Endogenous Activation of Adenosine A1 Receptors Accelerates Ischemic Suppression of Spontaneous Electrocortical Activity

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INTRODUCTION

It has been recognized for a long time that cerebral ischemia induces a rapid suppression of spontaneous brain rhythms prior to major alterations in ionic homeostasis reflected by the anoxic depolarization (Leão 1947). To date, the early factors responsible for the ischemic “shut-down” of the electrocortical activity remain poorly understood. During cerebral ischemia/hypoxia, the rapidly formed adenosine, resulting from the intracellular breakdown of ATP, may inhibit synaptic transmission via the A1 receptor subtype. The link between endogenous A1 receptor activation during ischemia and the suppression of electrocortical activity has not yet been established in the intact brain. The aim of this study was to investigate in vivo the effects of A1 receptor antagonism by 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) on the time to electrocortical suppression during global cerebral ischemia. Adult male Wistar rats under chloral hydrate anesthesia were subjected to 1-min transient “four-vessel occlusion” ischemic episodes, separated by 20-min reperfusion. The rats were injected intraperitoneally with either 1.25 mg/kg DPCPX dissolved in 2 ml/kg dimethyl sulfoxide (DMSO) or the same volume of DMSO alone, 15 min before the third ischemic episode. Time to electrocortical suppression was estimated based on the decay of the root mean square of two-channel electrocorticographic recordings. During the first two ischemic episodes, electrocortical suppression appeared after ~10 s in both groups. After DMSO administration, ischemic suppression remained unchanged. After DPCPX administration, the time to electrocortical suppression was increased by ~10 s, and bursts of activity were recorded during the entire ischemia. These effects disappeared within 15 h after DPCPX administration. Our data provide evidence that during cerebral ischemia endogenous activation of A1 receptors accelerates the electrical “shut-down” of the whole brain.

METHODS

Animals and experimental procedures

The study was carried out in 15 adult male Wistar rats (200–300 g) with free access to food and water. Surgical and experimental procedures were performed under chloral hydrate anesthesia (Sigma, 0.4 g/kg ip maintained with 0.1 g · kg⁻¹ · h⁻¹) with external body temperature regulation.

Our model to investigate the ischemic electrocortical suppression was recently described in detail (Ilie et al. 2006). Briefly, the rats were first implanted with two pairs of epidural electrodes for electrocorticographic (ECoG) recordings. After ~1 wk, the rats were subjected to a second surgery to induce transient, nonlethal, GCI by the “four-vessel occlusion” technique. The rats were injected intraperitoneally with either 1.25 mg/kg DPCPX dissolved in 2 ml/kg dimethyl sulfoxide (DMSO) or the same volume of DMSO alone, 15 min before the third ischemic episode. The rats were injected intraperitoneally with either 1.25 mg/kg DPCPX dissolved in 2 ml/kg dimethyl sulfoxide (DMSO) or the same volume of DMSO alone, 15 min before the third ischemic episode. Time to electrocortical suppression was estimated based on the decay of the root mean square of two-channel electrocorticographic recordings. During the first two ischemic episodes, electrocortical suppression appeared after ~10 s in both groups. After DMSO administration, ischemic suppression remained unchanged. After DPCPX administration, the time to electrocortical suppression was increased by ~10 s, and bursts of activity were recorded during the entire ischemia. These effects disappeared within 15 h after DPCPX administration. Our data provide evidence that during cerebral ischemia endogenous activation of A1 receptors accelerates the electrical “shut-down” of the whole brain.

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vessel occlusion" model (Moldovan et al. 2004b; Pulssellini and Brierley 1979; Zagrean et al. 1995). The animals were then left to recover overnight and subjected to another GCI episode. All rats were killed by cervical dislocation at the completion of experiments. The experiments were carried out with the approval of the local committee for animal research of Carol Davila University of Medicine and Pharmacy (Bucharest, Romania) in accordance with the American Physiological Society ethical policies and procedures regarding animal experimentation.

