Autonomic Neuroscience: Basic and Clinical 203 (2017) 67–73

Contents lists available at ScienceDirect

Autonomic Neuroscience: Basic and Clinical

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

# Perifornical hypothalamic pathway to the adrenal gland: Role for glutamatergic transmission in the glucose counter-regulatory response

A. Sabetghadam [b], W.S. Korim [b], A.J.M. Verberne [a,*]

[a] University of Melbourne, Clinical Pharmacology and Therapeutics Unit, Department of Medicine, Austin Health, Heidelberg, Victoria 3084, Australia
[b] Florey Institute of Neuroscience and Mental Health, University of Melbourne, Parkville, Victoria, Australia

ARTICLE INFO

*Article history:*
Received 16 December 2016
Received in revised form 31 January 2017
Accepted 1 February 2017

*Keywords:*
Adrenal
Perifornical hypothalamus
2-Deoxyglucose
Rostroventrolateral medulla
Sympathetic

ABSTRACT

Adrenaline is an important counter-regulatory hormone that helps restore glucose homeostasis during hypoglycaemia. However, the neurocircuitry that connects the brain glucose sensors and the adrenal sympathetic outflow to the chromaffin cells is poorly understood. We used electrical microstimulation of the perifornical hypothalamus (PeH) and the rostral ventrolateral medulla (RVLM) combined with adrenal sympathetic nerve activity (ASNA) recording to examine the relationship between the RVLM, the PeH and ASNA. In urethane-anaesthetised male Sprague-Dawley rats, intermittent single pulse electrical stimulation of the rostroventrolateral medulla (RVLM) elicited an evoked ASNA response that consisted of early (60 ± 3 ms) and late peaks (135 ± 4 ms) of preganglionic and postganglionic activity. In contrast, RVLM stimulation evoked responses in lumbar sympathetic nerve activity that were almost entirely postganglionic. PeH stimulation also produced an evoked excitatory response consisting of both preganglionic and postganglionic excitatory peaks in ASNA. Both peaks in ASNA following RVLM stimulation were reduced by intrathecal kynurenic acid (KYN) injection. In addition, the ASNA response to systemic neuroglucoprivation induced by 2-deoxy-D-glucose was abolished by bilateral microinjection of KYN into the RVLM. This suggests that a glutamatergic pathway from the perifornical hypothalamus (PeH) relays in the RVLM to activate the adrenal SPN and so modulate ASNA. The main findings of this study are that (i) adrenal premotor neurons in the RVLM may be, at least in part, glutamatergic and (ii) that the input to these neurons that is activated during neuroglucoprivation is also glutamatergic.



## 1. Introduction

Adrenaline is a hormone that is critically involved in the glucose counter-regulatory response especially in Type 1- and advanced Type 2 diabetes (Cryer, 1993; Verberne et al., 2016; Verberne et al., 2014). Electrical stimulation of the adrenal/splanchnic nerves (Edwards and Jones, 1993), systemic administration of the glucoprivic agent 2-deoxyglucose (2-DG) or insulin and haemorrhage (Mundinger et al., 1997; Vollmer et al., 2000), increase circulating catecholamines, while denervation of the adrenal medulla (Abbott et al., 2005) decreases catecholamine levels. Adrenal sympathetic preganglionic neurons (SPN) have been characterised anatomically (Edwards et al., 1996; Kesse et al., 1988) and physiologically (Cao and Morrison, 2000; Morrison and Cao, 2000). The cell bodies of rat adrenal SPNs project to the adrenaline and noradrenaline-synthesising chromaffin cells and are located in the intermediolateral cell column of the spinal cord between $T_4$–$T_{12}$ with the highest density at $T_8$ (Strack et al., 1988). Individual adrenal SPN target either adrenaline-synthesising or noradrenaline-synthesising chromaffin cells (Edwards et al., 1996; Kesse et al., 1988) but the conduction velocities of both types of adrenal SPNs are similar (~0.5–5.5 m/s) (Morrison and Cao, 2000). However, adrenal SPNs responded differentially to baroreceptor activation or 2-DG-induced glucoprivation (Morrison and Cao, 2000). Insensitivity to baroreceptor stimulation and activation following systemic 2-DG distinguished SPNs that target the adrenaline-synthesising chromaffin cells from the SPN that target the noradrenaline-synthesising cells (Morrison and Cao, 2000).

