BDNF and AMPA receptors in the cNTS modulate the hyperglycemic reflex after local carotid body NaCN stimulation

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ABSTRACT

The application of sodium cyanide (NaCN) to the carotid body receptors (CBR) (CBR stimulation) induces rapid blood hyperglycemia and an increase in brain glucose retention. The commissural nucleus tractus solitarius (cNTS) is an essential relay nucleus in this hyperglycemic reflex; it receives glutamatergic afferents (that also release brain derived neurotrophic factor, BDNF) from the nodose-petrosal ganglia that relays CBR information. Previous work showed that AMPA in NTS blocks hyperglycemia and brain glucose retention after CBR stimulation. In contrast, BDNF, which attenuates glutamatergic AMPA currents in NTS, enhances these glycemic responses. Here we investigated the combined effects of BDNF and AMPA (and their antagonists) in NTS on the glycemic responses to CBR stimulation. Microinjections of BDNF plus AMPA into the cNTS before CBR stimulation in anesthetized rats, induced blood hyperglycemia and an increase in brain arteriovenous (a-v) of blood glucose concentration difference, which we infer is due to increased brain glucose retention. By contrast, the microinjection of the TrkB antagonist K252a plus AMPA abolished the glycemic responses to CBR stimulation similar to what is observed after AMPA pretreatments. In BDNF plus AMPA microinjections preceding CBR stimulation, the number of *c-fos* immunoreactive cNTS neurons increased. In contrast, in the rats microinjected with K252a plus AMPA in NTS, before CBR stimulation, *c-fos* expression in cNTS decreased. The expression of AMPA receptors GluR2/3 did not change in any of the studied groups. These results indicate that BDNF in cNTS plays a key role in the modulation of the hyperglycemic reflex initiated by CBR stimulation.

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1. Introduction

Glucose is an essential metabolic substrate for the brain and other organs. Glucose sensors, distributed throughout the body, can detect small changes in glucose in the blood and the brain extracellular fluids (Koborsy et al., 2014). These sensors are essential to counterregulatory responses (Fehm et al., 2006) triggering homeostatic mechanisms to reverse elevations or depressions in plasma glucose concentration (Tesfaye and Seaquist, 2010; Verberne et al., 2014). The carotid body receptors (CBR) are well known for their role in detecting O_2, CO_2 and pH

levels in blood (Eyzaguirre and Zapata, 1984; González et al., 1994; Prabhakar, 2016). These receptors also sense glucose concentration and play an important role in glucose homeostasis (Álvarez-Buylla and de Álvarez-Buylla, 1988; Pardal and López-Barneo, 2002; García-Fernández et al., 2007). Carotid bodies' resection in dogs, or their desensitization by hyperoxia, drastically impairs the systemic counterregulatory responses to hypoglycemia (Koyama et al., 2000; Wehrwein et al., 2010). CBR responses to intermittent hypoxia also affect glucose metabolism increasing counterregulatory hormones; adrenaline, cortisol, glucagon and growth hormone (Wehrwein et al., 2010; Drager et al., 2010). Consistently, we have previously shown that the brief infusion of sodium cyanide (NaCN) through the local circulation of the CBR (CBR stimulation) induces a rapid hyperglycemic reflex and increases brain glucose retention (Álvarez-Buylla and Álvarez-Buylla, 1994). The CBR is strategically located at the initiation of the brain circulatory system. The glycemic reflex initiated by CBR stimulation could be part of the counterregulatory response to ensure proper supply of glucose to the brain during anoxia or/and hypoglycemia (Álvarez-Buylla and de Álvarez-Buylla, 1988).

