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Oxytocin, water intake, and food sodium availability in male rats

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Abstract

This study examined the effect of subcutaneous administration of the neurohormone oxytocin on water intake of *ad lib*-fed (with or without sodium availability in the diet) and food-deprived animals. Results of the first experiment showed that oxytocin increased water intake and urine excretion in food-deprived but not in *ad lib*-fed animals. However, oxytocin treatment did not modify the reduced water "balance" (fluid intake minus urine volume) resulting from food deprivation or the daily food intake (Experiment 1). The dose-dependent polydipsic effect of oxytocin on food-deprived rats was always preceded by an increase in sodium and fluid urine excretion (Experiment 2). Oxytocin also increased the water intake of animals fed *ad lib* with a low sodium diet (Experiment 3). These results suggest that the effect of oxytocin on water intake is dependent on the presence or absence of sodium in the diet and that the excretion of sodium is the main mechanism of oxytocinergic polydipsia in food-deprived male rats.

Keywords: Oxytocin; Water intake; Urine excretion; Natriuresis; Food deprivation; Sodium-deficient diet; Subcutaneous administration; Male rat

Introduction

The neurohormone oxytocin (OT) is secreted in the neurohypophysis from nerve terminals of the supraoptic nucleus and magnocellular component of the paraventricular nucleus (Armstrong, 1995; Binkley, 1995; Swaab et al., 1975; Swanson and Kuypers, 1980).

Recent studies have proposed the involvement of OT in regulatory processes (Kiss and Mikkelsen, 2005). Thus, it has been demonstrated that neurosecretory anatomic structures such as the supraoptic and paraventricular hypothalamic nuclei are activated in circumstances of osmotic and volemic thirst, stimulating secretion of OT and vasopressin into the bloodstream (Brimble and Dyball, 1977; Cheng and North, 1986; Dyball, 1968; Hattori et al., 1988; Kadekaro et al., 1995; Kiss and Mikkelsen, 2005; McKinley et al., 1994; Miyata et al., 2001; Robertson, 1987; Shibuki et al., 1988; Weitzman et al., 1978). However, the effects of OT on drinking behavior have been controversial, and the systemic administration of OT has been reported to increase, decrease, or even have no effect on fluid intake (Arletti et al., 1989, 1990; Stricker and Verbalis, 1987; Uvnäs-Moberg et al., 1996).

In this context, it has been demonstrated that the administration of OT in rats may have natriuretic effects (Conrad et al., 1986; Haanwinckel et al., 1995; Huang et al., 1994, 1995, 1996; Kadekaro et al., 1992, 1997; Verbalis et al., 1991; Windle et al., 1995, 1997), although the natriuretic potency of OT might depend on the availability of sodium in the diet (Balment et al., 1980; Verbalis et al., 1991). Furthermore, it is well established that water intake is fundamentally a sodium-related behavior in both osmotic (with high sodium levels) and volemic (induced by the loss of fluid and mineral salts) thirst (Fitzsimons, 1961, 1963, 1979, 1998; Johnson and Thunhorst, 1997; Phillips and Sumners, 1998; Share and Schneider, 2000; Stricker, 1966, 1981; Stricker and Sved, 2000; Verbalis and Stricker, 2000) and is therefore affected by both the intake and excretion of mineral salts.

The present experiments were designed to examine the effect of OT on water intake and to determine the potential relevance of sodium availability and sodium excretion. The effect of sodium was investigated by using standard *ad lib*-fed and food-deprived groups (Experiment 1) and animals fed *ad lib* with a

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sodium-deficient diet (Experiment 3) and by measuring sodium excretion (Experiment 2).

Materials and methods

Experiment 1

Subjects and groups

Thirty-two adult male Wistar rats from the breeding colony at the University of Granada (350–380 g) were used in this study. Animals were housed in individual cages in a room maintained at 21–23 °C on a 12/12-h light–dark cycle (lights on at 0800 h and off at 2000 h). Use and handling of the animals followed guidelines established by European Community Council Directive 86/609/EEC and Spanish Royal Law 223/1988.

After several days of habituation to the laboratory, the animals were randomly divided into four groups of 8 animals as follows: OT with food (OT/*ad lib*), Control with food (C/*ad lib*), OT without food (OT/deprived), and Control without food (C/deprived). They were then housed for a 24-h baseline period in metabolic cages (Tecniplast 3701MO-000) with *ad lib* access to water and food (Sandermus dry chow, Unión Alimentaria Sanders, Sanders S.A, Madrid).

Experimental procedure

This experiment lasted 72 h. At 24 h, measurements were recorded on the water and food intake and urine excretion volumes of each animal (baseline). During the following 48 h, food was withdrawn from the OT/deprived and C/deprived groups. The OT/*ad lib* and C/*ad lib* groups had *ad lib* access to food and water throughout the experiment.

