MINIREVIEW

Involvement of Calcium in Pineal Gland Function (43269)

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The pineal gland functions as a neurohumoral transducer, accepting photic information from the retinae and converting this neural message into chemical mediators, the best documented of which is melatonin (*N*-acetyl-5-methoxytryptamine) (1). The onset of darkness causes information to travel from the suprachiasmatic nuclei of the hypothalamus via the superior cervical ganglia to postganglionic sympathetic nerves that end in the pineal, where they release norepinephrine. The resultant stimulation of β-adrenergic receptors, possibly augmented by α-adrenergic stimulation, leads to an increased activity of *N*-acetyltransferase (NAT) (2), the rate-limiting enzyme involved in synthesis of melatonin (3). Serotonin, derived from dietary tryptophan taken up by pinealocytes from the blood, is *N*-acetylated by NAT, which is subsequently O-methylated by hydroxyindole-O-methyltransferase (HIOMT) to form melatonin, which is then released into the general circulation. Being a small lipophilic molecule, melatonin is generally considered to be released rapidly and passively following synthesis, although there is some evidence that short-term storage of melatonin by the pineal gland might occur (4). Melatonin's lipophilicity also ensures that it distributes widely to target tissues, which, therefore, receive photoperiodic information and can subsequently act with appropriate physiological responses (5).

The multiplicity of extracellular and intracellular events that interact to produce the melatonin message are not fully understood, although certain aspects have been reasonably well characterized. For example, it is well-known that β-adrenergic stimulation leads to activation of adenylate cyclase, and that the resultant increase in cAMP stimulates mRNA synthesis, leading to increased NAT activity (3). The α-adrenergic augmen-

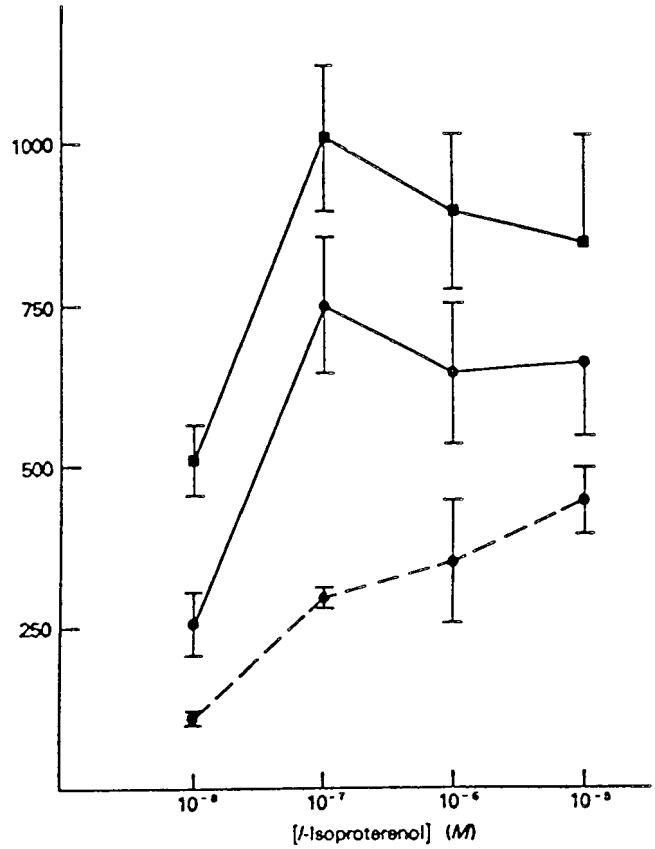

Figure 1. Induction of NAT by varying concentrations of isoproterenol in the presence of different concentrations of calcium. Solid squares indicate 7.5 m*M* $CaCl_2$, solid circles indicate 1.25 m*M* $CaCl_2$, and solid circles with dotted line indicates no $CaCl_2$ and 1 m*M* EGTA. Reprinted from Biochemical Pharmacology (Ref. 7), with permission.

