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Glibenclamide improves neurological function in neonatal hypoxia-ischemia in rats

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Abstract

Recent studies demonstrated that sulfonylurea receptor 1 (SUR 1) regulated nonselective cation channel, the NC_{Ca}-ATP channel, is involved in brain injury in rodent models of stroke. Block of SUR 1 with sulfonylurea such as glibenclamide has been shown to be highly effective in reducing cerebral edema, infarct volume and mortality in adult rat models of ischemic stroke. In this study, we tested glibenclamide in both severe and moderate models of neonatal hypoxia-ischemia (HI) in postnatal day 10 Sprague–Dawley rat pups. A total of 150 pups were used in the present study. Pups were subjected to unilateral carotid artery ligation followed by 2.5 or 2 h of hypoxia in the severe and moderate HI models, respectively. In the severe HI model, glibenclamide, administered immediately after HI and on postoperative Day 1, was not effective in attenuating short-term effects (brain edema and infarct volume) or long-term effects (brain weight and neurological function) of neonatal HI. In the moderate HI model, when injected immediately after HI and on postoperative Day 1, glibenclamide at 0.01 mg/kg improved several neurological parameters at 3 weeks after HI. We conclude that glibenclamide provided some long-term neuroprotective effect after neonatal HI.

Keywords

Glibenclamide; Neurological function; Infarction; Neonatal hypoxia-ischemia

1. Introduction

Perinatal cerebral hypoxia-ischemia is an important cause of brain injury in infants and children leading to mortality or lifelong impairments (Ferriero, 2004). Despite major advances in the understanding of the underlying mechanisms of a hypoxic-ischemic insult, including excitotoxicity, apoptosis and inflammation (Alkan et al., 2001; Calvert and Zhang, 2005), effective treatment strategies targeting perinatal HI are still lacking (Perlman, 2006).

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Edema often occurs in accompany with pathological conditions affecting the central nervous system (CNS), including cerebral ischemia and traumatic brain injury, and causes tissue swelling leading to worse neurological outcome and high mortality (Ayata and Ropper, 2002; Betz, 1996; Rosenberg, 1999). Malignant brain edema resulting from a massive middle cerebral stroke contributes to the high mortality of 60–80% of the patients (Ayata and Ropper, 2002). Therefore, it is very important to understand the molecular mechanisms involved in cerebral edema formation by pathological conditions in the CNS. Accumulating evidence indicates that a nonselective cation channel, the SUR 1-regulated NC_{Ca-ATP} channel, consisting of a regulatory subunit, sulfonylurea receptor 1 (SUR 1), and a pore-forming subunit, is crucially involved in development of cerebral edema in rodent models of stroke and in development of progressive hemorrhagic necrosis in a rodent model of acute spinal cord injury (Chen et al., 2003; Simard et al., 2006; Simard et al., 2007). The SUR 1-regulated NC_{Ca-ATP} channel is a 34 pS cation channel that conducts all inorganic monovalent cation, requires nanomolar concentrations of intracellular Ca²⁺ for opening, and is activated by depletion of intracellular ATP (Chen and Simard, 2001). Channel opening causes a strong inward current, cell depolarization, and results in cytotoxic edema, eventually in cell death (Simard et al., 2006). The channel is not constitutively expressed, but is expressed in the CNS by hypoxic or traumatic insult, and it has been identified upon its biophysical properties in freshly isolated reactive astrocytes obtained from the hypoxic inner zone of the gliotic capsule, in neurons in the core of an ischemic stroke in rats and in cultured human and mouse endothelial cells subjected to hypoxia (Chen and Simard, 2001; Chen et al., 2003; Simard et al., 2006; Simard et al., 2007). Block of SUR 1 with glibenclamide, a second generation sulfonylureas, reduced cerebral edema, infarct volume and mortality by 50% in rodent models of stroke (Simard et al., 2006).

