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Research report

Selective increase of dark phase water intake in neuropeptide-Y Y2 and Y4 receptor knockout mice

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Abstract

Neuropeptide-Y (NPY) is involved in the regulation of ingestive behaviour and energy homeostasis. Since deletion of the NPY Y2 and Y4 receptor gene increases and decreases food intake, respectively, we examined whether water intake during the light and dark phases is altered in Y2 and Y4 receptor knockout mice. The water consumption of mice staying in their home cages was measured by weighing the water bottles at the beginning and end of the light phase during 4 consecutive days. Control, Y2 and Y4 receptor knockout mice did not differ in their water intake during the light phase. However, during the dark phase Y2 and Y4 receptor knockout mice drank significantly more (46–63%, $P < 0.05$) water than the control mice. The total daily water intake over 24 h was also enhanced. The enhanced water intake during the dark phase was not altered by the β-adrenoceptor antagonist propranolol or the angiotensin AT1 receptor antagonist telmisartan (each injected intraperitoneally at 10 mg/kg). These data indicate that NPY acting via Y2 and Y4 receptors plays a distinctive role in the regulation of nocturnal water consumption. While β-adrenoceptors and angiotensin AT1 receptors do not seem to be involved, water intake in Y2 and Y4 receptor knockout mice may be enhanced because presynaptic autoinhibition of NPY release and inhibition of orexin neurons in the central nervous system are prevented.

Keywords: Neuropeptide-Y; Y2 receptor gene knockout; Y4 receptor gene knockout; Propranolol; Telmisartan; Nocturnal water consumption; Water homeostasis; Y2 autoreceptors

1. Introduction

Neuropeptide-Y (NPY) is involved in the regulation of food intake and energy homeostasis. For instance, deletion of the NPY Y2 and Y4 receptor gene increases and decreases food intake, respectively [14,25,30,31,33]. In contrast, the drinking of water provided ad libitum has not yet been systematically investigated in NPY receptor knockout mice. Thiele et al. [33] have reported that in an ethanol/water preference test Y2 receptor knockout (Y2−/−) mice drink more water, but less ethanol, than wild-type controls. When one of us (S.D.) noted that a colony of Y4 receptor knockout (Y4−/−) mice drank more water than the respective controls, we decided to carry out an explorative study of the spontaneous drinking behaviour in both Y2−/− and Y4−/− mice. Three particular aims were pursued. Firstly, we recorded the water intake during the light and dark phases and estimated the total daily intake of water in Y2−/− and Y4−/− mice relative to their controls. Secondly, we investigated whether the increase in dark phase water intake observed in Y2−/− and Y4−/− mice was inhibited by the β-adrenoceptor antagonist propranolol. This possibility was envisaged because β-adrenoceptor agonists stimulate water intake [20,23] and NPY is associated with noradrenergic neurons of the central and sympathetic nervous system [16,22]. Thirdly, we examined whether the enhanced water intake in Y2−/− and Y4−/−mice is normalized by the angiotensin AT1 receptor antagonist telmisartan, given that angiotensin II is a central messenger that stimulates drinking [3,12].

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2. Materials and methods

2.1. Experimental animals

This study was approved by an ethical committee at the Federal Ministry of Education, Science and Culture of the Republic of Austria, and conducted according to the Directive of the European Communities Council of 24 November 1986 (86/609/EEC). The experiments were carried out with female germline Y2−/− and Y4−/− mice and non-induced conditional Y2 and Y4 receptor knockout (FY2 and FY4) mice (Department of Pharmacology, Medical University of Innsbruck, Austria) weighing 16–24 g. The generation of Y2−/−, Y4−/−, FY2 and FY4 mice and the demonstration of the absence or presence of Y2 and Y4 receptors have been described previously [30,31]. Germline Y2−/− mice were generated from the same founders on the same mixed C57BL/6–129SvJ background as the conditional FY2 and FY4 knockout mice. Non-induced conditional FY2 and FY4 knockout mice were used as controls in all experiments and termed control mice throughout the paper.

2.2. Experimental protocols

The mice were housed in groups of three to four per cage under controlled temperature (21 °C) and a 12 h light/dark cycle (lights on at 6:00 a.m. and off at 6:00 p.m.). Tap water and standard laboratory food were provided ad libitum throughout the study. Since only a limited number of female control, Y2−/− and Y4−/− mice were available for the study, four experiments were carried out with most mice at 1 week intervals, if not stated otherwise. In experiment 1, the water intake during the light phase of 4 consecutive days and the three intervening periods of dark phase was estimated. To this end, the weight of the water bottles of each cage was determined at 7:45 a.m. and 4:30 p.m. The water bottles were cleaned and refilled every second day. Experiment 2 was carried out to test whether intraperitoneal (IP) injection of vehicle (physiological saline) would alter drinking during the dark phase. To this end the water intake during the dark phase of 3 consecutive days was estimated. On the second day, physiological saline (0.15 M NaCl, 2 ml/kg) was injected IP at 5:30 p.m. and 11:30 p.m. Experiments 3 and 4 were identical with the second experiment, except that instead of vehicle two doses of propranolol (each dose at 10 mg/kg; experiment 3) and telmisartan (each dose at 10 mg/kg; experiment 4) were injected IP at 5:30 p.m. and 11:30 p.m. An interval of 2 weeks was allowed between the third and fourth experiment to ensure complete washout of propranolol. The experiment involving telmisartan was repeated with naïve Y2−/−, Y4−/− and control mice in order to prove that previous treatment with vehicle and propranolol did not modify the effect of telmisartan (experiment 5). These additional mice were injected either with telmisartan (10 mg/kg IP at 5:30 p.m. and 11:30 p.m.) or its vehicle.

