ELSEVIER

Brain Research Bulletin 74 (2007) 14–20

BRAIN RESEARCH BULLETIN

www.elsevier.com/locate/brainresbull

Research report

DOCA stimulates salt appetite in Zucker rats: Effect of dose, synergistic action with central angiotensin II, and obesity

S.T. Omouessi [a,b], C. Falconetti [a,b], B. Fernette [a], S.N. Thornton [a,b,*]

[a] *EA 3453 SNCI, Université Henri Poincaré, Nancy, France*

[b] *U684 UHP-INSERM, Rigidité, Fibrose et Risque Cardiovasculaire, Faculté de Médecine, Université Henri Poincaré, Nancy, France*

Received 25 March 2007; accepted 3 April 2007

Available online 26 April 2007

Abstract

An enhanced sodium appetite is found in rats by the synergist interaction of peripheral mineralocorticoids, deoxycorticosterone acetate (DOCA), and central angiotensin II (AngII), the synergy theory. We used obese Zucker rats which have a predisposition to develop hypertension under appropriate salt conditions to examine this synergy response between AngII and different low doses of DOCA on 2% NaCl intake.

Obese and lean Zucker rats on low sodium food were treated systemically with 0.5, 1 and 2 mg/kg/day of DOCA for 3 days, before receiving i.c.v. AngII (10 pmol) on the fourth day. Food, fluid intakes and urine outputs were measured daily throughout. Plasma aldosterone levels were also analysed. Results showed that AngII alone increased water but not salt intake, whereas all three doses of DOCA by themselves enhanced daily salt intake during the treatment period. The lowest dose of DOCA plus AngII did not stimulate an enhanced sodium consumption. The 1 mg/kg was the threshold dose of DOCA for a synergistic response, and with 2 mg/kg DOCA the obese rats consumed nearly 2-fold more hypertonic NaCl solution than the leans. Moreover, obese baseline plasma levels of aldosterone were more elevated than the lean rats.

In conclusion, in adult Zucker rats a threshold level of mineralocorticoid is required for the salt stimulating action of central AngII. In the obese rat the synergistic effect is enhanced with higher doses of mineralocorticoid, suggesting that the plasma level of aldosterone could be a prominent factor, which may predispose the obese to salt-sensitivity and, possibly, subsequently to hypertension under appropriate conditions.

Keywords: Synergy; Sodium intake; Obesity

1. Introduction

Obesity is a significant risk factor for diseases such as hypertension, type II diabetes, coronary heart disease and a number of other conditions that adversely affect health [44]. Considerable evidence highlights the importance of obesity in the development of essential hypertension. In human and rodent studies, several components of the rennin–angiotensin–aldosterone system (RAAS) like angiotensinogen (AGT) [11,19,32], renin [54], angiotensin-converting enzyme (ACE) [11,43,55], and angiotensin II receptors [10,13,14] have been shown to be expressed in adipose tissue, suggesting the presence of a local RAAS in this tissue. Furthermore, circulating levels of these components are elevated in obese subjects [8,18]. An increased activity of adipose and systemic RAAS (AGT, renin, aldosterone, ACE) are associated with obesity-related hypertension [8,18].

To fully elucidate the mechanism(s) through which obesity can predispose an individual to the development of hypertension, suitable animal models are required and the obese Zucker rat is one that has been proposed [38].

The obese Zucker rat (fa/fa) has been reported to be a "pre- hypertensive" strain that has a predisposition to develop hypertension under appropriate salt conditions [40,41]; or to be hypertensive compared to lean (Fa/Fa or Fa/fa) control rats [1,34,68]. Furthermore obese Zucker rats were shown to be salt-sensitive in that they develop hypertension when placed on a high-salt diet [60]. This strain of obese rat seems to share many similarities with obese humans [70], and thus may provide additional insight into the etiology of obesity hypertension [1] which has become a serious health problem today.

* Corresponding author at: U684 UHP-INSERM, Rigidité, Fibrose et Risque Cardiovasculaire, Faculté de Médecine, B.P. 184, 54505 VANDŒUVRE Les Nancy Cedex, France. Tel.: +33 3 83 68 36 23; fax: +33 3 83 68 36 39.

E-mail address: Simon.Thornton@nancy.inserm.fr (S.N. Thornton).

0361-9230/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.brainresbull.2007.04.001

The obese Zucker rat has a significantly greater increase in blood pressure after intracerebroventricular (i.c.v.) injections of angiotensin II (AngII) compared to the lean rat [6]; and this strain exhibits a significant rise in systolic blood pressure after the start of mineralocorticoid (deoxycorticosterone acetate, a synthetic aldosterone analogue)-salt treatment [38]. This could appear as evidence that both AngII and mineralocorticoid might play a role in the maintenance of this hypertension. However, no investigation into the synergistic role of both hormones in the predisposition of the obese Zucker rat to salt-sensitivity and subsequently to hypertension, has been published.

AngII and mineralocorticoids are implicated also in the genesis of salt appetite. Acute i.c.v. injection of AngII after systemic pretreatment with mineralocorticoids, both at below threshold doses for salt appetite, induces a robust salt appetite in some rat strains [21,23,49,50]. At low doses, mineralocorticoids have a tendency to suppress the high salt intake in rats after adrenalectomy [24,37] whereas, at higher doses, they incite a salt appetite in adrenalectomized as well as non-adrenalectomized [24,31,67] animals.

In the present study our contribution was first, to examine whether such synergistic interaction between both AngII and mineralocorticoid might be one of several mechanism(s) by which the central nervous system can induce sodium appetite in the obese Zucker rat. Secondly, in order to refine our approach we have assessed what pharmacological dose of mineralocorticoids is necessary for activating salt intake after i.c.v. injection of AngII.

