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European Journal of Pharmacology 326 (1997) 229–236

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Pineal melatonin in rats: suppression by the selective α_2-adrenoceptor agonist medetomidine

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Received 5 December 1996; revised 3 March 1997; accepted 7 March 1997

Abstract

This study was done to clarify the role of α_2-adrenoceptors in the regulation of pineal melatonin synthesis. A selective α_2-adrenoceptor agonist, medetomidine, and antagonist, atipamezole, were injected subcutaneously into rats and their pineal melatonin contents were measured by radioimmunoassay. Medetomidine (120 μg/kg) suppressed melatonin at night to a similar extent during the rising and descending phase of melatonin synthesis, but it did not affect the daytime level. A dose of 12 μg/kg was ineffective; doses of 30–180 μg/kg suppressed nocturnal melatonin levels close to the daytime levels. Significant suppression was reached within 15 min and the effect started to fade 3 h after the injection (120 μg/kg). At midday, medetomidine did not inhibit isoproterenol-stimulated synthesis of melatonin. Atipamezole (0.4 or 1.2 mg/kg) had no effect alone, but it counteracted the medetomidine-induced suppression. The effects of α_2-adrenoceptor ligands on melatonin synthesis depend on the time of day and/or on the activity of the pineal sympathetic nerves.

Keywords: α_2-Adrenoceptor; Pineal gland; Melatonin; Circadian rhythm; Medetomidine; Atipamezole

1. Introduction

The synthesis of pineal melatonin is controlled by the sympathetic nervous system (reviewed, e.g., by Klein, 1993). Melatonin (5-methoxy-*N*-acetyltryptamine) is formed from serotonin, which is acetylated by the rate-limiting enzyme, *N*-acetyltransferase, and further methylated by hydroxyindole-*O*-methyltransferase. The activation of *N*-acetyltransferase depends on the activity of the sympathetic nerves originating from the superior cervical ganglia. Melatonin synthesis has a diurnal rhythm which is driven endogenously by an approximately 24-h clock located in the hypothalamic suprachiasmatic nuclei. Environmental light affects the oscillatory function of this clock through retinohypothalamic pathways. From the suprachiasmatic nuclei there are pathways via the brain stem to the sympathetic trunk.

In the rat pineal gland, the formation and activation of *N*-acetyltransferase are regulated by both α- and β-adrenoceptors, located on postsynaptic structures (Klein, 1993). Noradrenaline acts through α_1- and β_1-adrenoceptors, increasing the levels of pineal cyclic nucleotides (cAMP and cGMP) and thus stimulating *N*-acetyltransferase activity (Klein et al., 1983; Sugden et al., 1984). The activation of α_1-adrenoceptors potentiates β-adrenergic stimulation, but it has no effect alone.

There are α_2-adrenoceptors in the pineal gland (Schaad and Klein, 1992; Simonneaux et al., 1991), but their functions are still unclear. It has been shown that a presynaptic α-adrenergic system regulates the release of noradrenaline from pineal nerve endings (Pelayo et al., 1977). It has also been shown that an α_2-adrenoceptor agonist, clonidine, decreases nocturnal melatonin levels in humans (Lewy et al., 1986) and *N*-acetyltransferase activity in rats in vivo (Alphs et al., 1980). These findings are in agreement with the idea that there may be presynaptic α_2-adrenergic inhibition of noradrenaline release in the pineal gland. However, centrally conveyed inhibition is possible as well.

The preganglionic fibers to the superior cervical ganglia originate in the intermediolateral cell column of the spinal cord (Rando et al., 1981), which receives monosynaptic projections from the paraventricular nucleus of the hypothalamus and from the retrochiasmatic area (Hosoya et al., 1991; Swanson and Kuypers, 1980a,b). These areas receive in turn afferents from the suprachiasmatic nuclei (Berk and Finkelstein, 1981; Reuss, 1996). In autoradiographic studies, both the paraventricular nucleus and retrochiasmatic area have been shown to be rich in binding

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PII S0014-2999(97)00043-5

sites for some α_2-adrenoceptor ligands (Boyajian et al., 1987; Unnerstall et al., 1984). Thus, it is possible that the neural information originating in the suprachiasmatic nuclei can be modified by α_2-adrenergic manipulation on the way to the pineal gland.

