

DIURNAL VARIATION IN THE EFFECT OF PYROGENAL ON MITOTIC ACTIVITY IN THE CORNEAL EPITHELIUM

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The effect of intravenous injection of pyrogenal at various times of day and night on the mitotic activity of the corneal epithelium was studied in albino rats. The animals were killed 6 h (series I) and 24 h (series II) after injection of the substance. In both groups of experiments the results were basically similar. If injected during the morning (7 a.m.), pyrogenal stimulates cell division, but if injected during the evening (6 p.m.) or at midnight, it inhibited cell division. No changes in mitotic activity of the cells were found when pyrogenal was injected during the day (noon or 1 p.m.).

Many biologically active substances are known to produce different effects on cell division depending on the time of day or night. This has been observed by several workers who have studied the action of growth hormone [15], adrenalin [10], sarcocollin [5], cyclophosphamide [3], and so on. Determination of the optimal time for obtaining the maximal effect from administration of a preparation is of considerable interest to practical medicine.

The object of this investigation was to determine the effect of pyrogenal, which inhibits cell division in the epithelium [11, 12], when administered at different times of day and night.

EXPERIMENTAL METHOD

Noninbred female albino rats weighing 110-160 g were used. Pyrogenal (5 μ g/100 g body weight) was injected into the caudal vein. Animals receiving intravenous injections of physiological saline in equal volumes were used as the control. There were two series of experiments: in series I the animals were killed in the morning (7 a.m.), afternoon (noon, 1 p.m.), evening (6 p.m.), and night (midnight), 6 h after injection of the pyrogenal; in series II they were killed at 7 a.m. and 6 p.m., 24 h after injection of the pyrogenal. Each group of experiments, in which the pyrogenal was injected at different times of day and night, included 13-16 animals. Altogether 104 experiments were carried out. A total preparation was obtained from the cornea and stained with trioxymethine. Mitotic activity was determined by counting the number of mitoses in 100 fields of vision. The total number of mitoses was expressed in absolute figures and the ratio between the phases of mitosis in percent. Statistical analysis of the results was carried out by the Fisher-Student method.

EXPERIMENTAL RESULTS

The experiments showed marked fluctuations in mitotic activity in the cornea of the control animals during the 24-hour period, with a maximum in the morning and a minimum at night. Pyrogenal was found to affect the processes of cell division differently when given at different times of day or night (Fig. 1: D). In the experiments of series I, injection of pyrogenal at night (1 a.m.) led to a significant ($P < 0.02$) increase in mitotic activity at 7 a.m. The increase in the total number of dividing cells was not accompanied

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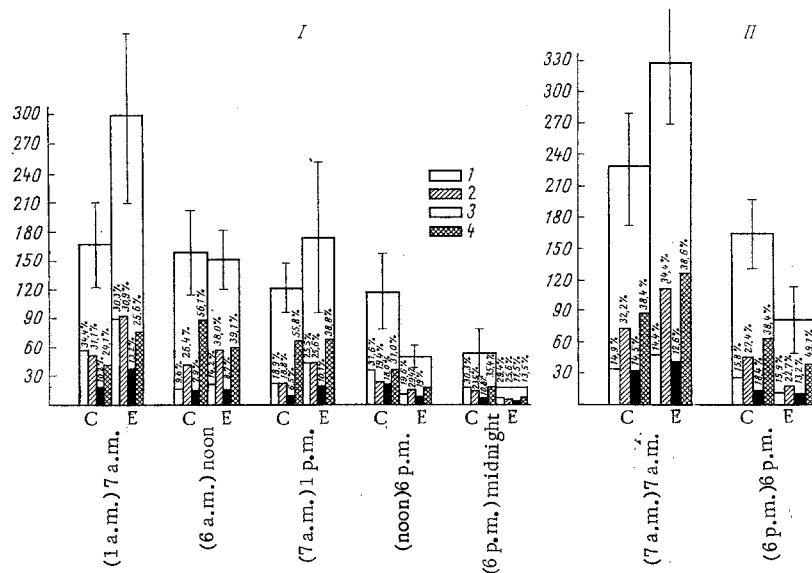


Fig. 1. Effect of pyrogenal on mitotic activity of the corneal epithelium after 6 h (I) and 24 h (II). C) control; E) experiment; 1) pro-phases; 2) metaphases; 3) anaphases; 4) telophases (in % of total number of mitoses). Ordinate, number of mitoses; abscissa, time of injection of pyrogenal (in parentheses) and time of sacrifice of animals.

by any change in the ratio between the phase of mitosis. Injection of pyrogenal in the morning (6, 7 a.m.) caused no significant changes in mitotic activity. When pyrogenal was given in the afternoon and evening a significant ($P < 0.03$) depression of mitotic activity was observed. The decrease in the total number of mitoses at 6 p.m. and midnight took place on account of a decrease in the number of pro-phases and meta-phases, evidence of delay in the commencement of mitosis by the cells.

The results of the experiments of series II (Fig. 1; II) showed that after longer exposure to pyrogenal its effect still depended on the time of its injection. Injection of pyrogenal at 6 p.m. had the result that 24 h later cell division was still depressed: the total number of mitoses in the experimental group was 50% less than in the control ($P < 0.02$). A statistically significant ($P < 0.02$) increase in the number of mitoses in the experimental animals compared with the control was observed 24 h after injection of pyrogenal in the morning (7 a.m.).

The variations in the response of the corneal epithelium to injection of pyrogenal in the morning and evening could be due to changes in reactivity of either the neuroendocrine system or the corneal cells during the 24-hour period. Pyrogenal is known to increase the secretion of hormones of the adrenal cortex and medulla [4, 7, 8], which are inhibitors of cell division [1, 2, 13, 14]. It is interesting to note that injection of pyrogenal into adrenalectomized rats and also into animals with adrenal insufficiency, even in the afternoon does not inhibit mitotic activity but actually stimulates it [12].

The absence of inhibition of mitotic activity after injection of pyrogenal in the morning may be partly explained by the fact that injection of ACTH in the morning does not increase the secretion of 11-hydroxy-ketosteroids in albino mice [6]. However, injection of pyrogenal in man, at whatever time of day or night, always increased the secretion of corticosteroids, although by a different degree [16].

The correlation between the absence of inhibition by pyrogenal when injected in the morning and changes in reactivity of the corneal cells is shown by observations that much larger doses of actinomycin D are required in the morning than in the afternoon to produce an antimetabolic effect.

The nature of the increase in mitotic activity during its morning peak under the influence of a stressor requires special study.

Pyrogenal and its analogs are used nowadays for the treatment of many diseases, especially psoriasis. One of the factors in the therapeutic effect of pyrogenal in the treatment of this disease is evidently the in-

hibition of the increased mitotic activity of the epithelium [9, 12]. The results described in this paper are evidence that for the optimal effect of pyrogenal to be obtained it should be injected at definite times of day or night.

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