2. Materials and methods

2.1. Animals and maintenance

The experiments were carried out on 42 adult male Zucker rats, approximately 28–32 weeks old at the start of the handling, consisting of lean (FA/?, $n = 19$) and obese (fa/fa, $n = 23$) rats weighing 345 ± 5 and 518 ± 13 g, respectively. They were bred in the lab and raised on standard lab chow (croquette A04, UAR, France). For the experiments, rats were housed individually in metabolic cages, with low sodium food (powdered A04, UAR, France, 0.02% Na^+) available in hoppers, and water and 2% NaCl both available in graduated burettes. Lab temperature was $22 \pm 2\,^{\circ}C$, and lighting was on from 7 to 19 h. Rats were accustomed to these conditions for 2 weeks before the beginning of experiments.

2.2. Surgical procedure

Rats were implanted stereotaxically under aseptic conditions with a guide cannula aimed at the third cerebral ventricle under ketamine anaesthesia (Imalgène, Rhône Mérieux, Lyon, France, 150 mg/kg, i.p.). The skull was levelled between bregma and lambda and the stainless steel guide cannula fixed to retaining stainless steel screws in the skull with dental acrylic.

The coordinates of the tip of the guide cannula were 0.8 mm posterior to Bregma, in the midline and 4.0 mm below the surface of the skull, according to the atlas of Paxinos and Watson [42]. The guide ended 1.0 mm above the third cerebral ventricle and the stainless steel 33-gauge injector was 2.0 mm longer than the guide thus ensuring that the injector reached the centre of the third cerebral ventricle. Rats were allowed to recover in a warmed cage 1–2 h after surgery.

Thereafter, rats were put back in their individual metabolic cage for at least a week postoperative period and handled daily to familiarize them with the injection procedure.

2.3. Experimental protocol

2.3.1. Experiment 1A: AngII infusion serve to testing guide cannula site

One week after the animals had recovered their preoperative weight, daily salt, water and food intakes were measured in all lean and obese Zucker rats. Five days of food, water and 2% NaCl intakes were taken as baseline measurements, before beginning central injections. Daily weight and urine output were also measured, and urine was collected to determine electrolyte (Na^+ and K^+) excretion throughout the experiment. Urine sodium and potassium were determined using an ion selective electrode system (AVL 9180 Electrolyte Analyzer).

Angiotensin II (10 ng ≈ 10 pmol) was dissolved in artificial cerebrospinal fluid (aCSF) and injected centrally in a volume of 300 nl. On the test day, intracerebroventricular injections of AngII were given in the morning after the 24 h measurements of food and fluid intake. The food cups and the graduated burettes with the water and salt solution were left in place on the cages, the animal taken out of the cage, the obturator removed and the injector inserted. The injector was connected by a length of polypropylene tubing to a 5 μl Hamilton syringe in an infusion pump (Harvard Apparatus, Syringe infusion pump 22). The 300 nl of solution were administered over 3 min, the injector removed, the obturator replaced and the animal returned to its home cage. Volumes consumed of both water and 2% NaCl were recorded 15, 30, 60 and 120 min later.

A criterion response of at least 5 ml of water intake in 15 min was required for participation in subsequent experiments. Three animals did not respond to this initial testing dose of AngII and were thus excluded from the experiment. The responding animals were selected and kept for the following experiments.

2.3.2. Experiment 1B: aCSF infusion serve as control for AngII

Animals received central injections of aCSF alone under the same conditions as in 1A. The 2 and 24 h-intakes of water and 2% NaCl after aCSF were compared to the corresponding AngII-induced intakes.

2.3.3. Experiment 2A: deoxycorticosterone acetate (DOCA)–salt intake

In this part, synergy between AngII and Aldosterone in evoking sodium appetite in obese Zucker rats was assessed. Thus, 17 lean and 22 obese Zucker rats which had responded positively to AngII, were divided into three groups which each received a different dose of DOCA.

Group Z0.5, consisted of 6 lean and 6 obese rats, which received subcutaneously 0.5 mg/kg/day of DOCA (Syncortyl, 10 mg/ml) for 3 days before i.c.v. AngII infusions on the fourth day. The groups Z1 and Z2 composed, respectively, by 5 lean and 10 obese, and 6 lean and 6 obese; received, respectively, 1 and 2 mg/kg/day of DOCA.

The DOCA effects on fluid intake induced by AngII were assessed by measuring water and salt intake during the 2 h after i.c.v. AngII infusions. And long-term effects were assessed by 24 h measurements of water and sodium intakes.

2.3.4. Experiment 2B

In this experiment, the groups Z0.5, Z1 and Z2 were treated for 3 days with equivalents volumes of sterile saline (0.9% NaCl) before receiving i.c.v. AngII infusions the day after (Fig. 1).

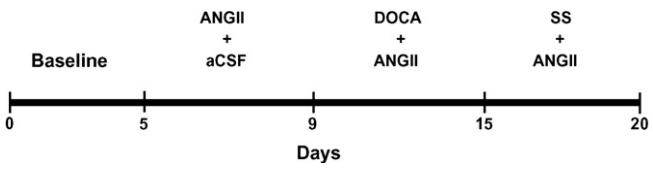

Fig. 1. Diagram of experiments in Zucker rats with successive periods for infusion of AngII or aCSF alone, and infusion of AngII combined with peripheral pre-treatment of DOCA or SS (0.9% NaCl). AngII and aCSF were infused into the third cerebral ventricle (intracerebroventricular) whereas DOCA and SS were administered subcutaneously. AngII, angiotensin II; aCSF, artificial cerebrospinal fluid; DOCA, deoxycorticosterone acetate; SS, sterile saline.