The Prostate 35:27–34 (1998)

Melatonin Receptors in Benign Prostate Epithelial Cells: Evidence for the Involvement of Cholera and Pertussis Toxins–Sensitive G Proteins in Their Signal Transduction Pathways

Eli Gilad,[1] Elah Pick,[1] Haim Matzkin,[2] and Nava Zisapel[1]*

[1]*Department of Neurobiochemistry, The George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel*

[2]*Department of Urology, Tel Aviv Medical Center, Tel Aviv, Israel*

BACKGROUND. Melatonin, the hormone secreted nocturnally by the pineal gland, binds to epithelial cells from the human benign prostate, and can reduce their growth and viability. The possible involvement of GTP binding proteins cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) in melatonin responses in these cells were investigated.

METHODS. The effects of melatonin on cAMP and cGMP were assessed in prostate cells untreated or pretreated with pertussis toxin (PTX) or cholera toxin (CTX).

RESULTS. Melatonin augmented cAMP but reduced cGMP in the epithelial cells (maximal responses at 10 nM). The increase in cAMP was attenuated by PTX, but not by CTX, whereas the decrease in cGMP was attenuated by CTX, but not by PTX. CTX, but not PTX, abolished the melatonin-mediated suppression of ^{3}H-thymidine incorporation. In addition, melatonin facilitated the CTX- and PTX-mediated ADP ribosylation of 44- and 41-kilodalton proteins, respectively. The cGMP analogue 8-bromo-cGMP, negated the melatonin-mediated decrease in 3H-thymidine incorporation, whereas H89, a protein kinase A inhibitor, did not inhibit melatonin's effect.

CONCLUSIONS. Melatonin receptors in the human benign prostate epithelial cells enhance cAMP and inhibit cGMP through PTX- and CTX-sensitive G proteins, respectively. The decrease in DNA synthesis may be secondary to the melatonin-mediated decrease in cGMP. *Prostate 35:27–34, 1998.*

KEY WORDS: **melatonin; prostate; receptor; G proteins; cAMP; cGMP**

INTRODUCTION

Melatonin, produced nocturnally by the pineal gland, plays a major role in coordination of seasonal reproduction and pubertal development in mammals [1,2]. The mediobasal hypothalamus and pars tuberalis of the pituitary have been implicated as the primary sites of melatonin action. However, accumulating evidence indicate the presence of melatonin receptors in peripheral organs in mammals [3].

We recently found that human benign prostate tissue contains melatonin binding sites primarily associated with the epithelial cells [4]. In culture, the benign prostate epithelial cells also display high affinity melatonin binding. Melatonin inhibited DNA and protein synthesis in these cells [5]. The effects of melatonin on 3H-thymidine incorporation in the prostate epithelial cells were transient: maximal responses were measured within 1 hr and the incorporation had returned to close-to-basal values within 24 hr of treatment [5]. Nevertheless, cells viability and growth were signifi-

*Correspondence to: Nava Zisapel, Department of Neurobiochemistry, The George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv 69978, Israel. E-mail: navazis@ccsg.tau.ac.il

Received 11 April 1997; Accepted 18 July 1997

cantly reduced in cells treated with melatonin for 1–4 days [5]. We have recently shown that melatonin receptors can be inactivated by protein kinase C (PKC) and that the transient response of the prostate cells to melatonin may result of a direct or indirect melatonin-induced activation of endogenous PKC [6]. These results indicate that human prostatic epithelial cells contain functional melatonin receptors that may undergo agonist-induced desensitization [5,6].

Several putative signal transduction pathways have been implicated in melatonin responses. Melatonin has been found to inhibit the forskolin-stimulated production of cAMP in ovine pars tuberalis, and rat pituitary without affecting basal cAMP levels [7,8], whereas in cultured murine melanoma cells, melatonin slightly decreased or increased basal cAMP, depending on cell density [9] and decreased cGMP [10]. Hence, we have explored the involvement of cAMP in the melatonin-mediated effects on the growth of the BPH epithelial prostate cells. This was pursued by assessing cAMP levels in the absence and presence of melatonin and the impact of H-89, a selective inhibitor of protein kinase A, on the melatonin-mediated effects on ^{3}H-thymidine incorporation.

Melatonin has been shown to enhance cGMP in the rat testis, rat medial basal hypothalamus, and golden hamster retina [11–13] but inhibit gonadotropin-releasing hormone (GnRH)-induced accumulation of cGMP in the neonatal rat pituitary [14]. Hence, we have investigated the effects of melatonin on cGMP in the cells and the impact of the cell-permeable cGMP analogue, 8-bromo-cGMP on the melatonin-mediated effects on ^{3}H-thymidine incorporation into these cells.