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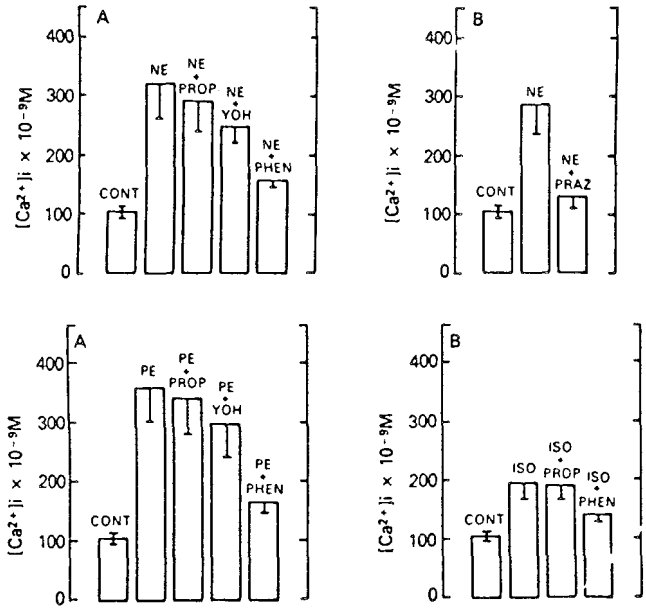

Figure 2. Influence of adrenergic agonists and antagonists on intracellular calcium content of pinealocytes. (Top panel A) Effects of propanolol (PROP), yohimbine (YOH), and phentolamine (PHEN) on the increase in intracellular calcium caused by norepinephrine (NE). (Bottom panel A) Effect of antagonists on phenylephrine (PE)-induced increase in intracellular calcium. (Top panel B) Effect of prazosin (PRAZ) on NE-induced increase in intracellular calcium. (Bottom panel B) Effect of PROP and PHEN on isoproterenol (ISO)-induced increase in intracellular calcium. Reprinted from the Journal of Biological Chemistry (Ref. 16), with permission.

tation of this process results from activation of protein kinase C. A number of stimulatory/regulatory processes involved in melatonin production have been associated with calcium, and the following discussion serves to review current understanding of the relationship between calcium and pineal gland function.

Induction and Inhibition of *N*-Acetyltransferase

Early work on the relationship between calcium ions and the pineal gland showed that extracellular calcium was necessary for complete induction of NAT activity in rats (6, 7). Thus, chelation of extracellular calcium led to a reduction in NAT activity by an indirect effect on the rate of protein synthesis (7), and stimulation of β-adrenergic receptors required extracellular calcium for full expression of the activity of the acetylating enzyme (6). These effects are well-illustrated when comparing NAT activity induced by isoproterenol in the presence of EGTA and varying concentrations of calcium (Fig. 1) (7). Long-term exposure to elevated calcium, however, leads to a reduction in activity of NAT (8); this may indicate an inhibitory role for calcium, in addition to its stimulatory activity. The

