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Short communication

Oestradiol and mirtazapine restore the disturbed tail-temperature of oestrogen-deficient rats

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Abstract

The purpose of the present study was to further evaluate the tail-temperature test as a tool to test potential steroidal and non-steroidal compounds for the treatment of hot flushes. Ovariectomized rats were implanted with a temperature sensitive probe. After a recovery period of 5 weeks, the effect of oestradiol (given via a silastic tube) and the 5-HT_2 receptor antagonist mirtazapine (10 mg/kg i.p.) on the tail-temperature in the active phase of the animals was measured. Oestradiol completely restored the disturbed tail temperature after 3 days. Treatment with mirtazapine also restored the oestrogen withdrawal-induced disturbed tail-temperature. The effect of mirtazapine was already seen on the first day of treatment. These experiments confirm and extend the idea that measuring the oestradiol withdrawal-induced disturbance of tail-temperature may be a useful tool to select compounds that might have beneficial effects in the treatment of hot flushes. Blockade of the 5-HT_{2A} receptors prevented or reduced the ovariectomy-induced disturbance of the rat tail-temperature, which may validate this model to evaluate the effect of compounds on hot flushes.

Keywords: Hot flush; Mirtazapine; Oestrogen; Tail-temperature; (Rat)

1. Introduction

Hot flushes are thought to be the result of oestrogen withdrawal since at menopause the oestrogen level in blood serum is strongly decreased and after treatment with oestrogens the frequency and intensity of hot flushes are reduced (Brook et al., 1997). They are shown to originate from the hypothalamus (Rebar and Spitzer, 1987), the center in which the body temperature is controlled (Lomax, 1991) and where oestrogen and progesterone concentrating neurons are present in high density (Bloom, 1991). However, the incidence, frequency and severity of hot flushes show a poor correlation with the blood oestrogen level per se (Hutton et al., 1978), suggesting that other mechanisms in the central nervous system may be triggered by a reduced oestrogen level.

Indeed, apart from the reproductive system, oestrogens have effects on neurotransmission, especially on serotonin (5-hydroxytriptamine, 5-HT) regulation (see for review Stahl, 1998, Stearns et al., 2002). Serotonin levels appear to be reduced to about 50% after ovariectomy and after the menopause, whereas after treatment with oestriol this 5-HT level is restored (Gonzales and Carillo, 1993). Platelet 5-HT_{2A} receptor levels have been shown to be dependent on circulating ovarian steroids across the menstrual cycle, with a minimum around midcycle and a maximal binding during the premenstrual period (Biegon, 1990). This suggests that low oestrogen levels cause a high sensitivity of 5-HT_{2A} receptors. 5-HT_{2A} receptors are thought to underlie the thermogenesis (Mazzola-Pomietto et al., 1995) and stimulation of these receptors may cause a change in set point temperature thereby activating autonomic functions to cool down the body. This has led to the hypothesis that hot flushes are the result of activation of oestrogen withdrawal-induced upregulated 5-HT_{2A} receptors (Berendsen, 2000). Blockade of the hypersensitive 5-HT_{2A} receptors in menopausal women then should prevent or reduce the occurrence of hot flushes. Indeed, a couple of preliminary clinical studies suggest that the 5-HT_2 receptor antagonists mianserin and mirtazapine are able to reduce the frequency and intensity of hot flushes in menopausal women (Takagi and Yanagisawa, 1986, Tome and Isaac, 1998, Waldinger et al., 2000).

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Recently, an animal model was developed in which it was shown that oestrogen withdrawal by ovariectomy or treatment with a gonadotrophine releasing hormone (GnRH) receptor antagonist affects the tail-temperature of rats in their active (dark) phase but not in their rest (light) phase (Berendsen et al., 2001). On the transition from the rest phase to the active phase, the tail-temperature of intact female rats drops from around 31 to around 25 °C whereas the tail-temperature from ovariectomized rats drops from around 31 to around 29 °C. After treatment with oestradiol or tibolone but not raloxifene, the tail-temperature drop of ovariectomized rats was restored to the level as seen in intact rats.

The purpose of the present experiments was to further evaluate the tail-temperature test as a tool to test compounds for their potential in the treatment of hot flushes. In the former experiments, oestradiol was given s.c. and it was seen that the compound was active after 3 days of treatment (Berendsen et al., 2001). In the present experiments, it was decided to administer oestrogen in a silactic tube in order to get a continuous release of the compound. This might give a shorter onset of action. Mirtazapine was included in these experiments because, as said before, preliminary clinical studies indicate that mirtazapine may have potential in the treatment for hot flushes.