Melatonin receptors coupled to pertussis-toxin (PTX) sensitive G protein(s) (Mel-1a) have been cloned [15]. However, in NIH-3T3 cells transfected with the Mel-1a clone, melatonin has been found to elicit both PTX-sensitive and insensitive responses [16]. The existence of additional, CTX-sensitive melatonin responses has been demonstrated, in melanoma and ovine pars tuberalis cells [9,10,17–19]. We have thus studied the effects of CTX and PTX on the melatonin-mediated effects on DNA synthesis and cyclic nucleotide responses.

MATERIALS AND METHODS

Materials

Melatonin, phenylmethane sulfonyl fluoride (PMSF), dihydrotestosterone (DHT), CTX, and pertussis toxin (PTX) were obtained from Sigma Chemical Co. (St. Louis, MO). Methyl-^{3}H-thymidine was obtained from Rotem Industries (Beer Sheva, Israel). The cGMP radioimmunoassay (RIA)kit were obtained from Amersham (Buckinghamshire, UK). ^{32}P-NAD and the cAMP RIA kit were obtained from DuPont NEN (Boston, MA).

Culture media (RPMI-1640) and RPMI-1640 without phenol red supplemented with L-glutamine (RPMI-P), newborn calf serum (NBS), and charcoal-stripped newborn calf serum (NBSC), insulin, transferrin, selenium, and antibiotics were purchased from Biological Industries (Beit Haemek, Israel).

Epithelial Cell Cultures

Human benign prostate tissue samples were obtained from patients undergoing trans-abdominal prostatectomies for benign prostatic hypertrophy (BPH). All patients (age 55–80, n = 24) were otherwise healthy. Approval for the use of the tissue was obtained from the local Ethical Committee. After removal, BPH was confirmed using histopathology. The epithelial cell cultures were prepared form the BPH tissue samples as described [5] and grown at 37°C in growth medium (RPMI containing 10% NBS, 10 ng/ml EGF, 5 ng/ml insulin, 5 ng/ml transferrin, 5 ng/ml selenium, 50 U/ml penicillin, 50 μg/ml streptomycin, 250 ng/ml amphotericin B, and 10 ng/ml DHT) in humidified atmosphere with 5% CO_2. Before each experiment, cells were harvested by trypsin and adjusted to a density of 106 cells/ml in culture medium (RPMI containing 10% NBSC, 10 ng/ml EGF, 5 ng/ml insulin, 5 ng/ml transferrin, 5 ng/ml selenium, 50 U/ml penicillin, 50 μg/ml streptomycin, and 10 ng/ml DHT) and replated in 24-well multiplates. After 24 hr, the medium was replaced with fresh culture medium containing 5% NBSC. Protein content was determined in each plate [2], and data of all experiments were normalized to protein content.

Assessment of Cells cAMP and cGMP Contents

Cells were washed twice with phosphate-buffered saline (PBS) and harvested by policeman in RPMI-P medium, containing 200 μM PMSF. After 10-min incubation at 37°C, melatonin (10^{-10}–10^{-6} M) or an equal volume of vehicle (0.1% ethanol) was added and incubation resumed for 10 min. The reaction mixture was then boiled for 4 min, then frozen and thawed. Samples were taken for protein determination. The mixture was then sonicated and soluble materials collected by centrifugation (10,000*g*, 10 min). The cAMP or cGMP contents of the supernatants were determined by radioimmunoassay. Data were normalized to cell protein content.

