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Effect of amylin in various experimental models of gastric ulcer

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Abstract

Subcutaneous administration of amylin (20–40 μg/kg) prevented, in a dose-dependent manner, reserpine- and serotonin-induced gastric damage, but the anti-ulcer effect was not present when lesions were induced by pylorus ligation. The protective effect of amylin was inhibited by pretreatment with capsaicin as well as CGRP-(8-37), a calcitonin gene-related peptide (CGRP) and amylin receptor antagonist, and was significantly reduced by domperidone, a dopamine D_2 receptor antagonist, or neostigmine, an inhibitor of acetylcholinesterase. Our data suggest that the gastroprotective activity of amylin in some experimental models of gastric ulcers involves capsaicin-sensitive fibers and CGRP receptors. Moreover, the peptide interferes, at least in part, with the dopaminergic and parasympathetic systems. © 1997 Elsevier Science B.V.

Keywords: Amylin; Ulcer; CGRP (calcitonin gene-related peptide), human; Capsaicin; Domperidone; Neostigmine

1. Introduction

Amylin is a 37-amino-acid peptide that is released from pancreatic β-cells in response to feeding, and has been proposed as an endocrine partner to insulin in the regulation of carbohydrate disposal and storage (Pittner et al., 1994). In addition to pancreas, amylin has been localized in rat stomach, intestine, lung, dorsal root ganglia and brain (Ferrier et al., 1989; Asai et al., 1990). Some evidence indicates that amylin, centrally or peripherally injected, reduces food intake in rats (Chance et al., 1991, 1993). Moreover, a possible function of amylin was suggested as a gastrointestinal peptide through interference with vagal activity and the dopaminergic system (Guidobono et al., 1994; Clementi et al., 1996). It shares 41–51% sequence identity with calcitonin gene-related peptide (CGRP), a neuropeptide that is widely distributed in the central and peripheral nervous system including hypothalamus and gastrointestinal tract (Mulderry et al., 1985). Both amylin and CGRP potently inhibit insulin-stimulated glycogen synthesis in rat skeletal muscle (Leighton and Cooper, 1988). Both have been reported to produce hypocalcemia, to be vasoactive, and to produce hypotension in vivo (Young et al., 1993). The effects of amylin and CGRP on the gastrointestinal function have been studied by several authors (Taché, 1992; Clementi et al., 1996). It has been shown, for example, that both peptides inhibit gastric secretion (Taché, 1992; Guidobono et al., 1994) and that CGRP can exert protective effects in various experimental models of gastric ulcer (Clementi et al., 1993). Our study was designed to verify the protective activity of single doses of rat amylin, given subcutaneously (s.c.), on pharmacologically or surgically induced ulcers as well as to elucidate possible mechanisms involved.

2. Materials and methods

2.1. General

Groups of 5 male Sprague–Dawley rats weighing 200–220 g were housed in individual cages under constant environmental conditions (22 ± 1°C; 65 ± 5% relative humidity; 12 h light/dark). The animals were fasted for 36 h before experiments with free access to tap water until 1 h before testing. Amylin was dissolved at a concentration of 1 ml/kg in normal saline and s.c. injected. Control animals received the same amount of vehicle.

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2.2. Drugs

Rat amylin and human-CGRP-(8-37) fragment were purchased from Peninsula Laboratories Europe (UK); reserpine, neostigmine methylsulfate and 5-hydroxytryptamine creatinine sulfate were purchased from Sigma Chemical (Italy), capsaicin and domperidone were purchased from Fluka Chemical (Italy) and Amersham (Italy), respectively.

2.3. Reserpine-induced gastric lesions

The method of Lau and Ogle (1981) was used. Reserpine was administered intraperitoneally (i.p.) at the dose of 25 mg/kg in 10 ml/kg of 0.5% acetic acid solution. Amylin was injected s.c. at different doses (20 to 180 μg/kg in 100 μl saline) immediately before reserpine administration. Four hours later the animals were killed by decapitation, the stomach was removed, opened along the greater curvature and examined under 3-fold magnification. The number and severity of lesions in the glandular mucosa were scored blind from 0 to 5 as follows:

0	no lesions
0.5	diffuse hyperemia
1	1 to 2 small ulcers
1.5	3 to 6 small ulcers
2	7 to 10 small ulcers
2.5	more than 10 small ulcers
3	1 marked ulcer plus 0 to 4 small ulcers
3.5	1 marked ulcer plus 5 or more small ulcers
4	2 marked ulcers plus 0 to 4 small ulcers
4.5	2 marked ulcers plus 5 or more small ulcers
5	3 or more marked ulcers

The anti-ulcer effect was expressed as percent protection vs. the control group, and the relative ED_{50} values were determined as the amylin concentrations that elicited a half-maximal response.

