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Control of Cell Division in the Cornea of Rats. III. Mitogenic Effect of Isoproterenol and Theophylline[1] (38910)

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Several investigators have noted that large doses of isoproterenol profoundly alter the circadian mitotic rhythm and significantly increase the rates of cell division of different mammalian tissues (1–7). Cyclic adenosine 3′–5′ monophosphate (cyclic AMP) has been proposed as a possible mediator of these isoproterenol effects (4–5, 8–14). As isoproterenol is a potent activator of adenylate cyclase and since theophylline inhibits cyclic nucleotide phosphodiesterase (8–14), their effect upon the circadian mitotic rhythm and rates of cell division were further investigated after the administration of these drugs either singly or in combination. The target tissue used for these studies was the corneal epithelium of the rat, which exhibits a high rate of cell division with a distinct circadian profile: maximum levels of cell division at 0700 and minimum levels at 1900 hr of the day. The results obtained offer tentative evidence that cyclic AMP may be involved in the drug induced alterations of the basic circadian mitotic rhythm.

Materials and Methods. Male Holtzman rats, 130–250 g, were used in the experiments. They were housed in a room with controlled temperature (23° ± 1), humidity (50%), and lighting schedule (lights on from 0600 to 1800). The animals were allowed food and water *ad libitum*. Drugs were given at 0700 hr ± 15 min, ip. Isoproterenol was dissolved in distilled water; theophylline was suspended in distilled water and maintained in suspension during dosage with a magnetic stirrer. The rats were sacrificed at the times indicated on the graphs. The eyes were removed, fixed and stained as previously described (5). One eye from each animal was used. Mitoses were counted with the use of a microscope (ocular 10×, objective 100×) with a micrometer grid inserted in the ocular piece. One hundred grid fields were counted in each cornea. All phases of mitosis were included with exceptions of those cells in early prophase or late telophase. Statistical analysis was done with an unpaired *t* test.

Results. Isoproterenol, 25 mg/kg, promptly abolished the high rates of cell division normally present in the early morning hours. This suppression of cell division to levels below that of control animals lasted about 10 hr (Fig. 1). Then, as the rhythm of cell division in control animals reached its nadir, there occurred in the isoproterenol treated animals a rise of mitotic activity, with a peak occurring at 2200. The increased rates of cell division remained above that of controls until 0100 hr, then sank, such that at the next zenith of the control rhythm, the drug-treated animals showed a depressed rate of cell division (Fig. 1).

At a dose of 10 mg/kg, isoproterenol showed a different effect (Fig. 3). As with the larger dose of the drug, there was a prompt inhibition of cell division, but this effect persisted only for 4 hr; there then occurred a burst of cell division which peaked at 1300, and remained above controls for 4 hr. Mitotic activity then fell again and reached trough levels at 1900 hr, a time coinciding with that of the trough for control animals. Thereafter, a late evening rise of cell division occurred in isoproterenol treated rats. This elevation was not as great as with the 25 mg/kg dose. Cell division then again dropped below control and was depressed at 0700 hr on the day following drug administration (Fig. 3).

Theophylline, 100 mg/kg, had effects which were initially similar to those of isoproterenol, 10 mg/kg: a suppression of cell division, with a subsequent surge which peaked at 1300 (Fig. 2). This initial surge subsided to a nadir at 1900 hr. There then

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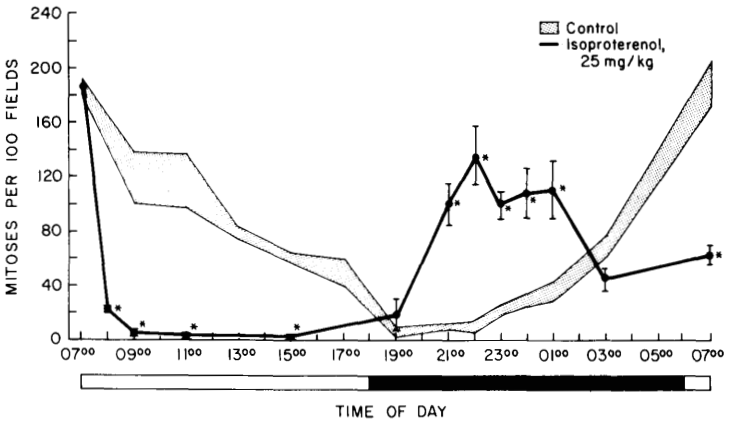

FIG. 1. Effect of isoproterenol, 25 mg/kg, ip, upon the circadian rhythm of cell division in rat cornea. The shaded area represents the control circadian rhythm of cell division; the width of the shaded area represents the mean number of mitoses per 100 microscope fields ± 1 SEM. The black and white bar at the bottom of the graph indicates the lighting schedule. The isoproterenol was given at 0700. The data points for isoproterenol represent means ± 1 SEM. Asterisks indicate drug different from control, $p < 0.05$; $n = 6$ or more animals.