In the present study, the effects of two potent and selective α_2-adrenoceptor ligands on melatonin synthesis were examined in rats in vivo. Medetomidine is an α_2-adrenoceptor agonist, and atipamezole an α_2-adrenoceptor antagonist. They have very high α_2/α_1 selectivity ratios in receptor binding experiments (1620 and 8526) compared to other α_2-adrenoceptor ligands like clonidine and yohimbine (220 and 40) (Virtanen et al., 1988, 1989). Medetomidine and atipamezole have not been commonly used for the pharmacological classification of α_2-adrenoceptor subtypes. Both drugs are imidazole derivatives and their effects may be partly conveyed through non-adrenergic imidazoline receptors (Hieble and Ruffolo, 1995). For simplicity, the term 'α_2-adrenoceptor' is used in this report without distinguishing between the possible non-adrenergic effects of medetomidine and atipamezole.

Experiments were carried out in order to determine the effects of medetomidine and atipamezole on pineal melatonin synthesis, by providing answers to the following questions: (1) Are the effects of the drugs time-of-day dependent? (2) What kind of dose dependence and time dependence can be found? Are these comparable to the other central or peripheral effects of the drugs? (3) After medetomidine was found to be a potent suppressor of pineal melatonin content at night, a further question emerged: whether a high rate of synthesis per se is a precondition for the suppressive effect. The influence of medetomidine on isoproterenol-stimulated melatonin synthesis was studied.

2. Materials and methods

2.1. Animals

Adult female Wistar rats weighing about 170 g were used in experiments 1, 2, 4 and 5, and adult male Wistar rats weighing about 270 g in experiments 3 and 6. The rats were kept under controlled lighting conditions (12-h light/12-h dark) from birth, with lights on at 6.00 h. Illumination during the light period, at the level of the cages, was 100–150 lux in the first experiment and 10–20 lux in all the other experiments. During the dark period there was dim red light of under 1 lux. Temperature and relative humidity were 23–26°C and 48–60%, respectively. The rats were given food and water ad libitum. The animals were decapitated by guillotine and the pineal glands were quickly removed and frozen on solid CO_2. The glands were stored at −24°C until the melatonin content was measured.

2.2. Experiments

2.2.1. Experiment 1

The effects of α_2-adrenoceptor stimulation or inhibition on the level of melatonin were studied, as well as any diurnal variations in these. There were three different time groups in the experiment: one group was injected and killed in the middle of the light period, one 4 h and one 10 h after the lights were off. Each experimental time group was divided into five subgroups ($n = 7$–8), one being a control group and receiving saline injections. Two different doses of medetomidine (12 and 120 μg/kg) or atipamezole (0.4 and 1.2 mg/kg) were given to the other subgroups 1 h before decapitation in an adjacent room. The pineal glands were quickly removed and frozen on solid CO_2. When the pineal glands were collected in the dark, a dim red light was used (< 1 lux).

2.2.2. Experiment 2

The experiment was designed to determine whether the decrease in the melatonin level produced by medetomidine was dose dependent, and whether atipamezole inhibited this effect. All injections were given at 22.00 h, 4 h after the beginning of the dark period. The rats were divided into seven groups ($n = 7$–8), and two injections were given simultaneously. The first group received two injections of saline. Groups 2, 3, and 4 received 30, 60 or 180 μg/kg of medetomidine and saline. Groups 5, 6, and 7 received 30, 60 or 180 μg/kg of medetomidine and 1.2 mg/kg of atipamezole. The samples were collected 1 h after the injections, as in experiment 1.

2.2.3. Experiment 3

This experiment was done to determine whether medetomidine's effect depended on the interval between injection and sampling. All injections were given at 22.00 h; the time of sampling varied. The rats were divided into eight groups ($n = 8$). Four groups received medetomidine (80 μg/kg) and four groups saline. They were decapitated 15, 30, 120, or 180 min after injection. The samples were collected as in experiment 1.

