# Effect of Amylin on Mast Cell Secretion as a Possible Mechanism Increasing Gastric Mucosa Resistance

**B. A. Umarova, E. A. Smirnova, G. V. Luk'yantseva, G. N. Kopylova, G. E. Samonina, S. V. German, and S. E. Zhuikova**

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 132, No. 10, pp. 372-374, October, 2001
Original article submitted July 2, 2001

Water-immersion restraint stress increased secretory activity of mast cells and led to the formation of erosive lesions in the gastric mucosa. Intraperitoneal administration of amylin in a dose of 0.5 μg/kg 1 h before stress suppressed degranulation of mast cells and decreased the severity of gastric mucosa damages. In *in vitro* experiments amylin abolished the activating effects of acetylcholine and bradykinin on mast cell degranulation. Amylin-induced stabilization of activated mast cells probably underlies its protective effects during ulceration.

**Key Words:** *amylin; mast cells; gastric ulcer; stress*

Amylin is a peptide hormone belonging to the calcitonin gene-related peptide family. It is synthesized and secreted by pancreatic β-cells and gastrointestinal endocrine cells. Amylin is a pancreatic islet hormone, which maintains (together with insulin and glucagon) glucose homeostasis, plays a role in calcium metabolism, and inhibits motor activity of the stomach [1]. Recent experiments on various models of ulcer formation revealed pronounced antiulcer activity of amylin [2,3,7].

Stress is a cause of gastric ulcer formation. Previous studies showed that stress factors markedly increase secretory activity of mast cells (MC) [5,10]. Activation of MC and release of the proinflammatory agent histamine disturb homeostasis, cause damages to the gastric mucosa (GM) and, therefore, play an important role in ulcer formation [6]. We hypothesized that the antiulcer effect of amylin is realized via suppression of secretory activity in MC. The effect of amylin on MC has not been studied yet. Here we studied the effects of amylin on secretory activity of rat MC.

Department of Human and Animal Physiology, Biological Faculty, M. V. Lomonosov Moscow State University. ***Address for correspondence:*** samonina@pisem.net. Samonina G. E.

## MATERIALS AND METHODS

Experiments were performed on male outbred albino and Wistar rats weighing 190-250 g. We used rat amylin (Bachem California), bradykinin, and acetylcholine. In *in vivo* experiments water-immersion restrain stress was used to induce ulcers. Twenty-four hours before the experiment Wistar rats were deprived of food, but had free access to water. The animals were immobilized in special tubes and immersed in water (16°C) in a vertical position for 3 h. Amylin in a dose of 0.5 μg/kg was injected intraperitoneally 1 h before stress. Control rats received physiological saline. After stress the animals were decapitated. The area of GM damages was estimated using an ocular micrometer.

Film preparations of the mesentery and subcutaneous fat were fixed in 0.1% formalin and stained with 0.1% toluidine blue. Secretory activity of MC was evaluated by the degranulation index (DI). DI was calculated as the ratio of degranulated to total cell count. The degree of degranulation was taken into account (minor, moderate, and pronounced). We analyzed 200 MC in each preparation [4].

We also studied *in vitro* effect of amylin on MC degranulation. Samples of the mesentery and subcutaneous fat were taken from outbred albino control rats

0007-4888/01/1324-0929$25.00 

after decapitation. Tissue samples were consecutively incubated in physiological saline (10 min) and $10^{-6}$ M acetylcholine or $3.2\times10^{-5}$ M bradykinin (10 min). Some samples were preincubated with $2\times10^{-9}$ M amylin. Degranulation of MC was evaluated using film preparations.

The results were analyzed by Student's *t* test.

## RESULTS

Stress increased secretory activity of MC in the mesentery and subcutaneous fat, which manifested in a 2-fold increase in DI ($p<0.05$, Fig. 1). The number of MC characterized by moderate and pronounced degranulation sharply increased. These changes were accompanied by the formation of numerous hemorrhagic erosions, which is typical of this stress model [8]. Intraperitoneal administration of amylin prevented the increase in secretory activity of MC with moderate and pronounced degranulation. In amylin-treated animals DI and distribution of MC by the degree of degranulation did not differ from the control (Fig. 1). Amylin decreased the area of GM erosions from 1.9 to 0.9 mm$^2$ ($p<0.05$).

In control rats (not subjected to stress) amylin had no effect on the functional state of MC. DI and distribution of MC by the degree of degranulation remained practically unchanged (Fig. 1).

Thus, amylin prevented degranulation of MC during stress, but did not modulate spontaneous secretion. However, it remains unclear whether these changes result from a direct effect of amylin on MC or they are mediated by other regulatory systems. Amylin receptors was previously found in various organs and system, *e.g.*, in brain structures [7].

To evaluate whether amylin directly affects MC we studied its *in vitro* effects on cell activation by bradykinin or acetylcholine. Incubation with bradykinin or acetylcholine led to a sharp increase in DI due to increased number of cells characterized by moderate and pronounced degranulation. Preincubation with amylin abolished the stimulatory effect of MC activators: the test parameters did not differ from the control.

