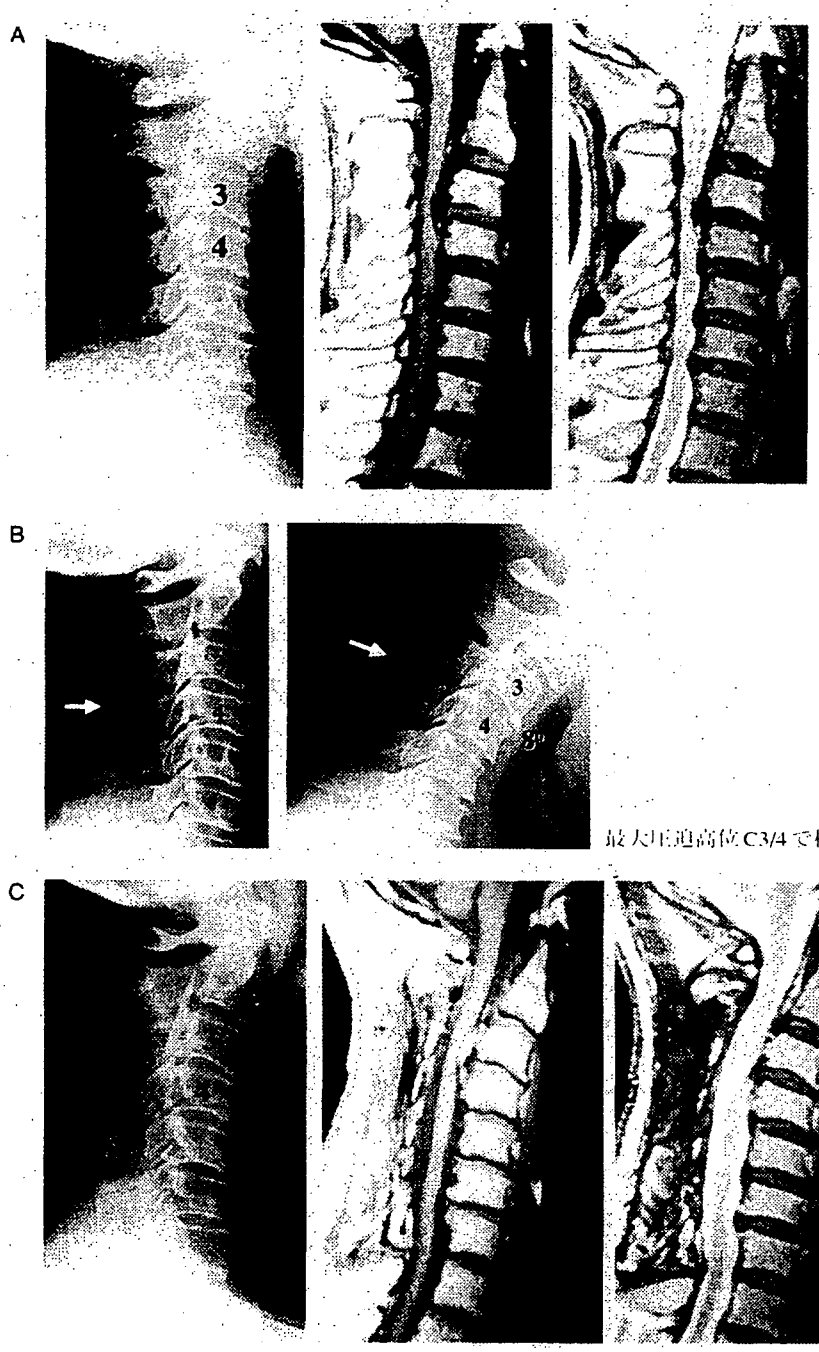

68歳，男性　OPLL（C3/4が最大圧迫高位），cJOA　6.5/17，椎弓形成術を選択

最大圧迫高位C3/4で椎間可動性が明瞭

椎弓形成術後にC3/4後弯増強，脊髄の後方移動不十分，cJOA－9/17，改善率＝21.7％

図2．椎弓形成術例

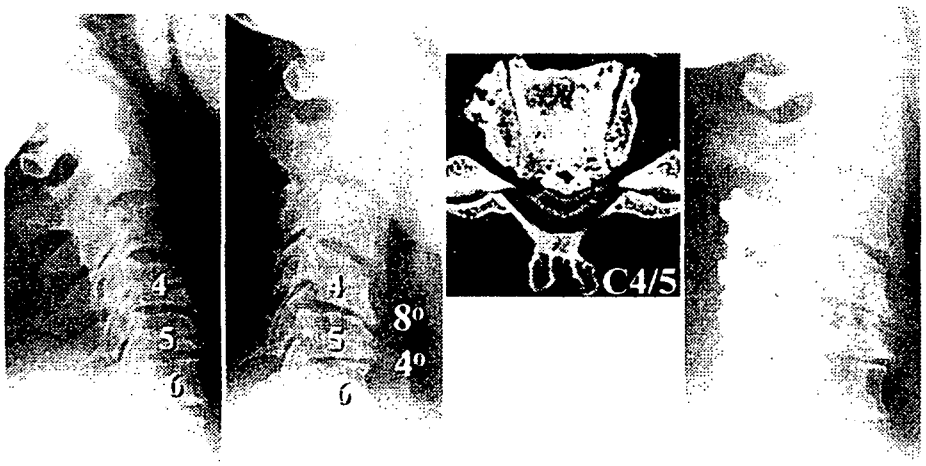

図3．後方除圧固定術施行例

後方除圧固定術の導入

今後の対策として，最大圧迫高位で椎間可動性が明瞭な後縦靭帯骨化症例に対しては，前方法の選択が望ましいと考えます．もし高齢などの理由で後方法を選択せざるを得ない場合，脊髄圧迫が残存する範囲に限り，後方instrumentation固定を追加する選択肢もあると考えます．instrumentation固定を併用することにより，脊柱を制動しマイクロモーションに起因する脊髄障害の発生を抑制することが可能と考えます．

我々は2003年以降，上記の理論のもとに，成績不良が予測される高齢の頸椎後縦靭帯骨化症例に対し，後方除圧固定術，すなわち，後方instrumentation固定を併用した椎弓形成術を行っており，現在までのところ良好な結果を得ています（図3）．今後さらにデータを積み重ね，後方除圧固定術の有用性を立証したいと考えています．

おわりに

高齢者頸髄症に対する手術では，脊髄症状が重度になる前に手術を行えば，青壮年者の頸髄症に対する手術成績に劣らぬ成績を期待することができます．前方法では良好な術後成績が期待できますが，入念な術中術後の管理が必要です．症例の多くは後方法で対処可能ですが，後弯，不安定性を有する例では成績が不良となる傾向があり，とくに後縦靭帯骨化症例では注意を払う必要があります．これらの成績不良因子を有しながらも，高齢という理由で後方法を選択せざるを得ない頸髄症例に対しては，今後，除圧にinstrumentation固定を併用する後方除圧固定術が有力な選択肢になると考えます．

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マウス脊髄圧挫損傷モデルにおける ヒト骨髄間質細胞由来神経幹細胞移植の検討

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Key words: Spinal cord injury（脊髄損傷）, Cell transplantation（細胞移植）, Neural stem cell（神経幹細胞）

〔目的〕　現在、脊髄損傷に対する治療の研究においては1次損傷に引き続きおこる2次損傷をおさえ、ひいては脊髄再生を促進するために主に薬物投与による治療と細胞移植による治療が研究されている。細胞移植については移植細胞の候補として神経幹細胞、嗅神経細胞、骨髄間質細胞など多種の細胞による移植が報告されているが、実際の臨床応用を考慮した場合、細胞の供給源としてES細胞や胎児由来細胞などのいくつかの細胞は倫理的問題から臨床応用が困難なことが考えられる。そこで我々は細胞の供給源の1つとして骨髄間質細胞に注目し、その脊髄損傷治療への有用性を過去に報告した[1]。今回我々はヒト骨髄間質細胞にNotchの細胞内ドメインを遺伝子導入することにより神経幹細胞へ誘導した細胞（以下hBMSC-NSC）を免疫不全マウスであるNOD/scidマウスの脊髄圧挫損傷モデルに移植し、その有効性について検討した。

〔方法〕　NOD/scidマウス（生後8週、雌、20-25g）をT9レベルでlaminectomyした後Precision Systems and Instrumentation社製Infinite Horizon impactorを用いて脊髄を圧挫損傷（60kdyn）し、脊髄損傷モデルを作製した。損傷1週後に、hBMSC-NSC（1.5×10^5個/3μl）を脊髄損傷部にガラスピペットを用いて髄注した（以下移植群）。また、同時にメディウム（3μl）のみを脊髄損傷部に髄注した対照群も作製した。hBMSC-NSCは出澤らのプロトコール[2]に従いヒト骨髄間質細胞から誘導した。すなわち、骨髄間質細胞にNotchの細胞内ドメイン(Notch ICD)を遺伝子導入し、薬剤耐性遺伝子により恒常的にNotch ICDを発現している細胞を選択した。損傷後の後肢運動機能評価をBasso Mouse Scale(BMS)[3]を用いて、損傷後1d、3d、1w、以後1週ごとに8週まで評価した。損傷後8週でsacrificeし損傷脊髄矢状断の凍結切片を作製、免疫染色を用いて組織を評価した。また、hBMSC-NSC移植群のうち2匹に損傷後8週の時点でジフテリアトキシン（以下DT）を腹腔内投与し、投与1週後に後肢運動機能評価を行った。これはマウスの細胞がヒト細胞に比べてDTに対する感受性が極度に低いことを利用して、移植したヒト細胞のみが消失することにより機能が変化するかどうかを検討するためである。