**Pharmacological investigations**

To account for individual variability (Pulssellini and Brierley 1979), we tested four consecutive 1-min GCIs, separated by 20-min reperfusion intervals (Ilie et al. 2006). We used the first two GCIs to establish a baseline and then injected intraperitoneally DPCPX (Sigma) dissolved in dimethyl sulfoxide (DMSO, Sigma), 15 min (Baumgold et al. 1992; Bissere et al. 1992) prior to the third GCI. We investigated the fourth GCI to test for peak effect and another GCI, (n = 5) received a similar volume of DMSO alone.

**Electrophysiological signals and statistics**

The ECoG signals from two bipolar occipito-frontal leads were recorded using a MP100 Biopac System (Biopac Systems) with EEG100A amplifiers (1- to 35-Hz band-pass filter, −3 dB). Simultaneously with ECoG, the electrocardiogram (ECG) was recorded from two Ag/AgCl electrodes attached to the forepaws using the same MP100 Biopac System. The heart rate (HR) was calculated off-line as previously described in detail (Moldovan et al. 2004a).

We recently introduced an automatic method to estimate $T_{ES}$ from ECoG during GCI (Ilie et al. 2006). Briefly, the root mean square of the signal (RMS) (calculated on consecutive 1-s epochs) was filtered through a low-pass third-order Butterworth filter using a zero-phase forward and reverse algorithm. The $T_{ES}$ was then calculated as the time between the clamping of the both common carotid arteries and the decay of the filtered signal <30%.

Signal processing was implemented in MATLAB (MathWorks). Numeric results are given as means ± SE. Nonparametric statistical comparisons were performed by Wilcoxon paired test (SPSS).

**RESULTS**

Our experimental setup allowed recording of large ECoG (Fig. 1) and ECG (Fig. 2) signals. During GCI there was a complete suppression of the electrocortical activity (Fig. 1). During each GCI we detected a slight (~25%) decrease in HR (Fig. 2A), which peaked after ~30 s (Fig. 2B and C). This

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**FIG. 1.** Representative experiments in control (A) and 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) group (B). Top: electrocorticographic (ECoG) recordings for 5 consecutive global cerebral ischemia episodes (GCIs) are presented at the same scale. Note the time lapse between the 4th and the 5th GCI; 1.25 mg/kg DPCPX in dimethyl sulfoxide (DMSO) vehicle, or DMSO alone, was administered 15 min prior to the 3rd GCI (J). Each 1-min GCI is detailed. * Spontaneous bursts of activity during ischemic suppression. Time origin is set 1 min prior to the 1st GCI.
ischemic sinus bradycardia (Fig. 2, D and E) recovered spontaneously prior to reperfusion with a time course that was reproducible within the same experiment (Fig. 2).

The ECoG (Fig. 1A) and ECG (data not shown) changes were unaffected by DMSO administration in control group. Prior to the GCI, DPCPX had also no apparent effect on either ECoG (Fig. 1B) or ECG (Fig. 2). During the GCI, DPCPX-induced alterations easily recognizable in all investigated rats: ECoG suppression appeared delayed and interrupted by bursts of activity that could be observed up to the end of investigated ischemia (Fig. 1B). After 1.25 mg/kg DPCPX, both ECoG (Fig. 1B) and ECG (Fig. 2) recovered fully after each GCI. Nevertheless, after 5 mg/kg DPCPX reperfusion consistently led to malignant arrhythmias (data not shown) and the experiments had to be discontinued.

The automatic $T_{ES}$ quantification after DPCPX administration was complicated by the need to distinguish the prolonged persistence of “background” ECoG activity from the “first burst” (Fig. 1B). We found that a 0.01-Hz RMS filter could reasonably predict visual $T_{ES}$ estimation both in control and DPCPX groups (Fig. 1). The differences in $T_{ES}$ estimation between the two ECoG channels never exceeded 2 s. For consistency we considered the representative $T_{ES}$ for one GCI as the shortest value between the two ECoG channels (Fig. 3A).

