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Action of GLP-1 (7-36) amide and exendin-4 on *Suncus murinus* (house musk shrew) isolated ileum

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Abstract

Glucagon-like peptide-1 (GLP-1) receptor agonists have been reported to modulate gastrointestinal motility but the mechanism is essentially unknown. In the present studies, we investigated the potency and mechanism of action of GLP-1 receptor ligands on the isolated ileum of *Suncus murinus*, an insectivore used in anti-emetic research. Ileal segments were mounted in organ baths containing Kreb's solution. Cumulative concentration–response curves to GLP-1 (7-36) amide (0.1–300 nM) and exendin-4 (0.1–100 nM) were constructed in the absence and presence of exendin (9-39) amide (0.3–3 nM). GLP-1 (7-36) amide and exendin-4 induced concentration-dependent contractions yielding pEC_{50} values of 8.4±0.2 and 8.4±0.4, respectively. Exendin (9-39) antagonized the action of both agonists in a non-competitive reversible manner, with apparent pK_B values of 9.5 and 9.7, respectively. Tetrodotoxin (1 μM), atropine (1 μM) and hexamethonium (500 μM) were used to determine the contractile mechanism of action of exendin-4. Tetrodotoxin and atropine significantly antagonized ($P<0.01$) the contractile action of exendin-4 (10 nM); hexamethonium (500 μM) had no action. These studies suggest that GLP-1 receptor agonists contract the ileum indirectly via postganglionic enteric neurones and an involvement of muscarinic receptors. These studies provide information relevant to the use of this species to estimate the therapeutic indexes of GLP-1 receptor agonists.

Keywords: GLP-1; Exendin-4; Exendin (9-39) amide; *Suncus murinus*; Ileum; Postganglionic enteric neurone; Nausea; Emesis; Diabetes

1. Introduction

Glucagon-like peptide-1 (GLP-1) (7-36) amide is a product of tissue-specific post-translational processing of proglucagon in intestinal L cells (Orskov et al., 1989). The process also leads to the production of two structurally related peptides, glucagon-like peptide-2 (GLP-2) and glucagon (Irwin, 2001). GLP-1 (7-36) amide is secreted into the circulation in response to nutrient ingestion (Kreymann et al., 1987) to potentiate glucose-stimulated insulin secretion and suppress glucagon release (Holst et al., 1987; Kreymann et al., 1987). GLP-2 and glucagon mainly play a role in energy homeostasis (Drucker, 2002). GLP-1 (7-36) amide exerts its biological actions via highly specific G-protein-coupled receptors, members of the glucagon receptor superfamily that also includes receptors for glucagon, GLP-2, glucose-dependent insulinotropic peptide, growth hormone-releasing hormone and secretin (Mayo et al., 2003).

GLP-1 receptor agonists such as exenatide are currently being explored for the treatment of hyperglycaemia in type-2 diabetic patients (Buse et al., 2004). However, treatment with these agonists is sometimes associated with nausea and vomiting, particularly at high doses (Perry and Greig, 2003). GLP-1 (7-36) amide profoundly affects the motor function of the gastrointestinal tract by inhibiting gastric emptying (Wettergren et al., 1993) and small bowel motility (Tolessa et al., 1998), thereby reducing metabolic demand in association with food consumption and appetite (Hellstrom and Naslund, 2001). It is not known if these actions contribute to nausea and emesis, but the reduced gastric emptying and increased satiety probably contributes to a decreased in food intake in healthy (Gutzwiller et al., 1999) and obese subjects (Naslund et al.,

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1998). However, it has been hypothesized that the side effects of GLP-1 treatment may also involve GLP-1 receptors located in the brain (Ritzel et al., 1995).

Up until now, most of the preclinical studies on GLP-1 function have used laboratory animals incapable of emesis. Unfortunately, therefore, the dose of a drug to affect plasma glucose status relative to inducing emesis is unknown until it enters clinical testing. *Suncus murinus* (house musk shrew) is an insectivore considered to be phylogenically closer to man than rodents (Novacek, 1992) and is used commonly in emesis research (Ueno et al., 1987). However, no studies have examined the action of GLP-1 receptor ligands in this species, even though it has been developed to model diabetes (Ohno et al., 1998). It would be useful to utilize this species in studies to identify the potential therapeutic limits of GLP-1 receptor ligands.

