EFFECT OF OPIOID RECEPTOR LIGANDS ON CELL DIVISION IN THE ALBINO RAT CORNEAL EPITHELIUM

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There is very little information on the effect of endogenous opiates on cell division.

The aim of this investigation was to study the effect of Dalargin, an enkephalin analog synthesized in the laboratory of peptide chemistry, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR (Director, Dr. Chem. Sci. M. I. Titov), and of naloxone, an opiate receptor antagonist, on cell division in the corneal epithelium in albino rats.

EXPERIMENTAL METHOD

Experiments were carried out on male rats weighing 280-300 g. In series I the direct action of the preparations on cell division was studied. For this purpose, 0.02 ml of 1 mg% solution of Dalargin or naloxone (Endo Laboratories, USA) was applied to the right eye by means of a microdoser. An equal volume of isotonic sodium chloride solution was applied to the left (control) eye. The preparations were applied three times, with an interval of 20 min, between 1 and 2 p.m. To assess the effect of repetition of the procedure, it was repeated daily for 5 days. Cell division was studied 4, 12, and 24 h after application of the preparation. By means of a microdoser 5 μmole of ^{3}H-thymidine was applied to both eyes 1 h before sacrifice. In series II Dalargin and naloxone were injected intraperitoneally in a dose of 10 μg/kg. either once or in 5 daily doses, at 2 p.m. Control animals received the same volume of isotonic saline intraperitoneally. Cell division was studied 24 h after injection of the preparations. The mitotic index (MI, in %) was determined, autoradiographs were prepared, and the index of labeled nuclei (ILN, in %) and the labeling intensity (LI, the mean number of grains of silver above the nucleus) were determined by methods adopted in the laboratory [2]. Altogether 126 rats were used in the experiments. The data were subjected to statistical analysis by Student's t test.

TABLE 1. Effect of Dalargin and Naloxone Application on Cell Division in the Corneal Epithelium of Albino Rats

Experimental conditions	Time, h								
	4			12			24		
	ILN, %	LI	MI, %	ILN, %	LI	MI, %	ILN, %	LI	MI, %
Control	6,9	23,1	3,7	5,3	16,7	5,4	4,8	24,2	9,5
Single application of Dalargin	5,0	24,2	3,4	5,7	38,1*	8,4*	4,1	22,1	7,3
Control	5,2	16,7	2,5	4,8	16,6	6,1	4,7	13,3	8,2
Single application of naloxone	6,9	34,6*	2,1	7,9*	34,7*	1,7*	8,6*	33,6*	5,4*
Control	4,9	18,9	2,5	5,9	18,0	6,2	5,7	26,1	8,1
Five applications of Dalargin	9,0*	22,9*	2,0	9,0*	22,7	8,4	5,8	20,7	8,7
Control	4,9	23,0	1,9	4,8	21,5	2,6	4,4	22,7	5,1
Five applications of naloxone	5,2	22,8	1,7	5,3	23,9	6,2*	5,1	23,6	7,3

Legend. Here and in Table 2 asterisk indicates that differences are significant.

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TABLE 2. Effect of Intraperitoneal Injection of Dalargin and Naloxone on Cell Division in the Corneal Epithelium of Albino Rats (24 h after injection)

Experimental conditions	ILN, %	LI	MI, %
Control	6,2	14,6	3,8
Single injection of naloxone	11,4*	24,4*	5,3
Single injection of Dalargin	11,2*	15,6	6,1
Control	5,8	29,4	3,6
Five injections of naloxone	12,3*	31,1	3,1
Five injections of Dalargin	11,7*	27,2	3,7

EXPERIMENTAL RESULTS

A single dose of naloxone stimulated cell division in the cornea at all three times of testing. This conclusion was based on an increase in LI of DNA, evidence for an increase in the rate of synthesis, 4 h after application of the drug. Besides an increase in LI, after 12 and 24 h there was also a significant increase in the number of DNA-synthesizing nuclei. A significant decrease in the number of dividing cells after 12 and 24 h may have been due to premitotic delay or an increase in the rate of mitosis itself. Evidence in support of the view that the rate of mitosis was increased was given by a significant increase in the number of prophases in these experiments. However, the final solution to this problem requires experimental investigations. A single application of Dalargin caused a significant increase in LI 12 h after administration. No other changes were observed in cell division processes after a single dose of DNA synthesis was observed 4 and 12 h after the final application. The absence of any significant increase in MI in these experiments can conjecturally be attributed to the same causes as in experiments with a single dose of naloxone. No significant changes in the parameters of DNA synthesis were found at any time of investigation after repeated applications of naloxone. The increase in MI 12 and 24 h after the 5th application was evidently explained by stimulation of DNA synthesis at times before the investigations were done.

Systematic administration of Dalargin and naloxone caused stimulation of proliferation. This was shown by a significant increase in the number of DNA-synthesizing nuclei after administration of one or five doses of both substances. After a single dose of Dalargin and naloxone, activation of DNA synthesis was accompanied by a corresponding increase in LI. No significant changes in LI were found after repeated doses, possibly because the investigation was undertaken only 24 h after administration (Tables 1 and 2).

It can be concluded from these data that both ligands of opioid receptors stimulate proliferation. Dalargin is a full δ-receptor and partial μ-receptor agonist. Although naloxone is a μ-receptor antagonist, its effect on cell division is largely identical to the action of Dalargin. It should be pointed out in this connection that the response in the experiments in [1] also was similar in type: growth of sympathetic ganglia in culture was stimulated under the influence both of opiate neuropeptides and of their antagonists. The absence of antagonism between naloxone and opioid peptides, according to the authors cited, does not rule out a receptor mechanism of peptide action, for naloxone does not always abolish the effect of opiate peptides. Indirect evidence in support of the receptor character of action of these substances is given by the fact that effective stimulation of cell division was achieved in our experiments with low doses. However, elucidation of this problem awaits experimental verification. Naloxone and Dalargin possess a broad spectrum of biological activity, but coincidence of the stimulating effect of direct application to the cornea with stimulation by systematic administration can be interpreted as evidence of the direct stimulating action of opioid receptor ligands on cell division. The ability of these ligands to block intracellular cAMP formation [5] (cAMP is an inhibitor of proliferation) would thus seem to be an important property of these ligands. The ability of Dalargin to stimulate cell division, together with its antistress properties [3], helps to explain the mechanisms of its cytoprotective action [4]. Stimulation of cell division under the

influence of naloxone necessitates the study of the effect of other opioid receptor ligands on cell division processes.

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