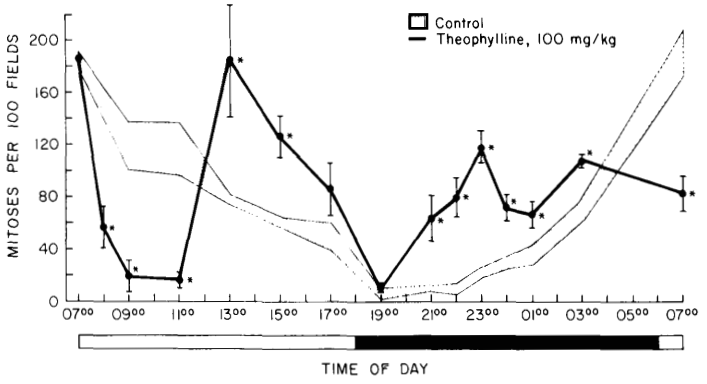

FIG. 2. Effect of theophylline, 100 mg/kg, ip, upon the circadian rhythm of cell division in rat cornea. The shaded area represents the control circadian rhythm of cell division; the width of the shaded area represents the mean number of mitoses per 100 microscope fields ± 1 SEM. The black and white bar at the bottom of the graph indicates the lighting schedule. The theophylline was given at 0700. The data points for theophylline represent means ± 1 SEM. Asterisks indicate drug different from control, $p < 0.05$; $n = 6$ or more animals.

occurred a late evening–early morning rise of cell division which persisted for 6 hr. This rise of mitotic activity was comparable in magnitude to that induced by isoproterenol, 25 mg/kg, but was more long lasting. As with isoproterenol, cell division was depressed to levels below that of controls 24 hours after drug administration.

Theophylline, 50 mg/kg, induced an alteration of the normal circadian rhythm comparable with that induced by isoproterenol, 10 mg/kg (Fig. 3). When isoproterenol, 10 mg/kg, and theophylline, 50 mg/kg, were given together, however, a different result was obtained (Fig. 3). The late evening–early morning peak of cell division was greatly magnified over effects produced by either drug alone. In addition, the suppression of cell division at 0700 hr on the day following administration of the two drugs was greater than that seen with any of the other drug treatments studied.

To verify whether the cardiovascular responses to isoproterenol were in any way related to the observed changes in the cell division rates and/or circadian mitotic

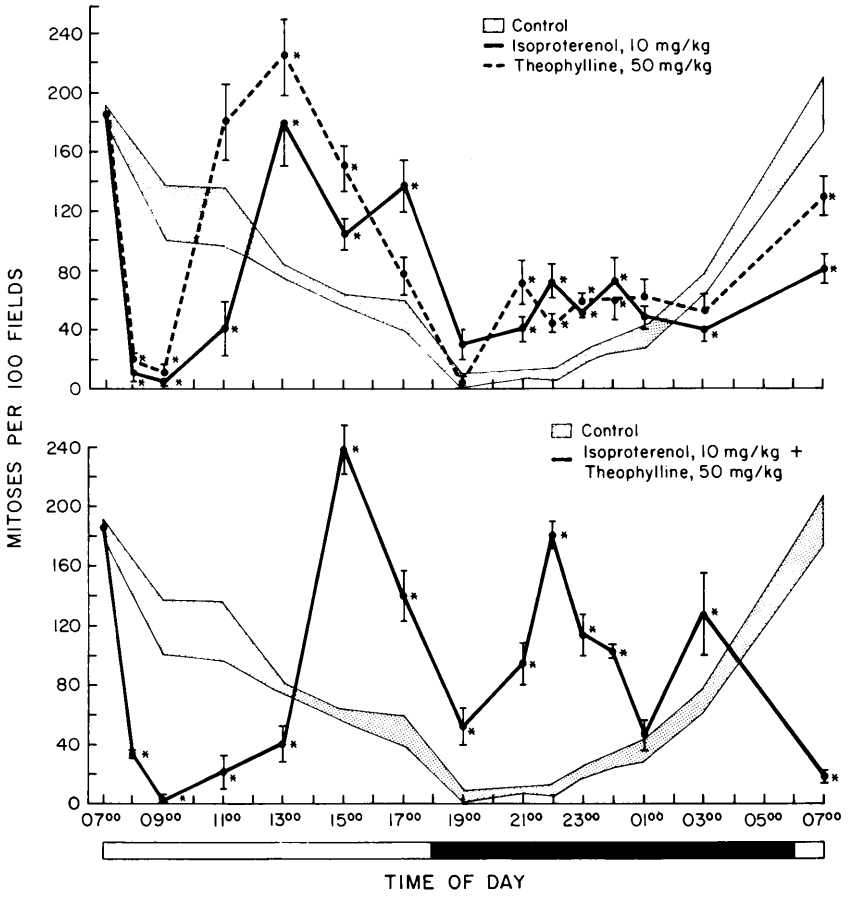

FIG. 3. Effect of isoproterenol, 10 mg/kg, ip, and theophylline, 50 mg/kg given alone (top panel) and given together (bottom panel) upon cell division in rat cornea. The shaded area represents the control circadian rhythm of cell division; the width of the shaded area represents the mean number of mitoses per 100 microscope fields ± 1 SEM. The black and white bar at the bottom of the graph indicates the lighting schedule. The drugs were given at 0700. The data points for the line graphs indicate means ± 1 SEM. Asterisks indicate drug different from control, $p < 0.05$; $n = 6$ or more animals.

