Swimming-Induced Suppression of Rat Pineal Melatonin is Prevented by Pretreatment with Calcium Channel Blockers[1] (42836)

DOUGAL J. MORTON, RUSSEL J. REITER,[2] AND GERALD R. BUZZELL
Department of Cellular and Structural Biology, The University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284-7762

Abstract. **Young adult male rats were treated with isoproterenol during the day to induce high levels of pineal *N*-acetyltransferase (NAT) activity and melatonin. Roughly 2 hr later when pineal NAT activity and melatonin levels were elevated, animals were given either an injection of a calcium channel blocker, i.e., either nifedipine or verapamil, or diluent. The rats were then forced to swim for 10 min in room temperature (22°C) water. Fifteen minutes after swimming onset, pineal glands were collected for measurement of NAT activity and melatonin. Swimming caused a dramatic reduction in pineal melatonin content without influencing NAT activity. Nifedipine substantially and verapamil completely blocked the drop in pineal melatonin levels due to swimming without influencing NAT activity. The results suggest that calcium may be somehow directly or indirectly involved in melatonin release from the rat pineal gland.** [P.S.E.B.M. 1989, Vol 190]

Pineal *N*-acetyltransferase (NAT) activity and melatonin levels have been shown to be affected by a variety of treatments which can be considered stressful, with the magnitude of the observed changes being influenced by the time during the light/dark cycle at which the stressor is applied (1–6). Application of stressors during the day appears to cause an increase in both pineal NAT activity and melatonin content (1, 2), effects probably resulting from increased catecholamine release (2).

In contrast to daytime stress, the exposure of animals to stressors at night or when pineal melatonin levels are high has been shown to have dichotomous effects on pineal NAT activity and melatonin content (5–11). Adrenalectomy has been shown to abolish the decrease in elevated NAT activity and pineal melatonin content caused by the stress of a hind leg saline injection (5) but this surgical procedure does not prevent the decrease pineal melatonin content observed following swimming (6); this indicates that the mechanisms responsible for the observed changes are different for these two stressors. The drop in pineal melatonin levels in rats forced to swim is believed to be a result of the rapid release of this constituent from the gland (6, 11).

Calcium has been suggested to play a role in release of pituitary hormones (12) and a recent report has intimated that a similar mechanism may be involved in pineal release of melatonin (13). Dihydropyridine calcium antagonists, but not verapamil, were shown to decrease circulating melatonin levels in baboons, effects attributed to a blockade of calcium entry into pineal cells leading to a suppression of melatonin synthesis and/or release (13).

As the decreased pineal melatonin content observed following swimming has been postulated to be the result of an increased efflux of the hormone from the gland (6–11), it was of interest to examine the effect of calcium entry blockers, which may alter melatonin release, on pineal melatonin content in rats forced to swim.

Materials and Methods

Male Sprague-Dawley rats (80–100 g; Harlan Sprague-Dawley, Houston, TX) were kept under a light/

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[2] To whom correspondence and requests for reprints should be addressed: Department of Cellular and Structural Biology, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78284-7762.

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dark cycle of 14 hr light/10 hr dark (lights on at 0600 hr) with unrestricted access to food and water. Isoproterenol HCl, verapamil HCl, and nifedipine were obtained from Sigma (St. Louis, MO). Nifedipine (1 mg/ml) and verapamil (10 mg/ml) were dissolved in a mixture of 1.5 ml of ethanol, 1.5 ml of polyethylene glycol 400, and 7 ml of normal saline before injection. Both drugs were weighed and dissolved under dark room conditions and stored prior to use wrapped in aluminum foil for protection from the light.

On the day of the experiment, each rat received a subcutaneous injection of isoproterenol HCl (1 mg/kg body wt) at 1500 hr (9 hr after light onset); this was done to stimulate high pineal NAT activity and melatonin content (11). One hour and 58 min later (at 1658 hr), animals were divided into three groups that received a subcutaneous injection of either diluent, nifedipine (1 mg/kg), or verapamil (10 mg/kg). Shortly after these injections at 1700 hr (2 hr following isoproterenol administration), half of the diluent-, nifedipine-, and verapamil-injected rats were forced to swim for 10 min as previously described (6). Briefly, the rats were placed in a stainless steel tank containing room temperature (22 ± 2°C) water; the water had a depth of approximately 22 cm so the animals could not touch the bottom without being submerged. At the end of the 10-min swimming period, the animals were returned to their home cage. Five minutes later (2 hr 15 min after isoproterenol and 15 min after swimming onset) all animals were decapitated. The pineal gland of each animal was removed with a minimum of delay, frozen on solid CO_2, and stored at −70°C until assayed for NAT activity and melatonin content. Within 3 days of tissue collection, the NAT and melatonin assays were performed using techniques previously described in this laboratory (14).

Data were statistically analyzed using an analysis of variance followed by a Student-Newman-Keuls test. Values were considered to be significantly different if the $P < 0.05$.

Results

Control animals treated with isoproterenol only had high levels of both NAT activity and melatonin at the conclusion of the study (Fig. 1). When isoproterenol-treated rats were forced to swim for 10 min, NAT activity remained high whereas pineal melatonin content dropped precipitously. Neither nifedipine nor verapamil treatment influenced either NAT activity or melatonin content of pineal glands of rats that did or did not swim. However, nifedipine partially prevented and verapamil totally prevented the drop in pineal melatonin content associated with a 10-min swim.

