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Functional Interaction of Melatonin Receptors and D1 Dopamine Receptors in Cultured Chick Retinal Neurons

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The possible interaction of melatonin receptors and D1 dopamine receptors was investigated in neural cells prepared from embryonic day 8 chick retinas and cultured for 6 d. Dopamine stimulated cAMP accumulation in cultured retinal cells. This effect of dopamine was antagonized by addition of dopamine receptor antagonists (haloperidol and SCH23390) or melatonin receptor agonists (melatonin, 2-iodomelatonin, and 6-chloromelatonin). The inhibition of dopamine-stimulated cAMP accumulation by melatonin was concentration dependent, with half-maximal inhibition at approximately 160 pM. Melatonin inhibited the effect of dopamine at all dopamine concentrations, suppressing the maximal response to the neurotransmitter by approximately 70%. Melatonin also inhibited the stimulation of cAMP accumulation by SKF 82958, a selective D1 dopamine receptor agonist. Pretreatment of cultures with pertussis toxin had no significant effect on dopamine-stimulated cAMP accumulation, but inhibited the response to melatonin. In contrast to its effect on cAMP accumulation, melatonin had no effect on dopamine-stimulated inositol phosphate accumulation. These results suggest that melatonin receptors are coupled to dopamine receptor-regulated adenylate cyclase via an inhibitory G protein, and demonstrate another mechanism, in addition to inhibition of dopamine release, through which melatonin can modulate dopaminergic neurotransmission.

[Key words: melatonin receptors, D1 dopamine receptors, cAMP, inositol phosphates, pertussis toxin, retina, receptor cross talk]

Melatonin and dopamine are putative neuromodulators with opposing roles in retinal physiology (Besharse et al., 1988). Melatonin is synthesized in and released from photoreceptor cells (e.g., Redburn and Mitchell, 1989; Iuvone et al., 1990; Cahill and Besharse, 1992; Zawilska and Iuvone, 1992; Thomas et al., 1993; Wiechmann and Craft, 1993), and dopamine is synthesized in amacrine and interplexiform cells (reviewed in Ehinger, 1977; Iuvone, 1986). In retina, melatonin activates rod photoreceptor disk shedding (Besharse and Dunis, 1983; Besharse et al., 1984), and promotes dark-adaptive retinomotor movements (Cheze and Ali, 1976; Pang and Yew, 1979; Pierce and Besharse, 1985). In contrast, dopamine inhibits rod photoreceptor disk shedding (Pierce and Besharse, 1986; Besharse et al., 1988) and promotes light-adaptive retinomotor movements (Pierce and Besharse, 1985; Dearry and Burnside, 1986). Melatonin inhibits electrically stimulated and light-evoked release of ACh (Mitchell and Redburn, 1991), while dopamine stimulates ACh release (Hensler and Dubocovich, 1986). Melatonin enhances the sensitivity of horizontal cells (Wiechmann et al., 1988), and decreases the trans-epithelial potential of retinal pigment epithelial (RPE) cells and the c-wave of the electroretinogram (ERG) (Nao-I et al., 1989; Rudolf et al., 1992). Dopamine decreases the response of horizontal cells to full-field stimulation (Mangel and Dowling, 1987), and increases the transepithelial potential of RPE cells and the c-wave amplitude (Gallemore and Steinberg, 1990; Rudolf et al., 1992). Melatonin is synthesized and released in retina in darkness, and in some species, under the influence of a circadian clock (Hamm and Menaker, 1980; Besharse and Iuvone, 1983; Redburn and Mitchell, 1989), while dopamine synthesis and release in retina is stimulated by steady or flickering light (Kramer, 1971; Iuvone et al., 1978; Dowling and Watling, 1981; Parkinson and Rando, 1983; Godley and Wurtman, 1988; Boatright et al., 1989). Melatonin inhibits dopamine synthesis and release (Dubocovich, 1983; Nowak, 1988; Nowak et al., 1992; Boatright et al., 1994), and melatonin synthesis and release are inhibited by dopamine (Iuvone and Besharse, 1986; Iuvone et al., 1987, 1990; Zawilska and Iuvone, 1989; Cahill and Besharse, 1991). Thus, melatonin and dopamine appear to form the basis of a neurochemical feedback loop that regulates opposing physiological processes.

