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Effect of Sedatin, a Synthetic Dermorphin Analog, on Cell Division in the Corneal and Lingual Epithelia of Rats

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Sedatin, an analog of the opioid peptide dermorphin, which is a mixed μ- and δ-receptor agonist, had no effect on DNA synthesis and mitotic activity in the corneal or lingual epithelium of rats in a single dose 10 μg/kg, but stimulated cell division in both epithelia in a single dose of 100 μg/kg and in the 10 μg/kg dose administered either once daily over a 21-day period or just once after a single injection of the opiate receptor antagonist naloxone.

Key Words: *dermorphin; DNA synthesis; cell division*

As shown in our earlier study [3], the synthetic analogs of dermorphin that are superselective μ-receptor agonists inhibit cell division in corneal and lingual epithelia. One of those analogs, provisionally called sedatin, also displayed a high affinity for δ-receptors as compared to the previously tested dermorphin analogs [4]. The biological properties of sedatin, which is thus a mixed μ- and δ-receptor agonist, are currently being explored. We have also shown [2] that ligands for different receptor subpopulations exert differential effects on cell proliferation. Thus, the μ-receptor agonists DAGO and dermorphin inhibit DNA synthesis in corneal epithelium, whereas dalargin and DADL, which preferentially bind to δ-receptors, stimulate cell division in a number of epithelia.

The present study was undertaken to test the dermorphin analog sedatin for its effects on cell division processes in corneal and lingual epithelia (for it was not possible to decide *a priori* how it would influence cell proliferation). We hoped that the results of such a study would be of help in the projected development of pharmaceutical preparations based on this bioactive peptide.

MATERIALS AND METHODS

The study was conducted on 115 male rats weighing 160-190 g. The dermorphin analog sedatin used was

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TABLE 1. Effect of a Single Intraperitoneal Injection of Sedatin at 10 μg/kg on Cell Proliferation in Corneal Epithelium

Time, h	Group	MI, ‰	NLI, %	LI, %
4	Control	8.5±0.75	10.4±1.17	26.6±1.45
	Test	7.7±1.20	9.6±1.05	25.6±1.23
24	Control	3.3±0.44	7.5±0.74	27.0±0.94
	Test	3.3±0.79	7.7±0.57	26.5±1.09

TABLE 2. Effects of a Single Intraperitoneal Injection of Sedatin at 100 μg/kg on Cell Proliferation in Corneal and Lingual Epithelia

Time, h	Group	Corneal epithelium			Lingual epithelium	
		MI, ‰	NLI, %	LI	NLI, %	LI
4	Control	4.5±0.75	13.1±0.88	18.0±1.21	12.9±0.79	45.2±1.74
	Test	7.4±0.84*	29.6±3.06*	17.7±0.75	16.2±0.52*	47.2±2.50
24	Control	7.3±0.21	8.0±1.45	15.4±2.04	13.5±0.45	47.3±1.71
	Test	10.5±1.30*	11.4±1.08*	17.2±0.37	17.2±0.22*	45.1±2.01

Note. Here and in Tables 3-5 the asterisk denotes a significant difference from the control (NaCl-treated) group.

synthesized in the Laboratory of Peptide Synthesis at the Cardiology Research Center, Moscow [4].

In single-dose tests, sedatin was administered intraperitoneally at 10 or 100 μg/kg body weight; rats injected with an equivalent volume of NaCl solution by the same route served as controls. Some rats were also given naloxone (Endo Laboratories) at 200 μg/kg intraperitoneally 20 min before sedatin in order to block the opiate receptors. At 4 h and 24 h after sedatin injection, 7-10 rats from the test and control groups were sacrificed to remove the corneal and lingual epithelia for examination.

In multiple-dose (chronic) tests, rats received daily intraperitoneal injections of sedatin at 10 μg/kg for a total of 21 days. These tests included two control groups, one of which consisted of intact rats and the other of rats injected with a 0.9% isotonic NaCl solution intraperitoneally over the same period. The rats of these three groups were sacrified 24 h after the last injection.

One hour before sacrifice, all rats received ^{3}H-thymidine with a specific activity of 1530 TBq/mol in an intraperitoneal dose of 0.6 μCi/g body weight and in a dose of 2 μCi applied to the cornea.

The procedures used to obtain histological preparations and autoradiographs of corneal and lingual epithelia and to determine the mitotic index (MI), nuclear labeling index (NLI), and labeling intensity (LI) were those commonly employed in our laboratory. The MI was expressed in promille, the NLI in percent, and the LI as the mean number of tracks over the nucleus.

The results were analyzed by Student's t test, setting statistical significance at the $p<0.05$ level.

RESULTS

The sedatin dose of 10 μg/kg had no effect on corneal cell proliferation at 4 or 24 h postinjection, as is indicated by the MI, NLI, and LI values in Table 1. The 100 μg/kg dose stimulated cell division in both epithelia (Table 2), with a 1.6-fold increase in the MI and a 2.3-fold increase in the NLI by hour 4 and a 1.4-fold increase in these two indexes by hour 24 in the corneal epithelium and 1.4- and 1.3-fold increases in the NLI by hours 4 and 24, respectively, in the lingual epithelium; the epithelial LI was not changed appreciably at either 4 or 24 h.

