Suppression of vagus-mediated pancreatic polypeptide release by the μ-opiate receptor agonist loperamide in man*

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1 Morphine suppresses the release of pancreatic polypeptide, a hormone under vagal cholinergic control. The intention of the study was to detect whether the μ-opiate receptor agonist loperamide is also able to inhibit pancreatic polypeptide release, and to define its site of action.

2 In groups of healthy subjects ($n = 6$ each) stimulation of pancreatic polypeptide was assessed in five different tests: (i) insulin-hypoglycaemia; (ii) modified sham feeding; (iii) intravenous infusion of the cholecystokinin analogue ceruletide; (iv) injection of corticotropin releasing hormone; (v) infusion of the muscarinic acetylcholine agonist bethanechol. All tests were performed after oral application of either a placebo or loperamide (16 mg), tests (ii) and (iii) were repeated with loperamide in smaller doses (2 and 6 mg), with loperamide plus naloxone, with naloxone alone, and with infusion of atropine. Plasma concentrations of pancreatic polypeptide were measured radioimmunologically.

3 Release of pancreatic polypeptide in test (i) to (iv) was completely blocked by 16 mg loperamide, whereas bethanechol-stimulated release (test 5) was not influenced. Tests (ii) and (iii) showed that the inhibition was dose-dependent and could be attenuated by naloxone. The inhibitory effect of loperamide was comparable with that of atropine.

4 We conclude that loperamide causes a dose-dependent inhibition of pancreatic polypeptide release mediated by vagal-cholinergic pathways, but does not have an atropine-like peripheral action.

Keywords atropine bethanechol ceruletide cholinergic system hypoglycaemia modified sham feeding pancreatic polypeptide vagus

Introduction

The μ-opiate receptor agonist loperamide is a widely used drug for symptomatic treatment of patients with diarrhoea [1]. Its antidiarrhoeal effect is primarily due to an inhibition of intestinal secretion and gut motility [2, 3]. Loperamide also inhibits basal and pentagastrin-stimulated gastric acid secretion [4, 5], meal-stimulated pancreatic enzyme secretion [6], and cholecystokinin (CCK)-induced gall bladder emptying [7].

The mode of action, however, has not been clearly defined. As loperamide hardly crosses the blood–brain barrier, it presumably acts on peripheral μ-opiate receptors [2, 8]. On the other hand, the inhibiting effects described above are similar to those of anticholinergic drugs. An anticholinergic action has also been reported for morphine [9, 10], which was found to suppress insulin- and meal-induced release of pancreatic polypeptide (PP), a hormone under vagal cholinergic control [11].

The intention of this study was to examine the hypothesis, that μ-opiate receptor agonists (like morphine and loperamide) display an anticholinergic action. For this purpose, we investigated the effects of loperamide on PP release in man after vagal stimulation and after application of a peripheral acting cholinergic drug. We also examined the influence of loperamide on CCK-induced PP release, which is known to be blocked by

atropine [12]. Bearing in mind that corticotropin releasing hormone (CRH) can cause PP release in man [14] and CRH receptors are synthesized and transported in the rat vagus nerve [13], loperamide was also given before application of CRH. A preliminary report on the results was presented at the 27th Annual Meeting of the European Society for Clinical Investigation, Heidelberg, Germany, 1993, and published as an abstract [33].

Methods

Subjects and test procedures

All tests described below were performed with groups of six young healthy males (aged 25–27 years). The test protocol was approved by the Ethics Committee of the Medical Faculty of the University of Munich. Each subject gave written informed consent.

Given orally, loperamide is absorbed slowly and peak plasma concentrations are reached 3–4 h after intake [2]. Therefore, the drug was applied at least 3 h before stimulating PP release. The highest dose given (16 mg) corresponds to the recommended maximum daily dose for treatment of diarrhoea. Blood samples for radioimmunological determination of PP were drawn frequently from a peripheral vein in ice-chilled 10 ml syringes (containing 37.1 μmol K_2-EDTA). The samples were immediately centrifuged at 4° C and the plasma stored at −20° C.

