EXPERIMENTAL BIOLOGY

Diurnal Rhythm of Cell Proliferation in the Lingual Epithelium of Mice Following Sialoadenectomy

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The biological role of epidermal growth factor (EGF) is currently the subject of intense study. Since it was first isolated from submandibular glands of mice by Cohen in 1962 [7,11], this growth factor been found to be synthesized in other tissues as well. The diversity of its synthetic sites, as well as the discovery that it also performs other functions in addition to being mitogenic, prompted investigators to undertake studies to define the place of EGF among biological regulators [1]. The biological role of EGF is difficult to assess because it elicits responses from cells of various origin, including keratinocytes [9], endothelial cells [8], smooth-muscle cells [6], hepatocytes, and fibroblasts, the last showing the greatest mitogenic activity in response to EGF [12]. In some studies, however, EGF was found not to induce mitosis in the target cells.

There are also conflicting reports of how much EGF is contained in tissues or organs. For example, in some studies rat liver was reported to be an organ rich in EGF [1], whereas in others the factor was found to be present in the liver of adult rats in concentrations too low to be detectable [2]. An important consideration for characterizing the physiological effects of EGF is that its receptors show affinity for the transforming growth factor p. However, the presence of many EGF receptors on the cell surface does not necessarily result in enhanced proliferation of the cells when they are acted upon by EGF [4]. Possibly, the conflicting evidence reported in the literature is due in part to the fact that most of the studies designed to identify biological effects of EGF used cell or organ cultures. Since cellular responses in culture may differ substantially from those *in vivo*, we deemed it desirable to evaluate the role EGF plays in establishing the diurnal rhythm of cell division in animal tissues, given that the regulatory role of a biologically active factor is manifested in its participation in the temporal organization of biological processes [5].

MATERIALS AND METHODS

Male CBA mice weighing 18-20 g had their submandibular glands removed under ether anesthesia - an operation that results in drastically reduced EGF concentrations in the blood [3]. After two weeks, the operated and control (intact) mice were injected with colchicine in a dose of 3 mg/kg body weight at 3-h intervals and killed 3 h later. One hour before sacrifice they were injected with ^{3}H-

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thymidine in a dose of 40 MBq/kg. Histological sections of their tongues 5-7 μ in thickness were prepared, covered with a photoemulsion of the M type (manufactured by the Research Institute Khimfotoproekt, Russia), developed, and stained, after which the number of labeled nuclei was counted in the basal epithelium of the upper lingual surface and the radioactivity index (RI) was calculated. The mitotic index (MI) was determined by adding the prophase index to the number of c-mitoses. The RI and MI were both expressed in pro milles (per thousand cells).

RESULTS

As follows from Table 1, the number of labeled nuclei in control mice was lowest between 10:00 and 16:00 h, higher between 19:00 and 22:00 h ($p<0.01$), and highest between 1:00 and 4:00 h. In test mice, the indexes of DNA synthesis in the lingual epithelium did not differ from those in control mice at the times when DNA synthesis in the latter mice declined (10:00 h) or was minimal (13:00 h). In contrast, after DNA synthesis started to increase in control mice, test mice exhibited lower levels of DNA synthesis than did control mice: at 16:00 and 19:00 h and at 4:00 and 7:00 h the number of their labeled nuclei was 25-35% lower. As a result, the amplitude of variation in RI during the diurnal rhythm in test mice was 3.1 vs. 3.7 in controls. The mean diurnal RI amounted to only 88.4% of its control level.

A similar but better defined pattern was noted in the diurnal rhythm of mitotic activity (Fig. 1). In test mice, the amplitude of variation in MI was 10.1 vs. 19.2 in controls and, as seen in the figure, the curve describing the rhythm was smoother.

In control mice, the proportion of cells that began to divide during the 24-h period was, on average, 21.93% of all cells, i.e., the time of epithelial renewal equaled 4.5 days. In test mice, which had lowered EGF concentrations in their blood, the number of cells that started to divide over that period was much lower, amounting to only 11.35% of all cells, or 51.7% of the control level, and the time of epithelial renewal was correspondingly longer (8.8 days). This indicates that when the EGF level is lowered, the processes of physiological regeneration proceed at slower rates.