2.2.4. Experiment 4

Medetomidine's effect in decreasing the level of melatonin was studied for different injection times, using one sampling time at 24.00 h. There were 12 groups ($n = 9$–10), including a separate control group for every time point. Medetomidine (120 μg/kg) or saline was injected 15, 30, 60, 120, 180, or 240 min before sampling. In order to determine the pineal melatonin content at the times of injection, untreated control samples were taken. The samples were collected as in experiment 1.

2.2.5. Experiments 5 and 6

These experiments were designed to determine whether medetomidine could inhibit the increase in melatonin

caused by isoproterenol. In experiment 5, medetomidine was administered prior to isoproterenol and in experiment 6 after the isoproterenol injection. Experiment 5 included four groups ($n = 9$–11). Each group received two injections at 15-min intervals. The first group received two saline injections, the second group received first saline, then isoproterenol (180 μg/kg). The third group received first medetomidine (120 μg/kg) and then saline, and the fourth one first medetomidine (120 μg/kg) and then isoproterenol (180 μg/kg). The rats were decapitated 60 min after the second injection at 13.00 h.

Experiment 6 included four groups ($n = 9$). The interval between the two injections was 60 min. One group received two saline injections. Three other groups received first isoproterenol (180 μg/kg); the second injections were saline, medetomidine at 12 μg/kg, or medetomidine at 120 μg/kg. The samples were collected 60 min after the second injection.

2.3. Measurement of melatonin

Melatonin was measured using a radioimmunological method described previously in detail (Vakkuri et al., 1984; Laakso et al., 1988). The unspecific binding in the assays was 5–7%. The lower detection limit, defined as the apparent concentration at two standard deviations from the counts at maximum binding, was lower than the lowest standard (19.5 pg/ml). Intra-assay variability was 6–8%, and the interassay variability of 21 assays, including the assays of this study, during 24 months was 10–12% depending on the concentration.

2.4. Statistics

One-way and two-way analyses of variance (ANOVA) and the Tukey-Kramer multiple comparisons test were used in the statistical evaluations. Logarithmic transformation of the data was used when Bartlett's test suggested differences among the variances.

2.5. Drugs

Medetomidine and atipamezole were synthesized by Farmos Group (Turku, Finland), and isoproterenol hydrochloride was purchased from Research Biochemicals International (Natick, MA, USA). The drugs were dissolved in saline and 100 μl was subcutaneously injected into each rat.

3. Results

3.1. Experiment 1

In daylight hours, medetomidine or atipamezole caused no significant effects on the melatonin level (Fig. 1). Both

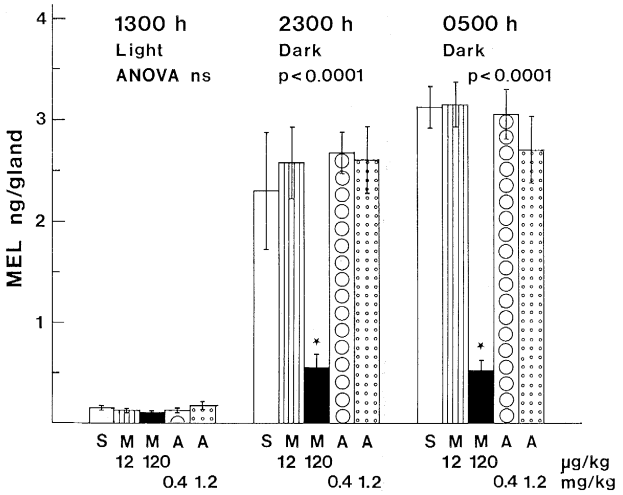

Fig. 1. Effects of medetomidine (M) and atipamezole (A) on pineal melatonin content in female rats at different times of the day. The results are means with S.E.M. for 7–8 rats/group. The animals were kept in 12/12-h light/dark conditions with lights on from 06.00 to 18.00 h. The drugs were injected subcutaneously 60 min before the sampling times shown above the columns. The doses are shown under the columns. The control rats (S) received saline. * Different from the control group and from the other drug-injected groups, $P < 0.001$ (Tukey-Kramer test).

at the beginning and the end of the dark period (at 23.00 and 05.00 h), the higher dose of medetomidine decreased melatonin in a similar way, to a fourth and to a sixth of the control level, whereas the lower dose had no effect. The two doses of atipamezole had no effect at any of the three time points.

