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Control of Cell Division in the Cornea of Rats
I. Interaction Between Isoproterenol And Dexamethasone[1] (38332)

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We have recently demonstrated that dexamethasone will (a) enhance the circadian synchronization of mitoses when administered at 1500 hr; (b) suppress mitoses when administered 2300 hr of the day (1). To assess the possible dependence of these effects upon a preexisting circadian mitotic rhythm, additional studies were carried out with the use of newborn rats in which, characteristically, a circadian rhythm does not develop before the 3rd week after birth (2, 3).

These studies revealed that the administration of dexamethasone at 1500 hr of either Day 11 or Day 12 after birth induces the appearance of an apparently normal circadian mitotic rhythm 2–4 days earlier than in normal animals (4). The data also indicate that dexamethasone can alter the circadian distribution of mitoses in the absence of a preestablished circadian mitotic rhythm.

How dexamethasone exerts the above effects is not known. However, a relationship has been suggested to exist between the prolonged suppression of mitoses and the steroid-produced inhibition of the hypothalamus–pituitary–adrenal exis (HPAA) (1). To test this proposition, an attempt was made to restore mitotic activity in the dexamethasone-suppressed animals with the administration of either pituitary hormones, glucosteroids, or a combination of both, at the time of the expected inhibition of the HPAA. Part of the results obtained in these studies are presented in this communication.

Materials and Methods. The following method was routinely used in these studies to suppress mitoses. Male Holtzman rats with weight ranging from 110 to 140 g were housed (six/cage) in a windowless room with temperature maintained at 23° ± 1°. Artificial lights were kept on from 0600 to 1800 hr. Purina lab chow and water were available *ad lib.* The animals were kept in this environment for at least 1 wk prior to their use in the experiments. A single 0.4-mg dose of dexamethasone[2] was administered to the appropriate groups of rats by the intraperitoneal route (ip), at 2300 hr of the day. The first group of rats was sacrificed 6–8 hr after drug administration, at 0500–0700 hr of the day. Subsequent groups of rats were sacrificed at 4-hr intervals and more often on Days 2 and 3 as the experiments required. The animals were sacrificed by decapitation. The corneas were collected, fixed, stained, and mounted for mitotic determinations as previously described (1). The above treatment (see Fig. 1) leads to reproducible alterations in the circadian distribution of mitoses in the corneal epithelium of the experimental animals with one outstanding characteristic: suppression of the second circadian mitotic cycle which follows the administration of the steroid. This suppressed mitotic cycle referred to in the remaining part of this work as suppressed circadian mitotic cycle (SCMC) is the focus of the experimental work presented below.

As shown in Fig. 1, the overall changes in the circadian mitotic cycles produced by the administration of a single 0.4-mg dose of dexamethasone were as follows: *first cycle* decreased levels of mitotic activity at 0700, 1100, and 1500 hr, without phase shift, *i.e.*, mitotic peak remained at 0700 hr; *second cycle* (SCMC), intensive suppression of mitoses with phase shift, *i.e.*, 6 to 8-hr delay of the remnant circadian mitotic peak; *third cycle* increased levels of mitotic activity above controls with a

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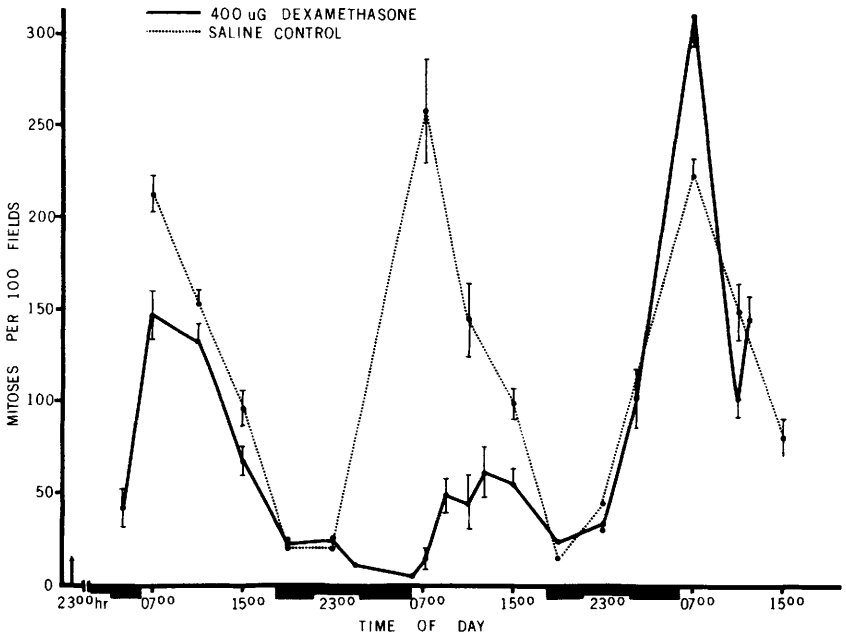