High-affinity melatonin receptors have been characterized in retina using 2-^{125}I-iodomelatonin as a radioligand (e.g., Dubocovich and Takahashi, 1987; Laitinen and Saavedra, 1990; Wiechmann and Wirsig-Wiechmann, 1991). Occupation of melatonin receptors in retinal membranes inhibits forskolin-stimulated adenylate cyclase (Niles et al., 1991). We recently reported that cultured neural cells prepared from chick embryo retina have 2-iodomelatonin binding sites, and that occupation of melatonin receptors in these cultures inhibits forskolin-stimulated cAMP accumulation (Iuvone and Gan, 1994a). Intact retina and retinal cell cultures are rich in D1 dopamine receptors, activation of which stimulates adenylate cyclase (Brown and Makman, 1972; Watling et al., 1979; DeMello et al., 1982). In this study, we examined the possible interaction of melatonin receptors and D1 dopamine receptors in cultured retinal cells, and report that these receptors appear to be colocalized on retinal neurons and have opposing effects on cAMP formation.

A preliminary report of some of these results has been published in abstract form (Iuvone and Gan, 1992).

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Materials and Methods

Cell cultures. Neuronal cell cultures were prepared from embryonic chick retina as described by Adler et al. (1984), with minor modifications (Avendano et al., 1990). Briefly, eggs of White Leghorn chickens (*Gallus domesticus*) were incubated at 37.5 ± 0.5°C in a humidified incubator. Cultures were prepared from embryonic day 8 neural retina. Neural retinas, apparently free of pigment epithelial or other cells, were dissected and dissociated by trypsinization (0.25% trypsin for 20 min at 37°C) and trituration. Cells were seeded into Falcon culture dishes at a density of 4.5×10^6 cells/60 mm dish. The culture dishes were pretreated with 0.1 mg/ml of polyornithine in 0.15 M sodium borate (pH 8.4), washed sequentially with H_2O and medium 199, and incubated with culture medium containing 20% defined fetal bovine serum (Hyclone, Logan, UT) before seeding. Cells were cultured for 6 d in 6 ml of medium 199 supplemented with 10% fetal bovine serum, linoleic acid–bovine serum albumin (110 μg/ml), 2 mM glutamine, and penicillin G (100 U/ml), at 37°C under an atmosphere of 5–6.5% CO_2 in air. All measurements were made after 6 d of culture. The following three cell types were found in the cultures: multipolar neurons, photoreceptors, and apparently undifferentiated round cells.

Assay of cAMP formation. The synthesis of ^{3}H-cAMP in cells prelabeled with ^{3}H-adenine was determined by a modification of the method of Shimizu et al. (1969). Culture medium was removed by aspiration and replaced by 3 ml of balanced salt solution (BSS; in mM: NaCl, 125.4; KCl, 3.6; $MgCl_2$, 1.2; $CaCl_2$, 1.15; $NaHCO_3$, 22.6; Na_2HPO_4, 0.4; NaH_2PO_4, 0.1; Na_2SO_4, 1.2; D-glucose, 10) containing 5 μCi of 2,8-^{3}H-adenine (20.7 Ci/mmol). Cells were returned to the incubator for 2 hr, after which the adenine solution was replaced by 2.85 ml of BSS, containing test compounds as indicated. After a 10 min preincubation, 150 μl of aqueous ascorbate (0.1 or 1 mM), with or without dopamine or SKF 82958 was added. All samples were incubated for 20 min. Melatonin and related drugs were present at the indicated concentrations during the 10 min preincubation and 20 min incubation periods. The incubation was terminated by addition of 250 μl of 77% trichloroacetic acid. Culture dishes were scraped with a spatula, and cells and medium were transferred to tubes. The dishes were washed with 0.5 ml of BSS, and the wash was added to the tubes. An aliquot (50 μl) of 10 mM cAMP was added as carrier. Samples were homogenized with a Tekmar Tissumizer, and centrifuged at 30,000 × *g* for 15 min. Aliquots (50 μl) of supernatant fraction were taken for determination of total radioactivity. ^{3}H-cAMP was isolated by sequential chromatography on Dowex 50W-X4 and alumina as described by Minneman et al. (1979), except that the bed dimension of the Dowex 50W-X4 columns was 0.8 × 3 cm. Columns were calibrated for recovery using external standards of ^{3}H-cAMP, and standards were run with each assay. The data were corrected for recovery and are expressed as percent conversion (^{3}H-cAMP × 100/total ^{3}H) or as percentage of dopamine-stimulated cAMP accumulation. Dopamine-stimulated cyclic AMP accumulation is defined as the increment of cAMP above basal levels elicited by dopamine (accumulation in the presence of dopamine minus basal accumulation in the absence of added neurotransmitter).