3.2. Experiment 2

As in the first experiment, medetomidine inhibited the increase in the melatonin level during the rising phase of melatonin synthesis, but there were no differences among the doses used (Fig. 2). Increasing the dose six times did not increase melatonin suppression. Thus, the lowest effective dose was less than 30 μg/kg, but more than 12 μg/kg, which was ineffective in experiment 1.

A constant dose of atipamezole counteracted the medetomidine-induced suppression, regardless of the dose of medetomidine. The level of melatonin was two to three times higher in groups injected with medetomidine and atipamezole than in groups injected with medetomidine and saline.

3.3. Experiment 3

Medetomidine had no effect on melatonin at the first time point at 22.15 h (15 min after the injection), where melatonin remained at the same daytime level as that of the control group (Fig. 3). At all the three other time points at 22.30, 24.00 and 01.00 h, there was not a great difference between the medetomidine-induced decrease in the level of melatonin. The suppression was 69, 58 and 48%

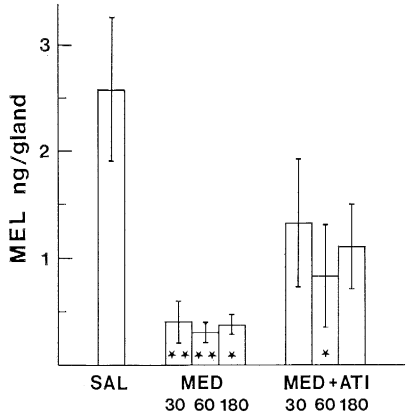

Fig. 2. Effect of different doses of medetomidine (MED) with and without concomitant administration of atipamezole (ATI, 1.2 mg/kg) on pineal melatonin content in female rats during the rising phase of melatonin synthesis. The results are means with S.E.M. for 7–8 rats/group. Injections were given subcutaneously at 22.00 h, 4 h after lights-off. Doses of medetomidine (μg/kg) are shown under the columns. The pineal samples were collected at 23.00 h. One-way ANOVA for all groups $P < 0.002$; two-way ANOVA for MED and MED + ATI groups, effect of atimamezole $P < 0.05$, dose of medetomidine NS, interaction NS. * Different from the control group (SAL), $P < 0.05$, ** $P < 0.01$ (Tukey-Kramer test).

of the control level, 30, 120 and 180 min after the injection, respectively. Thus, the effect lasted for the 3 h studied.

3.4. Experiment 4

In this experiment, the medetomidine-induced decrease in the level of melatonin was time dependent. Significant suppression occurred within 15 min. The lowest levels were in the samples collected 30–180 min after the injection. One to two hours after the injection, the levels were about 10% of the control level (Fig. 4). In the rats injected 4 h before sampling, the medetomidine-induced suppression was still evident; their pineal melatonin content was 35% of the content of saline-injected rats.

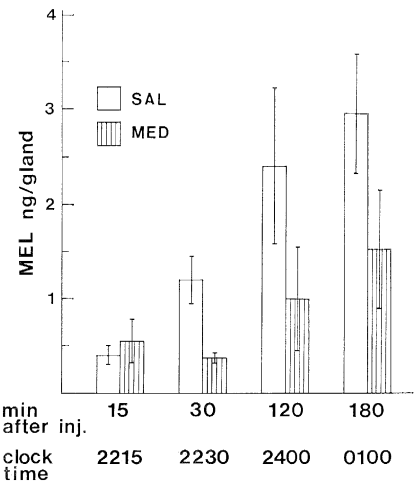

Fig. 3. Time-course of the effect of medetomidine injected at the beginning of the rising phase of melatonin synthesis on pineal melatonin content in male rats. The results are means with S.E.M. for 8 rats/group. The animals received saline (SAL) or medetomidine 80 μg/kg (MED) subcutaneously at 22.00 h, 4 h after lights-off. The samples were collected at different times (given under the columns) after the injection. Two-way ANOVA: saline vs. medetomidine-injected $P < 0.05$, time $P < 0.01$, interaction NS.

