Loperamide Effects on Anxiety Level and Feeding Behavior in Rats. Role of Vagal Afferentation

S. K. Sudakov, V. G. Bashkatova, A. A. Kolpakov, and N. N. Chernyaeva

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We investigated the role of vagal afferentation in the interaction of the peripheral and central parts of the endogenous opioid system, in the mechanisms of sensorial satiation and anxiety in rats. It has been established that vagotomized rats spent less time in open arm of the plus-maze in comparison with sham-operated animals. Peripheral administration of μ-opioid receptor agonist loperamide was shown to reduce anxiety level in sham-operated rats and had no effect on this parameter in vagotomized animals. Testing in a PhenoMaster module system showed that loperamide administration suppressed feeding behavior in sham-operated animals and partially suppressed it in vagotomized animals. Vagotomy virtually completely blocked the anxiolytic effect of loperamide and partially blocked the anorexigenic effect of the μ-opioid receptor agonist.

Key Words: *peripheral μ-opioid receptors; central opioid system; vagotomy; anxiolytic effect; feeding behavior*

The important role of the endogenous opioid system in the regulation of various physiological functions of the organism, including emotional and feeding behavior, was established in a number of studies [8-10,13]. Endogenous opioid system is present in CNS and in various peripheral organs and tissues [7,12]. Hypothesis of reciprocal relationships between the central and peripheral parts of the endogenous opioid system was proposed and substantiated at the Laboratory of Physiology of Reinforcement, P. K. Anokhin Institute of Normal Physiology, Russian Academy of Medical Sciences [5]. Our experiments demonstrated that peripherally administered μ-opioid receptor (μ-OR) agonists not crossing the blood-brain barrier suppress activity of the central opioid system, whereas peripherally administered μ-OR antagonists activate the system [2,3]. We previously reported that administration of μ-OR agonist into the stomach suppressed feeding behavior [6] and reduced anxiety in rats [3,4]. It was hypothesized that activation of opioid receptors in the stomach may occur under the effect of peptide fragments of alimentary proteins. In virtue of vagal afferentation, this information is transferred into CNS, which can be an important component of sensorial satiation.

To test this hypothesis, we studied the role of vagal afferentation in the interaction of the peripheral and central parts of the endogenous opioid system in the mechanisms of sensory satiation and anxiety in rats.

MATERIALS AND METHODS

The experiments were carried out on male Wistar rats (n=32) weighing ~200 g before the start of the experiment. The animals were kept in groups of 8 animals with unrestricted access to standard combined feed and water under 12:12 h light regimen before the experiment. The experiments were conducted in accordance with the Order No. 267 Ministry of Health of Russian Federation (19.06.2003) and “Rules of Studies on Experimental Animals” (approved by the Ethics Committee of the P. K. Anokhin Institute of Normal Physiology; protocol No. 1, 3.09.2005).

P.K.Anokhin Institute of Normal Physiology, Russian Academy of Medical Sciences, Moscow, Russia. ***Address for correspondence:*** s-sudakov@nphys.ru. S. K. Sudakov

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The animals were divided into 4 groups. Group 1 (n=8) comprised sham-operated rats receiving distilled water, group 2 (n=8) included vagotomized animals receiving distilled water, group 3 (n=8) consisted of sham-operated rats treated with μ-OR agonist loperamide (5 mg/kg, Sigma), and group 4 (n=8) comprised vagotomized rats receiving loperamide (5 mg/kg). Distilled water (groups 1 and 2) or loperamide (groups 3 and 4) was administered intragastrically through the tube.

The role of vagal afferentation was determined on vagotomized rats. Selective bilateral vagotomy was carried out as described elsewhere [1]. The anterior and posterior portions of the vagus nerve were cut below the hepatic and celiac branches, respectively. The sham-operated controls were exposed to the same intervention without nerve incision.

Anxiety level was evaluated in an elevated plus maze (EPM) and by behavior analysis [11].

Feeding behavior was evaluated using PhenoMaster module system (TSE), that appear as automated investigation system aimed at measuring behavioral and physiological phenotype in small laboratory animals in the home cage. PhenoMaster system is used for simultaneous testing of 8 rats; it has individual modules for measuring various parameters, including the module for measuring food consumption. During the experiment, each rat was individually placed into the system for 24 h. The experiment was carried out in several stages. At each stage the animals were divided into the control (n=4; administration of distilled water) and experimental (n=4; administration of μ-OR agonist loperamide 5 mg/kg) groups. Immediately after that, each rat was placed into individual "home" cage of the PhenoMaster system.