Insulin-hypoglycaemia-test (IHT)

Three hours before starting an IHT, the subjects received either 16 mg loperamide (Imodium®, Janssen, Neuss, Germany) or placebo orally. The IHT was performed by intravenous injection of recombinant human insulin (0.15 iu kg^{-1} body weight, Actrapid HM®, Novo, Mainz, Germany). Hypoglycaemia was regarded as adequate, when blood glucose levels decreased to below 2.2 mM and typical clinical symptoms were noted.

Modified sham feeding (MSF)

MSF was performed 5 h after oral intake of 2 mg, 6 mg, and 16 mg loperamide, respectively, or after application of placebo. In each series, the subjects had to chew pieces of a roll with jam over a 15 min period without swallowing. In order to investigate, whether the effect of loperamide is due to its opiate property, in another six subjects receiving 16 mg loperamide or a placebo, respectively, 0.8 mg naloxone (Narcanti®, DuPont Pharma, Bad Homburg, Germany) was injected intravenously, 20 min in advance and immediately before beginning of MSF. In a further series, MSF was performed 5 h after intravenous injection of 2.5 μg kg^{-1} body weight atropine (Atropinsulfat®, Braun, Melsungen, Germany) followed by an intravenous infusion of 5 μg kg^{-1} h^{-1} until the end of the experiment. Heart rate, blood pressure, and clinical symptoms of cholinergic blockade were recorded.

Ceruletide infusion

The CCK analogue ceruletide (40 ng kg^{-1} body weight; Takus®, Farmitalia, Freiburg, Germany) was infused intravenously over a 5 min period. Three hours before ceruletide infusion, either 2 mg, 6 mg, and 16 mg loperamide or placebo were applied orally. Similar to the procedure described in MSF, in two additional series with 16 mg loperamide or placebo, 0.8 mg of the opiate antagonist naloxone was injected intravenously 20 min before and immediately before infusion of ceruletide. In a further series, 3 h before ceruletide infusion, atropine was applied as described above.

Corticotropin releasing hormone (CRH) injection

Synthetic human CRH (100 μg, Bissendorf Peptide, Wedemark, Germany) was injected intravenously 3 h after oral application of 16 mg loperamide or of placebo.

Bethanechol infusion

Three hours after oral administration of 16 mg loperamide or of placebo, bethanechol infusions (Urecholine®, Merck Sharpe & Dohme, Westpoint, PA, USA) were administered over 40 min periods in gradually increasing doses (12.5, 25, and 50 μg kg^{-1} h^{-1}). Heart rate, blood pressure and clinical symptoms of cholinergic stimulation were recorded.

Radioimmunoassay of pancreatic polypeptide

Plasma PP concentrations were measured with a radioimmunoassay described previously [15]. The anti-porcine PP antiserum (K5418, Novo, Copenhagen, Denmark) showed a cross-reactivity of >95 % with human PP and none with other gastroenteropancreatic peptides. The detection limit of the PP assay was 1.1±0.3 pM. The intra- and inter-assay coefficients of variation were 11.1% at 12.9 pM and 13.1% at 14.3 pM, respectively.

Statistics and calculations

Changes of parameters were analysed by comparing the last value before stimulus application with those after stimulation using the Friedmann two-way analysis of variance. Integrated peptide values were calculated as the areas under the concentration curves. The integrated incremental responses (△) were obtained by subtraction of the baseline levels. The integrated incremental responses of the different series were compared and analysed with the U-test of Wilcoxon, Mann, and Whitney. $P \leq 0.05$ was considered statistically significant. All values are given in mean±s.e. mean.

Results

In all tests, basal plasma PP levels were significantly reduced by 16 mg loperamide and by atropine, 3 h after

application. The values were also significantly lower than in the placebo series.

