The Effect of μ- and δ-Opiate Receptor Agonists on Cell Division of Corneal and Tongue Epithelium in Albino Rats

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The effect of μ- and δ-opiate receptor agonists at 10 μg/kg on the processes of cell division in corneal and tongue epithelium of albino rats is studied using the method of autoradiography with ^{3}H-thymidine. The ligand of the μ-receptors causes the inhibition of DNA synthesis in corneal epithelium 4, 12, and 24 h later and lowers the mitotic index after 4 and 24 h. In contrast, in the tongue epithelium stimulation of the proliferative processes occurs. Ligand of the δ-receptors stimulates DNA synthesis in corneal epithelium after 12 and 24 h and cell division after 12 h. In the tongue epithelium DNA synthesis is activated after 4, 12, and 24 h and cell division after 12 h.

Key Words: *opiate receptor agonists; DNA synthesis; cell division*

A stimulatory effect on the processes of cell division was established for a number of opioid receptor ligands in epithelial tissues in our previous experiments [5]. One of them, the synthetic analog of leu-enkephalin dalargin (Tyr-D-Ala-Gly-Phe-Leu-Arg), proved to mediate the stimulatory effect via interaction with opiate receptors [3].

It is becoming increasingly evident that the grouping of various subpopulations of opiate receptors according to their analgesic effect on the organism is incorrect [6].

In a study of the effect of this paraopioid substance on proliferative activity dermorphin administration was found to decrease the number of DNA-synthesizing nuclei in corneal epithelium 1.5-1.8-fold [4]. Dermorphin is a ligand of μ-receptors [6]. Data on the inhibition of proliferation by another agonist of μ-receptors, methadone, are reported in the literature. In this connection a different role of opiate receptor subpopulations in the regulation of proliferative processes was suggested.

For assessment of the validity of this hypothesis the nature of the effect of selective μ- and δ-agonists, DAGO and DADLE, respectively, on cell division was studied in corneal and tongue epithelium of albino rats. The peptides were synthesized at the Laboratory of Peptide Chemistry, Research Center of Cardiology, Russian Academy of Medical Sciences.

MATERIALS AND METHODS

The experiments were carried out on male albino rats weighing 180-200 g. The agonists of μ- and δ-receptors (DAGO and DADLE) were administered once i.p. at 10:00 in a dose of 10 μg/kg. Control animals were injected with an equivolume of saline. The animals were killed 4, 12, and 24 h after administration of the preparation. For each period studied 7-8 control and test animals were selected. The total number of experimental animals was 118. For autoradiograph preparation one cornea was incubated in an ultrathermostat at 37°C in medium 199 with ^{3}H-thymidine (2 μCi/ml),

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TABLE 1. Effect of Selective Analogs of μ- and δ-Opiate Receptors DAGO and DADLE on Cell Division in Corneal and Tongue Epithelium in Albino Rats

Group of animals	Time after drug administration, h										
	4				12			24			
	Index of cell division										
	MIC, %	MI, ‰	ILN, %	IL	MI, ‰	ILN, %	IL	MIC, %	MI, ‰	ILN, %	IL
Corneal epithelium											
Control	23.2±1.0	15.3±0.9	9.7±0.44	18.4±1.4	20.1±1.8	13.0±1.5	23.1±2.0	23.2±1.0	15.3±0.9	9.7±0.5	18.4±1.42
DAGO	10.2±0.6*	8.1±0.97*	5.2±0.46	15.3±1.0	13.4±1.2*	8.0±0.5*	19.1±2.2	14.7±0.5*	6.4±0.7*	6.8±0.26	20.0±1.8
DADLE	23.1±0.9	15.1±1.5	11.4±0.2	18.2±1.5	30.5±2.4*	22.3±2.1*	24.4±3.0	23.3±0.9	11.2±0.3	12.7±0.7	13.2±1.1
Tongue epithelium											
Control	21.6±0.7	–	6.7±0.45	25.6±1.2	9.2±0.7	10.1±0.5	26.4±2.8	21.6±0.7	–	6.7±0.45	26.6±1.21
DAGO	33.3±1.4*	–	10.9±0.44	26.8±1.1	14.3±1.2*	17.9±0.8*	28.3±3.0	23.8±1.0	–	8.63±0.2	21.4±1.5
DADLE	22.9±1.4	–	16.45±1.45*	26.3±1.9	17.3±1.6*	17.4±2.1*	25.5±2.7	33.0±1.0*	–	9.64±0.75	20.7±0.5

