Brain Research 1649 (2016) 38–43

Contents lists available at ScienceDirect

Brain Research

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

Research Report

Chronic phenytoin treatment reduces rat carotid body chemosensory responses to acute hypoxia

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ARTICLE INFO

Article history:
Received 27 May 2016
Received in revised form
16 August 2016
Accepted 20 August 2016
Available online 21 August 2016

Keywords:
Phenytoin
Carotid body
Chemosensory activity
Petrosal ganglion
Peripheral ventilatory drive

ABSTRACT

Ventilation is peripherally controlled by afferent activity arising from the peripheral chemoreceptors. In the rat, chemosensory activity is conveyed to the central nervous system through axons of neurons located in the nodose-petrosal-jugular-complex. These neurons have distinct electrophysiological properties, including a persistent Na^+ current. Acute blockade of this current with phenytoin and other anti-epileptic drugs reduces normoxic chemosensory activity and responses to acute hypoxia. However, because anti-epileptic therapy is prolonged and there is no information on the effects of chronic phenytoin treatment on peripheral chemosensory activity, we studied the effects of long-lasting phenytoin treatment (~25 days) on afferent chemosensory activity, on a wide range of oxygen inspiratory fractions. Osmotic pumps containing dissolved phenytoin (166 mg/mL) or vehicle (daily flow: 60 μL) were implanted subcutaneously in male adult Sprague Dawley rats. At the end of the treatment, the animals were anesthetized and carotid sinus nerve activity was recorded in vivo. Afferent chemosensory activity in normoxia was not significantly different between control (71.2 ± 2.2 Hz) and phenytoin treated (95.4 ± 2.1 Hz) rats. In contrast, carotid body chemosensory responses to acute hypoxic challenges were markedly reduced in phenytoin treated rats, specifically in the lowest part of the hypoxic range (control 133.5 ± 18.0 Hz vs phenytoin treated 50.2 ± 29.4, at 5% F_IO_2). Chronic phenytoin treatment severely impaired the chemosensory responses to acute hypoxia, suggesting that long-term phenytoin treatment in patients may result in a reduced peripheral respiratory drive together with a reduction in the respiratory responses to hypoxic challenges.

1. Introduction

Phenytoin (5,5-diphenylimidazolidinedione; 5,5-diphenylhydantoin) is an anti-epileptic drug widely used for the treatment of epilepsy, which is generally treated with pharmacological agents intended to reduce neuronal excitability. Phenytoin is a Na^+ channel blocker that has no effect on fast activation and inactivation kinetics, responsible for the transient Na^+ current (I_{NaT}), but blocks the persistent Na^+ current (I_{NaP}), stabilizing the Na^+ channel in a non-conducting state (Catterall, 1999; Kuo and Bean, 1994) by accelerating its slow inactivation kinetics (Quandt, 1988; Colombo et al., 2013). The ability of phenytoin to block the I_{NaP} contributes to its anticonvulsant effect by decreasing membrane excitability and preventing the spread of the aberrant electrical activity from epileptogenic regions. Despite the known effects of phenytoin on epilepsy pathophysiology, less is known about its side effects on respiratory control. Donnelly and colleagues showed that acute treatment with phenytoin severely impaired normoxic ventilation and the hypoxic ventilatory response in rats (Faustino and Donnelly, 2006, 2006a). Furthermore, the same authors suggested that the carotid body chemoafferent pathway is a primary site of action of phenytoin. The carotid body (CB) is the main arterial chemoreceptor that peripherally drives ventilation in mammals. Afferent activity is generated by the synaptic drive of sensory nerve terminals of petrosal ganglion neurons by transmitters released by the CB receptor (glomus, Type-I) cells (Gonzalez et al., 1994; Iturriaga and Alcayaga, 2004; Nurse, 2014; Prabhakar, 2000). The generated afferent activity is conveyed to the central nervous system through sensory fibers of the

Abbreviations: I_{NaT}, transient Na^+ current; I_{NaP}, persistent Na^+ current; CB, carotid body; F_IO_2, oxygen inspiratory fraction; f_x, chemosensory discharge frequency; Δf_x, changes in chemosensory discharge frequency

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http://dx.doi.org/10.1016/j.brainres.2016.08.027
0006-8993/

glossopharyngeal nerve, projecting to the nucleus of the solitary tract. Although the transmitters released by glomus cells are considered essential in the generation of afferent chemosensory activity (Iturriaga and Alcayaga, 2004; Nurse, 2014; Piskuric and Nurse, 2013), the electrical characteristics of petrosal ganglion neurons may also influence the afferent activity (Donnelly et al., 1998). These neurons present fast action potentials with almost no overshoot, a short duration after hyperpolarization and they discharge tonically when depolarized by long-lasting intracellular current pulses (Donnelly, 1999). In the rat, chemosensory afferent activity is decreased by reduced extracellular Na^+ and TTX (Donnelly et al., 1998), and petrosal ganglion neurons present TTX-sensitive sodium currents, both transient and I_{NaP} types (Faustino and Donnelly, 2006a). Interestingly, acute inhibition of I_{NaP} by phenytoin reduces both basal discharge in normoxia and the maximal discharge induced by acute reduction of the oxygen inspiratory fraction (F_IO_2; from 21% to 12%) in identified chemosensory neurons in vitro (Faustino and Donnelly, 2006, 2006a). Thus, acute phenytoin treatment markedly alters CB-chemoafferent hypoxic responses. In contrast to what is known about the effects of acute phenytoin treatment on CB-mediated chemoreflex responses, no information is available about the effects of chronic phenytoin administration on CB chemosensory function. Indeed, understanding the outcome of chronic phenytoin treatment on CB function is of potential clinical value since most patients with epilepsy receive chronic prescription of the drug. Thus, the main purpose of this study was to determine in rats the long-lasting effects of phenytoin treatment on CB chemosensory activity at several oxygen levels.

