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Effects of nicorandil on the recovery of reflex potentials after spinal cord ischaemia in cats

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1 The pathophysiological significance of ATP-sensitive K^+ (K_{ATP}) channels in the central nervous system is not fully understood. In this study the effects of nicorandil (a hybrid vasodilator having a dual mechanism of action as a K^+ channel opener and a nitrate) on the recovery of the spinal cord reflex potentials after spinal cord ischaemia were examined and compared with those of pinacidil and nitroprusside in anaesthetized spinal cats.

2 Spinal cord ischaemia was produced by occlusion of the thoracic aorta and the bilateral internal mammary arteries for 10 min. Regional blood flow in the spinal cord was continuously measured with a laser-Doppler flow meter. The monosynaptic (MSR) and polysynaptic reflex (PSR) potentials, elicited by electrical stimulation of the tibial nerve, were recorded from the lumbo-sacral ventral root. The recovery process of spinal reflex potentials was reproducible when the occlusion was repeated twice at an interval of 120 min.

3 Pretreatment with nicorandil (30–100 $\mu g\ kg^{-1}$) accelerated the recovery of PSR potentials after spinal cord ischaemia. Such an accelerating effect on the recovery of PSR potentials was also shared by pinacidil (100 $\mu g\ kg^{-1}$), another K^+ channel opener. In addition, the accelerating effect of nicorandil (100 $\mu g\ kg^{-1}$) on the recovery of PSR potentials was abolished by co-administration of glibenclamide (3 $mg\ kg^{-1}$), a sulphonylurea K_{ATP} channel blocker. Nitroprusside (8 $\mu g\ kg^{-1}min^{-1}$) retarded rather than improved the recovery of PSR potentials after spinal cord ischaemia. All of these drugs failed to improve the spinal cord blood flow during ischaemia and reperfusion.

4 These results suggest that nicorandil promotes the recovery of polysynaptic reflex potentials after spinal cord ischaemia by opening the K_{ATP} channels of neurones rather than by increasing local blood flow. K^+ channel openers may exert a salutary effect on the functional recovery of the ischaemic spinal cord.

Keywords: Nicorandil; spinal cord; ischaemia; reflex potential; ATP-sensitive K^+ (K_{ATP}) channel; pinacidil; nitroprusside; glibenclamide

Introduction

ATP-sensitive K^+ channels were first described in cardiac cells by Noma (1983). Subsequently, the channels have been identified in various tissues, such as skeletal muscle (Spruce *et al.*, 1985), pancreatic β-cells (Rorsman & Trübe, 1985), vascular smooth muscles (Standen *et al.*, 1989) and central neurones (Bernardi *et al.*, 1988; Ashford *et al.*, 1988). The physiological roles of the ATP-sensitive K^+ channels are well understood in pancreatic β-cells and cardiac myocytes, where the channels are involved in the regulation of insulin release (Ashcroft 1988) and the action potential shortening in the ischaemic myocardium (Cole *et al.*, 1991; Nakaya *et al.*, 1991). However, the physiological significance of the ATP-sensitive K^+ channels in neuronal tissues is not fully understood. Several studies have suggested that activation of the channels may be involved in the inhibition of membrane depolarization and neurosecretion during anoxia (Ben-Ari, 1989; Ben-Ari *et al.*, 1990; Amoroso *et al.*, 1990; Murphy & Greenfield, 1991). Therefore, K^+ channel openers may influence neurological responses during anoxia or ischaemia in the central nervous system.

When traumatic forces such as contusion, compression and destruction are applied to the spinal cord, the resultant spinal cord ischaemia may produce irreversible injuries of neurones (Ducker *et al.*, 1978 a,b). Therefore, the search for a drug which can preserve spinal cord function during spinal cord ischaemia may be of importance. Nicorandil is a hybrid vasodilator with a dual mechanism of action as a K^+ channel opener and a nitrate (Taira, 1989). Experimental studies have indicated that nicorandil produces a cardioprotective effect by opening the ATP-sensitive K^+ channel in the ischaemic myocardium (Ohta *et al.*, 1991; Auchanpach *et al.*, 1992). Therefore, the present study was undertaken to examine effects of nicorandil on changes in reflex potentials during spinal cord ischaemia and reperfusion in anaesthetized spinal cats. Since nicorandil accelerated the recovery of polysynaptic reflex potentials after spinal cord ischaemia, the effect was compared with that of nitroprusside in this study.

Methods

Animal preparations and recordings of spinal cord reflex potentials

All experiments were performed under the regulations of the Animal Research Committee of School of Medicine, Chiba University. Adult cats of either sex weighing 2.1–3.5 kg were anaesthetized with urethane (600 $mg\ kg^{-1}$, i.p.) and α-chloralose (40 $mg\ kg^{-1}$, i.p.), and supplemental doses of anaesthetics were given when needed during experiments. The animal was spinalized at C1 level, and then artificially ventilated with room air through an endotracheal tube at a rate which maintained the end tidal CO_2 between 3.5 and 4%. The right femoral vein and the left femoral artery were cannulated for the administration of drugs and the measurement of blood pressure, respectively. Preparations with mean blood pressure less than 80 mmHg were discarded and not included in this study.