The RVLM contains PNMT immunoreactive and non-PNMT immunoreactive barosensitive premotor neurons that project to adrenal SPN (Strack et al., 1989; Wesselingh et al., 1989). Electrical stimulation of RVLM premotor neurons resulted in an increase in firing rate of both adrenaline and noradrenaline SPNs at different latencies (latencies ~ 29 ms and ~129 ms, respectively) implying the presence of two distinct populations of premotor neurons within the RVLM with different axonal conduction velocities (Morrison and Cao, 2000). This finding is consistent with single unit recordings made from RVLM medullospinal neurons which had distinct spinal axonal conduction velocities (Brown and Guyenet, 1985; Schreihofer and Guyenet, 1997).

* Corresponding author at: University of Melbourne, Clinical Pharmacology and Therapeutics Unit, Department of Medicine, Austin Health, Heidelberg, Victoria 3084, Australia.
*E-mail address:* antonius@unimelb.edu.au (A.J.M. Verberne).

http://dx.doi.org/10.1016/j.autneu.2017.02.002
1566-0702/

Chemical stimulation of the RVLM in the cat using excitatory amino acid injections elicits robust pressor responses accompanied by increases in plasma catecholamines (McAllen, 1986). Our laboratory identified slow-conducting (conduction velocity: <1 m/s), slow-firing, baroinsensitive, medullospinal neurons in the RVLM that were activated by glucoprivation suggesting that the RVLM may be involved in the adrenaline response to glucoprivation (Verberne and Sartor, 2010).

Adrenal catecholamine release can also be elicited by hypothalamic stimulation. Thus, electrical or chemical stimulation in the hypothalamus altered the blood glucose level or elicited adrenaline secretion (Folkow and Von Euler, 1954; Matsui, 1979; Verberne et al., 2014). Recently, a role for perifornical hypothalamic (PeH) neurons in the adrenaline response to systemic glucoprivation has been identified (Korim et al., 2014; Korim et al., 2016; Otlivanchik et al., 2014). In those studies it was proposed that PeH neurons couple the responses of hypothalamic glucose-sensing neurons to RVLM presympathetic neurons that control adrenaline secretion (Verberne et al., 2016). However, a neuropharmacological analysis of the pathway that connects the RVLM and adrenal sympathetic outflow has not been reported. The present study was designed to characterize the neural pathways between brain glucose-sensing neurons and the adrenal gland by electrical stimulation of the RVLM and PeH and recording ASNA.

In order to explore the connection between the RVLM and ASNA, we used intermittent electrical stimulation of the RVLM to construct post-stimulus neurograms in which we could identify sympathetic components with different latencies. In addition, the relative contribution of pre- and post-ganglionic activity was assessed by using the ganglion blocker hexamethonium. RVLM presympathetic neurons send projections to adrenal SPN (Morrison and Cao, 2000) and PeH neurons provide polysynaptic input to the adrenal sympathetic outflow (Kerman et al., 2007), Therefore, we examined the evoked response in the adrenal sympathetic outflow to electrical stimulation of the PeH as well as the RVLM. Some PeH neurons express vGlut2 mRNA, a marker for glutamatergic neurons (Rosin et al., 2003), and the increase in ASNA that occurs in response to systemic glucoprivation is blocked by inhibition of neurons in the PeH (Korim et al., 2014). Therefore, we tested the hypothesis that ionotropic glutamate receptors in the RVLM are involved in the excitatory responses in ASNA elicited by neuroglucoprivation using systemic 2DG. This was achieved by performing bilateral microinjections of kynurenic acid or vehicle into the RVLM. Several previous studies have also suggested that glutamate is the major excitatory neurotransmitter of RVLM neurons that project to SPN (Huangfu et al., 1994; Morrison, 2003) although this has not been established for the premotor pathway to the adrenal SPN. Therefore, the involvement of glutamate receptors at the level of the SPN was tested by administration of kynurenic acid intrathecally while recording evoked ASNA responses following RVLM stimulation.

## 2. Materials and methods

Experiments were performed according to the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* and were approved by the Animal Ethics Committee of Austin Health (Heidelberg, Victoria, Australia; Approval numbers 12/4764 and 14/5191).