In some experiments, cells were pretreated with 30 ng/ml of pertussis toxin, which was added directly to the culture medium on the fifth day *in vitro*. After 18 hr the culture medium containing pertussis toxin was removed and replaced with BSS without toxin. Samples were then processed as described above.

The experiments described in this article were conducted over a three year period with cells grown in the presence of four different lots of fetal bovine serum (Hyclone defined serum lots 991, 2034, 2053, 2280). Absolute values of cAMP accumulation varied with the different serum lots, but the responses to dopamine and melatonin were similar with all lots. In the earlier experiments of this study (see Figs. 1, 3; Tables 4, 5), incubations were conducted in the presence of 5 μM ascorbate, with or without dopamine. Under these conditions, 200 μM dopamine was required to elicit a large cAMP response. Subsequently, we observed that dopamine was more potent in the presence of 50 μM ascorbate, eliciting a maximal response at approximately 10 μM (see Fig. 2). All subsequent experiments were conducted with the higher concentration of antioxidant. cAMP responses to high or low concentrations of dopamine were antagonized by dopamine receptor blockers (see Fig. 1, Table 1), and melatonin produced the same response under both of these conditions.

Assay of inositol phosphate accumulation. Cells were prelabeled with ^{3}H-myo-inositol (0.67 μCi/ml) for 4–5 d. Inositol phosphate accumulation was determined by a modification of previously described methods (Berridge et al., 1982; Wilson et al., 1990). On the sixth day *in vitro*, culture medium was removed and washed with 3 ml of lithium-BSS (LiBSS): 10 mM LiCl, 115.4 mM NaCl, 3.6 mM KCl, 1.2 mM $MgCl_2$, 1.15 mM $CaCl_2$, 22.6 mM $NaHCO_3$, 0.4 mM Na_2HPO_4, 0.1 mM NaH_2PO_4, 1.2 mM Na_2SO_4, 0.029 mM EDTA; 10 mM D-glucose. LiBSS (2.85 ml) was added to each dish, and cells were preincubated for 10 min. Test compounds or vehicle were subsequently added in a volume of 0.15 ml and samples were incubated for 60 min. The incubation medium was then aspirated, and 0.75 ml of ice-cold methanol added. Cells were scraped from the dishes with a spatula and added to a tube containing 0.66 ml of chloroform. Dishes were washed with 0.66 ml of distilled water, which was added to the cell suspension. Samples were sonicated for 10 sec and centrifuged at 10,000 × *g* for 5 min to separate aqueous and organic phases. ^{3}H-Inositol phosphates were isolated from the aqueous phase by anion exchange chromatography and measured by liquid scintillation counting as described by Wilson et al. (1990). Aliquots of the organic phase were counted to determine ^{3}H-inositol incorporation into lipid. Percent hydrolysis was calculated by the dividing the amount of ^{3}H-inositol phosphates formed by the total ^{3}H-inositol incorporated.

Table 1. Dopamine-stimulated cAMP accumulation in cultured retinal neurons: antagonism by SCH23390

Addition	*N*	cAMP accumulation (% conversion)
Vehicle	5	0.19 ± 0.01
SCH23390 (20 μM)	6	0.15 ± 0.01
Dopamine (10 μM)	6	1.00 ± 0.03[a]
Dopamine + SCH23390	6	0.15 ± 0.01

cAMP accumulation was determined as described in Materials and Methods. SCH23390 was added 10 min prior to addition of dopamine.

[a] $p < 0.01$ versus all other groups.

Data analysis. Data are expressed as mean ± standard error of the mean. Comparisons of group means between two groups were made with the Student's *t* test, and among multiple groups with analysis of variance and Student-Newman-Keuls post hoc test. EC_{50} values were determined using the ALLFIT program (DeLean et al., 1978).

