Neuroscience Research 63 (2009) 95–99

Contents lists available at ScienceDirect

Neuroscience Research

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

Calmodulin and guanylyl cyclase inhibitors block the *in vivo* expression of gLTP in sympathetic ganglia from chronically stressed rats

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ARTICLE INFO

Article history:
Received 7 August 2008
Received in revised form 8 October 2008
Accepted 24 October 2008
Available online 7 November 2008

Keywords:
Calmodulin
W-7
Guanylate cyclase
LY-83583
Calmidazolium
gLTP
SCG

ABSTRACT

Previous work from this laboratory indicated that superior cervical ganglia from rats exposed to chronic psychosocial stress expressed ganglionic long-term potentiation (gLTP) *in vivo*. In the present study, we report additional pharmacological evidence indicating involvement of calmodulin and guanylyl cyclase in gLTP, and supporting the *in vivo* gLTP expression in ganglia from chronically stressed rats. Pretreatment with the calmodulin inhibitors W-7 (5 μM) or calmidazolium (5 μM) or with guanylyl cyclase inhibitor LY-83583 (5 μM) completely blocked HFS (20 Hz/20 s)-induced gLTP in superior cervical ganglia isolated from normal rats. Along with that, inhibition of apparent basal ganglionic transmission by W-7 (5 μM), calmidazolium (5 μM) or LY-83583 (5 μM) is observed in ganglia isolated from chronically stressed rats, but not in those from control rats, indicating *in vivo* expression of gLTP in ganglia isolated from stressed rats. The present results confirm the involvement of both calmodulin and GC activities in gLTP, and indicate that ganglia from stressed rats may have expressed gLTP *in vivo*, which is known to precipitate hypertension in these animals.

1. Introduction

Ganglionic long-term potentiation (gLTP) is a serotonin-dependent (Alkadhi et al., 1996), protracted enhancement of the nicotinic pathway that has been demonstrated in sympathetic ganglia both *in vivo* (Alonso-deFlorida et al., 1991; Bachoo et al., 1992; Bachoo and Polosa, 1992) and *in vitro* (Brown and McAfee, 1982; Briggs et al., 1985; Minota et al., 1991; Alkadhi et al., 1996, 2001a,b, 2005b; Alkadhi and Altememi, 1997; Gerges et al., 2002; Alzoubi et al., 2004). The gLTP manifests as long-term enhancement of evoked potential recorded from the postganglionic nerve fibers. Expression of gLTP is independent of activation of cholinergic, adrenergic (Briggs et al., 1985) or adenosine (Hogan et al., 1998) receptors and does not involve changes in acetylcholine (ACh) content of the ganglion (Briggs et al., 1985) or in the sensitivity of nicotinic ACh receptors (Briggs and McAfee, 1988; Briggs et al., 1988). Expression of gLTP is dependent upon activation of 5-HT_3 receptors (Alkadhi et al., 1996) by serotonin that is probably released by high frequency stimulation (HFS) from intracellular structures that may include serotonin-containing small intensely florescent (SIF) cells within the ganglion (Hadjiconstantinou et al., 1982).

The superior cervical ganglion (SCG) has been extensively used in the study of various aspects of synaptic transmission including synaptic plasticity. Most of the input to the SCG is provided by the cervical sympathetic preganglionic nerve fibers, which emerge through the ventral roots of the first to seventh thoracic nerves originating from cholinergic preganglionic neurons in the thoracic spinal cord. Two major postganglionic nerves emanate from the ganglion, the external and internal carotid nerves. In addition to principal neurons, the ganglion contains SIF cells that have catecholamines (mainly dopamine), serotonin (5-HT), substance P, endogenous opiates or histamine (Eränkö et al., 1986, for review).

Since $5HT_3$ receptor is highly permeable to calcium (Nichols and Mollard, 1996), it is possible that activation of the $5HT_3$ cationic channel–receptor complex in the ganglion creates a medium for a focused influx of calcium to increase its intracellular level to that required for activation of upstream enzymes, including calmodulin and calcium-calmodulin kinase II (CaMKII), needed for expression of gLTP. In addition, many lines of evidence suggest the involvement of nitric oxide (NO) (Sheng et al., 1993; Lin and Bennett, 1994; Alkadhi and Altememi, 1997; Altememi and Alkadhi, 1999) and carbon monoxide (CO) (Alkadhi et al., 2001a) in the expression of gLTP in mammalian sympathetic and avian ciliary ganglion. Nitric oxide, which is required for the maintenance phase of gLTP, activates guanylyl cyclase (GC), producing

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doi:10.1016/j.neures.2008.10.011

cyclic-guanine monophosphate (cGMP) in a cascade of events leading to expression of gLTP. Activation of GC leads to the accumulation of cGMP. In nerve cells, cGMP interacts with three classes of proteins. Firstly, it interacts with phosphodiesterases (PDEs), where it activates PDE II (Gs PDE) and inhibits PDE III (Gi PDE) (Beavo et al., 1994). Secondly, cGMP directly gates ion channels in many nerve cells (Schmidt et al., 1993; Bourgeois and Rakic, 1996; Meissirel et al., 1997). Thirdly, cGMP-stimulated protein kinase G (PKG) activates protein phosphatases (Scott, 1991), which through dephosphorylation of the calcium-dependent potassium channel enhance channel opening probability (Bennett, 1994).

