PHARMACOLOGY AND TOXICOLOGY

Mechanisms for the Effect of Arginine-Containing Dermorphin Analogue on Proliferative Processes in the Gastric Mucosa of Albino Rats

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Treatment with synthetic arginine-containing dermorphin analogue sedatin (100 mg/kg, 5 intraperitoneal injections) stimulated DNA synthesis in the gastric mucosa and decreased spontaneous and induced chemiluminescence in homogenates of the stomach from albino rats. Non-arginine sedatin analogue in the same dose had little effect on DNA synthesis and free radical oxidation. Fivefold treatment with NO synthase inhibitor L-NAME (9.3×10^{-5} mol/kg) suppressed DNA synthesis in the gastric mucosa. Sedatin did not modulate DNA synthesis against the background of L-NAME administration.

Key Words: *synthetic dermorphin analogues; DNA synthesis; arginine; free radical oxidation*

Sedatin is an arginine-containing dermorphin analogue and μ/δ-opioid receptor agonist. This drug in a wide doses range stimulates proliferative processes in various populations of cells, including those in the gastric mucosa (GM) [8]. Arginine-containing μ/δ-opioid receptor agonist dalargin have the same properties [7]. The structure of dalargin and sedatin differs by the position of arginine: C-position in dalargin and N-position in sedatin. Arginine can be cleaved from the molecule of dalargin during metabolism [4]. Arginine is a component of the NO synthase (NOS) system. NO not only stimulates proliferation, but also modulates free radical processes [10,12]. Reactive O_2 metabolites play a regulatory role in the maintenance of tissue homeostasis [3].

Here we studied the role of arginine in the effect of sedatin on DNA synthesis in GM of albino rats.

MATERIALS AND METHODS

Experiments were performed on male albino rats weighing 90-110 g. Sedatin (H-Arg-Tyr-D-Ala-Phe-Gly-OH) and its non-arginine analogue (H-Tyr-D-Ala-Phe-Gly-OH) synthesized at Peptos Research-and-Production Company were injected intraperitoneally in a dose of 100 μg/kg for 5 days. Control animals received an equivalent volume of isotonic NaCl. The rats were euthanized 24 h after the last injection. Since NOS system mediates the effects of arginine, the role of arginine in the realization of mitogenic activity was evaluated against the background of blockade of this system. To this end, L-NAME (9.3×10^{-5} mol/kg, ICN Biomedicals) was administered to group 1 animals. Group 2 rats re-

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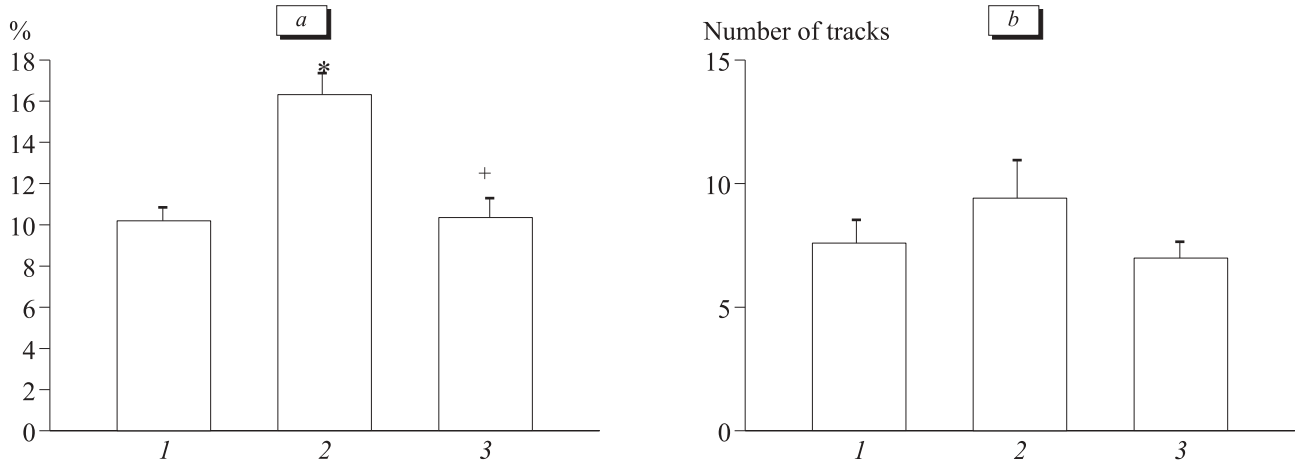