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The lumbo-sacral spinal cord was exposed by a laminectomy and immersed in a warm mineral oil pool maintained at 37.0±0.2°C. Body temperature was continuously monitored with a rectal thermistor and maintained constant with a thermostatically-controlled water pad. All the ventral roots from L6 to S3 were sectioned. Monosynaptic reflex (MSR) and polysynaptic reflex (PSR) potentials were recorded from the ipsilateral L7 or S1 ventral root with a bipolar silver electrode. The central end of the resected tibial nerve was stimulated with a bipolar platinum L hook electrode in a mineral oil pool at a rate of 0.2 Hz. The stimulus was a rectangular pulse of 0.2 ms duration at 8–10 times the threshold, which produced the maximum MSR potentials. The stimuli were delivered by an electronic stimulator (Nihon Kohden SEN-1101, Tokyo, Japan) through an isolation unit (Nihon Kohden SS-101J). Evoked potentials were amplified and displayed on an oscilloscope (Nihon Kohden VC-9) and five consecutive sweeps were superimposed on a Polaroid film.

Spinal cord blood flow was measured with a laser-Doppler flowmeter (Advance ALF2100, Tokyo, Japan). The probe was positioned over the spinal artery, and the blood flow through the artery was continuously monitored. The changes in the blood flow and blood pressure during ischaemia and reperfusion were recorded with a pen recorder (Yokogawa Technicorder F 3052, Tokyo, Japan).

Experimental protocol and drugs

In order to produce a lumbo-sacral spinal cord ischaemia, the aorta and the bilateral internal mammary arteries were occluded with bulldog clamps for 10 min. An occlusion of 10 min was repeated after an interval of more than 110 min. All cats were randomly divided into six groups: (1) Saline vehicle group ($n=10$), cats received saline infusion before the second spinal cord ischaemia; (2) nicorandil group, nicorandil at a dose of 10 μg kg^{-1} ($n=4$), 30 μg kg^{-1} ($n=4$) or 100 μg kg^{-1} ($n=5$) was intravenously administered 10 min before the second ischaemia; (3) pinacidil group ($n=9$), pinacidil (100 μg kg^{-1}) was intravenously administered 10 min before the second ischaemia; (4) glibenclamide-nicorandil group ($n=5$), glibenclamide (3 mg kg^{-1}) was injected 20 min prior to the second ischaemia and then 100 μg kg^{-1} of nicorandil was given 10 min before the occlusion; (5) glibenclamide group ($n=8$), glibenclamide at the same dose alone was given 20 min before the second ischaemia; (6) nitroprusside group, nitroprusside was infused at a rate of 5 μg kg^{-1} min^{-1} ($n=6$) or 8 μg kg^{-1} min^{-1} ($n=4$) from 10 min before the second occlusion to 90 min after reperfusion. The spinal cord reflex potentials were recorded priot to the spinal cord ischaemia (control) and various times after the reperfusion. Recovery of the reflex potentials was analyzed by measuring the amplitude of MSR potentials and the area of PSR potentials, which were the average of 5 consecutive potentials. The pre-occlusion reflex potentials served as control.

The drugs used in this study were as follows: nicorandil (Chugai Pharmaceutical Co., Tokyo, Japan), pinacidil (Shionogi Pharmaceutical Co., Tokyo, Japan), glibenclamide and sodium nitroprusside (Sigma Chemical, St. Louis, MO., U.S.A.). These drugs except for glibenclamide were dissolved in isotonic saline. Glibenclamide was dissolved in 1 ml of 0.1 N NaOH, and then pH of the solution was adjusted to 7.6 by adding 0.1 N HCl. The drug solution was diluted in 2 ml saline.

Statistical analysis

All data are expressed as mean±s.e. Analysis by Student's t test was performed for paired or unpaired observations. P values of less than 0.05 were considered significant.

Results

The spinal cord reflex potentials, recorded from the lumbo-sacral ventral roots of spinal cats, consisted of monosynaptic reflex (MSR) and subsequent polysynaptic reflex (PSR) potentials, as shown in Figure 1. These potentials were completely depressed within 2–3 min after the occlusion of the thoracic aorta and the internal mammary arteries. After the removal of the occlusion, the potentials reappeared gradually and returned to the control level within 110 min, as shown in Figure 1. It was confirmed that the time course changes in reflex potentials during reperfusion were reproducible when the spinal cord ischaemia of 10 min was repeated at an interval of 110 min or more in 10 cats.

Nicorandil *per se* did not significantly affect MSR or PSR potentials during the pre-ischaemic period. Pretreatment with nicorandil at a dose of 100 μg kg^{-1} significantly accelerated the recovery of PSR potentials after the second spinal cord ischaemia (Figure 1). At 15 min after reperfusion the area of the PSR potentials was 25±9% of the preocclusion level without any drug treatment (Control) while it was 65±10% after the administration of nicorandil ($P<0.05$, $n=5$, Figures 1 and 2). However, the recovery of MSR potentials was not significantly affected by nicorandil. The accelerating effect of nicorandil on the recovery of PSR potentials was dose-dependent, as shown in Figure 2. Nicorandil at a dose of 30 μg kg^{-1} slightly but significantly improved the recovery of PSR potentials ($n=4$, $P<0.05$) although the drug at a lower dose of 10 μg kg^{-1} failed to affect the recovery ($n=4$, NS). In 2 animals, 300 μg kg^{-1} nicorandil was administered prior to the second spinal cord ischaemia. However, the high dose of nicorandil markedly decreased the mean blood pressure to less than 50 mmHg and failed to accelerate the recovery of PSR potentials (data not shown).