2. Results

Fig. 1 depicts representative electroneurograms obtained from one sham operated rat and one phenytoin treated rat. Under normoxic conditions (F_IO_2=21%) CB chemosensory afferent discharges, over the background noise threshold, were not significantly different between sham and phenytoin treated rats (Fig. 1A, B). Increasing F_IO_2 to 100% for 20 s produced a marked reduction in the afferent activity (Fig. 1A, B), that recovered to

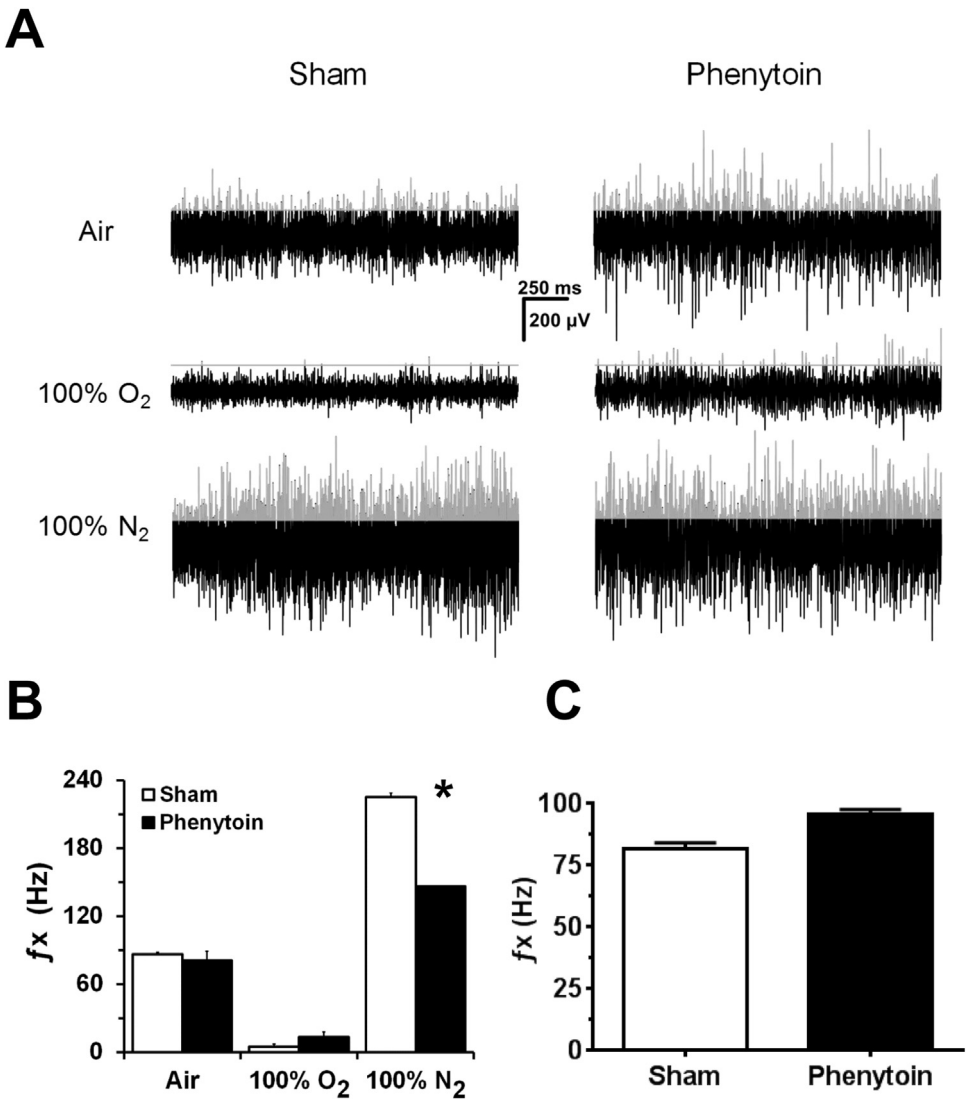

Fig. 1. Chemosensory frequency discharge (f_x) is modified by oxygen inspiratory fraction (F_IO_2) and phenytoin treatment. A) Chemosensory discharges recorded from the carotid nerve of one sham and one phenytoin treated rat are reduced in hyperoxia (100% O_2) and increased in hypoxia (100% N_2). Gray lines indicate the threshold for spike recognition and counted spikes. B) Mean chemosensory frequency discharges (f_x) computed in two consecutive one second intervals, from the upper recordings, during normoxia (Air; F_IO_2=21%), hyperoxia (F_IO_2=100% O_2) and hypoxia (F_IO_2=0%) in the sham (empty bars) and the phenytoin treated (filled bars) rat. * $P < 0.05$, Student´s *t*-test. C) Mean chemosensory frequency discharges in normoxia, computed during 5 normoxic intervals (30 s) on each animal prior to a change in F_IO_2, are not significantly different ($P > 0.05$, two way repeated measures ANOVA) between sham (n =9; empty bars) and phenytoin treated (n =7; filled bars) rats.