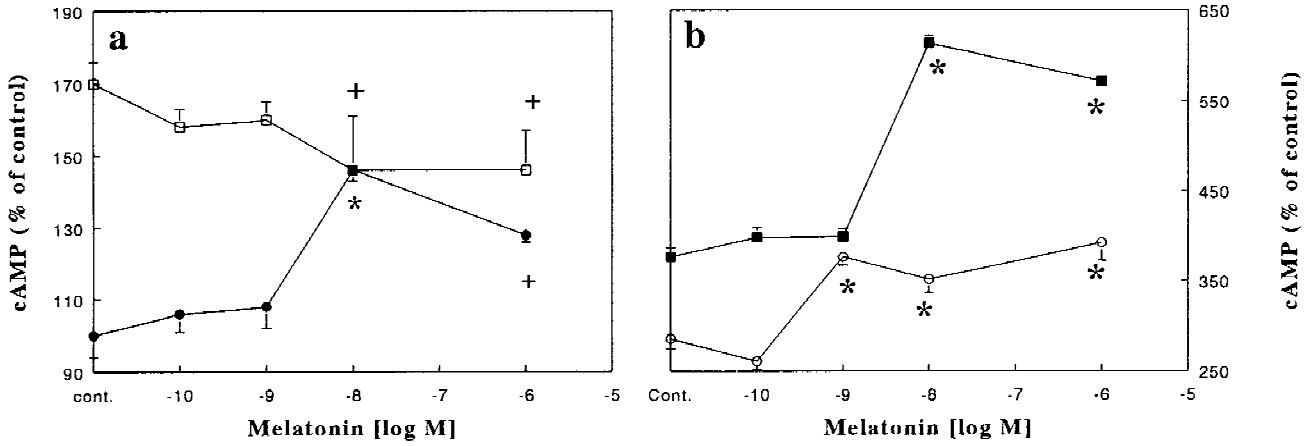

Fig. 1. Effects of melatonin on cAMP levels in human benign prostate epithelial cells. Cells were incubated with **(a)** buffer (filled circle) or **(b)** forskolin (1 μM) (open circle) for 10 min, or treated with (a) PTX (0.8 ng/ml) (open box) or (b) CTX (2 μg/ml) (filled box) for 24 hr. Cells were suspended in RPMI-P medium and incubated with melatonin (10^{-10}–10^{-6}M) or an equivalent volume of vehicle (cont.) for 10 min; cAMP was then determined by RIA. Results are mean ±SEM of three independent studies, run in triplicate, expressed as a percentage of cAMP content in cells treated with vehicle. *$P < 0.01$ and + $P < 0.05$ vs control without melatonin.

^{3}H-Thymidine Incorporation

^{3}H-thymidine incorporation was assessed as described [5]. Briefly, cells attached to the plates were incubated with buffer or melatonin for 1 hr at 37°C. ^{3}H-thymidine (60 Ci/mmol, 1 μCi/well) was then added and incubation resumed for 1 hr. Media were then discarded, the cells washed (2 × 2 ml) with ice-cold PBS and harvested by trypsin. Aliquots were retained for protein determination. Trichloroacetic acid (TCA) was added and insoluble materials collected by filtration on GF/C glass fiber filters. The amount of radioactivity determined by scintillation spectrometry.

In some studies, cells were pretreated with 50 nM H89, a protein kinase A blocker, or with 100 μM 8-Br-cGMP, a cGMP analogue, 15 min before assessment of ^{3}H-thymidine incorporation.

CTX and PTX Treatment

Cells were treated with CTX (2 μg/ml) or PTX (40 ng/ml), or an equivalent volume of buffer for 24 hr. The cells were then washed and used in the experiments. In some experiments, prostate epithelial cells, were homogenized and then incubated at 37°C with 50 mM Tris buffer, 5 mM $MgCl_2$, 30 mM DTT, 2.5 mM EDTA, 1 mM ATP, with or without 200 μM GTPΓS or GDPβS, CTX (2 μg/ml) or PTX (40 ng/ml), and in the absence and presence of melatonin (10 nM). CTX and PTX were activated by 20 mM DTT. ADP ribosylation reactions were initiated by the addition (^{32}P-NAD (5 μM), and were terminated after 10 minutes by addition of 300% v/v ethanol. Samples were stored over night at −20°C, proteins were sedimented by centrifugation (10,000*g*, 15 min), dissolved in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer [21]. The labeled proteins were resolved by PAGE and autoradiography.

Statistical Analyses

Results were compared by analysis of variance (ANOVA), followed by Student-Newman-Keul's test for multiple comparisons, significance at $P < 0.050$ [22].

RESULTS

cAMP

The effects of various melatonin concentrations (10^{-10}–10^{-6} M) on cAMP concentrations in the prostate epithelial cells are shown in Figure 1. Melatonin augmented the cells cAMP contents by ≤146 ± 3% of control values (Fig. 1a). Forskolin augmented the cells cAMP to 286 ± 10% of control values ($P < 0.001$); the cell's cAMP content was further augmented in the presence of melatonin (Fig. 1b). Maximal effects of melatonin on cAMP were observed at 10 nM in the absence of forskolin and at 1 nM in its presence (Fig. 1a,b).

PTX treatment increased cAMP content to 170 ± 6% of control values ($P < 0.0001$) and ablated the melatonin-mediated enhancement of cAMP (Fig. 1a). Interestingly, in the PTX-treated cells, melatonin slightly but significantly reduced cAMP content by 24% (Fig. 1a). CTX treatment increased cAMP content to 377 ±

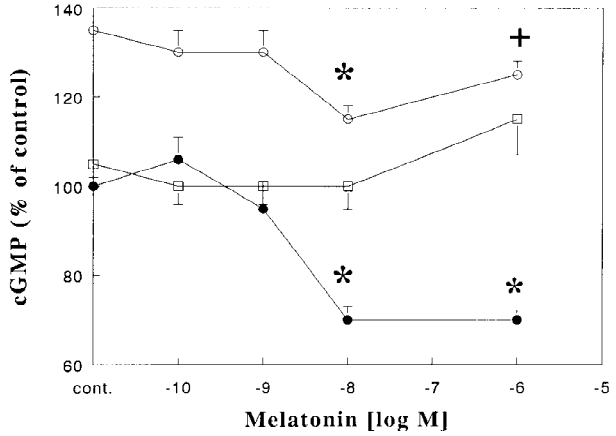

Fig. 2. Effects of melatonin of cGMP levels in human benign prostate epithelial cells. Cells were incubated with buffer (filled circle), CTX (2 µg/ml) (open box) or PTX (0.8 ng/ml) (open circle) for 24 hr. The cells were then suspended in RPMI-P medium and incubated with melatonin (10^{-10}–10^{-6} M) or equivalent volume of vehicle (cont.) for 10 min and cGMP was then determined by RIA. Results are mean ±SEM of three independent studies, run in triplicates, expressed as a percentage of cAMP content in cells treated with vehicle. *$P < 0.01$ and + $P < 0.05$ vs control without melatonin.

