Effects of Cholecystokinin-4 on Secretory Activity of Rat Mast Cells

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Intraperitoneal injection of cholecystokinin-4 in a dose of 100 mg/kg markedly increased secretory activity of mast cells in the mesentery and subcutaneous fat. These changes developed 5 min after treatment and progressed in time. Over the first 15 min we observed primarily merocrine secretion (granulolysis), while 60 min after cholecystokinin-4 administration apocrine secretion (degranulation) prevailed. *In vitro* cholecystokinin-4 had no effect on secretory activity of mast cells. Our findings suggest that stimulation of secretory activity of mast cells is determined by psychoemotional stress associated with activation of the sympathoadrenal and hypothalamic-pituitary-adrenal systems.

Key Words: *cholecystokinin-4; mast cells; psychoemotional stress*

Neuroendocrine peptide cholecystokinin (CCK) is now extensively studied. CCK is synthesized and secreted in various regions of the central nervous system and in the duodenal and jejunal mucosa. This peptide is involved in various physiological processes: stimulates gallbladder contractions and release of amylase from the pancreas, reduces food consumption and decelerates its evacuation from the stomach [4]. The CCK_{30-33} fragment (CCK-4) modulates food intake, cognitive behavior, and nociceptive response and enhances anxiety and panic reactions in humans [7,12] and animals [1,10]. Behavioral changes accompany autonomic and hormonal reactions typical of stress, including the increase in diastolic pressure and cardiac output and intensive secretion of corticotropin-releasing factor (CRF), ACTH, catecholamines, and prolactin [7,8,12,13]. Therefore, CCK-4 can be used for modeling of psychoemotional stress.

Previous studies showed that mast cells (MC) play a role in adaptive response to various stress factors [5,11]. Changes in secretory activity of MC during immobilization stress develop in time and are realized via the mechanism of general adaptation syndrome (activation of the sympathoadrenal and hypothalamic-pituitary-adrenal systems) [3].

It can be hypothesized that psychoemotional stress induced by CCK-4 is accompanied by changes in secretory activity of MC.

The effects of CCK and its fragments on MC are poorly studied. It was demonstrated that endogenous CCK stimulates secretory activity of mucosal MC in the duodenum [9]. The CCK-4 analogue tetragastrin intensifies secretion of hydrochloric acid and mucus in the stomach, which is associated with the release of histamine from peritoneal MC [6].

Here we studied functional state of MC in the connective tissue of the mesentery and subcutaneous fat in rats during psychoemotional stress induced by CCK-4.

MATERIALS AND METHODS

Experiments were performed on male outbred albino rats weighing 250-300 g. CCK-4 (ICN Biomedical Inc.) was injected intraperitoneally in doses of 10 and 100 mg/kg. The animals were decapitated 5, 15, and

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60 min after CCK-4 administration. Preparations of the mesentery and subcutaneous fat were fixed in 0.1% formalin for 30 min and stained with 0.15% toluidine blue.

Functional state of MC was estimated morphometrically under a light microscope [2]. We examined 200 cells in each preparation.

Secretory activity of MC was determined by the indexes of cell saturation with heparin, granulolysis, and degranulation (low, moderate, and intensive).

Specimens of the mesentery and subcutaneous fat were *in vitro* incubated with CCK-4 (3.2×10^{-12} M) at 37°C for 15 min. Control specimens were incubated in physiological saline. Film preparations were prepared and secretory activity of MC was determined.

The results were analyzed by ANOVA test.

RESULTS

Sixty minutes after injection of CCK-4 in doses of 10 and 100 mg/kg the indexes of saturation with heparin and granulolysis (merocrine secretion) did not differ from those in control animals (Fig. 1, *a*, *b*). The index of degranulation characterizing apocrine secretion of mediators (release of granules through the cell membrane) surpassed the control by 16% (Fig. 1, *c*). These changes were accompanied by an increase in the count of cells with moderate and intensive degranulation (Fig. 1, *d*).

Bearing in mind that epinephrine and ACTH are released immediately after administration of CCK-4 [8], we studied the dynamics of secretory activity of MC after intraperitoneal injection of 100 μg/kg CCK-4. Secretory activity of MC sharply increased 5 min post-injection. The index of saturation with heparin decreased by 74% (Fig. 2, *a*), while the indexes of granulolysis (Fig. 2, *b*) and degranulation (Fig. 2, *c*) increased by 160 and 19%, respectively. The count of cells with moderate and intensive degranulation also increased (Fig. 2, *d*). It should be emphasized that the index of granulolysis, *i.e.* the number of cells with merocrine secretion (intracellular lysis of granules and release of their content through the membrane) also increased.