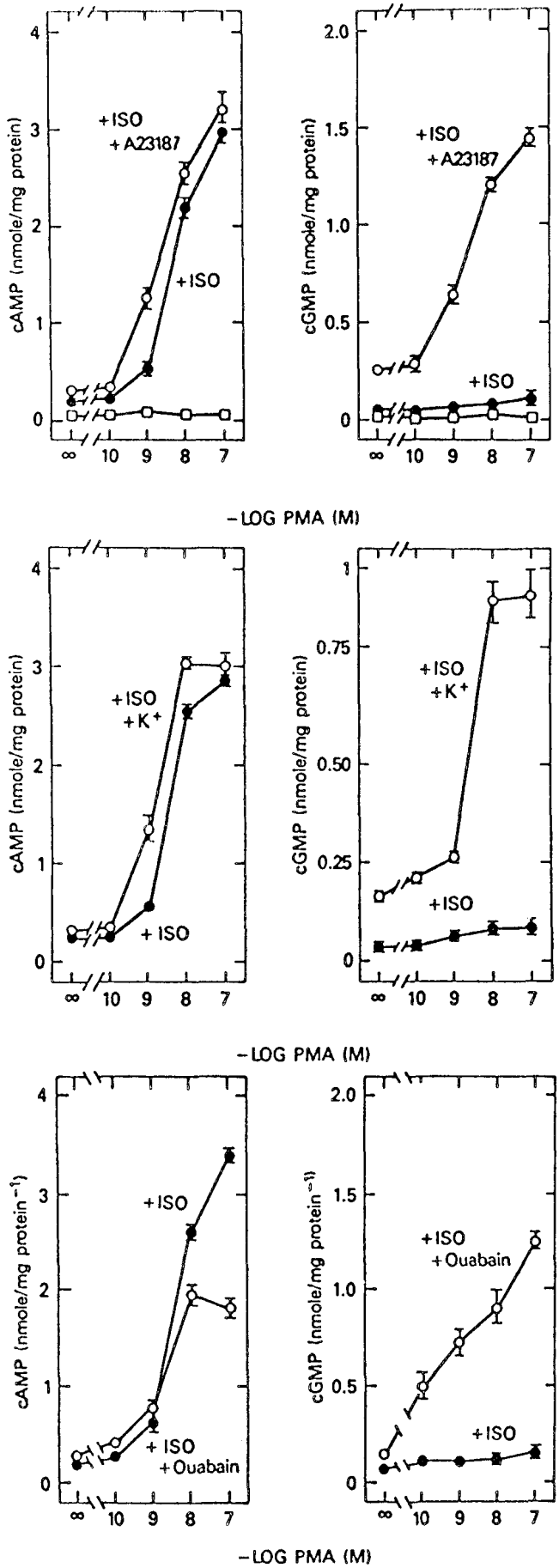

Figure 3. Effects of (top panel) A 23187, (middle panel) potassium (K^+), and (bottom panel) ouabain on 4 β-phorbol 12-myristate 13-acetate (PMA) stimulation of cAMP and cGMP content of pinealocytes stimulated with isoproterenol (ISO). Reprinted from the Journal of Biological Chemistry (Ref. 14), with permission.

Table I. Dose-Dependent Effects of Nitrendipine and Bay K 8644 on Nocturnal Melatonin Output[a]

	Nocturnal melatonin output (dpm/12 hr ± SD)
Nitrendipine (n*M*)	
0	2040 ± 80
3	1870 ± 70
10	1750 ± 52
30	1580 ± 56
100	1190 ± 9
Daytime control	650 ± 12
Bay K 8644 (n*M*)	
0	3650 ± 200
1	3530 ± 96
10	4380 ± 194
100	5370 ± 295
1[b]	5450 ± 114
Daytime control	1260 ± 36

[a] From Ref. 19.
[b] Value expressed in micromolars.

inhibitory effects of elevated intracellular calcium on rat pineal NAT have been suggested to be the result of activation of an NAT inhibitory substance in the pineal (9–11). Apart from these effects of calcium ions on the induction of NAT, the increase in rat pineal cGMP was also shown to be dependent on extracellular calcium ions, an effect presumed to be mediated at a presynaptic site, possibly involving α-adrenergic receptors (12).

Sympathetic Receptor-Mediated Intracellular Events

Recently, considerable attention has focused on the interrelationship between α- and β-adrenergic receptors in the rat pineal gland and the calcium dependency of various intracellular processes effected by receptor activation (13–18). An early hypothesis, that an increase in intracellular calcium was an α-adrenergic-mediated process responsible for activation of phospholipid hydrolysis which, in turn, led to potentiation of β-adrenergic stimulation of cAMP and cGMP production in the pineal gland (18), has been supported by subsequent findings (13–17). Whether the calcium-activated phospholipid hydrolysis potentiates cAMP production through a diacylglycerol-sensitive mechanism and cGMP production through a fatty acid-sensitive mechanism (18) remains to be verified. The fact that α-1 adrenergic receptors are involved in calcium ion influx in the pineal gland has been well-documented (15, 16), although there is contradictory evidence regarding the mechanism through which intracellular calcium ion concentration is increased (15, 16). Convincing evidence of α-adrenergic regulation of intracellular calcium ion concentrations comes from studies involving treatment of rat pinealocytes with a variety of adrenergic agonists and antagonists, the results being highly suggestive of α-, rather than β-, receptor involvement in the observed changes in calcium influx (Fig. 2) (16). One report discounts involvement of voltage-sensitive calcium channels and proposes either involvement of an ion exchange system or opening of a calcium ion gate (15); a more recent report proposes involvement of either voltage-dependent calcium channels or an ion exchange mechanism as being responsible for the observed calcium ion influx (16).

