Effect of the Arginine-Containing μ,δ-Opiate Receptor Agonist Sedatin on DNA Synthesis in the Epithelium of the Gastric Fundus in Albino Rats

M. Yu. Fleishman, A. V. Kuznetsov, V. I. Deigin, and S. S. Timoshin

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We studied the effect of arginine-containing μ,δ-opiate receptor agonist sedatin on DNA synthesis in the epithelium of the gastric fundus in albino rats. Fivefold intraperitoneal injection of sedatin in various doses increased the index of labeled nuclei. Similar changes were observed after intranasal and intraperitoneal treatment with sedatin in a dose of 10 μg/kg for 21 days. Administration of sedatin for 21 days prevented the decrease in weight gain induced by experimental stress. Our results suggest that arginine entering the composition of sedatin plays a role in its biological effects.

Key Words: *DNA synthesis; gastric epithelium; synthetic dermorphin analogue; sedatin*

Opiate receptor (OR) ligands maintain tissue homeostasis in various cell populations [8,10]. Opiate-induced changes in cell pressure depend on the type of tissues and affinity of the ligand for OR. Our previous experiments showed that μ-OR ligand dermorphin suppresses cell division in the ectodermal epithelium [5]. Synthetic dermorphin analogue sedatin (H-Arg-Tyr-D-Ala-Phe-Gly-OH) possesses affinity for δ-OR and μ-OR and stimulates cell division in the ectodermal epithelium [7]. The synthetic leu-enkephalin analogue dalargin induces similar changes in these tissues [4]. Dalargin and sedatin contain D-alanine. Another common characteristic of these opiates is the presence of arginine. Arginine is bound to the N-terminal fragment of dalargin. In sedatin molecule arginine is present in the C-terminal fragment.

The therapeutic effect of dalargin in patients with ulcer disease of the stomach and duodenum is associated with its ability to stimulate cell division. It was interesting to evaluate the effect of sedatin on cell division in the gastric fundus. The intensity of cell division is usually estimated after treatment with opiates in doses of 10-100 μg/kg. However, published data show that peptides in ultralow doses can modulate proliferation. Therefore, it is important to study the influence of peptides in various doses on DNA synthesis. Much recent attention is given to the effect of non-injection treatment with preparations (*e.g.*, intranasal administration) [2]. Here we studied the influence of intranasal sedatin on cell division in the gastric epithelium.

MATERIALS AND METHODS

Experiments were performed with sedatin synthesized by E. P. Yarovaya at the Research-and-Production Company Peptos. Sedatin in doses of 0.1, 1, 10, and 100 μg/kg and 1 mg/kg was injected intraperitoneally to 97 male rats (120-160 g) for 5 days. The animals were divided into groups of 8-9 specimens each. In chronic experiments the preparation in a daily dose of 10 μg/kg was administered for 21 days. The rats were decapitated 24 h after the last injection. The peptide was administered intraperitoneally or intranasally. In-

Central Research Laboratory, Far-Eastern State Medical University, Khabarovsk. ***Address for correspondence:*** timoshinss@yandex.ru. Timoshin S. S. f.marina@lycos.com. Fleishman M. Yu.

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TABLE 1. Effect of Intraperitoneal Treatment with Sedatin for 5 Days on DNA Synthesis (ILN, %) in the Epithelium of the Gastric Fundus in Albino Rats (*M*±*m*)

Group	Sedatin dose				
	0.1 μg/kg	1 μg/kg	10 μg/kg	100 μg/kg	1 mg/kg
Control	6.44±0.64	6.44±0.64	7.31±0.42	7.54±0.53	6.44±0.64
Experiment	10.73±1.13*	9.13±0.31*	11.10±0.51*	11.61±1.11*	13.02±1.57*

Note. *$p<0.05$ compared to the control.

tact animals and rats receiving an equivalent volume of physiological saline served as the control. We studied the effect of 5-fold treatment with sedatin in a dose of 100 μg/kg. Histamine content in homogenates of the gastric fundus was measured by the method described elsewhere [9] with modifications [6]. The measurements were performed on a Hitachi spectrofluorometer 4 h after the last injection. The rats intraperitoneally received ^{3}H-thymidine in a dose of 0.6 μCi/g (specific activity 84 Ci/mol) 1 h before decapitation. Samples of the gastric fundus were fixed in a mixture of 96% ethanol and acetic acid (3:1) ratio. Autoradiographs were prepared and the index of labeled nuclei (ILN) and labeling intensity (LI, mean number of tracks over the nucleus) were measured routinely. ILN was estimated in the generative zone (1700-2100 cells) and expressed in percents. In experiments with chronic administration of the preparation we evaluated the dynamics of weight gain. The results were analyzed by means of STATISTICA 5.0 software.

