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Short communication

Prolyl-leucyl-glycinamide, thyrotropin-releasing hormone and β-endorphin-(10–16), like antidepressants, antagonize melatonin-induced behavioural changes in rats

Kotaro Hatta [1], Gerrit Wolterink, Jan M. Van Ree *

Rudolf Magnus Institute for Neurosciences, Medical Faculty, University of Utrecht, Universiteitsweg 100, 3584 CG Utrecht, Netherlands

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Abstract

β-Endorphin-(10–16), as well as a variety of antidepressants, has been reported to block the behavioural changes induced by injecting melatonin into the nucleus accumbens. In the present study the influence of subcutaneously administered prolyl-leucyl-glycinamide (PLG) and thyrotropin-releasing hormone (TRH) on the behavioural changes induced by melatonin administration in the nucleus accumbens were investigated and compared with that of β-endorphin-(10–16). PLG and TRH were found to be as effective as β-endorphin-(10–16) in counteracting the melatonin-induced behavioural changes. The data suggest that these peptides may serve as a starting point for the development of a new class of antidepressants.

Keywords: β-Endorphin-(10–16); Prolyl-leucyl-glycinamide; TRH (thyrotropin-releasing hormone); Melatonin; Nucleus accumbens; Antidepressant

1. Introduction

Melatonin has been implicated in the aetiology of depression, and altered secretion of melatonin in depressed patients has been reported (for review see Van Ree et al., 1994). Animal studies have suggested that melatonin plays an important role in certain behavioural processes, especially in modulation of locomotor activity and emotionality (for review see Van Ree et al., 1994). Melatonin, when injected in low doses into the nucleus accumbens of rats, decreases locomotor activity and rearing while grooming and sniffing behaviour are increased. This melatonin effect is mimicked by similar administration of serotonin antagonists. Local application of serotonin or a variety of antidepressant drugs completely blocks the melatonin effect (Gaffori and Van Ree, 1985a,b). Furthermore, antidepressants block the action of melatonin in the nucleus accumbens after chronic systemic treatment (Durlach-Misteli and Van Ree, 1992). The fragment β-endorphin-(10–16) has been reported to antagonize behavioural responses elicited by melatonin following injection into the nucleus accumbens of rats (Gaffori and Van Ree, 1985b). Other fragments of β-endorphin (e.g. β-endorphin-(2–9), β-endorphin-(6–9), β-endorphin-(10–13), β-endorphin-(14–16)) were ineffective in this respect. β-Endorphin-(10–16) mimicked the action of antidepressants after chronic treatment as well (Van Ree et al., 1994). Thus, β-endorphin-(10–16) and a variety of antidepressant drugs possess common properties in this respect.

The C-terminal fragment prolyl-leucyl-glycinamide (PLG) of oxytocin has been reported to possess antidepressant properties in a number of clinical studies (Ehrensing and Kastin, 1974; Van der Velde, 1983; Ehrensing et al., 1994), although two other studies failed to confirm this (Ehrensing and Kastin, 1980; Levy et al., 1982). PLG has been shown to exert effects resembling those of antidepressants in animal studies using electrical self-stimulation (Dorsa and Van Ree, 1979), short-term social isolation (Niesink and Van

* Corresponding author. Tel. +31-30-2538801, fax +31-30-2539032.

[1] On leave of absence from the Department of Psychiatry, Tokyo Metropolitan Matsuzawa Hospital, 2-1-1-Kamikitazawa, Setagaya-ku, Tokyo 156, Japan.

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Ree, 1984), behavioural despair (Pulvirenti and Kastin, 1988) and chronic unpredictable stress (Pignatiello et al., 1989).

Thyrotropin releasing hormone (TRH) has been reported to exert antidepressant activity after intravenous administration, whereas oral administration is not effective in this respect (for review see Prange et al., 1987). In animal experiments TRH induces some effects which resemble those of antidepressants, for instance on short-term social isolation (Niesink and Van Ree, 1984) and behavioural despair (Ogawa et al., 1984).