2. Materials and methods

Animal handling was in accordance with the Dutch Law on Animal Experimentation and the European Directive for the protection of Vertebrate Animals used for Experimental and other scientific Purposes (European Union Directive #86/609/CEE). The Committee for Experiments on Animals (DEC) of NV Organon approved the experiments.

2.1. Animals

Naive female rats (HSD/Cpb:WU, Harlan Sprague Dawley, Horst, The Netherlands) weighing 250–330 g were used. The rats were individually housed in macrolon cages (38 × 22 × 15 cm) on a sawdust bedding, under controlled 14-h light–10-h dark cycle. Normally, the lights were turned on at 0600 h but, for practical reasons in the present experiments, the light–dark cycle was reversed (light on at 1930 h). Room temperature was between 20 and 21 °C. The rats had free access to standard food pellets and tap water. Each experimental group included at least seven animals.

2.2. Surgery

The rats were ovariectomized or SHAM operated and implanted with a TA10TA-F40 W/THERM probe (Data Sciences) under isoflurane (Forene®, Abbott) anaesthesia following the same procedure as described before (Berendsen et al., 2001). The tip of the adapted probe was placed in the rat tail 2 cm beyond the tail implant under the skin and fixed in place with 0.05 ml Vet Seal® (Braun Melsungen). The body of the probe was placed in the abdomen of the rats and fixed to the abdominal muscles with two stitches. Immediately after surgery, the rats were treated with the analgesic buprenorphine (Temgesic®, Schering-Plough, 0.6 μg/rat) and put back in their home cages. These cages were placed on receivers (RLA 1020, Data Sciences) for measuring the tail-temperature. The receivers were attached to a consolidation matrix (BCM 100) and thus to a data acquisition system (Dataquest IV).

2.3. Procedure

Five weeks after ovariectomy and temperature probe implantation, the rats were treated daily for 8 days intraperitoneally with mirtazapine 10 mg/kg at 1100 h, 90 min after the light in the animal room was turned off. During the injection period, the door of the animal room was left open in order to have enough light for correct injections. In the oestradiol experiment, the rats were implanted subcutaneously on day 1 with a silastic tube containing oestradiol plus cholesterol (1:100) via a trochar. The daily dose of oestradiol released from the tube caused a blood plasma level comparable to the amount normally circulating in the blood ($\pm$ 10 pg/ml plasma) as established in a separate experiment (not shown). The control groups were injected with vehicle (mirtazapine experiment) or implanted with a silastic tube containing only cholesterol (estradiol experiment). The tail-temperature of the rats was measured continuously, but for calculations of the treatment effects, the measurements between 1200 and 1400 h were used. During this period, the rats remained completely undisturbed. Thereafter, the rats could be cared for routinely. To be sure that the differences in tail-temperature between intact and ovariectomized rats were significantly different ($P < 0.05$), the last 3 days before compound treatment was started the tail-temperature of the rats was monitored. After completion of the experiments, the ovariectomized rats were killed and inspected for any remnants of the ovaries.

2.4. Drugs and solutions

The compounds used in this study were 17β-oestradiol and mirtazapine (Org 3770, Remeron®). The compounds were synthesised in the Medicinal Chemistry Department of NV Organon. 17β-Oestradiol was delivered via a silastic tube containing also cholesterol (1:100). Mirtazapine was suspended in an aqueous solution of 5% Mulgofen (EL 719; GAF) and 0.9% NaCl. The suspension was freshly prepared every second day. A dose volume of 5 ml/kg body weight was used. Control animals were implanted with a silastic tube containing only cholesterol (in the oestradiol experiment) or injected with an equivalent volume of vehicle.

2.5. Statistics

Tail-temperature of the rats was continuously measured each day for 2 h between 1200 and 1400 h. In this period, the tail-temperature for each rat was measured for 7 s every 5 min. The mean tail-temperature over this 2-h period was calculated for each rat. In addition, the group means were calculated together with the standard error of the mean (S.E.M.). The results were statistically evaluated with the analysis of variance (ANOVA).

3. Results

3.1. General

In the experiment with oestradiol, the mean tail-temperature of intact female rats in the active period of the animals was just below 25 °C at the start of the experiment (day 1) and increased slowly up to around 26 °C after 8 days (Fig. 1). In the experiment with mirtazapine, this tail-temperature fluctuated around 26 °C during the experiment (Fig. 2). The tail-temperature of the ovariectomized rats fluctuated between 28 and 29 °C over the experimental days in the experiment with oestrogen (Fig. 1) and between 30 and 31 °C in the mirtazapine experiment (Fig. 2) ($P<0.05$ in both experiments if compared to the intact placebo treated rats). Inspection of the ovariectomized rats for remnants of the ovaries revealed that the ovariectomy was correctly done. It was noticed that the uterus of the oestrogen-treated rats looked normal whereas those of the placebo-treated ones were strongly reduced. However, their exact weights were not measured.