10% of control values ($P < 0.0001$) but did not inhibit, and even extenuated, the melatonin-mediated increase in cAMP (Fig. 1b). Maximal enhancement of cAMP in the CTX-treated cells was observed at 10 nM melatonin (Fig. 1b).

cGMP

The effects of various melatonin concentrations (10^{-10}–10^{-6} M) on cGMP content of the prostate epithelial cells are shown in Figure 2. Melatonin significantly reduced the cells cGMP contents (by up to 30% of control values; Fig. 2) in a dose dependent manner. Maximal inhibition was observed at 10 nM melatonin (Fig. 2).

PTX treatment increased the cell's cGMP content by approximately 35% of the levels in untreated controls ($P < 0.001$) but did not abolish the melatonin-mediated inhibition of cGMP (Fig. 2). CTX treatment did not alter the cell's cGMP content but ablated the inhibitory effect of melatonin (Fig. 2).

ADP Ribosylation

The effects of melatonin on the ADP ribosylation of epithelial cell proteins in the absence and presence of CTX and PTX was studied, using ^{32}P-NAD (Fig. 3). Melatonin markedly augmented the CTX-mediated ADP ribosylation of a protein with an apparent molecular weight value of 44 kilodaltons (kDa) and the PTX-mediated ADP ribosylation of a 41-kDa protein. Melatonin did not significantly affect ADP ribosylation of these proteins in the absence of the toxins. The ADP ribosylation of an additional 37-kDa protein was also enhanced by melatonin (Fig. 3), but this enhancement was independent of the absence or presence of CTX or PTX (not shown).

^{3}H-Thymidine Incorporation

The involvement of PTX- and CTX-sensitive pathways in the effects of melatonin on ^{3}H-thymidine incorporation into the prostate epithelial cells was studied (Fig. 4). Treatment with melatonin (10^{-10}–10^{-6}M) for 1 hr resulted in a marked (up to 55%) decrease in ^{3}H-thymidine incorporation compared to values in control cells treated with vehicle. Maximal facilitation was observed at 10 nM melatonin. CTX treatment inhibited ^{3}H-thymidine incorporation. In addition, CTX abolished the inhibitory effects of melatonin on ^{3}H-thymidine incorporation, whereas PTX treatment attenuated but did not completely block this effect (Fig. 4).

The significance of the effects of melatonin on the cells cyclic nucleotide levels to its effects on ^{3}H-thymidine incorporation was investigated (Fig. 5). The protein kinase A inhibitor H89 attenuated the incorporation of ^{3}H-thymidine in the cells (by about 30%) but had no effect on the suppression by melatonin of ^{3}H-thymidine incorporation. The cGMP analogue, 8-Br-cGMP, also decreased ^{3}H-thymidine incorporation into the cells by approximately 30% of the values observed in its absence. However, 8-Br-cGMP abrogated the inhibitory effects of melatonin on ^{3}H-thymidine incorporation (Fig. 5).

DISCUSSION

The data presented in this paper indicate that melatonin receptors in human benign prostate epithelial cells can elicit multiple responses in the cells. These responses (increase in cAMP, decrease in cGMP and in ^{3}H-thymidine incorporation) are mediated by PTX- and CTX-sensitive G proteins, respectively. Maximal melatonin responses in these cells were attained with approximately 10 nM. Notably, nocturnal melatonin levels in the human plasma decline with age from 3 to 4 nM in infants to 0.2–0.5 nM in adults [23]. Hence, circulating melatonin levels comparable to those present at the prepubertal stage may potentially attenuate prostate epithelial cells growth.

The observed increase in cAMP in the prostate epithelial cells, differs from previous observations made with the cloned Mel1 melatonin receptor [15,16] and melatonin receptors in ovine pars tuberalis [19]. In both, melatonin alone did not affect basal cAMP con-

