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FORSKOLIN, AN ACTIVATOR OF ADENYLATE CYCLASE ACTIVITY, PROMOTES LARGE INCREASES IN N-ACETYL TRANSFERASE ACTIVITY AND MELATONIN PRODUCTION IN THE SYRIAN HAMSTER PINEAL GLAND ONLY DURING THE LATE DARK PERIOD

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SUMMARY: The exposure of organ cultured pineal glands of Syrian hamsters to forskolin, an adenylate cyclase activator, caused marked increases in serotonin N-acetyltransferase activity and melatonin content in a dose-related manner (1-100 μM) when glands were collected in the second half of the dark period. However, addition of forskolin to glands collected anytime during the light period or at the beginning of the dark period failed or only modestly stimulated either pineal N-acetyltransferase activity or melatonin levels. Similar results were obtained with isoproterenol. The results suggest that intrapinealocyte regulatory mechanisms may determine the nocturnal rise in the Syrian hamster pineal gland.

In the Syrian hamster, the nocturnal rise in pineal melatonin production is mediated by β-adrenergic receptors. Craft et al (1) have shown that the synthesis of catecholamines rises at night in the hamster as in the rat pineal while Lipton et al (2) found that the nighttime increase in pineal melatonin in the Syrian hamster was inhibited by propranolol, a β-adrenergic receptor blocker, just as in the rat. However, while rat pineal NAT activity can be induced by either NE or ISO, a β-adrenergic agonist, during either the day or at night (3,4), in the Syrian hamster both in vivo (5-7) and in vitro (8,9) studies have shown that NE and ISO are incapable of mediating a rise in pineal melatonin in hamster during the day. Furthermore, it has been reported that either NE or ISO stimulation of

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Abbreviations used are: NAT, N-acetyltransferase; ISO, isoproterenol; NE, norepinephrine.

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pineal melatonin production in the Syrian hamster is restricted to a brief interval during the latter half of the daily dark period; this responsiveness disappears if animals are exposed to light during the normal dark time period (6,10). Thus, β-adrenergic stimulation appears to be an absolute requirement to activate both rat and hamster pineal glands at night. However, since the Syrian hamster pineal gland only responses to ISO for a brief interval late in the dark period, in this species it seems something in addition to β-adrenergic stimulation is required to promote high melatonin levels.

This study addresses the problem of the control of melatonin synthesis by hamster pineals using in vitro techniques and forskolin. Forskolin is a diterpene that appears to act directly on the adenylate cyclase enzyme complex which consists of a guanyl nucleotide binding subunit and a catalytic subunit (11). Interaction of forskolin with adenylate cyclase not only increases enzyme activity but also appears to facilitate or augment activation by receptor agonists and/or guanyl nucleotide in a variety of tissues including rat pineal gland (12-14). Unexpectedly, it was found that forskolin was able to stimulate hamster pineal melatonin production only when the glands were collected late in the dark period.

METHODS

Male Syrian hamsters (80-100 g) were purchased from Sasco (Omaha, NE, USA). Upon arrival in the laboratory they were caged (4-5 per cage), given food and water ad libitum and exposed to a light-dark (LD) cycle of 14:10 (lights on daily from 06.00 h-20.00 h). Groups of 8 animals were killed by decapitation at the times indicated in each experiment. When animals were killed at night, they were previosly exposed to 50-70 $\mu W/cm^2$ white light for 30 min to suppress nighttime melatonin levels to basal daytime values (15).

Hamster pineal glands were cultured using a method described previously by Brammer et al (16). Glands were incubated at 37°C under a humidified atmosphere of 95% O2-5% CO2 in a water-jacketed incubator. The culture medium (BGJb Fitton-Jackson modification, Grand Island Biological Co., Grand Island, NY, USA) was supplemented with ascorbic acid (0.1 mg/ml), glutamine (0.2 mM), BSA fraction V (1 mg/ml), penicillin (100 units/ml) and streptomycin (100 μg/ml). Preincubated pineals (30 min) were cultured with either ISO or forskolin for 4 hours at the concentrations indicated. Both pineal glands and culture media were collected and quickly frozen on solid CO2 and stored at -70°C until assayed for NAT and melatonin. Forskolin was dissolved with dimethyl sulfoxide and serial dilutions were made with the culture media. The concentration of dimethyl sulfoxide in the culture medium was always lesser than 0.2% (vol/vol).

NAT activity assay was performed as described previously (17) and referred to pmol of N-acetyltryptamine produced/gland/h. Melatonin levels were estimated by radioimmunoassay according to technique previously described (18) and referred to either pg/gland or pg/ml/gland for gland and culture medium, respectively.

Data are expressed as means ± SEM and were analyzed using a ANOVA followed by a Student-Newman-Keuls multiple range test.