Fig. 1. Effect of 5-fold treatment with dermorphin analogues on DNA synthesis in the gastric epithelium of male albino rats. *a*: index of labeled nuclei; *b*: labeling intensity. Administration of physiological saline (*1*), sedatin (*2*), and non-arginine sedatin analogue (*3*). $p<0.05$: *compared to physiological saline; +compared to sedatin.

ceived sedatin (100 μg/kg) 30 min after administration of L-NAME in the same dose. Intact animals served as the control. The animals were euthanized 24 h after the last injection.

DNA synthesis was studied by ^{3}H-thymidine autoradiography. ^{3}H-thymidine in a dose of 0.6 μCi/g (1570 TBq/mol) was injected intraperitoneally 1 h before decapitation. Autoradiographs were prepared by the standard method with Kodak photoemulsions for autoradiography. The index of labeled nuclei was estimated by counting of 2500-3000 cells and expressed as a percent of S-phase cells in longitudinal gastric glands. Labeling intensity was calculated as the mean number of silver grains above 50 nuclei.

The method of chemiluminescence (CL) was used for integral evaluation of free radical oxidation in tissue homogenates. Spontaneous and Fe^{2+}-induced CL was recorded on a LS-50B luminescence spectrometer (Perkin Elmer) [2]. The following parameters were analyzed: total yield of spontaneous CL (S_{SP}) measured over 1 min correlating with the intensity of free radical generation, fast flash maximum (H1) of induced CL reflecting the content of lipid hydroperoxides, and total yield of CL recorded over 2 min after fast flash ($S1_{IND}$) reflecting the rate of lipoperoxide radical accumulation. Kinetic parameters of H_2O_2-induced luminol-dependent CL [9,11] were evaluated by the maximum luminescence (H2) reflecting the ability of biological object to undergo peroxidation and the yield of CL over 2 min ($S2_{IND}$) depending on activity of the antioxidant and antiradical defense system. Parameters of CL were calculated per 1 g wet tissue and expressed in relative units.

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

RESULTS

Fivefold treatment with sedatin significantly increased DNA synthesis in major glands of GM. The index of labeled nuclei increased by 1.6 times, while labeling intensity remained unchanged (Fig. 1). The non-arginine analogue had no effect on DNA synthesis. CL study also confirmed the important role of arginine in the effect of sedatin. Sedatin exhibited high antioxidant and antiradical properties, which was seen from the decrease in $S2_{IND}$ and H2 by 1.7 and 1.2 times, respectively. These changes contributed to inhibition of free radical generation (1.8-fold decrease in S_{SP}), reduction of lipid hydroperoxide content (1.5-fold decrease in H1),

TABLE 1. Effect of Bioactive Peptides on CL in Homogenates of the Stomach from Male Albino Rats (*M*±*m*)

Group	S_{SP}, rel. units	CL-1		CL-2	
		H1, rel. units	$S1_{IND}$, rel. units	H2, rel. units	$S2_{IND}$, rel. units
Control	1.330±0.093	2.280±0.077	2.882±0.141	2.150±0.106	1.090±0.099
Sedatin	0.739±0.057*	1.500±0.093*	1.896±0.128*	1.77±0.09*	0.660±0.053*
Non-arginine sedatin analogue	1.450±0.081	2.180±0.092	2.209±0.121*	2.020±0.143	1.060±0.094

Note. CL-1, Fe^{2+}-induced CL; CL-2, H_2O_2-induced luminol-dependent CL. *$p<0.05$ compared to the control.

