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Research Report

Idazoxan activates rat forebrain glycogen phosphorylase in vivo: A histochemical study

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Abstract

In vitro experiments show norepinephrine activates glycogen phosphorylase and glycogenolysis in forebrain glia. The present study used idazoxan (5 mg/kg) to elevate NE in vivo and examined patterns of active (aGP) and total (tGP) glycogen phosphorylase reactivity in selected neocortical, hippocampal, diencephalic, and striatal sites using a histochemical method. In somatosensory neocortex, aGP reactivity was highest in Layer 4 with consistent reactivity in the barrel fields in vehicle-treated brains. In the hippocampus, the stratum lacunosum moleculare was highly reactive, while cell layers were least reactive. The dentate gyrus and CA3 were more reactive for aGP than CA1. In the diencephalon, the medial habenula was most reactive followed by the reticular nucleus of the thalamus. In the striatum, globus pallidus was most reactive. Reactivity patterns for tGP were similar to those for aGP, but more intense. The neocortex had the highest overall reactivity for tGP. An estimate of the percentage of aGP relative to tGP suggested the regions sampled had similar levels of median basal activation (~65%). Idazoxan increased aGP reactivity in all regions of the neocortex assessed (Layers 3–6 of primary and secondary somatosensory cortex and the barrel fields). The neuropil layers, but not the cell layers, of hippocampus were more reactive following idazoxan treatment. Idazoxan also increased aGP reactivity in the laterodorsal, paraventricular, and reticular nuclei of the thalamus. The largest idazoxan-induced changes, as an estimated percentage of tGP, occurred in the hippocampus (~16% for stratum lacunosum moleculare and for CA1 stratum oriens). Increases ranged from ~3 to 6% in neocortex and were less than 3% in the diencephalic and striatal areas. These effects of idazoxan are consistent with a role for norepinephrine in activating forebrain glycogenolyis in vivo and supporting increased brain metabolism. They contrast with earlier evidence showing that idazoxan reduces 2-deoxyglucose uptake in these brain areas. Idazoxan, and norepinephrine, may preferentially recruit glycolytic over oxidative metabolism in the rat forebrain.

Theme: Other systems of the CNS
Topic: Brain metabolism and blood flow

Keywords: Norepinephrine; Locus coeruleus; Neocortex; Hippocampus; Diencephalon; Striatum; Brain metabolism; Glycogenolysis; 2-Deoxyglucose

1. Introduction

Glycogen is the primary energy store for the central nervous system [44]. Glycogen and its catabolic enzyme, glycogen phosphorylase (GP), are found mainly in astroctyes [46] where glycogenolysis can be activated in response to neuronal activation [40,48], consistent with the hypothesis of functional neuron/glial coupling. Glycogen is currently thought to play an important role in functional brain metabolism [5] and glycogen resynthesis is proposed to have a causal role in sleep [2].

Norepinephrine (NE) activates glycogenolyis in astrocytic cell cultures (EC_{50} = 20 nM), although high exogenous levels are reported to trigger glycogen synthesis (EC_{50} = 1 μM) [24]. Astrocytes that respond to noradrenergic stimulation are only a proportion of those cultured suggesting regional selectivity in vivo [33]. The glycogenolytic role of NE is well established for astrocytes in vitro [3,29,39,43], but there is a paucity of data on in vivo brain glycogenolysis in response to NE and no information on regional selectivity.

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While neither NE, nor isoproterenol, the two most effective adrenergic agonists in vitro cross the blood–brain barrier, indirect methods are available to elevate brain NE. In microdialysis studies, systemic and local idazoxan increases forebrain NE in a dose-dependent manner [41]. Idazoxan is an α2 receptor antagonist that reduces autoreceptor inhibition associated with NE release to increase terminal NE levels and, when given systemically, increases the firing rate of locus coeruleus neurons that are the source for NE in the forebrain [21].

Glycogenolysis is mediated by activation of GP. This enzyme exists in both a phosphorylated active state (aGP) and in a dephosphorylated inactive state. A protein kinase activated by NE switches the dephosphorylated inactive state to the phosphorylated active state [20]. Levels of both active and total (active + inactive) GP may be assessed histochemically using fresh frozen tissue.