Increased cAMP and cGMP levels in the pineal gland following α-1 adrenergic receptor stimulation involves activation of a calcium-dependent protein kinase C (17), an observation supported by the fact that the absence of an increase in intracellular calcium is accompanied by no change in cGMP despite activation of protein kinase C (14). A variety of treatments, all of which are associated with an increase in intracellular calcium, induce a rise in the cGMP content resulting from isoproterenol treatment of rat pinealocytes, whereas isoproterenol alone was without significant effect on cGMP accumulation (Fig. 3) (14). It is, therefore, apparent that an increase in intracellular calcium is mandatory for expression of α-1 adrenergic potentiation of β-adrenergic stimulation of cAMP and cGMP production and must be accompanied by an increased

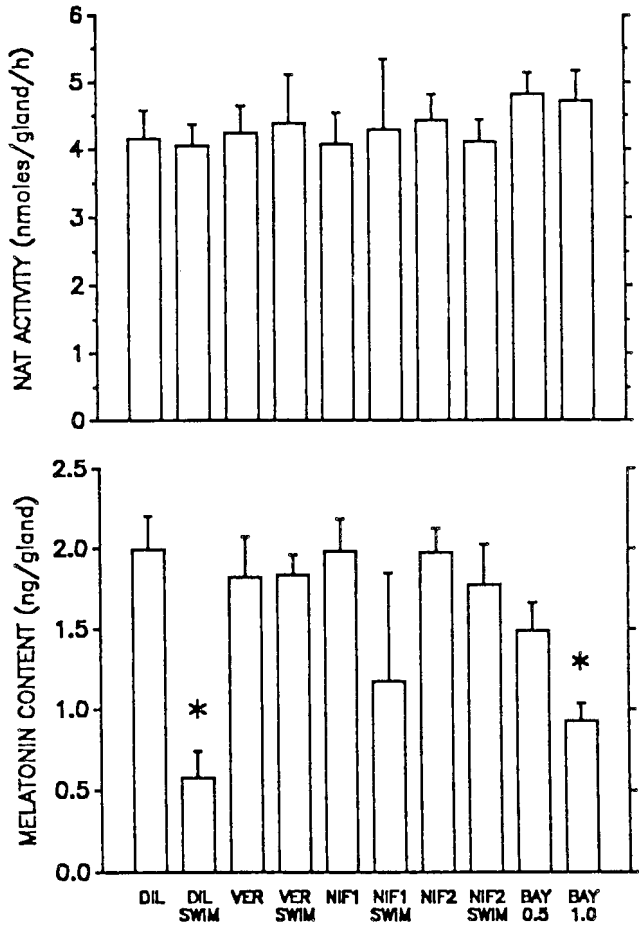

Figure 4. Pineal NAT activity and melatonin content in rats treated with isoproterenol (ISO) and nifedipine (NIF) or verapamil (VER). Some rats swam (SWIM) for 10 min before collection of pineal glands. Reprinted from the Proceedings of the Society for Experimental Biology and Medicine (Ref. 24), with permission.

