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Research Report

Altered expression of adenosine A_1 and A_{2A} receptors in the carotid body and nucleus tractus solitarius of adult male and female rats following neonatal caffeine treatment

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ABSTRACT

Neonatal caffeine treatment (adenosine receptor antagonist, 15 mg/kg/day, between postnatal days 3 and 12) affects respiratory patterns in adult male but not female rats as shown by an increase in the respiratory frequency in the early phase of response to hypoxia and an increase in the tidal volume in the late phase of response. Here, we tested the hypothesis that these changes are correlated with modified expression of adenosine receptors in the chemoreflex pathway. Carotid bodies, nucleus tractus solitarii, and superior cervical ganglia were collected from 3-month-old male and female rats that were either naive (not manipulated during the neonatal period) or treated with caffeine (NCT) or water (NWT) between postnatal days 3 and 12 by gavage. Western blot analysis was used to assess the expression of adenosine A_1 and A_{2A} receptors and tyrosine hydroxylase, the rate-limiting enzyme for dopamine synthesis. In male rats, there was a 37% increase in the level of A_{2A} receptor and a 17% decrease in tyrosine hydroxylase in the carotid body of NCT ($p<0.001$) as compared to NWT rats. In the nucleus tractus solitarius, we found a 13% and 19% decrease in A_1 receptor expression in NWT and NCT rats ($p<0.01$), respectively, compared to naive rats. In the superior cervical ganglion, there was no change in A_1 receptor, A_{2A} receptor, and tyrosine hydroxylase expression. In female rats, the only changes observed were decreases of 12% and 15% in A_1 receptor levels in the nucleus tractus solitarius of NWT and NCT rats ($p<0.01$), respectively, compared to naive rats. We conclude that NCT induces long-term changes in the adenosine receptor system. These changes may partially explain the modifications of the respiratory pattern induced by NCT in adults. The increased expression of the adenosine A_{2A} receptor (specific to male rats), combined with the decreased tyrosine hydroxylase expression in the carotid body, suggests that NCT affects adenosine–dopamine interactions regulating chemosensory activity.

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1. Introduction

To alleviate apnea, which is the most common respiratory disorder during the neonatal period in premature babies, caffeine is frequently used as an antagonist of adenosine A_1 and A_{2A} receptors (Fredholm et al., 2001a; Fredholm et al., 1999) to simulate breathing (Comer et al., 2001; Marchal et al., 1987). Such treatment does not seem to affect the neurodevelopmental outcome of 2-year-old infants (Schmidt et al., 2007). However, adult rats receiving caffeine for 10 consecutive days during the neonatal period (NCT; 15 mg/kg/day from postnatal days 3 to 12, P3–P12) had significant changes in respiratory pattern at baseline and in response to hypoxia (12% O_2) in comparison to vehicle (water) treated rats (NWT). These effects are sex-specific, as they are observed in male but not female rats, and are characterized by an increase in respiratory frequency response during the initial phase and an increase in tidal volume during the late phase of hypoxic exposure (Bairam et al., 2009; Montandon et al., 2008a). These physiological changes were associated with an increase in the mRNA levels of adenosine A_{2A} (but not A_1) receptors, dopamine D2 receptors, and tyrosine hydroxylase in the carotid body of male rats; in females, there was only a modest increase in A_{2A} receptor mRNA (Montandon et al., 2008a). However, it is still undetermined whether these modifications of gene expression are associated with changes in the final protein levels and whether similar changes also occur in central respiratory control areas. Using western blot analysis, this study evaluates in adult male and female rats the effects of NCT on protein levels of adenosine A_1 and A_{2A} receptors at the level of peripheral (carotid body) and central (nucleus tractus solitarius, a key region integrating the chemosensory fibers of the carotid sinus nerve (Housley et al., 1987; Housley and Sinclair, 1988; Finley and Katz, 1992) parts involved in respiratory control. Tyrosine hydroxylase, a key enzyme of dopamine synthesis, was also studied as an indirect indicator of adenosine–dopamine interaction (Franco et al., 2007; Fredholm et al., 2007; Fuxe et al., 2007). The superior cervical ganglion providing sympathetic innervation to the carotid bodies was also studied as a reference for peripheral, non-chemosensitive tissue.

2. Results

2.1. *Identification of adenosine A_1 and A_{2A} receptors*

Since no data are available in the literature concerning the expression level of adenosine receptors in chemoreflex organs

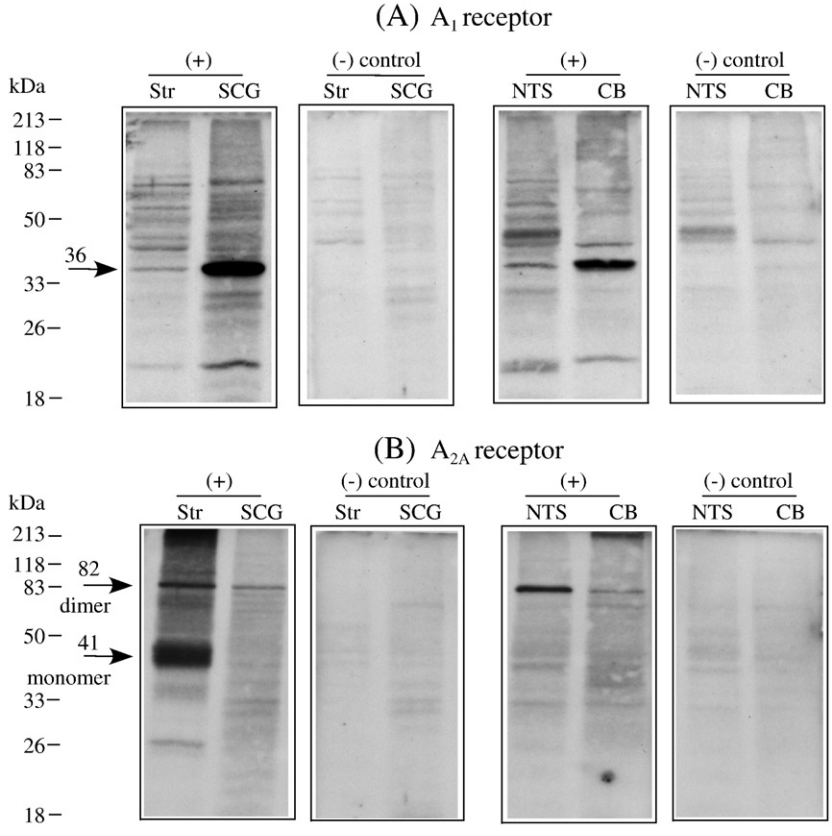

Fig. 1 – Representative western blots for adenosine A_1 and A_{2A} receptors in the striatum (Str), superior cervical ganglion (SCG), nucleus tractus solitarius (NTS), and carotid body (CB). Membrane protein was immunoblotted with specific antibodies for each receptor (Table 2). The striatum was arbitrarily chosen as the central reference tissue for technical purposes to determine the presence, molecular weight, and specific type of each protein tested. The adenosine A_1 receptor is detected at 36 kDa in all organs studied. For the adenosine A_{2A} receptor, both the dimeric and monomeric forms are detected in the striatum, while only the dimeric form is detected in the SCG, NTS, and CB. Negative control experiments that were performed using either peptide antigen (for A_1) or by omitting the primary antibodies (for A_{2A}) showed no signal at the expected molecular weight of each receptor.