (Harvard Apparatus, South Natick, MA, USA), recorded on a multicorder and sampled for digital analysis using the Windaq data acquisition system and a DI-2000 PGH card (Dataq Instruments, Akron, Ohio, USA). Contractions were expressed relative to the response induced by 60 mM KCl given at the end of the experiment.

2.2.3. Statistics

Data are represented as mean ± standard error of the mean (S.E.M.). Concentration–response curves were fitted by non-linear regression analysis using the Graphpad Prism software (San Diego, CA, USA) and the pEC_{50} value (negative logarithm of the concentration producing half maximal contraction) and the maximal contraction were calculated. Differences in pEC_{50} values and maximal contractions were analyzed by Student's unpaired *t*-test. A P value <0.05 was considered statistically significant.

3. Results

3.1. Contractile responses to ghrelin, GHRP-6 and D-Lys^3-GHRP-6 in the rat fundus

Administration of 10 μM ghrelin to the tissue bath induced a small contraction of 3.3 ± 1.3% ($P = 0.04$) relative to the response to 60 mM KCl (Fig. 1). At a 10-fold lower concentration, the

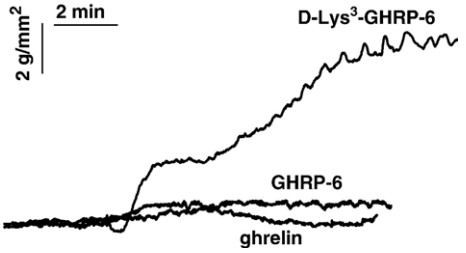

Fig. 1. Tracing showing the contraction to 10 μM ghrelin, GHRP-6 and D-Lys^3-GHRP-6 in strips from the rat fundus.

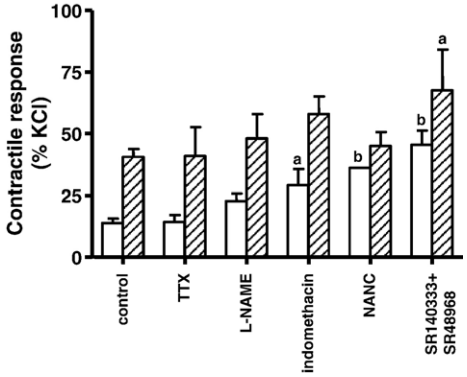

Fig. 2. Effect of blockers/receptor antagonists on the early phase (open bars) and late phase (hatched bars) contraction to D-Lys^3-GHRP-6 in the rat fundus. Strips were preincubated for 15 min with either TTX (3 μM), L-NAME (300 μM), indomethacin (10 μM), atropine (5 μM)+guanethidine (3 μM) or SR140333 (0.5 μM)+SR48968 (0.5 μM) before application of 10 μM D-Lys^3-GHRP-6. Data are represented as the mean±SEM (n=3) and are expressed as a percentage of the contractile response to 60 mM KCl. [a]P<0.05, [b]P<0.005 versus the control contraction.

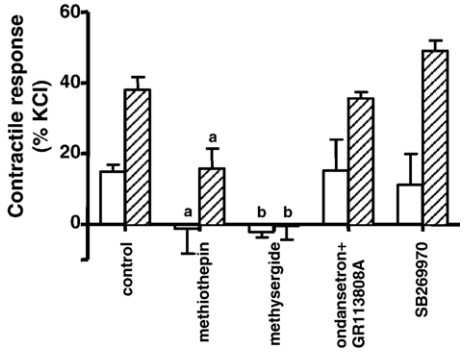

Fig. 4. Effect of 5-HT receptor antagonists on the early phase (open bars) and late phase (hatched bars) contraction to D-Lys^3-GHRP-6 in the rat fundus. Strips were preincubated for 15 min with either the 5-HT$_{1,2}$ receptor antagonists methiothepin (0.5 μM) or methysergide (0.1 μM), the 5-HT$_3$ receptor antagonist ondansetron (1 μM) and the 5-HT$_4$ receptor antagonist GR113808A (1 μM) or the 5-HT$_7$ receptor antagonist SB 269970 (0.1 μM) before application of 10 μM D-Lys^3-GHRP-6. Data are represented as the mean±SEM (n=3–5) and are expressed as a percentage of the contractile response to 60 mM KCl. [a]P<0.05, [b]P<0.001 versus the control contraction.

contraction amounted only 1.4±0.7% (P=0.09). GHRP-6 failed to induce a contraction that was significantly different from the baseline in the fundus neither at 10 μM (3.5±1.6%, P=0.07) nor at 1 μM (0.1±1.4%, P=0.9) (Fig. 1).