In the present studies, therefore, we decided to examine the potential of GLP-1 receptor ligands to modulate the contractility of the *S. murinus* isolated ileum. This was done to provide potency data of selected GLP-1 receptor ligands that would be necessary for planning more elaborate *in vivo* experiments. GLP-1 (7-36) amide and exendin-4 were selected as agonists for the GLP-1 receptor and exendin (9-39) amide was selected as an antagonist (Eng et al., 1992; Goke et al., 1993; Raufman et al., 1992). We also briefly investigated the mechanism of action of exendin-4 to affect the contractility of the ileum in comparative studies with nicotine and acetylcholine. Nicotine and acetylcholine are known to cause contractions of isolated ileal segments. Nicotine acts predominately at nicotinic receptors on ganglia in the enteric nervous system, whereas acetylcholine acts at ganglionic nicotinic receptors and also at muscarinic receptors located directly on smooth muscle (Galligan, 1999; Lecci et al., 2002; Schemann, 2005). A sodium channel blocker, tetrodotoxin, a ganglionic nicotinic receptor blocker, hexamethonium, and a muacarinic receptor antagonist, atropine, were used in these studies to determine if drug action involves the enteric nervous system and cholinergic receptors (Matsuo et al., 2002).

2. Materials and methods

2.1. Animals

Male *S. murinus* (45–65 g) were obtained from the Chinese University of Hong Kong and housed in a temperature-controlled room (24±1 °C). Artificial lighting was provided between 0600 and 1800 h, with humidity being maintained at 50±5%. Water and dry pelleted cat chow (Feline Diet 5003, PMI® Feeds, St. Louis, U.S.A.) was available *ad libitum*. All experiments were conducted under the license provided by the Government of the Hong Kong SAR and the Animal Research Ethics Committee, The Chinese University of Hong Kong.

2.2. Tissue preparation

Animals were fasted overnight then killed by cervical dislocation and the whole intestine was removed and immediately placed in freshly prepared Krebs' solution (composition in mM: NaCl 118, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ $7H_2O$ 1.2, $CaCl_2$ $2H_2O$ 2.5, $NaHCO_3$ 25 and glucose 10) and gassed with 95% O_2 and 5% CO_2 at room temperature. The ileum was identified (see Kanamori et al., 1989; Kiso et al., 1988; Kurohmaru et al., 1980) and a 1 cm segment was taken out and mounted longitudinally under a 0.5 g tension in a 10 ml organ bath containing Krebs' solution and gassed with 95% O_2 and 5% CO_2 at 37 °C. Only one ileal segment was used from one animal. After an equilibration period of 30 min, KCl (120 mM) was added to provide a reference contractile response followed by washout (Javid and Naylor, 1999a).

2.3. Application of drugs

Tissues were equilibrated alone or with exendin (9-39) amide (0.3, 1 and 3 nM) for 60 min, with regular washings every 20 min. The antagonist was present in the organ bath continuously during the construction of the concentration–response curves. Increasing concentrations of GLP-1 (7-36) amide (0.1–300 nM) and exendin-4 (0.1–100 nM) were added to the organ bath cumulatively using a 2–6 min dosing schedule. This procedure was found to produce optimal conditions for contractile responses. At the end of each concentration–response curve, KCl (120 mM) was added again, to check the viability of the tissues. In some experiments, the specificity of exendin (9-39) amide was investigated by adding a single concentration of nicotine (5 μM) or acetylcholine (0.5 μM) after the 60 min equilibration period. The potential of the reversibility of exendin (9-39) amide was also examined. In these experiments, the tissues were washed for 60 min after an initial 60 min equilibration period (Lew and Angus, 1984). Exendin-4 (10 nM) was then added to produce a contractile response; appropriate controls were also employed.

In a separate series of experiments, the mechanism of the exendin-4-induced responses were investigated. Tetrodotoxin (1 μM), atropine (1 μM) or hexamethonium (500 μM) was added to the organ bath 20 min prior to the addition of exendin-4 (10 nM), nicotine (5 μM) or acetylcholine (0.5 μM). The isometric contraction of the tissues was measured using Grass tranducers via a MacLab® system (ADInstruments Pty Ltd., New South Wales, Australia) connected to a Power Macintosh G3 computer (Apple Computer, Inc., California, U.S.A.). Analytical software (Chart, version 3.5/s MacLab®, New South Wales, Australia) was used to display and analyze the data.