〔結果〕　移植したhBMSC-NSCはヒト細胞由来であるので、ヒト細胞を認識する抗体として抗human mitochondria抗体を用いて損傷後8週の損傷脊髄の免疫染色を行ったところ、移植したhBMSC-NSCが残存しているのが確認された。hBMSC-NSCは損傷部周囲および損傷部からやや離れた部位では主に白質に残存していた。また、脊髄損傷後の瘢痕部を取り囲むreactive astrocyteのマーカーとして用いられる抗Glial Fibriary Acidic Protein抗体による免疫染色にて損傷面積を評価した結果、移植群では対照群に比べ損傷面積の割合が小さい傾向にあったが有意差を認めなかった（図1）。さらに、下行性軸索のマーカーとして用いられる抗serotonin抗体を用いて損傷部周囲のserotonin陽性線維の密度を評価した結果、移植群では対照群に比べ損傷中心部および損傷部より1000μm尾側部においてserotonin陽性線維の密度が有意に大きかった（図2）。損傷後後肢運動機能の回復は移植群において対照群よりも有意に良好であった（図3）。移植群のうち2匹に損傷後8週の時点でDTを腹腔内投与し、投与1週後に後肢運動機能評価を行ったが、後肢運動機能は不変であった。

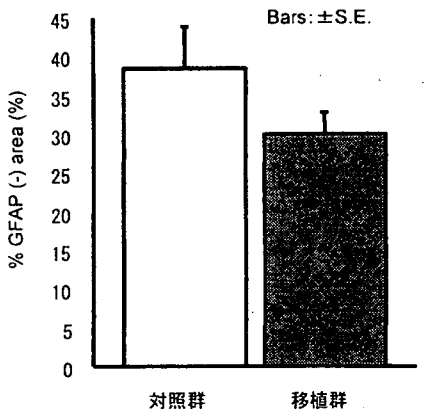

図1．　損傷脊髄GFAP陰性面積の割合

〔考察〕　今回の結果より、hBMSC-NSC移植により下行性軸索であるserotonin陽性線維のregenerationまたはsparingが促進し、後肢運動機能回復を促進した可能

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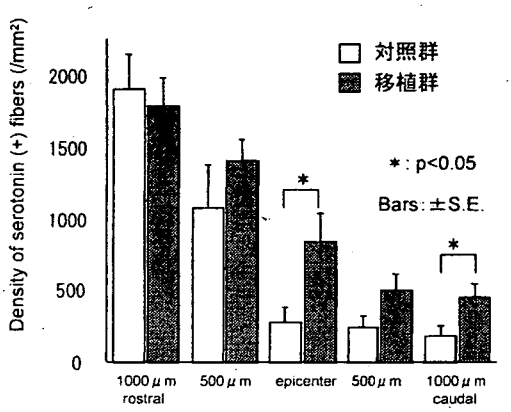

図２．　損傷脊髄 serotonin 陽性線維密度

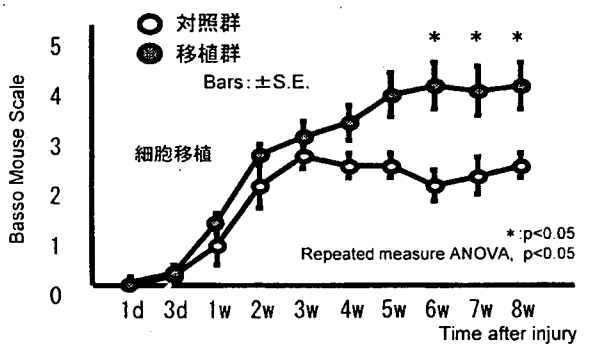

図３．　脊髄損傷後後肢運動機能

性が考えられた。hBMSC-NSC 移植マウス２匹に DT 投与したが後肢運動機能は明かな低下を認めなかったことから、hBMSC-NSC 移植の治療効果は主に移植後早期の trophic effect が主な要因である可能性、または残存細胞数が少なかったためヒト細胞が消失しても後肢運動機能に影響がなかった可能性が考えられた。

〔まとめ〕

・ヒト骨髄間質細胞由来神経幹細胞を免疫不全マウスの脊髄圧挫損傷モデルに移植し、移植細胞は損傷後８週で損傷脊髄内に残存していた。

・移植群では術後８週での脊髄損傷部および損傷尾側部の５HT 陽性線維の密度がコントロール群に比し有意に高かった。

・移植群では脊髄損傷後の後肢運動機能の回復がコントロール群に比し有意に促進された。

・ヒト骨髄間質細胞由来神経幹細胞が脊髄損傷に対する細胞移植治療において有用な選択肢の１つになりうることが示唆された。

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ラット脊髄圧挫損傷モデルにおける骨髄間質細胞(BMSC)移植とRhoキナーゼ阻害薬の併用による治療効果

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Key words: Spinal cord injury（脊髄損傷）, bone marrow stromal cell(骨髄間質細胞), Rho-kinase inhibitor(Rhoキナーゼ阻害薬)

〔背景〕近年の幹細胞生物学の進歩により、従来は再生不能とされてきた脊髄損傷に対し様々な実験的細胞移植が試みられている。一方で細胞移植のみでは臨床的に意義のある回復が得られないのが現状である。これらの観点から細胞移植に加え、薬物を投与する併用療法の必要性がクローズアップされてきている。現在までに我々は損傷脊髄に対する細胞移植として、骨髄より得られる造血幹細胞[1)2)]、骨髄由来分化細胞[3)]移植を行い比較的良好な成績を得ている。これらの結果をふまえ今回は骨髄間質細胞（Bone marrow stromal cell、BMSC)移植に薬剤投与を併用することにより、更なる行動回復が得られるかどうか検討した。

BMSCの利点は、自家移植が可能であること、旺盛な自己複製能を持つため培養が比較的容易であること、Stem cell likeな多分化能をもつことが挙げられる。特にBMSCの一部、間葉系幹細胞は中枢神経系の移植環境によっては neuronや gliaに分化するといわれ、胚葉を越えたtransdifferentiationがおこる可能性が示唆されている。また損傷脊髄において細胞が生着しなくとも細胞移植そのものが軸索伸展、抗アポトーシス作用を持つという報告も多い。今回併用療法として使用したRhoキナーゼ阻害薬(fasudil)は活性化Rhoを不活化することにより、神経軸索の退縮や細胞死を減少させることが知られ、当教室の西尾らは急性期ラット脊髄損傷に対し、くも膜下腔持続投与による行動回復を報告している[4)]。これらの知見を基にBMSC移植とfasudilの併用療法による効果が得られるかどうかを検討した。

〔対象および方法〕(BMSCの作製)GFP transgenicラットの両大腿骨・下腿骨より髄腔内容を採取し、付着性の細胞を継代培養しBMSCを作製した。

(脊髄圧挫モデルの作成)雌性SDラット(9週齢)の第9－10胸椎を椎弓切除後、Infinite Horizon Impactor (200Kdyn)を用いて脊髄圧挫モデルを作成した。

(fasudilの投与)第1腰椎を椎弓切除し、Alzet浸透圧ポンプを用いてfasudilのくも膜下腔持続注入(180μg/日)を損傷直後より4週間施行した。

(BMSCの移植)損傷から2週後、損傷部より2mm遠位と近位にBMSCを計2.5×10^6個髄注した。

BMSC移植にfasudil投与を行ったBMSC＋fasudil併用群に対し、対照としてBMSC単独群、fasudil単独群、および非治療コントロール群を作成し以下の比較検討を行った。

(下肢運動評価)損傷後8週間のBBBスコアによる行動評価、8週地点におけるFootprint analysis、11週時点での腓腹筋ヒラメ筋の定量評価を行った。

(下肢痛覚評価)Dynamic Plantar Anesthesiometerを用いた痛覚試験を行った。

(皮質脊髄路トレーシング)損傷後9週時点で大脳皮質運動野にBDAを注入、11週時点で灌流固定し染色線維を評価した。

(組織学的評価)組織学的評価として11週時点において抗GFP抗体にて移植細胞を染色し移植細胞の残存状態を確認した。

(統計)BBBスコアはRepeated measures ANOVAを用い各群の経時的変化を比較、Post-hoc testにはTurkey-Kramer法を用いた。その他の多群間の比較はOne-way ANOVAとTurkey-Kramer法を用いて検討した。

〔結果〕移植細胞の蛍光免疫染色ではNestin、Fibronectin、Vimentin、CD90各マーカー陽性であり、諸家のBMSCの報告と一致する細胞を得た。損傷後3日から8週間のBBBスコアの経時的変化を図1に示す。

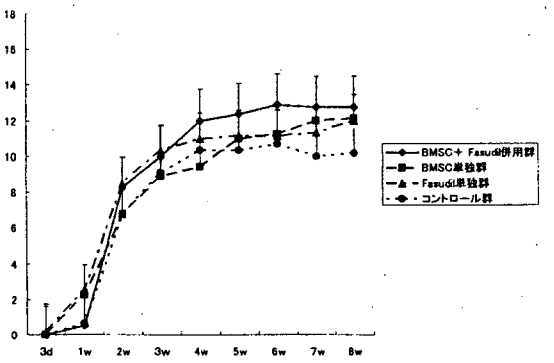

図1. BBBスコア

BBBスコアは、BMSC＋fasudil併用群(実線)で8週において12.8点と他群に比べ改善が得られたが有意差は認められなかった。併用群・単独群間において「相乗的な」効果は認められなかった。Footprint analysisでは両下肢幅、両足の外旋角度について有意差を認めなかった。腓腹筋、ヒラメ筋の定

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量評価において治療群はコントロール群と比べ高値を示したが有意差を認めなかった。下肢痛覚評価では潜時、閾値ともコントロール群と比べ併用群で有意に短かった。しかし併用群・単独群間において有意差を認めなかった。損傷部脊髄の免疫染色では併用群、BMSC 単独群で移植した BMSC は残存し、損傷部空洞周囲に多く存在していた。また同細胞は Astrocyte のマーカーGFAP を共発現していた。皮質脊髄路トレーシングでは損傷部を越えた線維は確認できなかった。

〔考察〕 下肢運動機能、痛覚評価にて今回検討した各項目では併用群と単独群の間に有意差を認めなかったが BBB スコアにおいて、併用群が単独群に勝る傾向は認められた。一方で期待した「相乗的な」効果は得られなかった。組織学的評価では移植細胞は損傷部空洞周囲に多く残存しており、空洞壁を形成した可能性が示唆された。他の報告と比較し細胞の生着が少なく移植法、時期、部位、量の再検討が必要であった。また、免疫抑制剤の投与法も見直しを要すると考えられた。

〔まとめ〕 ①ラット脊髄圧挫損傷モデルにおいて BMSC 移植と Rho キナーゼ阻害薬(fasudil)の併用による治療効果を検討した。

②併用群はコントロール群・単独群と比べ BBB スコアでの改善傾向がみられたが有意差は得られなかった。

③移植細胞は残存し、損傷部空洞周囲に多く存在していた。

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Hypothermia Suppresses Excitatory Synaptic Transmission and Neuronal Death Induced by Experimental Ischemia in Spinal Ventral Horn Neurons

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Study Design. Whole-cell patch-clamp recordings were performed from the ventral horn neurons obtained from the rat spinal cord slices.

