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Lack of Melatonin Response to Acute Administration of Nifedipine and Diltiazem in Healthy Men

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Summary

Calcium antagonists have been shown to influence some endocrinological processes in mammals. The use of calcium channel blockers in clinical practice is well documented. The current study monitored nocturnal melatonin, prolactin, and cortisol levels in 19 healthy volunteers before and after administration of calcium channel blockers. The effect of nifedipine was tested in 9 subjects, while diltiazem was administered in 10 men. The nocturnal profile of the given parameters was studied between 23:00 and 05:00 h. At midnight (zero time), the participants were given placebo, nifedipine (in a sublingual dose of 20 mg) or diltiazem (in a single dose of 90 mg). The hypothesis that calcium channel blockers decrease nocturnal melatonin secretion has not been confirmed. The mean nocturnal levels of melatonin between 01:00 and 05:00 h were: 78.1±8.8 (control study) *vs.* 82.4±10.2 ng/l (nifedipine study) and 73.0±5.3 ng/l (control study) *vs.* 75.1±5.1 ng/l (diltiazem study). In conclusion, the calcium channel blockers used in this study do not alter the nocturnal melatonin secretory process in healthy men.

Key words

Melatonin • Diltiazem • Nifedipine • Healthy volunteers

Introduction

Melatonin (5-methoxy-N-acetyltryptamine), the main hormone produced by the pineal gland, plays a significant role and exhibits wide variety of actions in the organism. There is, perhaps, no organic system that is not prone to its influence. For this reason, considerable attention has been paid to the effect of different drugs on melatonin production (Wakabayashi *et al.* 1991, Nathan *et al.* 1996). Calcium antagonists are widely used in clinical practice. It has been reported that organic and inorganic calcium channel blockers diminish melatonin release from chick cells *in vitro* (Zatz and Mullen 1988), as well as *in vivo* in rats (Zawilska and Nowak 1991) and in rainbow trout pineal organs (Gasser and Gern 1997). However, to the best of our knowledge, the effect of calcium antagonists on melatonin secretion in man has yet to be studied.

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Therefore, the present study has tested the effect of nifedipine and diltiazem on nocturnal melatonin secretion in healthy human male volunteers.

Methods

Subjects

Nineteen healthy male volunteers were investigated. Nine of them took part in the study with nifedipine, while 10 participated in the diltiazem study. None of the volunteers smoked or took any medication for at least two months before the study. Their age was 21.1±0.8 years (mean ± S.E.M.) in the nifedipine study and 22.3±0.5 years in the diltiazem study. All of the volunteers were fully informed about the purpose of the investigation and gave their written consent to participate. The study was performed with the approval of the local Ethical Committee of the Institute of Endocrinology in Prague.

Protocol

In each participant, two identical profiles of nocturnal melatonin, prolactin, and cortisol levels were measured. One of the profiles served as a control (in which a placebo was given) and during the other a single sublingual dose of 20 mg nifedipine or a single dose of 90 mg diltiazem was given. In half of the volunteers the control study was performed as the first test. For the others, the nifedipine or diltiazem study was applied first. The volunteers took a light meal at 18:00 h, and the test began at 21:00 h. All of the subjects were allowed to rest in the supine position for two hours in a quiet, dark room. During this period, a venous catheter was inserted into one cubital vein. Blood samples for the measurement of melatonin, prolactin, and cortisol concentrations were collected every hour from 23:00 h to 05:00 h, i.e. seven samples were obtained in each subject. At midnight, immediately after blood withdrawal, placebo (in the control study), nifedipine (Corinfar, Dresden, Germany) in a dose of 20 mg or diltiazem (Dilzem, Godecke, Germany) in a dose of 90 mg were given sublingually. Blood withdrawals were carried out under a red lamp.