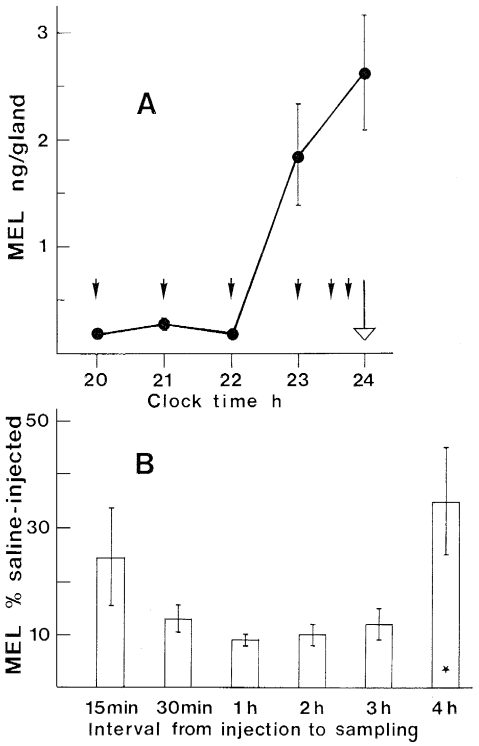

Fig. 4. Effect of medetomidine injected at different times before and during the rising phase of melatonin synthesis on pineal melatonin content in female rats. (A) Pineal melatonin content in noninjected control rats (means with S.E.M., $n = 8–10$/group). The curve shows the melatonin levels at the times of the injections (black arrows; white arrow = sampling time in B). (B) Pineal melatonin content in medetomidine-injected rats at 24.00 h, 6 h after lights-off. The animals received medetomidine 120 μg/kg subcutaneously at different times before sampling (times shown under the columns). The results are shown as percentages of the pineal melatonin content of saline-injected rats (means with S.E.M., $n = 9–10$/group). The saline-injected groups did not differ from each other, and a common reference value (2.82 ± 0.22 ng/gland, $n = 57$) was used for all groups for calculating the percentages. All the medetomidine-injected groups differed significantly from the saline-injected groups (Tukey-Kramer test). One-way ANOVA for the medetomidine-injected groups, $P = 0.02$. * Different from the 2-h group, $P < 0.05$ (Tukey-Kramer test).

3.5. Experiments 5 and 6

Isoproterenol increased the melatonin content to approx. six times the control level in 60 min, and 12 times in 120 min, in experiments 5 and 6, respectively (Fig. 5). In line with the results of experiment 1, medetomidine alone did not significantly influence the low daytime melatonin level (Fig. 5A). Nor did medetomidine significantly affect the