In the present study, we tested the effect of glibenclamide, a SUR 1 blocker, after neonatal hypoxia–ischemia in rats. We utilized a severe and a moderate neonatal hypoxia-ischemia (HI) model and glibenclamide was administrated immediately after HI and on postoperative Day 1. We hypothesize that glibenclamide will reduce cerebral edema, brain injury and improve neurological outcomes after neonatal HI brain injury in rats.

2. Results

2.1. Brain edema, infarct volume, mortality and blood glucose level

We tested the effect of glibenclamide at both 0.01 and 0.1 mg/kg on brain edema after severe HI. Both doses failed to reduce brain edema at 48 h after HI. In the vehicle- and glibenclamide-treated severe HI groups, the brain water content in the ipsilateral hemispheres increased significantly compared to the sham-operated group (vehicle-treated severe HI group 90.0±0.2%, glibenclamide at 0.01 mg/kg 89.4±0.4%, glibenclamide at 0.1 mg/kg 89.2±0.3%, versus sham-operated group 86.1±0.2%, $p<0.001$, Fig. 1A). As glibenclamide is a well-known anti-diabetic drug and may have effect on blood glucose level, we measured blood glucose in pups from the edema studies. Blood glucose at 70 min after subcutaneous injection was drastically decreased by glibenclamide at 0.1 mg/kg (91.5±7.8 versus 18.0±3.3 mg/dl, $p<0.001$, $n=4$), that of the treatment group with glibenclamide at 0.01 mg/kg at 70 min after drug injection was also decreased, but statistical

equivalent on ANOVA compared to blood glucose level before drug administration (106.8 ± 6.4 versus 91.5 ± 4.5 mg/dl, $p=0.1$, $n=4$). Glibenclamide at 0.01 mg/kg was considered appropriate dosage for rest of the experiments. We conjectured that the model could be too severe to achieve any effect of glibenclamide and therefore, we utilized a moderate neonatal HI model with 2 h hypoxia to assess the effect of the drug on brain edema. At 48 h, the brain water content in the vehicle-treated HI group was $89.4 \pm 0.4\%$ in the moderate HI model. Glibenclamide at 0.01 mg/kg did not reduce brain water content in the treatment group (Fig. 1B).

Glibenclamide had no effect on brain edema after severe and moderate neonatal HI. We then sought to determine the effect of glibenclamide on infarct volume. At 48 h, glibenclamide treatment resulted in no statistical difference in infarct volume compared to the vehicle-treated HI group in the severe HI model ($71.7 \pm 4.2\%$ versus $69.4 \pm 3.9\%$, $p=0.7$, Fig. 2A). In the moderate HI model, the mean infarct volume in the vehicle-treated HI group was $41.5 \pm 8.4\%$ of ipsilateral volume. Glibenclamide treatment slightly reduced the infarct volume, but there was no statistical difference compared to the vehicle-treated HI group ($36.3 \pm 5.9\%$ versus $41.5 \pm 8.4\%$, $p=0.5$, Fig. 2B).

In both severe and moderate HI models, in terms of mortality rat pups died during hypoxia period before they were randomly divided into vehicle- and glibenclamide-treated HI groups, or 48 h after they underwent HI procedure (long-term study). Thus only pups in long-term studies were considered in the mortality study. In the severe HI model, the mortality rate was 11.1% in the vehicle-treated HI group and 10% in the glibenclamide-treated HI group, respectively; in the moderate HI model, the mortality rate was 10% in both vehicle- and glibenclamide-treated HI groups. There were no statistical differences in mortality between vehicle- and glibenclamide-treated HI groups in both severe and moderate HI models.

2.2. Brain weight and histology

The top panel of Fig. 3A shows representative photographs of brains taken from sham-operated, vehicle- and glibenclamide-treatment groups, collected at 3 weeks after severe HI. There was significant loss of ipsilateral brain tissue in both vehicle- and glibenclamide-treatment groups, compared to the sham-operated group (Fig. 3A). In the moderate neonatal HI model, the ratios of the ipsilateral hemisphere compared to the contralateral hemisphere at 3 weeks after HI were as follows: sham-operated group 0.98 ± 0.02 , vehicle-treated HI group 0.54 ± 0.04 , glibenclamide-treated HI group 0.56 ± 0.01 . There was no statistical difference between the vehicle- and glibenclamide-treated HI groups (Fig. 3B).

