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Protection against ischemic damage by adenosine amine congener, a potent and selective adenosine A_1 receptor agonist

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Abstract

Although the selectivity and potency of adenosine amine congener (ADAC) at adenosine A_1 receptors are similar to other highly selective agonists at this receptor type, the chemical structure of the N^6 substituent is completely different. We now demonstrate that the characteristics of the therapeutic profile of ADAC are distinct from those observed during our previous studies of adenosine A_1 receptor agonist-mediated neuroprotection. Most significantly, chronic treatment with low microgram doses of ADAC (25–100 μg/kg) protects against both mortality and neuronal damage induced by 10 min bilateral carotid occlusion in gerbils. At higher chronic doses, the statistical significance of the protective effect is lost. Acute preischemic administration of the drug at 75–200 μg/kg also results in a statistically significant reduction of postischemic mortality and morbidity. These data indicate that, contrary to other adenosine A_1 receptor agonists whose chronic administration enhances postocclusive brain damage, ADAC may be a promising agent in treatment of both acute (e.g., cerebral ischemia) and chronic (seizures) disorders of the central nervous system in which adenosine A_1 receptors appear to be involved.

Keywords: Cerebral ischemia; Adenosine A_1 receptor; Therapy; Gerbil

1. Introduction

Clinical treatment of ischemic brain disorders with agonists of adenosine A_1 receptors has been often advocated in publications describing the positive outcome of their experimental administration (for reviews, see Rudolphi et al., 1992; Von Lubitz et al., 1995a; Jacobson et al., 1996). Yet, despite highly encouraging laboratory results, several factors, e.g., bradycardia and hypotension, mitigated against acute clinical implementation of adenosine A_1 receptor agonists (Williams, 1993). Moreover, the recently described phenomenon of treatment-dependent inversion of the in vivo therapeutic effect, where acute treatment leads to the outcome that is opposite to that seen after chronic exposure (Von Lubitz et al., 1994a,b; De Sarro et al., 1996), warranted equal caution in chronic application of this class of drugs. Interestingly, dependence of the therapeutic outcome on the treatment regimen (i.e., acute vs. chronic) has been encountered during studies involving adenosine A_{2A} and A_3 receptors as well (Von Lubitz et al., 1994b). Recently, several adenosine A_1 agonists unhampered by cardiovascular side effects have been described (Knutsen et al., 1995; Sheardown et al., 1995; Bischofberger et al., 1997). One of these drugs, adenosine amine congener (ADAC), proved to be highly effective in protecting the brain against ischemic damage when administered either pre- or postischemia at doses as low as 100 μg/kg. Moreover, some of the neuroprotective effects of ADAC were manifest even when the drug was administered as late as 12–18 h postischemia (Bischofberger et al., 1997 and unpublished observations). Although the currently available data indicate that ADAC, and possibly other compounds based upon the functionalized congener concept (Jacobson et al., 1985), may offer a very promising ground for the development of agents suitable for

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practical treatment of neurological diseases, it must be remembered that treatment of such disorders necessitates not only acute (e.g., stroke) but also chronic administration (e.g., seizures). Yet, despite a highly encouraging therapeutic profile in experimentally induced cerebral ischemia, the effect of chronic application of ADAC has not been studied. In view of the previously described (Jacobson et al., 1996) regimen-dependent inversion, such information may be exceedingly important when considering ADAC (and related compounds) for clinical use. Therefore, the present study describes the effects of different doses of ADAC administered either acutely or chronically on the recovery following severe cerebral ischemia in gerbils.

2. Materials and methods

2.1. Drug

Adenosine amine congener N^6-[4-[[[(2-aminoethyl)-amino] carbonylmethyl]phenyl]adenosine ADAC)was obtained from Research Biochemicals International (Natick, MA). The drug was dissolved in 20:80 (v/v) mixture of Alkamuls EL-620 (Rhône-Poulenc, Cranbury, NJ) and saline.

2.2. Drug administration

Adenosine amine congener was administered either acutely (15 min prior to ischemia) or chronically (once daily for 60 days, with one drug-free day immediately preceding the occlusion). In the acute treatment regimen, the drug was given at 25, 50, 75, and 200 μg/kg ($N = 20$/group), while in the chronic regimen, daily doses were administered at 10, 20, 25, 50, 75, 100, 150, and 200 μg/kg ($N = 20$/group). The drug was injected i.p. in the volume of 0.15 ml/injection. Controls ($N = 20$) were injected with the equivalent volume of the vehicle.

