Cholecystokinin type B receptor antagonist PD-136,450 is a partial secretory agonist in the stomach and a full agonist in the pancreas of the rat

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Abstract

Gastrin (cholecystokinin type B (CCK-B)) receptor antagonists may help to elucidate the physiological role of gastrin, have therapeutic potential as acid antisecretory drugs, and may be of use as adjuvant therapy for gastrin sensitive tumours. In binding studies, the gastrin receptor antagonist PD-136,450 had at least 1000 fold greater affinity for gastrin (CCK-B) than CCK-A receptors. In this study the biological activity of PD-136,450 was evaluated in conscious and anaesthetised rats. PD-136,450 antagonised gastrin stimulated acid secretion after subcutaneous (IC₅₀: 0.28 µmol/kg; conscious rats) and intravenous (IC₅₀: 0.17 µmol/kg; anaesthetised rats) administration. In basal secreting fistula animals, the compound stimulated acid output to 30 (5)% of the maximal response to gastrin. Stimulant activity was not caused by gastrin release. As an agonist PD-136,450 was about 350 times less potent than gastrin-17 on a molar basis. In addition, PD-136,450 was a powerful agonist of pancreatic secretion in anaesthetised rats. The specific gastrin antagonist L-365,260 inhibited the (partial) agonist activity of PD-136,450 in the stomach and the specific CCK-A receptor antagonist L-364,718 inhibited the agonist activity of PD-136,450 in the pancreas. It is concluded that the agonist effect of PD-136,450 is mediated via interaction with the gastrin (CCK-B) receptor in the stomach and the CCK-A receptor in the pancreas.

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Considerable progress has been made in elucidating the physiological and pathological roles of gastrin in recent years. This has been aided by the discovery of non-peptide antagonists of gastrin (cholecystokinin type B (CCK-B)) receptors, by molecular cloning of the gastrin receptor itself, and by identification of receptors for gastrin on enterochromaffin-like (ECL) cells from mastomys gastric tumours.^{1–10} Receptor antagonists have a potential use in peptic ulcer disease and as adjuvant therapy in gastrin receptor positive gastrointestinal tumours, since they not only antagonise acid secretion but also inhibit the trophic effect of gastrin on gastric mucosa, endocrine cell populations, and gastrin receptor positive malignant tumour cells.^{11 12} Gastric ECL cell hyperplasia and the possibility of inducing carcinoid tumours remain potential concerns during long term treatment with proton pump blockers.

CCK-A (pancreatic type) and CCK-B (gastric/

brain type) receptor antagonists are closely related in structure and pharmacological properties and most of the compounds are ligands for both CCK and gastrin receptors.^{7 13} Two CCK-B receptor antagonists have been the focus of recent attention – PD-136,450, also referred to as Cam-1189, is a dipeptoid analogue,^{1–5} while L-365,260 is a benzodiazepine derivative.^{6–8} These antagonists bind to CCK-B receptors in the stomach and brain where they display anti-secretory and anxiolytic properties respectively. Compared with L-365,260, PD-136,450 displays a higher binding affinity for CCK-B receptors (IC₅₀=0.6 nM in the guinea pig gastric mucosa and IC₅₀=0.7 nM in mouse cerebral cortex) and seems to be a more potent and longer acting antagonist of gastrin induced acid secretion *in vivo*.^{2 5 12 13} PD-136,450 has also been reported to inhibit ECL cell hyperplasia induced by chronic achlorhydria and endogenous hypergastrinaemia during treatment with a proton pump inhibitor.¹²

While studying antagonism of gastrin induced acid secretion by PD-136,450, we also observed an agonist response at higher doses in both the stomach and pancreas which prompted us to study the effects of PD-136,450 on acid and pancreatic secretions in detail.

Methods

ACID SECRETION STUDIES IN CONSCIOUS GASTRIC FISTULA RATS

Female Wistar rats weighing about 200 g were fitted with a chronic gastric fistula as described previously.¹⁴ The cannulas were implanted at least four weeks before the onset of acid secretory studies. At the start of experiments, the rats had a body weight of 240–260 g. During the entire study period, animals were kept under normal laboratory conditions with free access to water and standard rat chow. Over the course of the experiments, which lasted four to six weeks, the rats remained well and showed an average weight gain of 40 g.