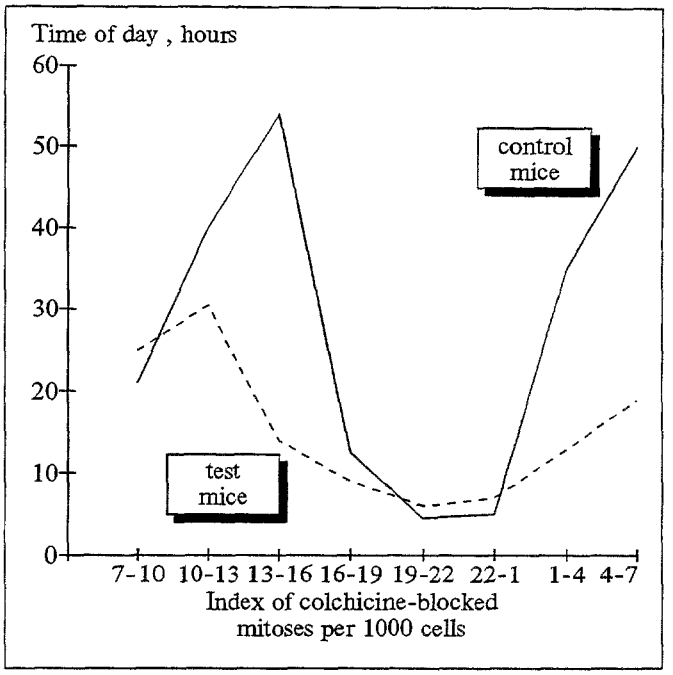

Fig. 1. Diurnal rhythm of mitotic activity in epithelium of upper lingual surface.

It is noteworthy that the level of mitotic activity in the test mice decreased to a greater extent than the level of DNA synthesis. This may be attributed to the fact that, in our experiments, all dividing cells were recorded by virtue of their being blocked with colchicine, so that the possible

TABLE 1. Mitotic Activity (MI) and DNA Synthesis (RI) in Epithelium of Upper Lingual Surface in Sialoadenectomized Mice. The Values are Means±SEM Expressed per Thousand Cells

Time of day (h)	Control mice		Test mice	
	MI	RI	MI	RI
10:00	60.78±3.53	21.35±0.91	64.46±7.65	24.40±3.71
13:00	42.87±3.05	38.73±2.14	44.41±2.38	30.50±4.21
16:00	68.41±5.90	54.35±4.81	44.91±2.59*	13.16±0.66*
19:00	83.02±7.75	12.44±0.52	59.52±6.78*	5.74±1.96*
22:00	83.57±4.23	2.83±0.27	91.27±2.29	3.01±0.91
1:00	133.50±6.38	4.85±0.91	137.11±2.28	6.57±1.11
4:00	158.73±6.43	35.70±3.79	119.96±6.44*	10.33±1.25*
7:00	118.39±4.01	49.18±4.32	100.76±3.70*	19.81±2.97*
Mean diurnal values	93.66	27.42	82.80 (88.4% of control)	14.19

Note. * Significant difference from control mice at $p<0.05$.

influence of EGF deficit on the mitotic rate was eliminated. It may be that the relatively high proportion of nuclei that incorporated ^{3}H-thymidine in the test mice was due to delayed DNA synthesis. Further studies are necessary to check this possibility.

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Optimization of the Process of Instrumental Conditioning with a Low Intensity of Conditioned Stimulus

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The influence of the probability of a random correct instrumental response (PRCR) on the rate of conditioned-reflex for different modes of reinforcement was previously shown by us [2,3]. The number of instrumental responses in the initial stage of training which are performed in connection with the conditioned signal and which are correspondingly reinforced depends on the level of PRCR. This in turn influences the interplay of information between the individual and the environment and, ultimately, the rate of reflex formation. It has been established that there are optimal and pessimal values of PRCR [2,3]; some mechanisms of the corresponding modulation of tentative-exploratory activity have been discovered [4,5], and a method of theoretical assessement of PRCR has been developed [1,5]. At the same time, all the above-mentioned studies were carried out under conditions of quite a high intensity of conditioned stimuli, known to exceed the threshold of sensory perception.

The aim of the present study was to investigate the possibility of optimizing the learning process at a low (near-threshold) intensity of the conditioned signal by selecting the appropriate PRCR value.

MATERIALS AND METHODS

Experiments were carried out on nonpedigree male rats weighing 250-310 g. The animals were as-

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