Effect of Amylin on the Tone of Rat Aorta Ring Preparation

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Amylin (10^{-10} M) induced relaxation of norepinephrine-precontracted rat aortic rings by more than 50%. This effect was preserved after blockade of NO-synthase and even after denudation of the vessel. Thus amylin-induced vasodilation is an endothelium-independent process not mediated by NO.

Key Words: *amylin; rat aorta; vasodilation*

Homeostasis of the gastrointestinal mucosa is an important mechanism maintaining structural and functional integrity of the organism. It depends on complex of neural and hormonal factors acting at the local and central levels. The imbalance of this system provokes the development of peptic ulcer. In view of high prevalence of this disease and the absence of universal therapeutic strategy the search for novel antiulcer agents is an urgent problem of modern gastroenterology.

Considerable recent attention was focused on pancreatic islet peptide amylin, possessing pronounced antiulcer activity [1,8]. This peptide is synthesized and secreted by pancreatic β-cells together with insulin [7,11] and participates in carbohydrate metabolism together with insulin and glucagon. In physiological concentrations amylin improves blood supply to the skin [6], brain [5], and intestine [12]. Amylin also takes a part in some other physiological processes: it modulates calcium metabolism, decelerates gastric evacuation, and acts as a satiety factor [3,8,10]. However, the study of versatile activity of amylin in living organism is far from being completed.

The protective antiulcer effect of amylin was demonstrated on various ulceration models based on ulcerogenic effects of ethanol, indomethacin, stress, pylorus ligation, and long-term vagal stimulation [1,2, 8,9,13]. In all these models amylin considerably reduced damage to the gastric mucosa induced by these different factors. However, the nature of this effect remains unclear. Possible effects of amylin on the protective systems in the gastric mucosa, aggressive properties of the gastric juice, as well as on the dynamics of inflammatory process, and regenerative capacities of the epithelium are discussed.

Adequate blood supply to the gastric mucosa is extremely important for its homeostasis. However, published data on the effect of amylin on circulation and blood pressure are controversial. Some authors explain the vasomotor effects of amylin by its direct action on CNS structures. The possibility of central effect of amylin is corroborated by the presence of amylin receptors in cerebral structures and the ability of this peptide to cross the blood-brain barrier [4].

Our aim was to test the possibility of direct effect of amylin on the tone of arterial vessels.

MATERIALS AND METHODS

Experiments were performed on aortic rings obtained from 4-month-old outbred albino male rats weighing 200-220 g.

The left aortic arch was isolated, rinsed in physiological saline, and a 2-mm long ring segment with an internal diameter of 1 mm was prepared. Two liga-

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TABLE 1. Effect of Amylin and Ach on NE-Induced Tone (Dilation in Percentage of Initial Tone) of Isolated Rat Aortic Rings (*M*±*m*)

Agent	Normal	L-NAME	Denudation
Ach, 10^{-7} M (*n*=8)	24.8±2.2	0	0
Amylin, 3.4×10^{-10} M (*n*=8)	57.8±8.3*	68.3±6.5	48.0±8.1

Note. *$p<0.005$ compared to Ach.

tures were threaded through the ring to fix it to the chamber and isometric contraction transducer. The ring preparation was placed in an 8-ml chamber perfused with warm (37°C) aerated (5% CO_2+95%O_2) Krebs—Henseleit solution at a rate of 5 ml/min. The contractions were recorded using an Isometric Muscle Transducer-0363 (Harvard Apparatus) and a Hitachi-056 automatic recorder. An initial load of 1 g was applied to the aortic segment, the duration of relaxation under load was 40-60 min, the measurements were performed for 4 h. The endothelium was mechanically removed with a glass rod. Denudation was verified by the absence of a dilator response to acetylcholine (Ach). The following agents were used: norepinephrine (NE, 5×10^{-7} M, Serva), Ach (10^{-7} M, Serva), N-nitro-L-arginine (L-NAME, 5×10^{-7} M, Sigma), and rat amylin (3.4×10^{-10} M, Bachem California).

RESULTS

Perfusion with NE induced a constrictor response of the aortic rings, which persisted for more than 2 h. In