PD-136,450 belongs to a class of recently described α-methyl-(R)-tryptophan dipeptoid gastrin receptor antagonists. It has the chemical name [R- (R*, R*)]-4-[[2-[[3-(1H-Indol-3-yl)-2methyl-1-oxo-2-[[[tricyclo[3.3.1.1^{3,7}]] dec-2-oxycarbonyl] amino] propyl]amino]-1-phenylethyl] amino]-4-oxo-2-butanoate N-methyl-D-glucamine and was kindly supplied by Professor J Hughes, Parke-Davis Neuroscience Research Unit, Cambridge, UK. Synthesis, chemical properties, receptor binding affinities, and preliminary biological activities, have been

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described recently.¹⁻⁵ The specific gastrin (CCK-B) receptor antagonist L-365,260 and the specific CCK-A receptor antagonist L-364,718 were kindly provided by Dr R M Freidinger, Merck Sharp & Dohme Research Laboratories, West Point, Pennsylvania, USA.

Studies were performed in a total of 36 different animals with a 'wash out' period of at least two days between experiments. Animals were fasted for 20 hours before studies. To study antagonist activity, rats were pretreated with PD-136,450 (4.5 mg/kg subcutaneously) or vehicle, and 15 minutes, two, four, or eight hours later 32 µg/kg gastrin (SHG-17, Fluka, Buchs, Switzerland) were injected subcutaneously. Agonist activity was studied in a separate group of rats by subcutaneous injection of either PD-136,450 (0.01–18 mg/kg) or gastrin (0.03–32 µg/kg) at six different doses per compound. A third set of rats was used to determine whether the specific gastrin antagonist L-365,260 inhibited the (partial) agonist activity of PD-136,450 on acid secretion. Animals were pretreated with L-365,260 (4.5 mg/kg subcutaneously) or vehicle and five minutes later, 4.5 mg/kg PD-136,450 were also given subcutaneously.

During experiments, animals were restrained in Bollmann cages. The stomach was rinsed with 0.15 mol/l saline solution (38°C) and allowed to drain for 30 minutes before collecting samples of gastric juice in 30 minute intervals. The pH was determined with a combined glass calomel pH electrode, GK 2321 C, after calibration with buffers at pH 4 and 7. Acidity was measured by titration using a TTT2-titrator and autoburette (Radiometer, Copenhagen, Denmark). Samples were titrated with 0.1 M NaOH to an end point of pH 7.4.

Plasma gastrin was also monitored every 30 minutes. Blood (0.5 ml) was collected from the tail vein into plastic tubes containing 20 µl heparin solution (corresponding to about 50 IU). The blood was centrifuged and the plasma was stored at -70°C. Gastrin was measured, as previously described,¹⁵ using antigastrin antiserum (a gift of Dr S R Bloom, Hammersmith Hospital, London, UK) and synthetic human gastrin I as a standard. The antibody bound and free hormones were separated by dextran coated charcoal, counted separately, and the percentage binding was calculated. The intraassay coefficient of variation was below 10%. To avoid interassay variation, all samples were analysed in the same run.

ACID AND PANCREATIC SECRETION STUDIES IN ANAESTHETISED RATS

Female Wistar rats (200–260 g) were fasted overnight but given free access to water. Anaesthesia was induced and maintained by intramuscular injection of urethane (1.5 g/kg). Body temperature was maintained at 36–37°C by a rectal thermistor and thermostatically controlled heated table. The trachea and an external jugular vein were cannulated.

For acid secretion studies, a flexible orogastric tube was introduced into the proximal portion of the stomach. A multi-orifice polyethylene tube

was then inserted into the antrum through an incision in the duodenum and was brought to the exterior through the flank. The stomach was perfused with saline via the orogastric tube at 7 ml/min and effluent was collected over 10 minute intervals from the pyloric outlet. Samples were titrated with 0.1 M NaOH to an end point of pH 7.4. Basal acid output was recorded for 30 minutes, then secretion was stimulated by intravenous injection of 1 µg/kg SHG-17. This control response was followed one hour later by subcutaneous injection of either vehicle or PD-136,450 (0.1–1.0 mg/kg). After a further 10 minutes, a second identical dose of gastrin was given and acid output was monitored for a further 60 minutes. Since there was no tachyphylaxis in the response to gastrin administered according to this protocol, the reduction of acid secretion by PD-136,450 was calculated as % of the initial response to gastrin.

For pancreatic secretion studies, pancreatic juice was collected from a fine polyethylene tube inserted into the main pancreatic duct and the volume output was measured gravimetrically. Basal pancreatic output was recorded for 30 minutes, then secretion was stimulated by intravenous injection of 0.5 µg/kg CCK-8. This control response was followed one hour later by a subcutaneous injection of 4.5 mg/kg PD-136,450. In a second set of experiments, we

Figure 1: Antagonist effect of PD-136,450 on gastrin stimulated acid secretion in conscious (A) and anaesthetised (B) rats. Gastrin stimulated acid secretion was reduced by 60 (5)% (mean (SEM)) in conscious rats and by 77 (3)% in anaesthetised animals (PD-136,450 v vehicle; $p < 0.001$). In the experiments shown, 4.5 mg/kg PD-136,450 and 32 µg/kg gastrin were injected subcutaneously in conscious rats while 1 mg/kg PD-136,450 and 1 µg/kg gastrin were injected intravenously in anaesthetised rats.