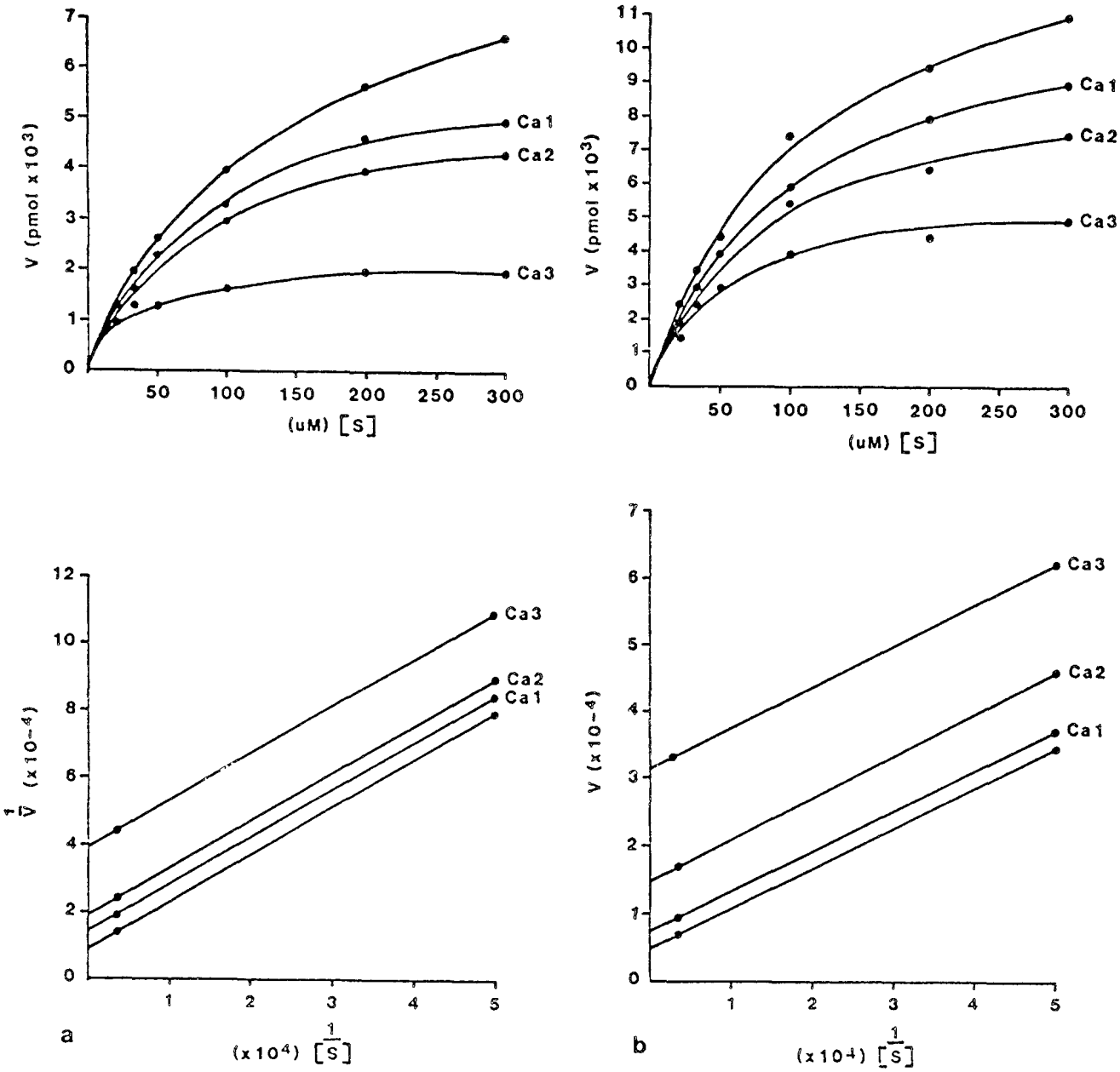

Figure 5. Velocity versus substrate concentration for HIOMT activity in the presence and absence of calcium. Double-reciprocal plots are shown to give an indication of the nature of the interaction. (a) Calcium effect on *S*-adenosylmethionine. (b) Calcium effect on *N*-acetylserotonin. (Ca 1 = 5 m*M*, Ca 2 = 10 m*M*, and Ca 3 = 25 m*M*.) Velocity measured in pmols of melatonin formed per incubate per hour at 42°C. Reprinted from the Journal of Pineal Research (Ref. 29), with permission.

activity of protein kinase C; calcium ions may influence translocation and/or activation of protein kinase C (15), the former hypothesis finding support from recently published observations (13). In addition, it has been suggested that stimulation of phospholipase C may also be a calcium-dependent process (15).

Pinealocyte Calcium Channels

Voltage-dependent calcium channels (L-type) appear to be involved in regulation of melatonin production by cultured chick pineal glands (19–22). Treatment of cultured chick pineals with the dihydropyridine calcium channel antagonist, nitrendipine, was associated with a dose-dependent decrease in melatonin output, while the calcium channel agonist, Bay K 8644, caused an increase in melatonin output (Table I) (19). Calcium influx in cultured chick pineal glands affects melatonin synthesis at some point distal to the circadian pacemaker (21) that appears to be at the level of cAMP production independent of the influence of light (20).

In addition to involvement of calcium in the modulation of synthetic activity of the pineal gland, there is a growing body of information suggesting a role for calcium in the release of melatonin (20, 21, 23, 24). Dihydropyridine calcium channel antagonists have been shown to reduce the amplitude of the nocturnal