Materials. ^{3}H-cAMP and ^{3}H-adenine were obtained from Du Pont/New England Nuclear (Boston, MA). ^{3}H-myo-inositol (20 Ci/mmol) was from American Radiolabeled Chemicals (St. Louis, MO). 2-Iodomelatonin and SKF 82958 were obtained from Research Biochemicals, Inc. (Natick, MA). 6-Chloromelatonin was provided by Lily Research Laboratories, Indianapolis, IN. Melatonin, dopamine, cAMP, Dowex 50W-X4 200–400 mesh, neutral alumina, linoleic acid–BSA, glutamine, polyornithine, and pertussis toxin were from Sigma Chemical Co. (St. Louis, MO). Defined fetal bovine serum was from Hyclone (Logan, UT).

Results

Melatonin receptor-mediated inhibition of dopamine-stimulated cAMP accumulation. Addition of dopamine markedly stimulated cAMP accumulation in cultured retinal cells (see Table 1, Fig. 1). The effect of dopamine was inhibited by the dopamine receptor antagonists SCH 23390 (Table 1) and haloperidol (Fig. 1). Melatonin (100 nM) had no significant effect on basal cAMP accumulation, but inhibited the stimulation elicited by dopamine by approximately 70% (Fig. 1). The D1-selective dopamine receptor agonist SKF 82958 (100 μM) also stimulated cAMP accumulation, and its effect was also inhibited by melatonin (Table 2). Melatonin reduced the maximal cAMP response to dopamine without significantly affecting its potency (Fig. 2).

The inhibitory effect of melatonin on dopamine-stimulated cAMP accumulation was concentration-dependent, with an estimated EC_{50} value (concentration producing a half-maximal effect) of 160 pM and a maximal inhibitory effect of 68% (Fig. 3). The melatonin receptor agonists, 2-iodomelatonin and 6-chloromelatonin, also reduced dopamine-stimulated cAMP ac-

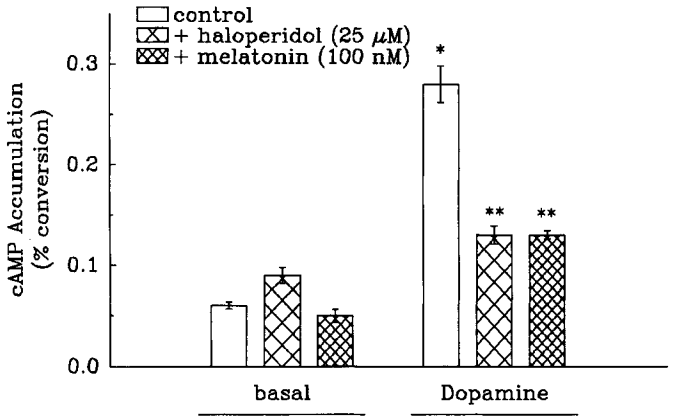

Figure 1. Inhibition of dopamine-stimulated cAMP accumulation by haloperidol and melatonin. cAMP accumulation was determined as described in Material and Methods. Haloperidol (25 μM) and melatonin (100 nM) were added 10 min prior to the addition of dopamine (200 μM). N = 6/condition. *, $p < 0.01$ vs basal; **, $p < 0.01$ vs dopamine.

cumulation, with estimated EC_{50} values of 35 pM and 260 pM, respectively (Fig. 3).

The experiments described above were performed in the absence of a cyclic nucleotide phosphodiesterase inhibitor, and the results could be due to either a stimulation of cAMP breakdown or an inhibition of its formation. The effect of melatonin was therefore reexamined in the presence of 3-isobutyl-1-methylxanthine (IBMX), to address this issue. IBMX (0.3 mM) significantly increased basal cAMP accumulation (Table 3). Melatonin (300 nM) had no effect on the increased accumulation caused by the phosphodiesterase inhibitor. Dopamine markedly stimulated cAMP accumulation in the presence of IBMX, and this stimulation was significantly attenuated by melatonin (Table 3). Thus, melatonin appears to inhibit the stimulation of cAMP *formation* caused by dopamine.

Pretreatment of cultures for 18 hr with pertussis toxin (30 ng/ml) had no effect on dopamine-stimulated cAMP accumulation, but significantly reduced the inhibitory effect of melatonin (Table 4).