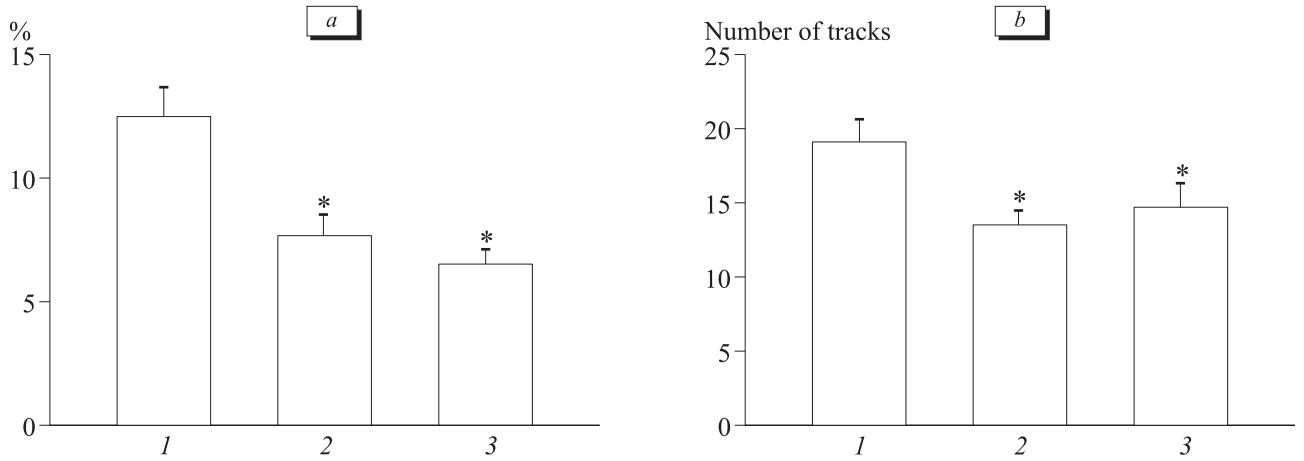

Fig. 2. Effect of 5-fold treatment with sedatin and L-NAME on DNA synthesis in the gastric epithelium of male albino rats. *a*: index of labeled nuclei; *b*: labeling intensity. Intact animals (*1*), L-NAME (*2*), and L-NAME+sedatin (*3*). **p*<0.05 compared to intact animals.

and prevention of peroxide radical formation and accumulation (1.5-fold decrease in $S1_{IND}$). Non-arginine analogue of sedatin only decreased the rate of free radical generation (1.3-fold decrease in $S1_{IND}$, Table 1).

Blockade of the NO—NOS system with L-NAME confirmed the role of arginine in the realization of mitogenic effect. Our experiments showed that the tonic effect of NO—NOS maintains tissue homeostasis in GM. NOS blockade significantly inhibited DNA synthesis in GM. Index of labeled nuclei decreased by 1.6 times compared to the control. The decrease in the number of DNA-synthesizing nuclei was accompanied by a significant decrease in labeling intensity attesting to deceleration of DNA synthesis. Our results are consistent with published data that L-NAME suppresses DNA synthesis in the myocardium of newborn rats [5,6]. However, DNA synthesis in skin epithelium and muscle layer of the intestine remained unchanged under these conditions. It should be emphasized that blockade of the NO—NOS system with L-NAME abolished the sedatin-induced activation of DNA synthesis (Fig. 2).

Our previous experiments showed that L-NAME abolished sedatin-induced activation of DNA synthesis in the epithelium and bronchi of newborn rats. The presence of arginine in synthetic analogues of opiate peptides does not determine a variety of their biological effects, but attachment of arginine to opioid receptor ligand leads to the appearance of some new properties.

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