During the first GCI episode, $T_{ES}$ was $12.2 \pm 0.3$ s in both control and DPCPX groups. No differences in $T_{ES}$ were observed between the first and the second GCI episode in either group (Fig. 3B). During the third GCI, 15 min after administration, 1.25 mg/kg DPCPX prolonged $T_{ES}$ to $21.3 \pm 1.2$ s ($P < 0.05$). This $T_{ES}$ prolongation was similar after 5 mg/kg DPCPX (Fig. 3B) in spite of the ECG alterations after the subsequent reperfusion. During the fourth GCI, 36 min after 1.25 mg/kg DPCPX administration, $T_{ES}$ was $20 \pm 1.2$ s ($P < 0.05$), which was not significantly different from $T_{ES}$ during the third GCI. At 15 h after DPCPX, $T_{ES}$ was no different from $T_{ES}$ prior to DPCPX administration (Figs. 1B and 3B).

**DISCUSSION**

We investigated the effect of systemic administration of the $A_1$R antagonist DPCPX on the suppression of spontaneous electrocortical activity during transient global cerebral ischemia in rat. We found that DPCPX reversibly prolonged $T_{ES}$. Consistent with previous in vitro observations from rat hippocampal slices (Canhão et al. 1994; Fowler 1989, 1990; Pearson et al. 2001), it is likely that DPCPX prevented the rapid synaptic depression caused by the ischemic release of adenosine (Dale et al. 2000; Valtysen et al. 1998; Van Wylen et al. 1986). Thus we report here the first evidence that ischemic activation of $A_1$Rs observed in vitro contributes to the
suppression of spontaneous ECoG activity recorded from the intact brain in vivo.

Ischemic suppression of spontaneous ECoG activity was found to occur within 15 s in various species including rats (Barzaghi et al. 1982; Ilie et al. 2006), cats (Hossmann et al. 1990), and humans (de Vries et al. 1998). The ultimate cause of the ischemic loss of ECoG activity is the widespread spread anoxic depolarization resulting from the failure of neuronal energy metabolism (Leão 1947). Nevertheless, during the “four-vessel occlusion” ischemia in rats, the suppression of spontaneous ECoG activity precedes anoxic depolarization (Matsumoto et al. 1990) with more than a minute (Halaby et al. 2004). Furthermore, at the onset of ischemic suppression of spontaneous ECoG, the cortical response to visual stimuli is preserved (Ilie et al. 2006). Thus even though our findings are in line with the known inhibitory effects of adenosine at synaptic level (Fowler 1989), we bring novel evidence that ischemic A₁R activation accelerates the suppression of the “whole brain” most likely by targeting the key cortico-thalamic circuit (Ochiishi et al. 1999) responsible for generation of spontaneous brain rhythms (reviewed in Steriade 2006).

The impairment in the ischemic ECoG suppression induced by DPCPX was easily identifiable at visual inspection (Fig. 1). In addition to the prolonged persistence of the “background” ECoG activity, DPCPX revealed bursts of activity which could be observed up to the end of the investigated ischemia (Fig. 1B). In this study, we could not directly address whether the persistence of spontaneous ECoG activity and the bursting activity were the consequence of the same altered circuit. In fact, in vivo recordings may suggest that the early ischemic bursting activity may be purely neocortical (Fleidervish et al. 2001). Therefore we adjusted the quantification method (Fig. 3A) to measure T_ES of only the background ECoG activity. While the simple filtered RMS decay may not be appropriate for detecting brief transients like “isolated” bursts, it could accurately distinguish the “first burst” from the suppression of the “background” ECoG activity (Figs. 1B and 3A). Furthermore, the same filter settings could reasonably estimate T_ES in control group (Fig. 1A) which ensured the consistency of the comparisons.