Objective. This study investigated the effects of hypothermia on excitatory synaptic transmission and ischemia-induced neuronal death.

Summary of Background Data. Hypothermia has long been recognized as a promising physical strategy against both ischemic and traumatic spinal cord injuries. However, the mechanism of hypothermia-mediated neuroprotective action in the spinal cord is still not fully understood at the single cell level.

Methods. Whole-cell patch-clamp recordings were performed from ventral horn neurons obtained from the spinal cord slices. Ischemia was simulated by superfusing an oxygen- and glucose-deprived medium [ischemia simulating medium (ISM)].

Results. When the temperature of the superfusing artificial cerebrospinal fluid solution was changed from normothermia (36°C) to hypothermia (32°C, 28°C, and 24°C), the frequency of spontaneous excitatory postsynaptic currents was significantly decreased in a temperature-dependent manner. Surperfusing the ISM generated an agonal inward current which consisted of a slow and subsequent rapid inward current in all of the neurons tested. The latencies of the slow and rapid inward currents after the ISM exposures were significantly longer at hypothermia than at normothermia. Hypothermia decreased the slope of the ISM-induced slow inward current, although it did not affect the slope of the rapid inward current. Moreover, the glutamate receptor antagonists slightly prolonged the latencies of the slow and rapid inward currents that were induced by ISM and significantly decreased their slopes.

Conclusion. These results suggest that hypothermia reduces the excitatory synaptic activities and ischemic neuronal death in the spinal ventral horn. This finding may help in achieving a better understanding of the mechanisms of hypothermia-mediated neuroprotection in the spinal cord.

Key words: spinal motoneurons, hypothermia, ischemia, cell death, electrophysiology. **Spine 2007;32:E741–E747**

The primary injury of the spinal cord is directed to the central gray matter, where frank destruction occurs. The primary injury is followed by the progressive autodestructive secondary injury that is believed to enlarge the area of neuronal death through necrosis and apoptosis.[1,2] The mechanism of the secondary injury includes a variety of processes such as ischemia, glutamate excitotoxicity, free radical-medicated cell death, ATP released from damaged tissues, and cytoskeletal degradation.[3] These processes trigger an inflammatory reaction that induces necrosis and apoptosis, thereby causing further destruction in the spinal cord. As the primary injury is immediate and irreversible, it is not well suited for therapeutic intervention. Because of the delayed processes, the secondary injury is the most appropriate target for therapeutic intervention.[4] A high-dose regimen of steroid drugs such as methylprednisolone is often administered to reduce the secondary injury.[5,6] However, the effect of steroid drugs is not satisfying and its complications cannot be ignored.[7] Many other neuroprotective interventions have been tested in both spinal cord injury patients and model animals.[4] Unfortunately, none of these interventions have resulted in any major improvement in the neurologic recovery or a meaningful increase in function, although much effort and resources have been expended. Hypothermia has long been recognized as a promising physical strategy against ischemic and traumatic spinal cord injury.[8–10] Paraplegia caused by ischemic spinal cord injury is one of the most serious complications during thoracic and thoracoabdominal aortic surgery. Hypothermia has been evaluated as a method of spinal cord protection during thoracic and thoracoabdominal aortic surgery,[11] and several techniques for spinal cord cooling have been developed providing clear evidence for its neuroprotective effects.[12,13] In addition, a revival of hypothermia for the treatment of traumatic brain injury in the last decade has encouraged a re-evaluation of the potential benefits of hypothermia in the treatment of traumatic spinal cord injury.[14–16] It

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has been demonstrated that systemic hypothermia induced 30 minutes after traumatic spinal cord injury and maintained for 4 hours significantly improved the locomotor function.[17] However, the mechanism of hypothermia-mediated neuroprotective action is still not fully understood at the single cell level. In the present study, we investigated the effect of hypothermia on excitatory synaptic transmission and ischemia-induced membrane dysfunction by performing whole-cell patch-clamp recordings from ventral horn neurons in the rat spinal cord slices. Ischemia was simulated by superfusing an oxygen- and glucose-deprived medium [ischemia simulating medium (ISM)], a process which has been well established in brain and spinal cord slices.[18–20]

■ Materials and Methods

All of the experimental procedures involving the use of animals were approved by the Ethics Committee on Animal Experiments, Wakayama Medical University, and were conducted in accordance with the UK Animals (Scientific Procedures) Act of 1986 and its associated guidelines.

Spinal Cord Slice Preparation. The methods used to obtain the rat spinal cord slice preparations have been described previously.[21] In brief, Sprague-Dawley rats at the postnatal age of 8 to 12 days were deeply anesthetized with pentobarbital sodium (60 mg/kg, intraperitoneal), then a lumbosacral laminectomy was performed. Next, the lumbosacral spinal cord (L1–S3) was removed and placed in a preoxygenated artificial cerebrospinal fluid (ACSF) solution at 1°C to 3°C. Immediately after the removal of the spinal cord, the rats were given an overdose of pentobarbital sodium and then were killed by exsanguination. The pia-arachnoid membrane was then removed after cutting all of the ventral and dorsal roots near the root entry zone. The spinal cord was placed on an agar block and mounted on a microslicer (DTK-1000; D.S.K., Kyoto, Japan), then a 500 μm-thick transverse slice was cut. The slice was placed in the recording chamber, which had a volume of 0.5 mL solution, and then was placed on the stage of an upright microscope equipped with an infrared-differential interference contrast (IR-DIC) system (BX51WI; Olympus, Tokyo, Japan). Next, the slice was superfused at a rate of 5 mL/min with ACSF solution saturated with 95% O_2 and 5% CO_2 at 36°C ± 1°C for more than 1 hour. The ACSF solution contained (in mM) 117 NaCl, 3.6 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 25 $NaHCO_3$, and 11 glucose. The temperature of the superfusing solution was continuously monitored with an auto-regulatory heat exchanger (TC324-B, Warner instruments, Hamden, CT). An in-line solution heater (SH-27B, Warner instruments) was automatically controlled to the set temperature (24°C, 28°C, 32°C, and 36°C), and the accuracy was typically better than ±1°C.

Patch-Clamp Recordings From Spinal Ventral Horn Neurons. The lamina regions were identified using a 5× objective lens, and the individual neurons were identified using a 40× objective lens under an IR-DIC microscope. The microscope was coupled with a CCD camera (C2741–C79; HAMAMATSU, Hamamatsu, Japan) and a video monitor screen. Whole-cell patch-clamp recordings were made from the lamina IX neurons in the spinal ventral horn with patch-pipette electrodes having a resistance of 4 to 8 MΩ.[22] The composition of the patch-pipette solution was as follows (in mM): 135 potassium gluconate, 5 KCl, 0.5 $CaCl_2$, 2 $MgCl_2$, 5 EGTA, 5 HEPES, 5 ATP-Mg, pH 7.2. The signals were acquired with an amplifier Axopatch 200B (Axon Instruments, Union City, CA). The data were digitized with an A/D converter (Digidata 1322A, Axon Instruments), then stored on a personal computer using a data acquisition software program (pCLAMP 9, Axon Instruments).

Ischemia Simulation and Drug Application. The slices were made "ischemic" by superfusing them with an ACSF solution equilibrated with 95% N_2 to 5% CO_2, then they were deprived of glucose by a bath application, which was replaced with sucrose isoosmotically (ISM). The drugs used in this study were 6-cyano-7-nitroquinoxaline-2,3-dion (CNQX) and D(-)-2-amino-5-phosphonopentanoic acid (AP5) from Tocris (Ballowin, MO). The drugs were dissolved in either the ACSF solution or ISM and then applied by perfusion via a 3-way stopcock without any change in the perfusion rate. The time necessary for the solution to flow from the stopcock to the surface of the spinal cord slice was approximately 20 seconds.

Statistical Analysis. All numerical data are expressed as the mean ± SEM. Statistical significance was determined as $P < 0.05$ using Student t test and analysis of variance followed by the Scheffe test. Regarding the electrophysiological data, n refers to the number of neurons studied.

■ Results

Whole-cell patch-clamp recordings were performed from 160 ventral horn neurons in the lamina IX of the spinal cord slices. The ventral horn neurons were viable for up to 12 hours in the slices perfused with the preoxygenated ACSF solution. However, all of the recordings were obtained within 4 hours in this study.

