Journal of Chemical Neuroanatomy 74 (2016) 47–54

Contents lists available at ScienceDirect

Journal of Chemical Neuroanatomy

journal homepage: www.elsevier.com/locate/jchemneu

Sodium and water intake are not affected by $GABA_C$ receptor activation in the lateral parabrachial nucleus of sodium-depleted rats

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ARTICLE INFO

Article history:
Received 17 September 2015
Received in revised form 13 February 2016
Accepted 5 March 2016
Available online 9 March 2016

Keywords:
Sodium intake
Water intake
$GABA_C$ receptor
Lateral parabrachial nucleus

ABSTRACT

The activation of GABAergic receptors, $GABA_A$ and $GABA_B$, in the lateral parabrachial nucleus (LPBN) increases water and sodium intake in satiated and fluid-depleted rats. The present study investigated the presence of the $GABA_C$ receptor in the LPBN, its involvement in water and sodium intake, and its effects on cardiovascular parameters during the acute fluid depletion induced by furosemide combined with captopril (Furo/Cap). One group of male Wistar rats (290–300 g) with bilateral stainless steel LPBN cannulas was used to test the effects of a $GABA_C$ receptor agonist and antagonist on the fluid intake and cardiovascular parameters. We investigated the effects of bilateral LPBN injections of trans-4-aminocrotonic acid (TACA) on the intake of water and 0.3 M NaCl induced by acute fluid depletion (subcutaneous injection of Furo/Cap). c-Fos expression increased ($P<0.05$), suggesting LPBN neuronal activation. The injection of different doses of TACA (0.5, 2.0 and 160 nmol) in the LPBN did not change the sodium or water intake in Furo/Cap-treated rats ($P>0.05$). Treatment with the $GABA_C$ receptor antagonist (*Z*)-3-[(aminoiminomethyl)thio]prop-2-enoic acid sulfate (ZAPA, 10 nmol) or with ZAPA (10 nmol) plus TACA (160 nmol) did not change the sodium or water intake compared with that for vehicle (saline) ($P>0.05$). Bilateral injections of the $GABA_C$ agonist in the LPBN of Furo/Cap-treated rats did not affect the mean arterial pressure (MAP) or heart rate (HR). The $GABA_C$ receptor expression in the LPBN was confirmed by the presence of a 50 kDa band. Although LPBN neurons might express $GABA_C$ receptors, their activation produced no change in water and sodium intake or in the cardiovascular parameters in the acute fluid depletion rats. Therefore, the $GABA_C$ receptors in the LPBN might not interfere with fluid and blood pressure regulation.

1. Introduction

Important inhibitory mechanisms that control water and sodium intake have been reported in the lateral parabrachial nucleus (LPBN) (Menani et al., 1996), a pontine structure that lies dorsolateral to the superior cerebellar peduncle. The LPBN is strategically connected to forebrain structures, such as the paraventricular nucleus of the hypothalamus and the amygdala (Feigenspan and Bormann, 1994b). In addition, the LPBN receives projections from the area postrema (AP) and the medial nucleus tractus solitary (mNTS) (Ciriello et al., 1984; Herbert et al., 1990; Jhamandas et al., 1996). These areas are involved in electrolyte balance and cardiovascular responses.

Gamma aminobutyric acid (GABA) is an inhibitory neurotransmitter that is widely distributed in the central nervous system (Bowery et al., 1987). A dense group of GABA-immunoreactive varicosities was reported in the parabrachial (PB) complex/Kolliker fuse nucleus, suggesting a strong GABAergic influence on the neuronal processes in this area, particularly the gustatory and visceral portion of the PB complex (Kobashi and Bradley, 1998).

Three main classes of GABA receptors exist and are termed $GABA_A$, $GABA_B$, and $GABA_C$ receptors (Bormann, 2000). Ionotropic $GABA_A$ receptors (bicuculline-sensitive) are ligand-gated Cl^- channels that form a heteropentameric structure. Metabotropic $GABA_B$ receptors couple to Ca^{2+} and K^+ channels via G proteins and are selectively activated by baclofen; these receptors do not respond to known $GABA_A$ receptor modulators, such as barbiturates and benzodiazepines (Bormann, 2000; Bowery, 1989). A third

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http://dx.doi.org/10.1016/j.jchemneu.2016.03.001
0891-0618/

type of GABA receptor, termed $GABA_C$, has been proposed, and it is distinct from the other known GABA receptors. Ionotropic $GABA_C$ receptors gate Cl^- currents in various parts of the vertebrate brain and are thought to be homo- or hetero-pentamers composed of ρ1, ρ2 and ρ3 subunits (Zhang et al., 2001). $GABA_C$ receptors are insensitive to the selective $GABA_A$ receptor antagonist bicuculline and some $GABA_A$ receptor modulators, and they are not activated by the $GABA_B$ agonist baclofen (Bormann, 2000). The function of $GABA_C$ receptors has mostly been studied in the retina (Feigenspan and Bormann, 1994a). Outside the retina, functional putative $GABA_C$ receptors have been detected in the superior colliculus, amygdala and brainstem (Boller and Schmidt, 2003; Delaney and Sah, 1999; Grabauskas and Bradley, 2001; Milligan et al., 2004). Ionotropic $GABA_C$ receptors are activated by cis-aminocrotonic acid and trans-4-aminocrotonic acid (TACA) and are selectively blocked by (1,2,5,6-tetrahydropyridin-4-yl) methylphosphinic acid and (*Z*)-3-[(aminoiminomethyl)thio]prop-2-enoic acid sulfate (ZAPA) (Woodward et al., 1992, 1993).