Laboratory methods

Melatonin concentrations were determined by slightly modified method of Fraser *et al.* (1983). The buffer (Tricine 0.1 M, pH 5.0), in addition to 0.1 % gelatine and 0.14 M NaCl, contained 5 mM EDTA, 0.1 % BSA and 0.01 % human gamma globulin. Sheep anti-melatonin antibody (batch No 704-8483) was obtained from Guild-Hay (University of Surrey, Surrey, U.K.) The assay parameters were as follows: sensitivity 3 pg/tube; slope of the curve 1.1; intra- and inter-assay coefficients of variation 7.2 % and 14 %, respectively. Prolactin was estimated by a commercial IRMA Adico kit from the Czech Republic, and determination of cortisol by an individual method described elsewhere (Hampl *et al.* 1988). The intra-assay coefficients of variation were as follows: 3.5 % for prolactin and 4.3 % for cortisol. The inter-assay coefficients of variation of the methods were: 8.2 % for prolactin and 9.2 % for cortisol.

Calculation and statistics

The results are presented as means ± SEM. Due to the fact that data were normally distributed, the Student's paired two-tailed test was used to compare the differences in the nocturnal melatonin, prolactin, and cortisol levels measured between 23:00 h and 05:00 h, before and after the administration of the calcium antagonists or placebo. The same test was used when comparing blood pressure before and after the administration of the drugs at hourly intervals. An unpaired t-test was used to compare the mean values in individual groups.

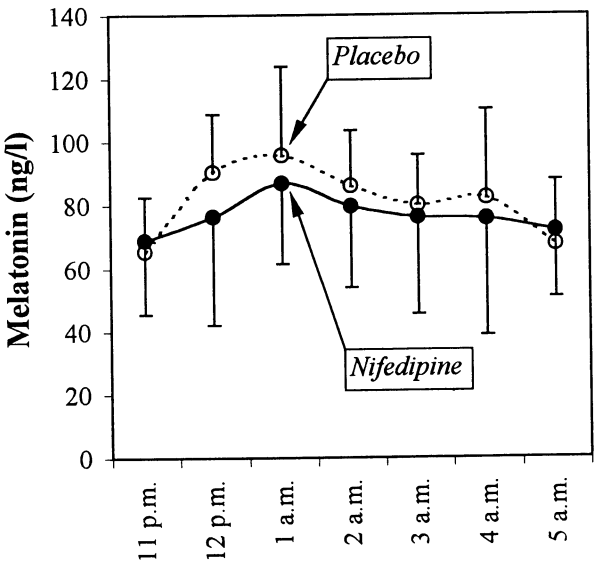

Fig. 1. *Nocturnal profile of serum melatonin (ng/l) in 9 healthy volunteers after placebo (broken line) and nifedipine (solid line) from 11 p.m. until 5 a.m.*

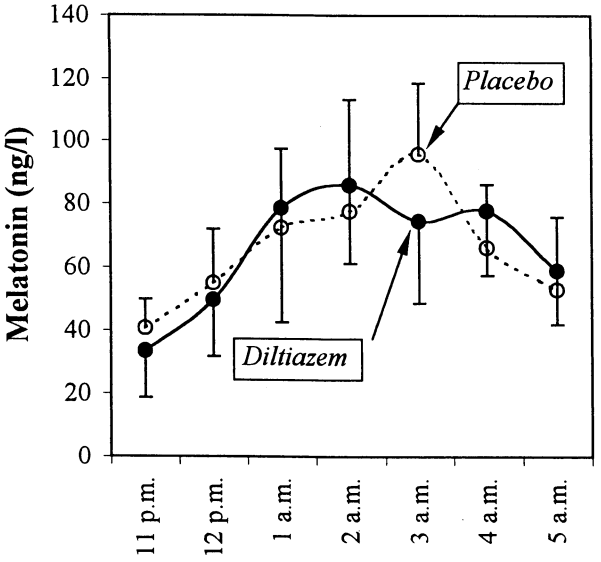

Fig. 2. *Nocturnal profile of serum melatonin (ng/l) in 10 healthy volunteers after placebo (broken line) and diltiazem (solid line) from 11 p.m. until 5 a.m.*

Results

Figure 1 shows the nocturnal profile of melatonin serum levels in healthy volunteers treated with placebo and nifedipine. The values obtained at various times did not differ significantly after nifedipine as compared to placebo. Similar results were obtained in subjects treated with diltiazem (Fig. 2).

It is evident from Table 1 that nifedipine does not change mean nocturnal melatonin, prolactin and cortisol levels. The same is true for diltiazem, which does not alter the nocturnal secretion of the aforementioned parameters (Table 2).