2.4. Data analysis

The contractile responses of the ileum were measured by the difference in change of tension (g) before and after the addition of drugs. Contractions were normalized and expressed as a percentage of the reference response produced by KCl (120 mM). pEC_{50} values were calculated by non-linear regression of the concentration–response curves using GraphPad Prism version 4.0 (GraphPad Software, Inc.Version, California, U.S.A.). When the antagonist caused non-parallel shifts of the concentration–response curves to the GLP-1

receptor agonists, with a depression of the maximal response (E_{max}), the apparent affinity of the antagonist was estimated by a method of Gaddum for non-competitive antagonism (Gaddum et al., 1955). A double reciprocal plot of equiactive agonist concentrations in the presence (1/[A]) and absence (1/[A']) of the antagonist ([B]) was constructed and the apparent pK_B value was estimated using the equation $K_B = [B]/(1 - \text{slope})$. Spontaneous activity in the absence and presence of exendin (9-39) amide was analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests. The significance of differences between data shown in the concentration–response curves were determined using a repeated measures two-way ANOVA followed by Bonferroni tests. An unpaired Student's *t*-test was used to determine the specificity and reversibility of exendin (9-39) amide and to determine if tetrodotoxin, atropine or hexamethonium altered spontaneous and contractile responses. All data were expressed as mean values ± s.e.m. Differences were considered statistically significant if $P < 0.05$.

2.5. Drugs

Acetylcholine chloride and (−)nicotine tartrate (Sigma, Saint Louis, USA) were dissolved in distilled water. Glucagon-like peptide-1 (7-36) amide, exendin-4 and exendin (9-39) amide (American Peptide Company, Sunnyvale, CA); hexamethonium bromide, atropine methyl nitrate and tetrodotoxin (Sigma, Saint Louis, USA) were dissolved in saline (0.9% w/v). The volume of drug solutions added to the organ bath was less than 0.3% of the total volume.

3. Results

3.1. General observations

All ileal segments exhibited regular spontaneous contractions with a mean tension of 0.53 ± 0.02 g and a frequency of 27.1 ± 0.3 cycles/min ($n = 43$); this is similar to data obtained by other investigators (Javid and Naylor, 1999b). KCl (120 mM) induced a rapid contraction that was maximal (2.3 ± 0.1 g, $n = 43$) within 5 s and was reversible on washing. Only one cumulative concentration–response curve was constructed from each tissue. The contractions induced by KCl were unaffected by the construction of the concentration–response curves (data not shown; $P > 0.05$; $n = 43$).

3.2. Effect of GLP-1 (7-36) amide and exendin-4 on ileal segments

GLP-1 (7-36) amide (0.1–300 nM) produced concentration-dependent contractions of the ileal segments yielding a pEC_{50} of 8.4 ± 0.2, with a maximum of 0.6 ± 0.1 g ($E_{max} = 28.4 \pm 3.1\%$ of KCl response) at 100 nM (Fig. 1A; $n = 6$). The potency of exendin-4 (0.1–100 nM) to contract the ileum was similar to that of GLP-1 (7-36) amide, with a pEC_{50} of 8.4 ± 0.4 and an E_{max} of 0.5 ± 0.1 g ($E_{max} = 20.8 \pm 5.0\%$ of KCl response) at 30 nM (Fig. 1B; also see Table 1; $n = 6$).