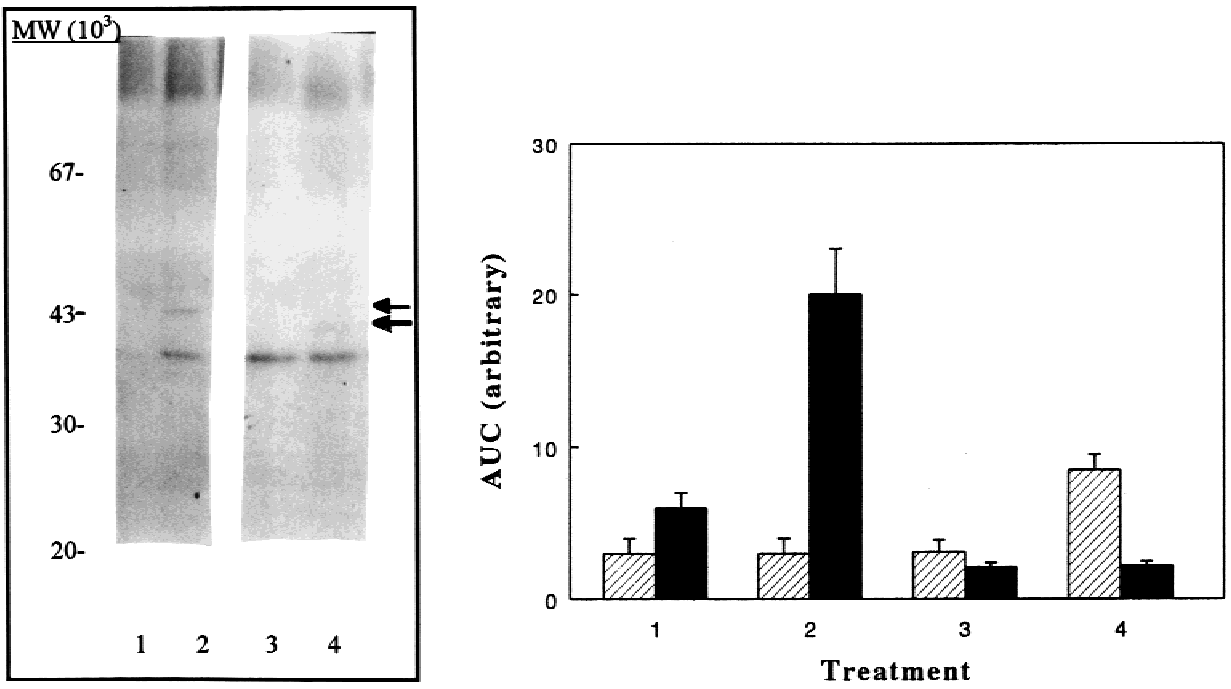

Fig. 3. Effects of melatonin, CTX, and PTX on protein ADP ribosylation in prostate epithelial cells. Cells were homogenized and incubated with ^{32}P-NAD in the presence of GTPΓS and CTX (1,2) or GDP and PTX (3,4) and in the absence (1,3) and presence (2,4) of melatonin (10 nM). **Left:** The labeled proteins were resolved by polyacrylamide gel electrophoresis and autoradiography. The apparent molecular-weight values of the proteins were related to those of protein markers run on the same gels. *Arrows,* 41- and 45-kDa bands. **Right:** Densitometric assessment of the incorporation of radioactive ADP ribose into 41-kDa *(striated bar)* and 45-kDa *(black bar)* proteins is presented as area under the curve (AUC). Results are mean ±SD values of four repetitive experiments.

centrations, but negated the increase in cAMP upon forskolin-mediated stimulation of adenylate cyclase. The fact that the enhancement by melatonin of cAMP in the benign prostate cells was maintained in the presence of forskolin suggests that the effects of melatonin were mediated by inhibition of phosphodiesterase, rather than by activation of adenylate cyclase activity.

The enhancement by melatonin of the cells cAMP was extenuated in CTX-treated cells but abolished by PTX treatment. Hence, the putative inhibition of phosphodiesterase activity by melatonin may be mediated by a PTX-sensitive G protein. Coupling of melatonin receptors to PTX-sensitive G proteins is compatible with the situation in the Mel-1a receptor and in pars tuberalis cells [15,16,19].

It is interesting to note that in PTX-treated cells, melatonin was able to inhibit cAMP. This suggests that besides the facilitation by melatonin of cAMP, which is mediated by a PTX-sensitive G protein, melatonin suppresses cAMP through a non-PTX-sensitive pathway. This response is compatible with recent observations indicating that melatonin receptors couple through a CTX-sensitive mechanism to inhibit cAMP in ovine pituitary [19].