The brainstem nucleus tractus solitarius (NTS) is the primary central target of peripheral visceral sensory neurons, including baro- and chemoreceptor afferents from CBR (Andresen et al., 2004; Cruz et al., 2010) via the nodose-petrosal ganglion complex (NPGC) (Brady et al., 1999; Martin et al., 2009). The commissural subnucleus of the NTS (cNTS) is key to glucose metabolism and feeding behavior (Wehrwein et al., 2010; Jeanneteau et al., 2012; Cuéllar et al., 2015; Cedernaes and Bass, 2016). BDNF is a potent modulator of synaptic transmission and glucose homeostasis, including for the neurons in the NPGC (Brady et al., 1999; Martin et al., 2009), which release BDNF in an activity-dependent manner (Balkowiec and Katz, 2000). Interestingly, low levels of circulating BDNF are associated with insulin resistance (Pedersen et al., 2009), human obesity and type 2 diabetes (Krabbe et al., 2007; Rosas-Vargas et al., 2011; Xu et al., 2003).

Glutamate is the excitatory neurotransmitter of NPGC afferent terminals in cNTS (Vardhan et al., 1993; Ohta and Talman, 1994; Aylwin et al., 1997), but the function of glutamate in the processing of afferent information during the hyperglycemic reflex initiated by CBR stimulation remains poorly understood. Previous results from our laboratory show that BDNF microinjection in the cNTS a few minutes before NaCN application to the CBR increases arterial glucose concentration and brain glucose retention (Montero et al., 2012). In contrast, AMPA microinjection in the cNTS, before the CBR stimulation with NaCN, inhibits the increase in arterial glucose and brain glucose retention (Cuéllar et al., 2015). Since BDNF has been shown to attenuate glutamatergic AMPA currents in neurons of the cNTS (Balkowiec et al., 2000), we tested here whether BDNF modified the effects of AMPA in cNTS. We microinjected BDNF followed by AMPA (or antagonists) into the cNTS, before application of NaCN into the CBR, and measured changes in glucose concentrations in arterial and venous blood. Results show that BDNF prevented the inhibitory effects that AMPA injections have in cNTS on the glycemic response induced by the local application of NaCN in CBR.

2. Methods

2.1. Animals and general surgery

All procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals, from the US National Institutes of Health and the Bioethics and the Biosecurity Committee of the University of Colima (No. 2013-02).

Fifty-two male Wistar rats (280–300 g) were used. They were housed individually under a 12:12-h light-dark cycle at 22–23 °C and fed a rodent laboratory diet with water ad libitum, but food was removed 12 h before surgery. Procedures for surgery: catheters implantation and the local NaCN application to the CBR (CBR stimulation) was as previously described (Álvarez-Buylla and Bencosme, 1981; Álvarez-Buylla and de Álvarez-Buylla, 1988). Briefly, rats were anesthetized with sodium pentobarbital (i.p.) (3.3 mg/100 g/saline, Pfizer, Mex.), and maintained by i.p. infusion of sodium pentobarbital (0.063 mg/100 g/min/saline). Buprenorphine analgesia (0.03 mg/kg, Temgesic, Schering-Plough, Mex.) was provided before the surgical procedures. Rats were artificially ventilated and respiratory rate and tidal volume were adjusted to maintain the pO2, pCO2 and pH values. Body temperature was also kept constant at 37.5 ± 1 °C. Silastic catheters were implanted into the carotid artery, abdominal aorta and into the jugular sinus without interrupting circulation in these vessels (Álvarez-Buylla and Bencosme, 1981). Correct placement of catheters was verified at the end of each experiment. NaCN was administered locally into the left CBR (CBR stimulation), which were isolated briefly from the systemic circulation (ICS) to prevent systemic effects of NaCN (see below).

Microinjections of drugs into the cNTS were done using a stereotaxic frame (Stoelting Co., Wood Dale, IL, USA). The incisor bar was adjusted until the heights of lambda and bregma skull points were equal (flat skull position) (Paxinos and Watson, 1986). Occipital craniotomy was performed with a 1/32-in. burr to expose the brain surface after cutting the dura mater membrane; access to cNTS was made with a glass micropipette (40–50 μm tip diameter), choosing lambda as midpoint, inserted into the cNTS region (the lambda coordinates were: AP = −5.1 mm, L = −0.1 mm, V = 8.1 mm). The rats were allowed to stabilize for at least 30 min after cannulae implantations in order to minimize effects of surgery on the experimental results.

