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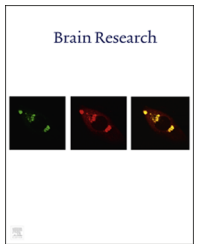

Research Report

Hydralazine administration activates sympathetic preganglionic neurons whose activity mobilizes glucose and increases cardiovascular function

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ABSTRACT

Hypotensive drugs have been used to identify central neurons that mediate compensatory baroreceptor reflex responses. Such drugs also increase blood glucose. Our aim was to identify the neurochemical phenotypes of sympathetic preganglionic neurons (SPN) and adrenal chromaffin cells activated following hydralazine (HDZ; 10 mg/kg) administration in rats, and utilize this and SPN target organ destination to ascribe their function as cardiovascular or glucose regulating. Blood glucose was measured and adrenal chromaffin cell activation was assessed using c-Fos immunoreactivity (-ir) and phosphorylation of tyrosine hydroxylase, respectively. The activation and neurochemical phenotype of SPN innervating the adrenal glands and celiac ganglia were determined using the retrograde tracer cholera toxin B subunit, in combination with *in situ* hybridization and immunohistochemistry. Blood glucose was elevated at multiple time points following HDZ administration but little evidence of chromaffin cell activation was seen suggesting non-adrenal mechanisms contribute to the sustained hyperglycemia. 16±0.1% of T4-T11 SPN contained c-Fos and of these: 24.3±1.4% projected to adrenal glands and 29±5.5% projected to celiac ganglia with the rest innervating other targets. 62.8±1.4% of SPN innervating adrenal glands were activated and 29.9±3.3% expressed PPE mRNA whereas 53.2±8.6% of SPN innervating celiac ganglia were activated and 31.2±8.8% expressed PPE mRNA. CART-ir SPN innervating each target were also activated and did not co-express PPE mRNA. Neurochemical coding reveals that HDZ administration activates both PPE+SPN, whose activity increase glucose mobilization causing hyperglycemia, as well as CART+SPN whose activity drive vasomotor responses mediated by baroreceptor unloading to raise vascular tone and heart rate.

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1. Introduction

Immunoreactivity for the protein c-Fos has been used to identify neurons in the central nervous system activated by drugs that cause hypotension (Badoer et al., 1993; Burman et al., 2004; Chan and Sawchenko, 1994; Stornetta et al., 2001) or hypertension (Dampney and Horiuchi, 2003; Li and Dampney, 1994). Activated neurons were found in the nucleus of the solitary tract, the ventrolateral medulla and the lateral horn of the spinal cord, which contains sympathetic preganglionic neurons (SPN), therefore it was postulated that these stimuli identify neurons participating in baroreceptor reflex mediated changes that restore hemodynamic homeostasis (Chan and Sawchenko, 1994; Dampney and Horiuchi, 2003; Minson et al., 1996b, 1997). It should be noted that non-blood pressure related drug effects were never tested in these studies. Nevertheless, in response to hypotensive stimuli c-Fos immunoreactivity (ir) was found in the spinal cord only in SPN, predominantly in T5 to T13 spinal segments (Burman et al., 2001; Fenwick et al., 2006; Minson et al., 1996a, 1996b, 2002). The targets of these activated SPN include the adrenal medulla (Minson et al., 1996a) but must also include the vasculature and heart in order to convey the baroreflex mediated effects. However, hypotensive stimuli also evoke increases in plasma glucose and this seems independent of the hypotensive agent used for example: hydralazine (Sanbar and de Romero, 1969; Satoh et al., 1980), sodium nitroprusside (Boquist, 1989; Staquet et al., 1965), and diazoxide (Altszuler et al., 1977). Increased plasma glucose mediated by sympathetic activation arises predominantly from stimulation of the splanchnic nerve. This nerve directly innervates the adrenal medulla to increase catecholamines and via the celiac ganglia innervates the pancreas, to release glucagon and inhibit insulin secretion, and the liver (Yamaguchi, 1992; Yi et al., 2010). Sympathetic activation of these effectors increases hepatic glycogenolysis and gluconeogenesis (Yamaguchi, 1992; Yi et al., 2010). SPN responsible for both the sympathetically mediated hemodynamic responses and also the hyperglycemia evoked by hydralazine administration have not been neurochemically identified or differentiated.

