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ORIGINAL PAPER

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Melatonin attenuates α-adrenergic-induced contractions by increasing the release of vasoactive intestinal peptide in isolated rat penile bulb

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Abstract The effects of melatonin on α-adrenergic-induced contractions caused by electrical field stimulation (EFS) or the α_1-adrenoceptor agonist phenylephrine (Phe) were investigated in isolated rat penile bulb. Melatonin as well as melatonin receptor agonists N-acetylserotonin and 2-iodomelatonin and melatonin antagonist luzindole attenuated the EFS-induced contractions and the concentration-response curve to Phe. The effect of melatonin on Phe-induced contractions was completely reversed by treatment with tetrodotoxin, guanethidine or vasoactive intestinal peptide (VIP) antagonist. On the other hand, pretreatment with N-methyl-l-arginine, atropine, and luzindole did not reverse the effect of melatonin. Thus, we demonstrated that melatonin at nanomolar concentrations inhibits the α-adrenergic responses in isolated rat penile bulb. Since α-adrenoceptor blocking agents are known to interfere with detumescence of the erect penis, serum levels or administration of this pineal hormone may affect erectile function. This effect of melatonin may be the result of its allosteric interaction with the presynaptic receptors on VIPergic neurons, which are affected by sympathetic transmission, and then an increase in VIP release from these neurons.

Keywords Melatonin · α-adrenergic contractions · Rat penile bulb

Introduction

Penile erection is a hemodynamic event mediated by the relaxation of smooth muscle cells of the cavernous tissue and its associated arterioles resulting from the activity of cholinergic and nonadrenergic-noncholinergic (NANC) systems [2, 4]. Although penile erection is mainly achieved by neurally released nitric oxide (NO) [7], vasoactive intestinal peptide (VIP) is also known to be involved [8]. Detumescence results predominantly from the activity of α_1-adrenoceptors by increasing the intracellular Ca^{2+} concentration of corporal smooth muscle [5]. Electrical field stimulation (EFS) of isolated corporal smooth muscle has been demonstrated to cause frequency-dependent contractions mediated by postjunctional α_1-adrenoceptors [14].

Melatonin, a primary hormone of the pineal gland, regulates many biological processes, such as circadian rhythm, sex maturation, immune responses, etc. [3]. Reports have described age-related changes in the amplitude of the melatonin rhythm in adults [12]. Although not licensed as a drug, melatonin is widely sold as a nutritional supplement in the USA and is used especially in patients with sleep disorders, which is another important problem in the elderly [13]. On the other hand, the effect of melatonin administration or decreased serum melatonin levels with age on erectile function remains unknown. Since α-adrenoceptor blocking agents can potentially interfere with the detumescence of the erect penis, leading to prolonged erection [11], and melatonin can suppress sympathetic nerve function [9] and block adrenergic receptors [1], melatonin administration or serum melatonin levels may also affect the erectile function.

This study was designed to investigate the effects of melatonin on α-adrenergic-induced contractions by using EFS and an α_1-adrenergic agonist phenylephrine (Phe) in isolated rat penile bulb.

Materials and methods

Male Wistar rats weighing 250–300 g were placed in a quiet, temperature (21 ± 2°C) and humidity (60 ± 5%) controlled room in which a 12:12 h light:dark cycle was maintained.

Rats were killed by a sharp blow to the head which was followed by exsanguination. Penile bulbs were rapidly removed with surrounding bulbospongiosum muscle. After the removal of this

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muscle and the underlying tunica albuginea, the penile bulbs were opened by making two lateral incisions.

Tissues were mounted in 40-ml organ baths containing Krebs-Henseleit buffer (in mmol l^{-1}: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11, and pH 7.4) maintained at 37°C and oxygenated with a 95% O_2, 5% CO_2 mixture. The preparations were suspended under 0.5 g resting tension, which was determined in the baseline studies, and equilibrated for 30 min with changes in the bathing fluid every 10 min. Isometric tension studies were performed using a Harvard model 60-2998 transducer and Harvard Universal pen-recorder.