2.3. Animals and ischemia

Female gerbils (70 g, Tumble Brook Farms, Massachussetts) were used in the study. As in the previous experiments (e.g., Von Lubitz et al., 1996a), the animals were screened for the incidence of spontaneous seizures, and the convulsing gerbils were eliminated from further experiments.

Animals were anesthetized with halothane (2%) in a mixture of O_2/NO_2. Following cessation of pain reflexes (pinch), a ventral midline incision of the neck was made (1 cm) and both carotid arteries were exposed using blunt dissection methods. Cerebral ischemia was induced as described previously (Von Lubitz et al., 1994a, 1996a), i.e., by ligating both common carotid arteries for 10 min. In similarity with the previous experiments, pre- intra, and postischemic rectal temperatures were monitored, while the tympanic temperature was measured immediately after ischemia using a modified Exergen (Natick, MA) tympanic temperature monitor. Moreover, in order to preclude the possibility that the acute effects of the drug were related to its possible influence on the temperature regulation, five non-ischemic gerbils were injected with the highest studied dose of ADAC (i.e., 200 μg/kg) and their rectal and tympanic temperatures were monitored at 15-min intervals for the subsequent 60 min. Following release of the ligature, the wound was closed with 9 mm autoclips, and the animals were returned to their home cages for recovery.

2.4. Histology

Two weeks after ischemia, the surviving animals were heavily anesthetized with Nembutal (50 mg/kg) and perfused through the ascending aorta with a solution of paraformaldehyde in phosphate-buffered (pH 7.4) saline. Serial cryostat sections were cut at 25 μm, mounted on glass slips (three sections per slip) and stained using the Nissl's method. Fifteen stained sections selected randomly from the sector located between 1.6 and 1.9 mm behind bregma (Loskota et al., 1974) were selected from each brain, and the number of surviving neurons in the stratum pyramidale was counted at 400× magnification along the 500 μm length of the medial part of the CA1 sector.

2.5. Statistics

End-point survival data were analyzed using Fisher's exact test. The neuronal preservation data were analyzed using analysis of variance (ANOVA) followed by Dunnett's test. $P < 0.05$ was considered significant.

3. Results

3.1. Effects of the drug on body / brain temperature

At the highest tested dose, the drug had no effect on either body (37.9 ± 0.4°C) or brain (37.6 ± 0.3°C) temper-

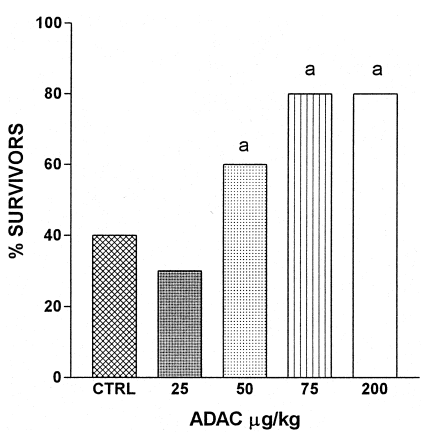

Fig. 1. The effect of various low doses of ADAC ($N = 20$ animals/group) administered acutely 15 min prior to 10 min bilateral carotid artery occlusion on survival 14 days after the insult. Abbreviations: a, $P > 0.05$; CTRL, controls.

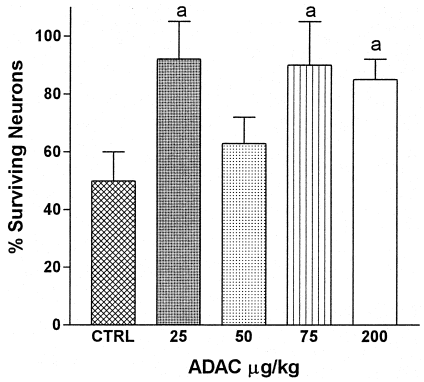

Fig. 2. Acute preischemic administration of ADAC: effect on neuronal preservation in the hippocampal CA1 sector 14 days after 10 min bilateral carotid occlusion. Abbreviations as in Fig. 1.

ature. The body/brain temperature of animals injected chronically with ADAC at 200 μg/kg did not differ significantly from that seen in the acutely treated group.

3.2. Acute administration

In all groups, the highest mortality rate was observed within the initial 24 h of recovery. End-point survival of control animals (40%, Fig. 1) was similar to that reported in our previous studies of ADAC (Bischofberger et al., 1997). Survival of animals treated with either 25 or 50 μg/kg administered 15 min prior to ischemia (30 and 60%, respectively) did not differ statistically from the controls. On the other hand, 80% of gerbils treated with either 75 or 200 μg/kg survived to the end of the monitoring period, i.e., 14 days postischemia ($P < 0.05$).

