EXPERIMENTAL BIOLOGY

Effect of Synthetic Analogs of Dermorphin on Mitosis in the Corneal and Lingual Epithelium of Albino Rats

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A study of the effects of synthetic analogs of dermorphin which are prime agonists of the μ-opiate receptors, on cell division in the corneal and lingual epithelium of albino rats showed that both analogs depressed DNA synthesis in the corneal and lingual epithelium 4 h after administration. In the lingual epithelium DNA synthesis and the mitogenic index were still depressed 24 h after drug administration. In the cornea cell division parameters had normalized by this time.

Key Words: *dermorphin; DNA synthesis; mitosis*

Our previous studies revealed that, in contrast to numerous ligands of opiate receptors stimulating proliferative processes, the paraopioid dermorphin (DM) depressed cell mitosis in the corneal epithelium [3]. Hypotheses on the different role of subpopulations of opiate receptors in mitosis regulation were proposed to explain the difference between the effects of DM and those of dalargin and β-endorphin. DM is a μ-receptor agonist, whereas the stimulating effect of dalargin on proliferation is mediated by interaction with δ-receptors [2].

Two synthetic analogs of DM that are selective agonists of μ-receptors were investigated. This study is interesting from the standpoint of applied research, because the analogs in question are being assayed as potential drugs.

MATERIALS AND METHODS

Experiments were carried out with male albino rats weighing 160 to 190 g. Synthetic analogs of DM A10 (H-Tyr-D-Orn-Phe-Gly-OH) and A43 (D-Arg-Tyr-D-Arg-Phe-D-Ala-NH_2) were injected intraperitoneally in a dose of 10 μg/kg. The substances were synthesized at the Laboratory of Peptide Chemistry, All-Russian Cardiology Research Center, Russian Academy of Medical Sciences [4].

To control animals similar volumes of isotonic solution of sodium chloride were injected intraperitoneally. Colchicine was injected in a dose of 2.0 μg/kg intraperitoneally 2 h before sacrifice. The experimental and control animals were euthanized 4 and 24 h after drug injection. Groups consisting of 6-7 control and experimental animals were examined at each period of the study. One hour before sacrifice the rats were intraperitoneally injected ^{3}H-thymidine with a specific activity of 87 Ci/mmol in a dose of 0.6 μCi. In addition, 2 μCi ^{3}H-thymidine was applied to the cornea. Histologic preparations of the cornea and tongue and radioautographs were made routinely, and the mitogenic index (MI), index of labeled nuclei (ILN), mitotic index after colchicine treatment (MIC), and label intensity (LI) were estimated using our own laboratory technique [1]; the MI and MIC were

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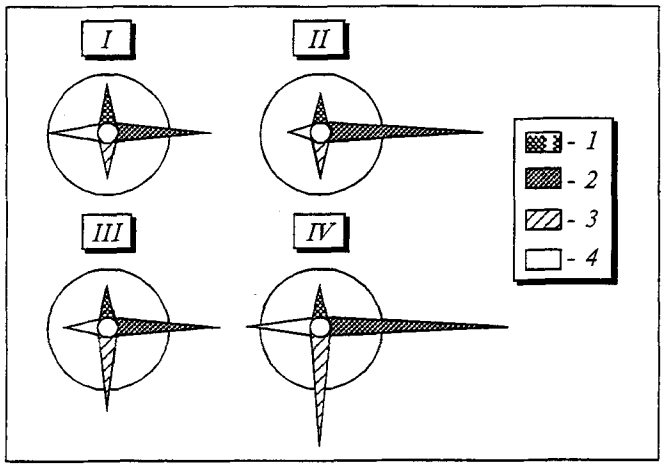

Fig. 1. The length of the radius reflects the ratio of mitosis phases in control animals. Ratio of prophases (*1*), metaphases (*2*), anaphases (*3*), and telophases (*4*), %. Ratio of mitosis phases: 4 (*I*) and 24 h (*II*) after injection of A10; 4 (*III*) and 24 h (*IV*) after injection of A43.

expressed in promille, ILN in percent, and LI by the mean number of tracks above the nucleus. A total of 75 animals were used in the experiment.

RESULTS

As in our previous experiments with DM, both analogs caused an increase of the mitogenic index in the corneal epithelium 4 h after drug injection. Results of experiments with colchicine confirmed the hypothesis that the increase of MI 4 h after DM injection is due to prolongation of mitosis proper. The MIC in experiments with A43 and colchicine reliably dropped 1.6-fold, and it showed a tendency to drop in experiments with A10. Further proof of mitosis slowdown was obtained in the results of investigation of the ratio of mitosis phases (Fig. 1). In experiments with A43 a reliable twofold reduction of the specific weight of prophases was observed 4 h after injection, with metaphases increasing 1.5-fold. Changes in the phase ratio after injection of A10 were unreliable at this point in the investigation.