Similar results were also obtained when the cats were treated with pinacidil, another K^+ channel opener. Pretreatment with pinacidil (100 μg kg^{-1}) prior to the second occlusion did not affect the reflex potentials during the pre-ischaemic period. Pinacidil also accelerated the recovery of PSR potentials after the second spinal cord ischaemia. At 15 min reperfusion, the area of PSR potentials was 59±7% of the preocclusion level, which was significantly larger than that of the control recovery (34±4% at 15 min, $P<0.05$, $n=9$, Figure 2). Thus, K^+ channel openers all accelerated the recovery of PSR potentials after the spinal cord ischaemia in anaesthetized cats.

The accelerating effect of nicorandil on the PSR potential recovery was abolished by co-administration of glibenclamide, a sulphonylurea which blocks ATP-sensitive K^+ channels in various tissues. (Edwards & Weston, 1993). Glibenclamide (3 mg kg^{-1}) was administered intravenously 10 min before the injection of 100 μg kg^{-1} nicorandil, and then the second spinal cord ischaemia was produced. At 15 min reperfusion the area of PSR potentials was 21±7% of the preocclusion level, which was not significantly different from that observed after the first spinal cord ischaemia (Figures 2 and 3). Thus, combined treatment with glibenclamide and nicorandil failed to accelerate the recovery of PSR potentials after the second spinal cord ischaemia. However, glibenclamide (3 mg kg^{-1}) *per se* did not retard the recovery of PSR potentials after spinal cord ischaemia. In glibenclamide-treated cats, the area of PSR potentials at 15 min reperfusion was 30±3% of the preocclusion level ($n=8$), which was not significantly different from that after the control spinal cord ischaemia (Figure 2).

In the following experiments effects of nitroprusside, a vasodilator which increases cyclic GMP in various cells (Ahlner *et al.*, 1991), on the recovery of PSR potentials after spinal cord ischaemia were examined. Continuous infusion of nitroprusside at a rate of 5 μg kg^{-1} min^{-1} failed to improve the recovery of PSR potentials after spinal cord ischaemia (Figure 2). Further increase in the infusion rate of nitroprusside to 8 μg kg^{-1} min^{-1} significantly retarded rather than improved the recovery of PSR potentials. Therefore, the ATP-sensitive K^+ channel-opening action rather than the nitrate action of nicorandil might be important for the accelerated recovery of PSR potentials after spinal cord ischaemia.

Regional blood flow (RBF) of the spinal cord was continuously measured with a laser-Doppler flowmeter. During

