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Amylin given by central or peripheral routes decreases gastric emptying and intestinal transit in the rat

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Abstract. The effect of rat amylin on gastric emptying and intestinal transit in the rat was examined. Amylin administered intracerebroventricularly (1, 2, 2.5 or 4 μg/rat) produced the maximal decrease in gastric emptying and intestinal transit at the dose of 2.5 μg/rat. Higher doses produced a lower effect. Peripheral administration (25, 50 or 100 μg/kg) produced dose-dependent effects. Pre-treatment with neostigmine blocked the effect of amylin when it was centrally injected, while the effect of amylin given peripherally was partially reduced. Pre-treatment with domperidone decreased the inhibitory effect of peripherally injected amylin, but no effect was observed when the peptide was centrally injected.

Key words. Amylin; gastric emptying; intestinal transit; neostigmine; domperidone.

Amylin (AMY) is a 37-amino-acid protein, with 50% homology with the calcitonin gene-related peptide. It is secreted by pancreatic β-cells, in response to secretagogues that evoke insulin secretion, including arginine and glucose[1]. Although AMY is co-released with insulin, it inhibits insulin release[2] and appears to oppose the metabolic effect of insulin[3]. It has been shown that AMY is not only located in the pancreas but also in the rat's stomach, intestine, lung, dorsal root ganglia and brain[4,5]. It has been suggested that this peptide, centrally or peripherally injected, is able to cause anorectic effects[6,7] and decrease gastric acid secretion[8]. These results indicated that this peptide can exert a biological function at the gastrointestinal level[8]. The purpose of the present study is to investigate the possible influence of different doses of AMY, centrally or peripherally injected, on gastric emptying and intestinal transit in the rat.

Materials and methods

Groups of five male Sprague Dawley rats weighing 190–200 g were used for all the experiments. The animals were kept under constant environmental conditions (22 ± 2 °C, 65 ± 5% relative humidity, 12-h light/dark cycle). They were starved for 24 h before use, having access only to tap water. Seven days before the experimental sessions some groups of animals were implanted with permanent metallic cannulae in their lateral ventricle (intraventricular foramen, König and Klippel, A6360). The operation was performed according to the method described by Brakkee et al.[9], under anesthesia obtained by injection of 0.6 mg/kg Hypnorm (Duphar, the Netherlands).

Gastric emptying test. Animals were injected intracerebroventricularly (i.c.v.) or subcutaneously (s.c.) with AMY 1, 2, 2.5 or 4 μg/rat and 25, 50, 100 μg/kg, respectively. The peptide was given either immediately before or 5 min before the standard meal. For the assessment of gastric emptying, the method of Croci et al.[10], with modifications, was used. Rats were starved 24 h before testing, and were placed in cages with a wire-mesh bottom, with water freely available. A 3-ml bolus of a standard meal at 25 °C was given by gavage. The meal contained 2 ml $BaSO_4$ (113% w/v, Prontobario Esofago, Bracco s.p.a., Italy) plus 1 ml 0.5% carboxymethylcellulose and 0.05% phenol red. The meals had a viscosity of about 1900 mPa s^{-1} at 25 °C. The rats were killed by cervical dislocation immediately (t = 0) or 20 min after the meal. The pylorus and the esophageal junction were clamped before excision of the stomach. The organs were minced and vigorously stirred for 15 min in 20 ml 0.1 N NaOH. After centrifugation (3000 rpm for 15 min), 1 ml of supernatant was diluted with 9 ml 0.1 N NaOH and the samples were read for absorbance at 560 nm. The absorbance of the samples was considered as inversely proportional to the extent of gastric emptying. Gastric emptying for each rat was calculated as 1 − (absorbance at t = 20 min/mean absorbance at t = 0); subsequently, results were expressed as percent inhibition of gastric emptying in treated vs control animals.