(-)-isoproterenol (ISO) was purchased by Sigma Chemical Co. (St. Louis, MO, USA); forskolin was purchased by Calbiochem (San Diego, CA, USA). Others reagents were obtained from comercial sources.

RESULTS

Experiment 1: The induction of NAT activity and melatonin production by forskolin was studied in pineal glands from hamsters killed in the second half of the normal dark period (02.30 h). Pineals were incubated with either forskolin (1, 10 or 100 μM) or ISO (1 μM). Additional pineals were incubated in the absence of these drugs. As shown in figure 1, ISO stimulated NAT activity and melatonin production, in agreement with earlier observations (8). Likewise, forskolin markedly increased melatonin levels in both pineal glands and culture media in a dose-related manner. However, NAT activity did not increase with doses of 1 or 10 μM forskolin, exhibiting a significant increase only at 100 μM forskolin.

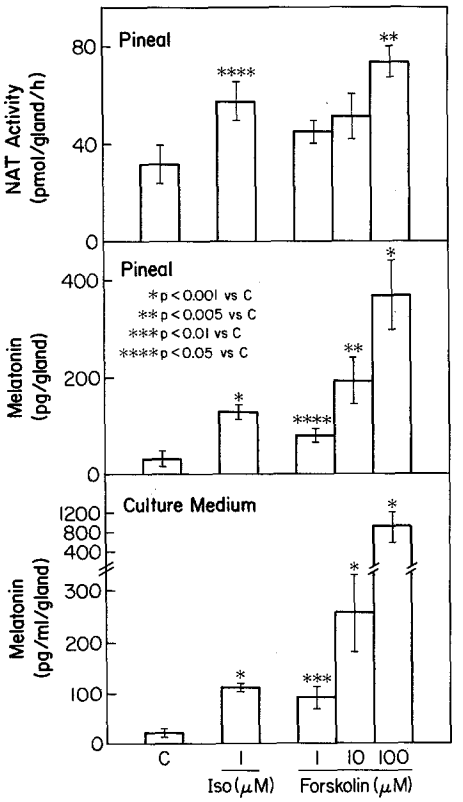

Fig. 1. Effect of ISO or different doses of forskolin on the in vitro stimulation of NAT activity and melatonin production. Pineal glands were collected from hamsters killed at 02.30 h (late dark phase) and incubated for 4 h. Control values (C) were obtained by incubating glands in the absence of the drugs. Values are expressed as the mean ± SEM of 8 pineal glands.

Experiment 2: To investigate the response of the hamster pineal gland to forskolin during the day, pineal glands were collected at 16.00 h, during the normal light period and incubated with either 50 μM forskolin or 1 μM ISO. A control group of pineal glands was incubated in the absence of drugs. Since both 10 and 100 μM concentration of forskolin were able to stimulate markedly pineal melatonin production, an intermediate dose was chosen in this experiment. Figure 2 shows that ISO was incapable of inducing either enzyme activity or melatonin production. These results are in accordance with those previously described by Santana et al (8) and Vaughan et al (9). Surprisingly, forskolin, which acts directly on the adenylate cyclase enzyme, was also unable to stimulate NAT activity and melatonin production in pineal glands collected during the day.

Experiment 3: Since the results reported above (Fig. 2) showed that forskolin was unable to promote either NAT activity or melatonin levels in hamster pineal glands collected during the day, this experiment was performed to investigate the sensitivity of the hamster pineal gland to forskolin at four different times throughout a 24-hour period. Pineal glands were collected at 10.00 h (early light phase), 16.00 h (late light phase), 22.00 h (early dark phase) and 02.00 h (late dark phase) and incubated in the presence of 50 μM forskolin. As shown in figure 3, clearly at both times during the day (10.00 and 16.00 h) forskolin had no effect on either NAT activity or pineal or media melatonin levels. Likewise, early in the dark phase (22.00 h) forskolin did not increase either pineal NAT activity or melatonin levels in culture media, although a slight increase in pineal melatonin content was observed. However, in pineal glands collected at 02.00 h, both NAT activity and melatonin rose as a consequence of the incubation with forskolin.

DISCUSSION

It has been clearly established in rats that the stimulation of pineal NAT activity and melatonin synthesis can be induced by NE and ISO in pineals collected either during the daytime or at night after the donor animals are exposed to light (3,4,8,19). However, in the Syrian hamster it is not possible to stimulate pineal melatonin by either NE or ISO during the daily light period (6-10).