Effect of melatonin on inositol phosphate accumulation. Dopamine (200 μM) produced a small, but statistically significant stimulation of inositol phosphate accumulation (Table 5), which was inhibited by haloperidol and SCH 23390 (data not shown). Melatonin had no effect on either basal or dopamine-stimulated inositol phosphate accumulation (Table 5). Carbachol, a muscarinic cholinergic receptor agonist, produced a much larger stimulation of inositol phosphate accumulation than did dopamine (Table 5); the effect of carbachol was also unaffected by melatonin.

Discussion

Previous studies have demonstrated that melatonin modulates dopaminergic neurotransmission in retina by inhibiting dopamine release (Dubocovich, 1983; Nowak, 1988; Boatright et al., 1994). The present results indicate that melatonin may also modulate retinal dopaminergic neurotransmission at a postsynaptic site by inhibiting the cAMP response to D1 dopamine receptor activation. Melatonin, 2-iodomelatonin, and 6-chloromelatonin inhibited dopamine-stimulated cAMP accumulation, a well established response to D1 dopamine receptor activation in retina (Brown and Makman, 1972). The EC_{50} values for inhibition of

Table 2. Melatonin inhibits SKF82958-stimulated cAMP accumulation

Addition	N	cAMP accumulation (% conversion)
Vehicle	5	0.38 ± 0.04
Melatonin (100 nM)	6	0.31 ± 0.03
SKF82958 (100 μM)	6	0.92 ± 0.19[a]
Melatonin + SKF82958	5	0.59 ± 0.04[b]

[a] $p < 0.01$ versus vehicle.
[b] $p < 0.05$ versus SKF82958.

dopamine-stimulated cAMP accumulation by melatonin and related agonists are similar to those for inhibition of chick retinal dopamine release (Dubocovich and Takahashi, 1987) and to the K_i values for inhibition of 2-^{125}I-iodomelatonin binding to chick brain slices (Siuciak et al., 1991) and retinal neurons (Iuvone and Gan, unpublished observation). The EC_{50} for inhibition of dopamine-stimulated cAMP accumulation by 2-iodomelatonin (35 pM) is virtually identical to the equilibrium dissociation constant (K_D) for 2-^{125}I-iodomelatonin binding to chick retinal neurons in culture (42 pM; Iuvone and Gan, 1994a). Thus, melatonin and its analogs inhibit dopamine-stimulated cAMP accumulation by activating melatonin receptors similar to those described pre-

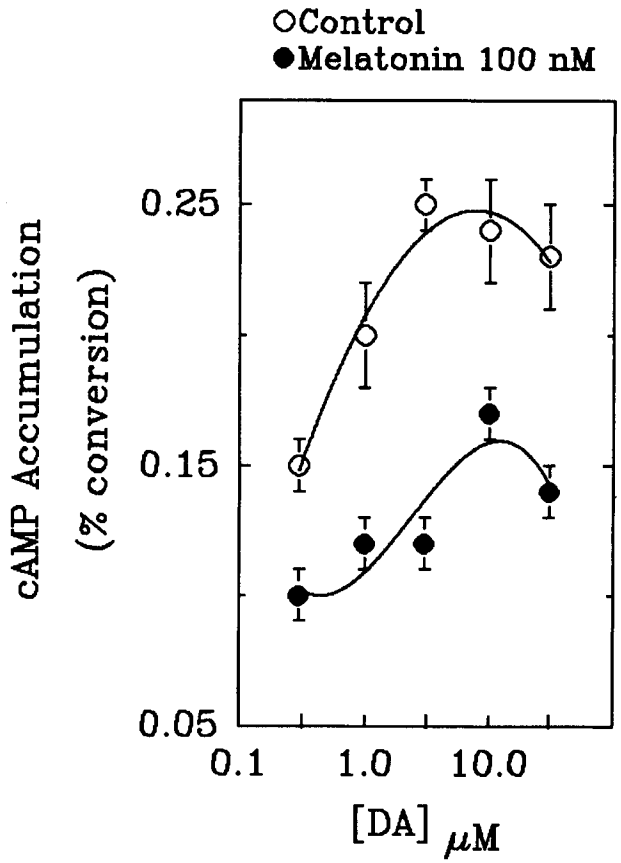

Figure 2. Concentration–response analysis of dopamine-stimulated cAMP accumulation in the presence and absence of melatonin (100 nM). Melatonin reduced the maximal response to dopamine but had no significant effect on its potency; EC_{50} values were 0.74 and 1.1 in the absence and presence of melatonin, respectively ($p > 0.05$). Melatonin significantly ($p < 0.01$) reduced cAMP accumulation at all dopamine concentrations. N = 7–8/data point.