FIG. 1. The effect of dexamethasone (0.4 mg/ip) administered at 2300 hr (arrow in the Figure) upon mitosis in the cornea of rats. The results represent the means (six or more animals/mean) number of mitoses ± standard error of the mean/100 microscopic fields, as found in dexamethasone and saline-treated rats. The shaded area under the abcissa represents the hr of darkness of a lighting regimen in which the lights were kept on from 0600 to 1800 hr.

return of the circadian mitotic peak to 0700 hr.

With the use of the above system for creating SCMC, the animals were then treated with either a second dose of dexamethasone, or growth hormone, or isoproterenol, or certain combinations of the above, as described below in Table I.

Experimental. In previous work we interpreted the suppression (SCMC) seen after dexamethasone (Fig. 1) as a delayed effect possibly related to suppression of the hypothalamus–pituitary–adrenal axis (1). From such an interpretation, replacement of the suppressed glucosteroids and/or ACTH, would be expected to lead to partial or total reestablishment of mitotic activity in the SCMC. To test this hypothesis animals were treated with an additional dose of dexamethasone at 1500 hr of the immediate day after the first dose (period of the day at which maximum levels of corticosterone are found in the plasma of normal animals). The results obtained are presented in Fig 2 below and in Table I, Group B.

The results obtained did not show any increase in mitotic activity (Table I, Group B, and Fig. 2 above) and could be interpreted as due to: (a) inadequate dose or inappropriate time for the administration of dexamethasone; (b) ineffectiveness of dexamethasone (under the above conditions) as substitute for corticosterone; (c) suppression (by the first dose of dexamethasone) of hormones and/or factors other than those of the adrenal–pituitary axis (5, 6); (d) alteration of tissue levels of cyclic AMP.

Altered levels of cyclic AMP could be secondary to dexamethasone suppression of the pituitary gland, as pituitary hormones exert their effect through the adenyl cyclase system (7–11). Also, the interaction that has been demonstrated between dexamethasone and cyclic AMP, in regulating specific liver enzyme synthesis (14), lend support to the propositions (c) and (d) above and suggest that dexamethasone administered alone was not able to restore mitotic activity due to the absence of additional factors (? cyclic AMP) required to drive the cell cycle. These considerations led us to look for a possible interaction between these two systems (glucosteroid–cyclic AMP) within the over-all mechanism which controls the circadian mitotic cycle.

In an additional series of experiments, attention was given first to items (c) and (d) above and therefore, the second dose of dexamethasone was administered either in association with an

TABLE I. Hour of Drug Administration with the Influence on SCMC of Corneal Epithelium.

Group	Day 1 suppressive treatment[a]	Day 2 replacement treatment[b]			Day 3 response[c]
	DEX	ISO	GH	DEX	
A (Fig. 1)	2300[d]	—	—	—	SCMC
B (Fig. 2)	2300	—	—	1500[d]	None
C	2300	—	1500	—	None
D	2300	—	1300	1500	None
E	2300	1500	—	—	None
F_1 (Panel C, Fig. 3)	2300	1300	—	1330	Increased mitoses
F_2 (Panel D, Fig. 3)	2300	1300	—	1330	Increased mitoses
		1500	—	1530	

[a] Dexamethasone was administered to all groups of rats, to obtain a SCMC on Day 3.

