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Research Report

The selective A_{2A} receptor antagonist SCH 58261 protects from neurological deficit, brain damage and activation of p38 MAPK in rat focal cerebral ischemia

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ABSTRACT

We investigated the protective effect of subchronic treatment of the A_{2A} receptor antagonist, SCH 58261 (0.01 mg/kg, i.p.), administered 5 min, 6 h and 15 h after permanent right middle cerebral artery occlusion (MCAo). Twenty-four hours after ischemia, an extensive pallid area, evaluated by cresyl violet staining, is evident in the vascular territories supplied by the MCA, the striatum and the sensory motor cortex. The pallid area reflects the extent of necrotic neurons. Soon after waking, rats showed a definite contralateral turning behavior which was significantly reduced by SCH 58261 treatment. Twenty-four hours after MCAo, SCH 58261 significantly improved the neurological deficit and reduced ischemic damage in the striatum and cortex. Phospho-p38 mitogen-activated protein kinase (MAPK), evaluated by Western Blot, increased by 500% in the ischemic striatum 24 h after MCAo. SCH 58261 treatment significantly reduced phospho-p38 MAPK by 70%. Microglia was immunostained using the OX-42 antibody. Phospho-p38 MAPK and OX-42-immunoreactive cells are localized in the ventral striatum and frontoparietal cortex. Furthermore, both OX-42 and phospho-p38 MAPK-immunoreactive cells have overlapping morphological features, typical of reactive microglia. SCH 58261 reduced phospho-p38 MAPK immunoreactivity in the striatum and in the cortex without changing the microglial cell morphology. These results indicate that the protective effect of the adenosine antagonist SCH 58261 during ischemia is not due to reduced microglial activation but involves inhibition of phospho-p38 MAPK and suggest that treatment with the A_{2A} antagonist from the first hour to several hours after ischemia may be a useful therapeutic approach in cerebral ischemia.

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1. Introduction

Adenosine is an important transmitter in the central nervous system (CNS) (Fredholm et al., 2005; Latini and Pedata, 2001; Stone, 2002). Its tissue levels increase dramatically during ischemia as a consequence of energy metabolism failure (Rudolphi et al., 1992). Of the four so far identified receptors, A_1, A_{2A}, A_{2B} and A_3 (Fredholm et al., 1994), evidence indicates

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that the A_{2A} receptor plays an important role in ischemia. Although adenosine is a metabolic regulator of cerebrovascular resistance and may contribute to cerebral vasodilation acting on this receptor (Phillis, 2004), most evidence indicates that A_{2A} antagonists are protective in different animal models of cerebral ischemia (Bona et al., 1997; Gao and Phillis, 1994; Monopoli et al., 1998b; von Lubitz et al., 1995) and excitotoxicity (Jones et al., 1998a,b). Indeed, A_{2A} knockout mice are protected against cerebral infarction and against the neurological outcome of focal ischemia (Chen et al., 1999).

We have recently demonstrated (Melani et al., 2003) that in the focal ischemia model induced by right medial cerebral artery occlusion (MCAo), the A_{2A} selective antagonist SCH 58261, administered i.p. 5 min after MCAo, significantly reduces ischemia-evoked glutamate and aspartate outflow from the striatum and completely inhibits acute motor disturbance characterized by contralateral turning to the ischemic side during the 4 h after ischemia. Moreover, 24 h after ischemia, SCH 58261 reduces the infarct volume in the cortex, without improving neurological outcome (Melani et al., 2003). Protection from lesion size is not always correlated with protection from neurological deficit in different rodent models (Rogers and Hunter, 1997). Nevertheless, both improvement of functional impairment and recovery of neurological outcome are important corollaries for assessing the putative neuroprotective effect of drugs aimed at predicting clinical studies that use measures of functional and neurological outcome.

Although most evidence indicates that A_{2A} antagonists are protective during ischemia by reducing the excitotoxic cascade initiated by NMDA receptor stimulation, other mechanisms may also account for their protective effects. Excitotoxicity is an early event after focal ischemia followed, over hours or days, by microglia cell activation which leads cell phenotypic changes (Bruce-Keller, 1999). Microglia cells start to proliferate and migrate toward the site of damage (Marks et al., 2001; Stoll et al., 1998), and, by production of cytotoxic substance and cytokines, they may contribute to the inflammatory response that follows ischemic insult, thus aggravating brain damage (see Dirnagl et al., 1999). Besides neurons, adenosine A_{2A} receptors are located on microglia cells (Fiebich et al., 1996) and astrocytes (Lee et al., 2003) where they can modulate production of proinflammatory and inflammation products (Brodie et al., 1998; Fiebich et al., 1996; Flavin and Ho, 1999).