At the time of perfusion, only 50% of CA1 neurons showed intact morphology in control animals. Although numerically slightly better than in controls (60%), neuronal sparing in the group that received ADAC at 50 μg/kg did not differ from that seen in controls. In all remaining treatment groups (i.e., 25, 75, and 200 μg/kg), the number of neurons with the intact morphological appearance was significantly higher (85–90%, $P < 0.05$, Fig. 2) than in the controls.

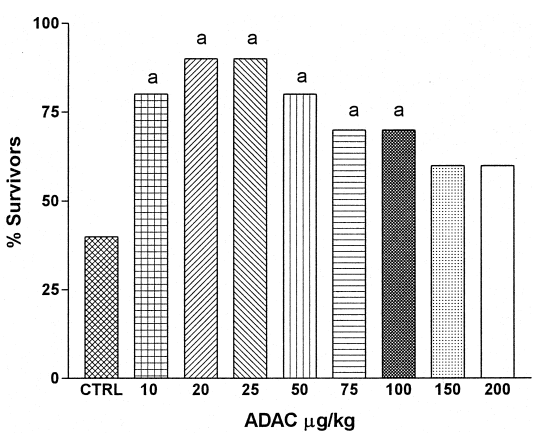

Fig. 3. The effect of chronic (60 days) treatment with different microgram doses of ADAC: survival 14 days after 10 min bilateral occlusion. Abbreviations as in Fig. 1.

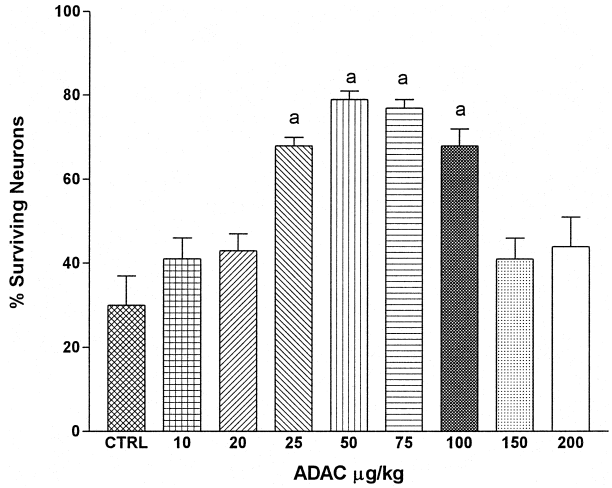

Fig. 4. Chronic treatment with various low doses of ADAC and its effect on neuronal preservation in the CA1 sector of the hippocampus 14 days after 10 min forebrain ischemia. Abbreviations as in Fig. 1.

3.3. Chronic administration

As compared to the acute treatment, chronic administration of the vehicle did not affect survival of the controls (40% at the end of the monitoring period). Chronic treatment with doses ranging between 10 and 100 μg/kg resulted in the end-point survival ranging between 70 and 90% ($P < 0.05$), with the highest survival values typical of the lower doses of ADAC (Fig. 3). Despite numerical improvement, the survival effect of chronic ADAC given at 150 and 200 μg/kg (60% survivors) showed no statistical significance.

A statistically significant ($P < 0.05$) increase in the number of intact neurons was observed in animals receiving chronic ADAC treatment at 25–100 μg/kg (Fig. 4). Despite numerical improvement, neuronal preservation in all other treatment groups did not differ statistically from that seen in control animals.

4. Discussion

The neuroprotective effects of low doses of either acutely or chronically administered ADAC are clearly not the result of drug-induced hypothermia. Furthermore, as compared to an earlier study in which N^6-cyclopentyladenosine (CPA) was used (Von Lubitz et al., 1994a), the present results indicate both similarities and striking differences in the protective impact of ADAC. Thus, although the overall effect of the acute treatment with ADAC given at 75–200 μg/kg (present study and Von Lubitz et al., 1996b) on both survival and neuronal preservation is highly

comparable to that seen following CPA administration, the latter had to be administered at a dose that was significantly higher (i.e., 1 mg/kg). Moreover, chronic treatment with ADAC at low microgram doses resulted in a very high degree of protection in both measures and contrasted that seen after chronic exposure to CPA (Von Lubitz et al., 1994a).