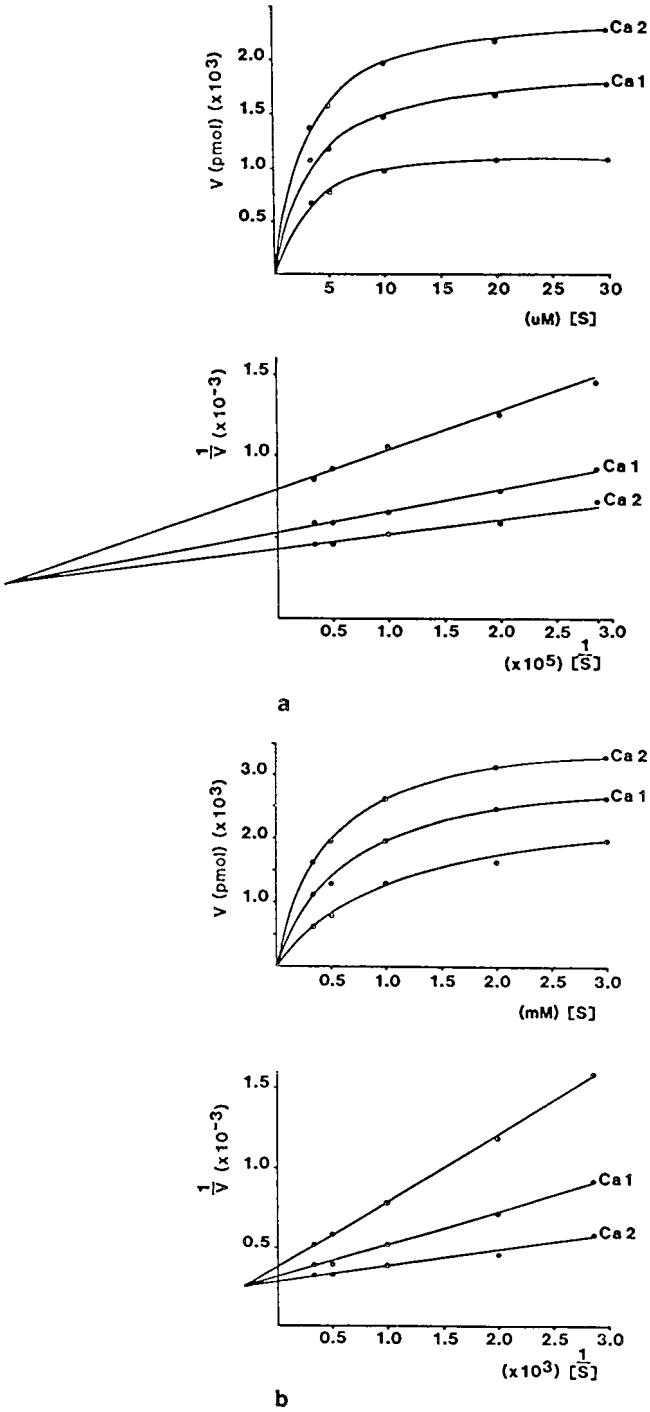

Figure 6. Velocity versus substrate concentration for NAT activity in the presence and absence of calcium. Double-reciprocal plots are shown to give an indication of the nature of the interaction. (a) Calcium effect on acetyl coenzyme A. (b) Calcium effect on tryptamine. (Ca 1 = 2.5 m*M* and Ca 2 = 5 m*M*.) Reprinted from the Journal of Neural Transmissions (Ref. 30), with permission.

rise in plasma melatonin in baboons, an effect proposed to be due to decreased synthesis and/or release of melatonin by the pineal gland (23). As calcium channel antagonists have been shown to prevent the swimming-induced decrease in high pineal melatonin levels at night without an effect on NAT activity, it seems likely that calcium channels are involved in melatonin release under these conditions (Fig. 4) (24). Results reported for melatonin output from cultured chick pineal glands show an increased output with enhanced calcium channel activity, and a decreased output when calcium channels are blocked (Table I) (20, 21). Although these results have been discussed in terms of a calcium effect on synthesis, as pineal melatonin content was not evaluated together with the concentration in culture medium, calcium effects on synthesis and release cannot be separated. As presented, these results, in addition to confirming the necessity of calcium for synthesis of melatonin (20, 21), also favor a role for calcium channels in the release of melatonin from the pineal gland.

The observation that calcium channel blockade resulted in inhibition of normal nocturnal increase in NAT activity in hen pineal gland, an effect prevented by concomitant administration of a calcium channel agonist, underlines the importance of calcium channels for the full expression of NAT activity (22). In addition, enhancement of nocturnal NAT activity by calcium channel agonist treatment (22) suggests that the α-adrenergic augmentation of β-adrenergically stimulated NAT induction (15, 16) is the result of enhanced calcium channel-mediated calcium influx.

