EFFECT OF DERMORPHINE ON CELL DIVISION IN THE CORNEAL AND LINGUAL EPITHELIUM OF ALBINO RATS

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Endogenous ligands of opiate receptors and their synthetic analogs stimulate cell division in vivo [1, 3, 4, 9]. It was decided to study the character of the effect of dermorphine, a paraopioid derivative [2], on cell division in the albino rat corneal and lingual epithelium. The study of this problem is of great applied importance in connection with the development of drugs based on dermorphine.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 180-200 g. Three groups of animals were used in the experiments. The rats of control group 1 received an intraperitoneal injection of 0.9% sodium chloride solution, animals of group 2 received dermorphine by the same method in a single dose of 10 μg/kg 4 or 24 h before sacrifice, and the rats of group 3 received a dermorphine antagonist in a single dose of 10 μg/kg 4 and 24 h before sacrifice. The rats were given an intraperitoneal injection of ^{3}H-thymidine with specific activity of 87 Ci/mmole, in a dose of 0.6 μCi/g body weight 1 h before sacrifice. In addition, 0.02 ml of a solution of ^{3}H-thymidine (2 μCi/ml) was applied to the cornea. The animals were killed humanely between 12 noon and 1 p.m. Histological sections of the cornea and tongue, and autoradiographs were prepared; the mitotic index (MI), index of labeled nuclei (ILN), and the labeling intensity (LI) were determined by the usual laboratory methods. MI was expressed in promille, ILN in per cent, and LI as the average number of tracks above the nucleus. Altogether 49 animals were used. Dermorphine and its antagonist were synthesized in the Laboratory of Peptide Chemistry, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR.

EXPERIMENTAL RESULTS

The results show that 4 h after injection of dermorphine ILN was significantly reduced (by 1.6 times; Table 1) in the corneal epithelium compared with the control. LI, which reflects the rate of DNA synthesis, showed no significant changes. Lowering of ILN was accompanied by a paradoxical increase of 1.5 times in MI. This phenomenon can be explained either by lengthening of the duration of mitosis itself or by the ability of dermorphine, not only to reduce ILN, but also to accelerate passage of the cells through the premitotic period. The results of the experiments with the dermorphine antagonist confirm the regular character of the increase in MI following injection of the paraopioid peptide. The dermorphine antagonist caused a decrease in MI, which may have been the result of deprivation of the stimulating action of endogenous dermorphine on the premitotic period. No changes in ILN were observed under these circumstances. MI in the corneal epithelium of the experimental animals 24 h after injection of dermorphine was significantly lower (by 1.5 times) than the control parameters. This decrease was evidently determined by reduction of ILN, which took place in response to

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TABLE 1. Effect of Dermorphine on Cell Division in Corneal and Lingual Epithelium of Albino Rats

Group of animals	Time after injection of substance, h					
	4			24		
	MI, %	ILN, %	LI	MI, %	ILN, %	LI, mean number of tracks above
			Cornea			
1- Dermorphine	12,6±1,13	10,4±0,73	13,0±1,0	12,6±1,13	10,4±0,73	13,0±1,0
2-Dermorphine antagonist	18,5±0,96*	6,5±0,26*	12,0±0,87	8,3±0,32*	5,7±0,23*	12,6±1,1
3- Dermorphine antagonist	7,1±0,69*	11,6±0,7	16,0±2,9	9,8±0,82	10,8±0,75	17,7±1,4
			Tongue			
1 Dermorphine	3,1±1,3	6,6±0,4	28,3±1,8	3,1±0,3	6,6±0,4	28,3±1,8
2- **Dermophine**	7,5±0,52*	7,2±0,4	29,4±0,9	2,3±0,22	4,5±0,3	24,3±1,4
3- Dermorphine antagonist	5,7±0,57	7,2±0,3	31,4±1,7	4,0±0,28	6,75±0,4	23,5±1,5

Legend. * indicates statistically significant differences compared with control.

injection of dermorphine 4 h before sacrifice. Just as in the previous group of experiments, a decrease in the number of DNA-synthesizing nuclei was observed. ILN in the experimental animals was 1.8 times lower than in the control. No significant changes were observed in LI. The dermorphine antagonist caused no significant changes in the parameters of proliferation at this stage of the investigation.