Separate experiments were carried out with amylin at the dose of 40 μg/kg s.c., after pretreatment with neostigmine (0.2 mg/kg, s.c. in 0.5 ml water) 30 min before reserpine, with capsaicin (125 mg/kg, s.c.in 0.5 ml saline containing 2% Tween 80) divided into 3 injections over 24 h and 7 days before, with domperidone (2.5 mg/kg p.o. suspended in 0.5 ml water), or with human-CGRP-(8-37) fragment (1 μg/kg i.p. in 200 μl saline). The controls received neostigmine, capsaicin, domperidone or human-CGRP-(8-37) fragment alone at the same time.

2.4. Serotonin-induced ulcers

The method described by Hashizume et al. (1978) was used to induce gastric damage. Amylin (20 to 180 μg/kg, s.c.) was given 1 h before serotonin creatinine sulfate (106 mg/kg s.c., equivalent to 50 mg serotonin free base) in 5 ml/kg of saline. The rats were killed by decapitation after 4 h and the stomach was examined for ulcers as above.

2.5. Gastric damage in pylorus-ligated rats

Pylorus-ligated rats modified from Shay et al. (1945), were carefully standardized to the following protocol: animals were fasted, with ad libitum access to water, for 36 h, starting at 10.00 h; the rats did not have access to bedding or feces to reduce the amount of solid residue in the stomach. Ligation of the pylorus was carried out through a small midline incision under light, short-duration ether anesthesia. Various doses of amylin (20 to 180 μg/kg) or saline were given s.c. at the time of pylorus ligation. After 3 h, the animals were killed by decapitation, the cardia was ligated, the stomach was removed and opened along the greater curvature over a glass funnel for collection of gastric juice in a graduated centrifuge tube, and volume was measured; the pH of gastric juice was determined with a glass electrode pH-meter. Mucosal lesions were scored as described above.

2.6. Statistical analysis

All results are expressed as means ± S.E. Ulcer scores were compared using Kruskal–Wallis analysis of variance on ranks followed by Dunn's test. Gastric secretion data were compared by analysis of variance followed by the Student–Newman–Keuls multiple comparisons test. Statistical significance was set at $P < 0.05$. Relative ED_{50} values and their 95% confidence limits were calculated according to Lichtfield and Wilcoxon (1949).

3. Results

Amylin s.c. injected reduced, in a dose-dependent manner, the reserpine-induced gastric ulceration. Gastroprotective activity was significantly present (31%) at the dose of 30 μg/kg, was maximal (59%) at a dose of 40 μg/kg, while higher doses produced smaller or no effects (Table 1).

This protective effect was inhibited after pretreatment with human-CGRP-(8-37) fragment (85%) or capsaicin (90%), and was significantly decreased by domperidone (66%) or neostigmine (73%) (Fig. 1).

In serotonin-induced gastric ulceration the peptide administered at a dose of 30 μg/kg significantly reduced (48%) mucosal lesions, and the maximal activity (65%) was present at a dose of 40 μg/kg (Table 1). The relative ED_{50} values were 28 (20–37) μg/kg for reserpine-induced ulcers, corresponding to 29.5% inhibition, and 25 (18–33) μg/kg for serotonin-induced ulcers, corresponding to 32.5% inhibition.

