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Laboratory Studies

The neuroprotective effect of diazoxide is mediated by mitochondrial ATP-dependent potassium channels in a rat model of acute subdural hematoma

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ABSTRACT

Acute subdural hematoma (ASDH) results in neuronal death due to mitochondrial dysfunction and a subsequent cascade of apoptotic and necrotic events. We previously demonstrated that mitochondrial ATP-dependent potassium ($mitoK_{ATP}$) channels have a major role in cerebral ischemic preconditioning *in vivo* and *in vitro*. However, the role of the $mitoK_{ATP}$ channel has not been investigated in the context of ASDH. Thus, the purpose of this study was to determine whether the $mitoK_{ATP}$ channel mediates neuroprotection in a rat model of ASDH. Male Wistar rats were subjected to subdural infusion of 400 μL autologous venous blood. The rats were assigned to four experimental groups pretreated intraventricularly 15 minutes before ASDH with (1) vehicle ($n = 10$); (2) the $mitoK_{ATP}$ channel agonist diazoxide ($n = 9$); (3) diazoxide plus the selective $mitoK_{ATP}$ channel antagonist 5-hydroxydecanoate (5-HD) ($n = 6$); or (4) 5-HD alone ($n = 6$). Infarct volume was assessed at 4 days after ASDH. Brain edema formation was also measured. Pretreatment with diazoxide significantly reduced infarct volume and brain edema formation after ASDH. However, the effects of diazoxide were abolished by co-treatment with 5-HD. 5-HD alone increased infarct volume. These data suggest that the $mitoK_{ATP}$ channel is an important mediator of the neuroprotective effects of cerebral preconditioning in a rat model of ASDH.

1. Introduction

Acute subdural hematoma (ASDH) is a common mass lesion in traumatic brain injuries. Reduction in local cerebral blood flow around the hematoma after ASDH leads to secondary ischemic brain damage and significant neurologic dysfunction.[1] This process is mediated at least in part by mitochondrial dysfunction and subsequent alterations in excitatory amino acids and reactive oxygen species (ROS), and disruption of Ca^{2+} homeostasis.[2] Previous studies have suggested that the neuroprotective preconditioning effect against ischemic insult is mediated by mitochondrial adenosine triphosphate-dependent potassium ($mitoK_{ATP}$) channels in the brain.[3,4] Indeed, application of the selective $mitoK_{ATP}$ channel agonist diazoxide induces protective effects similar to ischemic preconditioning, while application of the selective $mitoK_{ATP}$ channel antagonist 5-hydroxydecanoate (5-HD) abolishes ischemic tolerance in the brain.[5] Recent findings suggest that diazoxide prevents glutamate release from presynaptic terminals,[6] prevents the generation of ROS,[7] and inhibits apoptosis.[8,9] The purpose of the present study was to determine whether $mitoK_{ATP}$ channels mediate neuroprotection by chemical preconditioning in a rat model of ASDH.

2. Materials and methods

This animal study was conducted in accordance with the guidelines approved at the 80th General Assembly of the Japan Science Council (1980).

Adult male Wistar rats (weight 300–340 g; age 8–12 weeks) were used. Animals were anesthetized with chloral hydrate and mechanically ventilated. The femoral artery was cannulated for blood pressure monitoring and for withdrawal of blood samples for blood gas analysis. A femoral vein was cannulated to obtain venous blood for subdural infusion. Animals were fixed in a stereotaxic frame and a 3-mm burr hole was drilled at a distance of 1 mm posterior to the bregma and lateral to the midline suture. For subdural blood infusion, an L-shaped, blunted needle was inserted underneath the dura and fixed with cyanoacrylate glue. To induce ASDH, 400 μL of autologous unheparinized blood was injected at a flow rate of 50 μL/min. After injection, the catheter was removed, and the scalp was sutured.[10]

Thirty-one rats were randomly assigned to 1 of 4 groups: a vehicle-treated group that received either saline ($n = 5$) or 0.1 mol/L NaOH ($n = 5$); a diazoxide-treated group ($n = 9$); a diazoxide plus 5-HD-treated group ($n = 6$); and a 5-HD-treated group ($n = 6$). In all groups, drugs were injected into the right cerebral ventricle (stereotactic coordinates, AC anteroposterior −0.8 mm, lateral

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1.5 mm, and dorsoventral 4.0 mm).[11] Diazoxide and 5-HD were purchased from Sigma Chemical Co. Diazoxide was dissolved in 0.1 mol/L NaOH, and a total of 15 μL diazoxide (2 mmol/L) was administered 15 minutes before induction of ASDH. The 5-HD was dissolved in saline, and 10 μL of a 100 mmol/L solution was injected intracerebroventricularly 15 minutes before ASDH. Vehicle-treated animals received either saline or 0.1 mol/L NaOH administered before ischemia.[5] Because the groups that received vehicle did not differ in infarct volume, these rats were combined into one vehicle group. Drugs were administered intracerebroventicularly rather than intraperitoneally to minimize the potential side effects of a high drug dose in other organs.

Rats were returned to individual cages and killed 4 days after surgery. This time period was chosen because both acute necrotic cell death and delayed necrotic cell death contribute to infarcts at 4 days after ASDH insult.[10] Each rat was perfusion-fixed with 4% paraformaldehyde (pH 7.4) under deep anesthesia, and the brain was carefully removed from the skull. Brains were embedded in paraffin, and coronal sections (3 μm thick) were cut so as to span the parietal region containing the infarct. Sections were stained with hematoxylin and eosin. Infarct size was measured histologically using a light microscope connected to a calibrated image-analysis system, which consisted of a Sony charge-coupled device camera (Sony, Tokyo, Japan) and Optimas 6.51 software (Optimas Corporation, Seattle, WA, USA). Infarct area was evaluated using serial sections of 200-μm steps. Infarct volume was calculated and expressed in cubic millimeters (mm^3).