Calcium/Enzyme Interactions

A circadian rhythm in the calcium content of the pineal gland, with a peak at the onset of darkness, has been reported (25), and, although the physiological significance of this observation was not apparent at the time, in terms of current understanding of the calcium dependency of melatonin production (6, 7), a high calcium content at lights out may be necessary for the processes leading to the nocturnal increase in melatonin production. It is possible to speculate that a negative feedback mechanism may exist between calcium ions and melatonin, considering the evidence that an increase in intracellular calcium enhances melatonin production (15–18), while a rise in plasma melatonin leads to a reduction in circulating plasma calcium levels (26, 27). Because the doses of melatonin used in these studies in order to elicit a significant change in plasma calcium levels were far greater than physiological levels, it is not possible to conclude that a negative feedback mechanism is involved in normal processes regulating melatonin and calcium homeostasis, but this does not eliminate the possibility.

At a molecular level, calcium ions have been shown to inhibit the activity of the pineal (HIOMT) (28, 29) and to enhance the activity of NAT (28, 30), such inferences being made on the basis of changes to the double-reciprocal plots of the Michaelis-Menten kinetic function (Figs. 5 and 6) (29, 30). Calcium is apparently an uncompetitive inhibitor of HIOMT, binding to the enzyme/substrate complex, which leads to formation of a catalytically inactive complex (29). Activation of NAT by calcium results from binding of the cation to

the enzyme; this leads to an increased affinity of serotonin for binding to the NAT molecule, resulting in enhanced catalytic activity (30). This mechanistic scheme for calcium activation of NAT would imply that the cation bridge must be in place before catalysis can occur; therefore, it is assumed that the enzyme is dependent on the presence of calcium for the production of melatonin (30). To what extent the intervention of calcium at a molecular level influences pineal function is not known, and progress is hampered by difficulties encountered in purification of NAT for detailed studies.

Concluding Remarks

From the foregoing discussion, it is apparent that calcium is an important component in numerous pineal processes, although many of these have not been fully defined. Of particular interest is the possible linkage between α-adrenergic receptors and calcium channels; this area certainly warrants further investigation. As calcium often functions in physiological systems in concert with other cations, future studies should address the possibility of such synergistic/antagonistic interactions. The results from such work should further enhance understanding of the complex biochemical regulatory processes in the pineal, possibly shedding light on important mechanisms involved in the dissemination of photoperiodic information throughout the organism.