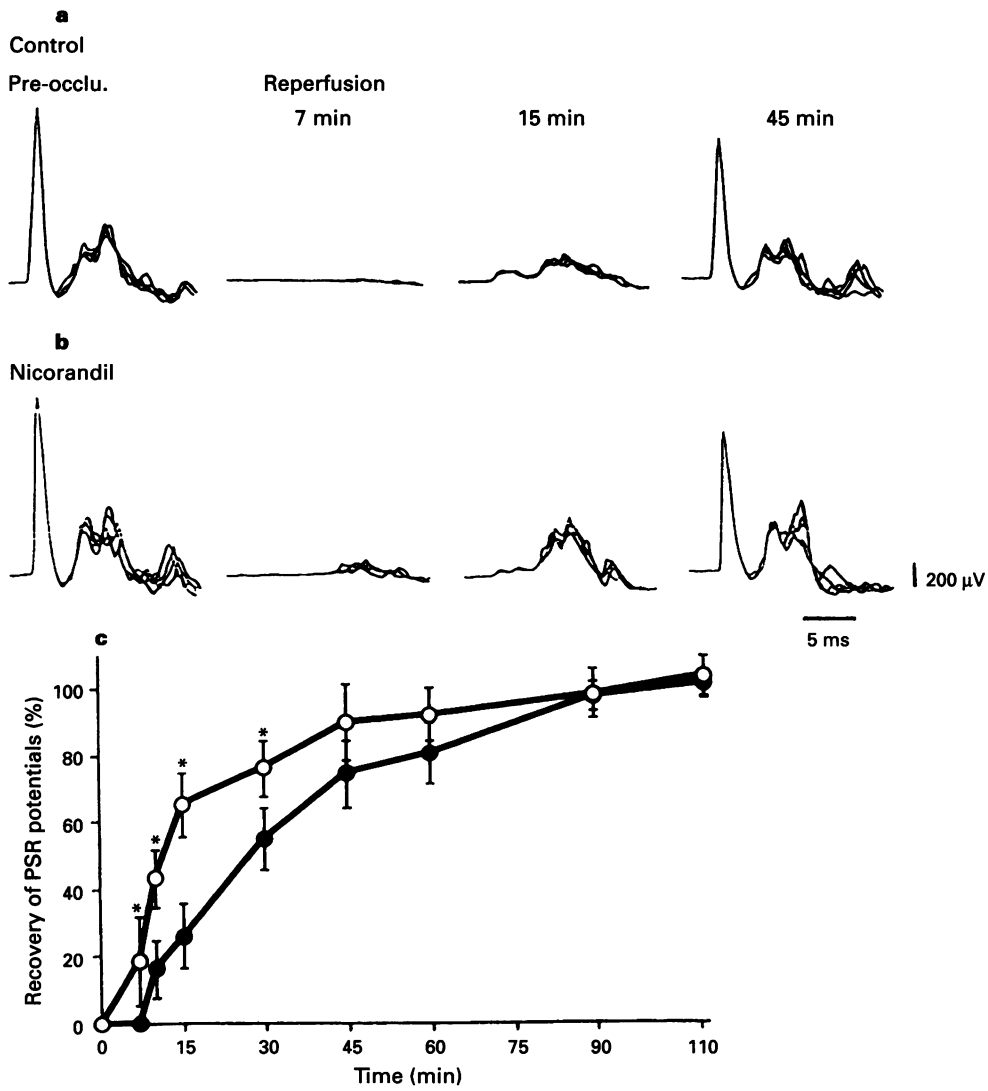

Figure 1 Effects of nicorandil on the recovery of reflex potentials after spinal cord ischaemia in cats: (a) and (b) are actual records of reflex potentials after the 1st control and 2nd post-nicorandil spinal cord ischaemia in a spinal cat; (c) indicates the recovery of polysynaptic reflex (PSR) potentials following spinal cord ischaemia before (●) and after the administration of 100 μg kg^{-1} nicorandil (○). The percentage recovery of PSR potentials and the time elapsed after reperfusion are indicated on the ordinate scale and the abscissa scale, respectively. Values are expressed as mean ± s.e. of 5 experiments. *$P < 0.05$ compared to the recovery after the 1st control ischaemia.

10 min spinal cord ischaemia RBF was decreased to 16 ± 3% of the preocclusion control. Upon reperfusion RBF was increased over the preocclusion level, and reactive hyperaemia was observed (Table 1). RBF reached its peak value around 5–15 min after reperfusion, and then gradually decreased to its preocclusion level around 45 min. Although mean blood pressure (BP) decreased from 91 ± 9 mmHg to 65 ± 5 mmHg immediately after injection of 100 μg kg^{-1} nicorandil, BP returned to the control level at 10 min after injection (Table 2). Nicorandil slightly increased RBF just prior to the second ischaemia (at 10 min) although the change was statistically insignificant. Neither RBF during spinal cord ischaemia nor reperfusion was significantly affected by nicorandil. Pinacidil and nitroprusside failed to increase RBF at these time points. BP was significantly decreased by nitroprusside infusion and increased by glibenclamide. Glibenclamide alone did not influence RBF during spinal cord ischaemia and reperfusion. Pretreatment with glibenclamide slightly attenuated the nicorandil-induced decrease in BP but hardly affected RBF change during spinal cord ischaemia and reperfusion (Tables 1 and 2). Thus, the accelerating effects of nicorandil and pinacidil on the recovery of PSR potentials were not associated with an increase in RBF during spinal cord ischaemia and reperfusion.

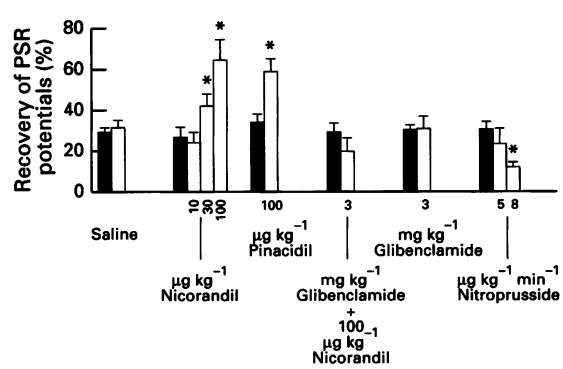

Figure 2 Effects of various drugs on the recovery of polysynaptic reflex (PSR) potentials after spinal cord ischaemia. Solid and open columns indicate the recovery of PSR potentials after the 1st control and 2nd post-drug spinal cord ischaemia, respectively. The recovery at 15 min after reperfusion is expressed as % area of preocclusion PSR potentials. Values are expressed as mean ± s.e. of 4–10 experiments. There was no significant difference in the recovery after the 1st spinal cord ischaemia among the subgroups. *$P < 0.05$ compared to the recovery of PSR potentials after the 1st control spinal cord ischaemia.

Discussion

A number of studies have indicated that nicorandil protects the heart from ischaemia/reperfusion injuries in experimental animals (Lamping *et al.*, 1984; Gross *et al.*, 1987; Grover *et al.*, 1990; Auchampach *et al.*, 1992). However, effects of nicorandil on ischaemic injuries in the central nervous system have not been thoroughly examined. Recently Kurihara *et al.* (1993) reported that nicorandil prevented post-ischaemic decrease in baroreflex sensitivity in a canine model of global cerebral ischaemia. This study has demonstrated that nicorandil accelerate the functional recovery from transient spinal cord ischaemia.