Nissl histological staining of the coronal brain sections demonstrated massive tissue loss in the ipsilateral cortex and hippocampus in both vehicle- and glibenclamide-treated groups after severe HI. In the moderate HI model, increased neuronal cell death was observed in CA1 hippocampal regions of the ipsilateral hemisphere at 3 weeks in both vehicle- and glibenclamide-treated HI groups (Fig. 4).

2.3. Neurobehavioral studies

In the severe neonatal HI model, T-maze testing for spontaneous alternation demonstrated a significantly worse performance in the vehicle- and glibenclamide-treated HI groups at 3 weeks after HI, compared to the sham-operated group (Fig. 5A). In the foot-fault test, limb misplacements in the vehicle- and glibenclamide-treated HI groups were more than that of the sham-operated group (Fig. 5B). In the postural reflex test and the forelimb placement test, pups in the vehicle- and glibenclamide-treated HI groups had significantly lower neurological value at 3 weeks after severe HI, compared to the sham-operated pups (Fig. 6A).

In the moderate neonatal HI model, pups in the vehicle- and glibenclamide-treated HI groups performed significantly worse in the T-maze test and forelimb placement test at 3 weeks after HI, compared to the sham-operated pups (Figs. 5C and 6B). In the foot-fault test and postural reflex test, pups in the vehicle-treated HI group demonstrated a significantly worse performance, whereas glibenclamide significantly improved the motor performance in these two tests (Figs. 5D and 6B).

3. Discussion

As a potent SUR 1-regulated $\text{NC}_{\text{Ca-ATP}}$ channel blocker, glibenclamide has been shown to have beneficial effects in adult animal models of CNS ischemia and injury (Simard et al., 2006; Simard et al., 2007; Simard et al., 2008). It has been widely and safely used in humans for treatment of type 2 diabetes mellitus. Clinical studies showed that sulfonylureas improve outcome in patients with type 2 diabetes and acute ischemic stroke (Kahn et al., 2006; Kunte et al., 2007). In the present study, we found that glibenclamide treatment at 0.01 mg/kg, in the moderate neonatal HI model in rats, was associated with an improvement of motor functions in the foot-fault test and postural reflex test at 3 weeks after HI brain injury, but with no beneficial effect on brain edema, infarct volume or brain tissue loss. In addition, treatment with glibenclamide in the severe neonatal HI model conferred no benefit to brain edema, infarct volume, brain weight or neurological function.

Since glibenclamide treatment may cause hypoglycemia, we measured blood glucose before and after drug administration in pups. Glibenclamide treatment at 0.1 mg/kg resulted in a significant reduction in blood glucose from 91.5 ± 7.8 to 18.0 ± 3.3 mg/dl, which may exacerbate ischemic brain injury. Glibenclamide at 0.01 mg/kg only slightly decreased the blood glucose (106.8 ± 6.4 before versus 91.5 ± 4.5 mg/dl after drug administration), and such levels of blood glucose are not associated with altered outcome in cerebral ischemia (Li et al., 1994; Wass and Lanier, 1996). Thus we considered glibenclamide at 0.01 mg/kg as the appropriate dosage for our experiments.