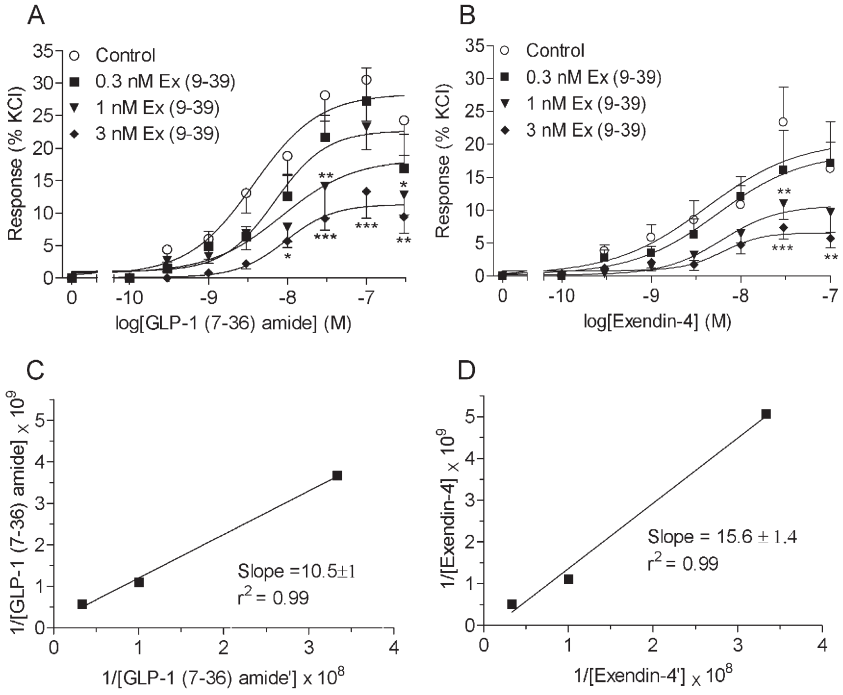

Fig. 1. Effect of exendin (9-39) amide (Ex (9-39)) on GLP-1 (7-36) amide- and exendin-4-induced contractions in *Suncus murinus* isolated ileum. (A) Cumulative concentration–response curves to GLP-1 (7-36) amide at 0.1–300 nM in the absence (control) and presence of Ex (9-39) (0.3, 1, and 3 nM). (B) Cumulative concentration–response curves to exendin-4 at 0.1–100 nM in the absence (control) and presence of Ex (9-39) (0.3, 1 and 3 nM). (C) Double reciprocal plot for GLP-1 (7-36) amide in the presence of Ex (9-39) (3 nM). (D) Double reciprocal plot for exendin-4 in the presence of Ex (9-39) (3 nM). Each data point represents means ± s.e.m. of 6 experiments. Significant differences relative to controls are indicated as $*P < 0.01$, $**P < 0.01$, $***P < 0.001$ (repeated measures two way ANOVA followed by post hoc Bonferroni tests).

3.3. Effect of exendin (9-39) amide on GLP-1 (7-36) amide- and exendin-4-induced contractions

Exendin (9-39) amide at 0.3, 1, and 3 nM alone failed to modify the spontaneous contractile activity of the ileum (control 0.62±0.08 g; 0.3 nM 0.54±0.06 g; 1 nM 0.56±0.07 g; 3 nM 0.67±0.08 g; $P>0.05$; $n=12$). However, it antagonized the contractile effects induced by GLP-1 (7-36) amide and exendin-4. Thus, exendin (9-39) amide caused a progressive rightward shift of the concentration–response curves to GLP-1 (7-36) amide and exendin-4, with a depression of the maximal responses indicating non-competitive antagonism. Exendin (9-39) amide at 1 and 3 nM significantly reduced the maximal contraction induced by GLP-1 (7-36) amide by approximately 36% ($P<0.05$) and 60% ($P<0.01$), respectively, compared to controls ($n=6$), whereas 3 nM significantly reduced the maximal contraction of the exendin-4-induced responses by 68% ($P<0.05$; $n=6$) (Fig. 1A and B). Double reciprocal plots of equiactive concentrations of GLP-1 (7-36) amide in the presence and absence of exendin (9-39) amide at 3 nM yielded a correlation coefficient, slope, and apparent pK_B of 0.99, 10.5±0.6 and 9.5, respectively (Fig. 1C; Table 1). Double reciprocal analysis of exendin (9-39) amide with exendin-4 yielded a correlation coefficient, slope, and apparent pK_B of 0.99, 15.6±1.4 and 9.7, respectively (Fig. 1D; Table 1). However, exendin (9-39) amide at 3 nM neither reduced nicotine-induced (5 μM) (Fig. 2; $P>0.05$; $n=5$) nor acetylcholine-induced contractions (0.5 μM) (Fig. 2; $P>0.05$; $n=5$). The reversibility of GLP-1 receptor antagonism produced by exendin (9-39) amide was then examined after a 60 min wash out period prior to challenge with exendin-4. The action of exendin-4 at 10 nM was not altered by exposure to exendin (9-39) amide (control=0.7±0.1 g; exendin (9-39) amide=0.7±0.1 g; $P=0.4$, $n=8$).

3.4. Effect of tetrodotoxin, atropine and hexamethonium on exendin-4-, nicotine- and acetylcholine-induced contractions

Exendin-4 at 10 nM, nicotine at 5 μM and acetylcholine at 0.5 μM contracted the ileum producing 0.7±0.1, 1.2±0.1 and 0.9±0.2 g tensions, respectively ($n=18$). Exendin-4-induced contractions were antagonized by tetrodotoxin and atropine ($P<0.05$). Representative tracings are shown in Fig. 3.