EFS, using a Harvard 50-8952 model stimulator, was accomplished by means of two platinum plate electrodes, positioned on either side of the tissue. Each stimulation lasted 10 s at 0.5–50 Hz and 50 V, with a1 ms pulse duration. In a separate series of experiments, EFS was repeated in the presence melatonin (10^{-9}–10^{-7} M), melatonin agonists N-acetylserotonin (10^{-6}–10^{-4} M) and 2-iodomelatonin (10^{-7}–10^{-5} M) and melatonin antagonist (mt_1/MT_2) luzindole (2×10^{-6} M). The neural selectivity of the stimulating pulse was confirmed by sensitivity to tetrodotoxin (10^{-6} M).

A cumulative concentration-response curve to Phe (10^{-8}–10^{-4} M) was established. In a separate series of experiments, Phe-induced contraction responses were repeated in the presence of melatonin (10^{-9}–10^{-7} M), N-acetylserotonin (10^{-6}–10^{-4} M), 2-iodomelatonin (10^{-7}–10^{-5} M) and luzindole (2×10^{-6} M). To evaluate the effect of melatonin, a concentration-response curve to Phe in the presence of 10^{-8} M melatonin was made in the presence of tetrodotoxin (10^{-6} M), VIP antagonist (3×10^{-6} M), guanethidine (10^{-5} M), atropine (10^{-5} M), N-methyl-l-arginine (L-NAME, 10^{-5} M) or luzindole (2×10^{-6} M).

Cumulative concentration-response curves to $CaCl_2$ (10^{-5}–10^{-2} M) were obtained by a stepwise increase in the presence of K^+ depolarizing Ca^{+2}-free Krebs solution (in mmol l^{-1}: NaCl 71, KCl 52, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11). It was repeated in the presence of melatonin (10^{-8} and 10^{-5} M).

Contractile responses were measured as force (g). All values were expressed as the percentage of 50 Hz-induced contractions for EFS experiments or maximal contractions by Phe.

All experiments in this study were performed in accordance with the "Principles of laboratory animal care" (NIH publication no. 86-23, revised 1984) and were approved by the Commitee on Animal Research at Inonu University.

Drugs

Phe, guanethidine, atropine, melatonin, 2-iodomelatonin, N-acetylserotonin, luzindole, L-NAME, VIP antagonist ([D-p-Cl-Phe^6, Leu^{17}]-vasoactive intestinal peptide) were purchased from Sigma (St. Louis, Mo., USA). Tetrodotoxin was purchased from Alomone Labs (Israel). All drugs were dissolved in distilled water except for melatonin, 2-iodomelatonin and luzindole which were dissolved in absolute ethyl alcohol. The maximum final concentration of absolute alcohol in the bath was about 1%, which did not alter the optimal tension or the responses to EFS or Phe.

Statistics

All data were expressed as the arithmetic mean ± SEM of the number (n) of experiments; $P < 0.05$ was considered to be statistically significant. For the analysis of Phe- and EFS-induced contractions, one-way analysis of variance followed by LSD multiple comparisons test was used.

Results

EFS produced frequency-dependent contractile responses in the rat penile bulb. The contractions were abolished by the neuronal sodium channel blocker tetrodotoxin (data not shown). EFS-induced contractions were reduced by melatonin (Fig. 1) and the melatonin receptor agonists N-acetylserotonin and 2-iodomelatonin (data not shown). However, melatonin receptor (mt1/MT2) antagonist luzindole (2×10^{-6}M) also reduced the EFS-induced contractions but did not reverse the effect of melatonin on these contractions (Fig. 2).