Figure 2: The upper panel (A) shows dose-responses for the antagonist effect of PD-136,450 on gastrin induced acid secretion in conscious and anaesthetised rats. IC_{50} values were $0.28 \mu\text{mol}$ (0.23 mg/kg) and $0.17 \mu\text{mol}$ (0.14 mg/kg) in conscious and anaesthetised rats respectively. (B) shows dose-responses for the agonist effect of PD-136,450 on acid secretion in comparison with gastrin in conscious gastric fistula rats. PD-136,450 increased acid output to 30 (5)% of the maximal gastrin induced acid output (PD-136,450 v vehicle: $p < 0.0001$). The ED_{50} of PD-136,450 was about $0.25 \mu\text{mol}$ (0.2 mg/kg) and the response was maximal at $5.6 \mu\text{mol}$ (4.5 mg/kg).

determined whether the specific CCK-A or CCK-B antagonists could block stimulation of pancreatic secretion by PD-136,450. Rats were pretreated with vehicle, L-365,260, or L-364,718 (both at 4.5 mg/kg subcutaneously) and five minutes later, 4.5 mg/kg PD-136,450 was injected subcutaneously.

The significance of differences was determined by unpaired t test. Probability values of $p < 0.05$ were regarded as significant.

Results

PD-136,450 antagonised gastrin-induced acid secretion by 60 (5)% (mean (SEM), $n = 12$) at a dose of 4.5 mg/kg subcutaneously in conscious gastric fistula rats and by 77 (3)% at a dose of 1 mg/kg intravenously in anaesthetised rats by comparison with vehicle controls (Figs 1A and B). Dose-response studies of the antagonist effect of PD-136,450 in conscious and anaesthetised rats produced IC_{50} values of $0.28 \mu\text{mol}$

Figure 3: Duration of antagonist and agonist actions of PD-136,450 in the conscious rat. The effects decreased over time with a half life of four to six hours. In the antagonist study, rats were pretreated with 4.5 mg/kg subcutaneous PD-136,450 and challenged with $32 \mu\text{g/kg}$ subcutaneous gastrin 0, 2, 4, or 8 hours later. In the agonist study, the acid stimulatory effect of PD-136,450 was measured directly after subcutaneous injection of 4.5 mg/kg .

(0.23 mg/kg) and $0.17 \mu\text{mol}$ (0.14 mg/kg) respectively (Fig 2A). PD-136,450 displayed a partial agonist action in as much as acid output in basal secreting conscious rats increased to 30 (5)% of the maximal response to gastrin itself. Dose-response studies of the stimulation of acid secretion by PD-136,450 in conscious animals showed a maximal response at $5.6 \mu\text{mol}$ (4.5 mg/kg) (Fig 2B). Gastrin-17 displayed greater efficacy and was about 350 fold more potent on a molar basis. The agonist and antagonist activities decreased with time, although the duration of action of PD-136,450 was fairly lengthy after subcutaneous dosing (Fig 3). Pretreatment with PD-136,450 did not change the basal plasma gastrin value and did not modify the pharmacokinetics of injected gastrin (Fig 4). As shown in Figure 5, the stimulation of gastric acid secretion was completely inhibited by pretreatment with the gastrin (CCK-B) receptor antagonist L-365,260.

PD-136,450 (4.5 mg/kg subcutaneously) caused a sustained increase in pancreatic volume

Figure 4: Effect of PD-136,450 on plasma gastrin values. Subcutaneous injection of 4.5 mg/kg PD-136,450 did not change basal gastrin concentrations (PD-136,450 first, vehicle 15 minutes later). Plasma values after subcutaneous injection of $32 \mu\text{g/kg}$ gastrin were unchanged after pretreatment with PD-136,450 compared with vehicle.

Figure 5: In basal secreting animals, the gastrin receptor antagonist L-365,260 inhibited the (partial) agonist activity of PD-136,450 on acid secretion (L-365,260 v vehicle: $p < 0.001$). Vehicle or 4.5 mg/kg L-365,260 were injected subcutaneously and five minutes later, 4.5 mg/kg PD-136,450 were also injected subcutaneously.

output from 5.4 (1.3) to 33.1 (4.6) $\mu\text{l}/10$ min, which was slightly higher than the maximal response to an intravenous injection of CCK-8 and was maintained for at least two hours (Fig 6A). The increase in pancreatic flow in response to PD-136,450 was not affected by pretreatment with L-365,260 but was strongly inhibited by the CCK-A antagonist L-364,718 (Fig 6B).