On day 2, OT/*ad lib* and OT/deprived groups received 1 ml of OT (Oxytocin, 10 IU/ml distilled water, Laboratorios Iven, Madrid) in two 0.5 ml injections, the first at 0830 h and the second at 1430 h. OT was subcutaneously (s.c.) administered at the midline of the lumbar region of the animal (Uvnäs-Moberg et al., 1996). The selection of the dose and two-injection procedure was based on previous studies (Stricker and Verbalis, 1987) and took account of the short half-life of OT (Binkley, 1995). Following the same procedure and in order to maintain the osmotic state of the animals, C/*ad lib* and C/deprived groups in this initial study were injected with 1 ml of physiological saline.

On days 2 and 3 of the experiment, the water intake and urine excretion of all animals and the food intake of the OT/*ad lib* and Control/*ad lib*-fed animals were measured daily at 0830 h.

Statistical analysis

The body weight of the 2×2 groups before each s.c. administration was analyzed by means of an analysis of variance (ANOVA).

Water intake and urine excretion volumes were analyzed by using a multivariate analysis of variance (MANOVA) for 2×2 groups during the 3 days. An ANOVA was also performed on fluid intake minus urine volume (water "balance").

The amount of food ingested during the 3 days by *ad lib*-fed animals was analyzed by using an ANOVA test. Significant effects were analyzed by means of planned comparisons.

Experiment 2

Subjects and groups

Forty-eight adult male Wistar rats from the breeding colony at the University of Granada (290–330 g) were used in this study. Animals were housed in individual cages in a room under the same conditions as in Experiment 1.

After several days of habituation to the laboratory, the animals were randomly divided into six groups of 8 animals as follows: Control with food (C/*ad lib*), Control without food (C/deprived), and four food-deprived groups treated with 2.5, 5.0, 7.5, or 10.0 IU of OT, respectively. They were then housed during a 24-h baseline period in metabolic cages (Tecniplast 3701MO-000) with *ad lib* access to water and standard food (Sandermus dry chow, Unión Alimentaria Sanders, Sanders S.A, Madrid).

Experimental procedure

At 24 h, water and food intake and the volume of urine excretion of each animal during baseline were measured. During the following 24 h, food was withdrawn from the C/deprived and OT/deprived groups. The C/*ad lib* group had *ad lib* access to food and water throughout the experiment.

On day 2, the four OT groups received their dose of OT (2.5, 5.0, 7.5, or 10.0 IU) in two 0.5 ml injections, as described in the above experiment. Following the same procedure, C/*ad lib* and C/deprived groups were injected with distilled water.

Water intake, urine excretion, and urinary sodium concentration of all animals were measured at 6 and 24 h after the first injection. Urinary sodium concentration (mEq/l) was measured by using an automatic analyzer (Beckman Instruments, Synchron CX7 Delta, USA).

Experiment 3

Subjects and groups

Twenty-four adult male Wistar rats from the breeding colony at the University of Granada (290–330 g) were used in this study. Animals were housed in individual cages in a room under the same conditions as in the above experiments.

After several days of habituation to the laboratory, the animals were randomly divided into three groups of 8 animals as follows: OT 10 IU, OT 5 IU, and Control. They were then housed for a 24-h baseline period in individual cages with *ad lib* access to water and standard food (Sandermus dry chow, Unión Alimentaria Sanders, Sanders S.A, Madrid).

Experimental procedure

After the 24-h baseline period with standard food, food was withdrawn from the cages and replaced with a sodium-deficient diet (0.04% Na^+) (Dieta Mucedola, Debiomed S.L., Barcelona). At 0830 h on day 3, after 48 h of adaptation to the low sodium diet (Verbalis et al., 1991), OT (10 and 5 IU) or distilled water was administered as described in the above experiment.

Water and food intake of groups were measured daily at 0830 h.

Results

Experiment 1

Body weights of groups before s.c. administration of substances

Results obtained show no differences in body weight among groups before the first or second s.c. administration of substances (370±2.8 g for C/*ad lib*, 365±3.9 g for OT/*ad lib*, 368±3.9 g for C/deprived, and 375±2.2 g for OT/deprived at 0830 h; and 362±3.5 g for C/*ad lib*, 354±3.8 g for OT/*ad lib*, 357±4.5 g for C/deprived and 362±3.8 g for OT/deprived at 1430 h).

Water intake and urine excretion

Results obtained showed that water intake and urine volumes were significantly dependent on food deprivation, OT administration, and the day (1, 2, or 3) on which measurements were taken (Rao's R (4,25)=6.26; $p<0.01$).

No significant differences were found between OT/deprived and C/deprived groups on day 1 (baseline). On day 2, water intake (Fig. 1) and urine excretion (Fig. 2) volumes were larger in the OT/deprived group than in the C/deprived group ($F(1,28)=7.59$, $p<0.02$ for water intake and $F(1,28)=23.79$, $p<0.01$ for urine volume). On day 3, urine volume was larger in OT/deprived than in C/deprived animals ($F(1,28)=7.51$, $p<0.02$) (Figs. 1 and 2).