Effect of Hypothermia on Excitatory Synaptic Transmission

At a holding potential of −70 mV, spontaneous excitatory postsynaptic currents (sEPSCs) were observed in all of the ventral horn neurons examined (Figure 1A). The sEPSCs recorded from 19 neurons at normothermia (36°C) had an average frequency and amplitude of 10.2 ± 0.98 Hz (range: 1.9–18.5 Hz) and 20.2 ± 1.5 A (range: 11.6–42.2 pA), respectively. When the temperature of the superfusing ACSF solution was changed from normothermia (36°C) to hypothermia (32°C, 28°C, and 24°C), the sEPSC frequency significantly decreased in a temperature-dependent manner (Figure 1A). When measured for 30 seconds at 32°C, 28°C, and 24°C, the averages in sEPSC frequency were decreased to 54.7% ± 6.6% (n = 6), 34.2 ± 6.4% (n = 6), and 18.9 ± 2.9% (n = 7), respectively (Figure 1B). The change in the temperature of the superfusing ACSF solution from 36°C to hypothermia (32°C and 28°C) did not significantly affect the sEPSC amplitude (Figure 1B). The average changes in sEPSC amplitude were 95.9% ± 3.7% (32°C, n = 6) and 97.0% ± 3.9% (28°C, n = 6). On the other hand, the sEPSC amplitude was significantly decreased when the

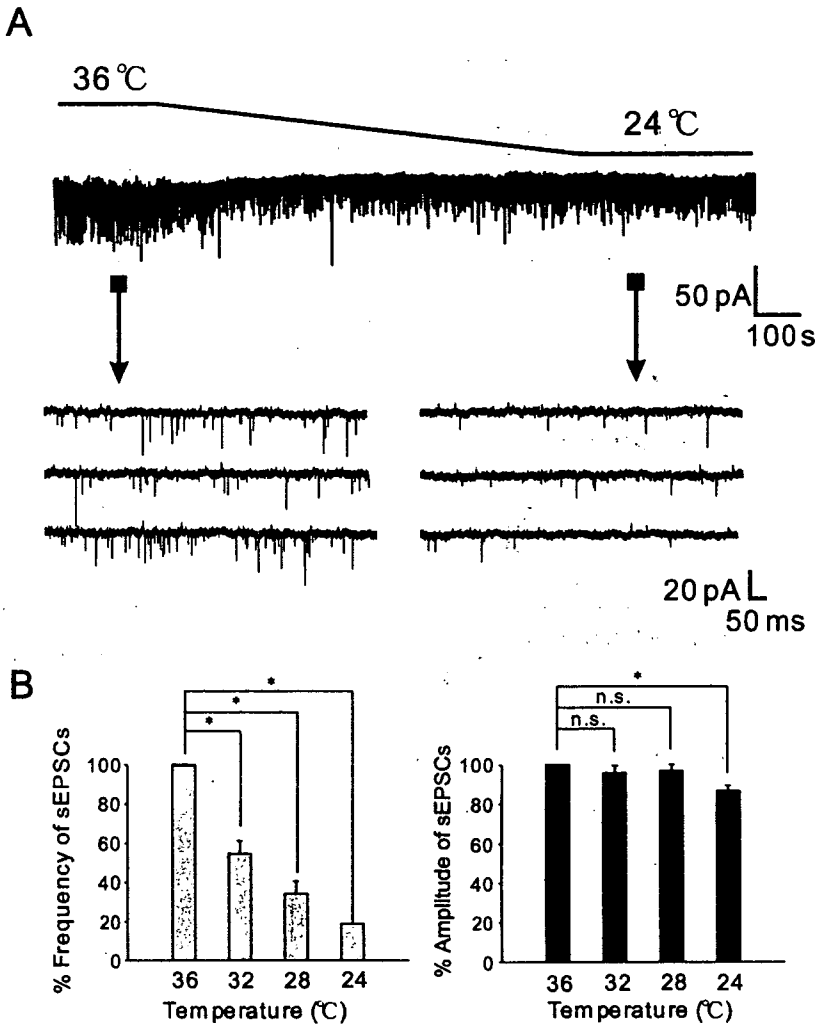

Figure 1. Hypothermia suppressed glutamatergic excitatory synaptic transmission in the spinal ventral horn neurons. **A**, A continuous chart recording of glutamatergic spontaneous excitatory postsynaptic currents (sEPSCs) before, during, and after the temperature of the superfusing ACSF solution was changed from 36°C to 24°C (upper). Three consecutive traces of sEPSCs are shown in an expanded scale in time, before (lower left) and after (lower right) the temperature of the superfusing ACSF solution was changed from 36°C to 24°C. Note that both the frequency and amplitude of the sEPSCs were significantly decreased at 24°C. **B**, Summary of the sEPSC frequency (left) and amplitude (right) when the temperature of the superfusing ACSF solution was changed from 36°C to 32°C, 28°C, and 24°C, relative to those at 36°C. Vertical lines accompanied by bars show SEM; statistical significance between the data shown by bars is indicated by an asterisk; $*P < 0.05$. The holding potential (V_H) used was −70 mV.

temperature of the superfusing ACSF solution was changed from 36°C to 24°C (Figure 1B). The average changes in sEPSC amplitude were 86.9% ± 2.8% (24°C, n = 7).

The Latency of the Agonal Inward Current Induced by Superfusing ISM

As previously described,[23] the ISM for a several minutes generated an agonal inward current in the ventral horn neurons recorded at normothermia (36°C). This agonal inward current consisted of a slow and subsequent rapid inward current (Figure 2A). When continuously superfused with the ISM, the synaptic activity disappeared and then the holding current became unstable and irreversible even if oxygen and glucose were reintroduced in the ventral horn neurons, thus indicating that the ISM exposure apparently produced an irreversible membrane dysfunction. The latency of the slow inward current was measured from the onset of superfusion with the ISM to the onset of the agonal inward current (Figure 2A). The latency of the rapid inward current was measured from the onset of superfusion with the ISM to the onset of the rapid inward current, as estimated by extrapolating the slope of the rapid inward current to the slope of the slow current (Figure 2A). At normothermia (36°C), the average latencies of the slow and rapid inward currents were 262 ± 10 seconds and 386 ± 12 seconds in 59 ventral horn neurons recorded, respectively (Figure 3A). Although the ISM generated an agonal inward current at hypothermia (32°C, 28°C, and 24°C) and normothermia, the latencies of the slow and rapid inward currents were significantly prolonged, when compared with those at normothermia (Figure 2B). The average latencies of the slow and rapid inward currents were 546 ± 36 seconds and 737 ± 32 seconds (32°C, n = 32), 759 ± 39 seconds and 1109 ± 30 seconds (28°C, n = 23), and 1263 ± 65 seconds and 1705 ± 72 seconds (24°C, n = 27), respectively (Figure 3A). Furthermore, the slope of the slow inward current at hypothermia (32°C, 28°C, and 24°C) was significantly decreased when compared with that at normothermia (Figure 3B). The average slope of the slow inward currents was 0.59 ± 0.07 pA/s (36°C, n = 59), 0.17 ± 0.03 pA/s (32±C, n = 32), 0.12 ± 0.02 pA/s (28°C,

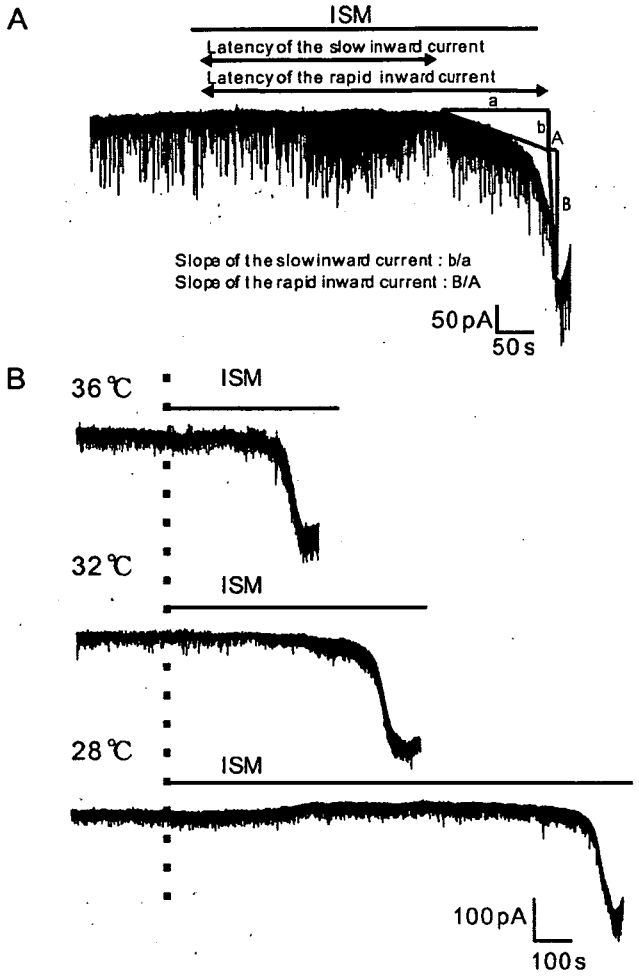

Figure 2. Hypothermia prolonged the latency of the ISM-induced agonal inward current, which consisted of a slow and subsequent rapid inward current. **A**, A continuous chart recording of the sEPSCs before and during the application of the ischemia simulating medium (ISM) at 36°C. The ISM produced an agonal inward current which consisted of a slow and subsequent rapid inward current. The onset of the rapid inward current was estimated by extrapolating the slope of the rapid inward current to the slope of the slow inward current. The amplitude of the rapid inward current was measured between the peak and the onset current. **B**, A continuous chart recording of the sEPSCs before and during the application of the ISM at 36°C (upper), 32°C (middle), and 28°C (lower) at the same scale. V_H used was −70 mV.

n = 23), and 0.05 ± 0.01 pA/s (24°C, n = 27). On the other hand, hypothermia (32°C, 28°C, and 24°C) did not significantly affect the slope of the rapid inward current induced by the ISM (Figure 3B). The average slope of the slow inward currents was 6.60 ± 1.55 pA/s (36°C, n = 59), 7.17 ± 1.77 pA/s (32°C, n = 32), 8.89 ± 1.48 pA/s (28°C, n = 23), and 3.78 ± 1.19 pA/s (24°C, n = 27).