The experimental design entailed that the water intake per cage (i.e., per three to four mice) was determined. Since, in addition, the weight of the mice was recorded at the beginning of each experiment, the water intake during the light and dark phases was expressed relative to body weight. The total intake of water per day was calculated by summing up the water intake of one light phase and the following dark phase.

2.3. Drugs

(±)-Propranolol hydrochloride (Sigma, Vienna, Austria) was dissolved in saline at a concentration of 5 mg/ml and injected IP at a volume of 2 ml/kg. Telmisartan (gift of Boehringer Ingelheim, Biberach, Germany) was dissolved in 0.15 M NaOH, the pH of this solution being adjusted with 0.15 M HCl to 9.5 [12]. The injection solution contained 5 mg/ml telmisartan and was administered IP at a volume of 2 ml/kg. The vehicle solution was prepared in an analogous manner.

2.4. Statistics

Statistical evaluation of the results was performed on Statistica (StatSoft Inc., Tulsa, OK, USA) with two-way analysis of variance (ANOVA) for repeated measures (to identify differences between genotypes over time) or three-way ANOVA for repeated measures (to identify differences between genotypes and treatments over time). Since no significant effect of time and treatment and no significant interaction between time, genotype and treatment were found, the data were reevaluated with one-way ANOVA and significant differences between the genotypes identified with the Holm–Sidak method (SigmaStat, SPSS Inc., Chicago, IL, USA). All data are presented as means ± S.E., n referring to the number of cages in the respective group. Probability values of $P < 0.05$ were regarded as significant.

3. Results

The water intake of control (FY2 and FY4) mice during the light phase was in the range of 0.057–0.077 ml/g (Fig. 1A) while that during the dark phase was in the range of 0.112–0.119 ml/g (Fig. 1B) and significantly higher ($P < 0.05$) than during the light phase. Y2−/− and Y4−/− mice did not significantly differ from control mice in their water intake during the light phase (Fig. 1A). During the dark phase, however, Y2−/− mice drank significantly more water than the control mice as recorded over 3 consecutive days (Fig. 1B). The total daily (24 h) intake of water was also significantly increased in Y2−/− mice during this observation period (Fig. 2). Y4−/− mice likewise drank more water during the dark phase than control mice, but this difference was statistically significant only on the second and third

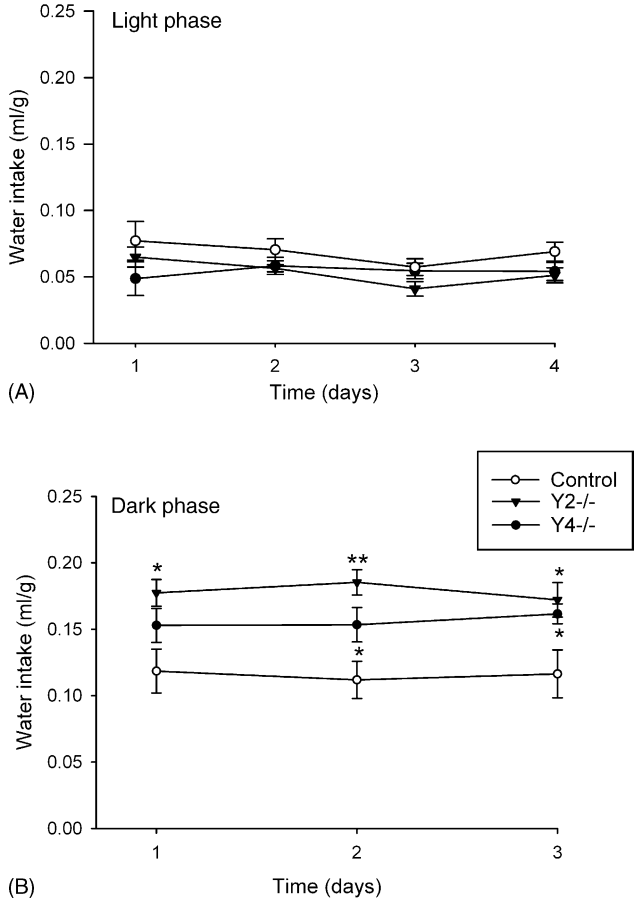

Fig. 1. Water intake in control, Y2−/− and Y4−/− mice during the light phase (A) of 4 consecutive days and the three intervening periods of dark phase (B). The water intake is expressed as ml/g body weight. Means ± S.E., $n = 6$. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. control.