The reason for the daytime insensitivity of the hamster pineal gland to NE or its agonists remains unknown although it has been suggested that it might relate to a possible rhythm in the number of β-receptors on the pinealocyte membrane (6) such that, during the day, hamster pinealocyte β-receptors are down-regulated and thus the gland is unresponsive to

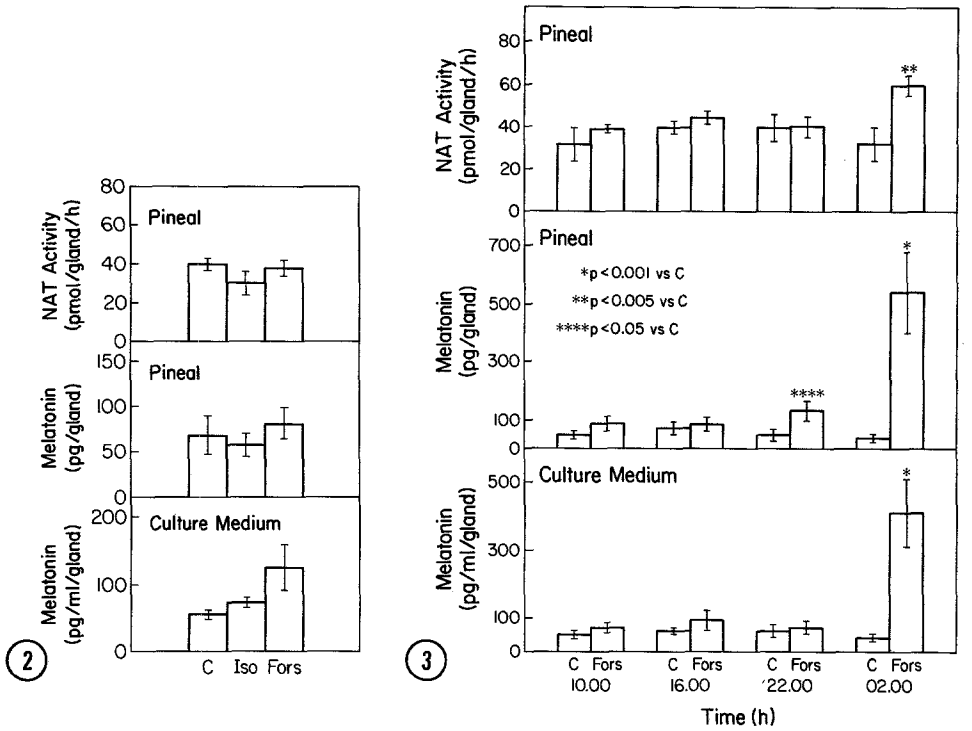

Fig. 2. Effect of 1 μM or 50 μM forskolin (FORS) on the in vitro stimulation of NAT activity and melatonin production. Pineal glands were collected from hamsters killed at 16.00 h (late light phase) and incubated for 4 h. Control values (C) were obtained by incubating glands in the absence of the drugs. Values are expressed as the mean ± SEM of 8 pineals.

Fig. 3. Effect of 50 μM forskolin (FORS) on the in vitro stimulation of NAT activity and melatonin production. Pineals were collected from hamsters at the indicated times and incubated for 4 h. Control values (C) were obtained by incubating glands in the absence of the drug. Values are expressed as the mean ± SEM of 8 pineal glands.

β-agonists. In the rat, for example, an obvious rhythm in pineal β-receptors density has been described with maximal number of receptors being present during darkness (20,21). In the present study, forskolin caused a large rise of pineal NAT activity and melatonin production at night but not during the day. Since the action of forskolin does not depend upon the β-adrenergic receptor, the number of binding sites for β-adrenergic agonists may not be the primary basis for the regulation of the 24 rhythm in sensitivity of the hamster pineal gland. The point of convergence in the actions of ISO and forskolin is the activation of adenylate cyclase. ISO activates adenylate cyclase by a mechanism involving its interactions with the β-adrenergic binding sites. Forskolin, unlike β-adrenergic agonists, exerts a direct action on the adenylate cyclase enzyme complex. However, the interaction of forskolin with adenylate cyclase not only increases enzyme

activity but also appears to facilitate or augment activation by receptor agonists and/or guanyl nucleotides (12,13). Thus, forskolin augments the response to the β-adrenergic agonist ISO in different brain regions and augments the response to vasoactive intestinal peptide in cerebral cortical slices (13).

It is also known that the basal activity of adenylate cyclase may be markedly influenced by a variety of factors including inhibitory and stimulatory modulation by guanyl nucleotide binding subunits (11) which could contribute to the magnitude of the forskolin response. These factors may explain the lack of response of the Syrian hamster pineal gland to forskolin and ISO during the day.

Another possibility is that the regulatory mechanisms involved in the changes in sensitivity of the hamster pineal gland are located at a site beyond the adenylate cyclase enzyme complex. Further studies concerning the effect of forskolin or β-adrenergic agonists on the induction of cyclic AMP production in the hamster pineal gland are required to clarify the mechanisms involved in the regulation of Syrian hamster melatonin production. However, the present results indicate that mechanisms distal to the β-receptors in the pinealocyte membrane may be involved in determining the nocturnal increase in pineal melatonin production in the Syrian hamster.

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