The data were processed by ANOVA.

RESULTS

Vagotomized rats spent significantly less time in EPM open arms than sham-operated animals (Fig. 1). Peripheral administration of μ-OR agonist loperamide 5 mg/kg decreased anxiety in sham-operated animals (Fig. 1), but had virtually no effect on the time spent in open arms by vagotomized rats. The time spent in open arms by sham-operated and vagotomized rats following loperamide administration was 45±11 and 16.5±7.0 sec, respectively (Fig. 1). Thus, loperamide had anxiolytic effect on sham-operated animals and did not affect anxiety level in vagotomized rats. In this study, we showed in details that normal afferentation from the stomach through the vagus nerve produces substantial anxiolytic and antistress effects. Alongside with opioid receptor activation in the gastrointestinal tract, loperamide apparently intensifies vagus afferentation, which probably explaines its anxiolytic effect.

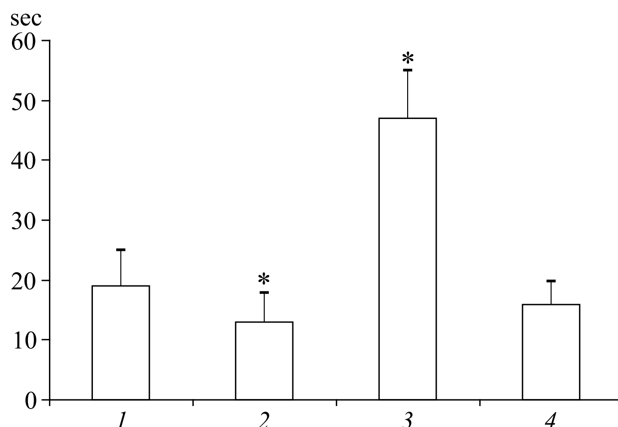

Fig. 1. Effects of vagotomy and loperamide on rat behavior in EPM. Ordinate: time spent in open arms. *$p<0.05$ in comparison with sham-operated rats. Here and in Fig 2: *1*) sham-operated animals; *2*) vagotomized animals; *3*) sham-operated animals+loperamide; *4*) vagotomized animals+loperamide.

Evaluation of feeding behavior in PhenoMaster module system showed that food consumption tended to decrease in vagotomized rats during the light time in comparison with sham-operated group (Fig. 2). During the dark time, sham-operated and vagotomized rats consumed the same amount of food. Meanwhile, peripheral administration of μ-OR agonist loperamide in a dose of 5 mg/kg suppressed feeding behavior in both vagotomized and sham-operated animals (Fig. 2).

Thus, the experiments showed that loperamide while activating the opioid receptors of the gastrointestinal tract apparently increases vagus afferentation, which can explain its anxiolytic effect. The anxiolytic effect of loperamide is partially mediated by vagus afferents. However, the greatest contribution is ap-

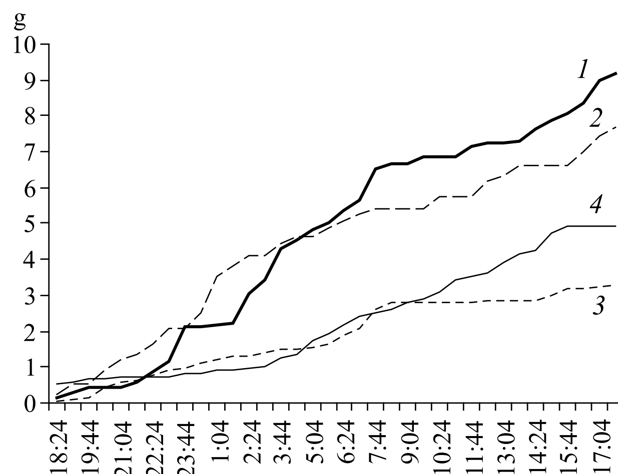

Fig. 2. Effects of vagotomy and loperamide on food consumption. Abscissa: observation time (40-min interval). Light time from 8 a.m. to 8 p.m. Ordinate: food consumption (cumulative curve, the next 40 min are summed up with the previous value).

parently made by other mechanisms associated with glucose release from the depot into circulation.

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