In the present study the influences of subcutaneous administration of the neuropeptides TRH and PLG on the melatonin-induced changes in locomotor and sniffing activity were investigated and compared with those of β-endorphin-(10–16).

2. Materials and methods

2.1. Animals and surgical procedure

Male Wistar rats (TNO, Zeist, Netherlands) weighing 140–160 g at the time of operation were used. They were kept under standard conditions (room temperature 22 ± 1°C, light on from 5:00 a.m. till 7:00 p.m.), were housed in groups of three and received food and water ad libitum. The rats were anaesthetized with Hypnorm (1 ml/kg body weight i.m.) and were secured in a stereotaxic instrument. Stainless steel cannulas (0.6 mm outer diameter, 0.3 mm inner diameter) were implanted on each side of the brain and were aimed at the nucleus accumbens. The coordinates for implantation into the nucleus accumbens area were 2.6 mm anterior to the bregma, 2.7 mm lateral to the midline, 6.1 mm below the dura. The upper incisor bar was at the level of the interaural line and the cannulas were inserted at an angle of 12°. The rats were allowed to recover from the operation for at least 6 days.

2.2. Behavioural procedure

Experiments were carried out between 8:00 a.m. and 5:00 p.m. in a sound-attenuated room. Two injections were given, spaced in time by 60 min; peptide solution or saline was injected first subcutaneously (pretreatment) followed by an injection with melatonin or placebo (1 μl saline containing 1% acetic acid and 2% ethanol) bilaterally into the nucleus accumbens (treatment). The rats were placed at 30 min after the last injection in a circular Perspex test cage (diameter 19.5 cm, height 28.5 cm), the bottom of which was divided into four equal sections, and locomotor activity (number of sections explored) and the duration of sniffing were assessed for 3 min. Table 1 presents the combined data that were derived from three separate experiments, each of which always included groups of saline-placebo- and saline-melatonin-treated animals, with 5–6 animals per group.

Table 1
Interaction between subcutaneously administered PLG, TRH or β-endorphin-(10–16) and intra-accumbally injected melatonin

Treatment		n	Small open field	
−90 min	−30 min		Locomotion (units)	Sniffing (s)
Saline	Placebo	16	18.4 ± 0.9	21.6 ± 1.8
Saline	Melatonin	18	12.2 ± 0.6 [a]	35.9 ± 2.8 [a]
PLG				
1.5 μg/kg	Melatonin	6	13.3 ± 0.8	26.0 ± 3.3
5 μg/kg	Melatonin	5	13.8 ± 1.8	27.4 ± 5.4
15 μg/kg	Melatonin	6	20.5 ± 1.8 [b]	19.0 ± 3.7 [b]
TRH				
1.5 μg/kg	Melatonin	6	11.3 ± 1.2	33.2 ± 4.9
5 μg/kg	Melatonin	6	12.3 ± 1.4	27.8 ± 6.1
15 μg/kg	Melatonin	6	17.3 ± 2.3 [b]	20.0 ± 5.6 [b]
β-Endorphin-(10–16)				
1.5 μg/kg	Melatonin	6	13.3 ± 1.3	33.3 ± 5.7
5 μg/kg	Melatonin	6	16.5 ± 1.4 [b]	25.0 ± 2.4
15 μg/kg	Melatonin	6	18.2 ± 0.7 [b]	20.8 ± 3.0 [b]

Behavioural activity (± S.E.M.) of rats treated s.c. with saline, PLG, TRH or β-endorphin-(10–16), 90 min before testing and treated with placebo or melatonin (10 ng) bilaterally in the nucleus accumbens 30 min before testing. [a] Different from saline, saline ($P < 0.05$). [b] Different from saline, melatonin ($P < 0.05$).

2.3. Histological control

After experimentation the injection sites were evaluated histologically. The rats were killed and the brains were fixed in 4% formalin. Serial sections were cut with a thickness of 100 μm on a cryostat. The sites of injection were determined microscopically. The data of animals with injection sites outside the nucleus accumbens were discarded from further analysis.