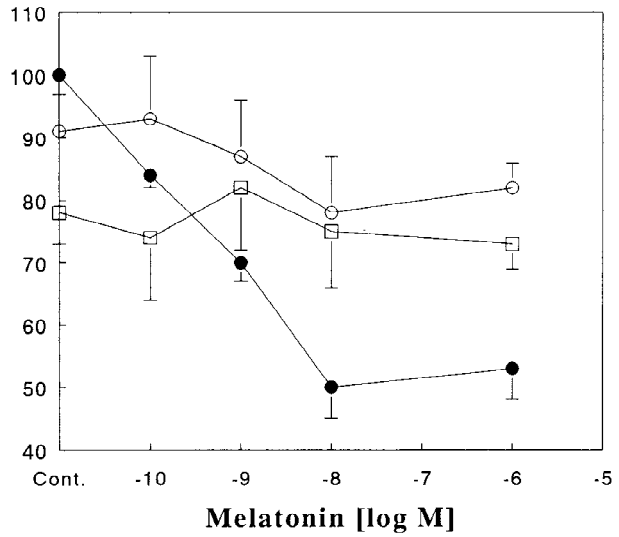

Fig. 4. Effects of PTX and CTX on the melatonin-mediated suppression of ^{3}H-thymidine incorporation in prostate epithelial cells. Cells were incubated with buffer (filled circle), or CTX (2 μg/ml) (open box) or PTX (0.8 ng/ml) (open circle) for 24 hr, and then incubated with various melatonin concentrations or the same volume of vehicle (cont.) for 1 hr. The incorporation of ^{3}H-thymidine was then assessed. Results are mean ±SEM of three independent studies, run in quintuplicate, expressed as a percentage of incorporation into the control vehicle-treated cells.

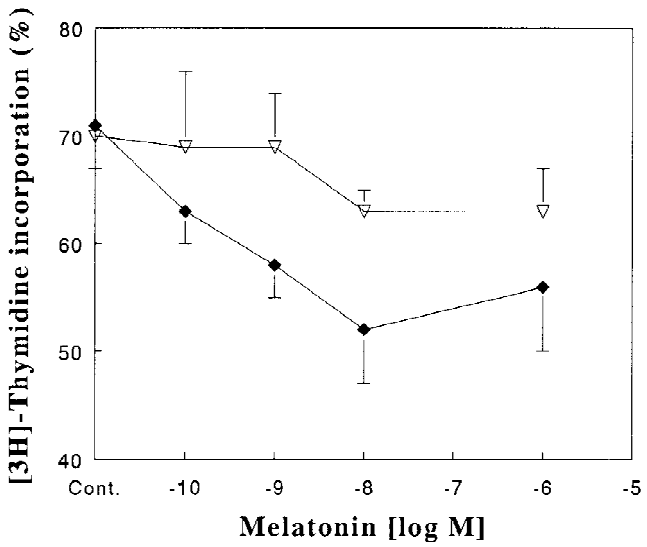

Fig. 5. Effects of melatonin on ^{3}H-thymidine incorporation in human BPH epithelial cells. Cells were incubated with H89 (50 nM) (filled diamond) or 8-Br-cGMP (100 μM) (open inverted triangle) for 15 min and then incubated with various melatonin concentrations or the same volume of vehicle (cont.) for 1 hr. The incorporation of ^{3}H-thymidine was then assessed. Results are mean ±SEM of three independent studies, run in quintuplicate, expressed as a percentage of incorporation into the control vehicle-treated cells.

Inhibition by melatonin of cGMP in the prostate epithelial cells was ablated by CTX and not by PTX. This finding indicates that melatonin either suppresses guanylate cyclase or activates phosphodiesterase activity in these cells, through a CTX-sensitive G protein. A CTX-sensitive activation of phosphodiesterase is compatible with the extenuation of the melatonin-mediated increase in cAMP in CTX-treated cells, as well as with the melatonin-mediated decrease in cAMP in the PTX-treated cells. The mechanism by which a CTX-sensitive protein activates a phosphodiesterase activity (or suppresses guanylate cyclase activity) is unknown, nor is it known how melatonin receptors communicate with this protein. It should be noted, that activation of CTX-sensitive G proteins has been shown to elicit, besides cAMP production, cellular effects such as regulation of ion channels and phospholipase activity [24].

Inhibition by melatonin of the calmodulin-dependent cGMP phosphodiesterase has been documented. However, this effect was attributed to inhibition by melatonin of calmodulin [25]. This process may not be of critical importance in the prostate cells, as in these cells melatonin reduced rather than enhanced cGMP concentrations.

The inhibitory effect of melatonin on cGMP in the prostate cells is different from the commonly described enhancement by melatonin of cGMP in the rat testes and hypothalamus and in the golden hamster retina [11–13] but is compatible with suppression by melatonin of cGMP in the rat pituitary [4]. It is thus possible, that the relative abundance of calmodulin-dependent cGMP phosphodiesterase and of other phosphodiesterases in the cells (which may be directly or indirectly be affected by PTX-and CTX-sensitive G proteins), will determine if the overall effect of melatonin on cGMP will be an enhancement or suppression.

Melatonin augmented both CTX- and PTX-catalyzed ADP-ribosylation of specific protein substrates in the cells. The 45 and 41 kilodalton protein bands are compatible with the α-subunits of Gs- and Gi-type G proteins, which are known to become specifically ADP ribosylated by CTX and PTX, respectively [26]. Hence, the effects of melatonin on cAMP and cGMP in the cells may be effected by activation of Gs and Gi type G proteins. These results may suggest that melatonin receptors are coupled to multiple GTP binding proteins. Alternatively, melatonin may provoke some primary response, which subsequently leads to enhancement of GTP exchange and activation of multiple GTP binding proteins. These conclusions are compatible with recent observation showing activation by melatonin of multiple GTP binding proteins in murine melanoma cells [18], involvement of PTX- and CTX-sensitive G proteins in melatonin's effects on cAMP in the ovine pituitary [19], and induction by melatonin of PTX-sensitive and -insensitive signal transduction pathways in cells transfected with the melatonin Mel-1a receptor clone [16]. Altogether, our data suggest that melatonin inhibits a cAMP phosphodiesterase activity by a PTX-sensitive pathway and enhances a cGMP/cAMP phosphodiesterase activity by a CTX-sensitive pathway.