Interestingly, the blockade of LPBN neurons with bilateral injections of the selective $GABA_A$ receptor agonist muscimol increases arterial pressure and induces a high intake of a hypertonic sodium solution and a slight intake of water in rats depleted of fluid by furosemide + captopril (Furo/Cap) (Callera et al., 2005; de Oliveira et al., 2007). In addition, a recent study showed that activation of $GABA_B$ receptors through the administration of baclofen in the LPBN also causes water and sodium intake in fluid replete rats (De Oliveira et al., 2011), suggesting that an LPBN GABAergic mechanism is involved in controlling sodium intake.

Although previous studies showed that the activation of $GABA_A$ and $GABA_B$ receptors in the LPBN causes the intake of water and 0.3 M NaCl solution in fluid-depleted rats and increases 0.3 M NaCl intake in Furo/Cap-treated rats, the possible effects of $GABA_C$ receptor activation in the LPBN on sodium depletion-induced NaCl intake had not been tested. Therefore, in the present study, we investigate the effects of a $GABA_C$ agonist on the sodium and water intake and cardiovascular alterations of rats that were depleted of sodium by Furo/Cap.

2. Materials and methods

2.1. Animals

Male Wistar rats weighing 290–310 g were used. The animals were housed in individual stainless steel cages with free access to food containing a standard level of sodium (Guabi Rat Chow, Paulinia, SP, Brazil), water and 0.3 M NaCl solution. The positions of the bottles containing the water and the 0.3 M NaCl were rotated daily to avoid place preference. The room temperature was maintained at 23 ± 2 °C, and the humidity was maintained at 55 ± 10% with a 12:12 light–dark cycle with light onset at 07:30 AM. The experiments were approved by the local Institutional Animal Research Ethics Committee (process number 1332/2008). All efforts were made to minimize animal discomfort and the number of animals used, and the experiments complied with the recommendations of the Brazilian College of Animal Experimentation (COBEA) and the American National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publications No. 80-23, 1996, USA).

2.2. Drugs

Furosemide (Furo), captopril (Cap) and muscimol HBr were purchased from Sigma (Saint Louis, MO, USA); TACA and ZAPA were purchased from Tocris (Ellisville, MO, USA). Furosemide was dissolved in alkaline saline (pH adjusted to 9.0), and all other drugs were dissolved in 0.15 M NaCl, which served as the vehicle. Up to our knowledge, this is the first study to use in vivo injection of $GABA_C$ agonist and antagonist in the LPBN, thus, there is no consensus or reports on literature about the dose of these drugs for this use. However, we chose $GABA_C$ receptor agonist and antagonist doses based on previous publications about $GABA_A$ receptors agonists and antagonists (Callera et al., 2005; de Melo e Silva et al., 2013; Kimura et al., 2008) because $GABA_C$ receptors is an ionotropic receptor such as $GABA_A$ receptor. Initially, we used TACA dose similar to muscimol dose (0.5 nmol) (Callera et al., 2005; de Melo e Silva et al., 2013; Kimura et al., 2008). Previous studies that evaluated the agonist profiles of electrophysiological experiments the Xenopus oocytes observed that potent agonists of these receptors were muscimol and TACA, which were approximately equipotent (Hosie and Sattelle, 1996). Muscimol and TACA were the most potent agonists of RDLac homo-oligomers and were full agonists, equipotent with GABA. Moreover, the ZAPA antagonist (Feigenspan et al., 1993) is approximately equipotent with muscimol (agonist) in certain unidentified locust and cockroach neurons (Taylor et al., 1993).

2.3. Brain surgery

Rats were anesthetized with an intraperitoneal (i.p.) injection of ketamine (80 mg/kg of body weight [b.w.]) combined with xylazine (7 mg/kg b.w.) and placed in a stereotaxic instrument (Kopf, USA). The skull was leveled between bregma and lambda. Stainless steel guide cannulas (12 × 0.6 mm o.d.) were implanted bilaterally with their tips ending 2 mm above the LPBN using the following coordinates: 9.4 mm caudal to bregma, 2.2 mm lateral to the midline, and 3.8 mm below the dura mater. The cannulas were fixed to the cranium using dental acrylic resin and jeweler screws and were filled with 30-gauge metal obturators between tests. After the surgery, the rats received intramuscular injections of the analgesic cetoprophen (1%, 0.03 mL) and a prophylactic dose of the antibiotic penicillin (30,000 IU). The rats were allowed to recover for 5 days before the ingestion tests began, and during this period, they had free access to water, 0.3 M NaCl solution, and food containing 2.7 mg/kg of sodium.

2.4. Injections in the LPBN

Bilateral injections in the LPBN were made using 10 μL Hamilton syringes connected via polyethylene tubing (PE 10) to 30-gauge injection cannulas. At the time of testing, the obturators were removed, and the injection cannula (2 mm longer than the guide cannula) was carefully inserted into the guide cannula. For bilateral injections, the first injection was performed on one side, the needle was removed and repositioned at the contralateral side, and then the second injection was administered. Therefore, injections were performed ∼1 min apart. A volume of 0.2 μL was injected in the LPBN at each site. The obturators were reinstalled after the injections, and the rats were returned to their cages.

2.5. Experimental procedures

2.5.1. Water and 0.3 M NaCl intake of Furo/Cap-treated rats

The rats were tested in their home cages. Water and 0.3 M NaCl were provided in burettes with 0.1 mL divisions that were fitted with metal drinking spouts. In one group of rats, water and 0.3 M NaCl intake (two-bottle test) was induced by the treatment with s. c. Furo (10 mg/kg b.w.) plus Cap (5 mg/kg b.w.). The rats received the s.c. Furo/Cap treatment and were returned to their home cages, which lacked water and 0.3 M NaCl solution. One hour later, water and 0.3 M NaCl, but not food, were made available to the animals,