Injecting sedatin at 10 μg/kg 20 min after the opiate receptor blocker naloxone gave results similar to those recorded after the 100 μg/kg dose. Thus, unlike in the rats given sedatin alone, both the MI and NLI significantly increased 2.2-fold and 1.3-fold, respectively (Table 3).

DNA synthesis in the corneal and lingual epithelia was also stimulated by sedatin in the chronic tests, where it was injected at 10 μg/kg once daily for 21 days. As shown in Table 4, the sedatin-treated group has significantly higher NLI values in the two epithelia than the intact and NaCl-treated groups (1.6 and 1.5 times higher, respectively, in the corneal epithelium and 1.9 and 1.6 times higher in the lingual). LI values do not differ appreciably between the groups, while the MI in the corneal epithelium of the sedatin-treated group is much lower than in the other two groups. The significant fall in the MI can be explained

TABLE 3. Effect of a Single Intraperitoneal Injection of Sedatin at 10 μg/kg after Naloxone Injection at 200 μg/kg by the Same Route on Cell Proliferation in Corneal Epithelium

Group	MI, ‰	NLI, %	LI
Control	3.3±0.18	7.5±1.03	35.1±2.42
Naloxone+Sedatin	7.2±0.79*	10.1±1.12*	40.4±3.15
Sedatin	3.3±0.18	7.7±2.07	37.2±1.37

TABLE 4. Effect of Chronic Intraperitoneal (21-Day) Administration of Sedatin at 10 μg/kg on Cell Proliferation in Corneal and Lingual Epithelia

Group	Corneal epithelium			Lingual epithelium	
	MI, ‰	NLI, %	LI	NLI, %	LI
NaCl-treated	4.5±0.84	8.4±0.42	20.1±1.45	8.4±0.82	22.6±2.4
Test	2.5±0.37*	12.5±0.34*	18.5±0.8	13.5±0.35*	23.6±1.7
Intact	5.3±0.49	7.8±0.27	23.0±2.62	7.2±0.53	21.6±1.26

by the accelerated rate of mitosis under the influence of sedatin. We observed a similar decrease of this index in our earlier experiment with colchicine.

Sedatin also stimulated body weight gains with chronic administration (Table 5). Thus, the total and daily weight gains in the sedatin-treated group were similar to those in the intact controls, but 1.3 times greater than in the NaCl-treated group, where daily injections of the isotonic NaCl solution led to a 40% loss of body weight, apparently as a result of the stress produced by this intervention.

We interpret the maintenance of body weight gains by sedatin-treated animals at the control level as a manifestation of its antistress activity — an activity which is characteristic of opioid peptides in general.

The results from studies of dermorphin and its analogs with different affinities for μ- and δ-receptors have enabled us to explain the ultimate effects of these ligands on cell division. Dermorphin analogs A10 and A43, which have lower affinities for δ-receptors than dermorphin, produce greater and more intense inhibitory effects [3]. Sedatin has the same affinity for μ-receptors as A10 and A43, but a much higher affinity for δ-receptors than these. Sedatin in the dose of 10 μg/kg had no effect on cell proliferation in the cornea, whereas A10 and dermorphin inhibited it at this dose level. The interaction of sedatin with both receptor subpopulations (μ and δ) appears to have resulted in its inhibitory effect being balanced by its stimulatory activity. This balance was upset in favor of the stimulatory effect when sedatin was administered in a much higher dose (100 μg/kg) or at 10 μg/kg but after naloxone. Raising the dosage of opioid peptides that are mixed μ- and δ-receptor agonists is generally believed to result in increased numbers of low-affinity bonds which are typical of δ-receptors. Such an increase may also occur with chronic administration of sedatin. Of interest in this context is the finding that dalargin, a peptide preferentially interacting with δ-receptors, increasingly activated DNA synthesis in the cornea as its dose was raised from 0.1 μg/kg to 100 μg/kg [1].

TABLE 5. Effect of Chronic (21-Day) Intraperitoneal Administration of Sedatin at 10 μg/kg on Body Weight in Rats

Group	Weight gain	
	total	daily
Control	43.3±3.33**	2.1±0.16**
Test	55.0±3.44*	2.6±0.16*
Intact	60.0±3.42	2.9±0.16

Note. **Significant difference from the intact group.

As regards the stimulation of corneal cell proliferation by sedatin injected at 10 μg/kg after naloxone, it should be noted that since the affinity of the latter agonist for μ-receptors is approximately 20 times that for δ-receptors [6], the predominant blocking of μ-receptors by it probably augmented sedatin's ability to exert its stimulatory effect via δ-receptors.

In seeking to explain the differential effects produced by agonists of different opiate receptor subpopulations, we took into account published data indicating that the response of the system of cyclic nucleotides and Ca^{2+} fluxes to μ agonists is the opposite of its response to δ agonists [5,7]. In order to understand why cell division was stimulated by sedatin in the chronic tests, the reported high degree of homology between opiate receptors and factors of cell adhesion and contact needs to be taken into consideration [8]. Thus, the long-continued exposure to sedatin could have altered the adhesive cell properties, which play an important part in the regulation of cell proliferation.

In conclusion, our observation that the effects of such a mixed μ and δ agonist as sedatin on proliferation processes depends on the size and number of its doses helps explain the modulating influences of opiate receptor ligands on these processes.

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