The Effect of Glutamate Receptor Antagonists on the Agonal Inward Current Induced by Superfusing ISM

To elucidate the contribution of the accumulation of glutamate to the agonal inward current induced by superfusing the ISM, the effect of the glutamate receptor antagonists was examined. In the presence of the glutamate receptor antagonists, CNQX (10 μmol/L) and AP5 (50 μmol/L), the sEPSCs completely disappeared at a holding potential of −70 mV (Figure 4A). Although the ISM still generated an agonal inward current in the presence of CNQX (10 μmol/L) and AP5 (50 μmol/L) at normothermia, the latencies of the slow and rapid inward currents were significantly prolonged in the presence of CNQX and AP5 at normothermia, when compared with those in the absence of CNQX and AP5 (Figure 4A). The average latencies of the slow and rapid inward currents were 305 ± 18 seconds and 478 ± 19 seconds (36°C, n = 24), respectively (Figure 4B). In addition, the slopes of the slow and rapid inward currents were significantly decreased in the presence of CNQX and AP5, when compared with those in the absence of CNQX and AP5. The average slopes of the slow and rapid inward currents were 0.38 ± 0.05 pA/s and3.43 ± 0.79 pA/s (36 C, n = 24), respectively (Figure 4B).

■ Discussion

The efficiency of synaptic transmission depends on a variety of pre- and postsynaptic processes involving vesicular release and recruitment as well as the postsynaptic response to the neurotransmitter. The rate of spontaneous neurotransmitter release at the frog neuromuscular junction was first reported to depend on the temperature[24] and this finding has subsequently been well documented. In the present study, we observed that a decrease in the glutamatergic sEPSC frequency depends on a decrease in temperature, thus suggesting that hypothermia inhibits the glutamate release from the presynaptic terminals innervated onto the spinal ventral horn neurons. Consistently, recent studies have demonstrated the EPSC frequency to significantly increase at normothermia, when compared with that at hypothermia in brain slices and autaptic cultures of hippocampal neurons.[25,26] The increase in spontaneous neurotransmitter release seems to represent a decreased energy requirement for Ca^{2+}-independent fusion of the synaptic vesicle in the presynaptic processes, possibly because of an increase in the fluidity of the membrane lipid bilayer.[26] On the other hand, the shape of the sEPSC is likely to be due to the changes in the postsynaptic processes. An increased open probability or conductance of the postsynaptic receptor would account for the increase in EPSC amplitude and charge.[26,27] Interestingly, the amplitude of the sEPSC was observed to decrease at deep hypothermia (24°C), but it did not change at mild hypothermia (28°C and 32°C) in the present study. Therefore, the presynaptic process in the excitatory synaptic transmission may be more sensitive to changes in temperature than the postsynaptic process.

The spinal ventral horn neurons are very vulnerable to ischemia.[23] In the present study, ischemia was simulated by superfusing an oxygen- and glucose-

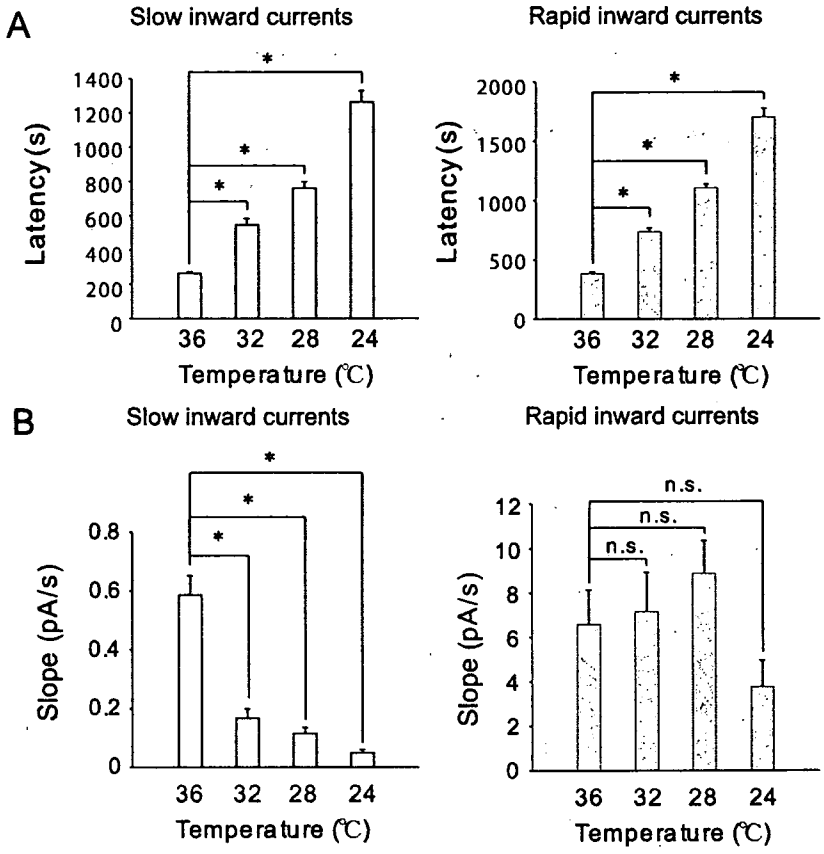

Figure 3. Effect of hypothermia on the ISM-induced agonal inward current. **A**, Summary of the latencies of the slow (left) and rapid inward current (right) after ISM exposures at 36°C (n = 59), 32°C (n = 32), 28°C (n = 23), and 24°C (n = 27). **B**, Summary of the slopes of the slow (left) and rapid inward current (right) after ISM exposures at 36°C (n = 59), 32°C (n = 32), 28°C (n = 23), and 24°C (n = 27). Vertical lines accompanied by bars show SEM; statistical significance between the data shown by bars is indicated by an asterisk; $*P < 0.05$. V_H used was −70 mV.

deprived medium (ISM), which has been well established in spinal and brain slices.[18–20] The ISM for several minutes at normothermia induced an agonal inward current in the spinal ventral horn neurons, as we previously demonstrated.[23] The ISM-induced agonal inward current consisted of a slow and subsequent rapid inward current in the spinal neurons. It has been considered that an agonal inward current consists of pharmacologically distinct components in the hippocampal neurons.[18] The slow inward current was partially suppressed by CNQX and AP5, suggesting that the activation of non-NMDA and NMDA receptors by glutamate accumulation may be involved in the slow inward current. On the other hand, the rapid inward current is probably due to a nonselective increase in permeability to all participating ions; this may occur only under pathologic conditions. In the present study, hypothermia significantly prolonged the onset of the slow and rapid inward currents induced by ISM in the spinal ventral horn neurons, which is consistent with the experiments in hippocampal slices. These results were consistent with those from other animal models of ischemic and traumatic spinal cord injury and with clinical studies,[13–17] which indicate that hypothermia improves the neurologic outcome in spinal cord injury patients. Moreover, the slope of the inward current was significantly decreased at hypothermia, whereas that of the rapid inward current did not change in the present study. These results suggest that glutamate accumulation after spinal ischemia may be suppressed at hypothermia, which results in delaying the latency of the ISM-induced membrane dysfunction in the spinal ventral horn neurons.

Various ischemic injuries, such as hypoxia, stroke, and trauma, can disrupt the synaptic function leading to the accumulation of extracellular glutamate and an excessive stimulation of these receptors.[28] The excessive stimulation of the glutamate receptor/ion channel complexes triggers calcium flooding and a cascade of intracellular events which results in apoptosis and/or necrosis. Although the glutamate receptor antagonists, CNQX and AP5, could block the glutamatergic excitatory synaptic transmission, the ISM still generated an agonal inward current in the spinal ventral horn neurons examined. In addition, the latencies of the slow and rapid inward currents were slightly, but significantly, prolonged in the presence of the glutamate receptor antagonists, when compared with those in their absence. These results indicate that the activation of the glutamate receptors is involved in the generation and maintenance of the ISM-induced membrane dysfunction in the spinal ventral horn neurons. However, the hypothermia-induced neuroprotection against ischemic membrane dysfunction may involve not only the activation of the glutamate receptors, but also other various mechanisms, such as reducing the energy demand, attenuating ATP depletion and intra-