Bioavailability of the treatment by calci n channel blockers was controlled by the decline of syste and diastolic blood pressure (Figs 3 and 4).

Table 1: Nocturnal serum levels (from 01:00 to 05:00 h) of melatonin, prolactin and cortisol after placebo or nifedipine administration.

Parameter	Placebo	Nifedipine
Melatonin (ng/l)	78.1±8.8	82.4±10.2
Prolactin (ng/ml)	10.6±0.6	10.7±0.5
Cortisol (nmol/l)	196.7±17.3	252.9±22.1

Data are means ± S.E.M. Evaluated by Student's paired t-test (n=9).

Table 2: Nocturnal serum levels (between 01:00-05:00) of melatonin, prolactin and cortisol after placebo or diltiazem administration.

Parameter	Placebo	Diltiazem
Melatonin (ng/l)	75±5.1	73.0±5.3
Prolactin (ng/ml)	11.9±0.5	11.5±0.7
Cortisol (nmol/l)	167.8±23.2	172.8±21.9

Data are means ± S.E.M. Evaluated by Student's paired t-test (n=10).

Discussion

It is known that calcium influx is necessary for melatonin production (Zatz 1989). It has been proven that the dihydropyridine agonist of calcium channels Bay K8644 enhances melatonin output. On the other hand, a calcium channel blocker of a similar chemical structure, nitrendipine, markedly diminished melatonin release from chick pineal cells (Zatz and Mullen 1988). In addition, Meyer *et al.* (1986) reported that nifedipine in a dose of 1.2 mg/kg depressed the amplitude of plasma melatonin in baboons. On this basis, one would expect that calcium channel blockers would also suppress melatonin secretion *in vivo* in humans. However, the data presented here failed to confirm this hypothesis.

Although it was demonstrated that after comparable oral doses of the respective drugs (Hermann *et al.* 1983, Raemsch and Sommer 1983), the peak of plasma levels is reached between 30-60 min (for nifedipine) and 60-240 min (for diltiazem), mean nocturnal melatonin secretion was not diminished in the present study. Such unresponsiveness to highly effective calcium channel blockers was described previously in other electrically excitable hormonal systems, especially in the

adenohypophysis (Struthers *et al.* 1983, Žofková *et al.* 1983). A possible explanation of this phenomenon lies in the contra-regulatory factors (dependent on species differences, the dose and chemical structure of the calcium antagonists), which may compensate for the calcium channel blockade at the subcellular level (Raeburn 1987, Schneider *et al.* 1988, Schulz *et al.* 1989, McDonald *et al.* 1989, Yajima *et al.* 1990). On the other hand, the activation of some systemic regulators under sustained channel blockade is, less plausible (Blahoš *et al.* 1989).