Chronic psychosocial stress is associated with the onset and aggravation of ischemic heart disease, and produces a greater rise in blood pressure in patients with labile hypertension than in normotensive subjects (Esler et al., 1977; Boone, 1991; McEwen, 1998). Stress is also related to sustained hypertension and increased risk of coronary heart disease due to enhanced activation of the sympathetic nervous system (Siegrist, 2001). Both genetic and stress-induced hypertension involves a significant neural component. Elevation of blood pressure in chronically stressed rats can be reversibly prevented or normalized by treatment with $5HT_3$ receptor antagonists, which block gLTP (Alkadhi et al., 2005b). Similar results were obtained in spontaneously hypertensive rats (SHR) and obese Zucker rats where a stress component of the hypertension was blocked by $5HT_3$ receptor antagonists (Alkadhi et al., 2001b; Gerges et al., 2002; Alzoubi et al., 2008b). Recently, we have presented evidence indicating stress-generated sustained increase in central sympathetic outflow to ganglia provides repetitive activation of preganglionic nerve, resulting in the expression of gLTP, in vivo, during chronic stress conditions (Alkadhi et al., 2005a,b; Alkadhi and Alzoubi, 2007; Alzoubi et al., 2008a,b). In this study, we investigated the effect of calmodulin or GC inhibitors on basal as well as activity-dependent (gLTP) synaptic transmission in ganglia isolated from normal as well as chronically stressed rats.

2. Materials and methods

2.1. Animals

All animal experiments were carried out in accordance with the NIH Guides for care and use of laboratory animals and approved by the university of Houston's Institutional Animal Care and Use Committee. Adult male Wistar rats (Harlan, Indianapolis, IN) weighing 350–400 g were housed on a 12:12 h light/dark schedule (lights on at 7 a.m.) in Plexiglas cages (6 rats per cage) at 25 °C with *ad libitum* access to standard rodent chow and water. All rats were allowed 1 week after arrival at the research facility before manipulations began.

2.2. Drugs

Drugs were dissolved in Locke solution and superfused on ganglia using a peristaltic pump. LY-83583 and W-7 were obtained from Research Biochemicals International (Natick, MA). Calmidazolium was purchased from Sigma–Aldrich Biochemical's (Natick, MA).

2.3. Induction of psychosocial stress as a model of in vivo gLTP expression

Psychosocial stress was induced using an intruder method (Gerges et al., 2001, 2003; Alkadhi et al., 2005b). Two groups of animals were housed in two cages. Each group remained with the same cage mates for at least 1 week to allow animals to establish a social hierarchy within each group. The stress procedure was generated by daily random switching of two animals from one cage to the other for a period of 4–6 weeks. This switching disrupts the social hierarchy, therefore, rats need to continually adapt to new stressful situations. The procedure is known to produce stress; indicated by a significant increase in blood pressure measured directly from the aorta or by using tail-cuff (Szilagyi, 1991; Alkadhi and Alzoubi, 2007; Alkadhi et al., 2005b) and a 50% increase in blood corticosterone level (Gerges et al., 2001).

2.4. Electrophysiological recording from isolated superior cervical ganglion

Superior cervical ganglia (SCG) were rapidly dissected out and carefully desheathed in oxygenated (95% O_2, 5% CO_2) Locke solution (pH 7.4; NaCl 136 mM, KCl 5.6 mM, $CaCl_2$ 2.2 mM, $MgCl_2$ 1.2 mM, NaH_2PO_4 1.2 mM, $NaHCO_3$ 16 mM, glucose 11 mM) under a microscope. The ganglion was placed in a constant temperature (32 + 1 °C) chamber (3 ml) and continuously superfused (1.3 ml/min) with Locke solution. The cervical sympathetic preganglionic and the internal carotid postganglionic nerves were drawn into capillary stimulating and recording bipolar suction electrodes respectively. Square wave supramaximal test stimuli (duration, 0.3 ms) at 0.017 Hz were used to evoke compound action potentials (CAPs) using a Grass S-88 stimulator. Amplified CAPs were visualized on an oscilloscope as well as on the monitor of a computer based acquisition system (Digidata 1322A, Axon Instruments, CA) in conjunction with pclamp 8.2 software (Axon Instruments, CA). After stabilization for about 30 min, baseline CAPs were recorded for 20 min and gLTP was evoked by a train of high frequency stimuli (HFS; 20 Hz/20 s). Then test stimuli were resumed and CAPs were recorded and averaged every 2 min for the first 10 min and every 5 min thereafter. Changes in CAP amplitude were expressed as percentage of baseline CAP recoded before the train.

2.5. Statistical analysis

Unpaired *t*-test was used to compare groups. Significant difference was set at p values set at <0.05. All values are represented as mean ± S.E.M.

3. Results

3.1. Inhibition of HFS-induced gLTP by calmodulin inhibitors

In untreated ganglia, HFS evoked a well-maintained, robust gLTP. However, in ganglia treated with the calmodulin inhibitor W-7 or calmidazolium, HFS failed to induce gLTP. The drugs prevented the induction of gLTP and partially blocked post-tetanic potentiation (Fig. 1A and B). One hour after HFS, the amplitude of CAP in ganglia superfused with W-7 or calmedazolium were 102.7 ± 4.4% (Fig. 1A; $n = 6$), 101.2 ± 7.2% (Fig. 1B; $n = 7$) of baseline CAP, respectively, compared to 131.3 ± 6.1% ($n = 10$) in untreated ganglia. Removal of the drugs by washing for 25–30 min did not reverse the effects of these agents, which suggested that inhibition of calmodulin, prevented induction of gLTP.

3.2. Effect of guanylyl cyclase (GC) inhibitor on HFS-induced gLTP

To ascertain the involvement of GC in the mechanism responsible for gLTP, we tested the effect of pretreatment with the GC inhibitor LY-83853 on the expression of gLTP in the SCG. One hour after HFS, the amplitude of CAP in ganglia superfused