[b] Single doses of either dexamethasone, 0.4 mg (DEX), or growth hormone 1 mg/kg (GH), or isoproterenol 25 mg/kg (ISO), administered independently or in combination (as indicated in Table) to the different groups.

[c] Changes in mitotic activity (none or increased) at 0500, 0600, 0700, 0800, 0900, and 1000 hr of the day when compared with SCMC. *N* was = or greater than five rats for each of the different subgroups of rats (time of sacrifice).

[d] Hours when DEX, GH, or ISO administered.

agent which promotes the synthesis of cyclic AMP, or with growth hormone (see Table I).

In experiments in which growth hormone was administered (1 mg/ip), either alone or 1 or 2 hr preceding the administration of the second dose of dexamethasone (Table I, Groups C and D), no increased levels of mitotic activity were observed. Whether different doses of either dexamethasone or growth hormone might eventually prove effective in reestablishing the normal sequence of mitotic cycles in the system above is not known and will be subject of future work. For the present, however, we chose to proceed with the use of isoproterenol, a drug with recognized growth and cyclic AMP stimulatory effects (7–11). Thus, in eight different experiments (Table I, Group E), a single dose of isoproterenol (10 or 25 mg/kg/body wt) was administered alone at either 1300 or 1500 hr of the day preceding the SCMC. Again, no stimulation of cell division was observed in this group of animals when compared with suppressed (Group A, Table I) controls. In the next series of 14 experiments, the administration of isoproterenol (25 mg/kg) was followed in 15–75 min by a 0.4-mg dose of dexamethasone. This combination of isoproterenol and dexamethasone was administered twice to the same animals in some of these experiments. Either combination type of treatment led to partial reestablishment of mitotic activity in the suppressed mitotic cycle. The results obtained in some of these experiments

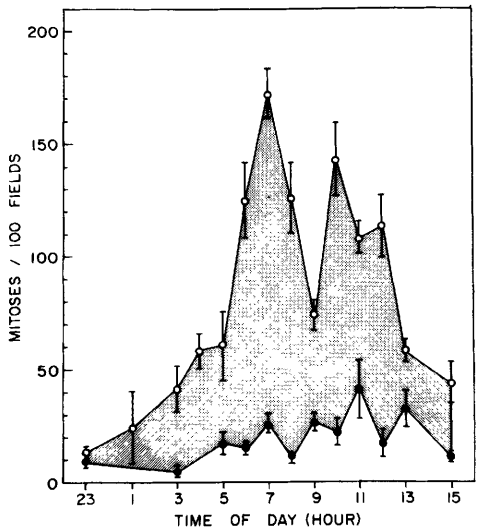

FIG. 2. The effects of two doses of dexamethasone upon a circadian mitotic cycle of the corneal epithelium of rats. The results represent the mean (six or more rats/mean) number of mitoses ± standard error of the mean/100 microscopic fields. Untreated controls and dexamethasone-treated animals were sacrificed at the hr indicated in the abcissa. The shaded area between controls and dexamethasone-treated animals, illustrates the extent and intensity of mitotic suppression by the administration of two doses of dexamethasone. See Table I, Group B, for the time sequence of drug administration.

(representatives of the series) are presented in Fig. 3 (also Table I, Groups F_1 and F_2).

In Fig. 3, we compare the results obtained with the administration of two 0.4-mg doses of dexamethasone with those observed when the second 0.4-mg dose of dexamethasone was administered in combination with a 25-mg dose of isoproterenol (panels B and C, respectively). Increased mitotic activity is apparent in the group of animals treated with the drug combination. However, it should be noted that the overall levels of mitotic activity are still lower in this group of animals (panel C) when compared with that of untreated controls (panel A). To ascertain whether this mitogenic effect of the drug combination could be enhanced, an additional group of rats was treated twice with the isoproterenol–dexamethasone combination. The results presented in panel D indicate an apparent additive effect for this type of treatment (compared with panel C), with restoration of mitotic activity to levels close to that of untreated controls (panel A).