Intestinal transit test. A modification of the method described by Jausseu and Jagineau was used[11]. Animals were given 2 ml of an aqueous suspension of 10% charcoal and 5% gum acacia by stomach tube. After 60 min the rats were killed by cervical dislocation, followed by the opening of the abdominal cavity and the removal of

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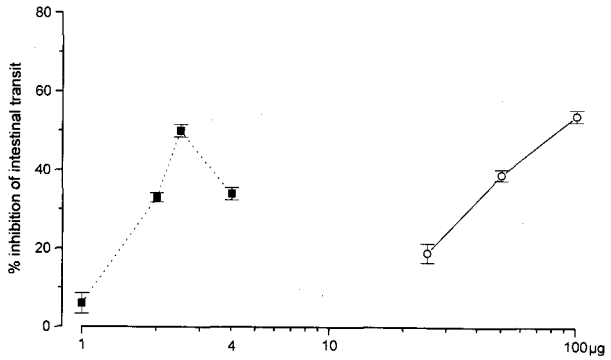

Figure 1. Dose-related inhibition of intestinal transit of a charcoal meal by amylin given i.c.v. (µg/rat, dotted line) or s.c. (µg/kg, solid line). Bars indicate ±SEM of five animals.

the small intestine. The total length of the intestine (pyloric sphincter to ileo-caecal junction) was measured. The distance the charcoal meal had travelled from the pylorus was measured, and this distance expressed as the percentage of the total length of the small intestine from gastropyloric junction to the ileocaecal junction (intestinal transit). In order to study the effect of AMY on intestinal transit, the peptide was injected i.c.v. in doses of 1, 2, 2.5 or 4 µg/rat, or s.c. in doses of 25, 50 or 100 µg/kg, immediately before or 5 min before the charcoal meal. Control animals received saline injections, i.c.v. or s.c., immediately or 5 min before the charcoal meal.

In other experiments, groups of animals (n = 5) received neostigmine injected s.c. at a dose of 0.1 mg/kg, 30 min before the charcoal meal, plus AMY 2.5 µg/rat (i.c.v.) or 100 µg/kg (s.c.) at the times described above. Other groups of animals (n = 5) received domperidone, injected s.c. at a dose of 5 mg/kg, 1 h before i.c.v. or s.c. AMY. Controls received neostigmine or domperidone 30 or 60 min before the charcoal meal and saline.

Statistical analysis. Results were expressed as means ±S.E. and compared using one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls test, with significance set at $p < 0.05$.

Results

AMY i.c.v. injected at the dose of 2.0 µg/rat significantly decreased (33%) intestinal transit. Maximal activity (50%) was observed at a dose of 2.5 µg/rat, while higher doses produced smaller effects (fig. 1). The peptide injected s.c. produced a dose-dependent decrease of intestinal transit time (fig. 1). Pre-treatment with neostigmine methylsulphate completely blocked the effect of AMY injected i.c.v. (fig. 2), while when the peptide was peripherally injected the effect on intestinal transit was blocked only in part (fig. 2). Pre-treatment with domperidone partially prevented the inhibitory effect of AMY injected s.c., without showing any effect on i.c.v. AMY (fig. 2).

The effects of the peptide on gastric emptying were similar to those exerted on intestinal transit. I.c.v. administration produced a bell-shaped dose-response curve, with significant inhibition (62%) at the dose of 2 µg/kg, and maximal inhibition (76%) at 2.5 µg/rat, while higher doses produced a smaller effect (fig. 3). Subcutaneous administration gave rise to dose-dependent inhibition (fig. 3).

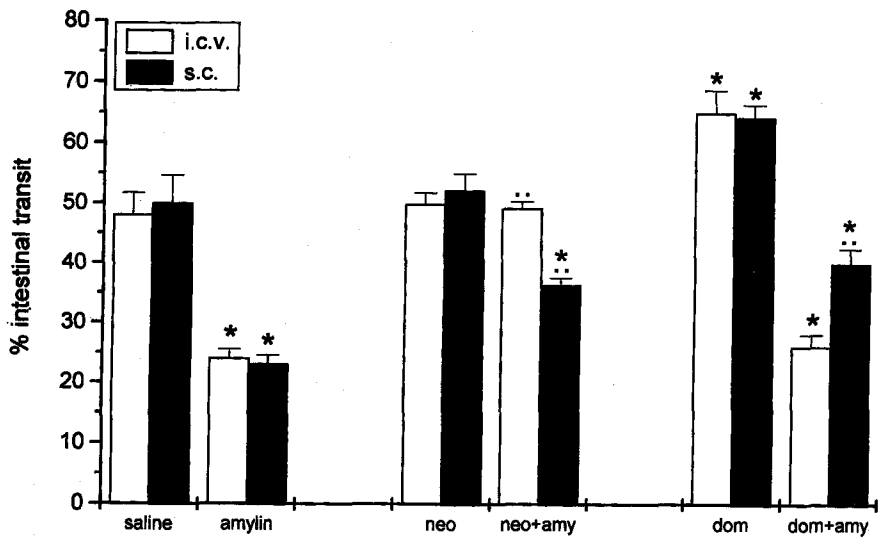

Figure 2. Effect of amylin (2.5 µg/rat i.c.v. or 100 µg/kg s.c.), alone or in association with neostigmine (neo) 0.1 mg/kg or domperidone (dom) 5 mg/kg, on intestinal transit of a charcoal meal in the rat. Bars indicate ±SEM of five animals. *$p < 0.05$ vs saline, **$p < 0.05$ vs. AMY.