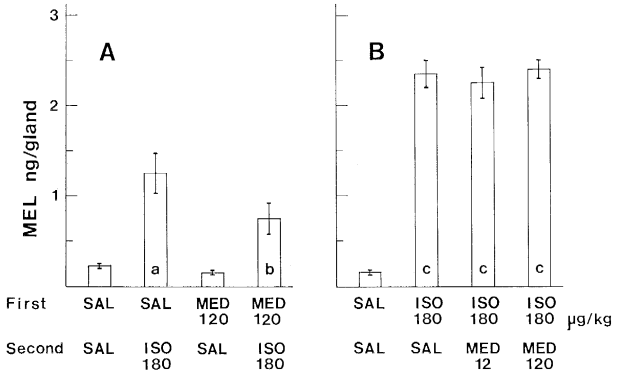

Fig. 5. Combined effects of isoproterenol and medetomidine on pineal melatonin content in rats. The results are means with S.E.M. for 9–11 animals/group. (A) Female rats received saline (SAL) or medetomidine 120 μg/kg (MED) subcutaneously at noon. Fifteen minutes later they received saline or isoproterenol 180 μg/kg (ISO). The samples were collected 60 min after the second injection. (B) Male rats received saline or isoproterenol 180 μg/kg at noon. Sixty minutes later they received saline or medetomidine 12 or 120 μg/kg. The samples were collected 60 min after the second injection. One-way ANOVAs for both experiments $P < 0.0001$. Tukey-Kramer tests: (A) [a] different from group SAL/SAL and from group MED/SAL $P < 0.001$; [b] different from group SAL/SAL $P < 0.05$ and from group MED/SAL $P < 0.01$; (B) [c] different from group SAL/SAL $P < 0.001$.

isoproterenol-induced stimulation of melatonin synthesis. When medetomidine preceded isoproterenol, the melatonin content in the medetomidine-pretreated rats tended to be lower than in the control rats injected with saline and isoproterenol, but the difference was not statistically significant (Fig. 5A). When the dose of medetomidine followed the dose of isoproterenol, there was not even a tendency to a decrease (Fig. 5B).

4. Discussion

The results of this study show that medetomidine, a selective α_2-adrenoceptor agonist, is a potent suppressor of pineal melatonin synthesis at night. After the 120 μg/kg dose, the melatonin content in many rats was suppressed close to daytime levels. This result is in line with earlier observations that clonidine, another drug with high selectivity for α_2-adrenoceptors, reduces pineal *N*-acetyltransferase activity in rats in vivo (Alphs et al., 1980) and nocturnal serum melatonin levels in humans (Lewy et al., 1986).

Although the adrenergic regulation of melatonin synthesis has been extensively studied, and its principles and several details are known, the physiological role of α_2-adrenoceptors is obscure in mammals. In the chicken pineal gland, there are postsynaptic α_2-adrenoceptors, which are involved in the inhibition of *N*-acetyltransferase activity (Pratt and Takahashi, 1987; Voisin and Collin, 1986). In mammals, if postsynaptic α_2-adrenoceptors have any role, they are stimulatory rather than inhibitory for melatonin synthesis, as shown in in vitro experiments (Alphs et al., 1980; Schaad and Klein, 1991). However, the overall effect of α_2-adrenoceptor stimulation in rats in vivo seems to be inhibition of melatonin synthesis.

The first experiment showed that medetomidine reduced the pineal melatonin content in a similar fashion during the rising and descending phases of nocturnal melatonin synthesis, but the daytime base level was not affected. This suggests that α_2-adrenoceptors may participate in the regulation of melatonin synthesis only at night, when synthesis is stimulated by sympathetic activation. The same conclusion can be drawn by comparison of the effects of equal doses of medetomidine in experiments 3 and 4. An injection given at midnight suppressed the high melatonin level significantly within 15 min, while an injection given at 22.00 h had no effect, because the rise in melatonin synthesis had not yet started by the time of sampling at 22.15 h.

The α_2-adrenoceptor antagonist atipamezole had no effect on the pineal melatonin content during the day, nor did it alone affect the night-time content. However, it was able to antagonize the medetomidine-induced suppression. It is possible that the rate of melatonin synthesis cannot be increased after it has reached its maximum at night. Therefore blocking the α_2-adrenoceptors at night had no detectable consequences. The fact that neither stimulating nor blocking the α_2-adrenoceptors during the day influenced the melatonin level supports the interpretation that the receptors do not play a role in regulating melatonin synthesis under normal midday conditions. The effects of α_2-adrenoceptor ligands on melatonin synthesis may well be circadian time dependent: it has been shown that clonidine injections cause phase shifts in the wheel-running activity rhythm of hamsters and the extent of the shift depends on the time of injection (Rosenwasser et al., 1995).