The temporal profile of edema formation was measured using the wet–dry weight method. Brains were quickly removed at 48 hours after ASDH from rats in the vehicle-treated group ($n = 8$), the diazoxide-treated group ($n = 5$), the diazoxide plus 5-HD-treated group ($n = 6$), and the 5-HD-treated group ($n = 5$), and were separated into the ipsilateral and contralateral hemispheres. The hemispheres were then dried at 100 °C in an oven for 24 hours. Tissue weight before (wet weight) and after drying (dry weight) was measured, and hemispheric water content was calculated for each animal. All data are expressed as mean ± standard error. Statistical analyses were performed using one-way analysis of variance (ANOVA) on ranks. Differences between values were considered significant when $P < 0.05$. All statistical analyses were performed using Sigma-Stat software (Jandel Scientific, Erkrath, Germany). The investigator was blinded to treatment until after histological analysis.

3. Results

There were no significant differences noted in arterial pO_2, pCO_2, pH, rectal temperature, or mean arterial blood pressure among the groups (Table 1).

All rats studied had a cortical infarct. Infarct volume was significantly lower in the diazoxide-treated group (39.54 ± 5.14 mm^3) than in the vehicle-treated group (83.31 ± 14.79 mm^3). However, this reduction in infarct volume was abolished by co-treatment with 5-HD (82.32 ± 13.36 mm^3) (Figs. 1, 2). In the group treated with 5-HD alone, the infarcts were significantly larger (99.67 ± 9.74 mm^3) than those in the vehicle-treated group.

At 48 hours after ASDH, water content was significantly lower in the diazoxide-treated group (ipsilateral hemisphere, 78.7 ± 0.3%; contralateral hemisphere, 78.2 ± 0.2%) than in the vehicle-treated group (79.9 ± 0.3% and 79.2 ± 0.2%, respectively). However, this reduction in edema was abrogated by co-treatment with 5-HD (79.7 ± 0.3, 79.1 ± 0.2%, respectively) (Fig. 3). There was no statistical difference in the extent of edema between the diazoxide plus 5-HD group and the 5-HD alone group (79.8 ± 0.5%, 79.4 ± 0.3%, respectively) (Fig. 3).

4. Discussion

ASDH is a common complication of traumatic brain injury and is associated with poorer outcomes. The clinical outcome in patients with ASDH is often worse than that which would be expected as a result of the mass effect of the hematoma alone. Since ASDH induces a range of degenerative processes, understanding the nature of the insult is important when selecting treatment. In a previous study involving a rat model of ASDH, injection of 400 μL blood to induce a hematoma resulted in a transient increase in intracranial pressure to >70 mmHg and then a rapid decrease to ~20 mmHg.[12] These acute changes resulted in development of an infarct a few hours after induction of ASDH. However, the lesion size increased further despite only moderate intracranial hypertension and hypoperfusion in most rat models.[13] Indeed, the ischemic area can increase even if cerebral perfusion pressure is normalized.[10] Thus, better treatment options are needed in order to prevent secondary ischemic cell death.

Severe brain injury is followed by excitotoxicity, oxidative stress, inflammatory events, and mitochondrial dysfunction. The inflammation and apoptosis that follow ischemia and traumatic brain injury are important factors contributing to delayed cell death, and caspase-dependent mechanisms play a crucial role in delayed cell death in ASDH.[10] Further, mitochondrial damage leads to a cascade of apoptotic and necrotic events and plays a central role in the growth of the secondary infarct.[14]

4.1. Ischemic preconditioning model

Preconditioning with sublethal ischemia induces tolerance to subsequent lethal ischemia in the brain.[15,16] The neuroprotective

Table 1
Physiological variables before and after acute subdural hematoma (ASDH) in the four treatment groups

	MABP (mmHg)	Brain temperature (°C)	Glucose (mg/dL)	pH	PO_2 (mmHg)	PCO_2 (mmHg)
Vehicle						
Before ASDH	101.3 ± 0.5	36.7 ± 0.03	92.3 ± 5.7	7.417 ± 0.01	125.4 ± 2.8	42.4 ± 0.5
After ASDH	99.5 ± 0.4	36.9 ± 0.02	90.5 ± 6.1	7.402 ± 0.01	124.2 ± 2.4	41.2 ± 0.9
Diazoxide						
Before ASDH	97.5 ± 0.3	36.8 ± 0.03	90.8 ± 6.1	7.422 ± 0.01	129.6 ± 2.9	42.4 ± 0.7
After ASDH	98.2 ± 0.4	36.7 ± 0.04	92.7 ± 5.8	7.435 ± 0.01	128.9 ± 3.5	40.6 ± 0.8
Diazoxide+5-HD						
Before ASDH	102.9 ± 0.3	36.8 ± 0.03	88.7 ± 6.3	7.412 ± 0.01	126.8 ± 3.6	40.5 ± 0.6
After ASDH	104.3 ± 0.4	36.9 ± 0.04	90.9 ± 5.7	7.434 ± 0.01	125.1 ± 3.7	39.5 ± 0.8
5-HD						
Before ASDH	101.6 ± 0.5	36.8 ± 0.02	91.0 ± 4.9	7.423 ± 0.01	124.8 ± 4.9	41.4 ± 0.7
After ASDH	103.1 ± 0.4	36.7 ± 0.03	88.9 ± 6.2	7.419 ± 0.02	119.8 ± 5.8	39.7 ± 1.0

5-HD = 5-hydroxydecanoate, MABP = mean arterial blood pressure. Data are expressed as mean ± standard error of the mean. There were no statistically significant differences for any physiological variable between any of the four groups.