2.2. Drugs and drug application

The following drugs were used: (a) sodium cyanide (NaCN) (Fluka Biochemika, Sigma, Mex. - 5 μg/100 g/100 μL saline in the circulatory isolated carotid sinus) (Álvarez-Buylla and de Álvarez-Buylla, 1988); (b) freshly prepared artificial cerebrospinal fluid (aCSF containing 145 mM NaCl, 2.7 mM KCl, 1.0 mM MgCl, 1.2 mM CaCl$_2$, 2.0 mM ascorbate, 2 mM NaH$_2$PO$_4$ (pH 7.3–7.4)); (c) brain-derived neurotrophic factor, BDNF (1 ng/100 nL of aCSF) (Clark et al., 2011); (d) TrkB receptor antagonist, K252a (25 ng/100 nL aCSF in the cNTS (Shen et al., 2006)); (e) α-amino-3-hydroxy-5-methylisoxazole-4-acid propionic (AMPA) (Sigma, Mex.), selective AMPA receptor agonist (2 μM/100 nL saline (Mueller et al., 2005)); and (f) 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[*f*]quinoxaline-7-sulfonamide disodium (NBQX) (Sigma, Mex.), selective AMPA receptor antagonist (2 mM/100 nL aCSF) (Mueller et al., 2005). The same volume of aCSF was injected into the cNTS in the control experiments.

2.3. Experimental protocol and number of animals in each experiment

The rats were randomized as follows: (a) the aCSF + aCSF control group received aCSF two times at $t = -4$ min and $t = -2$ min (100 nL into the cNTS) followed by NaCN (5 μg/100 g/100 μL saline) at $t = 0$ into the isolated carotid sinus ($n = 11$) (Fig. 1A); (b) the BDNF + aCSF group received injections of BDNF (1 ng/100 nL) and aCSF (100 nL) into the cNTS, 4 and 2 min previous to NaCN, respectively (5 μg/100 g/100 μL saline into the isolated carotid sinus) ($n = 6$) (Fig. 1A); (c) the BDNF + AMPA group received injections of BDNF (1 ng/100 nL) and AMPA (2 μM/100 nL) into the cNTS, 4 and 2 min previous to NaCN, respectively (5 μg/100 g in 100 μL saline into the isolated carotid sinus) ($n = 8$) (Fig. 1B); (d) the BDNF + NBQX group received the injections of BDNF (1 ng/100 nL) and NBQX (2 mM/100 nL) into the cNTS, 4 and 2 min previous to the NaCN (5 μg/100 g in 100 μL saline into the isolated carotid sinus), respectively ($n = 8$) (Fig. 1B); (e) the K252a + AMPA group received the injections of K252a (25 ng/100 nL) and AMPA into the cNTS, 4 and 2 min previous to the NaCN (5 μg/100 g in 100 μL saline into isolated carotid sinus), respectively ($n = 8$) (Fig. 1B); and (f) the K252a + NBQX group received injections of K252a and NBQX (same dose as above) into the cNTS, 4 and 2 min previous to the NaCN (5 μg/100 g in 100 μL saline into isolated carotid sinus), respectively ($n = 8$) (Fig. 1B).

In addition to the above, 2 groups (3 rats in each) were used for BDNF protein measure (ELISA) in the cNTS: (g) aCSF + aCSF injected in the cNTS and saline in the isolated carotid sinus; (h) as in (g) but instead of saline, NaCN was injected in the carotid sinus.

2.4. Carotid chemoreceptor stimulation (CBR)

The local application of sodium cyanide (NaCN) to the carotid body receptors (CBR) (CBR stimulation) was carried out by application of NaCN into the carotid sinus (3 s) which was temporarily isolated from the systemic circulation (Serani et al., 1983). NaCN has been previously used as a tool for peripheral chemoreflex activation (Haibara et al., 1999; Cruz et al., 2010). Briefly, 5 μg/100 g NaCN/100 μL saline/3 s was injected into the local circulation of the in vivo isolated left carotid sinus, avoiding baroreceptor stimulation. Because our intention was to locally stimulate only one carotid sinus with a micro-dose of NaCN,