Nicorandil is a hybrid vasodilator exerting a dual mechanism of action as a K^+ channel opener and a nitrate (Taira, 1989). However, the regional blood flow of the spinal cord during ischaemia and reperfusion was not significantly affected by nicorandil. Therefore, a direct effect of nicorandil on neurones rather than an indirect effect via vascular dilatation might be important for the accelerating effect of nicorandil on the recovery of the reflex potentials. In this study, the K^+ channel opener, pinacidil, also accelerated the recovery of the reflex potentials after spinal cord ischaemia. In addition, the beneficial effect of nicorandil on PSR potentials was abolished by co-administration of glibenclamide. These findings indicate that activation of the K_{ATP} channels in neuronal tissues might

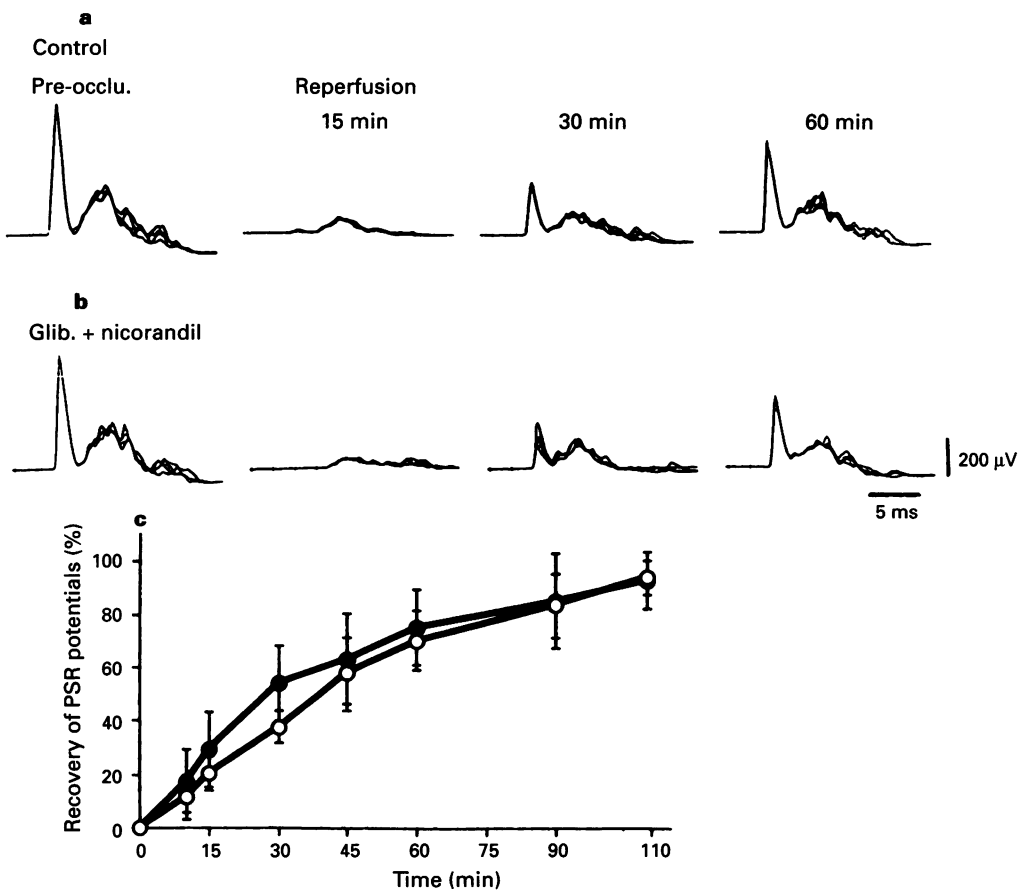

Figure 3 Effects of nicorandil combined with glibenclamide on the recovery of reflex potentials after spinal cord ischaemia in cats: (a) and (b) are actual records of reflex potentials after the 1st control and 2nd post-drugs spinal cord ischaemia in a spinal cat, (c) indicates the recovery of PSR potentials following spinal cord ischaemia before (●) and after the combined administration of 3 mg kg^{-1} glibenclamide and 100 μg kg^{-1} nicorandil (○). The recovery, expressed as % of the area of the preocclusion PSR potentials, is indicated on the ordinate scale. Values are expressed as mean ± s.e. of 5 experiments.

Table 1 Changes in regional blood flow of the spinal cord during 1st and 2nd ischaemia and reperfusion

	Ischaemia		*Reperfusion*			*Post-drug*	*Ischaemia*	*Reperfusion*	
	Pre	*10 min*	*5 min*	*15 min*	*Pre*	*10 min*	*10 min*	*5 min*	*15 min*
Saline	100	16 ± 3*	251 ± 23*	270 ± 10*	96 ± 4	102 ± 5	19 ± 3*	269 ± 34*	301 ± 30*
Nicorandil 10 μg kg^{-1}	100	17 ± 9*	289 ± 33*	264 ± 30*	96 ± 2	94 ± 5	18 ± 9*	267 ± 41*	308 ± 43*
30 μg kg^{-1}	100	18 ± 6*	241 ± 27*	270 ± 38*	97 ± 6	94 ± 7	21 ± 7*	237 ± 23*	257 ± 32*
100 μg kg^{-1}	100	18 ± 4*	253 ± 13*	268 ± 39*	110 ± 3	115 ± 6	19 ± 5*	248 ± 12*	248 ± 16*
Pinacidil 100 μg kg^{-1}	100	18 ± 2*	273 ± 25*	306 ± 25*	96 ± 4	94 ± 6	21 ± 4*	282 ± 19*	294 ± 36*
Glib 3 mg kg^{-1} + Nic 100 μg kg^{-1}	100	21 ± 9*	283 ± 33*	261 ± 30*	104 ± 12	106 ± 15	26 ± 7*	299 ± 57*	247 ± 47*
Glibenclamide 3 mg kg^{-1}	100	17 ± 4*	271 ± 20*	258 ± 24*	103 ± 8	107 ± 5	21 ± 4*	292 ± 20*	246 ± 18*
Nitroprusside 5 μg kg^{-1} min^{-1}	100	22 ± 4*	257 ± 20*	275 ± 20*	108 ± 7	106 ± 7	24 ± 8*	206 ± 31*	212 ± 15*†
8 μg kg^{-1} min^{-1}	100	16 ± 6*	221 ± 17*	287 ± 28*	96 ± 5	90 ± 15	10 ± 2*	128 ± 13*†	167 ± 31*†

Values are expressed as percentage (mean ± s.e.) of baseline value (pre) before 1st control occlusion.
*$P < 0.05$ vs each pre-value before the 1st control ischaemia. †$P < 0.05$ vs changes observed at each respective time point during 1st control ischaemia and reperfusion. Numbers of experiments are 4–10.