2.4. Drugs and peptides

Melatonin was obtained from Fluka, Buchs, Switzerland. The following peptides, donated by Organon International, Oss, Netherlands, were used: β-endorphin-(10–16), prolyl-leucyl-glycinamide (PLG, MIF-1) and thyrotropin-releasing hormone (TRH). The drugs and peptides were stored in dry form under conditions recommended by the supplier. They were dissolved in saline (0.9% NaCl), except for melatonin which was dissolved in saline containing 1% acetic acid and 2% ethanol, immediately prior to use.

2.5. Data analysis and statistics

Group means and S.E.M. were calculated. The data were analysed using a one-way analysis of variance

(ANOVA) followed by the Newman-Keuls procedure when the outcome revealed a statistically significant effect ($P < 0.05$).

3. Results

The sites of injection appeared to be bilateral and in the middle and anterior part of the nucleus accumbens. None of the studied peptides significantly influenced the behaviour of placebo-treated rats (data not shown). Melatonin (10 ng), injected bilaterally into the nucleus accumbens area, decreased the locomotion score and increased the duration of sniffing (Table 1). All of the studied peptides dose dependently inhibited these effects of melatonin. A dose of 15 μg/kg of each peptide completely antagonized the melatonin-induced hypomotility. β-Endorphin-(10–16) antagonized the effect of melatonin on locomotor activity already at a dose of 5 μg/kg.

4. Discussion

In the present study we found that the peptides TRH, PLG and β-endorphin-(10–16) antagonized the melatonin-induced behavioural responses following injection into the nucleus accumbens, an effect which has already been reported for β-endorphin-(10–16) (Gaffori and Van Ree, 1985b). In this respect the action of these peptides resembles that of antidepressants (Gaffori and Van Ree, 1985a; Durlach-Misteli and Van Ree, 1992). It is worth noting that both β-endorphin-(10–16) and antidepressant drugs are not capable of blocking the behavioural effects induced by low doses of apomorphine injected in the nucleus accumbens (Gaffori and Van Ree, 1985b; Durlach-Misteli and Van Ree, 1992), and the dopamine antagonists haloperidol and sulpiride do not affect the melatonin-induced behavioural effects (Gaffori and Van Ree, 1985a), suggesting that the interaction with melatonin in the nucleus accumbens may be specific for antidepressant drugs.

The three peptides antagonized the reduction in locomotion activity as well as the increase in sniffing, all being effective at a dose of 15 μg/kg. Since the three peptides do not differ much in size, PLG and TRH are tripeptides and β-endorphin-(10–16) consists of seven amino acids, they are more or less equipotent in this respect.

The peptides TRH and PLG are active in a number of animal models allegedly detecting antidepressant actions. Furthermore, both peptides have antidepressant activity in some patients (Prange et al., 1987; Ehrensing and Kastin, 1974; Van der Velde, 1983; Ehrensing et al., 1994). Interestingly, PLG was clinically effective within a few days after the start of treatment as opposed to the typical delayed onset of improvement seen with tricyclic antidepressant (Ehrensing and Kastin, 1974; Van der Velde, 1983). Thus, although PLG may share some activities with antidepressants, the underlying processes through which they exert their action may differ.

As far as the mode of action of these peptides is concerned, we can only speculate. Recently, it has been suggested that the development of dopamine receptor supersensitivity might be the mechanism of action by which the therapeutic effect of antidepressant drugs is mediated (Durlach-Misteli and Van Ree, 1992; Pulvirenti and Kastin, 1988). Further studies at a behavioural and biochemical level are necessary to clarify this matter.

In conclusion, the present study shows that β-endorphin-(10–16), PLG and TRH are equipotent in blocking the behavioural responses induced by acute treatment with melatonin in the nucleus accumbens. In this respect these peptides resemble antidepressant drugs and it is tempting to speculate that the blockade of the action of melatonin in the nucleus accumbens is essential for the antidepressant effects. Further research into the effects of β-endorphin-(10–16), PLG and TRH may lead to a better understanding of the processes that are involved in the aetiology of depression and to the development of a new class of antidepressant drugs.

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