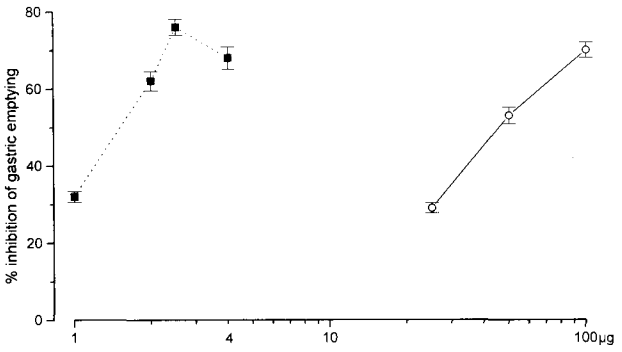

Figure 3. Dose-related inhibition of gastric emptying by amylin i.c.v. (µg/rat, dotted line) or s.c. (µg/kg, solid line). Bars indicate ±SEM of five animals.

Discussion

The presence of mRNA for AMY has been detected in the stomach[5], where the peptide itself has also been demonstrated, with the highest concentration in the pyloric antrum. Moreover, in the gastrointestinal tract, AMY-containing cells are located in the basal layers of the mucosa, with a distribution which is similar to those of gastrin and somatostatin[12]. These facts, and other evidence such as the observation of an anorectic effect[6,7] and an inhibitory effect on acid gastric secretion[8] have prompted some authors to suggest that this peptide plays a role in gastrointestinal function[8,12]. Our results are in agreement with this hypothesis. In fact, the peptide, injected i.c.v. or s.c., is able to decrease gastric emptying and intestinal transit. AMY given i.c.v. produced an inhibition of gastric emptying and intestinal transit that was nearly equal to that produced by the most active s.c. dose, which was approximately 8 times larger. The effect is dose-dependent with the peripheral administration, but when the peptide is injected i.c.v. the maximal decrement is present at a dose of 2.5 µg/rat, and higher doses produce lower effects.

Since gastrointestinal activity is well documented to be principally coordinated by the parasympathetic system, we have evaluated the inhibitory effect of AMY after pre-treatment with peripheral administration of neostigmine, a drug that enhances the effect of cholinergic nerves. Neostigmine, at the dose used, is slow to penetrate the brain[13], thus the actions of this agent were probably confined mainly to peripheral structures. Our results show that neostigmine abolished the inhibitory effect on the intestinal transit of AMY injected i.c.v., whereas when the peptide was injected s.c. the inhibitory effect was reduced to about 50%. It is known that AMY, centrally or peripherally injected, increases dopaminergic transmission[6,7], and since dopamine decreases the gastrointestinal transit in the rat[14], it is possible that this inhibitory effect of the peptide is dependent, at least in part, on interference with the dopaminergic pathway. This hypothesis is supported by the evidence that administration of domperidone, a D2 receptor antagonist, decreased the inhibitory activity of AMY on intestinal transit to about 38% when it was injected s.c., whereas when AMY was centrally injected domperidone did not interfere with the inhibitory effect of the peptide.

These results suggest that AMY injected i.c.v. decreases gastrointestinal activity through a central mechanism that prinicipally involves the parasympathetic nerve fibres. This hypothesis is in agreement with the data of Guidobono et al. that suggested that the inhibitory effect of AMY, injected i.c.v., could be dependent on the decrease of vagal activity[8]. The fact that neostigmine only partially prevents the inhibitory effect of the peptide when it is injected s.c. suggested that in this case the inhibitory effect of the peptide involves other mechanisms besides the parasympathetic fibres. Our data suggest that dopaminergic transmission can exert a role in the inhibitory effect of AMY when it is peripherally injected.

In conclusion, our study has shown that AMY, injected i.c.v. or s.c., inhibits gastric emptying and intestinal transit in the rat. The effectiveness of the peptide when injected i.c.v., at the low doses utilized, supports the hypothesis suggested by some authors[8] that AMY may play a role as a neuromodulator.

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