Table 2 Changes in mean arterial blood pressure after various drugs

	Nicorandil			*Pinacidil*	*Glib + Nic* 3 mg kg^{-1} +	*Glibenclamide*	*Nitroprusside*	
	10	*30*	*100* μg kg^{-1}	*100* μg kg^{-1}	*100* μg kg^{-1}	*3 mg* kg^{-1}	*5*	*8* μg kg^{-1} min^{-1}
Pre-drug	91.0±6.4	88.2±7.0	91.0±9.4	104.0±8.4	89.4±5.2	90.4±3.7	90.0±7.0	99.0±14.8
10 min	90.2±5.0	88.0±3.5	90.0±10.1	95.0±6.1	117.3±13.0	131.8±4.7*	67.5±4.7*	48.5±5.0*

Values of mean arterial blood pressure before (pre-drug) and 10 min after various drugs (10 min) are expressed as mean±s.e. of 4–8 experiments. *$P<0.05$ vs pre-drug value by paired t test. Note that nitroprusside decreased but glibenclamide increased arterial blood pressure.

be involved in the acceleration of PSR potentials after spinal cord ischaemia. This concept is also supported by the present findings that nitroprusside, a donor of nitric oxide (NO), failed to accelerate the recovery of PSR potentials. Moreover, a higher dose of nitroprusside significantly retarded the recovery. We cannot exclude the possibility that the slowed recovery of PSR potentials after the high dose of nitroprusside might stem from the decrease in RBF associated with a marked reduction of blood pressure. However, it is interesting to note several studies showing that NO production caused deleterious effects on neuronal injuries during cerebral ischaemia and reperfusion (Nowicki *et al.*, 1991; Kader *et al.*, 1993; Faraci & Brian, 1994). Therefore, the nitrate action of nicorandil might partly offset the beneficial action resulting from the K_{ATP} channel-activating property.

Although the specific mechanisms underlying the beneficial effect of K^+ channel openers on the functional recovery after spinal cord ischaemia remains unknown, some speculation may be permissible. High affinity [^{3}H]-glibenclamide binding sites, putative neuronal K_{ATP} channels, were observed not only in the brain (Bernardi *et al.*, 1988; Mourre *et al.*, 1989) but also in the spinal cord (Miller *et al.*, 1991; Jiang *et al.*, 1992). It was reported that in hippocampal neurones, anoxia induced an early hyperpolarization which was blocked by glibenclamide (Ben-Ari 1989; Mourre *et al.*, 1989). In addition, K^+ channel openers reduced the depolarization, which might lead to release of glutamate, following the early hyperpolarization. Therefore, K^+ channels openers may reduce ischaemic injuries by counteracting the neurotoxic action of excitatory amino acids. Recently it was also reported that K^+ channel openers including nicorandil and pinacidil were shown to inhibit γ-aminobutyric acid (GABA) release from slices of substantia nigra (Amoroso *et al.*, 1990; Schmid-Antomarchi *et al.*, 1990). Previous reports from our and other laboratories indicated that acute spinal ischaemia followed by reperfusion reduced the GABA level of the spinal cord probably by increasing GABA release (Homma *et al.*, 1979; Martiniak *et al.*, 1991; Zhang *et al.*, 1994). Since GABA receptor stimulation inhibited the recovery of PSR potentials after spinal cord ischaemia (Suzuki *et al.*, 1995), nicorandil and pinacidil might inhibit the depolarization-induced GABA release from spinal cord neurones and enhance the recovery of PSR potentials. Another possibility may be that K^+ channel openers counteract the depression of synaptic potentials resulting from the decrease of the inward calcium current in the presynaptic terminals. Somjen and his coworkers reported that hypoxia produced a failure of synaptic transmission in the isolated spinal cord of mice potentially due to slight depolarization of the resting membrane and depression of calcium-dependent action potentials (Urban & Somjen, 1990; Czeh & Somjen, 1990). The K^+ channel openers may have maintained the calcium current by some mechanism(s) under ischaemic conditions. However, these considerations are only speculative and further studies are needed to clarify the precise underlying mechanisms.

In the present study, glibenclamide *per se* failed to affect the recovery of PSR potential after spinal cord ischaemia although the drug significantly antagonized the effect of nicorandil. One possible explanation may be that openings of the K_{ATP} channel during spinal cord ischaemia might be minimal and that the K_{ATP} channels might be significantly activated by the administration of K^+ channel openers. In the present study, the recovery of MSR potentials after spinal cord ischaemia was not significantly affected by the drugs examined in this study. It has long been known that spinal interneurones are more susceptible to ischaemic insults than motoneurones (Murayama & Smith, 1965; Homma *et al.*, 1979). Therefore, MSR potentials, in which interneurones are not involved, might be less sensitive to pharmacological modulation. In addition, it has been reported that glibenclamide binding sites, i.e. K_{ATP} channels, are higher in the posterior horn than in the anterior horn of rat spinal cord (Mourre *et al.*, 1990).