Insulin-hypoglycaemia-test

The IHT caused a marked and long-lasting augmentation of plasma PP concentrations from 3.9 ± 1.0 to 102.6 ± 14.7 pM. The increase was prevented by loperamide (peak concentration 10.9 ± 4.1 pM). Accordingly, IHT-induced incremental integrated plasma PP was significantly suppressed by loperamide (Figure 1).

Modified sham feeding

After placebo, MSF caused a prompt rise of plasma PP levels to a peak of 48.8 ± 7.2 pM at 15 min. After 2 and 6 mg loperamide, this increase was significantly reduced (peaks of 31.1 ± 10.9 and 13.5 ± 5.5 pM) and after 16 mg it was nearly nullified (5.5 ± 1.1 pM) (Figure 2a). The suppression achieved with 16 mg loperamide was significantly attenuated by naloxone (21.5 ± 10.2 pM). Naloxone, applied alone, did not significantly influence PP release (39.1 ± 5.0 pM; Figure 2b). Atropine suppressed MSF-induced increase of plasma PP concentrations to the same extent as 16 mg loperamide (4.2 ± 0.7; Figure 2b). Incremental integrated plasma PP was decreased by loperamide in a dose-dependent manner ($y = -0.064 \times + 0.937$, $r = -0.62$, $P = 0.001$). Again, naloxone partly, but significantly attenuated this effect. When given alone, naloxone had no significant influence on incremental integrated PP. Atropine reduced incremental integrated PP significantly and to a similar extent as 16 mg loperamide (Figure 3). Apart from a small increase of the heart rate during atropine infusion, no side effects were recorded in the MSF series.
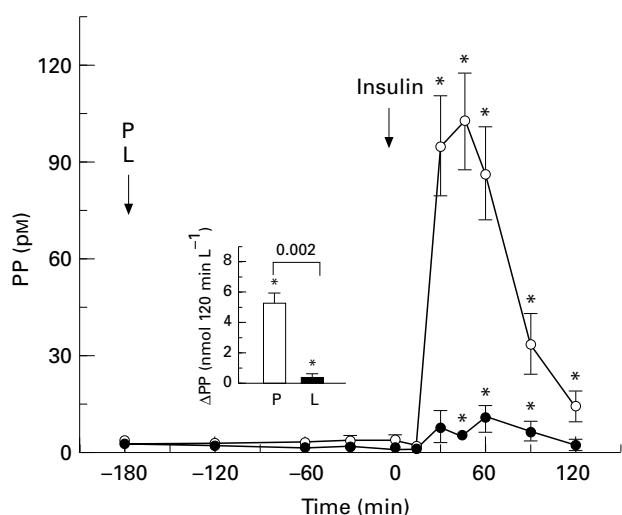

Figure 1 Stimulation of PP release by insulin-hypoglycaemia-test: Effect of orally applied placebo (P, ○) and of 16 mg loperamide (L, ●) on plasma PP increase and on incremental integrated plasma PP (small graph; $n = 6$). Asterisks indicate significant increases of plasma PP concentrations and of integrated PP compared with the respective prestimulatory values at 0 min.
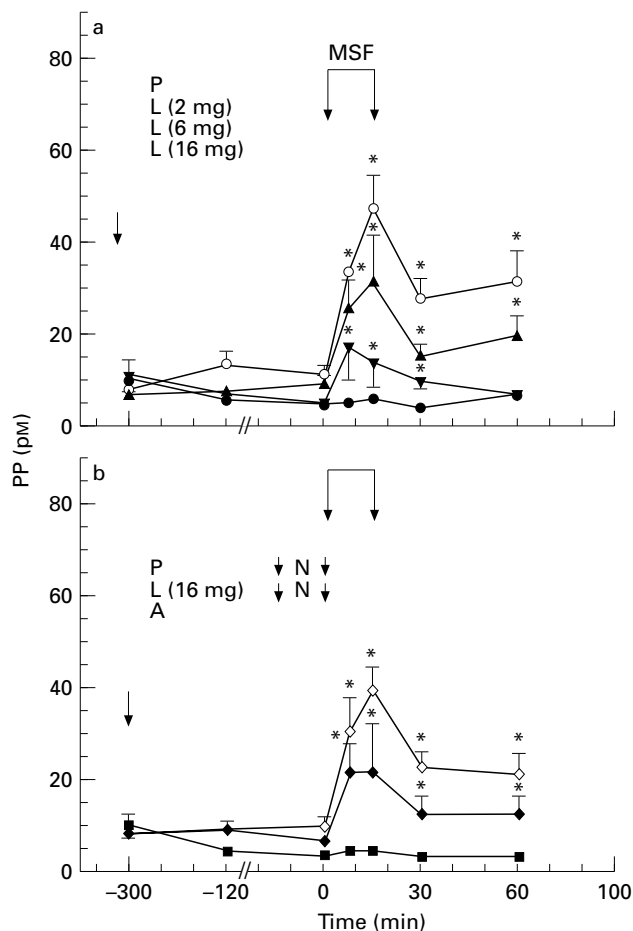