Discussion

PD-136,450 belongs to a new class of orally effective gastrin antagonists that act on central and peripheral CCK-B receptors.¹⁻⁵ In vitro, PD-136,450 displays a nanomolar affinity for the CCK-B receptor, with over 1000 fold selectivity

compared with CCK-A sites and a wide range of other receptors.² PD-136,450 was developed by minor modifications from the older CCK-B receptor antagonist PD-134,308 which is currently being evaluated clinically for its anxiolytic properties. Our studies in conscious and anaesthetised rats, confirm that this compound is an effective inhibitor of gastrin induced acid secretion. We found that PD-136,450 was some three times less potent than reported previously, which is probably a reflection of the differences in experimental design. Thus, previous studies^{2,5} used an intravenous infusion of pentagastrin and also administered PD-136,450 by intravenous infusion, whereas in the present experiments animals were pretreated with a bolus dose of PD-136,450 and gastrin-17 was administered 15 minutes later at submaximal bolus doses of 32 $\mu\text{g}/\text{kg}$ in conscious rats and 1 mg intravenously in anaesthetised rats. PD-136,450 showed a sustained duration of action with a half-life of about four hours, which is clearly longer than that described for L-365,260 which seems to be less than one hour.^{13,16}

Although the affinity of PD-136,450 for the CCK-B receptor in vitro is in the same range as for gastrin, it behaves as a partial agonist in vivo. However, the compound shows lower efficacy and considerably lower potency than gastrin as a stimulant of acid secretion in the rat. Agonist activity amounted to about 30% of the maximal gastrin response. This seems to be a direct effect since PD-136,450 did not cause the release of endogenous gastrin and did not alter the pharmacokinetics of injected gastrin. The maximal response to PD-136,450 occurred 30–60 minutes after administration, similar to the time at which peak behavioural effects were observed.³ Agonist and antagonist effects displayed similar sensitivity and duration of action, and there was no dose at which these two actions could be dissociated. L-365,260, which did not affect basal acid secretion, inhibited the agonist activity of PD-136,450 in the stomach. This further supports the suggestion that stimulation of acid secretion by PD-136,450 is mediated through direct interaction with gastrin receptors on parietal or ECL cells. PD-136,450 is therefore not an optimal antagonist for studying the role of gastrin. Nevertheless, in long term experiments, high doses of PD-136,450 almost completely abolished the gastrin induced increase in mucosal height, ECL cell density, and histamine concentration in the oxyntic mucosa.¹²

In binding studies, PD-136,450 was found to be highly selective for the CCK-B receptor with a K_d value some 1100 times lower than for CCK-A receptors.² In vivo, however, PD-136,450 displayed powerful stimulation of pancreatic secretion. This finding is consistent with the observation that PD-136,450 caused a noticeable increase of pancreatic weight after chronic administration at even higher doses of 18 mg/kg, three times daily.¹⁷ Moreover, the present experiments show very closely that PD-136,450 exerts an effect on the pancreas via CCK-A receptors as demonstrated by the fact that the response could be antagonised by L-364,718. This action on pancreatic CCK-A receptors seems to be direct in as much as PD-136,450 is

Figure 6: Agonist effect of PD-136,450 on pancreatic secretion in anaesthetised rats is shown in the upper panel (A). PD-136,450 increased pancreatic secretion from mean (SEM) 5.4 (1.3) to 33.1 (4.6) $\mu\text{l}/10$ min, which exceed the output after intravenous injection of 0.5 mg/kg cholecystokinin-8 (CCK-8). (B) The CCK-B receptor antagonist L-365,260 did not affect the response to PD-136,450. In contrast, the CCK-A receptor antagonist L-364,718 inhibited the agonist activity of PD-136,450 on pancreatic secretion (L-364,718 v vehicle: $p < 0.01$). Vehicle, 4.5 mg/kg L-365,260 or 4.5 mg/kg L-364,718 were injected subcutaneously and five minutes later 4.5 mg/kg PD-136,450 was also injected subcutaneously.

known to be effective in an isolated gland preparation and does not release CCK in vivo.¹⁷

In conclusion, PD-136,450 seems to be capable of exerting effects through both CCK-A and CCK-B receptors in vivo. It antagonises gastrin induced acid secretion, but exerts a partial agonist effect in the stomach. In the pancreas, PD-136,450 behaves as a powerful stimulant of fluid secretion via a CCK-A receptor mechanism. Studies of the binding, distribution, and metabolism of PD-136,450 in the rat in vitro and in vivo are necessary to clarify apparent discrepancies between the in vitro binding data and pharmacodynamic behaviour of the compound in vivo. Although PD-136,450 was designed for optimal activity at CCK-B receptors, further structural modification seems necessary in order to achieve selectivity of action in this series of compounds in vivo.

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