When traumatic forces are applied to the spinal cord, resultant spinal cord ischaemia seriously injures the neurones. The present study has demonstrated for the first time that K^+ channel openers accelerate neurological recovery after spinal cord ischaemia although the precise mechanism of spinal protection is still obscure. In addition, it remains undetermined whether K^+ channel openers can retard neuronal death after longer spinal cord ischaemia. Further studies are required to evaluate the effects of K^+ channel openers on ischaemic injuries of the central nervous system including the spinal cord.

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References

AHLNER, J., ANDERSSON, R.G.G., TORFGARD, K. & AXELSSON, K.L. (1991). Organic nitrate esters: clinical use and mechanisms of actions. *Pharmacol. Rev.*, **43**, 351–423.

AMOROSO, S., SCHMID-ANTOMARCHI, H., FOSSET, M. & LAZDUNSKI, M. (1990). Glucose, sulfonylureas, and neurotransmitter release: role of ATP-sensitive K^+ channels. *Science*, **247**, 852–854.

ASHCROFT, F.M. (1988). Adenosine 5'-triphosphate-sensitive potassium channels. *Annu. Rev. Neurosci.*, **11**, 97–118.

ASHFORD, M.L.J., STURGESS, N.C., TROUT, N.J., GARDNER, N.J. & HALES, C.N. (1988). Adenosine-5'-triphosphate-sensitive ion channels in neonatal rat cultured central neurones. *Pflügers Arch.*, **412**, 297–304.

AUCHAMPACH, J.A., CAVERO, I. & GROSS, G.J. (1992). Nicorandil attenuates myocardial dysfunction associated with transient ischemia by opening ATP-dependent potassium channels. *J. Cardiovasc. Pharmacol.*, **20**, 765–771.

BEN-ARI, Y. (1989). Effect of glibenclamide, a selective blocker of an ATP-K^+ channel, on the anoxic response of hippocampal neurones. *Pflügers Arch.*, **414** (Suppl. 1), S111–S114.

BEN-ARI, Y., KRNJEVIC, K. & CREPEL, V. (1990). Activators of ATP-sensitive K^+ channels reduce anoxic depolarization in CA3 hippocampal neurons. *Neurosci.*, **37**, 55–60.

BERNARDI, H., FOSSET, H. & LAZDUNSKI, M. (1988). Characterization, purification and affinity labelling of the brain [^{3}H] glibenclamide-binding protien, a putative ATP-regulated K^+ channel. *Proc. Natl. Acad. Sci. U.S.A.*, **85**, 9816–9820.

COLE, W.C., MCPHERSON, C.D. & SONTAG, D. (1991). ATP-regulated K^+ channels protect the myocardium against ischaemia/reperfusion damage. *Circ. Res.*, **69**, 571–581.

CZEH, G. & SOMJEN, G.G. (1990). Hypoxic failure of synaptic transmission in the isolated spinal cord, and the effects of divalent cations. *Brain Res.*, **527**, 224–233.

DUCKER, T.B., SALCMAN, M., LUCAS, J.T., GARRISON, W.B. & PEROT P.L. (1978a). Experimental spinal cord trauma, II: Blood flow, tissue oxygen, evoked potentials in both paretic and plegic monkeys. *Surg. Neurol.*, **10**, 64–70.

DUCKER, T.B., SALCMAN, M., PEROT, P.L. & BALLANTINE, D. (1978b). Experimental spinal cord trauma, I: Correlation of blood flow, tissue oxygen and neurologic status in the dog. *Surg. Neurol.*, **10**, 60–63.

EDWARDS, G. & WESTON, A.H. (1993). The pharmacology of ATP-sensitive potassium channels. *Annu. Rev. Pharmacol. Toxicol.*, **33**, 597–637.

FARACI, F.M. & BRIAN, J.E. (1994). Nitric oxide and the cerebral circulation. *Stroke*, **25**, 692–703.

GROSS, G.J., WARLTIER, D.C. & HARDMAN, H.F. (1987). Comparative effects of nicorandil, a nicotinamide nitrate derivative, and nifedipine on myocardial reperfusion injury in dogs. *J. Cardiovasc. Pharmacol.*, **10**, 535–542.

GROVER, G.J., SLEPH, P.G. & PARHAM, C.S. (1990). Nicorandil improves postischemic contractile function independently of direct myocardial effects. *J. Cardiovasc. Pharmacol.*, **15**, 698–705.

HOMMA, S., SUZUKI, T., MURAYAMA, S. & OTSUKA, M. (1979). Amino acid and substance P contents in spinal cord of cats with experimental hind-limb rigidity produced by occlusion of spinal cord blood supply. *J. Neurochem. (Lond)*, **32**, 691–698.

JIANG, C., XIA, Y. & HADDAD, G.G. (1992). Role of ATP-sensitive K^+ channels during anoxia: major differences between rat (newborn and adult) and turtle neurons. *J. Physiol.*, **448**, 599–612.