### 2.1. General preparation

Male Sprague-Dawley rats (250–300 g) were anaesthetised with 2% isoflurane, tracheostomised and connected to a rodent ventilator (50–60 breaths/min; 10 ml/kg) supplying isoflurane (1.5–1.7%/100% $O_2$). The depth of anaesthesia was monitored regularly and confirmed by the absence of responses to toe pinch and corneal probing. Rectal temperature was maintained at 36.5–37.5 °C by a thermocouple-controlled heating pad. The jugular vein and carotid artery were cannulated for intravenous drug injection and measurement of blood pressure, respectively. After completion of all surgery, the isoflurane anaesthesia was discontinued and urethane (1.4 g/kg, i.v.) was administered. Urethane was chosen for its minimal effects on blood glucose (Verberne and Sartor, 2010), and supplemented when necessary. The animal was paralysed with pancuronium bromide (2 mg/kg, i.v. with 0.2 mg/kg supplement if required) before performing electrical stimulation. After neuromuscular blockade, anaesthesia was maintained at a level in which paw pinch produced minimal changes in blood pressure (<10 mm Hg).

### 2.2. Intrathecal cannulation

The animal was placed into a stereotaxic apparatus with the incisor bar at −3.3 mm below the intra-aural line. The atlanto-occipital membrane was exposed, a fine polyethylene cannula (SP-8, O.D 0.5 mm, ID 0.2 mm) filled with artificial cerebrospinal fluid (aCSF) was inserted through a small opening in the dura mater, and its tip was positioned at the end of the $T_9$–$T_{10}$ thoracic vertebra. Kynurenic acid (KYN) (0.5 μmol in 10 μl) or aCSF mixed with fluorescent latex microspheres was slowly administered followed by a 10 μl flush with aCSF (Verberne et al., 1990). The effect of intrathecal KYN or vehicle on the ASNA responses to RVLM stimulation was tested.

### 2.3. Extracellular single-unit recording of RVLM barosensitive neurons

A partial parietal craniotomy was performed to access the rostroventrolateral medulla (RVLM) using a transcerebellar approach. The procedure was performed as described previously (Brown and Guyenet, 1985) using transcerebellar insertion of a glass microelectrode (4–8 MΩ impedance at 30 Hz), filled with a solution of Pontamine sky blue (2%/0.5 M sodium acetate), into the ventrolateral medulla at the level of the facial motor nucleus (coordinates: 3.0 mm caudal to lambda, 2.0 mm lateral to midline, 8.0–9.0 mm ventral to the surface of cerebellum). A band pass amplifier (Model WDR 420, Fintronics Inc., Orange, Connecticut, USA) was used to filter and amplify the field potentials of facial motor neurons during facial nucleus mapping (50 Hz–8 kHz) and a bandpass of 400 Hz–4 kHz was used for recording RVLM premotor neurons.

Facial field potentials were recorded in response to electrical stimulation of the mandibular branch of the facial nerve (0.5 Hz, 0.1 ms, 1.0–2.0 mA). RVLM presympathetic neurons are found immediately caudal, medial and ventral to the caudal pole of the facial nucleus (Verberne et al., 1999b) extending caudally for 500 μm. RVLM presympathetic neurons are barosensitive and have spinally-projecting axons (Brown and Guyenet, 1985; Verberne et al., 1999b) or are baroinsensitive, spinally-projecting and are activated by neuroglucoprivation (Verberne and Sartor, 2010). Since these two populations are intermingled, we used the location of a barosensitive neuron to guide placement of the stimulation electrode. Spontaneously active RVLM neurons were tested for barosensitivity by increasing arterial blood pressure using the selective $\alpha_1$-adrenoceptor agonist phenylephrine (10 μg/kg, i.v.), as well as phenylbiguanide (10 μg/kg, i.v.), a $5HT_3$ agonist and activator of the von Bezold–Jarisch reflex (Verberne and Guyenet, 1992a). Once an RVLM barosensitive neuron was found, the recording electrode was replaced with a monopolar, stainless steel stimulation electrode at identical stereotaxic coordinates.

### 2.4. Adrenal and lumbar sympathetic nerve activity

Levels of circulating adrenaline, but not noradrenaline levels, correlate with adrenal preganglionic sympathetic nerve activity (ASNA) during systemic neuroglucoprivation (Korim et al., 2014). Therefore, ASNA seems to be a reliable index for studying adrenal gland catecholamine release in experimental hypoglycaemia or neuroglucoprivation.

The left or right adrenal gland was exposed using a retroperitoneal approach. The translucent nerve bundles in between the suprarenal ganglion (Celler and Schramm, 1981) and adrenal gland were carefully