In contrast, D-Lys^3-GHRP-6 at 10 μM but not at 1 μM, induced a clear, biphasic contraction (Fig. 1). The early phase contraction reached 13.9±1.8%, the late phase contraction 40.5±3.2% of the response to high KCl. At higher concentrations (0.1 mM) the biphasic contraction was further increased to 49.7±9.3% and 62.9±0.5% respectively.

3.2. Pharmacology of D-Lys^3-GHRP-6 induced contractions

The biphasic contraction to D-Lys^3-GHRP-6 was unaffected by tetrodotoxin (3 μM) (Fig. 2). Pretreatment with the NO synthase blocker, L-NAME (300 μM), was also ineffective in modulating the contraction (Fig. 2). Under non-adrenergic non-cholinergic conditions (atropine 5 μM, guanethidine 3 μM), the early phase contraction to D-Lys^3-GHRP-6 was significantly (P<0.005) augmented from 13.9±1.8% to 36.1±0.07%. Similar increases in the early phase contraction to D-Lys^3-GHRP-6 were observed after pretreatment of the strips with the non-selective cyclooxygenase inhibitor, indomethacin (29.0±6.5%, P<0.05), or the NK$_1$ and NK$_2$ receptor antagonists, SR140333 and SR48968 (45.4±5.9%, P<0.005) (Fig. 2). Only the latter receptor antagonists also significantly increased the late phase contraction to D-Lys^3-GHRP-6 from 40.5±3.2% to 67.6±16.5% (P<0.05).

The tracings in Fig. 3 indicate that the biphasic contraction to D-Lys^3-GHRP-6 was mediated through interaction with 5-HT receptors as the 5-HT$_{1,2}$ receptor antagonists methiothepin (0.5 μM) or methysergide (0.1 μM) both abolished the early phase contraction and reduced (from 40.5±3.2% to 15.7±5.6%; P<0.05) or abolished the late phase contraction respectively (Fig. 4). The specificity of the interaction of D-Lys^3-GHRP-6 with 5-HT$_{1,2}$ receptors was investigated by pretreatment of the strip preparations with other 5-HT receptor antagonists, such as a combination of 5-HT$_3$ (1 μM ondansetron) and 5-HT$_4$ receptor antagonists (1 μM GR113808A) or a 5-HT$_7$ receptor antagonist

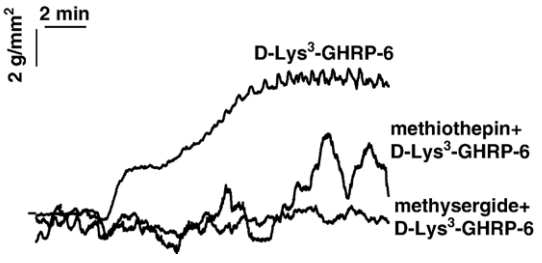

Fig. 3. Tracing showing the effect of the 5-HT$_{1,2}$ receptor antagonists, methiothepin and methysergide, on the biphasic contraction to D-Lys^3-GHRP-6 in the rat fundus. Strips were preincubated for 15 min with either methiothepin (0.5 μM) or methysergide (0.1 μM) before application of 10 μM D-Lys^3-GHRP-6.