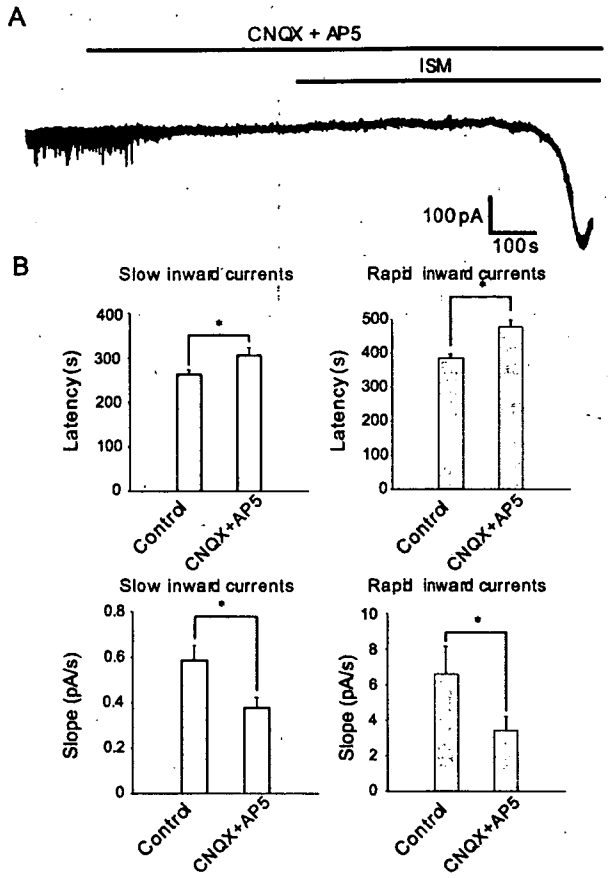

Figure 4. Effect of glutamate receptor antagonists on the ISM-induced agonal inward current. **A**, A continuous chart recording of the sEPSCs before and during the application of ISM in the presence of the glutamate receptor agonists, CNQX (10 μmol/L) and AP5 (50 μmol/L). Note that the glutamate receptor agonists completely blocked the sEPSCs and prolonged the latency of the ISM-induced agonal inward current. **B**, The upper histograms show the summary of the latencies of the slow (left) and rapid inward current (right) after ISM exposures in the absence and presence of CNQX and AP5. The lower histograms show the summary of the slopes of the slow (left) and rapid inward current (right) after ISM exposures in the absence and presence of CNQX and AP5. Vertical lines accompanied by bars show SEM; statistical significance between the data shown by bars is indicated by an asterisk; $^*P < 0.05$. V_H used was −70 mV.

cellular acidosis, and inhibiting the intracellular protein kinases.

■ Key Points

- Hypothermia suppressed the glutamatergic excitatory synaptic transmission.
- Hypothermia significantly prolonged the latency of the ISM-induced agonal inward current which consisted of a slow and subsequent rapid inward current.
- Hypothermia decreased the slope of the ISM-induced slow inward current.
- The glutamate receptor antagonists slightly prolonged the latencies of the slow and rapid inward currents that were induced by ISM and significantly decreased their slopes.

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Selective Vulnerability to Ischemia in the Rat Spinal Cord

A Comparison Between Ventral and Dorsal Horn Neurons

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Study Design. Whole-cell patch-clamp recordings were performed from ventral horn (VH) and dorsal horn (DH) neurons obtained from the rat spinal cord slices.

Objective. This study investigated which is more vulnerable to ischemia, spinal VH neurons or DH neurons.

Summary of Background Data. Spinal cord ischemia or injury sometimes causes a greater loss of motor function than of sensory function in patients. However, it is difficult to evaluate whether spinal motor neurons are more vulnerable than sensory neurons because of the anatomic complexity and a variety of physiologic factors in the spinal cord.

Methods. Whole-cell patch-clamp recordings were performed from VH and DH neurons obtained from the spinal cord slices. Ischemia was simulated by superfusing an oxygen- and glucose-deprived medium (ischemia simulating medium [ISM]).

Results. Perfusion with ISM generated an agonal depolarization in all VH and DH neurons recorded in current-clamp mode. Following ISM superfusion, an agonal inward current was produced at a holding potential of −70 mV in all VH and DH neurons tested in voltage-clamp mode. The agonal inward current consisted of a slow and subsequent rapid inward current. The average latency of the rapid inward currents after ISM exposures in VH neurons was significantly shorter than that in DH neurons. The average amplitude of the agonal inward currents in VH neurons was significantly bigger than that of DH neurons. Moreover, the recovery ratio by the reintroduction of oxygen and glucose in VH neurons was smaller than that in DH neurons.

Conclusions. These results suggest that VH neurons are more vulnerable to ischemia than DH neurons. This finding may help in achieving a better understanding of the difference between motor and sensory disturbance in spinal cord ischemia or injury patients.

Key words: spinal neurons, ischemia, cell death, patch-clamp, electrophysiology. **Spine 2007;32:1060–1066**

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Specific regions are prone to develop neuronal damage after brain ischemia or injury, and this phenomenon is called "selective vulnerability in the brain."[1–3] Hippocampal CA1 and neocortical III, V, and VI are particularly vulnerable to ischemia or hypoxia.[2,4] The precise mechanisms responsible for such selective vulnerability are still not fully understood, although it is assumed to be related with the facilitation of excitatory synaptic transmission as well as the enhancement of the energy metabolism in the brain.[3] Although the pathologic aspects and the topographic distribution of ischemic lesions have been extensively studied in the brain,[1,2] little is known about the distribution of ischemic changes in the spinal cord. Although it is generally accepted that the gray matter in the watershed area of the midthoracic level is particularly vulnerable in the spinal cord,[5,6] it has recently been reported that the lumbosacral cord is more vulnerable to ischemia than other levels of the spinal cord.[7] Traumatic spinal cord injury usually induces the swelling of the spinal cord, thus resulting in spinal ischemia; and it thus becomes a common cause of secondary damage.[8] There are cases such as Frankel Grade B spinal cord injury in which motor disturbance does not improve, although some feeling remains or the sensory disturbance improves in some spinal cord injury patients.[9] Spinal cord injury after a successful operation on the thoracic aorta is a disastrous complication in humans.[10] The mechanism of the spinal cord injury has been thought to also be involved in spinal ischemia, and spinal motor neurons are thus suggested to be vulnerable to ischemia.[11] Although several spinal cord injury animal models have been developed, it remains very difficult to evaluate whether spinal sensory neurons are more vulnerable to ischemia or injury than motor neurons at the cellular level because of the anatomic complexity and a variety of physiologic factors in the spinal cord. In the present study, in order to investigate which is more vulnerable to ischemia, namely, spinal ventral horn (VH) neurons or dorsal horn (DH) neurons, the occurrence of ischemia-induced membrane dysfunction in VH and DH neurons of rat spinal cord slices was recorded using whole-cell patch-clamp technique. Ischemia was therefore simulated by superfusing an oxygen- and glucose-deprived medium (ischemia simulating medium [ISM]), which has been well established in such brain slices.[12,13]

■ Materials and Methods

All experimental procedures involving the use of animals were approved by the Ethics Committee on Animal Experiments,

Wakayama Medical University, and were conducted in accordance with the U.K. Animals (Scientific Procedures) Act 1986 and associated guidelines.

Spinal Cord Slice Preparation. The methods used to obtain rat spinal cord slice preparations have been described previously.[14] In brief, Sprague-Dawley rats at the postnatal age 8 to 12 days were deeply anesthetized with pentobarbital sodium (60 mg/kg, intraperitoneal), and then a lumbosacral laminectomy was performed. The lumbosacral spinal cord (L1–S3) was removed and placed in preoxygenated artificial cerebrospinal fluid (ACSF) solution at 1°C to 3°C. Immediately after the removal of the spinal cord, the rats were given an overdose of urethane and then were killed by exsanguination. The pia-arachnoid membrane was removed after cutting all the ventral and dorsal roots near the root entry zone. The spinal cord was put on an agar block and mounted on a Vibratome (DTK-1000; D.S.K., Kyoto, Japan) and then a 500-μm-thick transverse slice was cut. The slice was placed in the recording chamber, which had a volume of 0.5 mL solution, and then was placed on the stage of an upright microscope equipped with an infrared-differential interference contrast (IR-DIC) system (BX51WI; Olympus, Tokyo, Japan). Next, the slice was superfused at a rate of 5 mL/min with ACSF solution saturated with 95% O_2 and 5% CO_2 at 36 ± 1°C. The ACSF solution contained (in mmol/L) 117 NaCl, 3.6 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 25 $NaHCO_3$, and 11 glucose, and osmolarity was 300 mOsm.

Patch-Clamp Recordings From Spinal Neurons. The lamina regions were identified using a 5× objective lens, and individual neurons were identified using a 40× objective lens under an IR-DIC microscope. The microscope was coupled with a CCD camera (C2741–C79; Hamamatsu, Hamamatsu, Japan) and a video monitor screen. Whole-cell patch-clamp recordings were made from VH neurons in the lamina IX and DH neurons in the lamina II with patch-pipette electrodes having a resistance of 4 to 8 MΩ.[15] The composition of the patch-pipette solution was as follows (in mmol/L): 135 potassium gluconate, 5 KCl, 0.5 $CaCl_2$, 2 $MgCl_2$, 5 EGTA, 5 HEPES, 5 ATP-Mg, pH 7.2. The signals were acquired with an amplifier Axopatch 200B (Axon Instruments, Union City, CA). The data were digitized with an A/D converter (Digidata 1322A, Axon Instruments) and then were stored on a personal computer using a data acquisition program (pCLAMP 9, Axon Instruments).

Ischemia Simulation and Drug Application. The slices were made "ischemic" by superfusing them with ACSF solution equilibrated with 95% N_2/5% CO_2 and then they were deprived of glucose by a bath application, which was replaced with sucrose iso-osmotically ISM. The solution contained (in mmol/L) 117 NaCl, 3.6 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 25 $NaHCO_3$, and 11 sucrose, and osmolarity was 300 mOsm. The drugs were dissolved in ACSF solution and then were applied by perfusion *via* a 3-way stopcock without any change in the perfusion rate. The time necessary for the solution to flow from the stopcock to the surface of the spinal cord slice was approximately 20 seconds. The drugs used in this study were 6-cyano-7-nitroquinoxaline-2,3-dion (CNQX) and D(-)-2-amino-5-phosphonopentanoic acid (AP5) from Tocris (Ballowin, MO).

Statistical Analysis. All numerical data were expressed as the mean ± SEM. Statistical significance was determined as $P < 0.05$ using Student t test to compare the latency and amplitude of the inward current and Spearman's ranked correlation test to investigate the correlation between the membrane capacitance and the latency of the inward current. Regarding the electrophysiologic data, n refers to the number of neurons studied.