Phe induced concentration-dependent contractions in this tissue. The concentration-response curve to Phe was attenuated by melatonin (Fig. 3) and melatonin receptor agonists N-acetylserotonin and 2-iodomelatonin (data not shown), resulting in a shift of the curve to the right without affecting the maximal response. Luzindole also reduced the Phe-induced contractions, but it was statistically significant for only one concentration of Phe (3×10^{-7} M) (Fig. 4). The effect of melatonin on Phe-induced contractions in rat penile bulb was reversed and returned to control levels by treatment with tetrodotoxin (10^{-6} M), guanethidine (10^{-5} M) and VIP antagonist (3×10^{-6} M) (Fig. 5, 6). On the contrary, luzindole (2×10^{-6} M), L-NAME (10^{-5} M) and atropine (10^{-5} M) did not reversed the effect of melatonin (Fig. 4, 6).

Melatonin did not alter the extracellular calcium-dependent contractions in the smooth muscle of rat penile bulb (data not shown).

Discussion

We demonstrated that melatonin at nanomolar concentrations significantly attenuated the α-adrenergic-induced contractions caused by either EFS or Phe in isolated rat penile bulb. It has been reported that innervation of the penile bulb, the proximal part of corpus spongiosum, is very similar to the corpus cavernosum which plays a major role in erection [6]. This effect of melatonin did not increase at concentrations

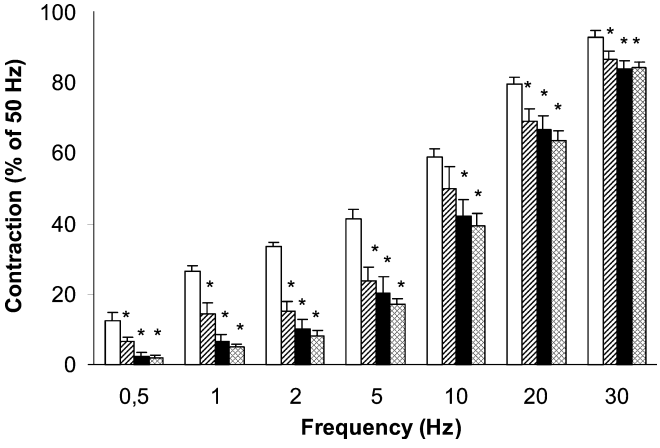

Fig. 1 Contractile responses of rat penile bulb elicited by electrical field stimulation in the absence (*open bars*) or the presence of melatonin (10^{-9} M: *hatched bars*, 10^{-8} M: *solid bars*, 10^{-7} M *cross-hatched bars*). Each point represents the mean ± SEM of five experiments. * $P < 0.05$ indicates significantly different from the control group

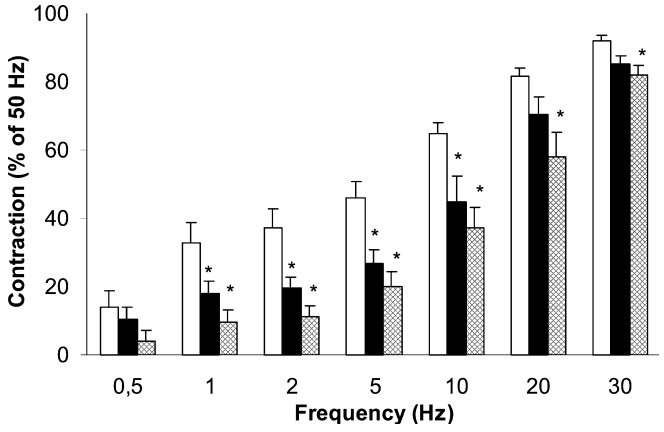

Fig. 2 Contractile responses of rat penile bulb elicited by electrical field stimulation in the absence (*open bars*) or the presence of luzindole 2×10^{-6} M (*solid bars*) or luzindole 2×10^{-6} M and melatonin 10^{-8} M (*cross-hatched bars*). Each point represents the mean ± SEM of five experiments. * $P < 0.05$ indicates significantly different from the control group