In contrast, though amylin reduced gastric secretion in

Table 1
Effects of amylin on gastric lesions induced by reserpine or serotonin in rats ($n = 5$)

Treatment	Dose (μg/kg)	Reserpine-induced lesions		Serotonin-induced lesions	
		ulcer index (mean ± S.E.)	inhibition (%)	ulcer index (mean ± S.E.)	inhibition (%)
Saline	—	3.90 ± 0.19	—	4.60 ± 0.24	—
Amylin	20	3.60 ± 0.29	9	3.80 ± 0.40	17
	25	3.10 ± 0.19	20	3.20 ± 0.25 [a]	30
	30	2.70 ± 0.12 [b]	31	2.40 ± 0.29 [b]	48
	35	1.90 ± 0.29 [b]	51	2.00 ± 0.16 [b]	56
	40	1.50 ± 0.16 [b]	59	1.60 ± 0.19 [b]	65
	45	2.40 ± 0.19 [b]	38	2.10 ± 0.29 [b]	54
	100	3.50 ± 0.27	9	4.15 ± 0.31	—
	180	3.95 ± 0.33	—	4.75 ± 0.28	—
ED50 (95% confidence limits)		28 (20.8–37.7) μg/kg		25 (18.8–33.2) μg/kg	

Amylin or saline were injected s.c. immediately before reserpine (25 mg/kg i.p.) or 1 h before serotonin (50 mg/kg s.c.). The ulcer index was evaluated 4 h after ulcerogenic agents.
[a] $P < 0.05$, [b] $P < 0.01$ vs. respective controls.

the 20 to 100 μg/kg dose range, with maximal activity at 40 μg/kg, inducing a substantial decrease in gastric juice volume (75%) and acidity (61%), it did not show any significant protective activity against pylorus ligation-induced gastric lesions; moreover, at the dose of 180 μg/kg the peptide was also ineffective to modify gastric acid secretion (Table 2).

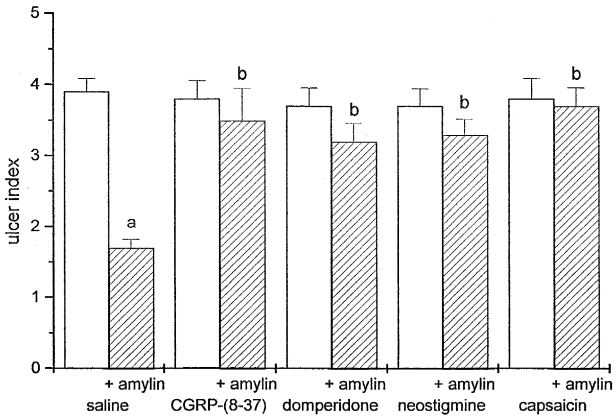

Fig. 1. Effect of amylin (40 μg/kg s.c.), alone or in association with CGRP-(8-37) fragment (1 μg/kg i.p.), domperidone (2.5 mg/kg p.o.), neostigmine (0.2 mg/kg s.c.) or capsaicin (125 mg/kg s.c.) on reserpine-induced gastric damage. (a) $P < 0.05$ vs. controls; (b) $P < 0.05$ vs. amylin.

Table 2
Effects of amylin at different doses on gastric juice volume, pH and ulcer formation in 3 h pylorus ligated rats ($n = 5$)

Treatment	Dose (μg/kg)	Volume (ml)	pH	Ulcer score (mean ± S.E.)
Saline		2.61 ± 0.20	1.36 ± 0.08	3.00 ± 0.20
Amylin	20	1.40 ± 0.14 [a]	2.02 ± 0.10 [a]	2.85 ± 0.15
	40	1.03 ± 0.11 [a]	2.39 ± 0.08 [a]	3.06 ± 0.22
	100	1.55 ± 0.17 [a]	2.01 ± 0.06 [a]	2.97 ± 0.18
	180	2.65 ± 0.24	1.29 ± 0.07	2.86 ± 0.28

[a] $P < 0.05$ vs. respective control.

4. Discussion

The results presented in this paper demonstrate that amylin exerts different effects on gastric ulcers produced in various experimental models in the rat. The peptide s.c. injected prevented in a dose-dependent manner (20 to 40 μg/kg), the reserpine- and serotonin-induced gastric mucosal damage. Higher doses (45, 100 or 180 μg/kg) showed little or no anti-ulcer activity. In contrast, the anti-ulcer activity was not present at any dose from 20 to 180 μg/kg when lesions were induced by pylorus ligation.