Table 1
Maximal effect (E_{max}) and pEC_{50} values of GLP-1 receptor agonists in the absence (control) and presence of exendin (9-39) amide (3 nM) in *Suncus murinus* isolated ileum

Agonists	Control		Exendin (9-39) amide 3 nM		
	E_{max} (% KCl)	pEC_{50}	E_{max} (% KCl)	pEC_{50}	pK_B
GLP-1 (7-36) amide	28.4±3.1	8.4±0.2	11.4±1.5[a]	8.0±0.2	9.5
Exendin-4	20.6±5.0	8.4±0.4	6.6±1.0[b]	8.2±0.2	9.7

Antagonist affinity is expressed as apparent pK_B value and is estimated by a double reciprocal plot of equiactive agonist concentrations in the presence (1/[A]) and absence (1/[A′]) of the antagonist ([B]). Each value represents means±s.e.m. of 6 experiments. Significant differences relative to control are indicated as [a]$P<0.01$, [b]$P<0.05$ (one way ANOVA followed by post hoc Dunnett's multiple comparison test).

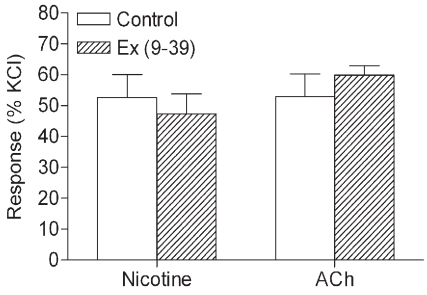

Fig. 2. Effect of exendin (9-39) amide (Ex (9-39)) (3 nM) on nicotine (5 μM)- and acetylcholine (ACh) (0.5 μM)-induced contractions in *Suncus murinus* isolated ileum. Each column represents means±s.e.m. of 5 experiments. There were no significant differences between the data ($P>0.05$) (unpaired Student's *t*-test).

Tetrodotoxin at 1 μM also modified spontaneous activity (control 0.52±0.11 g; tetrodotoxin 0.17±0.10 g; $P<0.05$; $n=5$) and reduced significantly the contractions induced by exendin-4 and nicotine by 100% (Fig. 4A; $P<0.001$; $n=5$) and 79% (Fig. 4A; $P<0.01$; $n=5$), respectively. In contrast, tetrodotoxin was ineffective to modify the acetylcholine-induced contractions (Fig. 4A; $P>0.05$; $n=5$). Atropine at 1 μM reduced spontaneous activity (control 0.29±0.05 g; atropine 0.10±0.04 g; $P<0.05$; $n=5$) and completely blocked contractions induced by exendin-4 and acetylcholine (Fig. 4B; $P<0.001$; $n=5$); it also reduced significantly the nicotine-induced contractions by 78% (Fig. 4B; $P<0.05$; $n=5$). Hexamethonium at 500 μM failed to modify spontaneous activity (control 0.71±0.10 g; hexamethonium 0.53±0.07 g; $P>0.05$; $n=8$), but reduced contractions induced by exendin-4 by approximately 36% (Fig. 4C; $P>0.05$; $n=8$) and significantly reduced the nicotine-induced contractions by approximately 74% (Fig. 4C; $P<0.05$; $n=8$). In contrast,