Figure 2 Stimulation of PP release by modified sham feeding (MSF): a) effect of orally applied placebo (P, ○) vs 2 (▲), 6 (▼), and 16 (●) mg loperamide (L) on plasma PP increase. b) effect of naloxone (N; 2×0.8 mg, injected intravenously), given with placebo (◇) and with 16 mg loperamide (◆), and of atropine (A (■); 2.5 μg kg^{-1} intravenous bolus injection, followed by an infusion of 5 μg kg^{-1} h^{-1}) on plasma PP increase ($n = 6$). Asterisks indicate significant increases of plasma PP concentrations compared with the respective prestimulatory values at 0 min.

Ceruletide

Ceruletide caused a steep rise of plasma PP levels to a peak of 103.2 ± 21.3 pM at 7.5 min in the placebo series. The effect was significantly and dose-dependently reduced by 2 and 6 mg loperamide and almost negated by 16 mg loperamide (59.1 ± 10.4, 33.3 ± 7.6, and 8.0 ± 1.3 pM, respectively). The blockade of PP release by 16 mg loperamide was partly but significantly reversed by naloxone (51.4 ± 13.5 pM). Naloxone alone, however, did not influence ceruletide-induced PP increase (112.9 ± 27.3 pM).

Integrated plasma PP was significantly increased by ceruletide in all series, except after 16 mg loperamide (Figure 4). Incremental integrated plasma PP was dose-dependently decreased by loperamide ($y = -0.079 \times + 1.15$, $r = -0.66$, $P = 0.0004$). Addition of naloxone partly but significantly counteracted the suppressive effect of 16 mg loperamide. When applied alone, naloxone did not influence incremental integrated plasma PP. Atropine significantly suppressed ceruletide-induced PP release and incremental integrated PP, as
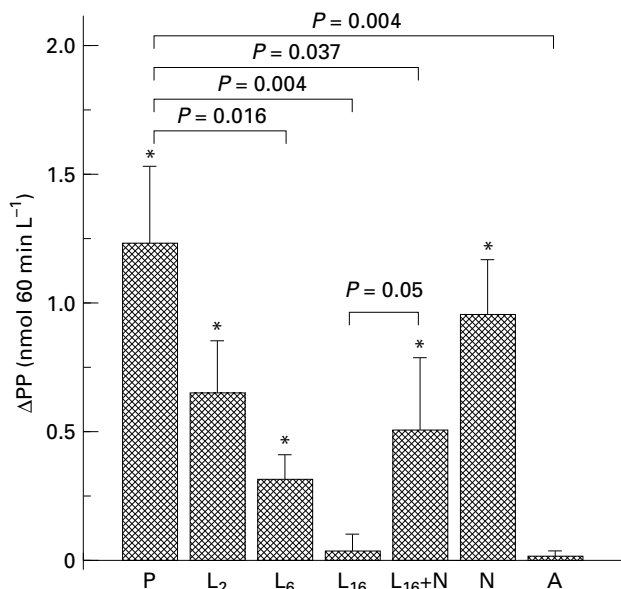
Figure 3 Stimulation of PP release by modified sham feeding: Effect of orally applied placebo (P), of 2, 6, and 16 mg loperamide (L), of naloxone (N; 2×0.8 mg, injected intravenously), with and without 16 mg loperamide, and of atropine (A; $2.5 \mu g \, kg^{-1}$ intravenous bolus injection followed by an infusion of $5 \mu g \, kg^{-1} \, h^{-1}$) on incremental integrated plasma PP ($n = 6$). Asterisks indicate significant increases of integrated PP compared with the respective prestimulatory values at 0 min.