A twofold increase in MI was observed in the lingual epithelium 4 h after injection of dermorphine. Injection of the antagonist also was followed by a significant increase (by 1.8 times) in MI. No changes were observed in the parameters of DNA synthesis in either case. No significant changes in the parameters of cell division likewise were observed in the lingual epithelium 24 h after injection of dermorphine.

Injection of dermorphine thus led to a brief increase of mitotic activity in the corneal and lingual epithelium of albino rats 4 h after its injection. This stimulation of cell division was evidently due to the more rapid passage of the cells through the G_2-period. There is also an important difference in the reaction of proliferative processes in the corneal and lingual epithelium to injection of dermorphine. In the corneal epithelium, besides transient stimulation of mitotic activity, dermorphine also induced a lasting (for 24 h) inhibition of DNA synthesis, whereas in the lingual epithelium DNA synthesis was not significantly changed. The response of proliferative processes to injection of dermorphine, which occurred in the corneal epithelium, differed from the pattern which we observed previously after injection of endorphins and enkephalins, which caused activation of cell division and DNA synthesis [1, 3, 4]. One possible explanation of the difference in the character of response of proliferative processes to dermorphins, endorphins, and enkephalins may be the fact that dermorphins are selective agonists of mu-receptors [8], whereas dalargin reacts mainly with delta-receptors [7]. In this connection there are some interesting data [11, 12] showing inhibition of cell division in a neuroblastoma, and also in cerebellar neurons of six-day-old rats under the influence of the mu-receptor agonist met-enkephalin. Information on the inhibitory effect of methadone and morphine — mu-receptor agonists — is given in [10]. The cause of the increase in MI 4 h after injection of dermorphine, observed in the corneal and lingual epithelium, remains unclear. Evidence of the regular occurrence of this phenomenon is given by the opposite effect, namely reduction of MI in response to injection of the dermorphine antagonist. The question of absence of correlation between the action of dermorphine and its antagonist on cell division in other groups of experiments requires further examination. One possible explanation of this phenomenon, with particular reference to naloxone, was given by the writers previously [5]. The doses of the antagonist must also be analyzed and its stability and efficacy evaluated. The absence of an inhibitory effect in the lingual epithelium under the influence of dermorphine can be explained on the grounds that the mu-receptor population in it is extremely small. In the generally accepted view, mu-receptors are more closely related to pain perception, whereas delta-receptors are involved in the regulation of visceral functions [6]. The opinion is held [12] that opiate receptors, mediating the action of endorphins and enkephalins on proliferation, differ in their properties from those generally known. The authors cited suggest calling them zeta-receptors, from the greek word "zoe" meaning life [12].

The results of this investigation show that the paraopioid substance dermorphine inhibits DNA synthesis in the corneal epithelium 4 and 24 h after its injection, and also reduces MI 24 h after injection. An increase in MI in the corneal and lingual epithelium took place 4 h after injection of dermorphine. To explain the role of the different subpopulations of opiate receptors in the regulation of cell division further investigations are needed.

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ADRENALIN UPTAKE BY RAT BRAIN SYNAPTOSOMES: EFFECT OF PSYCHOTROPIC DRUGS

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Investigations have shown that adrenalin (AD) may perhaps play the role of neurotransmitter in the CNS. The following facts may be regarded as proof of this statement: 1) phenylethanolamine-methyltransferase, which synthesizes AD [6], is found in mammalian brain neurons; 2) 6-hydroxydopamine lowers the concentrations of noradrenalin (NA) and AD in the hypothalamus [14]; 3) besides inducing exhaustion of vesicular NA and dopamine (DA), reserpine also reduces the brain AD content [13]; 4) depolarization of neurons in the hypothalamus (potassium, veratridine) leads to release of AD from that structure [5]; 5) a receptor for AD, linked with adenylate cyclase, is found in the brain [15]. Thus AD is synthetized, stored in nerve endings of the brain, and released from them under the influence of depolarization, in agreement with the criteria defining a neurotransmitter. The problem of its inactivation still remains unclear. It can be tentatively suggested that AD, like other monoamines and neurotransmitter amino acids, is inactivated in the synaptic space by reuptake into nerve endings.

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