We found that glibenclamide improved several neurological parameters, but failed to attenuate brain edema, infarct volume or brain tissue loss. These findings are somewhat surprising given that glibenclamide has been shown to be neuroprotective in in vitro and vivo studies under hypoxic/ischemic condition (Chen et al., 2003; Simard et al., 2006). In addition, work from the same laboratory demonstrated that block of SUR 1 by glibenclamide was highly effective in ameliorating vasogenic edema and cell death after

subarachnoid hemorrhage (Simard et al., 2008). Several factors may explain this discrepancy. The first possible reason relates to the drug dosage. The reduction in blood glucose level by glibenclamide in our model confirmed that serum levels of glibenclamide were sufficient to affect pancreatic SUR 1 and suggests that the drug reached therapeutic levels in the rat pups after drug administration. Because previous study showed that subcutaneously injected low-dose glibenclamide reached peri-infarct regions of cerebral ischemia (Simard et al., 2006), we believe that glibenclamide administered in our experiments also reached the targeted areas of cerebral hypoxic-ischemic injury. It is possible that multiple post-HI doses of glibenclamide may have resulted in a beneficial effect on brain edema and infarct volume. The second possible reason relates to the animal models. In the present study, we utilized both severe and moderate models of neonatal hypoxia-ischemia. After unilateral carotid artery ligation, the pups were subjected to 2.5 or 2 h hypoxia in the severe and moderate models, respectively. The mean infarct size in the severe HI model was greater than that of the moderate HI model. However, the brain water content in the ipsilateral hemisphere was nearly the same in both models. Since glibenclamide exerts its neuroprotective effect by blocking the SUR1-regulated $\text{NC}_{\text{Ca-ATP}}$ channel and attenuating brain edema, it is possible that our moderate HI model may still be too severe and glibenclamide treatment may have provided protective effect in a HI model with less brain edema formation. Another possible explanation for our findings relates to the $\text{NC}_{\text{Ca-ATP}}$ channel expression under hypoxic condition in the immature rat brain. Xia and colleagues found that whereas the adult rat brain decreased the number of Na^+ channels during chronic hypoxia, the immature brain tended to show the opposite response (Xia and Haddad, 1999). Although, in the present study, we did not evaluate the expression of SUR 1-regulated $\text{NC}_{\text{Ca-ATP}}$ channel in the immature rat brain after hypoxic-ischemic brain injury, it is possible that, in contrast to adult rat brain, the absence of upregulation of the SUR 1-regulated $\text{NC}_{\text{Ca-ATP}}$ channel in the immature rat brain after HI is the reason why glibenclamide failed to reduce the brain edema. Lastly, SUR 1 is not only involved with $\text{NC}_{\text{Ca-ATP}}$ channels but also a regulatory subunit that incorporates with Kir6.x pore-forming subunits to form potassium ATP channel (K_{ATP}). The K_{ATP} channel plays important physiological roles in brain and couples cellular energy status to membrane potential. K_{ATP} channels may contribute to seizure prevention in ATP-depleted metabolic states such as hypoxia (Ballanyi, 2004; Yamada and Inagaki, 2005). Sun and colleagues showed that expression of Kir6.2 channels prevents prolonged depolarization of neurons resulting from acute hypoxic or ischemic insults and thus protects these central neurons from the injury (Sun et al., 2007). Brockhaus and Deitmer reported a developmental downregulation of ATP-sensitive potassium conductance in astrocytes and found that diazoxide activated K_{ATP} currents in 57% of the astrocytes in rats aged 8–11 days (Brockhaus and Deitmer, 2000). In the present study, we employed rat pups at age postnatal 10 days. Thus it is possible that K_{ATP} channels are expressed abundantly in this developmental stage in the CNS. Therefore, treatment with glibenclamide to block the SUR 1-regulated $\text{NC}_{\text{Ca-ATP}}$ channel and attenuate cerebral edema may also inhibit the K_{ATP} channels in the CNS and thereby abolish the neuroprotective effects of the drug under hypoxic/ischemic condition, although we did not investigate the function of K_{ATP} channels specifically in this study.

In the present study, we showed that glibenclamide was not effective in reducing short-term effects of neonatal HI, but we did observe some positive effects of glibenclamide on several neurological outcomes, which may result from subtle glibenclamide-induced neuroprotection by inhibiting NC_{Ca}-ATP channels. Future studies of glibenclamide and neonatal HI will address the following issues: whether multiple doses of glibenclamide will result in a beneficial effect on brain edema and infarct volume and whether glibenclamide will be more effective in a neonatal HI model with less brain edema. Overall, we conclude that glibenclamide treatment is associated with improved several neurological outcomes after moderate neonatal hypoxia-ischemia, but not severe neonatal hypoxia-ischemia in rats.