The results of autoradiographic analysis once again demonstrate the depression of the proliferative processes under the influence of the studied synthetic DM analogs. ILN reliably decreased 4 h after injection of A10 and A43 by 1.9 and 1.4 times, respectively.

The cause of the MI increase 24 h after injection of A10 was the same as that 4 h postinjection, namely the extension of mitosis in time. Both the drop of MIC and the results of analysis of the ratio of mitosis phases confirm this. The specific weight of prophases in this experimental series (Fig. 1) declined 2.9-fold, and the content of metaphases increased 2.3-fold. No changes in the ILN or LI were observed in this period.

Our findings on the effects of synthetic DM analogs on mitosis in the lingual epithelium differed from the data of experiments with DM. DM exerted no noticeable effect on the proliferative processes in the tongue (the parameters of DNA synthesis remained stable at both times of investigation), whereas both analogs tested caused a reliable drop of ILN 4 and 24 h after agent administration (Table 1). After injection of A10 ILN decreased 1.4-fold 4 h postinjection and twofold 24 h postinjection. Administration of A43 resulted in a decrease of ILN by 1.5 and 1.7 times 4 and 24 h postinjection, respectively.

These data indicate that the depression of proliferation in the tongue is even more pronounced than in the cornea. A reliable drop of ILN was observed not only 4 h postinjection, as in the experiments with the cornea, but 24 h postinjection as well. The fact that the MI drop not always coincided with a drop of ILN may be explained by a change in the rate of mitosis, as occurred in the cornea.

Depression of the proliferative processes in the lingual epithelium under the influence of DM ana-

TABLE 1. Effect of DM on Cell Division Processes in the Corneal and Lingual Epithelium of Albino Rats

Group of animals	Time postinjection, h							
	4				24			
	MIC, ‰	MI, ‰	ILN, %	LI	MIC, ‰	MI, ‰	ILN, %	LI
				Cornea				
1 (control)	30.95±2.9	5.6±0.9	5.1±0.04	42.6±6.2	30.95±2.99	5.7±0.8	3.5±0.2	32.6±5.14
2 (DM A10)	24.3±2.8*	16.8±0.6*	2.7±0.3*	39.1±1.9	22.7±4.2	9.6±0.7*	3.5±0.5	42.83±5.42
3 (DM A43)	18.9±1.3*	15.6±1.2*	3.5±0.3*	40.3±3.9	34±2.43	6.2±0.9	3.36±0.4	33.67±4.11
				Tongue				
1 (control)	–	31.1±1.9	11.19±1.9	39.2±3.4		31.1±1.92	11.1±1.1	38.2±4.1
2 (DM A10)		21.9±1.2*	7.7±0.8*	35.1±3.6		20.8±0.65*	5.5±0.6*	41.1±5.3
3 (DM A43)		22.8±1.6*	7.6±0.5*	39.5±4.1		29.8±2.86	6.4±0.6*	40.1±4.6

Note. Asterisk shows reliable differences from the control.

logs merits special analysis. Let us recall that in our previous studies DM, while depressing mitosis in the corneal epithelium, had no appreciable effect on the proliferative processes in the tongue. Another selective agonist of μ-receptors, DAGO, like DM, depressed proliferation in the cornea, but at the same time activated proliferative processes in the lingual epithelium.

Some studies help explain the differences in the ratio of μ- and δ-opioid activities of DM and its analogs [4]. Besides μ-opioid activity, DM is characterized by a certain activity towards δ-receptors. The same properties are intrinsic to DAGO. A10 and A43 have at least one order of magnitude less binding to δ-receptors in comparison with DAGO and DM. Our previous studies showed that the δ-receptor agonist dalargin markedly stimulates the proliferative processes.

The results of the present study indicate that the tested DM analogs, μ-receptor agonists, have an inhibitory effect on cell division.

A10 and A43 are analogs of an endogenous ligand of opiate receptors. The results not only confirmed the capacity of DM to exert an inhibitory effect, but demonstrated the possibility of extending the spectrum of the inhibitory effect in parallel with a reduction of the capacity to bind to δ-receptors. Hence, there are two subsystems in the family of opioid peptides, one of which (δ-agonists) stimulates the mitotic processes in the epithelium, and the other (μ-agonists) inhibits them.

REFERENCES

1. G. M. Kalivetskaya, S. S. Timoshin, and A. L. Lykov, *Byull. Eksp. Biol. Med.*, **93**, № 4, 92 (1982).
2. T. D. Pan'kova and S. S. Timoshin, *Ibid.*, **110**, № 7, 96 (1990).
3. M. I. Radivoz, E. I. Mel'nik, S. S. Timoshin, *et al.*, *Ibid.*, **112**, № 8, 162 (1991).
4. E. P. Yarova and V. I. Deigin, "Synthesis and study of the activity of dermorphin and its analogs", in: *Proc. All-Union Symposium on Peptide Biochemistry* [in Russian], Riga (1992), p. 70.