■ Results

The spinal neurons in all laminae were viable for up to 12 hours in the slices perfused with preoxygenated ACSF solution. However, all recordings were obtained within 4 hours in this study. Whole-cell patch-clamp recordings were performed from VH neurons in the lamina IX and DH neurons in the lamina II of the spinal cord slices. VH neurons were relatively bigger than DH neurons on a monitor screen under the IR-DIC system (Figure 1A). In current-clamp mode, superfused with ISM for a several minutes caused changes in membrane potential consisting of a slow and subsequent rapid depolarization in all of VH and DH neurons tested (Figure 1B). When continuously superfused with ISM, these neurons did not repolarize and the membrane continued to depolarize progressively to 0 mV (n = 7).

Latency of the Agonal Inward Current Induced by Superfusing ISM

In voltage-clamp mode, spontaneous excitatory postsynaptic currents (sEPSCs) were observed in both VH and DH neurons at a holding potential of −70 mV. In the presence of CNQX (10 μmol/L) and AP5 (50 μmol/L), sEPSCs completely disappeared in both the VH and DH neurons (data not shown). ISM exposure for several minutes generated an agonal inward current in all of VH and DH neurons recorded (Figure 1B). This agonal inward current consisted of a slow and subsequent rapid inward current. When continuously superfused with ISM, the synaptic activity disappeared and then the holding current became unstable and irreversible even if oxygen and glucose were reintroduced in all the VH and DH neurons examined, thus indicating that the ISM exposure apparently produced an irreversible membrane dysfunction (Figure 1B). As previously described,[12] the latency of the rapid inward current in VH and DH neurons was measured from the onset of superfusion with ISM to the onset of the rapid inward current, as estimated by extrapolating the slope of the rapid inward current to the slope of the slow current (Figures 2A, 3A). The average latencies of the rapid inward currents were 477 ± 11 seconds in 161 VH neurons and 603 ± 20 seconds in 115 DH neurons. The average latency of the rapid inward currents in the VH neurons was significantly shorter than that in the DH neurons (Figure 2A). In addition, the membrane capacitance of the VH and DH neurons was examined. The average membrane capacitances were 70 ± 3 pF in 57 VH neurons and 39 ± 1 pF in 54 DH neurons. The average membrane capacitance in the VH neurons was significantly bigger than that in the DH neurons. In addition, the membrane capacitance in VH neurons demonstrated a significantly negative correlation with the latency of the

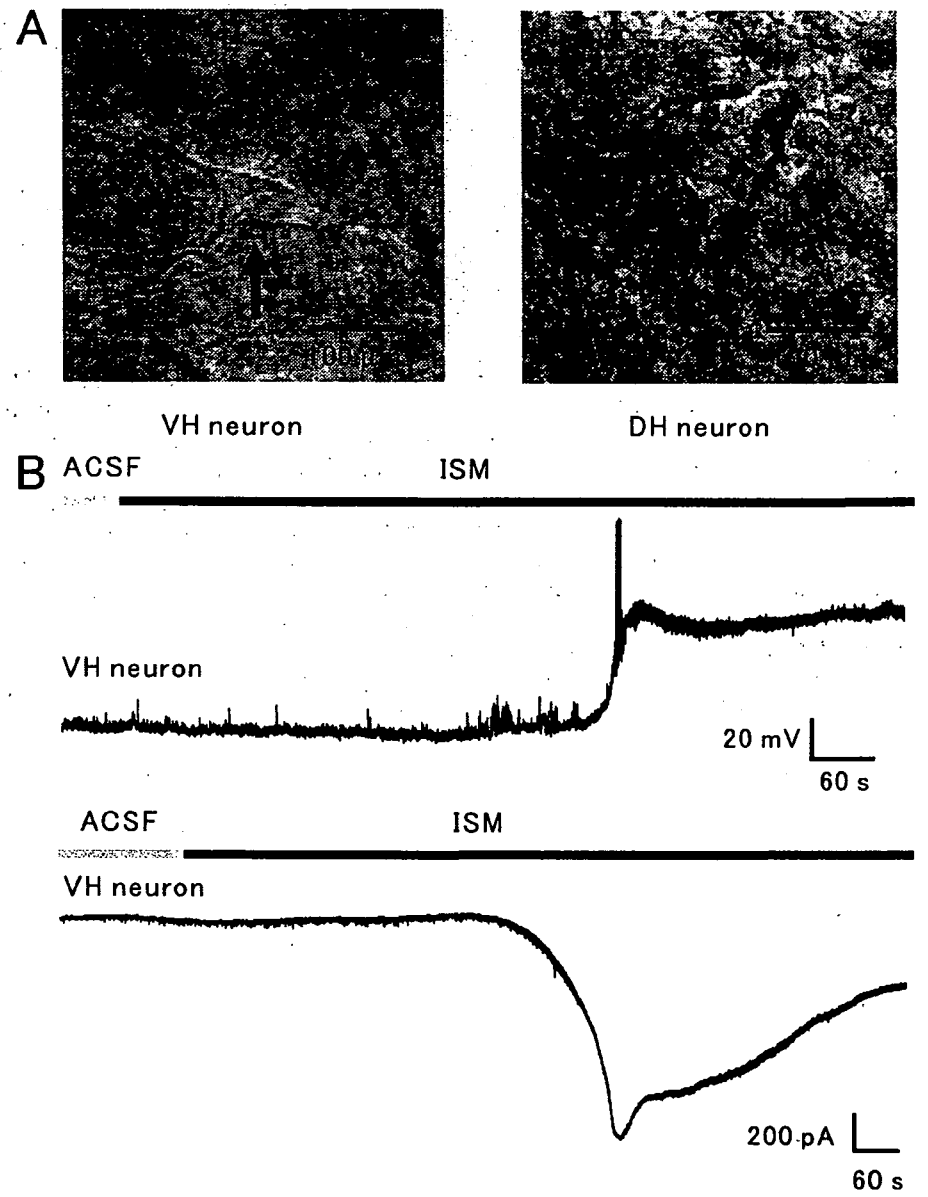

Figure 1. The ISM-induced an agonal depolarization and inward current in spinal neurons. **A**, IR-DIC images of a VH (left image) and a DH neuron (right image). The VH neurons were relatively big compared with the DH neurons. **B**, In current-clamp mode, the perfusion with ischemia simulating medium (ISM) produced an agonal depolarization (upper trace). The resting membrane potential was −60 mV. In voltage-clamp mode, the perfusion with ISM generated an agonal inward current (lower trace) at a holding potential of −70 mV in all of the VH and DH neurons recorded.

rapid inward current after ISM exposure, but that in DH neurons did not have a significant correlation (Figure 2B).

Amplitude of the Agonal Inward Current Induced by Superfusing ISM

The superfusion with ISM produced an agonal inward current, which consisted of a slow and subsequent rapid inward current in all the VH and DH neurons tested. The amplitudes of the agonal, slow and rapid inward currents were measured, as previously described.[12] The amplitude of the rapid inward current was measured between the peak and onset current (Figure 3). The average amplitudes of the slow inward currents were 119 ± 14 pA in 33 VH neurons and 91 ± 12 pA in 33 DH neurons. Although the average amplitude of the slow inward currents in VH neurons was bigger than that in DH neurons, no significant difference was observed between the average amplitudes of the slow inward currents in VH and DH neurons (Figure 3B). The average amplitudes of the rapid inward currents were 468 ± 27 pA in 33 VH neurons and 365 ± 28 pA in 33 DH neurons. The average amplitude of the rapid inward currents in the VH neurons was significantly larger than that in the DH neurons (Figure 3B). In addition, the average amplitudes of the agonal inward currents were 588 ± 34 pA in 33 VH neurons and 456 ± 32 pA in 33 DH neurons. The average amplitude of the agonal inward currents in the VH neurons was also significantly larger than that in the DH neurons (Figure 3B).

Recovery of the Holding Current by the Reintroduction of Oxygen and Glucose

The recovery ratio followed by the reintroduction of oxygen and glucose was compared between the VH and DH neurons. We considered the neurons to have fully recovered when the holding current and sEPSCs were restored, and their levels were similar to those before the superfusion with ISM. When ISM was stopped and ACSF was readministered immediately after the peak of the agonal inward