4. Experimental procedures

4.1. Neonatal hypoxia-ischemia model

The neonatal hypoxia-ischemia protocol used in this study was approved by the Institutional Animal Care and Use Committee (IACUC). Timed pregnant female Sprague-Dawley rats were purchased from Harlan Labs, Indianapolis, IN, USA and housed in individual cages. After birth, pups were housed with their dam under a 12 h light/dark cycle, with food and water available *ad libitum* throughout the study. We used a modified Rice-Vannucci model (Calvert et al., 2002; Rice et al., 1981) on postnatal day 10 pups. Both male and female rat pups were used in this study and the pups were anesthetized by inhalation with isoflurane (2.5% in 30% O₂ and 70% medical air) and kept at a temperature at 37 °C during the surgical procedure. The right common carotid artery of each pup was exposed, carefully separated from the vagus nerve and jugular vein, and permanently ligated with 5–0 surgical silk through a near-midline incision. The wound was closed and the pups were returned to their cages with their dam in a stable thermal environment. The pups were then allowed to recover with their dam for 1 h. After recovering, the pups were placed in a glass chamber, which was submerged in a water bath at a stable temperature of 37 °C, and subjected to humidified and prewarmed gas mixture of 8% O₂ and 92% N₂. We utilized both severe and moderate models of neonatal hypoxia-ischemia in the present study. The hypoxic period lasted for 2.5 or 2 h in the severe and moderate models, respectively. After hypoxia, the pups were returned to their dams and the ambient temperature was maintained at 37 °C for 24 h. Sham-operated animals underwent anesthesia and incision only. The rat pups were euthanized under deep anesthesia at 48 h or 3 weeks after hypoxia-ischemia.

4.2. Glibenclamide treatment

In the severe neonatal HI model, glibenclamide (Sigma, St. Louis, MO, USA), a potent SUR 1 blocker, was administered subcutaneously in two dosages of 0.1 mg/kg and 0.01 mg/kg after HI. Two doses were given in the treatment groups: the first dose was injected immediately after HI; the second one on postoperative Day 1. In the moderate neonatal HI model, glibenclamide at 0.01 mg/kg was given immediately after HI and on postoperative Day 1. A stock solution of glibenclamide was made by placing 12.5 mg glibenclamide into 10 ml dimethylsulfoxide (DMSO) and the injection solution was made by placing stock into PBS (0.1 mg/kg and 0.01 mg/kg). The vehicle-treated HI group received DMSO diluted with PBS at the same volume as the treatment group (approximately 100 µl per injection).

4.3. Brain water content

Rat pups were sacrificed under deep anesthesia at 48 h after HI and the brains were removed. Following methods described by Xi et al. (2002), the hemispheres were separated by a midline incision and weighted on a high precision balance with a sensitivity of 0.001 g (Denver Instrument) (wet weight). After drying in an oven at 100 °C for 48 h, the hemispheres were weighted again (dry weight). The percentage of brain water content was calculated as [(wet weight–dry weight)/wet weight]×100%.

4.4. Infarct volume

At 48 h after HI, pups were euthanized and the brains were removed and sectioned into 2 cm slices. 2,3,5-triphenyltetrazolium chloride monohydrate (TTC) staining was performed to assess the infarct volume as previously described (Yin et al., 2003). The brain sections were immersed into 2% TTC solution at 37 °C for 5 min, washed in PBS and then immersed in 10% formaldehyde. The infarct volume was traced and analyzed by Adobe Photoshop software (Version 6.0).