Figure 4 Stimulation of PP release by ceruletide ($40 \, ng \, kg^{-1}$, infused intravenously in 5 min): Effect of orally applied placebo (P), of 2, 6, and 16 mg loperamide (L), of naloxone (N; 2×0.8 mg, injected intravenously), with and without 16 mg loperamide, and of atropine (A; $2.5 \mu g \, kg^{-1}$ intravenous bolus injection followed by an infusion of $5 \mu g \, kg^{-1} \, h^{-1}$) on incremental integrated plasma PP ($n = 6$). Asterisks indicate significant increases of integrated PP compared with the respective prestimulatory values at 0 min.
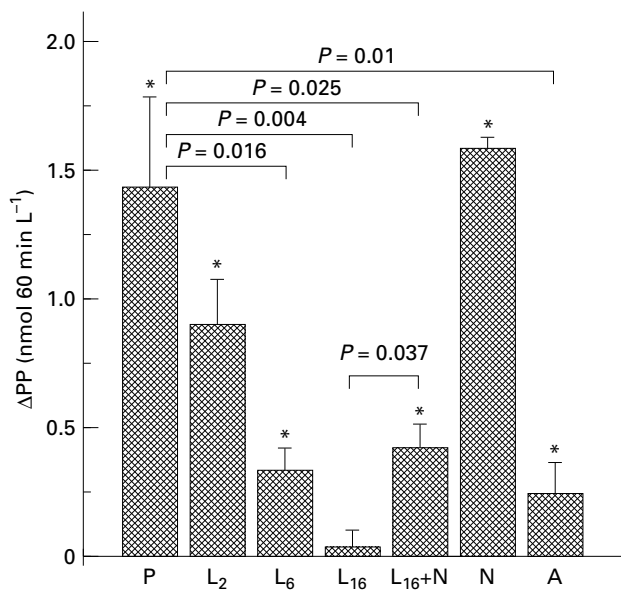
well. Its effects were comparable with those observed after 6 mg loperamide (Figure 4).

During ceruletide infusion half of the subjects complained of sweating and mild abdominal gripes. Apart from a small increase of heart rate during atropine

infusion, no further side effects were noted in the ceruletide series.

Corticotropin releasing hormone (CRH) injection

Intravenous injection of $100 \, \mu g$ CRH caused a prompt and significant rise of plasma PP concentrations to a peak value of 21.6 ± 7.4 pM. The increase was nearly nullified by 16 mg loperamide (3.4 ± 1.1 pM). Accordingly, incremental integrated plasma PP was also significantly suppressed (713 ± 344 *vs* 66 ± 25 pmol l^{-1} 120 min; $P = 0.01$).