Our findings indicate that amylin *in vitro* inhibits MC stimulation induced by acetylcholine and bradykinin.

Incubation of samples with amylin alone did not change DI. *In vitro* and *in vivo* experiments showed that amylin had no effect on spontaneous MC degranulation.

Thus, amylin stabilizes MC and decreases their activity. This probably contributes to the amylin-induced inhibition of MC degranulation in response to stress. Suppressed production of proinflammatory mediators by MC is a possible mechanism underlying the protective effect of amylin during GM ulceration.

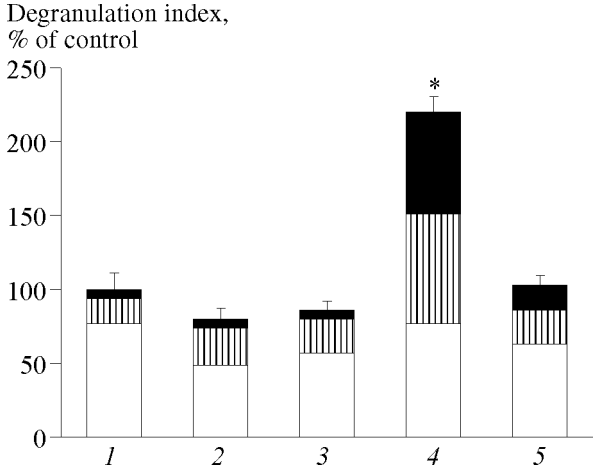


**Fig. 1.** *In vivo* effects of amylin on spontaneous and stress-induced secretory activity of mast cells: intact animals (*1*), physiological saline (*2*), 0.5 μg/kg amylin (*3*), stress (*4*), and stress+amylin (*5*). Here and in Fig. 2: minor (light bars), moderate (shaded bars), and pronounced degranulation (dark bars). *$p<0.05$ compared to the control.

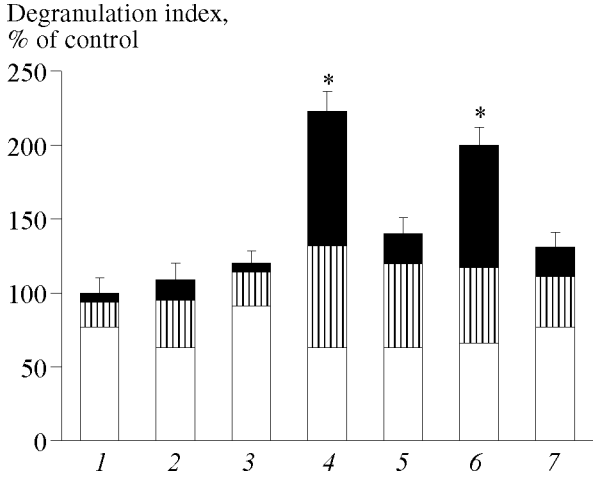


**Fig. 2.** *In vitro* effects of amylin on spontaneous and acetylcholine- or bradykinin-induced secretory activity of mast cells: intact animals (*1*), physiological saline (*2*), amylin (*3*), bradykinin (*4*), amylin+bradykinin (*5*), acetylcholine (*6*), and amylin+acetylcholine (*7*).

This work was supported by the Russian Foundation for Basic Research (grant No. 00-04-48086).

## REFERENCES

1. S. V. German, *Klin. Med*., **73**, No. 5, 7-11 (1995).
2. S. V. German, G. Dzh. Kuper, G. E. Samonina, *et al*., *Ros. Zh. Gastroenterol., Gepatol., Proktol.*, **7**, No. 5, 93-97 (1997).
3. S. V. German, G. Dzh. Kuper, G. N. Kopylova, *et al*., *Vestn. Ros. Akad. Med. Nauk*, No. 4, 10-12 (1998).
4. D. P. Lindner, I. A. Poberii, M. Ya. Rozkin, and V. S. Efimov, *Arkh. Pat*., **42**, No. 6, 60 (1980).
5. B. A. Umarova, F. B. Shapiro, and S. M. Strukova, *Vestn. Mosk. Gos. Univ. Ser. 16, Biol*., No. 3, 18-24 (1994).

6. M. Barszyk, W. Debek, and L. Chyczewski, *Rocz. Akad. Med. Bialymst*., **40**, No. 1, 36-57 (1995).
7. F. Guidobono, F. Pagani, C. Ticozzi, *et al*., *Br. J. Pharmacol*., **120**, No. 4, 581-586 (1997).
8. D. E. Hernandez and G. B. Glavin, *Neurobiology of Stress Ulcer*, New York (1990).
9. D. Lagunoff, T. W. Martin, and G. Read, *Ann. Rev. Pharmacol. Toxicol*., **23**, 331-351 (1983).
10. J. B. Overmier and R. Murison, *Behav. Brain Res*., **110**, 161-174 (2000).