In the present study, idazoxan was administered systemically in a dose (5 mg/kg, i.p.) sufficient to significantly elevate brain NE based on previous microdialysis studies (see Methods). Brains were fresh frozen and processed histochemically to provide data on the localization and intensity of active and total GP reactivity in selected forebrain regions of idazoxan-treated and control hemispheres.

2. Methods

2.1. Subjects

Subjects were 14 male, Sprague–Dawley rats weighing approximately 250 g and obtained from Memorial University's Vivarium. Animals were maintained on a 12:12 light/dark cycle and provided with Purina Rat Chow and water ad libitum. Experimental procedures were performed in accordance with the Canadian Council of Animal Care guidelines and followed a protocol approved by the Institutional Animal Care Committee.

2.2. Procedure

Idazoxan (5 mg/kg) was dissolved in physiological saline (0.9% NaCl) and injected at a volume of 5 mg/ml intraperitoneally (i.p.) into seven subjects. The 5 mg/kg dose was selected since earlier work suggested it maximally occupies α2 receptors without impinging on α1 adrenoceptor function [42]. Microdialysis experiments also indicated that 3 mg/kg idazoxan was just at threshold for significant increases in NE in neocortex in awake rats [7] and NE levels were significantly lower with 3 mg/kg than with 5 mg/kg [7]. NE increases with 5 mg/kg did not differ from increases induced with higher doses up to 20 mg/kg (2–2.5× basal levels) [7]. Similar increases in NE are produced by 5 mg/kg idazoxan in the hippocampus [41] and in diencephalon [28]. In these studies [7,28,41], peak NE occurred at 40 min or sooner following the idazoxan injection and remained elevated for ~60 min.

Corresponding volumes of the vehicle, saline, were administered, i.p., to seven control rats. The order of injections was counterbalanced such that brains from idazoxan- and vehicle-injected rats could be processed as pairs. Injections were performed between 11:30 a.m. and 2:30 p.m. to minimize circadian effects [14]. Forty-five minutes after idazoxan or saline administration, rats were injected with chloral hydrate (0.5 ml/100 g, 80 mg/ml, i.p.) and decapitated rapidly after losing consciousness (~2 min). Brains were fresh-frozen by immersion in methyl butane cooled to −70 °C to preserve enzymatic activity.

2.3. Sectioning

A drug-treated and a control brain were sectioned along the midsagittal axis and complementary halves joined to construct a chimeric brain. Coronal sections (30 um) were taken of the resulting chimera. Three consecutive sections were mounted on three slide sets for aGP histochemistry, tGP histochemistry, and Nissl staining (0.1% cresyl violet). Each slide contained at least 4 chimeric brain slices, separated by ~seven consecutive slices. As well, 5 slices were taken to measure background staining intensity by omitting the enzyme incubation step (see below). Slides were refrigerated until sectioning was complete and then warmed at room temperature for ~10 min prior to histochemical processing. This reduced the drop in water bath temperature (37 °C) with slide immersion.

2.4. Histochemical procedures

2.4.1. Active and total GP histochemistry

Incubation medium for aGP histochemistry was prepared fresh. It consisted of 45 ml of 0.1 M sodium acetate buffer (pH 5.6) to which was added 100 mg disodium ethylenediaminetetra-acetate, 80 mg sodium fluoride, 2 g dextran, and 400 mg α-D-glucose-1-phosphate (disodium salt). The resulting solution was adjusted to pH 6.0 and brought to 50 ml with distilled H_2O. For tGP slides, the same medium was prepared with the addition of 40 mg of AMP. The medium was placed in a water bath at 37 °C for at least 1 h prior to slide incubation. Slides were immersed in the following solutions consecutively: incubation medium (30 min; note this step was omitted for unincubated slides), 95% ethanol (3 min), Lugol's iodine (4 min), and physiological saline (5 s). Slides were allowed to air dry for at least 15 min following immersion in incubation medium and ethanol. Following immersion in Lugol's iodine, slides were placed in physiological saline to remove excess iodine. Slides were then air-dried overnight prior to coverslipping with Microkit. This protocol is a minor variation of that described in Woolf et al. [48].

2.5. Relative optical density measurements

The MCID system (BRS v. 1.3 software, St. Catherine's, Ontario) was used to collect images from slides and take relative optical density (ROD) measurements. Chimeric