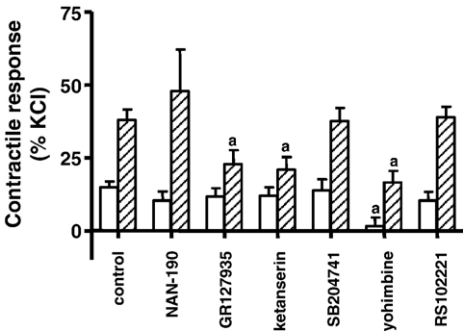

Fig. 5. Effect of specific 5-HT$_1$ and 5-HT$_2$ receptor antagonists on the early phase (open bars) and late phase (hatched bars) contraction to D-Lys^3-GHRP-6 in the rat fundus. Strips were preincubated for 15 min with either the 5-HT$_{1A}$ receptor antagonists NAN-190 (0.1 μM), the 5-HT$_{1B,1D}$ receptor antagonist GR127935 (0.1 μM), the 5-HT$_{2A}$ receptor antagonist ketanserin (0.5 μM), the 5-HT$_{2B}$ receptor antagonists SB204741 (0.5 μM) and yohimbine (1 μM) or the 5-HT$_{2C}$ receptor antagonist RS102221 (0.1 μM) before application of 10 μM D-Lys^3-GHRP-6. Data are represented as the mean±SEM (n=3–9) and are expressed as a percentage of the contractile response to 60 mM KCl. [a]P<0.005 versus the control contraction.

Table 1
Effect of 5-HT receptor antagonists on 5-HT induced concentration–response curves in the rat fundus

	pEC_{50}	% KCl
5-HT	7.81±0.07	71.4±5.6
0.1 μM GR127935+5-HT	8.05±0.08	48.5±15.9
0.5 μM ketanserin+5-HT	8.00±0.09	53.9±13.3
0.5 μM SB204741+5-HT	7.61±0.07	77.5±8.7
1 μM yohimbine +5-HT	5.87±0.13[a]	76.4±3.4

[a] $P<0.0001$ compared to 5-HT.

(0.1 μM SB269970) (Fig. 4). All these receptor antagonists were without effect (Fig. 4).

In order to elucidate which 5-$HT_{1,2}$ receptor subtype is involved, 5-HT_{1A} (0.1 μM NAN-190), 5-$HT_{1B,D}$ (0.1 μM GR127935), 5-HT_{2A} (0.5 μM ketanserin), 5-HT_{2B} (0.5 μM SB204741 or 1 μM yohimbine) and 5-HT_{2C} (0.1 μM RS102221) receptor antagonists were tested upon their ability to block the D-Lys^3-GHRP-6 induced biphasic contraction. The early phase contraction was only significantly ($P<0.005$) reduced by 89.3% after pretreatment with yohimbine while the late phase contraction was reduced by 39.8%, 44.9% or 56.2% after pretreatment with GR127935, ketanserin, or yohimbine respectively (Fig. 5). NAN-190, SB204741 and RS102221 were without effect.

3.3. Characterization of 5-HT receptor subtypes in the rat fundus

The existence of 5-$HT_{1,2}$ receptor subtypes in the rat fundus was verified by testing the effect of selective 5-HT_1 and 5-HT_2 receptor antagonists on the contraction to 5-HT and by evaluating the contractile response to 5-HT_1 and 5-HT_2 agonists.

The dose–response curve to 5-HT (pEC_{50}: 7.81±0.07) was shifted to the right by pretreatment with the 5-HT_{2B} receptor antagonist, yohimbine (pEC_{50}: 5.87±0.13) while the shift induced by the 5-HT_{2B} receptor antagonist, SB204741 (pEC_{50}: 7.61±0.07, $P=0.06$) just failed to reach significance (Table 1). The 5-HT_{2A} receptor antagonist, ketanserin and the 5-$HT_{1B,1D}$ receptor antagonist, GR127935 were without effect. Neither of the compounds affected the maximal contraction to 5-HT.

The 5-HT_2 agonist, α-methyl-5-HT, and the 5-HT_{2B} agonist, BW 723C86, were equipotent to 5-HT to induce contractions in the fundus but their intrinsic activity was lower (Table 2). In contrast the pEC_{50} value but not the intrinsic activity of the 5-HT_1 agonist, 5-carboxamidotryptamine, was significantly lower compared to 5-HT. Pretreatment of the strips with TTX (3 μM) only shifted the concentration–response curve to 5-HT to the left but did not affect the contractile response to 5-carboxytryptamine, α-methyl-5-HT or BW 723C86 (Table 2). Because our data suggest that D-Lys^3-GHRP-6 probably interacts with 5-HT_{2B} receptors, we investigated whether a single dose of BW 723C86 could mimic the biphasic contraction to D-Lys^3-GHRP-6. As illustrated in Fig. 6, the contraction to BW 723C86 at 10^{-5} M was monophasic and reached 56.8±3.8% of the response to 60 mM KCl.