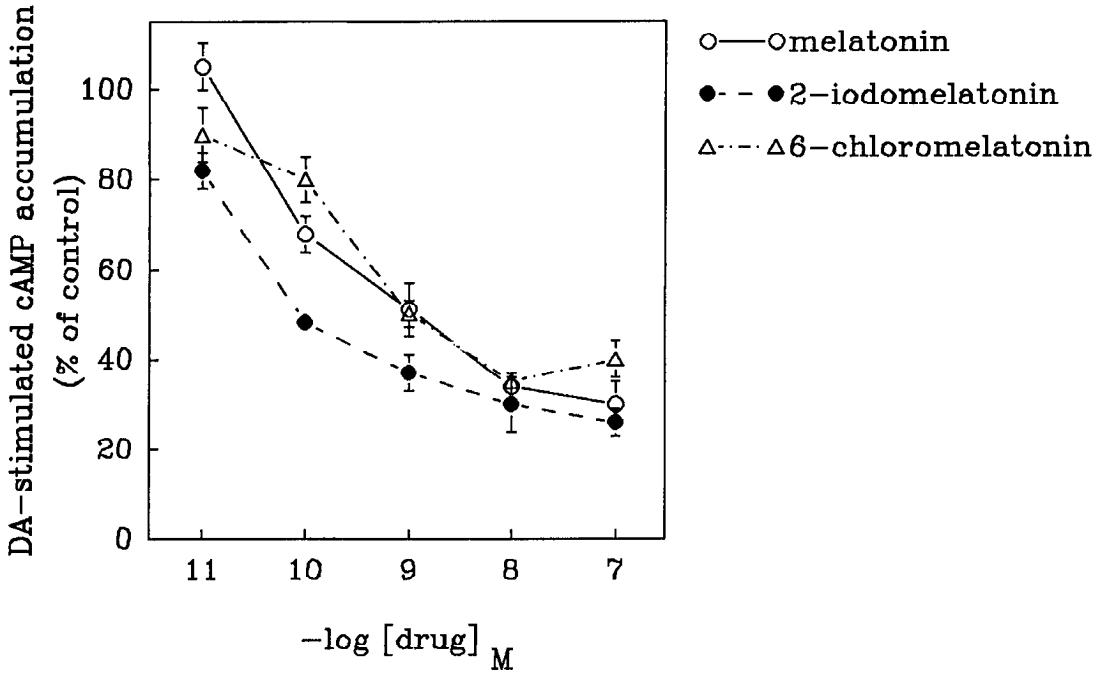

Figure 3. Concentration–response analysis for inhibition of dopamine-stimulated cAMP accumulation by melatonin and halogenated analogs. The rank order of potency for inhibition of dopamine-stimulated cAMP accumulation was 2-iodomelatonin > melatonin ≥ 6-chloromelatonin (see text for EC_{50} values). Sample sizes: melatonin, 9–11/data point; 2-iodomelatonin, 4–6/data point; 6-chloromelatonin, 4–5/data point.

viously using other functional or binding assays. Furthermore, the inhibitory effect of melatonin on dopamine-stimulated cAMP accumulation was blocked by pretreatment with pertussis toxin, which also attenuates the inhibition by melatonin of forskolin-stimulated cAMP accumulation in retinal cells (Iuvone and Gan, 1994), brain (Niles et al., 1991), and pars tuberalis (Morgan et al., 1990). Thus, we suggest that melatonin stimulates receptors located on neurons that also contain D1 dopamine receptors, and that the melatonin receptors activate a G_i-like guanyl nucleotide binding protein that inhibits the effect of G_s activation by D1 dopamine receptor stimulation.

