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GLIBENCLAMIDE REDUCES SECONDARY BRAIN DAMAGE AFTER EXPERIMENTAL TRAUMATIC BRAIN INJURY

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Abstract—Following traumatic brain injury (TBI) SUR1-regulated $NC_{Ca\text{-}ATP}$ (SUR1/TRPM4) channels are transcriptionally up-regulated in ischemic astrocytes, neurons, and capillaries. ATP depletion results in depolarization and opening of the channel leading to cytotoxic edema. Glibenclamide is an inhibitor of SUR-1 and, thus, might prevent cytotoxic edema and secondary brain damage following TBI. Anesthetized adult Sprague–Dawley rats underwent parietal craniotomy and were subjected to controlled cortical impact injury (CCI). Glibenclamide was administered as a bolus injection 15 min after CCI injury and continuously via osmotic pumps throughout 7 days. In an acute trial (180 min) mean arterial blood pressure, heart rate, intracranial pressure, encephalographic activity, and cerebral metabolism were monitored. Brain water content was assessed gravimetrically 24 h after CCI injury and contusion volumes were measured by MRI scanning technique at 8 h, 24 h, 72 h, and 7 d post injury. Throughout the entire time of observation neurological function was quantified using the ''beam-walking'' test. Glibenclamide-treated animals showed a significant reduction in the development of brain tissue water content(80.47% ± 0.37% (glibenclamide) vs. 80.83% ± 0.44% (control); $p < 0.05$; $n = 14$). Contusion sizes increased continuously within 72 h following CCI injury, but glibenclamide-treated animals had significantly smaller volumes at any time-points, like 172.53 ± 38.74 mm^3 (glibenclamide) vs. 299.20 ± 64.02 mm^3 (control) ($p < 0.01$; $n = 10$; 24 h) or 211.10 ± 41.03 mm^3 (glibenclamide) vs. 309.76 ± 19.45 mm^3 (control) ($p < 0.05$; $n = 10$; 72 h), respectively. An effect on acute parameters, however, could not be detected, most likely because of the up-regulation of the channel within 3–6 h after injury. Furthermore, there was no significant effect on motor function assessed by the beam-walking test throughout 7 days. In accordance to these results and the available literature, glibenclamide seems to have promising potency in the treatment of TBI.

Key words: traumatic brain injury, glibenclamide, secondary brain damage, brain edema, cerebral metabolism, epileptic seizures.

INTRODUCTION

Despite significant improvements in intensive care management, severe traumatic brain injury (TBI) still remains the leading cause of death and long-term disability and dependency in young patients. Brain edema, leading to uncontrollable increased intracranial pressure (ICP), is predominantly responsible for the development of secondary brain damage characterized by enlarged contusion volumes, and hence, deteriorated neurological outcome. Treatment guidelines, thus currently focus on the control and the reduction of brain swelling (Brain Trauma Foundation, 2007).

Osmotherapy has been the mainstay of pharmacological therapy and is typically administered as an escalating treatment scheme (Brain Trauma Foundation, 2007; Walcott et al., 2012). Among others, a novel treatment target for cerebral edema might be the SUR1-regulated $NC_{Ca\text{-}ATP}$ (SUR1/TRPM4) channel (Simard et al., 2006; Walcott et al., 2012). Recently, this non-specification channel has been described in ischemic astrocytes, neurons, and capillaries regulated by sulfonylurea receptor 1 (SUR1). Depletion of ATP, as it can frequently be observed in the peri-contusional area following trauma, causes depolarization and opening of this channel. This finally leads to an uncontrolled influx of cations and, according to the osmotic pressure, of water resulting in cell ''blebbing'' that is characteristic of cytotoxic edema (Simard et al., 2006). In situations of mechanical stress, inflammation, and hypoxia an up-regulation of the ABcc8 gene that encodes for SUR1 receptors could be observed (Simard et al., 2012a).

SUR-1 receptors can specifically be blocked by the administration of glibenclamide, explaining the fact, that patients with diabetes type II receiving glibenclamide seem to be in favor to reach a better neurological outcome after stroke quantified by the Modified Ranking Scale and the National Institute of Health Stroke Scale in comparison to controls (Kunte et al., 2007). Furthermore, in a rodent model of cerebral stroke, blocking of SUR-1 receptors with low-dose glibenclamide has significantly reduced cerebral edema, infarct volume, and mortality rate (Simard et al., 2006, 2009a; Zhou et al., 2009).

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Abbreviations: CCI, controlled cortical impact injury; EEG, electroencephalography; HF, heart rate; ICP, intracranial pressure; MANOVA, multivariate analysis of variance; MAP, mean arterial blood pressure; MD, microdialysis; TBI, traumatic brain injury.

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In experimental models of subarachnoid hemorrhage glibenclamide seems to have positive effects on inflammation and edema formation (Simard et al., 2009b). In experimental spinal cord injury, glibenclamide diminishes the development of hemorrhagic necrosis, leading to improved neurological outcome (Simard et al., 2007a, 2012b,c; Popovich et al., 2012). The inhibition of the up-regulated SUR1 receptor by low-dose glibenclamide displays furthermore a key role in the prevention of developing a progressive secondary hemorrhage following TBI (Simard et al., 2009c).