The inhibition by melatonin of ^{3}H-thymidine incorporation may represent a slow down of the cell cycle or a specific slowdown of DNA elongation in the S phase. Whether and how this inhibition leads to the attenuation of cell growth is important issue for the ultimate understanding of how melatonin attenuates prostate cells growth. The possibility exists, that the effect of melatonin on ^{3}H-thymidine incorporation is secondary to its effects of cyclic nucleotides in the cells. In rat and human prostate cancer cells, cAMP has been shown to serve as a growth inhibitor [27,28]. The fact that CTX (which enhances cell cAMP) reduced ^{3}H-thymidine incorporation in the prostate epithelial cells is compatible with this notion. On the other hand, CTX, which elevates cAMP, has been demonstrated to be a potent growth stimulator of rodent and human prostate normal and malignant cells when present continuously in the cultures [29,30]. The apparent discrepancy between the different studies may perhaps be explained by the fact that CTX might

induce other cellular effects, besides the increase in cAMP production [24]. In addition, depletion of alpha subunit of the Gs type GTP binding protein may occur upon continuous CTX treatment [31].

The relationship between the melatonin-induced elevation of cAMP and the attenuation by melatonin of ^{3}H-thymidine incorporation is plausible. This is because CTX, which did not prevent the enhancement by melatonin of cAMP, did prevent the suppression by melatonin of ^{3}H-thymidine incorporation. Moreover, PTX, which did prevent the facilitation by melatonin of cAMP, did not completely block the inhibition by melatonin of ^{3}H-thymidine incorporation. Nor did blockade of protein kinase A activity by H-89. Hence, the effects of melatonin on cAMP may not explain the inhibition of ^{3}H-thymidine incorporation induced by this hormone.

The possibility that the decrease in cGMP mediates the suppression of ^{3}H-thymidine incorporation cannot be ruled out. CTX, which blocked the melatonin-mediated inhibition of cGMP also blocked the effects of melatonin on ^{3}H-thymidine incorporation. Moreover, the cGMP analogue, 8-Br-cGMP, was able to abrogate the inhibitory action of melatonin on ^{3}H-thymidine incorporation. By contrast, elevation of cGMP have been associated with suppression of growth in a number of cell types [32,33]. The fact that the cGMP analogue and, to a lesser extent, PTX treatment (both increased cGMP content) did reduce ^{3}H-thymidine incorporation, is compatible with this notion. Thus, an association of a decrease in cGMP with suppression of ^{3}H-thymidine incorporation as effected by melatonin, is apparently paradoxical. One possible explanation is that the changes in cGMP are compartmentalized and the responses generated may depend on the specific compartment involved. Some support for this notion comes from recent findings indicating that the distribution of cGMP within the cells is changing with culture growth state [34].