D1 dopamine receptors in brain have been reported to be coupled to phospholipase C (Undie and Friedman, 1992), activation of which stimulates inositol phosphate formation and activation of protein kinase C (Nishizuka, 1992). We recently demonstrated that dopamine stimulates inositol phosphate accumulation in cultured neural retinal cells by activating a receptor with the pharmacological characteristics of a D1 dopamine receptor (Iuvone and Gan, 1994b). Remarkably, melatonin inhibits the cAMP response to dopamine but not dopamine-stimulated inositol phosphate accumulation. Thus, melatonin receptors may be colocalized with D1 receptors that are coupled to adenylate cyclase, but not with D1-like receptors that are coupled to phospholipase C. Alternatively, all three receptor-effector complexes may be colocalized, with melatonin receptors capable of inhibiting G_s-mediated adenylate cyclase activation, but not the stimulation of phospholipase C by G_q or related G proteins. In retina, D1 dopamine receptors coupled to both adenylate cyclase and phospholipase C may be colocalized on horizontal cells. cAMP mediates the effects of dopamine on horizontal cell gap junction permeability and on glutamate-gated cation conductance (Dowling, 1989), while the effects of dopamine on horizontal cell neurite extension and spinule formation may involve activation of phospholipase C (Dos Santos Rodrigues and Dowling, 1990; Weiler et al., 1991). These observations suggest a novel mechanism for modulation of dopaminergic neurotransmission, whereby melatonin interferes with some, but not all of the consequences of dopamine receptor activation.

Table 3. Melatonin inhibits dopamine-stimulated cAMP accumulation in the presence of IBMX

Addition	cAMP accumulation (% conversion)
Vehicle	0.07 ± 0.004
IBMX (0.3 mM)	0.29 ± 0.02[a]
IBMX	
+Melatonin (300 nM)	0.29 ± 0.01[a]
+Dopamine (10 μM)	1.88 ± 0.04[b]
+Melatonin and dopamine	1.34 ± 0.06[c]

IBMX was added, where indicated, during the preincubation prior to stimulation with dopamine. N = 5–6/condition.

[a] $p < 0.01$ versus vehicle control.

[b] $p < 0.01$ versus IBMX control.

[c] $p < 0.01$ versus IBMX + dopamine.

Melatonin also failed to alter the stimulation of inositol phosphate accumulation in retinal cell cultures in response to activation of muscarinic cholinergic receptors with carbachol. This result is consistent with a preliminary report that melatonin does not inhibit carbachol-stimulated inositol phosphate accumulation in chick brain slices (Fang et al., 1990). Melatonin also failed to alter AlF_4-induced inositol phosphate accumulation in ovine pars tuberalis (Morgan et al., 1991), suggesting that melatonin receptors are not negatively coupled to G protein–stimulated phospholipase C.

Dopamine–melatonin interactions are not unique to retina, but occur in other parts of the CNS as well. Melatonin inhibits hypothalamic dopamine release (Zisapel and Laudon, 1983) and

Table 4. Pretreatment with pertussis toxin blocks the inhibition of dopamine-stimulated cAMP accumulation by melatonin

Addition	cAMP accumulation	
	Without toxin	+Pertussis toxin
Vehicle	0.46 ± 0.02	0.51 ± 0.03
Dopamine (200 μM)	0.84 ± 0.02[a]	0.79 ± 0.02[c]
Dopamine + melatonin (300 nM)	0.57 ± 0.03[b]	0.74 ± 0.03[d]

Cultures were pretreated with pertussis toxin (30 ng/ml) for 18 hr, as described in Materials and Methods, prior to labeling cells with ^{3}H-adenine. N = 5–6/condition.

[a] $p < 0.01$ versus vehicle control.

[b] $p < 0.01$ versus dopamine control.

[c] $p < 0.01$ versus vehicle + pertussis toxin.

[d] $p < 0.05$ versus dopamine.

reduces the dopamine content of the neurointermediate lobe of the pituitary gland (Alexiuk and Vriend, 1993). Dopamine and melatonin may interact to regulate pituitary gonadotropin release (Acuña-Castroviejo et al., 1993). Furthermore, it has been suggested that interactions of dopamine and melatonin in the nucleus accumbens may play a role in the behavioral response to antidepressant drugs (Durlach-Misteli and Van Ree, 1992).