A range of neurochemicals have been described in SPN, particularly in those projecting to the adrenal gland and celiac ganglia, including pituitary adenylate cyclase activating polypeptide (PACAP) mRNA, pre-proenkephalin (PPE) mRNA, nitric oxide synthase (NOS), cocaine and amphetamine-regulated transcript (CART) and calretinin (Edwards et al., 1996; Fenwick et al., 2006; Hinrichs and Llewellyn-Smith, 2009; Kumar et al., 2010; Parker et al., 2013). We have shown previously that glucoprivation, induced by 2-deoxy-D-glucose, which does not change blood pressure, activates all SPN projecting to the adrenal glands and celiac ganglia that express PPE mRNA and only activates adrenergic chromaffin cells (Parker et al., 2013) suggesting PPE mRNA codes SPN regulating glycemic but not cardiovascular responses. This is in keeping with the findings that enkephalinergic terminals preferentially target adrenergic chromaffin cells (Holgert et al., 1995, 1996, 1998; Pelto-Huikko et al., 1987) and that adrenal SPN responsive to glucopenia were not responsive to baroreceptor reflex activation (Cao and Morrison, 2000). Conversely PPCART mRNA was not found in any adrenal or celiac ganglia projecting SPN activated following glucoprivation (Parker et al., 2013) suggesting these SPN are not involved in regulating glycemia. This was not surprising as it has been suggested that CART-ir SPN predominantly have cardiovascular targets (Gonsalvez et al., 2010) including noradrenergic chromaffin cells and post ganglionic neurons innervating the vasculature. It is perhaps surprising that adrenal chromaffin cells activated in response to hypotensive stimuli have not been identified particularly considering hypotensive stimuli have been correlated with increases in both plasma catecholamines, at least at early time points (Altszuler et al., 1977; Madden et al., 2006; Staquet et al., 1965; Vollmer et al., 2000).

The objective of this study was therefore to determine the effects of hydralazine on the sympathoadrenal and sympathoceliac pathways. The major aim was to describe the neurochemical phenotype of SPN that project to the adrenal medulla and celiac ganglia activated following hydralazine administration. We hypothesized that SPN projecting to the adrenal gland activated following hydralazine would include those which express PPE mRNA and those which express CART-ir in order to regulate the release of adrenaline and noradrenaline, respectively. Similar phenotypes would also distinguish SPN that project to the celiac ganglia to influence glucose mobilization or hemodynamic homeostasis. Multilabel immunohistochemistry, in combination with *in situ* hybridization, was used to investigate for the first time the expression of neurochemicals in activated SPN with known projection targets following hydralazine administration. We also assessed the activation of adrenal chromaffin cells using c-Fos-ir and sensitive measures of catecholamine synthesis, i.e. phosphorylation of tyrosine hydroxylase which indicate increased capacity for catecholamine release (Damanhuri et al., 2012) across multiple time points.

2. Results

2.1. *HDZ administration increased blood glucose over time*

In order to confirm that blood glucose levels increased following hydralazine (HDZ) administration as described previously (Sanbar and de Romero, 1969; Satoh et al., 1980), measurements were made following injection at: 20 min (HDZ, 13.1±1.4 mmol/l; $n=5$; vs control 10.2±0.6 mmol/l; $n=5$, $p<0.08$), 60 min (HDZ 16.3±1.1 mmol/l; $n=6$; vs control 9.8±0.6 mmol/l; $n=6$, $p<0.001$) and 120 min (23.8±2.8 mmol/l; $n=7$; vs 10.6±0.8 mmol/l; $n=7$ $p<0.001$).

2.2. *Plasma catecholamines were not elevated 120 min following HDZ administration*

Several previous studies have demonstrated that plasma catecholamines are increased early following HDZ administration (Madden et al., 2006; Vollmer et al., 2000) so in the current study plasma catecholamines were determined 120 min after injection (adrenaline HDZ 0.4±0.2 ng/ml; $n=7$ vs saline 0.5±0.1 ng/ml; $n=7$; noradrenaline HDZ 0.4±0.1 ng/ml; $n=7$ vs saline 0.6±0.1 ng/ml; $n=7$).