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COMPARATIVE EFFECT OF DIFFERENT LIGANDS OF OPIOID RECEPTORS ON CELL DIVISION IN THE ALBINO RAT LINGUAL EPITHELIUM

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UDC 612.312.3.014.2:612.6].014.467:615.31:[547.95:547.943

KEY WORDS: endorphins; lingual epithelium; DNA synthesis; cell division.

Ligands of opiate receptors include regulatory peptides, with a modulating influence on the function of various systems. In previous investigations the writers showed that following direct application and systemic administration of dalargin, a synthetic enkephalin analog, and naloxone, an antagonist of opiate receptors, cell division is stimulated in the corneal epithelium.

The aim of this investigation was to compare the effects of different ligands of opioid receptors on cell division in the lingual epithelium.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 150-180 g. β-Endorphin, Leu-enkephalin (from Serva, West Germany), naloxone (from Endo Laboratories, USA), and Dalargin (All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR) were injected intraperitoneally (0.1 ml of a $2 \cdot 10^{-9}$ M solution of the test ligands/100 g body weight). The preparations were injected at 2 p.m. Animals receiving an intraperitoneal injection of the same volume of isotonic saline served as the control. Cell division was studied 24 h after injection of the preparations. An intraperitoneal injection of ^{3}H-thymidine (molar activity 60 Cu/mmole) was given to the animals in a dose of 0.6 μCi/g body weight 1 h before sacrifice. Autoradiographs and histologic sections were prepared, and the index of labeled nuclei (ILN, %), intensity of DNA labeling (IL, average number of grains

Central Research Laboratory, Khabarovski Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR S. S. Debov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 9, pp. 354-355, September, 1987. Original article submitted October 17, 1986.

TABLE 1. Effect of Ligands of Opiate Receptors on Cell Division in the Albino Rat Lingual Epithelium 24 h after Injection of Preparations

Preparation	DNA synthesis		Mitotic activity, ratio of phases of mitosis, %				MI %
	ILN %	IL	prophase	metaphase	anaphase	telophase	
Isotonic saline (control)	6,9±0,5	29,5±1,0	9	62	6	23	2,6±0,6
Dalargin	11,6±0,6*	29,9±1,4	6	67	7	20	4,6±0,3*
β-Endorphin	10,4±0,8*	37,6±2,2*	12	56	11	21	3,1±0,3
μ-Enkephalin	8,2±0,8	31,2±1,5	7	64	10	19	3,4±0,2
Naloxone	10,3±0,9*	32,2±2,9	5	68	8	19	3,3±0,6

Legend. Asterisk indicates that differences relative to control are significant.

of silver above a labeled nucleus), and the mitotic index (MI, %) were determined by the method adopted in the laboratory [3]. The numerical results were subjected to statistical analysis by Student's test and are given in Table 1.

EXPERIMENTAL RESULTS

Analysis of changes in cell division processes in the lingual epithelium shows that Dalargin, β-endorphin and naloxone caused activation of DNA synthesis. ILN in the lingual epithelium of animals receiving these preparations was increased by 1.5-1.7 times. After injection of β-endorphin, the increase in ILN was accompanied by a significant increase in IL, evidence of an increase in the rate of DNA synthesis under these conditions. It is an interesting fact that administration of Leu-enkephalin, which is a relatively selective analog of δ-receptors, caused no changes in cell division in the lingual epithelium. Dalargin is closely similar in its chemical structure to Leu-enkephalin: the Gly^2 in the latter is replaced by $D\text{-}Ala^2$, and arginine is added to the C-terminal region [6]. Dalargin binds mainly with δ-receptors and partially with μ-receptors, and this is responsible for its marked cytoprotective effect [6].

β-Endorphin also interacts with μ- and δ-receptors. The absence of any stimulating effect after administration of Leu-enkephalin, which also binds with δ- and μ-receptors, can be explained by its instability and its more rapid degradation compared with Dalargin. The fact that administration of naloxone, an antagonist of opiate receptors, had a similar stimulating effect on DNA synthesis, is at first glance evidence against a receptor mechanism of the stimulating action of these ligands of opioid peptides. However, it must be recalled that naloxone selectively blocks only μ-receptors, and that in order to block δ-receptors doses 10 times higher than those which we used are necessary [8]. Support for the view that the stimulating effect of the ligands studied is realized through a receptor binding mechanism in these experiments are given indirectly by the extremely small doses of the preparations used.

The increase in ILN in the experiments with Dalargin was accompanied by an adequate increase in MI. The absence of any significant increase in the number of dividing cells in the lingual epithelium after injection of β-endorphin and naloxone may be attributed to the acceleration of mitosis itself, and this conclusion is supported by the increase in the percentage of prophases in the experiments with β-andorphin. Another probable explanation may be changes in the circadian rhythm of mitosis under the influence of these preparations, or the presence of premitotic delay. The explanation of these problems must await experimental analysis.

The results of this investigation, together with data obtained previously on stimulation of cell division through the action of Dalargin and naloxone suggest that endogenous opioid peptides are an important stage in the regulation of cell division. This is confirmed by data in the literature on stimulation of growth processes in sympathetic ganglia in culture under the influence of endorphins [2]. Other investigators [9] found that bombesin is a mitogenic factor for resting 3T3 cells. β-Endorphin potentiates the proliferative response of rat spleen cells to mitogens [7].

It was demonstrated previously that repeated and chronic exposures to stress induce activation of cell division [1, 4, 5]. The mechanism of stimulation of cell division in

chronic stress is little understood. Hormones of the pituitary-adrenal system, increased secretion of which occurs in stress, are inhibitors of cell division. Besides reduction of the cell population due to death of cells during stress, an essential role in activation of proliferation processes is evidently played by endogenous opiates, production of which is increased in the general adaptation syndrome. This hypothesis, however, requires experimental verification.

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