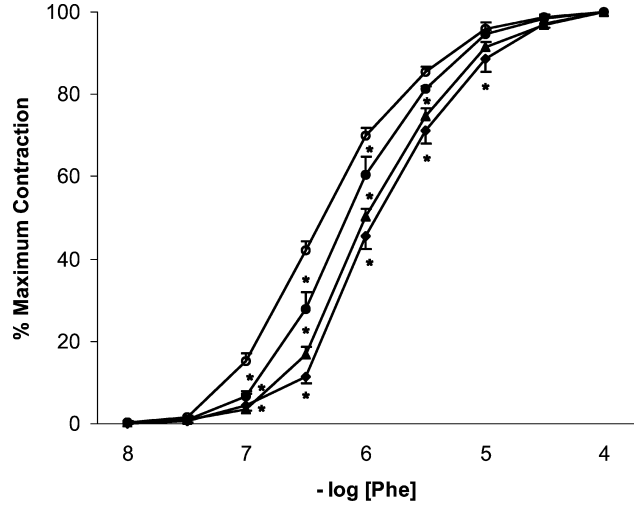

Fig. 3 Contractile responses of rat penile bulb induced by pheylephrine in the absence (*open circles*) or the presence of melatonin (10^{-9} M: *solid circles*, 10^{-8} M: *solid triangles*, 10^{-7} M: *solid diamonds*). Each point represents the mean ± SEM of five experiments. * $P < 0.05$ indicates significantly different from the control group

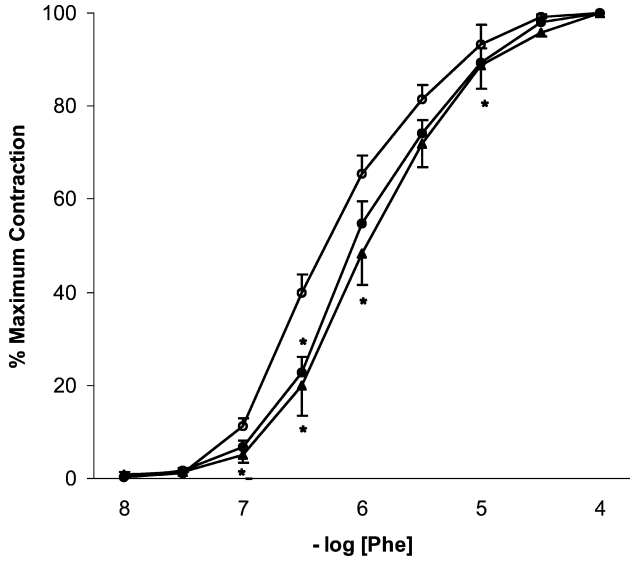

Fig. 4 Contractile responses of rat penile bulb induced by pheylephrine in the absence (*open circles*) or the presence of luzindole (2×10^{-6} M: *solid circles*) or luzindole (2×10^{-6} M) and melatonin (10^{-8} M) (*solid triangles*). Each point represents the mean ± SEM of five experiments. * $P < 0.05$ indicates significantly different from the control group

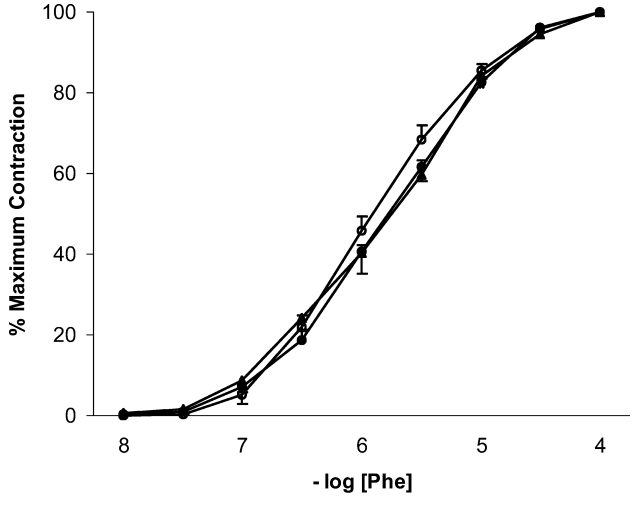

Fig. 5 Effects of tetrodotoxin (10^{-6} M) on pheylephrine-induced contractions in the absence (*solid circles*) or the presence of melatonin (10^{-8} M) (*solid triangles*) in rat penile bulb. *Open circles* represent the data obtained from the control. Each point represents the mean ± SEM of five experiments