1. Reiter RJ. The pineal and its indole products: Basic aspects and clinical applications. In: Foa PP, Cohen MP, Eds. The Brain as an Endocrine Organ. New York: Springer, 96–149, 1988.
2. Pangerl B, Pangerl A, Reiter RJ. Circadian variations of adrenergic receptors in the mammalian pineal gland: A review. J Neural Transm **81**:17–30, 1990.
3. Klein DC. Photoneural regulation of the mammalian pineal gland. In: Photoperiodism, Melatonin and the Pineal. London: Pitman, 38–51, 1985.
4. Reiter RJ, Vaughan MK. Dynamic organization of endocrine networks: The neuroendocrine-reproductive axis and the pineal gland as examples. Ann NY Acad Sci **618**:11–27, 1991.
5. Reiter RJ. The melatonin message: Duration versus coincidence hypotheses. Life Sci **46**:2119–2131, 1987.
6. Wilkinson M. The sensitivity of pineal gland beta-receptors appears to be dependent upon calcium ions. Pflugers Arch **373**:209–210, 1976.
7. Zatz M, Romero JA. Effects of calcium-free medium on the induction of serotonin N-acetyltransferase in the rat pineal. Biochem Pharmacol **27**:2549–2553, 1978.
8. Wilkinson M. Inhibition of the noradrenergic induction of pineal N-acetyltransferase by dibutyryl cyclic guanosine monophosphate and by ionophore X-537A. Neurosci Lett **2**:29–33, 1978.
9. Chan A, Ebadi M. Evidence for existence of a serotonin N-acetyltransferase inactivating substance in rat pineal gland. Endocr Res Commun **8**:205–227, 1981.
10. Khoory R, Schloot W. Inhibition of night time, but not isoproterenol-stimulated pineal N-acetyltransferase activity by an unidentified pineal compound. J Neural Transm **66**:211–219, 1986.
11. Lerchl A, Reiter RJ, Howes KA, Nonaka KO, Stokkan K-A. Extremely low frequency Ca^{2+}-cyclotron resonance: Depression of pineal melatonin synthesis in vitro. Neurosci Lett **124**:213–215, 1991.
12. O'Dea RF, Zatz M. Catecholamine stimulated cyclic GMP accumulation in the rat pineal: Apparent presynaptic site of action. Proc Natl Acad Sci USA **73**:3398–3402, 1976.
13. Chik CL, Ho AK, Klein DC. Alpha-adrenergic potentiation of vasoactive intestinal peptide stimulation of rat pinealocyte adenosine 3′,5′-monophosphate and guanosine 3′,5′-monophosphate: Evidence for a role of calcium and protein kinase C. Endocrinology **122**:702–708, 1988.
14. Ho AK, Chik CL, Klein DC. Protein kinase C is involved in adrenergic stimulation of pineal cGMP accumulation. J Biol Chem **262**:10059–10064, 1987.
15. Sugden AL, Sugden D, Klein DC. Essential role of calcium influx in the adrenergic regulation of cAMP and cGMP in rat pinealocytes. J Biol Chem **261**:11608–11612, 1986.
16. Sugden LA, Sugden D, Klein DC. Alpha 1-adrenoceptor activation elevates cytosolic calcium in rat pinealocytes by increasing net influx. J Biol Chem **262**:741–745, 1987.
17. Sugden D, Vanecek J, Klein DC, Thomas TP, Anderson WB. Activation of protein kinase C potentiates isoprenaline-induced cyclic AMP accumulation in rat pinealocytes. Nature **314**:359–361, 1985.
18. Vanecek J, Sugden D, Weller JL, Klein DC. See-saw signal processing in pinealocytes involves reciprocal changes in the alpha 1-adrenergic component of the cyclic GMP response and the beta-adrenergic component of the cyclic AMP response. J Neurochem **47**:678–686, 1986.
19. Harrison NL, Zatz M. Voltage-dependent calcium channels regulate melatonin output from cultured chick pineal cells. J Neurosci **9**:2462–2467, 1989.
20. Zatz M. Relationship between light, calcium influx and cAMP in the acute regulation of melatonin production by cultured chick pineal cells. Brain Res **477**:14–18, 1989.
21. Zatz M, Mullen DA. Does calcium influx regulate melatonin production through the circadian pacemaker in chick pineal cells? Effects of nitrendipine, Bay K 8644, Co^{2+}, Mn^{2+}, and low external Ca^{2+}. Brain Res **463**:305–316, 1988.
22. Zawilska JB, Nowak JZ. Calcium influx through voltage-sensitive calcium channels regulates in vivo serotonin N-acetyltransferase (NAT) activity in hen retina and pineal gland. Neurosci Lett **118**:17–20, 1990.
23. Meyer AC, Nieuwenhuis JJ, Kociszewska VJ, Joubert WS, Meyer BJ. Dihydropyridine calcium antagonists depress the amplitude of the plasma melatonin cycle in baboons. Life Sci **39**:1563–1569, 1986.
24. Morton DJ, Reiter RJ, Buzzell GR. Swimming-induced suppression of rat pineal melatonin is prevented by pretreatment with calcium channel blockers. Proc Soc Exp Biol Med **190**:105–108, 1989.
25. Piechowiak H, Schnizer W. On electrolyte determinations in the pituitary and pineal gland: Is there a diurnal rythym? Endocrinologie **67**:S51–59, 1976.
26. Csaba G, Bokay J. The effect of melatonin and corpus pineale extract on serum electrolytes in the rat. Acta Biol Acad Sci Hung **28**:143–144, 1977.
27. Morton DJ. Effect of methoxyindole administration on plasma cation levels in the rat. J Pineal Res **6**:141–147, 1989.
28. Morton DJ. Effect of various cations on the activity of pineal gland N-acetyltransferase (EC 2.3.1.5) and hydroxyindole-O-methyltransferase (EC 2.1.1.4) in vitro. J Pineal Res **4**:161–167, 1987.
29. Morton DJ. Mechanism of inhibition of bovine pineal gland hydroxyindole-O-methyltransferase (EC 2.1.1.4) by divalent cations. J Pineal Res **4**:295–303, 1987.
30. Morton DJ. Possible mechanisms of inhibition and activation of rat pineal N-acetyltransferase (EC 2.3.1.5) by cations. J Neural Transm **75**:51–64, 1989.