REFERENCES

1. Reiter RJ: Pineal melatonin: Cell biology of its synthesis and of its physiological interaction. Endocrine Rev 1991;12:151–180.
2. Waldhauser F, Boepple PA, Schemper M, Mansfield MJ, Crowley WF Jr: Serum melatonin in central precocious puberty is lower in age-matched prepubertal children. J Clin Endocrinol Metab 1991;73:793–796.
3. Pang SF, Dubocovich ML, Brown GM: Melatonin receptors in peripheral tissues: A new area of melatonin research. Biol Signals 1993;2:177–180.
4. Laudon M, Gilad E, Matzkin H, Braf Z, Zisapel N: Putative melatonin receptors in benign human prostate tissue. J Clin Endocr Metab 1996;81:1336–1342.
5. Gilad E, Laudon M, Matzkin H, Pick E, Sofer M, Braf Z, Zisapel N: Functional melatonin receptors in human prostate epithelial cells. Endocrinology 1996;137:1412–1417.
6. Gilad E, Matzkin H, Zisapel N: Inactivation of melatonin receptors by protein kinase C in human prostate epithelial cells. Endocrinology 1997;138:4255–4261.
7. McNulty S, Ross AW, Barrett P, Hastings MH, Morgan PJ: Melatonin regulates the phosphorylation of CREB in ovine pars tuberalis. J Neuroendocrinol 1994;6:523–553.
8. Vanecek J, Klein DC: Mechanism of melatonin signal transduction in neonatal rat pituitary. Neurochem Int 1995;27:273–278.
9. Bubis M, Zisapel N: Modulation by melatonin of protein secretion from melanoma cells: Is cAMP involved? Mol Cell Endocrinol 1995;112:169–175.
10. Bubis M, Zisapel N: Involvement of cGMP in cellular melatonin responses. Biol of the Cell 1998 (in press).
11. Kano T, Miyachi Y: Direct action of melatonin on testosterone and cGMP production using rat testis tissue in vitro. Biochem Biophys Res Commun 1976;72:969–975.
12. Vacas MI, Sarmiento MI, Cardinali DP: Melatonin increases cGMP and decreases cAMP levels in rat medial basal hypothalamus in vitro. Brain Res 1981;225:207–211.
13. Faillace MP, Keller Sarmiento MI, Rosenstein RE: Melatonin effect on the cGMP system in the golden hamster retina. Brain Res 1996;711:112–117.
14. Vanecek J, Vollrath L: Melatonin inhibits cAMP and cGMP accumulation in the rat pituitary. Brain Res 1989;505:157–159.
15. Reppert M, Weaver DM, Godson C: Melatonin receptors step into the light: Cloning and classification of subtypes. Trends Pharmacol Sci 1996;17:100–102.
16. Catherine G, Reppert SM: The Mel1 melatonin receptor is coupled to parallel signal transduction pathways. Endocrinology 1997;138:397–404.
17. Bubis M, Zisapel N: Facilitation and inhibition of G protein regulated protein secretion by melatonin. Neurochem Int 1995; 27:177–183.
18. Bubis M, Anis Y, Zisapel N: Enhancement by melatonin of GTP exchange and ADP ribosylation reactions. Mol Cell Endocrinol 1996;123:139–148.
19. Morgan PJ, Perry B, Hazlerigg D, Milligan G, Lawson W, MacLean A, Davidson G: Melatonin receptors couple through a cholera toxin-sensitive mechanism to inhibit cyclic AMP in ovine pituitary. J Neuroendocrinol 1995;7:361–369.
20. Markwell MAK, Haas SM, Bieber LC, Talbert NE: A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. Anal Biochem 1978;87: 206–210.
21. Laemmli UK: Cleavage of structural proteins during assembly of the head bacteriophage T4. Nature 1970;227:680–685.
22. Sokal RR, Rholf FJ: ''Biometry.'' San Francisco: Freeman and Co., 1969.
23. Waldhauser F, Weiszenbacher G, Tatzer E, Gisinger B, Waldhauser M, Schemper M, Frisch H: Alterations in nocturnal serum melatonin levels in humans with growth and aging. J Clin Endocrinol Metab 1988;66:648–652.
24. Neer EJ: Heterotrimeric G proteins: Organizers of transmembrans signals. Cell 1995;80:249–257.
25. Benitez-King G, Huerto-Delgadillo L, Anton-Tay F: Melatonin modifies calmodulin cell levels in MDCK and N1E-115 cell lines and inhibits phosphodiesterase activity in vitro. Brain Res 1991;557:289–292.
26. Birnbaumer L, Abramowitz J, Brown AM: Receptor-effector coupling by G proteins. Biochim Biophys Acta 1990;1031:163–224.
27. Steiner MS, Wand GS, Barrack ER: Effects of Transforming growth factor beta-1 on the adenylyl cyclase-cAMP pathway in prostate cancer. Growth Factors 1994;11:283–290.

28. Bang YJ, Kim SJ, Danielpour D, O'Reilly MA, Kim KY, Myers CE, Trepel JB: Cyclic AMP induced transforming growth factor beta 2 gene expression and growth arrest in the human androgen-independent prostate carcinoma cell line PC3. Proc Natl Acad Sci USA 1992;89:3556–3560.
29. McKeenan WL, Adams PS, Rosser MP: Direct mitogenic effects of insulin, epidermal growth factor, glucocorticoid, cholera toxin, unknown pituitary factors and possibly prolactin, but not androgen, on normal rat prostate epithelial cells in serum-free primary cell culture. Cancer Res 1984;44:1998–2010.
30. Kabalin JN, Peehl DM, Stamey TA: Clonal growth of human prostatic epithelial cells is stimulated by fibroblasts. Prostate 1989;14:251–263.
31. White BH, Klein DC: Stimulation of cyclic GMP accumulation by sodium nitroprusside is potentiated via a Gs mechanism in intact pinealocytes. J Neurochem 1995;64:711–717.
32. Yang W, Ando J, Korenaga R, Toyo-oka T, Kamiya A: Exogenous nitric oxide inhibits proliferation of cultured vascular endothelial cells. Biochem Biophys Res Commun 1994;203:1160–1167.
33. Wolf G, Ziyadeh FN, Stahl RA: Atrial natriuretic peptide stimulates the expression of transforming growth factor-beta in cultured murine mesangial cells: Relationship to suppression of proliferation. J Am Soc Nephrol 1995;6:224–233.
34. Sorci G, Spreca A, Donato R, Rambotti MG: Detection of membrane-bound guanylate cyclase activity in rat C6 glioma cells at different growth states following activation by natriuretic peptides. Brain Res 1995;683:51–58.