Based on the present results, we propose the following working hypothesis for modulation of dopaminergic transmission by melatonin (Fig. 4). Melatonin may inhibit dopamine release at a presynaptic site (Dubocovich, 1983; Boatright et al., 1994). The effect of melatonin on dopamine release is dependent on GABAergic neurotransmission, as it is completely blocked by GABA receptor antagonists (Boatright et al., 1994). It is unclear if melatonin acts to increase the synaptic concentration of GABA or to enhance the response to GABA at a postsynaptic site. In addition, melatonin inhibits the stimulation of adenylate cyclase by D1 dopamine receptor activation at a postsynaptic site. We propose that melatonin receptors and cyclase-coupled D1 dopamine receptors are colocalized in the same neurons, but can not exclude the possibility that melatonin acts on other cells, which release a neurotransmitter that modulates dopamine-stimulated adenylate cyclase activity. In contrast, melatonin has no effect on dopamine-stimulated activation of phospholipase C. It is unclear at present if the cyclase-coupled dopamine receptors and the D1-like receptors linked to phospholipase C are co-localized or exist on different neurons. It is also not known if melatonin modulates responses to D2 dopamine receptor activation. Melatonin maximally inhibits endogenous dopamine release by 50–70%. Thus, in the presence of melatonin, dopaminergic transmission may be modulated by a combination of reduced dopamine release and a selective reduction of some postsynaptic responses to dopamine receptor activation.

We emphasize the hypothetical nature of this model, which is intended to present a framework for the design of additional experiments. The functional interaction of melatonin receptors and D1 dopamine receptors has only been investigated in cell cultures of embryonic retinal neurons. The cell types generating the observed responses are not yet identified. Additionally, it will be important to determine if a similar functional interaction occurs in intact, adult retina.

Table 5. Melatonin does not inhibit inositol phosphate accumulation in retinal neurons

Condition	Inositol phosphates (% hydrolysis)	
	Without melatonin	+Melatonin
Experiment I		
Basal	6.4 ± 0.3	5.7 ± 0.8
Dopamine 200 μM	10.0 ± 0.8[a]	10.1 ± 0.9[a]
Experiment II		
Basal	7.4 ± 0.2	6.8 ± 0.2
Carbachol 500 μM	20.4 ± 0.8[a]	21.0 ± 0.8[a]

Melatonin concentration was 100 nM in experiment I and 300 nM in experiment II. Inositol phosphate accumulation was determined as described in Materials and Methods. N = 5–6/condition.

[a] $p < 0.05$ versus basal.

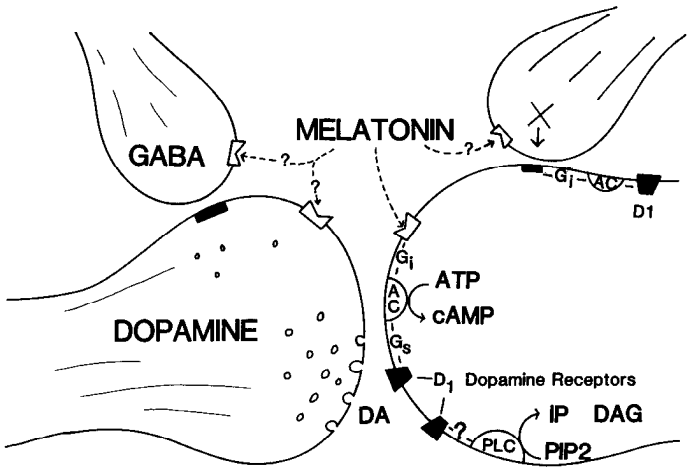

Figure 4. Hypothetical model for the modulation of dopaminergic neurotransmission by melatonin. Melatonin receptor activation inhibits dopamine release by a mechanism that involves GABAergic neurotransmission (Boatright et al., 1994). Postsynaptically, D1 dopamine receptors are coupled to adenylate cyclase (*AC*) by the stimulatory guanyl nucleotide-binding protein G_s. Melatonin receptor activation inhibits dopamine-stimulated AC activity via a pertussis toxin–sensitive G protein, such as G_i. We propose that this functional interaction occurs via melatonin receptors and dopamine receptors that are colocalized on the same postsynaptic membrane. However, the possibility that melatonin acts on other neurons, which release a neurotransmitter (X) that modulates dopamine-stimulated adenylate cyclase, cannot be excluded. D1-like dopamine receptors also appear to activate phospholipase C (*PLC*) (Iuvone and Gan, 1994b), which converts phosphatidylinositol 4,5 bisphosphate (*PIP2*) into two second messengers, inositol trisphosphate (*IP3*) and diacylglycerol (*DAG*). Melatonin has no effect on this response. Thus, melatonin may modulate dopaminergic transmission by a combination of reducing dopamine release and inhibiting some of the postsynaptic responses to dopamine receptor activation.

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