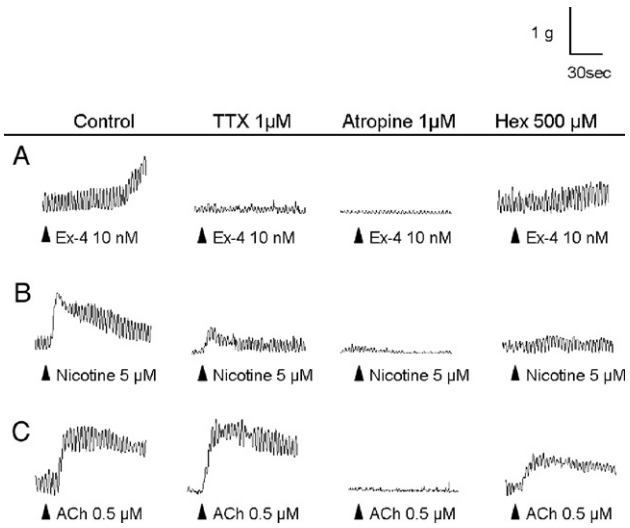

Fig. 3. Typical tracings illustrating the contractile responsiveness of *Suncus murinus* isolated ileum to exendin-4 (Ex-4) (10 nM), nicotine (5 μM) and ACh (0.5 μM) in the absence (control) and presence of other drugs. (A) Effect of exendin-4 in the absence and presence of tetrodotoxin (TTX) (1 μM), atropine (1 μM) and hexamethonium (Hex) (500 μM). (B) Effect of nicotine in the absence and presence of TTX (1 μM), atropine (1 μM) and Hex (500 μM). (C) Effect of ACh in the absence and presence of TTX (1 μM), atropine (1 μM) and Hex (500 μM).

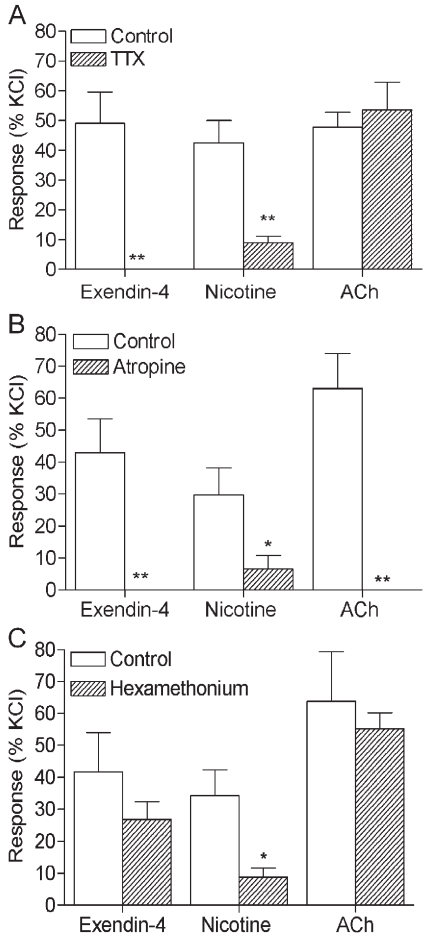

Fig. 4. Contractile responsiveness of *Suncus murinus* isolated ileum to exendin-4 (10 nM), nicotine (5 μM) and ACh (0.5 μM) in the absence (control) and presence of other drugs. (A) Effect of exendin-4, nicotine and ACh in the absence and presence of TTX (1 μM). (B) Effect of exendin-4, nicotine and ACh in the absence and presence of atropine (1 μM). (C) Effect of exendin-4, nicotine and ACh in the absence and presence of Hex (500 μM). Each column represents means±s.e.m. of 5–8 experiments. Significant differences relative to controls are indicated as $*P<0.05$, $**P<0.01$ (unpaired Student's *t*-test).

hexamethonium had no effect on acetylcholine-induced responses (Fig. 4C; $P>0.05$; $n=8$).

4. Discussion

GLP-1 receptors are expressed in the gastrointestinal tract of rats, pig and man (Bullock et al., 1996; Eissele et al., 1992; Kieffer et al., 1996). However, to the authors' knowledge, only one previous study has examined the action of GLP-1 receptor ligands on isolated tissues from the gastrointestinal tract. The previous studies used isolated smooth muscle cells from the human colon and GLP-1 (7-36) amide at 0.1–1 nM induced a weak contractile response that was abolished by exendin (9-39) amide (Ayachi et al., 2005). In our studies, GLP-1 (7-36) amide (0.1–300 nM) and exendin-4 (0.1–100 nM) induced contractions of *S. murinus* isolated ileum in a concentration-dependent manner yielding pEC_{50} values of 8.4. The potency of the agonists on *S. murinus* ileum is comparable with their potency to induce cAMP production in other studies where GLP-1 (7-36) amide stimulates cAMP production in rat insulinoma-derived beta-cells (RINm5F) with an EC_{50} of 2.98 nM (Goke et al., 1989), and exendin-4 induces cAMP production in rat parietal cells with an EC_{50} value of 0.7 nM (Schepp et al., 1994). In non-functional studies, GLP-1 (7-36) amide and exendin-4 displace ^{125}I GLP-1 (7-36) amide from RINm5F cells with K_d values of 0.3 nM and 0.1 nM, respectively (Goke et al., 1993).