4.5. Brain weight

As previously described (Calvert et al., 2002), pups were euthanized under deep anesthesia and the brains were removed at 3 weeks after HI. The cerebellum and brain stem were dissected from the forebrain. The hemispheres were then separated by a midline incision and weighted on a high precision balance with a sensitivity of 0.001 g. Brain tissue loss was expressed as the mass ratio of the ipsilateral hemisphere compared to the contralateral hemisphere.

4.6. Nissl staining

At 3 weeks after HI, animals were perfused transcardially with PBS under deep anesthesia, followed by 4% paraformaldehyde. The brains were then removed and post-fixed in formalin. Coronal brain sections with 10 µm thickness were prepared by cryostat (CM3050S, Leica Microsystems). Nissl staining followed the standard protocol (Ostrowski et al., 2006).

4.7. Neurobehavioral testing

All neurobehavioral tests were performed in a blinded set-up.

4.7.1. T-maze test—The T-maze spontaneous alternation task has been shown to test exploratory behavior and working memory by hippocampal dysfunction (Gerlai, 2001; Ishibashi et al., 2003). Prior to sacrifice at 3 weeks after HI, the rats were tested for spontaneous alternation on a T-shaped maze as previously described (Matchett et al., 2007). The T-maze measured 40 (stem)×46 (arm)×10 (width) cm and was constructed from acrylic and silicon glue. The rats were placed in the stem of the T-maze and allowed to freely explore the two arms of the maze, throughout a 10-trial continuous alternation session. Once an arm was chosen, the rat was placed in the stem of the maze again, and the trial was repeated. Absolute numbers of left and right choices were recorded, and the spontaneous alternation rate was calculated as the ratio of the alternating choices to the total number of the choices.

4.7.2. Foot-fault test—In the foot-fault test, the rats were placed on a horizontal grid floor (wire diameter 0.4 cm) for 2 min. Foot-fault was defined as when the animal inaccurately placed a fore- or hindlimb, and it fell through one of the openings in the grid. The numbers of foot-faults for each animal were recorded. Previous study has shown that intact animals place their paws on the grid frame or foot holds while moving around on the grid (Barth and Stanfield, 1990). Limb misplacements in intact animals were infrequent.

4.7.3. Postural reflex test—As previously described (Yonemori et al., 1998), in the postural reflex test, the rats were held by the tail and fore-/hindlimb flexion and body twisting were evaluated as: value 100, normal; value 50, slight twisting and limb flexion; value 0, marked twisting and limb flexion.

4.7.4. Forelimb placement test—In the forelimb placement test, the rats were held by the examiner, and the forelimb placement after visual and proprioceptive stimuli was recorded as: value 100, immediate and correct paw placing; value 50, delayed and/or incomplete correction; value 0, no placing. Intact animal showed consistently high forelimb placement value (Bona et al., 1997; Wang et al., 2006).

4.8. Statistical analysis

Data were expressed as mean±SEM. Statistical differences among groups were analyzed by using one-way ANOVA followed by Tukey post-hoc analysis (Systat Software, Richmond, CA). P value <0.05 was considered statistically significant.

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Abbreviations:

NC_{Ca-ATP} channel	Ca ²⁺ -activated
[ATP]_i	sensitive nonspecific cation channel
HI	Hypoxia-Ischemia
PBS	Phosphate buffered saline
SUR 1	sulfonylurea receptor 1
TTC	2,3,5-triphenyltetrazolium chloride

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Fig. 1 -

Brain water content in the ipsilateral and contralateral hemispheres at 48 h after severe and moderate HI. Glibenclamide at 0.01 mg/kg was administered immediately after HI and on postoperative Day 1. Data is mean $\pm$ SEM of 8 pups in each group. (A) In the ipsilateral hemisphere, the brain water content was markedly increased in the severe HI group compared to the sham-operated group. Glibenclamide did not reduce the ipsilateral hemisphere water content. (B) The brain water content in the ipsilateral hemisphere in the moderate HI group and glibenclamide treatment group was significantly higher than that of the sham-operated group. * indicates significance compared to the sham-operated group, $p < 0.001$. There was no statistical difference among the groups in contralateral hemisphere water content.