The peptide showed a bell-shaped dose–response relationship for inhibition of gastric lesions induced by reserpine and serotonin, as well as for reduction of gastric juice outflow and acidity after pylorus ligation. Such behavior was observed previously for amylin (Guidobono et al., 1994) as well as for other gastrointestinal peptides (Walsh, 1988), and might be explained as the effect of the interaction with two or more receptors involving opposite actions (Rovati and Nicosia, 1994) or by the existence of multiple-state cell signaling pathways (Pliska, 1994). A better characterization of amylin receptors is needed to allow better understanding of this effect.

The reasons for lack of amylin activity on ulcers induced by pylorus ligation is not known at present. However, we may speculate that it could depend on the different mechanisms involved in the induction of gastric damage. In fact, though it is believed that pylorus ligation induces the formation of gastric mucosal lesions through an increase of acid secretion, it is known that not all drugs which reduce acid output are protective in this respect, and that drugs which enhance acid secretion can also significantly reduce mucosal lesions (Improta and Broccardo, 1992). Amylin is known to reduce both volume and H^+ content of gastric secretion (Guidobono et al., 1994), as confirmed by our results, but the lack of protective effect on gastric lesions after pylorus ligation suggests that either

these lesions are not dependent on an increased acid output, or that amylin-induced reduction of gastric secretion is insufficient to prevent mucosal damage.

It has been suggested that the inhibitory effect of amylin on gastric secretion is dependent on a decrease of vagal activity (Guidobono et al., 1994). Moreover, we have shown that amylin, centrally or peripherally injected, decreases intestinal transit, suggesting that this activity can be dependent, at least in part, on a decrease in cholinergic activity (Clementi et al., 1996).

On the other hand, reserpine is known to induce gastric mucosal damage through various mechanisms. Sandor and Cuparencu (1977), among others, claimed that the main pathogenic factor in reserpine-induced ulceration was the depression of adrenergic activity with an increase of cholinergic tone. Kagoshima and Suguro (1982) suggested that reserpine-induced gastric lesions depend on the hypermotility of the stomach rather than on hypersecretion, and that atropine or vagotomy was able to completely inhibit reserpine-induced hypermotility as well as to reduce gastric erosions. Considering this evidence, we believe it possible that the prevention of gastric mucosal damage by amylin can be due, at least in part, to interference on vagal fibers. Our hypothesis is supported by the evidence that pretreatment with peripheral administration of neostigmine, a drug that enhances the acetylcholine content at the cholinergic receptor level, partially reduced (73%) the gastroprotective effect of amylin. The fact that neostigmine only partially prevents the gastroprotective activity of amylin suggests that the effect of the peptide involves other paths besides the parasympathetic fibers.

It has been suggested that amylin, centrally or peripherally injected, increases dopaminergic transmission (Chance et al., 1991, 1993), and since it has been suggested that dopamine reduces experimental gastric lesions (Hernandez et al., 1987), it is possible that the gastroprotective effect of the peptide can be partly dependent on interference with the dopaminergic system. This hypothesis is supported by the evidence that administration of domperidone, a dopamine D_2 receptor antagonist, decreased the gastroprotective activity of amylin by about 66%.

On the other hand, it is known that the effects of amylin might reflect cross-reaction with CGRP receptors rather than actions transmitted through amylin receptors (Zhu et al., 1991). Our results suggest an involvement of CGRP receptors, in fact the gastroprotective effect of amylin is inhibited by the human-CGRP-(8-37) fragment, a CGRP receptor antagonist.

It has been shown that capsaicin-sensitive afferent neurons are involved in the mechanism of gastric mucosal defensive ability against acid or noxious stimuli. In the gut, CGRP is predominantly present in these neurons, and many gastrointestinal activities of CGRP involve capsaicin-sensitive neurons. Our results suggest that the gastroprotective effect of amylin involves the capsaicin-sensitive afferent neurons. In fact, the gastroprotective effect of amylin is prevented by pretreatment with capsaicin, that administered in high doses induces selective degeneration of non-adrenergic and non-cholinergic neurons innervating, among others, the gastrointestinal tract (Sternini et al., 1987).

In conclusion, our study has shown that amylin, peripherally injected, exerts a gastroprotective effect in some models of ulcer. This activity involves the same receptors of CGRP, and it appears to be mediated by capsaicin-sensitive fibers; moreover, amylin activity is mediated, at least in part, by the dopaminergic and parasympathetic systems. However, we cannot exclude the possible involvement of specific amylin receptors.

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