higher than 10^{-8} M. Since α-adrenoceptor blockers have been reported to cause penile tumescence and prolonged erection [14], decreased melatonin levels with age or melatonin administration for its purported beneficial effects such as sleep-promoting or antioxidant activity [3, 13] may affect erectile function.

Although melatonin receptor agonists N-acetylserotonin and 2-iodomelatonin also decreased the EFS- and Phe-induced contractions in isolated rat penile bulb, a melatonin antagonist luzindole did not reverse but mimicked the effect of melatonin. Thus, it seems unlikely that this effect of melatonin is mediated by melatonin receptors. Similarly to our results, Li et al. [10] demonstrated that luzindole mimicked the modulatory effect of melatonin on gamma-aminobutyric (GABA) receptor-mediated currents on isolated carp retinal neurons. They speculated that this modulatory effect may be due to an allosteric action caused by melatonin binding to a modulatory site on the receptors. Accordingly, we can speculate that the inhibitory effect of melatonin on α-adrenergic-induced contractions may be the result of an allosteric interaction with melatonin and presynaptic receptors on NANC neurons in the smooth muscle of isolated rat penile bulb.

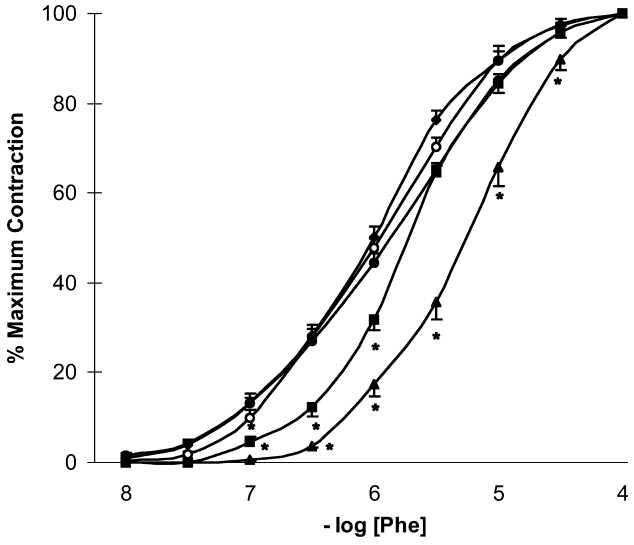

Fig. 6 Effects of vasoactive intestinal peptide (VIP) antagonist (3x 10^{-6} M: *solid circles*), atropine (10^{-6} M: *solid triangles*), L-NAME (10^{-5} M: *solid squares*) and guanethidine (10^{-6} M: *solid diamonds*) on phenylephrine-induced contractions in the presence of melatonin (10^{-8} M) in rat penile bulb. *Open circles* represent the data obtained from the control. Each point represents the mean ± SEM of five experiments. * $P < 0.05$ indicates significantly different from the control group

NO and VIP, as inhibitory NANC neurotransmitters, are known to relax corporal smooth muscle [2, 4, 7, 8]. In this study, VIP antagonist completely reversed the effect of melatonin on Phe-induced contractions, while L-NAME, which blocks nitric oxide synthase, did not change the effect of melatonin except at the higher concentrations of Phe. Consistent with our findings, VIP was shown to attenuate adrenergically-mediated contractions in corporal smooth muscle [14]. Interestingly, the neuronal sodium channel blocker tetrodotoxin and adrenergic neuron blocker guanethidine also completely antagonized the inhibitory effect of melatonin on Phe-induced contractions. These findings together may indicate that melatonin can modulate the effect of sympathetic neurotransmission on VIPergic neurons in isolated rat penile bulb. Similarly to our results, melatonin was shown to cause a dose-dependent relaxation of precontracted rat aorta, an effect which was blocked by preincubating the vessels with VIP antagonist, tetrodotoxin, lidocaine or 6-hydroxydopamine, which destroys the sympathetic nerve terminals in the vascular wall [15].

