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Age-related changes in cerebellar phosphatase-1 reduce Na,K-ATPase activity

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Abstract

We evaluated whether changes in protein content and activity of PP-1 and PP-2A were the mechanism underneath the basal age-related reduction in $\alpha_{2/3}$-Na,K-ATPase activity in rats cerebella and whether this occurred through the cyclic GMP-PKG pathway. PP1 activity, but not its expression, increased with age, whereas PP-2 was not changed. The activity of $\alpha_{2/3}$-Na,K-ATPase varied with age, and there was a negative association between the PP-1 and $\alpha_{2/3}$-Na,K-ATPase activities. In young rats, the inhibition of PP-1 and PP-2A by okadaic acid (OA) increased in a dose-dependent manner α_1- and $\alpha_{2/3}$-Na,K-ATPase, but had no effect on Mg-ATPase activity. A direct stimulation of PKG with 8-Br-cyclic GMP did not surmount the effect of OA. This analogue of cyclic GMP inhibited PP-1 activity only, indicating that at least part of the increase in α_1- and $\alpha_{2/3}$-Na,K-ATPase activity induced by OA was mediated by the cyclic GMP-PKG-PP-1 cascade. Taking into account that PP1 inhibition increased $\alpha_{2/3}$-Na,K-ATPase activity, we propose that an age-related increase in PP-1 activity due to a decrease in cyclic GMP-PKG modulation plays a role for the age-related reduction of $\alpha_{2/3}$-Na,K-ATPase activity in rat cerebellum.

Keywords: Glutamate; Phosphatase; Na,K-ATPase; Cyclic GMP

1. Introduction

Three isoforms of the α-subunit of rat cerebellar Na,K-ATPase have been described, and they can be functionally differentiated by ouabain (Sweadner, 1989). The α_1-Na,K-ATPase isoform is thousand times less sensitive to the cardiac glycoside than the $\alpha_{2/3}$ isoform. Recently, we demonstrated that age-related decline in $\alpha_{2/3}$-Na,K-ATPase activity in rat cerebellum is a result of changes in the cyclic GMP-PKG pathway (Scavone et al., 2005). We observed that the amount of protein ($\alpha_{1,2,3}$-Na,K-ATPase) is not changed between 4, 12 and 24 month old rats, whereas the enzyme activity is progressively reduced. Na,K-ATPase is responsible for maintaining the electrochemical gradient of sodium and potassium ions and plays an important role in the regulation of ionic homeostasis in tissue and cells (Beal et al., 1993). This enzyme can also act as a signal transducer and a transcription activator by interacting with neighboring membrane

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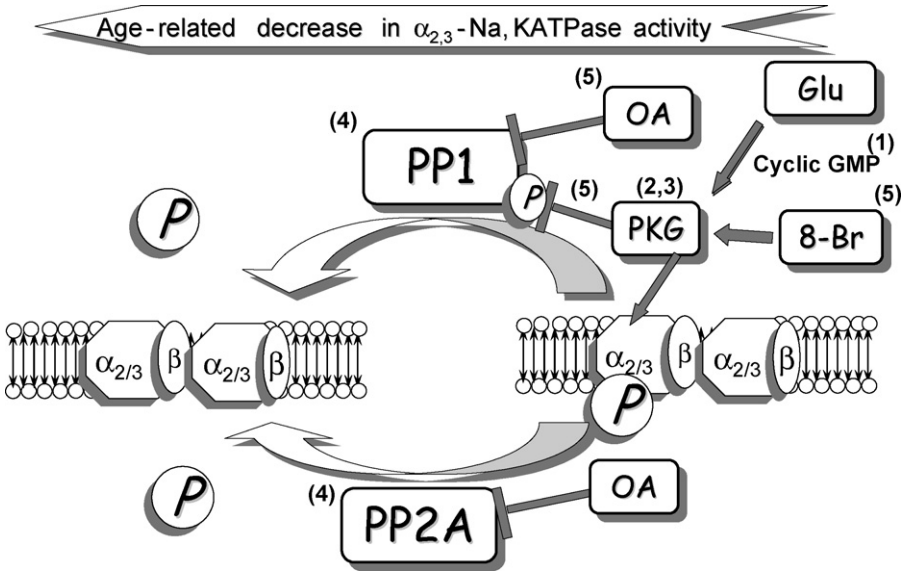