In a more recent series of experiments it was determined that the mitogenic effects of isoproterenol can be significantly enhanced by theophilline and aminophylline (12, 13). The results of these experiments with the phosphodiesterase inhibitors suggest that cyclic AMP might indeed be a key factor in the control of the circadian mitotic cycle of the corneal epithelium of rats (King, Kauker, and Cardoso, in preparation for publication).

In summary and conclusion, the combination of dexamethasone and isoproterenol promotes restoration of mitotic activity in the suppressed circadian mitotic cycle (SCMC), while in this system either drug is entirely devoid of mitogenic activity when administered alone.

While we do not know the mechanism of the above effects, we feel that the main goal of these studies has indeed been reached: the reestablishment of cell division in the dexamethasone-suppressed circadian mitotic cycle. With it, we

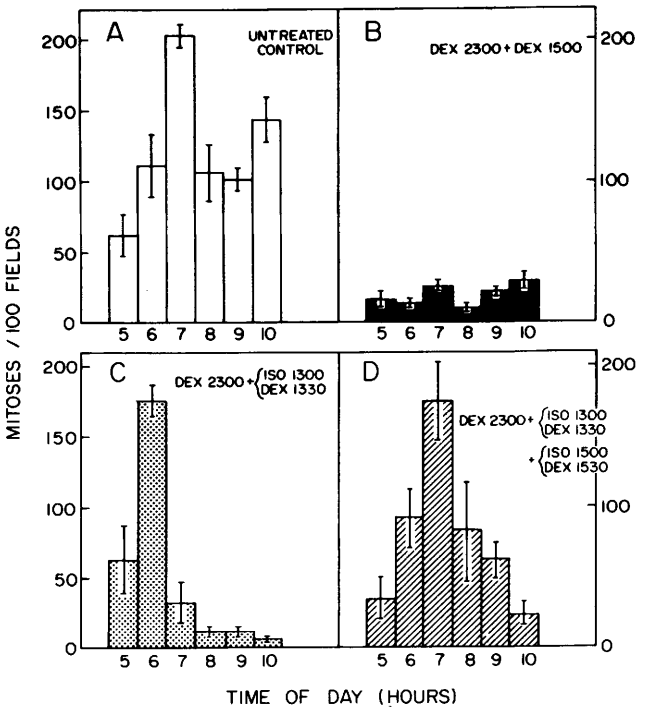

FIG. 3. The mitogenic interaction of dexamethasone–isoproterenol upon a dexamethasone-suppressed circadian mitotic cycle. The bars in the Figure represent the mean number of mitoses per 100 fields ± standard error or the mean. *N* for the different groups varied from a minimum of five rats per time point (panels C and D), to a maximum of 31 rats per time point (Panel B at 0700 hr). The doses of isoproterenol and dexamethasone were always 25 mg/kg of body weight and 0.4 mg/rat, respectively. These doses were repeated in some experimental groups, as indicated in the panels. See also Table I for the time sequence of drug administration.

now have at hand the means to pharmacologically suppress and reestablish cell division *in vivo*. Current efforts are being dircted toward the acquisition of evidence to either support or reject the following propositions: (a) that the mechanism of this synergistic effect of dexamethasone and isoproterenol is similar to that of the combination dexamethasone–cyclic AMP (transcription and translation, respectively) upon the synthesis of hepatic enzymes (12); (b) that the combination dexamethasone–isoproterenol promotes the flow of cells from the G_1 to the S phase of the cell cycle; (c) that under homeostatic conditions, equivalent hormones and/or mechanisms control the time sequence of the circadian mitotic cycle in normal but not in neoplastic cells (15).

Summary. With the use of corneal epithelim of rats we have demonstrated (a) that cell division can be suppressed (circadian mitotic cycle) with the administration of a single dose of dexamethasone; (b) that this suppression can be partially overcome with a second dose of dexamethasone administered in combination with isoproterenol; (c) that this mitogenic affect of the drug combination is absent in animals treated with either drug alone.

The method for obtaining suppression of one full circadian mitotic cycle is presented. Also discussed is the possible significance of the results toward the understanding of the over-all mechanisms which control the circadian mitotic cycle.

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