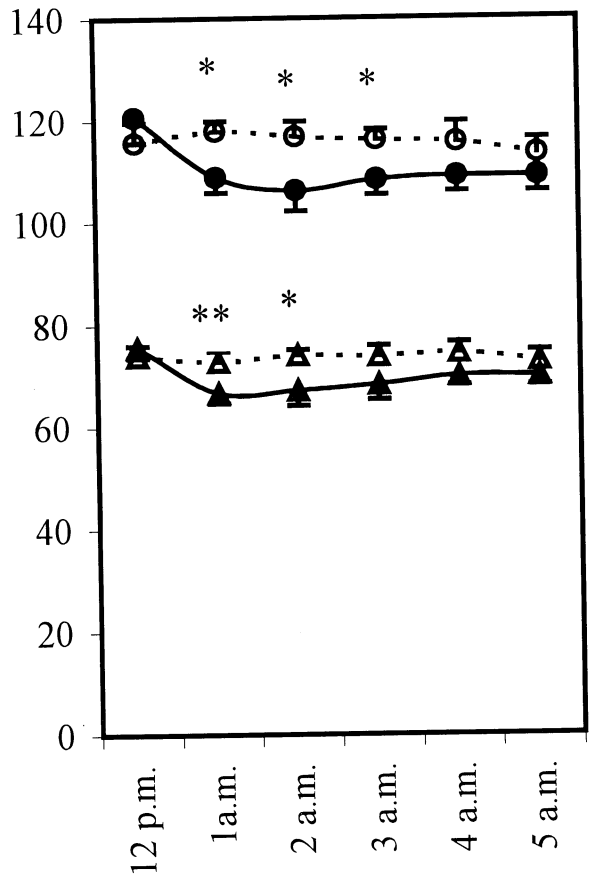

Fig. 3. *Nocturnal values of systolic and diastolic blood pressure in 9 healthy volunteers after placebo or nifedipine treatment. Dashed line with empty circles – systolic blood pressure after placebo, solid line with full circles - systolic blood pressure after nifedipine, dashed line with empty triangles – diastolic blood pressure after placebo, solid line with full triangles - diastolic blood pressure after nifedipine. * p<0.05, ** p<0.01 (evaluated by Student's paired t-test).*

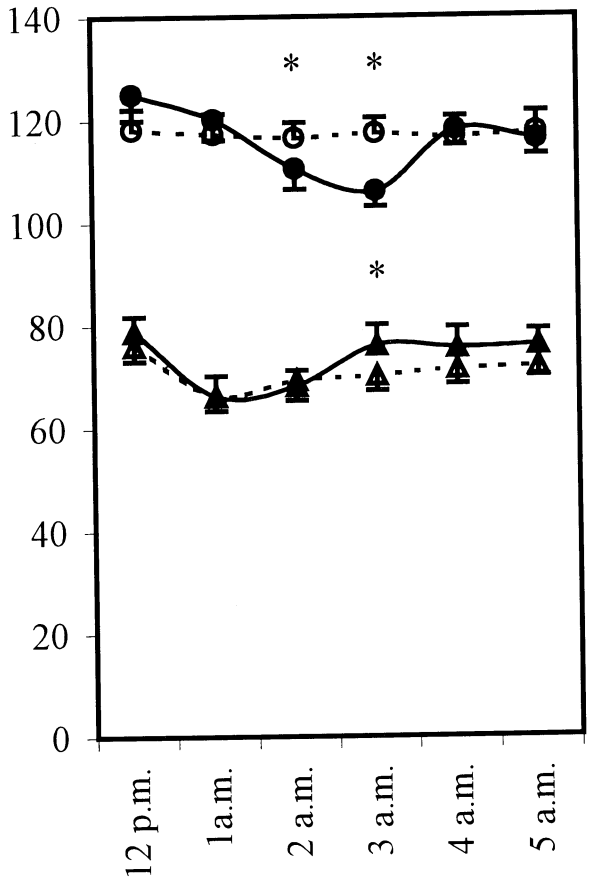

Fig. 4. *Nocturnal values of systolic and diastolic blood pressure in 10 healthy volunteers after placebo or diltiazem treatment. Dashed line with empty circles – systolic blood pressure after placebo, solid line with full circles - systolic blood pressure after diltiazem, dashed line with empty triangles – diastolic blood pressure after placebo, solid line with full triangles - diastolic blood pressure after diltiazem. * p<0.05, ** p<0.01 (evaluated by Student's paired t-test).*

The present study, however, has some limitations. Firstly, the time of administration of calcium channel blockers might be important. Zawilska and Nowak (1991) demonstrated the stimulatory effect of calcium antagonist BAY K8644, when administered before the time of light offset, on N-acetyltransferase activity in rats. Therefore, it might be possible to explain the insensitivity of the hormonal system by the fact that administration of the drug

occurred after the initiation of signal transduction. The second limitation concerns the doses used, which were significantly lower than those given by Meyer *et al.* (1986) to baboons. However, the aim of the present study was to test the clinical impact of calcium channel blockers given in the recommended pharmacological doses. The decrease in blood pressure following nifedipine and diltiazem administration documents the effectiveness of the treatment.

Although the mechanism(s) determining the insensitivity of hormonal systems, including the pineal gland, to nifedipine and diltiazem remain to be elucidated, this study indicates that a single pharmacological dose of the tested drugs (administered according to the protocol used), which effectively influenced the hemodynamics in humans, had no effect on nocturnal melatonin secretion. The latter observation is important in view of the generally beneficial impact of melatonin in human physiology. Further clinical investigations to clarify the phenomenon are undoubtedly necessary.

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