KADER, A., FRAZZINI, V.I., SOLOMON, R.A. & TRIFILETTI, R.R. (1993). Nitric oxide production during focal cerebral ischemia in rats. *Stroke*, **24**, 1709–1716.

KURIHARA, J., OCHIAI, N. & KATO, H. (1993). Protection by nicorandil against the dysfunction of the central vagal baroreflex system following transient global cerebral ischaemia in dogs. *Br. J. Pharmacol.*, **109**, 1263–1267.

LAMPING, K.A., CHRISTENSEN, C.W., PELC, L.R., WARLTIER, D.C. & GROSS, G.J. (1984). Effects of nicorandil and nifedipine on protection of ischemic myocardium. *J. Cardiovasc. Pharmacol.*, **6**, 536–542.

MARTINIAK, K., SAGANOVA, K. & CHAVKO, M. (1991). Free and peptide-bound amino acids as indicators of ischemic damage of the rabbit spinal cord. *J. Neuropathol. Exp. Neurol.*, **50**, 73–81.

MILLER, J.A., VELAYO, N.L., DAGE, R.C. & RAMPE, D. (1991). High affinity [^{3}H] glibenclamide binding sites in rat neuronal and cardiac tissue: localization and developmental characteristics. *J. Pharmacol. Exp. Ther.*, **256**, 358–364.

MOURRE, C., BEN ARI, Y., BERNARDI, H., FOSSET, M. & LAZDUNSKI, M. (1989). Antidiabetic sulfonylureas: localization of binding sites in the brain and effects of the hyperpolarization induced by anoxia in hippocampal slices. *Brain Res.*, **486**, 159–164.

MOURRE, C., WIDMANN, C. & LAZDUNSKI, M. (1990). Sulfonylurea binding sites associated with ATP-regulated K^+ channels in the central nervous system: autoradiographic analysis of their distribution and ontogenesis, and of their localization in mutant mice cerebellum. *Brain Res.*, **519**, 29–43.

MURAYAMA, S. & SMITH, C.M. (1965). Rigidity of hind limbs of cats produced by occlusion of spinal cord blood supply. *Neurology*, **15**, 565–577.

MURPHY, K.P.S.J. & GREENFIELD, S.A. (1991). ATP-sensitive potassium channels counteract anoxia in neurones of the substantia nigra. *Exp. Brain Res.*, **84**, 355–358.

NAKAYA, H., TAKEDA, Y., TOHSE, N. & KANNO, M. (1991). Effects of ATP-sensitive K^+ channel blockers on the action potential shortening in hypoxic and ischaemic myocardium. *Br. J. Pharmacol.*, **103**, 1019–1026.

NOMA, A. (1983). ATP-regulated K^+ channels in cardiac muscle. *Nature*, **305**, 147–148.

NOWICKI, J.P., DUVAL, D., POIGNET, H. & SCATTON, B. (1991). Nitric oxide mediates neuronal death after focal cerebral ischemia in the mouse. *Eur. J. Pharmacol.*, **204**, 339–340.

OHTA, H., JINNO, Y., HARADA, K., OGAWA, N., FUKUSHIMA, H. & NISHIKORI, K. (1991). Cardioprotective effects of KRN 2391 and nicorandil on ischemic dysfunction in perfused rat heart. *Eur. J. Pharmacol.*, **204**, 171–177.

RORSMAN, P. & TRUBE, G. (1985). Glucose dependent K^+-channels in pancreatic beta-cells are regulated by intracellular ATP. *Pflügers Arch.*, **405**, 305–309.

SCHMID-ANTOMARCHI, H., AMOROSO, S., FOSSET, M. & LAZDUNSKI, M. (1990). K^+ channel openers activate brain sulfonylurea-sensitive K^+ channels and block neurosecretion. *Proc. Natl. Acad. Sci. U.S.A.*, **87**, 3489–3492.

SPRUCE, A.E., STANDEN, N.B. & STANFIELD, P.R. (1985). Voltage-dependent ATP-sensitive potassium channels of skeletal muscle membrane. *Nature*, **316**, 736–738.

STANDEN, N.B., QUAYLE, J.M., DAVIES, N.W., BRAYDEN, J.E., HUANG, Y. & NELSON, M.T. (1989). Hyperpolarizing vasodilators activate ATP-sensitive K^+ channels in arterial smooth muscle. *Science*, **245**, 177–180.

SUZUKI, T., OKAMOTO, Y., SEKIKAWA, T., NEMOTO, T., MORIYA, H., MURAYAMA, S. & NAKAYA, H. (1995). Effects of GABAergic drugs on the recovery reflex potentials after spinal cord ischemia in cats. *Jpn. J. Pharmacol.*, (in press).

TAIRA, N. (1989). Nicorandil as a hybrid between nitrates and potassium channel activators. *Am. J. Cardiol.*, **63**, 18J–24J.

URBAN, L. & SOMJEN, G.G. (1990). Reversible effects of hypoxia on neurons in mouse dorsal root ganglia in vitro. *Brain Res.*, **520**, 36–42.

ZHANG, A.L., HAO, J.X., SEIGER, A., XU, X.J., WIESENFELD-HALLIN, Z., GRANT, G. & ALDSKOGIUS, H. (1994). Decreased GABA immunoreactivity in spinal cord dorsal horn neurons after transient spinal cord ischemia in the rat. *Brain Res.*, **656**, 187–190.

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