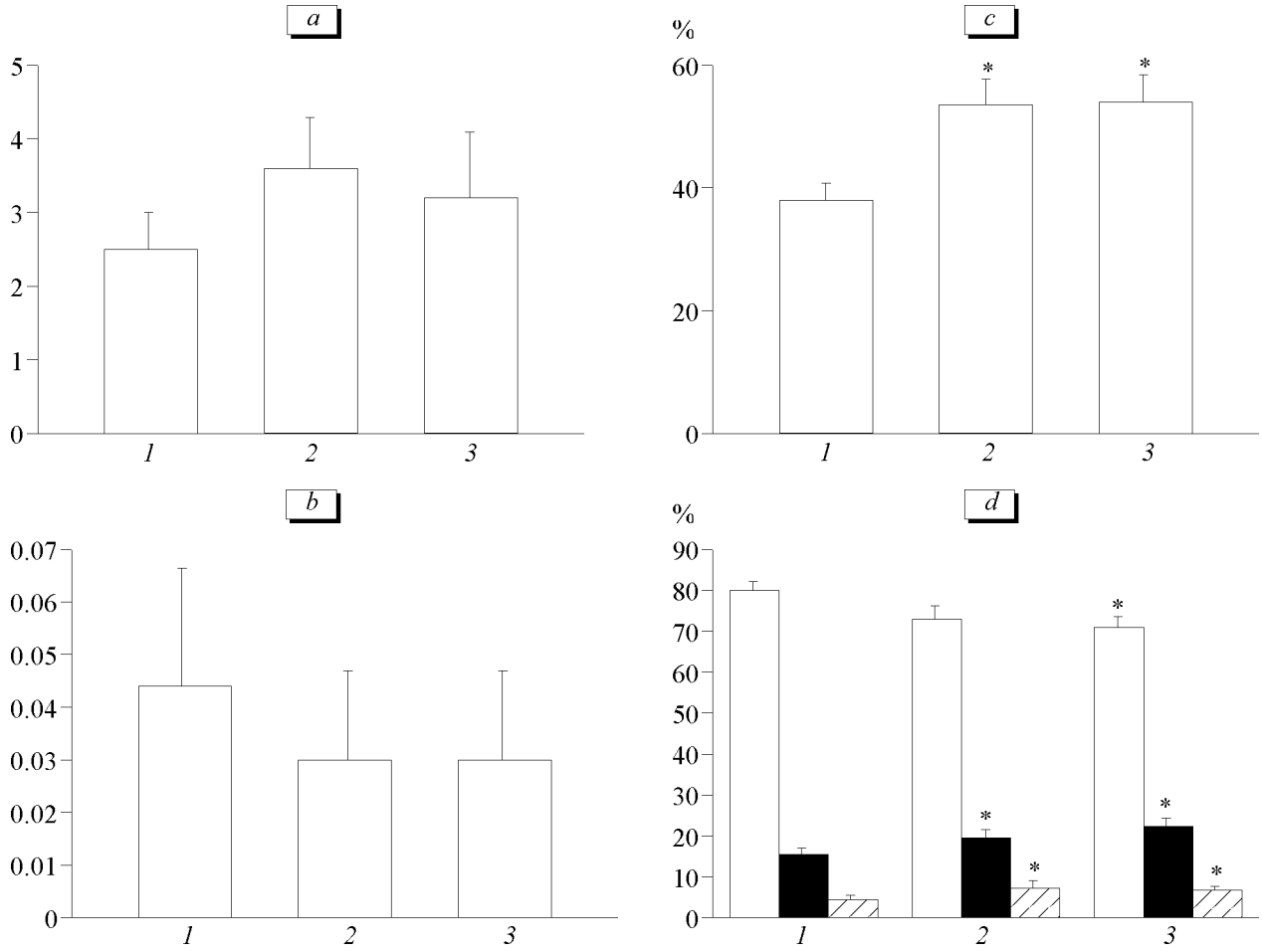

Fig 1. Secretory activity of mast cells 60 min after injection of physiological saline (*1*) or cholecystokinin-4 in doses of 10 (*2*) and 100 mg/kg (*3*). Here and in Fig. 2: indexes of saturation with heparin (*a*), granulolysis (*b*), and degranulation (*c*). Low (light bars), moderate (shaded bars), and intensive degranulation (dark bars, *d*). *$p<0.05$ compared to *1*.