Note. An asterisk denotes $p<0.05$.

while a wholemount was prepared from another cornea for the evaluation of the mitotic index (MI, %). All procedures, namely, the preparation of autoradiographs, determination of the index of labeled nuclei (ILN, %), the index of intensity of labeling (IL), preparation of wholemounts, and determination of MI, were described elsewhere [1]. To prevent the variation of mitotic time the tests were performed with colchicine at 2 μg/kg administered i.p. 2 h before sacrifice. The tests with colchicine were carried out 4 and 24 h after the administration of opiate receptor agonists. In this case, MI was determined for blocked metaphase (MIC, %). The results were treated statistically by the Student *t* test.

RESULTS

The results of the investigations attest that DADLE produced a stimulative effect on the proliferative processes in epithelium of the cornea and tongue (Table 1). In tongue epithelium a 2.4-fold increase in ILN was found after 4 h, whereas after 12 h this index rose 1.7-fold in corneal and tongue epithelium. The ILN increase of 1.3 and 1.4 times after 24 h was insignificant. An adequate increase of the number of dividing cells in test epithelia was also found 12 h after peptide administration. A 1.5- and 1.8-fold rise of MI was found in the cornea and tongue, respectively. In tongue epithelium a 1.5-fold increase of MIC occurred 24 h after DADLE administration. It should be noted that the maximum stimulatory effect of dalargin (a synthetic analog of leu-enkephalin), interacting mainly with δ-opiate receptors, was noted 12 h after its administration [2]. DAGO exhibited different effects on cell division in epithelium of the cornea and tongue. In this case, an inhibition of proliferation was observed in corneal epithelium, just as with the administration of other μ-agonist dermorphin. The MI decreased reliably 1.8-fold 4 h after drug administration. Simultaneously MIC reliably diminished 2.2-fold as well. Moreover, a 1.8-fold decrease of ILN occurred. This reduction of ILN, combined with the MI drop, was also noted in corneal epithelium 12 h after drug administration. The decrease in the number of dividing cells still persisted in the cornea 24 h after DAGO administration, whereby MI and MIC were 2.3- and 1.5-fold lower, respectively, than the control indexes. Although ILN was 1.4-fold lower than in the control, the differences were insignificant. Some discrepancies between DAGO and dermorphin in the nature of inhibition of cell division may be due to a particularity in the pharmacokinetics of the compared substances. The tongue epithelium did not react differentially to the μ- and δ-agonist: DAGO, like DADLE, induced stimulation of cell division. Regarding the ability of DAGO to inhibit the proliferative process in the cornea, the data on the depression by this opioid of the preimplantation development of mouse embryos are of interest. DADLE did not produce such an effect on this process.

Thus, the results of the present investigation confirm the previous idea of a possible differential reaction to opiate receptor agonists of various subpopulations. The inhibition of cell division in corneal epithelium by μ-agonists and its stimulation by δ-agonists is additional evidence of the diverse role of opiate receptor subpopulations in the regulation of physiological functions.

Our data explain to a certain extent the contradictory reports on the effect of opiates on proliferative processes. It may be assumed that the nature of the end result of the effects depends on the nature of the opiate ligand as well as on the representation or ratio of various subpopulations of receptors in tissues. Zagon suggests that the receptor structure which mediates the response of proliferative processes differs from the classic μ-, δ-, σ, and κ-opioid receptors [7]. Additional analysis is required for elucidation of the causes of the stereotypical response of proliferation of tongue epithelium to the action of μ- and δ-agonists.

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