In this study, the presence of atropine or L-NAME, which block muscarinic receptors and nitric oxide synthase, respectively, did not reverse the effect of melatonin on α-adrenergic-induced contractions, suggesting that muscarinic receptors and NO may not be involved in this effect of melatonin. In addition, melatonin did not change the concentration-response curve to calcium, indicating that melatonin has no effect on voltage-gated calcium channels in this tissue.

In conclusion, we demonstrated that melatonin significantly inhibits the α-adrenergic responses in isolated rat penile bulb. As the stimulation of α-adrenergic receptors is known to cause penile flaccidity and detumescence, serum levels or administration of this pineal hormone may affect erectile function. The effect of melatonin was probably due to its allosteric interaction with the presynaptic receptors on VIPergic neurons, which are affected by the sympathetic transmission followed by an increase in VIP release from these neurons. Further studies should be done, especially under in vivo conditions, in order to determine the effect of melatonin on erectile function.

References

1. Abete P, Bianco S, Calabrese C, Napoli C, Cacciatore F, Ferrara N, Rengo F (1997) Effects of melatonin in isolated rat papillary muscle. FEBS Lett 412: 79
2. Andersson KE, Wagner G (1995) Physiology of penile erection. Physiol Rev 75: 191
3. Brzezinski A (1997) Melatonin in humans. New Engl J Med 336: 186
4. Burnett AL (1997) Nitric oxide in the penis: physiology and pathology. J Urol 157: 320
5. Fovaeus M, Andersson KE, Hedlund H (1987) Effects of some calcium channel blockers on isolated human penile erectile tissues. J Urol 138: 1267
6. Hedlund P, Larsson B, Alm P, Andersson KE (1995) Distribution and function of nitric oxide-containing nerves in canine corpus cavernosum and spongiosum. Acta Physiol Scand 155: 445
7. Ignorro LJ, Bush PA, Buga GM, Wood KS, Fukuto JM, Rajfer J (1990) Nitric oxide and cyclic GMP formation upon electrical field stimulation cause relaxation of corpus cavernosum smooth muscle. Biochem Biophys Res Commun 170: 843
8. Kim YC, Kim CH, Davies MG, Hagen PO, Carson CC (1995) Modulation of vasoactive intestinal polypeptide (VIP)-mediated relaxation by nitric oxide and prostanoids in the rabbit corpus cavernosum. J Urol 153: 807
9. K-Laflamme A, Wu L, Foucart S, Champlain DJ (1998) Impaired basal sympathetic tone and alpha-1-adrenergic responsiveness in association with the hypotensive effect of melatonin in spontaneously hypertensive rats. Am J Hypertens 11: 219
10. Li GL, Li P, Yang XL (2001) Melatonin modulates gamma-aminobutyric acid (A) receptor-mediated currents on isolated carp retinal neurons. Neurosci Lett 301: 49
11. Lue TF, Tanagho EA (1989) Physiology of erection and pharmacological management of impotence. J Urol 137: 829
12. Sack RL, Lewy AJ, Erb DL, Vollmer WM, Singer CM (1986) Human melatonin production decreases with age. J Pineal Res 3: 379
13. Sack RL, Lewy AJ, Hughes RJ (1998) Use of melatonin for sleep and circadian rhythm disorders. Ann Med 30: 115
14. Saenz de Tejada I, Kim N, Lagan I, Krane RJ, Goldsteine I (1989) Regulation of adrenergic activity in penile corpus cavernosum. J Urol 142: 1117
15. Weekley LB (1995) Pharmacologic studies on the mechanism of melatonin-induced vasorelaxation in rat aorta. J Pineal Res 19: 133