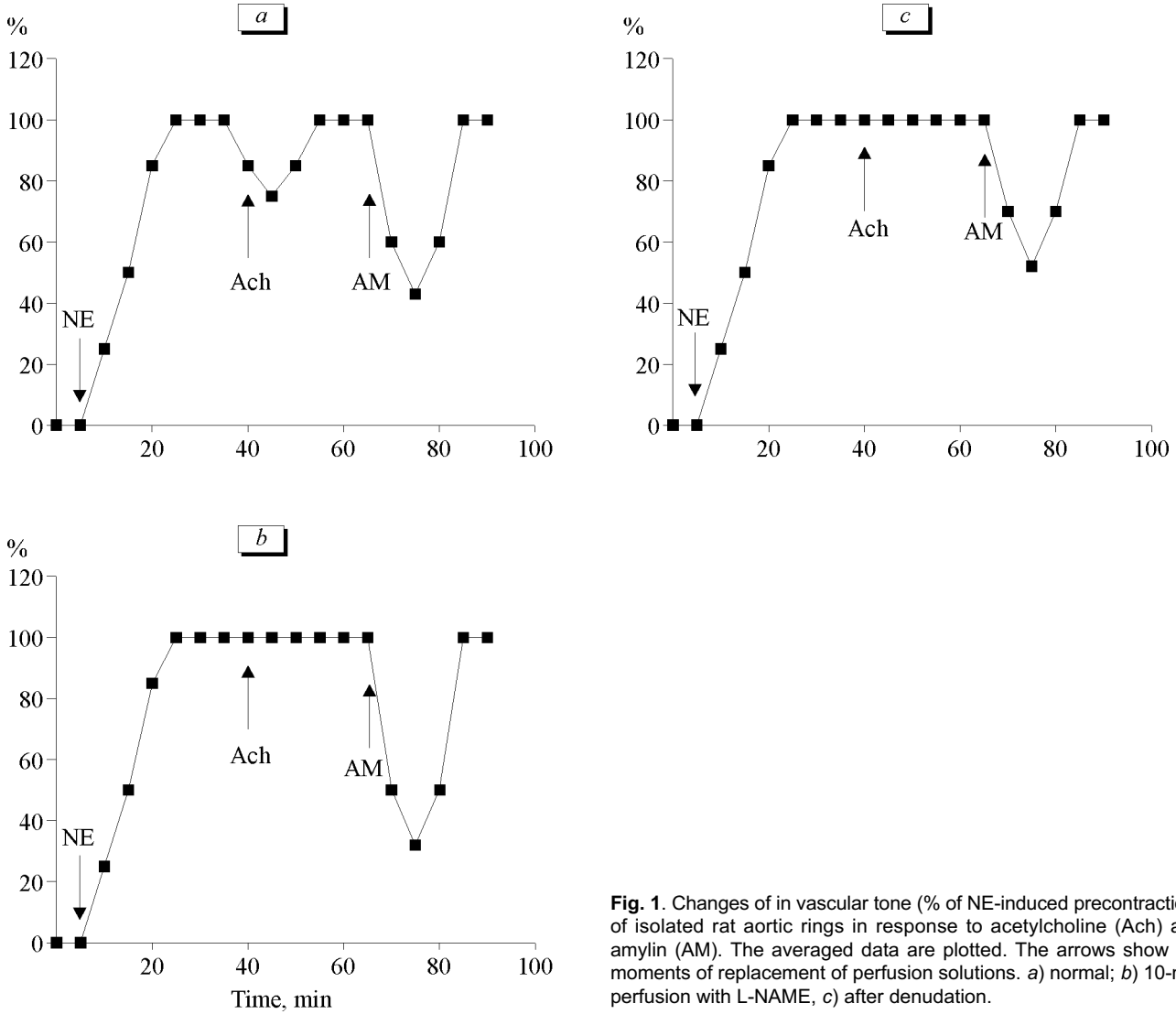

Fig. 1. Changes of in vascular tone (% of NE-induced precontraction) of isolated rat aortic rings in response to acetylcholine (Ach) and amylin (AM). The averaged data are plotted. The arrows show the moments of replacement of perfusion solutions. *a*) normal; *b*) 10-min perfusion with L-NAME, *c*) after denudation.

each experiment the dilator response to NE was taken as 100%. Dilatory response to Ach confirmed intactness of the endothelium and proper preparation of the aortic ring (Table 1). Amylin reduced the tone of the vascular ring, which attested to its potent dilatory activity (Fig. 1, *a*)

Taking into consideration the fact that the effects of many endogenous vasodilators are mediated via NO synthesis in the endothelial cells, we examined the effect of NO-synthase blocker L-NAME on amylin-induced dilator response. Perfusion with L-NAME for 10 min completely blocked the Ach-induced dilation, while the effect of amylin did not differ from the initial (Fig. 1, *b*; Table 1). Hence, the vasodilatory effect of amylin does not depend on NO synthesis. To exclude possible effects of other vasoactive substances released by the endothelium in response to amylin, a special experimental series was performed on denuded aortic rings. Denudation completely abolished the effect of Ach, but had no effect on the dilator response to amylin (Fig. 1, *c*; Table 1). This fact suggests that vasodilatory effect of amylin is an endothelium-independent process and is probably determined by a direct action of this peptide on smooth muscle cells in blood vessels.

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