Fig. 2 -

Infarct volume, assessed by TTC staining at 48 h after severe and moderate HI.

Glibenclamide at 0.01 mg/kg was administered immediately after HI and on postoperative Day 1. The top panels of A and B show representative TTC stained coronal brain sections from vehicle- and glibenclamide-treated HI groups. The bottom panels demonstrate quantitative analysis of infarct volume of 7–10 pups in each group. (A) In the severe HI model, glibenclamide was not effective in reducing infarct volume. (B) In the moderate HI model, infarct volume was slightly decreased in the glibenclamide treatment group, but there were no statistical differences compared to the vehicle-treated HI group.

Fig. 3 -

Brain weight at 3 weeks after severe and moderate HI. Glibenclamide at 0.01 mg/kg was administered immediately after HI and on postoperative Day 1. The top panels of A and B show representative pictures of brains taken from sham-operated, vehicle- and glibenclamide-treated HI groups. The bottom panels demonstrate quantitative analysis of brain weight of 6–9 pups in each group, expressed as the mass ratio of the ipsilateral hemisphere compared to the contralateral hemisphere. In the severe (A) and moderate (B) HI model, there was significant loss of ipsilateral brain tissue in both vehicle- and glibenclamide-treated HI groups. * Indicates significance compared to the sham-operated group, $p < 0.05$.

Fig. 4 -

Nissl histology from hippocampus and CA-1 region in coronal sections of the brain at 3 weeks after severe and moderate HI. Glibenclamide at 0.01 mg/kg was administered immediately after HI and on postoperative Day 1. In the severe HI group, massive tissue loss was observed in the ipsilateral cortex and hippocampus in both vehicle- and glibenclamide-treated groups. Nissl staining of the brain in moderate HI model demonstrated brain damage and cell death in both vehicle- and glibenclamide-treated HI groups.

Fig. 5 -

Spontaneous alternation in T-maze test and limb misplacements in foot-fault test at 3 weeks after severe and moderate HI (6–9 pups in each group). Glibenclamide at 0.01 mg/kg was administered immediately after HI and on postoperative Day 1. (A) Sham-operated animals tended to alternate $74.1 \pm 5.5\%$ of the time when presented with two choices in the T-maze. Animals in the vehicle- and glibenclamide-treated severe HI groups performed worse than sham-operated animals (vehicle-treated severe HI group, $44.4 \pm 6.9\%$; glibenclamide-treated severe HI group, $45.7 \pm 8.2\%$). (B) In the severe HI model, animals in the vehicle- and glibenclamide-treated HI groups demonstrated more limb misplacements than sham-operated animals. (C) In the moderate HI model, animals in the vehicle- and glibenclamide-treated HI groups fared worse than sham-operated animals. (D) In the moderate HI model, more limb misplacements were recorded in the vehicle- and glibenclamide-treated HI groups than these in the sham-operated group. Animals with glibenclamide treatment had significantly less limb misplacements than animals in the vehicle-treated HI group. * Indicates significance compared to the sham-operated group, $p < 0.05$, and # indicates significance compared to the vehicle-treated HI group, $p < 0.05$.

Fig. 6 -

The postural reflex test and forelimb placement test at 3 weeks after severe and moderate HI (6–9 pups in each group). Glibenclamide at 0.01 mg/kg was administered immediately after HI and on postoperative Day 1. (A) In the severe HI model, animals in the vehicle- and glibenclamide-treated HI groups had lower neurological value than the sham-operated animals. (B) In the moderate HI model, animals in the vehicle- and glibenclamide-treated HI groups performed worse in the postural reflex test and forelimb placement test, than the sham-operated animals. However, animals with glibenclamide treatment had significantly better motor performance in the postural reflex test than the vehicle-treated HI animals. * indicates significance compared to the sham-operated group, $p < 0.05$, and # indicates significance compared to the vehicle-treated HI group, $p < 0.05$.