Fig. 1. Simplified model of modulation of Na,K-ATPase by cyclic GMP-PKG cascade and protein phosphatase (PP) in rat cerebellum and its implications in aging process. Age-related change in the regulation of cerebellum $\alpha_{2/3}$-Na,K-ATPase activity. Glutamate activates Na,K-ATPase through cyclic GMP-PKG pathway. Basal activity of $\alpha_{2/3}$-Na,K-ATPase is reduced due to an impairment of cyclic GMP production (1), as well as a reduction in its ability to activate PKG pathway (2), while glutamate stimulation of cerebellar $\alpha_{2/3}$-Na,K-ATPase is reduced due to an impairment at the PKG signaling (3). In this study we test if age-related changes in cerebellum PP-1 and/or PP-2A tissue activity and/or content could be the mechanism underneath the age-related reduction in $\alpha_{2/3}$-Na,K-ATPase cerebellar activity (4). In addition, we also evaluated if the activity of cerebellar $\alpha_{2/3}$-Na,K-ATPase can be regulated by PP-1and/or PP2A through the cyclic GMP–PKG pathway by using OA, an inhibitor of PP, and/or 8-Br-cyclic GMP, an activator of PKG (5).

proteins and organized cytosolic cascades of signaling proteins (Xie and Askari, 2002). Aging-related reduction in Na, K-ATPase activity results in partial membrane depolarization in some tissues and increases production of reactive oxygen species (Mattson and Liu, 2002). Specially in neurons, the decrease in the sodium pump activity may be the cause of many age-related changes and some neurodegenerative processes.

The activity of Na,K-ATPase has been shown to be partially regulated by a mechanism dependent on cyclic AMP, cyclic AMP-dependent protein kinase (PKA), as well as, cyclic GMP and PKG (Greengard et al., 1998; Mckee et al., 1994). Actually, nitric oxide (NO) signaling, as well as carbon monoxide (CO), occurs through the activation of soluble guanylyl cyclase and therefore the activation of the cyclic GMP-PKG pathway, which among several actions inhibits protein phosphatase (PP) activity (Beltowski et al., 1998; De Oliveira Elias et al., 1999; Eklof et al., 2001; Liang and Knox, 1999; Mckee et al., 1994; Scavone et al., 1995; Syrén, 1997). Our previous studies showed that aged-induced decrease in basal cerebellar $\alpha_{2/3}$-Na,K-ATPase activity is a consequence of the decrease in cyclic GMP-PKG signaling (Scavone et al., 2005). This pathway enhances the sodium pump activity either by a direct phosphorylation of some isoforms of Na,K-ATPase or by a decrease in PP activity (Beltowski et al., 1998; De Oliveira Elias et al., 1999; Eklof et al., 2001; Liang and Knox, 1999; Mckee et al., 1994; Scavone et al., 1995; Syrén, 1997) (Fig. 1). PP-1 and PP2A isoforms are immunohistochemically localized in Purkinje cell (Hashikawa et al., 1995), therefore they are putative targets for changes related to aging (Fig. 2).

The first aim of this work was to verify whether age-related changes in protein content and activity of PP-1 and PP-2A were the mechanism underneath the basal age-related reduction in $\alpha_{2/3}$-Na,K-ATPase activity in rats cerebella. In addition, we also evaluated the relationship between PP-1 regulation by cyclic GMP-PKG pathway upon $\alpha_{2/3}$-Na,K-ATPase activity by using OA, an inhibitor of PP, and 8-Br-cyclic GMP, an activator of PKG (Fig. 1).

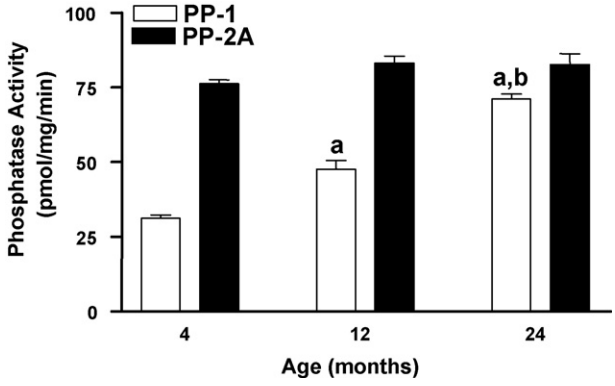

Fig. 2. Age-related change in the activity of PP1 and PP-2A in rat cerebellum. PP-1 and PP-2A activities were measured in tissue homogenates in the absence (basal) of drugs. Values shown are the mean ± S.E.M. of three individual experiments. $^{a}p < 0.05$ vs. 4-month-old basal sample, $^{b}p < 0.01$ vs. 4 and 12-month-old sample (one-way ANOVA followed by Newman–Keuls test).