In our studies, the ability of exendin (9-39) amide to antagonize the action of GLP-1 (7-36) amide and exendin-4, with similar apparent pK_B values of 9.5 and 9.7, respectively, suggests that they act at a common binding site. However, exendin (9-39) amide behaved as a non-competitive antagonist as exemplified by non-parallel shifts of the concentration–response curves to both GLP-1 (7-36) amide and exendin-4, with progressive decreases in the maximal responses. The effect of exendin (9-39) amide appeared reversible after 60 min washout and had no appreciable action to inhibit the action of nicotine and acetylcholine to indicate some degree of specificity. However, in other studies utilizing the guinea pig ileum, exendin (9-39) amide appeared to behave in a competitive manner, with a reported K_B value of 3.5 nM (Eng et al., 1992); its IC_{50} is 3.2 nM in COS-7 cells (Beinborn et al., 2005). It is possible that the reversible non-competitive nature of exendin (9-39) amide at *S. murinus* GLP-1 receptors is unique and the result of species differences.

We decided to investigate the potential mechanism of action of exendin-4 to induce contractions of the ileum in more detail. This was done in comparison with acetylcholine and nicotine, which have both been shown to contract the ileum of this species at low micromolar concentrations (Muraki et al., 1988). Exendin-4 was slower to contract the ileum compared to nicotine and acetylcholine, but the action of all three agonists was antagonized by atropine, to indicate an involvement of muscarinic receptors (Galligan, 1999). Conversely, tetrodotoxin antagonized the action of nicotine and exendin-4, but not acetylcholine. This suggests that both nicotine and exendin-4 act via neurones to induce contractions, whereas acetylcholine's action is predominately on smooth muscle (Matsuo et al., 2002). Certainly, this seems likely, since hexamethonium had no action to modify significantly the contractions induced by acetylcholine, but it predictably reduced the action of nicotine.

The failure of hexamethonium to reduce the action of exendin-4 may indicate that GLP-1 receptors are on postganglionic nerves, rather than on the preganglionic sensory side of the system (Chan and Rudd, 2006). However, the mechanism of action of nicotine compared to exendin-4 appears different. Thus, the ability of nicotine to contract the ileum was not prevented completely by any of the pre-treatments that we used (i.e. treatments only reduced responses by ~75%), whereas tetrodotoxin and atropine abolished the action of exendin-4. There are nicotinic receptors on ganglia, but some also exist on, or near to nerve terminals and cause transmitter release when activated via tetrodotoxin insensitive mechanisms (Galligan, 1999; Schneider and Galligan, 2000). It is possible that the nicotine-induced tetrodotoxin-resistant responses that we observed, that were partially resistant to hexamethonium and

atropine, involve a non-cholinergic component (see Galligan, 1999). Alternatively, nicotine may have a direct action on smooth muscle, or mediate part of its contractile action via other undefined mechanisms. Nevertheless, we did observe that both tetrodotoxin and atropine reduced the spontaneous activity of the ileum, which is similar to the effects of these drugs on isolated segments of guinea pig colon (Spencer et al., 2002).

In conclusion, the ability of exendin (9-39) amide to antagonize the contractile action of GLP-1 (7-36) amide and exendin-4 provides evidence for the existence of GLP-1 receptors in *S. murinus* isolated ileum. These receptors are probably located on postganglionic cholinergic nerves, rather than directly on smooth muscle. The non-competitive and reversible nature of exendin (9-39) amide appears unique and may indicate a species differences in the pharmacology of GLP-1 receptors. Nevertheless, our experiments validate exendin (9-39) amide, GLP-1 (7-36) amide and exendin-4 as useful tools to probe GLP-1 receptors in *S. murinus*. It is tempting to speculate the nausea and vomiting associated with GLP-1 treatment is a consequence of the action of GLP-1 receptor ligands to modulate gastrointestinal motility, in addition to hypothesized action in the central nervous system (Ritzel et al., 1995). Further, the present *in vitro* studies have provided some initial dose-response and potency data of the GLP-1 receptor ligands to aid in selecting the most appropriate pharmacological tools for *in vivo* studies. The employment of appropriate *in vivo* studies using a species that is capable of vomiting such as *S. murinus* are vital prior to clinical evaluation of the risk of side effects of nausea and emesis associated with GLP-1 treatments in type-2 diabetes.

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