The rapid suppression of spontaneous ECoG activity after cerebral ischemia was proposed to be a neuroprotective response (Hossmann et al. 1990; Nagashima 1994). During ischemia/hypoxia lethal neuronal injury occurs largely due to increased levels of glutamate and the subsequent activation of N-methyl-D-aspartate (NDMA) receptors (Simon et al. 1984). In vitro, endogenous adenosine release was found to efficiently prevent glutamate release via presynaptic A₁Rs (Arrigoni et al. 2005; Coelho et al. 2000; Hershkowitz et al. 1993). Furthermore, recovery of synaptic transmission in hippocampal slices subjected to prolonged ischemia was largely impaired after DPCPX (Sebastião et al. 2001). We found that in vivo, DPCPX did not alter ECoG recovery after 1-min GCI (Fig. 1B). Although in vitro A₁R antagonists may slightly shorten the delay to anoxic depolarization (Lee and Lowenkopf 1993), several minutes of anoxic depolarization may be required to induce neuronal damage in the most ischemic-sensitive neurons (Halaby et al. 2004; Sorimachi et al. 1999). Thus it is unlikely that DPCPX induced a significant acute neuronal damage during the 1-min transient ischemic episodes used in this study.

Consistent with a low basal adenosine tone (Fulga and Stone 1998) we found that DPCPX administration had no effect on the ECoG amplitude outside the GCI. Whereas cerebral A₁Rs are primarily neuronal (Ochiishi et al. 1999), expression of A₁Rs on several nonneuronal tissues, most notably in the heart (for review, see Fredholm et al. 2001), may confound the neuronal interpretation of T_ES changes induced by systemically administered DPCPX. At doses of 1.25 mg/kg, we found that DPCPX prolonged T_ES (Figs. 1 and 3) without altering the basal HR (Fig. 2). Furthermore, during “four-vessel occlusion” we detected a very slight sinusal bradycardia with a time course that was unaffected by DPCPX (Fig. 2). The observed decrease in HR normalized before the onset of repuffusion, resembling the transient reflex hypoxic bradycardia (Giussani et al. 1993) that was also found to be unaffected by DPCPX.
(Koos and Maeda 2001). Therefore we consider it unlikely that cardiovascular actions of DPCPX contributed to the prolongation of $T_{ES}$ reported here.

During our repeated ischemia paradigm we found that 5 mg/kg DPCPX had no ECG effects prior to, or during the following 1-min cerebral ischemia; however, it led to malignant arrhythmias early during reperfusion. These cardiac effects were not detected at 1.25 mg/kg DPCPX (Fig. 2). Adenosine is a known modulator of ventricular automaticity (for review, see Hernandez and Ribeiro 1995). We may speculate that the high concentrations of DPCPX impaired the anti $\beta$-adrenergic effect of adenosine and increased the ventricular vulnerability to fibrillation (Lubbe et al. 1978). Further investigations should be carried out to explore this antiarrhythmic, potentially protective, role of A1 Rs in the context of cerebral ischemia.

In spite of the differences in the cardiac response, $T_{ES}$ prolongation remained unchanged after as much as four times increase in DPCPX concentration (Fig. 2B). We recently reported, on the same ischemic model, that under energy-stress conditions (such as those occurring during rapid repeated cerebral ischemia and kainate-induced seizures), $T_{ES}$ could be prolonged to a remarkably similar “plateau” (Ilie et al. 2006). Consistent with the “depletable adenosine pool hypothesis” formulated in vitro (Pearson et al. 2001), it is likely that the maximal $T_{ES}$ prolongation reflects the limit in the plasticity of adenosine release during ischemia (reviewed in Pearson et al. 2003). Thus our simple experimental model used to investigate changes in $T_{ES}$ may offer a new in vivo window into the ischemic adenosine release and its contribution to the electrical “shut-down” of the whole brain.

ACKNOWLEDGMENTS

We thank T. Vladoiu for taking part in some experimental procedures and two anonymous reviewers for very constructive comments.

GRANTS

The project was supported by Carol Davila University of Medicine and Pharmacy (Bucharest, Romania) and grants from Viasan National Research Program, Academy of Medical Sciences, Romania.

REFERENCES


Baumgold J, Nikodijevic O, and Jacobson KA. Adenosine A(2A) receptors mediate cardiovascular actions of DPCPX contributed to the prolongation of $T_{ES}$ reported here.