In this current study the effect of glibenclamide on the development of secondary brain damage after experimental TBI is investigated emphasizing the influence on brain edema, contusion volume, cortical activity, and cerebral metabolism.

EXPERIMENTAL PROCEDURES

Animals

A total of 68 Sprague–Dawley rats (Charles River, Germany, 350–380 g) were subjected to this study. Guidelines for laboratory animal care were strictly followed. Animals had free access to food and water throughout the study and all experiments were performed under deep anesthesia with isoflurane.

Experimental groups

A total of three experimental sections were investigated: In sections 1 ($n = 20$), acute (patho-) physiological changes, encompassing mean arterial blood pressure (MAP), heart rate (HF), body temperature, ICP, encephalographic activity, and cerebral metabolism (microdialysis (MD)) were monitored continuously over 180 min following trauma. In section 2 ($n = 28$), brain water content was assessed gravimetrically 24 h after controlled cortical impact injury (CCI), and, in section 3 ($n = 20$), contusion volumes by MRI scanning and neurological assessment were quantified 8 h, 24 h, 72 h, and 7 d following trauma, respectively. In all sections animals were randomized either to a treatment-, receiving glibenclamide, or to a control group that received placebo. In section 1, furthermore a sham-operated group was added. In this group animals were not traumatized, but only received a craniotomy.

Anesthesia and trauma application

All experiments were performed under deep anesthesia using 1.8–2.2% isoflurane, 68% N_2O and 30% O_2. During the entire time of anesthesia a catheter in the tail artery digitally monitored systemic MAP and HF. Furthermore, blood gases were controlled in regular intervals. In order to control and maintain body temperature at 37.0 ± 0.5 °C, rats were positioned on a feedback-controlled heating pad. As previously described, experimental TBI was performed using a "Controlled Cortical Impact" injury (CCI) device (Zweckberger et al., 2003, 2006). Briefly, after induction of anesthesia, the head was fixed in a stereotactic frame and, using a micro-drill under permanent cooling with saline, a temporo-parietal craniotomy was performed. Thereby special attention was paid to leave the dura intact. The trauma was performed perpendicular to the surface of the brain (90 degrees) by an impactor that was adjusted at an angle of 35 degrees. The diameter of the flat impactor (with rounded edges) was 5 mm, the velocity 7.5 m/s, the impact depth 1.5 mm, and the impact duration 300 ms. In order to avoid any decompressive effects created by the craniotomy itself, the bone-flap was replaced immediately after CCI and fixed with dental cement.

Drug administration

15 min after CCI, a loading dose of glibenclamide (10 μg/kg) was administered subcutaneously, and additionally, osmotic pumps (Alzet®, Model 2ML1; ALZET Osmotic Pumps, DURECT Corporation, Cupertino, CA, USA) were implanted subcutaneously in the left flank region for continuous delivery of the drug throughout 7 days (10 μl/h). All rats, randomized either to the treatment or the control group, have received the same total volume. According to Simard et al. [13], a stock solution of 25 mg of glibenclamide (Sigma, St. Louis, MO, USA) in 10 ml dimethylsulfoxide (DMSO) was prepared. The infusion/injection solution was made by mixing 200 μl stock in 25 ml un-buffered normal saline (0.9%NaCl), thus, administering a total concentration of 200 ng/h.

ICP and electro-encephalography (EEG)

ICP was measured using an intraparenchymal micro-ICP probe (Codman® MicroSensor Basic Kit; Raynham, MA 02767) that was inserted in the cortex via a borehole 2 mm rostral from the anterior edge of the craniotomy. Monitoring started 10 min prior to CCI receiving a physiological baseline, and was continued throughout the entire time of observation following CCI injury (180 min).

In order to monitor encephalographic activity (EEG) two stainless screws were inserted in two boreholes fronto-lateral and parieto-occipital referred to the craniotomy. Bipolar recording was amplified using a Bio Potential Amplifier Module type 675 (Hugo Sachs Electronic-Harvard Apparatus GmbH, March-Hugstetten, Germany). Filter limits were set to 0.003–100 Hz. To avoid electrical interference at 50 Hz a notch filter was applied. These records were screened for epileptiform activity by one of the investigators who was blinded to the entire treatment procedure. Quantitative measurements of the EEG signals, such as root mean square values, delta (1–3 Hz), theta- (4–7 Hz), alpha- (8–15 Hz), and beta (15–30 Hz) power, as well as the mean frequency, were derived from the artifact-free recordings 10 min before and up to 180 min after CCI (Sakowitz et al., 2002).

MD

Following CCI, the MD catheter (CMA12: length 14 mm; length of the permeable membrane 2 mm; diameter 0.5 mm; molecular cut-off 100,000 Dalton) was inserted