The divergence in the level of protective effects induced by either ADAC or CPA is baffling in view of the high similarity of their receptor affinities and selectivity determined in binding assays (Jacobson et al., 1985; Maillard et al., 1993). On the other hand, significant differences in the chemical structure of these two drugs (Daly and Jacobson, 1995) may affect their pharmacokinetic properties such as their ability to cross the blood–brain barrier. Presently, it is unknown whether pharmacokinetics of ADAC differ substantially from those of CPA. Nonetheless, the fact that a tenfold higher dose of CPA administered prior to ischemia is required to attain parity with the effects produced by ADAC (Von Lubitz et al., 1996b; present study) indicates such possibility. One may, therefore, speculate that preischemic treatment even with low doses of ADAC results in a perineuronal concentration of the drug that is sufficient to activate at least some of the adenosine A_1 receptors in the regions most susceptible to the ischemic damage. Thus, even a partial activation of adenosine A_1 receptors prior to the insult would lead to attenuation of a number of processes (e.g., calcium influx, neurotransmitter release, NMDA receptor hyperactivation—for the reviews, see Rudolphi et al., 1992; Von Lubitz and Jacobson, 1995; Von Lubitz et al., 1995a) and prevent them from reaching their destructive intensity during the subsequent arrest of the cerebral blood flow. If a similar level of receptor occupancy by CPA can only be attained following ischemia-induced opening of the blood–brain barrier, the resulting delay in adenosine A_1 receptor-mediated modulation of glutamate-evoked cytotoxic cascade (Rudolphi and Schubert, 1996) could provide an explanation for the baffling differences in the outcome observed when either ADAC or CPA are given at small doses.

Compared to CPA, the results of chronic treatment with ADAC are equally contrasting. Thus, contrary to CPA, chronic treatment with ADAC at doses below 150 μg/kg protects against postischemic mortality and morbidity. Nonetheless, the statistical significance of ADAC-mediated protection is lost when the drug is given chronically at 150–200 μg/kg, indicating that these amounts may constitute the border zone of the regimen-dependent inversion (for a review, see Jacobson et al., 1996). Therefore, one cannot exclude the possibility that, in similarity to CPA (Von Lubitz et al., 1994a), chronic treatment with higher doses of ADAC may exacerbate cerebral damage as well.

Regimen-dependent loss of the therapeutic effect of selective adenosine A_1 receptor agonists has been frequently reported in the recent years (see Jacobson et al., 1996). Moreover, similar phenomena have been also described for A_{2A} and A_3 receptors (Von Lubitz et al., 1994b; Ceruti et al., 1996). Although the detailed nature of the involved mechanisms is still elusive, receptor desensitization and/or downregulation have been cited as responsible for the differences seen following acute and chronic exposure of adenosine A_1 receptors to their ligands both in vitro and in vivo (reviewed by Hoppe and Lohse, 1995; Jacobson et al., 1996).

Substantial evidence obtained from non-neural tissues demonstrates adenosine A_1 receptor downregulation following protracted exposure to their agonists (e.g., Parsons and Stiles, 1987; Longabaugh et al., 1989; Liang and Donovan, 1990). Nonetheless, studies in which cerebral adenosine A_1 receptors have been chronically exposed to either agonists (Von Lubitz et al., 1994a) or non-selective antagonists (Georgiev et al., 1993) provided contradictory data indicating that, contrary to the expectations, chronic treatment with drugs acting at adenosine A_1 receptors does not result in the decrease of the receptor density.

The striking pattern of the dependence of physiological and therapeutic effects of adenosine receptor stimulation on the dose (Ceruti et al., 1996) and/or duration of exposure to the stimulating agent (present study; Von Lubitz et al., 1994a, 1995b) may be related to desensitization of receptor-coupled G proteins. Chronic exposure of rat adipocytes to a selective adenosine A_1 agonist *R*-phenylisopropyladenosine (*R*-PIA) results in a profound loss of immunoreactivity for G proteins, particularly $G_{i\alpha 1}$ and $G_{i\alpha 3}$, both in vitro and in vivo (Longabaugh et al., 1989). Moreover, Palmer et al. (1995) have recently demonstrated that prolonged exposure of A_3 receptors to 5′-*N*-ethylcarboxamidoadenosine (NECA) extensively depresses the expression of $G_{i\alpha 3}$ and β subunits in a dose and duration of stimulation-dependent manner. Interestingly, while the dose-dependent decrease of $G_{i\alpha 3}$ and β subunits is relatively straightforward, the time dependence is much more complex, with the β subunits showing a biphasic decline.

In summary, although the evidence provided by this and our former studies (Bischofberger et al., 1997 and the present study) indicate several issues that need experimental resolution, the available data also show that ADAC deserves further consideration as a therapeutic agent in treatment of both acute and chronic neurological disorders.

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