In the second experiment there was no dose dependence in the suppression of melatonin by medetomidine. The possibility of there being an on/off effect seems to be, however, improbable, because the clonidine-induced reduction of plasma melatonin in humans appears to be dose dependent (doses 2, 2.5, and 3 μg/kg; Lewy et al., 1986), and so are clonidine-induced phase shifts in hamster activity rhythms (doses 0.25–10.0 mg/kg; Rosenwasser et al., 1995). In the current study, a 12 μg/kg dose of medetomidine was ineffective, and already 30 μg/kg caused a suppression equal to that elicited by the highest 180 μg/kg dose. Dose dependence is, of course, possible in both the 12–30 μg/kg range, and beyond 180 μg/kg. In addition, the large interindividual variation of the pineal melatonin levels found could require a larger sample population for detecting smaller differences among the groups.

The doses of medetomidine and atipamezole used in this study were selected in light of previously known effects of the drugs in rats in vivo. The peripheral effects of medetomidine, e.g., hypotension, slowing of the heart rate, and mydriasis, are produced by a dose lower than 12 μg/kg, which was ineffective in reducing melatonin lev-

els in this study (Savola et al., 1986; Virtanen et al., 1988). Although the effects, mentioned above, on peripheral functions are probably of central origin, higher doses such as 30 or 100 μg/kg are needed to get local influences in the central nervous system, such as sedation and decreased turnover rates of biogenic amines (MacDonald et al., 1988).

Atipamezole, at the doses used in this study, is a potent, selective and specific competitive antagonist of both the central and peripheral effects of medetomidine (Scheinin et al., 1988; Virtanen et al., 1989). A dose of 1 mg/kg increases the mean arterial pressure in pithed rats (Virtanen et al., 1989) and produces the first signs of an increased turnover rate of serotonin in the brain (Scheinin et al., 1988). However, higher doses, > 3 mg/kg, are necessary for demonstrating any behavioral effects (vocalization, hostility, rapid breathing, piloerection), or increased turnover of noradrenaline in the brain (Scheinin et al., 1988). The inability of atipamezole to produce changes in pineal melatonin content in this study may be explained by inadequate doses. However, the interpretation that α_2-adrenoceptor-mediated effects depend on circadian time cannot be disproved, because medetomidine was ineffective during the day.

The location of the $\alpha_2$adrenergic regulation of melatonin synthesis is not known. If the suppression of melatonin by medetomidine is of central origin, the relatively high dose of medetomidine needed for the suppression suggests that the mechanism is not directly comparable, for example, to that responsible for the cardiovascular effects. Another possibility is that medetomidine acts peripherally in the pineal gland, reducing the release of noradrenaline through presynaptic α_2-adrenoceptors, as suggested previously for clonidine (Alphs et al., 1980; Lewy et al., 1986; Pelayo et al., 1977). The possible direct effect at the pineal level may require higher doses than centrally conveyed autonomic effects. The rat pineal α_2-adrenoceptors belong mainly to the pharmacological subtype 2D (Schaad and Klein, 1992), while in the rat brain, pharmacological heterogeneity of α_2-adrenoceptors is evident (Boyajian et al., 1987; Boyajian and Leslie, 1987; Wamsley et al., 1992).

A significant suppression of melatonin by medetomidine appeared within 15 min. After the 120 μg/kg s.c. dose, the effect was at its greatest 30–180 min after the injection, and started to fade after this. A rapid onset of effects is typical for this highly lipophilic compound (Savola et al., 1986; MacDonald et al., 1988). After a 100 μg/kg dose, sedation has been reported to attenuate in 90 min, although changes in cerebral amines and their metabolites can still be found after 4 h (MacDonald et al., 1988). In this experiment, sedation had disappeared from medetomidine-injected rats already by 4 h, although the pineal melatonin content was only 35% of the control level. The inhibition of melatonin synthesis by medetomidine is thus not directly related to its sedative effect, but it can be associated with the altered metabolism of cerebral amines.