Bethanechol infusion

As in the other series, basal plasma PP levels were significantly reduced by 16 mg loperamide (from 15.8 ± 3.3 pM to 6.3 ± 1.3 pM) and remained unchanged after placebo. Bethanechol significantly and dose-dependently enhanced PP release during placebo (from 15.0 ± 1.4 to a maximum of 36.3 ± 7.9 pM) as well as during loperamide (from 6.3 ± 1.3 to a maximum of 25.0 ± 5.9 pM). The absolute plasma concentrations of PP after loperamide remained significantly below those after placebo, but the difference in increases did not significantly differ (values at the end of the highest dose of bethanechol: 19.0 ± 7.8 *vs* 18.7 ± 5.0 pM). Accordingly, incremental integrated plasma PP values were not significantly different (Figure 5). Side effects (salivation, sweating, mild abdominal gripe, and need to pass water) occurred during the infusion of the high dose of bethanechol with and without loperamide to a comparable extent.
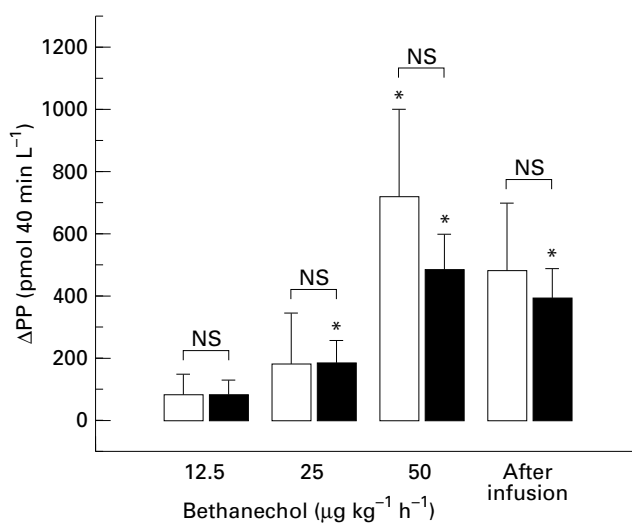

Figure 5 Stimulation of PP release by bethanechol (12.5, 25, and 50 $\mu g \, kg^{-1} \, h^{-1}$, each dose infused intravenously over 40 min): Effect of orally applied placebo (□) and of 16 mg loperamide (■) on incremental integrated plasma PP ($n = 6$). Asterisks indicate significant increases of integrated PP compared with the respective prestimulatory values at 0 min.

Discussion

PP is a hormone under vagal cholinergic control [11]. PP release induced by an IHT in man is suppressed by morphine [9]. We observed a similar suppressive effect for the μ-opiate receptor agonist loperamide. Apart from the suppressive effect on IHT-induced PP release, it was shown for the first time that loperamide also counteracts PP release induced by MSF, ceruletide, and CRH. In contrast, PP release stimulated by the muscarinergic agonist bethanechol was not influenced.

In adults, 60 mg loperamide is the threshold dose for producing side effects [16]. Accordingly, no side effects of loperamide were observed in our study (maximal dose applied was 16 mg).

The IHT is an established procedure to stimulate vagal cholinergic mechanisms [17]. The concomitant PP release [11] is independent of adrenergic mechanisms [18]. The IHT performed in this study caused a marked and long-lasting PP release, which was prevented by 16 mg loperamide. PP release stimulated by MSF was completely blocked by 16 mg loperamide. The effect was comparable with that of atropine. Moreover, the suppressive effect of loperamide on MSF-induced PP release was dose-dependent. As both IHT- and MSF-induced PP release is blunted by vagotomy [19], central vagal stimulation and efferent vagal fibres seem to be involved. Therefore, loperamide may exert its inhibitory action on efferent vagal pathways. It cannot be ruled out, however, that central vagal structures may also represent a target of loperamide, as traces of the drug can be detected in the brain [2]. A direct action of loperamide on the PP-producing F cells of the pancreas can be excluded.

PP release induced by physiological doses of CCK or CCK analogues, like ceruletide, is dependent on cholinergic pathways, since it can be blocked by atropine [12]. In our study, infusion of a pharmacological dose of ceruletide caused a significant rise of plasma PP concentrations—comparable with post-prandial peak concentrations [20]—and of integrated PP. This PP release was dose-dependently inhibited by loperamide. Atropine used in a dose sufficient to inhibit acetylcholine-mediated gastrointestinal functions [21] exerted a certain suppressive effect on ceruletide-induced PP release similar to 6 mg loperamide, but did not negate it completely. In a previous study [12] using the same dose of atropine, ceruletide-induced PP release was blunted. The dose of ceruletide applied was lower than in our study, as verified by peak plasma levels of CCK-like immunoreactivity (approximately 20 pM *vs* 233 ± 43 pM; personal observation). On the other hand, 16 mg loperamide completely blocked the PP response to the pharmacological dose of ceruletide applied in our study. In rats, expression and transport of CCK receptors in vagal fibres has been shown recently [22]. Furthermore, striatal acetylcholine release was stimulated by peripherally administered ceruletide via vagal afferent impulses [23], and brain stem neurons in the area postrema and in the nucleus of the tractus solitarius, primarily regions where sensory vagal afferent fibres terminate, might be activated by CCK [24]. Therefore, CCK- or ceruletide-stimulated PP release may be mediated by binding of these peptides to afferent sensory vagal fibres followed by central activation of efferent vagal cholinergic fibres.