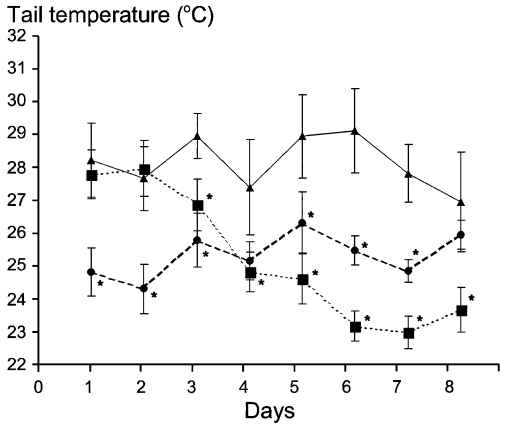

Fig 1. Effect of oestradiol on tail temperature of ovariectomized rats. Oestradiol was administered via a silastic tube with cholesterol (1:100) s.c. implanted. Rats were housed under reversed light–dark cycle: lights on at 1930 h. Tail temperature was measured daily from 1200 till 1400 h. Given are the mean temperature in this period ± standard error of the mean (S.E.M.). Number of animals in each treatment group was 7. ● Intact rats placebo treated; ▲ ovariectomized rats placebo treated; ■ ovariectomized rats treated with oestradiol. *$P<0.05$ if compared to the ovariectomized rats treated with placebo.

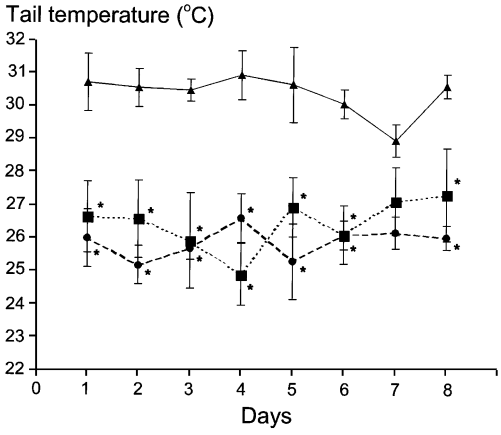

Fig 2. Effect of mirtazapine on tail temperature of ovariectomized rats. Mirtazapine 10 mg/kg was daily injected intraperitoneally between 1100 and 1130 h. Tail temperature was measured from 1200 till 1400 h. Rats were housed under reversed light–dark cycle: lights on at 1930 h. Given are the mean temperatures in this period ± standard error of the mean (S.E.M.). Number of animals in each treatment group was 7. ● Intact rats placebo treated; ▲ ovariectomized rats placebo treated; ■ ovariectomized rats treated with mirtazapine 10 mg/kg. *$P<0.05$ if compared to the ovariectomized rats treated with placebo.

3.2. Effect of oestradiol on tail-temperature of ovariectomized rats

Implantation of ovariectomized rats with a silastic tube containing only cholesterol (placebo) had no effect on tail-temperature of these rats. Implantation of the rats with a silastic tube containing a mixture of oestradiol and cholesterol (1:100) caused a fall in tail-temperature from the third day on ($P<0.05$). On day 4, the tail-temperature of these rats was equal to the tail-temperature of the intact rats and dropped somewhat further to around 24 °C on days 6, 7 and 8 (Fig. 1).

3.3. Effect of mirtazapine on tail-temperature of ovariectomized rats

Injection of ovariectomized rats with mirtazapine 10 mg/kg i.p. caused a fall in tail-temperature of the ovariectomized rats to the level of intact rats. This fall in tail-temperature was already seen on the first day of treatment ($P<0.05$ if compared to the ovariectomized rats treated with placebo) and remained at this level during the experimental period of 8 days (Fig. 2). In former experiments, it was seen that mirtazapine 10 mg/kg had no effect on body temperature of normal intact rats.

4. Discussion

Ovariectomy prevented (partially) the fall in tail-temperature at the transition from the rest phase to the active phase of the animals. This disturbed fall in tail-temperature could

be restored by the implantation of a silastic tube containing oestradiol and cholesterol (1:100). The amount of oestradiol released from the tube (causing a plasma level of about 10 pg/ml being about the normal physiologic level of oestradiol (Haruyama et al., 2002) was enough to restore completely the disturbed tail-temperature. In former experiments using the same procedure, it was shown that a dose of 5 μg/kg given twice daily caused the same effect with the same onset of action (Berendsen et al., 2001). This indicates that the route of administration of oestradiol is not important for its effect as long as normal plasma levels are reached. This is in contrast to the effect of oestradiol in the morphine withdrawal model for hot flushes. In the latter model, only a dose of oestradiol 100–300 times higher was able to reach a complete block of the morphine withdrawal-induced rise of tail-temperature (Merchenthaler et al., 1998). This may also indicate that the effect of oestradiol in both models is caused by different mechanisms.