Because medetomidine reduced the pineal melatonin content only at night, during its enhanced synthesis, an experiment was done to find out whether the high rate of synthesis per se was a precondition for suppression. The daytime formation of melatonin was stimulated by isoproterenol, which acts directly on rat pinealocyte β-adrenoceptors increasing *N*-acetyltransferase activity and maintaining it in an active form (Deguchi and Axelrod, 1972; Deguchi, 1973). The isoproterenol-elevated pineal melatonin level was not reduced by medetomidine. This result makes it improbable that medetomidine would inhibit melatonin synthesis in pinealocytes downstream from the β-adrenoceptors, or via α_2-adrenoceptors on the pinealocytes.

The existence of α_2-adrenoceptors locally in the mammalian pineal gland has been shown in two independent studies (Schaad and Klein, 1992; Simonneaux et al., 1991). Both in bovine and rat pineal membrane preparations, the binding site was pharmacologically classified as an α_{2D} subtype, but conclusions concerning its synaptic locus were different. An α_2-adrenoceptor antagonist, SKF 104078, which is considered to be selective for postjunctional sites, had only low affinity for bovine receptors, suggesting that they would be presynaptic (Simonneaux et al., 1991). In contrast, in rat pineal preparations, a postsynaptic location for the receptors was suggested, because the elimination of neural elements by preincubating the glands for 48 h in the culture medium did not change the α_2-adrenergic binding sites (Schaad and Klein, 1992). However, the nerve endings in the rat pineal gland also contain functional α_2-adrenoceptors. The potassium-elicited release of noradrenaline in organ culture can be reduced by the α_2-adrenoceptor agonists clonidine and oxymetazoline and enhanced by the antagonist yohimbine (Pelayo et al., 1977).

The functions of presynaptic and postsynaptic α_2-adrenoceptors seem to be opposite in the rat pineal gland. The inhibition of transmitter release by presynaptic receptors results in diminished melatonin synthesis, while exposure of pineal preparations to α_2-adrenoceptor agonists in vitro may increase *N*-acetyltransferase activity (Alphs et al., 1980; Schaad and Klein, 1991). If the α_2-adrenoceptor ligands administered in vivo act directly at the pineal level, presynaptic mechanisms seem to predominate, resulting in reduced melatonin production.

Light is the most effective suppressor of melatonin at night. Exposure to light during the enhanced melatonin synthesis inhibits the release of noradrenaline from sympathetic nerve endings in the gland (Wurtman et al., 1967). It is possible that α_2-adrenoceptor stimulation is involved in the suppressive effect of light. It has, however, been shown that a selective α_2-adrenoceptor antagonist, yohimbine, is ineffective in protecting *N*-acetyltransferase against inactivation by light, although yohimbine does counteract the inactivation of *N*-acetyltransferase caused by clonidine (Alphs et al., 1980). If the α_2-adrenoceptor-mediated regu-

lation of pineal melatonin synthesis has biological significance in mammals, it could be associated with other functions such as insulating the pineal gland from general sympathetic influence.

In summary, the results of this study confirm earlier findings suggesting that α_2-adrenoceptor agonists inhibit pineal melatonin synthesis in mammals in vivo (Alphs et al., 1980; Lewy et al., 1986). Pineal melatonin synthesis is somewhat less sensitive to medetomidine than other autonomic functions. The site of this inhibitory action seems to be outside the pinealocytes. In this respect the mammalian regulatory system differs from the avian system. The inhibition can be produced by presynaptic mechanisms at the pineal level or by mechanisms higher in the neural regulatory pathways. The former possibility is supported by the existence of α_2-adrenoceptors in the pineal gland preparations (Schaad and Klein, 1992; Simonneaux et al., 1991), by the existence of functional presynaptic α_2-adrenoceptors at pineal nerve endings (Pelayo et al., 1977), and by the finding of this study that melatonin levels are reduced by medetomidine only when pineal nerves are active. However, the activity of these pineal nerves is controlled by the hypothalamic circadian clock and by the pathways conveying information about environmental lighting. There are α_2-adrenergic binding sites in these structures as well (Unnerstall et al., 1984; Boyajian et al., 1987). The biological significance of the α_2-adrenoceptor-mediated inhibition of melatonin synthesis remains to be evaluated.

Acknowledgements

Medetomidine and atipamezole were provided by Farmos Group Ltd. (Turku, Finland).

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