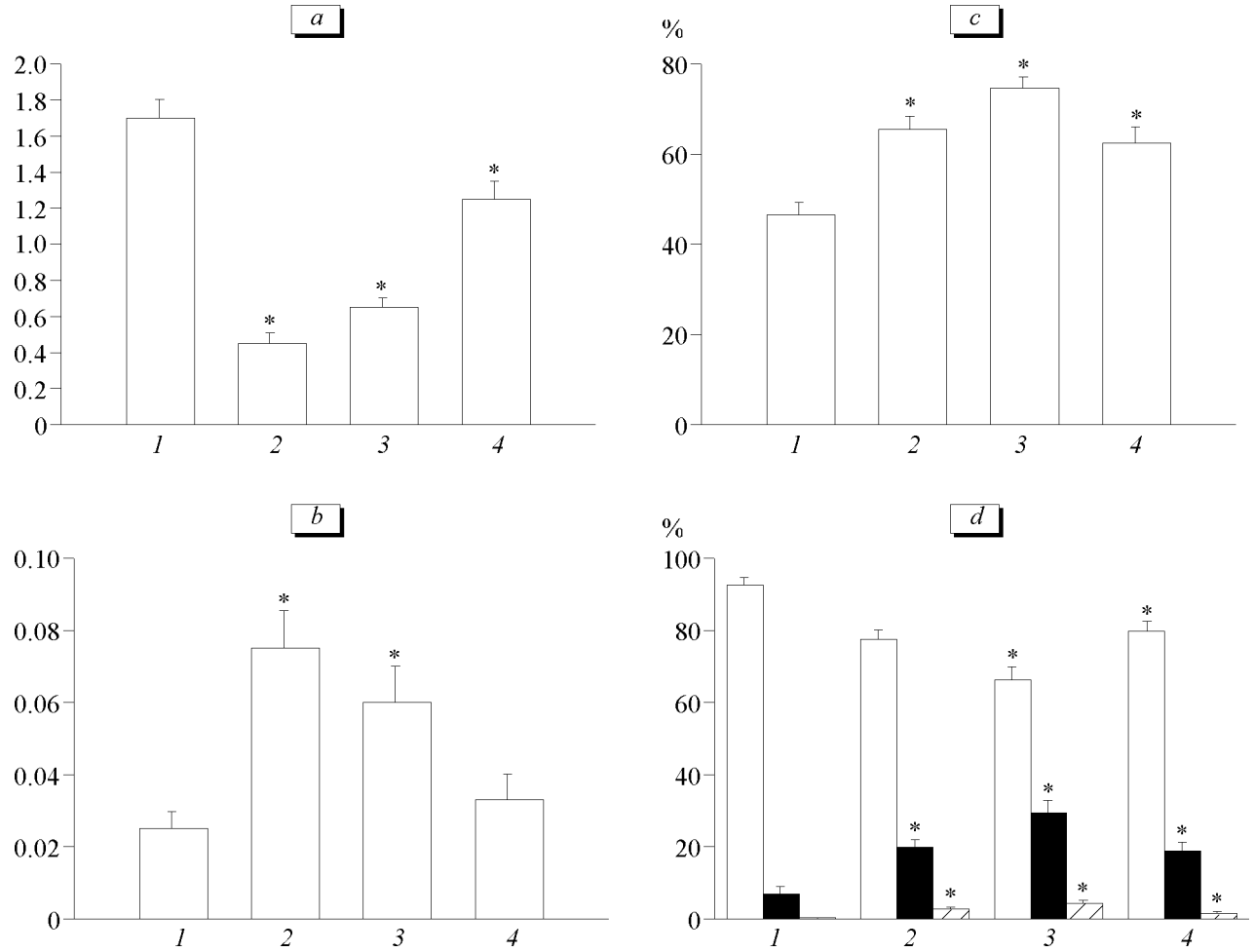

Fig. 2. Secretory activity of mast cells 5 (*2*), 15 (*3*), and 60 min after injection of cholecystokinin-4 (*4*). Intact animals (*1*).

The intensity of secretion remained high by the 15th minute. The intensity of granulolysis decreased, while the index of degranulation and the count of cells with moderate and intensive degranulation increased. Sixty minutes after treatment the intensity of granulolysis returned to normal, while the index of degranulation and the count of cells with moderate and intensive degranulation remained high (Fig. 2, *c*, *d*). These data attested to rapid activation and termination of merocrine secretion, whereas apocrine secretion developed slowly.

Our previous studies showed that the intensity of merocrine secretion increases over the first minutes of immobilization stress. The index of granulolysis increased, while the index of cell saturation with heparin decreased. Apocrine secretion prevailed after 60-min immobilization: the index of degranulation increased [3].

Our findings indicate that secretory activity of MC sharply increases during psychoemotional stress produced by CCK-4. The reaction of MC develops 5 min after CCK-4 administration and progresses in time. It cannot be excluded CCK-4 plays a role in pathophysiological reactions associated with the MC activation.

In vitro incubation of mesentery preparations with CCK-4 (3.2×10^{-12} M) did not modulate secretory activity of MC. Therefore, CCK-4 produced no direct effect on these cells.

Our results suggest that the increase in secretory activity of MC after intraperitoneal injection of CCK-4 is related to the development of psychoemotional stress and activation of the sympathoadrenal and hypothalamic-pituitary-adrenal systems.

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