In the cat μ-opiate receptors have been detected in presynaptical parts of vagal afferents terminating within a restricted region of the nucleus of the tractus solitarius [25]. Furthermore, μ-opiate receptors are also found on capsaicin-sensitive afferent vagal fibres [26] and on dorsal spinal root fibres [27] of the rat. Therefore, binding of loperamide to opiate receptors on afferent vagal fibres might block impulses elicited by CCK. CCK applied in a pharmacological dose to prelabelled rat pancreatic lobules caused a release of [³H]-acetylcholine [28] and most probably also caused PP release. PP, however, was not monitored in this study. Thus, an additional inhibitory action of loperamide on intrapancreatic cholinergic neurones cannot be ruled out.

CRH applied in a pharmacological dose has also been shown to cause PP release [14]. We confirmed this finding, but the peak plasma PP concentrations were distinctly lower than those obtained with ceruletide. We can only speculate on the mode of action of CRH. Vagal pathways may be involved as CRH receptors have been found to be present in the vagus nerve of the rat [13]. The suppression of CRH-induced PP release by loperamide shown in our study also points to the vagus nerve as a target of loperamide.

No data are available concerning the action of μ-opiate receptor agonists on the PP-producing F cells. Therefore, bethanechol, an acetylcholine analogue binding to muscarinic receptors only, was applied in order to stimulate PP release at the cellular level. Bethanechol caused a dose-dependent increase of plasma PP which was not influenced by loperamide. Therefore, this drug does not exert an atropine-like peripheral anticholinergic action.

We conclude from these results that loperamide exerts its inhibitory action on PP release via afferent and efferent vagal pathways. An additional action on central vagal structures or on intrapancreatic cholinergic neurons and on enteropancreatic cholinergic pathways cannot be ruled out. As basal PP release is dependent on a vagal cholinergic tone [11, 29], the suppressive effect of loperamide on basal PP levels shown in this study also indicates that vagal pathways may be involved.

The mechanisms of the inhibitory action of loperamide on PP release have not yet been investigated. In the present study, naloxone, a central and peripheral acting morphine antagonist attenuated loperamide-induced suppression of MSF- and ceruletide-stimulated PP release. The effect did not seem to be a matter of dosage. Using the same doses of loperamide (16 mg) and naloxone (1.6 mg) as in this study, we were able to show that the inhibitory action of loperamide on ceruletide-stimulated ACTH relase could be blunted by naloxone [30]. In a previous study [9], intravenous infusion of a three times higher dose of naloxone only partially reversed morphine-induced suppression of IHT-stimulated PP release. Naloxone applied alone did not modify basal and stimulated PP release in our study and in others [9, 10, 31]. Therefore, the opiate property of

loperamide is only partially responsible for the suppressive effect. This is in accordance with the results of a study on human isolated taenia coli muscles [32] showing that naloxone could only partially reverse the inhibitory effect of loperamide on acetylcholine release evoked by electrical field stimulation. The naloxone-insensitive portion may have been mediated via non-morphine-specific receptors capable of binding loperamide [2].

It has not yet been investigated whether endogenously released opiates are also able to exert an inhibitory action on the vagal cholinergic system under physiological or pathological conditions. However, in dogs bombesin-induced PP release was distinctly suppressed by the endorphine methionine-enkephalin [31].

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