Indeed, the morphine withdrawal model, first introduced by Simpkins et al. (1983) and Katovich et al. (1986), is based on the similarities that are seen after morphine withdrawal in rats and hot flush symptoms in man. In rats, a strong rise in tail-temperature, an increase in heart rate and a surge in luteinising hormone (LH) are seen. Hot flushes in man are accompanied with a rise in peripheral skin temperature, palpitations and a LH surge (Simpkins et al., 1983).

The model we recently developed (Berendsen et al., 2001) and used here is based on the idea that a hot flush is caused by an oestradiol-withdrawal-induced lowered serotonin level thereby creating an increased number of 5-HT_{2A} receptors in the hypothalamus (Biegon, 1990). Activation of this hypersensitive 5-HT_{2A} receptors by a mild in- or external stress (coffee, alcohol, light ambient temperature, physical contact, anxiety, etc.) results in a disturbance of the thermoregulatory system in the hypothalamus (Berendsen, 2000). Increasing the oestradiol level in turn then causes a rise in 5-HT level and a down regulation of 5-HT_{2A} receptors. This down regulation of the increased 5-HT_{2A} receptors takes some time and this may be the cause of the delayed effect of oestrogen treatment on tail temperature in our experiments and the delayed efficacy of oestrogen on hot flushes in man (Haas et al., 1988).

If this is true then a treatment that blocks the supersensitive 5-HT_{2A} receptors directly should cause an immediate effect on hot flushes. In a couple of preliminary studies in man, the 2-HT_2 receptor antagonist mirtazapine was shown to reduce both the intensity and the number of hot flushes in man (Tome and Isaac, 1998, Waldinger et al., 2000) and that the effect indeed occurs on short term. In the present experiments, we also see that already after the first treatment with mirtazapine the disturbed drop of tail-temperature is restored, suggesting that direct blockade of 5-HT_2 receptors is responsible for this effect.

Mirtazapine is a mixed 5-HT_{2A} and 5-HT_{2C} receptor antagonist with strong α_2-adrenoceptor blocking properties (De Boer et al., 1995). An effect of the compound on hot flushes via its α_2-adrenoceptor blocking properties however, is not likely since the selective α_2-adrenoceptor agonist clonidine has some hot flush reducing properties (Tulandi et al., 1983, Nagamani et al., 1987) and restores the tail temperature drop (Berendsen et al., 2001). An effect of mirtazapine via its 5-HT_{2C} receptor blocking properties is also not likely since it was shown before that in case of effects of a compound via the 5-HT_{2A} and 5HT_{2C} receptors the effect via the 5-HT_{2A} receptors prevails (Berendsen and Broekkamp, 1990). It is thus plausible that the effects of mirtazapine seen here are mediated via the 5-HT_{2A} receptors.

The neurotransmitter serotonin plays an important role in thermoregulation (Sheard and Aghajanian, 1967) and especially the 5-HT_{1A} and 5-HT_{2A} receptor subtypes are associated with temperature control. Stimulation of 5-HT_{1A} receptors causes a lowering of core body temperature both in rodents and man (Berendsen and Broekkamp, 1990, Cryan et al., 1999, Cleare et al., 1998), whereas blockade of 5-HT_{2A} receptors completely prevented the hyperthermia in an animal model of the 5-HT syndrome (Nisijima et al., 2001) and activation of these receptors in rodents induces hyperthermia (Salmi and Ahlenius., 1998). In man, it was seen that activation of 5-HT_{2A} receptors with relatively high doses of *m*-chlorophenylpiperazine (*m*-CPP) induces "sweating and hot and cold flashes" (Charney et al., 1987), "flushes" (Murphy et al., 1989), "palpitations and sweating" (Kahn et al., 1990), and "hot flushes and cold chills" (Klaassen et al., 1998). A prominent role for serotonin in the pathophysiology of hot flushes was therefore suggested (Berendsen, 2000, Stearns et al., 2002).

In conclusion, the experiments presented here confirm and extend the idea that the method of measuring the tail-temperature of ovariectomized rats may serve as a useful tool for selection of compounds that are of potential use in the treatment of hot flushes. Moreover, blockade of the (hypersensitive) 5-HT_{2A} receptors prevents or reduces the disturbance of rat tail-temperature in the rat which may validate this experimental model to evaluate the effect of compounds on hot flushes.

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