rhythm, blood pressure was recorded (Grass polygraph and Statham pressure transducer) in several animals for periods up to 5 hr after drug administration. The doses administered to the animals ranged from 0.1 mg/kg up to 100 mg/kg of body weight. Blood pressure was also recorded in animals receiving theophylline (either 50 or 100 mg/kg). An abrupt fall in the blood pressure, averaging 50 mm of Hg of mean blood pressure, was observed in all animals receiving isoproterenol by the ip route. A return to normal levels was observed in all animals, within 30 min to 3 hr after drug administration, respectively for the lower and higher doses. Tachycardia accompanied hypotension in all animals receiving isoproterenol. On the other hand, no significant fall in the blood pressure was observed in animals receiving either 50 or 100 mg/kg of theophylline.

Discussion. While the mechanism through which isoproterenol alters both the rate of cell division and circadian mitotic rhythms has not been elucidated, a direct effect upon the target cells, possibly mediated through cyclic AMP has been suggested by many investigators (1–8). On the other hand, the role of profound cardiovascular alterations which occur in response to the administra-

tion of high doses of isoproterenol (used to conduct these as well as other studies in the literature) have not been ruled out as possible primary or intermediate determinant factors of the observed cell division responses. Our studies indicated that the cardiovascular responses to isoproterenol, very likely, do not play a major role in the activation of cell division as theophylline induces identical changes (both in intensity and circadian phase in which the response occurs) without the cardiovascular component of the isoproterenol response.

Regardless of mechanisms, the alterations which can be observed both in the rates and in the circadian profile of cell division, are quite typical, reproducible and dose dependent. A prompt, profound and long lasting suppression of cell division follows the administration of isoproterenol 25 mg/kg. Thus, the subsequent rise in mitotic activity (12–16 hr after drug administration) might seem to represent a rebound phenomenon subsequent to the initial suppression of cell division (see Fig. 1). The data collected with isoproterenol 10 mg/kg, with the two doses of theophylline and with the combination isoproterenol plus theophylline, also showed the initial suppression of cell division followed by a rebound-like surge of cell division which peaked (earlier than with isoproterenol 25 mg/kg) at 4–6 hr postdrug administration. There then followed a second drug induced increase of cell division, 12–16 hr after drug administration (see Figs. 2 and 3). In each treatment, the rate of cell division 24 hr after drug administration was significantly depressed when compared with controls. These results suggest (a) that the first drug induced peak (4–6 hr after drug administration) might represent a rebound activity following the initial suppression, (b) that the second drug induced peak might represent a displacement with an advance in time of the subsequent mitotic peak (see Figs. 2 and 3).

The role of cyclic AMP in cell division remains, at best, obscure, with some investigators favoring the view that cell division is controlled by the nucleotide and with others favoring the view that there is a negative correlation between the nucleotide and cell proliferation (8–14). Our data could indicate either possibility, as both suppression and rise in the circadian rates of cell division occur within the 24 hr which follow the administration of drugs which increase tissue levels of cyclic AMP. Our data also stress the importance of frequent sampling of the target tissue within the entire 24 hr after drug administration. Without it, a partial view only would have been obtained in our experiments and with it the sequential suppression and rises in the rates of cell division would have been either lost or not entirely appreciated. This in itself could be the reason for the controversial situation which prevails to date, insofar as the responses to isoproterenol and cyclic AMP are concerned. In addition, it is expected that the following experiments, now in progress, will bring additional information and fuller understanding of the mechanisms through which isoproterenol and theophylline lead to the described changes in the rates of cell division and alterations in the circadian profile of mitoses: A) circadian measurements of cyclic AMP levels in the cornea after drug treatment and B) the search for a naturally occurring rhythm of the nucleotide in the cornea as correlated with the normal circadian rhythm of cell division in animals not given the drug.

Summary. The effects of isoproterenol, theophylline and the combination of the two drugs were studied on the circadian mitotic rhythm in rat corneal epithelium. The drugs induced profound alterations in the normal circadian mitotic pattern. The alterations included an initial suppression of cell division, followed by two distinct rises above control; the first immediately following suppression, the second 12–16 hr following drug administration. The second peak of cell division occurred at the circadian trough in controls. The simultaneous administration of isoproterenol and theophylline greatly magnified the effects seen with either drug alone.

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