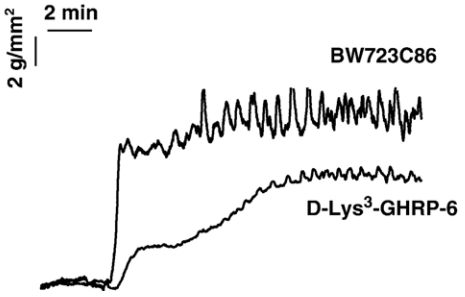

Fig. 6. Tracing showing the contractile response to a single dose (10 μM) of D-Lys^3-GHRP-6 or of the 5-HT_{2B} agonist, BW 723C86, in the rat fundus.

4. Discussion

In order to evaluate the mismatch between the inhibitory effects observed with D-Lys^3-GHRP-6 in vivo on gastric emptying and food intake and the lack of significant changes observed when comparing the phenotype of the ghrelin knockout mice with their wild type equivalents, we investigated the pharmacology of D-Lys^3-GHRP-6 in vitro in the rat fundus.

The ghrelin receptor antagonist, D-Lys^3-GHRP-6, induced profound biphasic smooth muscle contractions in the fundus. In contrast neither ghrelin, nor the synthetic ghrelin agonist, GHRP-6, induced contractions in the absence of electrical field stimulation thereby excluding the presence of ghrelin receptors on smooth muscle cells but not on the nerves which should be preferentially studied in the presence of electrical field stimulation. Indeed, previous studies indicated that ghrelin increased electrically-evoked cholinergic neural contractions in rat and mice but not in rabbit stomach strips (Dass et al., 2003; Depoortere et al., 2003, 2005; Kitazawa et al., 2005). In addition morphological studies showed expression of the ghrelin receptor in the enteric nervous system but not in smooth muscle tissue of rat stomach and guinea pig ileum (Dass et al., 2003; Xu et al., 2005).

The profound smooth muscle response to D-Lys^3-GHRP-6 was only markedly reduced by 5-$HT_{1,2}$ antagonists. The

Table 2
Concentration–response curves to 5-HT and 5-HT_1, 5-HT_2, 5-HT_{2B} agonists in the rat fundus in the absence and presence of TTX (3 μM)

	pEC_{50}	pEC_{50}+TTX	% KCl	% KCl+TTX
5-HT	7.81±0.07	8.28±0.04[c]	71.4±5.6	67.4±8.0
5-carboxamidotryptamine	7.16±0.12[b]	7.03±0.14[b]	58.5±3.2	61.2±8.1
α-methyl-5-HT	7.94±0.18	7.80±0.28	55.3±4.1[a]	66.9±13.6
BW 723C86	7.91±0.08	8.12±0.03[a]	44.3±5.2[b]	48.5±5.7

[a] $P<0.05$, [b] $P<0.005$ compared to 5-HT, [c] $P<0.01$ compared to the response in the absence of TTX.

presence of a contractile receptor in the rat gastric fundus with extreme sensitivity to serotonin has been well documented for over 30 years. Pharmacological studies attempting to characterize the contractile serotonergic receptor in the rat fundus demonstrated that the contractile response to 5-HT was not mediated by interaction with receptors identical with the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D}, 5-HT_2 or 5-HT_3 receptors (Clineschmidt et al., 1985; Cohen and Fludzinski, 1987; Baez et al., 1990). These data supported the idea that the 5-HT receptor in the rat fundus was unique and distinct from known 5-HT binding sites. Finally, a receptor, the 5-HT_{2B} receptor (originally called 5-HT_{2F}) was cloned from the rat stomach (Foguet et al., 1992; Kursar et al., 1992). Receptor binding studies of cells expressing the cloned rat 5-HT_{2B} receptor revealed that the affinity of ketanserin (Ki=3559±175 nM) to bind to the receptor was much lower compared to yohimbine (Ki=53.1±4.6 nM) and methysergide (6.28±0.91 nM) respectively (Wainscott et al., 1993). On the basis of the low receptor antagonist affinity of ketanserin but not of yohimbine for 5-HT induced contractions in rat fundus longitudinal muscle, Baxter et al. (1994) concluded close pharmacological identity between 5-HT receptors in the rat fundus and the cloned 5-HT_{2B} receptor. In analogy with these studies we also found that yohimbine but not ketanserin profoundly affected 5-HT and D-Lys^3-GHRP-6 induced contractile responses in the rat fundus. The inability of the 5-HT_{2B} receptor antagonist, SB 204741, to block 5-HT and D-Lys^3-GHRP-6 induced contractions may question the effectiveness of SB 204741 to block 5-HT_{2B} receptors in the gastrointestinal tract.