Between-group differences were significant at $p<0.05$.

RESULTS

Sedatin in doses of 0.1-1.0 mg/kg increased the number of DNA-synthesizing nuclei (Table 1). ILN increased by 1.42-2.02 times. LI reflecting the rate of DNA synthesis remained practically unchanged in both series.

Doses of 0.1 and 1.0 μg/kg correspond to concentrations of 1.3×10^{-10} and 1.3×10^{-9} mol/kg, respectively. They are comparable with ultralow doses of immunoactive preparations stimulating proliferation processes [1]. Dalargin induced similar changes in the corneal epithelium of albino rats. However, dalargin in a dose of 1 mg/kg had no effect on the number of DNA-synthesizing cells. This is related to the "escape" phenomenon observed after administration of neuropeptides. Another common characteristic of sedatin and dalargin is their ability to increase histamine content in the gastric mucosa. Histamine content increased by 2.1 times 4 h after the last injection of sedatin in a dose of 100 μg/kg. In stomach tissue of control and sedatin-treated rats histamine concentration was 0.110±0.005 and 0.23±0.1 μmol/g, respectively. It should be emphasized that dalargin stimulates DNA synthesis and increases histamine concentration in the stomach. According to modern notions, the increase in stomach histamine content within the physiological limits improves blood supply and stimulates proliferative processes. Intraperitoneal or intranasal administration of 0.9% NaCl for 3 weeks reduced weight gain (compared to intact animals, Fig. 1). Intranasal and intraperitoneal administration of sedatin prevented the decrease in weight gain observed in animals receiving physiological saline. Sedatin possesses anabolic activity. Control experiments were performed with animals receiving isotonic NaCl (no intact control rats). Our results suggest that sedatin produces an antistress effect (similarly to opiate peptides) [3]. Intraperitoneal or intranasal administration of sedatin in a dose of 10 μg/kg for 21 days induced more significant changes in DNA synthesis in the gastric fundus compared to 5-fold treatment with the preparation. ILN increased by 2.6 and 1.8 times after intraperitoneal and intranasal administration of sedatin, respectively (Table 2).

These data show that sedatin in various doses produces a potent mitogenic effect (similarly to another

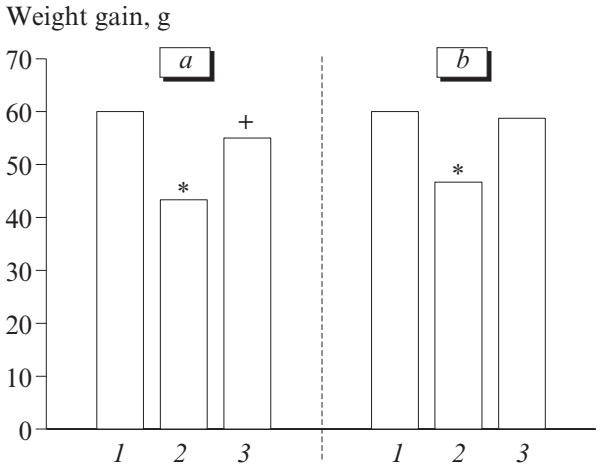

Fig. 1. Effect of sedatin for 21 days in a dose of 10 μg/kg on weight gain in albino rats: intraperitoneal (*a*) and intranasal administration (*b*). Intact rats (*1*), control (*2*), and sedatin (*3*). $p<0.05$: *compared to intact rats; +compared to the control.

TABLE 2. Effect of Treatment with 10 μg/kg Sedatin for 21 Days on DNA Synthesis (ILN, %) in the Epithelium of the Gastric Fundus in Albino Rats (*M*±*m*)

Administration	Intact rats	Control (NaCl)	Sedatin
Intraperitoneal	5.44±0.26	4.68±0.13	12.10±0.55*+
Intranasal	5.44±0.26	7.14±0.73*	13.02±0.52*+

Note. $p<0.05$: *compared to intact rats; +compared to the control.

μ,δ-OR agonist dalargin). Intraperitoneal and intranasal administration of sedatin induces similar changes. It should be emphasized that sedatin and dalargin are arginine-containing peptides. It is important to evaluate the role of arginine in the realization of biological effects of both peptides. Preclinical trials with the peptide are currently performed and a new pharmacological preparation will be synthesized from the peptide.

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