Adenosine, activating different intracellular signalling pathways depending on the receptor subtype, may stimulate both cell survival (Fredholm, 1997; Neary, 1996; Vitolo et al., 1998) and death (Jacobson et al., 1999). Activation of adenosine A_{2A} receptors increases intracellular cAMP with ensuing protein kinase A activation (Gubitz et al., 1996), stimulates phosphatidylinositol, with ensuing protein kinase C activation and increases intracellular Ca^{++} (Goncalves et al., 1997; Gubitz et al., 1996). The intracellular pathways that may be involved in neuronal survival, brought about by adenosine A_{2A} antagonism during ischemia, are still to be fully elucidated. Recent reports demonstrate that members of the adenosine receptor family stimulate or inhibit in different tissues or cell preparations mitogen-activated protein kinases (MAPKs) (for review, see Schulte and Fredholm, 2003). MAPKs comprise four subfamilies, the extracellular-signal regulated protein kinases (ERK1/2 or p44/42 MAPK), c-jun N-terminal kinases (JNKs or SAPKs), p38 MAPK (Koistinaho and Koistinaho, 2002) and big MAPK 1 or ERK5 (BMK1/ERK5) (Zhou et al., 1995), which are signal transduction pathways that serve several different functions at the cellular level.

Activation of MAPKs is reported during ischemia (see Nozaki et al., 2001). In particular, following focal cerebral ischemia in the rat, activation of both ERK and p38 MAPK up to 24 after ischemia was reported (Irving et al., 2000). While most evidence indicates that ERK activation is associated with the regulation of cell proliferation and differentiation (see Tibbles and Woodgett, 1999), JNK and p38 MAPK activations are involved in responses to environmental stress, cell suffering and death (Kummer et al., 1997; Takeda and Ichijo, 2002; Xia et al., 1995). In regard to p38 MAPK, data agree on protection by p38 inhibition against focal ischemia-induced infarct, neurological deficit and expression of inflammatory cytokines (Barone and Feuerstein, 1999; Barone et al., 2001).

In this study, the selective adenosine A_{2A} antagonist SCH 58261 was subchronically administered up to 15 h after focal ischemia induced by MCAo by the suture technique (Longa et al., 1989; Melani et al., 1999). We report that the A_{2A} antagonist significantly protects against neurological deficit, infarct size and activation of p38 MAPK.

2. Results

2.1. *Animal survival rate*

The mortality at 24 h after MCAo was 3 of 16 vehicle-treated rats (18.7%) and 2 of 16 SCH 58261-treated rats (12.5%). No mortality was observed in sham-operated rats 24 h after operation.

2.2. *Subchronic administration of SCH 58261 improved the neurological deficit and antagonized turning behavior induced by MCAo*

The neurological score of vehicle-treated, SCH 58261-treated and sham-operated rats is reported in Table 1. Sham-operated rats had a neurological score of 17.5. The neurological score of MCAo vehicle-treated rats was significantly reduced (by 50%) in comparison to sham-operated rats, confirming our previous results (Melani et al., 1999, 2003). SCH 58261, subchronically administered intraperitoneally 5 min, 6 h and 15 h after

Table 1 – Effect of SCH 58261 (0.01 mg/kg, i.p., 5 min, 6 h and 15 h after occlusion) on neurological deficit evaluated 24 h after permanent MCAo

Treatment	Neurological score
Vehicle (n = 13)	8.8 ± 0.5*,†
SCH 58261 (n = 14)	10.8 ± 0.4*
Sham operated (n = 12)	17.5 ± 0.2

Data are the mean ± SE of "n" rats. Number of rats in parenthesis. One-way ANOVA followed by Newman–Keuls multiple comparison test: *$P < 0.001$ vs. sham-operated rats. †$P < 0.001$ versus SCH 58261-treated rats.

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