The existence of 5-HT_{2B} receptors on the smooth muscle cells of the rat fundus preparation was confirmed by the contractile effects of the 5-HT_2 agonist, α-methyl-5-HT, and the 5-HT_{2B} agonist, BW 723C86 in the presence of TTX. However, a single dose of BW 723C86 could not mimic the biphasic contraction to D-Lys^3-GHRP-6. Shallow or overtly biphasic agonist concentration–effect curves for tryptamines are commonly observed in the rat fundus (Buchheit et al., 1986; Clineschmidt et al., 1985) and have been cited as evidence for multiple 5-HT receptor subtypes (Foguet et al., 1992). Because the contraction to D-Lys^3-GHRP-6 was somehow affected by the 5-$HT_{1B,D}$ agonist GR127935 it is not unlikely that our preparation also contains 5-HT_1 receptors, as evidenced by the effects of 5-carboxytryptamine. 5-HT has also been shown to evoke a contractile response in the muscularis mucosae taken from the fundus region of the stomach (Horn and Zweifach, 1963). As the muscularis mucosa was stripped away in our preparation we eliminated the possible contribution of a mucosal receptor.

All previous studies have been performed on longitudinal strips of the rat fundus. It is therefore worth re-iterating that the character of the receptor which mediates a contractile response to 5-HT in the circular muscle of the fundus was up till now still unknown (Vane, 1959). We provided evidence that the contraction to 5-HT in the circular muscle is also mediated via a 5-HT_{2B} receptor subtype but the potency (pEC_{50}) of 5-HT (7.81), α-methyl-5-HT (7.94) and 5-CT (7.16) was somewhat lower than the potencies previously reported for these compounds in the longitudinal muscle (5-HT: 8.64, α-methyl-5-HT: 8.42, 5-CT: 7.98) (Kursar et al., 1992; Baxter et al., 1994). Thus a component of the longitudinal muscle which lies perpendicular to the circular muscle may contribute to the complex behavior, certainly if the potencies of both receptors differ.

From a structural point of view D-Lys^3-GHRP-6 (His-DTrp-DLys-Trp-DPhe-Lys-NH_2) contains 2 tryptophan residues that can mimic the effects of 5-HT. However, when the basic amino acid, DLys, at position 3 is replaced by the nonpolar Ala as in the ghrelin agonist GHRP-6 (His-DTrp-Ala-Trp-DPhe-Lys-NH_2), the biphasic smooth muscle contraction that involves 5-HT receptor interaction is not observed. Probably the $DLys^3$ is essential to expose the Trp residues in a conformation, favoring 5-HT receptor interaction.

The observation that D-Lys^3-GHRP-6 interacts with 5-HT_{2B} receptors may have functional consequences for the effects observed with D-Lys^3-GHRP-6 in vivo. The dose needed in vivo to delay gastric emptying and to inhibit food intake was 200 nmol/mouse (Asakawa et al., 2003; Kitazawa et al., 2005). This means that the order of magnitude of the concentration reached in the blood is similar to the concentration of D-Lys^3-GHRP-6 used in our in vitro study. Contraction of the fundus may impair gastric accommodation and induce early satiety leading to decreased food intake (Tack et al., 1998). This may question the importance of endogenous ghrelin in the effects observed with D-Lys^3-GHRP-6 on food intake in vivo. More specific ghrelin receptor antagonists need to be developed to address this issue.

Acknowledgments

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