Discussion

The observation that NAT activity was unaffected by treatment with the calcium antagonists implies that any changes in pineal gland melatonin content are probably not attributable to altered synthesis of the indole. A previous report has shown that inhibition of calcium influx does not affect pineal production of cyclic nucleotide following isoproterenol stimulation (15); the present report confirms these findings indirectly and, furthermore, it shows that no subsequent step in the production of NAT is affected by treatment with the calcium antagonists used. This finding is particularly interesting as nifedipine has been shown to prevent the nocturnal increase in retinal NAT activity (16), suggesting that the mechanisms controlling production of this enzyme may be different in the retina and pineal gland.

The dramatic and well-documented decrease in pineal gland melatonin content following swimming (6, 10) was prevented by calcium antagonists with verapamil being more potent than nifedipine (Fig. 1). This apparent difference in potency of the calcium antagonists might be dose related, although the doses used would have been expected to be more or less equivalent

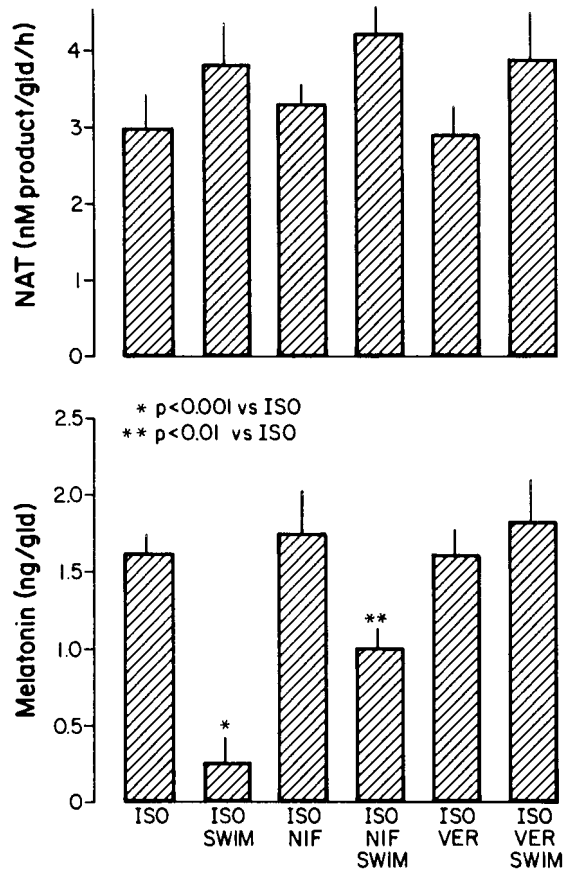

Figure 1. Mean (±SEM) pineal NAT activity and melatonin levels in rats treated with isoproterenol (ISO) and either one of two calcium channel blockers, i.e., nifedipine (NIF) or verapamil (VER). Some animals swam (SWIM) for 10 min before their pineal glands were collected for analysis. The calcium channel blockers either greatly reduced (in the case of nifedipine) or totally prevented (in the case of verapamil) the drop in pineal melatonin normally associated with swimming.

in terms of their ability to block calcium influx (17, 18). Alternatively, the different efficacies of the drugs in this case may be related to differences in the mode of action of these compounds (19). The latter suggestion is possibly the more plausible as nifedipine exhibits greater potency as a peripheral vasodilator compared with verapamil (19) and increased blood flow through the pineal gland may lead to enhanced melatonin efflux and, hence, to lower pineal levels of the indole. Differences in the activities of verapamil and nifedipine have been noted previously when other end points were used (13, 20, 21). Verapamil was shown to inhibit the release of anterior pituitary hormones, but nifedipine was without effect (20, 21). Nifedipine, but not verapamil, caused a marked decrease in circulating plasma melatonin, possibly a consequence of the reduced synthesis and/or release of the hormone (13). This finding suggest that nifedipine, acting as a vasodilator, does not lead to a pronounced efflux of melatonin from the pineal gland, at least not in the baboon. Two distinct types of calcium channels have been identified, potential operated and receptor operated (19), and it is possible, therefore, that differences in selectivity of the calcium antagonists for these channel populations may be responsible, at least in part, for the differences noted.

Since neither calcium antagonist had an effect on pineal NAT activity nor on the melatonin content in the non-swimming groups, it is presumed that these drugs were without effect on the ability of the pineal gland to produce melatonin. The drop in melatonin content following swimming may, therefore, be related to the release of melatonin from the pinealocytes. Indeed, when circulating melatonin levels are measured in rats after a short swimming interval, values are increased (6, 11). Nifedipine pretreatment did not completely prevent the decrease in pineal melatonin content following swimming, but the magnitude of the decline was far less than in the nontreated controls that swam. Both nifedipine and verapamil were obviously effective in curtailing the drop in pineal melatonin content due to swimming; however, the latter drug was clearly more effective in this regard.

On the basis of the results presented, it is suggested that swimming enhances the efflux of melatonin from the pineal gland and the blockade of calcium channels with either nifedipine or verapamil prevents the melatonin decline by reducing its release. Whether melatonin efflux occurs through calcium channels per se or whether calcium channel activation leads to secretion of melatonin by some other mechanism is unknown but warrants further investigation.

The mechanism of the drop in elevated pineal melatonin during swimming obviously remains unknown. The reduction is not prevented by adrenalectomy or by hypophysectomy and it does not involve the sympathetic innervation to the pineal gland (14). We have recently proposed that possibly atrial natriuretic factor (ANF) may be involved in this process (10). Certainly during excessive exercise, such as swimming, ANF is released from cardiac muscle (22) which could, in turn, cause the discharge of melatonin from the pineal gland by mechanisms that are yet unknown; ANF receptors have been identified in the rat pineal gland (23). If exercise-related ANF release is part of this process, the calcium channel blockers may have prevented the discharge of this peptide from the heart and, thereby, only indirectly overcome the reduction in pineal melatonin levels.

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