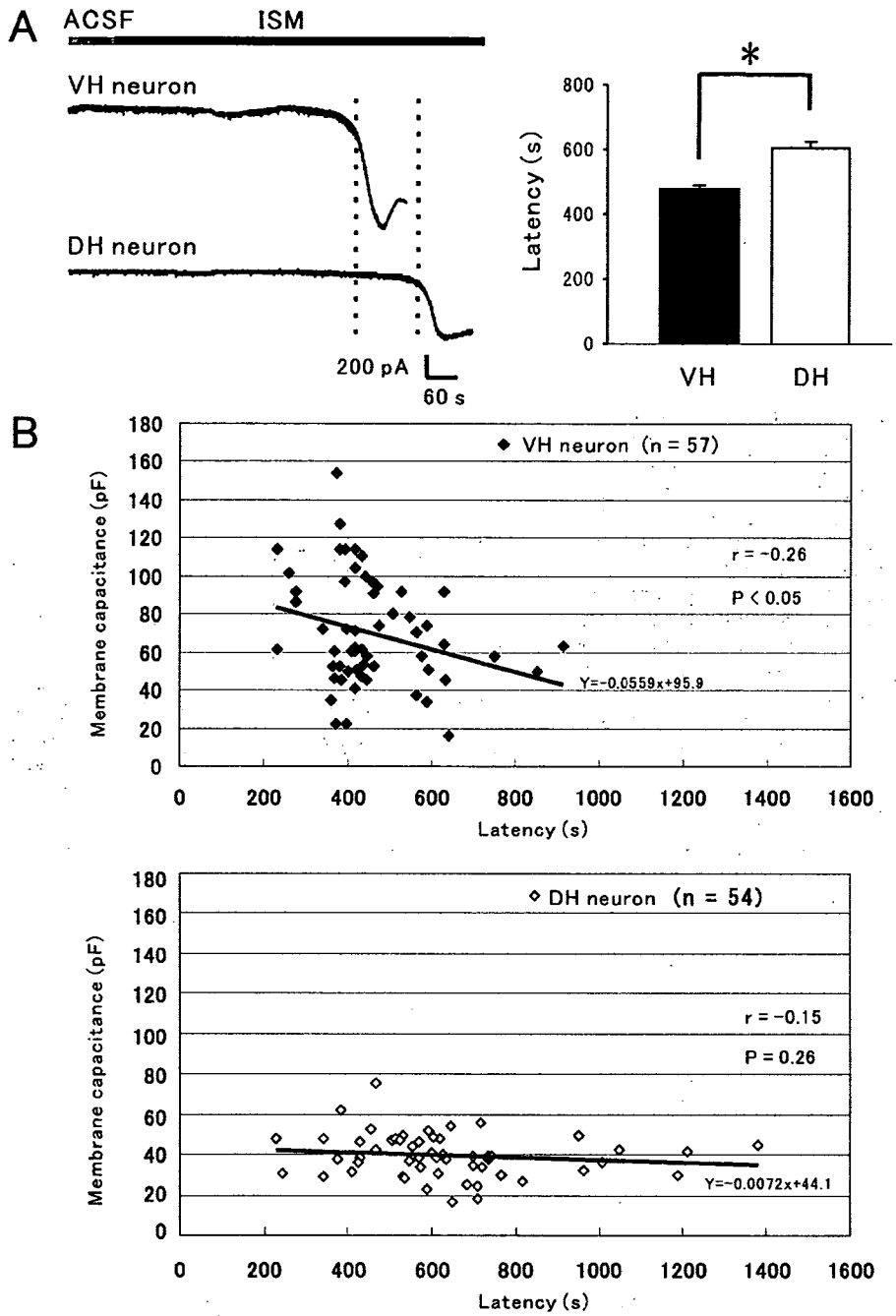

Figure 2. Comparison of the latency of the rapid inward current produced by superfusion with ISM. **A**, Example traces of the ISM-induced inward currents in a VH (upper left trace) and a DH neuron (lower left trace). Comparison of the average latency of the rapid inward current between the VH and DH neurons (right histogram). The average latency in the VH neurons was significantly shorter than that in the DH neurons. $*P < 0.05$. **B**, Correlation between the membrane capacitance and the latency of the rapid inward current as generated by the perfusion with ISM in VH (upper histogram) and DH neurons (lower histogram). A negative correlation was observed in VH neurons. $*P < 0.05$.

current, then 60% of the VH neurons (6 of 10 neurons) and 75% of the DH neurons (9 of 12 neurons) were fully recovered (Figure 4). During the reperfusion of ACSF after the peak of the agonal inward current was prolonged, the recovery ratio decreased in both the VH and DH neurons. When ACSF was readministered at 0.5, 1, and 1.5 minutes after the peak of the agonal inward current, 40% (4 of 10), 18% (2 of 11), and 8% (1 of 12) of the VH neurons were fully recovered, respectively (Figure 4B). On the other hand, when ACSF was reperfused at 0.5, 1, and 1.5 minutes after the peak of the agonal inward current, then 54% (6 of 11), 33% (4 of 12), and 27% (3 of 11) of the DH neurons were fully recovered, respectively (Figure 4). The recovery ratio of DH neurons tended to be higher that of VH neurons when ACSF was readministered 0, 0.5, 1, and 1.5 minutes after the peak of the agonal inward current. On the other hand, neither the VH (n = 10) nor the DH neurons (n = 11) ever recovered when ACSF was reperfused at 2 minutes after the peak of the agonal inward current (Figure 4).

■ Discussion

The present study demonstrated that ISM generated an agonal inward current, which consisted of a slow and subsequent rapid inward current in all of VH and DH neurons recorded. It has been considered that an agonal inward current consists of pharmacologically distinct components

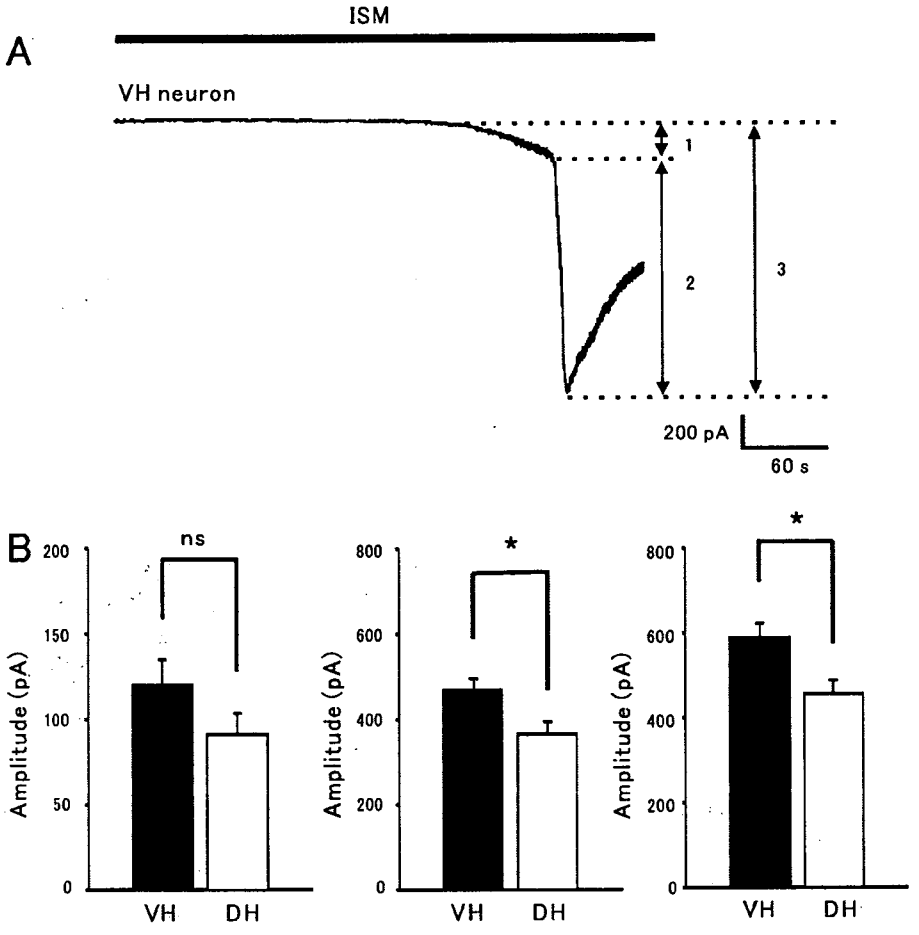

Figure 3. Comparison of the agonal inward current amplitude produced by superfusion with ISM. **A**, The measured amplitudes of the slow (*1*), rapid (*2*), and total inward current (*3*). The onset of the rapid inward current, as estimated by extrapolating the slope of the rapid inward current to the slope of the slow inward current. The amplitude of the rapid inward current was measured between the peak and onset current. **B**, Comparisons of the average amplitude of the slow inward current (left histogram), the rapid inward current (middle histogram), and the total inward current (right histogram) between the VH and DH neurons.

in hippocampal neurons. The slow inward current was partially suppressed by CNQX and AP5, suggesting that activation of non-NMDA and NMDA receptors by glutamate accumulation may be involved in the slow inward current.[12] On the other hand, the rapid inward current is probably due to a nonselective increase in permeability to all participating ions; this may occur only in pathologic conditions.[12] In the present study, the latency of the irreversible membrane dysfunction after ISM exposures in the VH neurons was significantly shorter than that in the DH neurons. In addition, the recovery ratio after the reintroduction of oxygen and glucose in the DH neurons tended to be higher than that in the VH neurons. Transient ischemia has consistently been histologically demonstrated to result in selective motor neuron death in the lumbar regions of the rabbit spinal cord.[11] The amplitude of compound muscle action potentials either decreased or disappeared after the ligation of the thoracoabdominal aorta or intercostal arteries, while that of the spinal somatosensory-evoked potentials did not change.[16] This result indicated that the anterior spinal cord or motor tract is more vulnerable to ischemia than the posterior spinal cord. In clinic practice, such cases as Frankel Grade B spinal cord injury are encountered in which the observed motor disturbance does not improve, even though some sensation remains or a sensory disturbance improves.[9] A few reports have described that the motor score based on the extent and severity of neurologic deficits is less than the sensory score in both traumatic and ischemic spinal cord injury patients.[17] However, it remains unclear as to which is more vulnerable to ischemia, spinal motor neurons or sensory neurons at a single cell level. This is the first electrophysiological report showing spinal VH neurons to be more vulnerable to ischemia than DH neurons.

The ISM-induced rapid inward current in the VH neurons was significantly larger than that in the DH neurons. Interestingly, the membrane capacitance in the VH neurons demonstrated a significant correlation to the latency of the rapid inward currents induced by superfusion with ISM, but that in DH neurons did not have a significant correlation in the present study. It has been previously demonstrated that the association of the cell volume and the enzyme activity to vulnerability to ischemia in the thoracic and lumber spinal cord.[18] On the other hand, it remains controversial regarding whether or not larger cells are easier to be damaged in the brain. Previous studies have suggested low vulnerability of small interneurons, while large motor neurons are more sensitive to ischemia in the brain.[19] In addition, large neurons are more sensitive to the destruction caused by a viral infection than small neurons in the neocortex.[20] In contrast, several opposite findings have also been reported, namely, that smaller cells are more vulnerable to ischemia in brain. The large CA3 pyramidal cells have high resistance to brain ischemia.[21] Astrocytic demise precedes neuronal death following permanent middle cere-

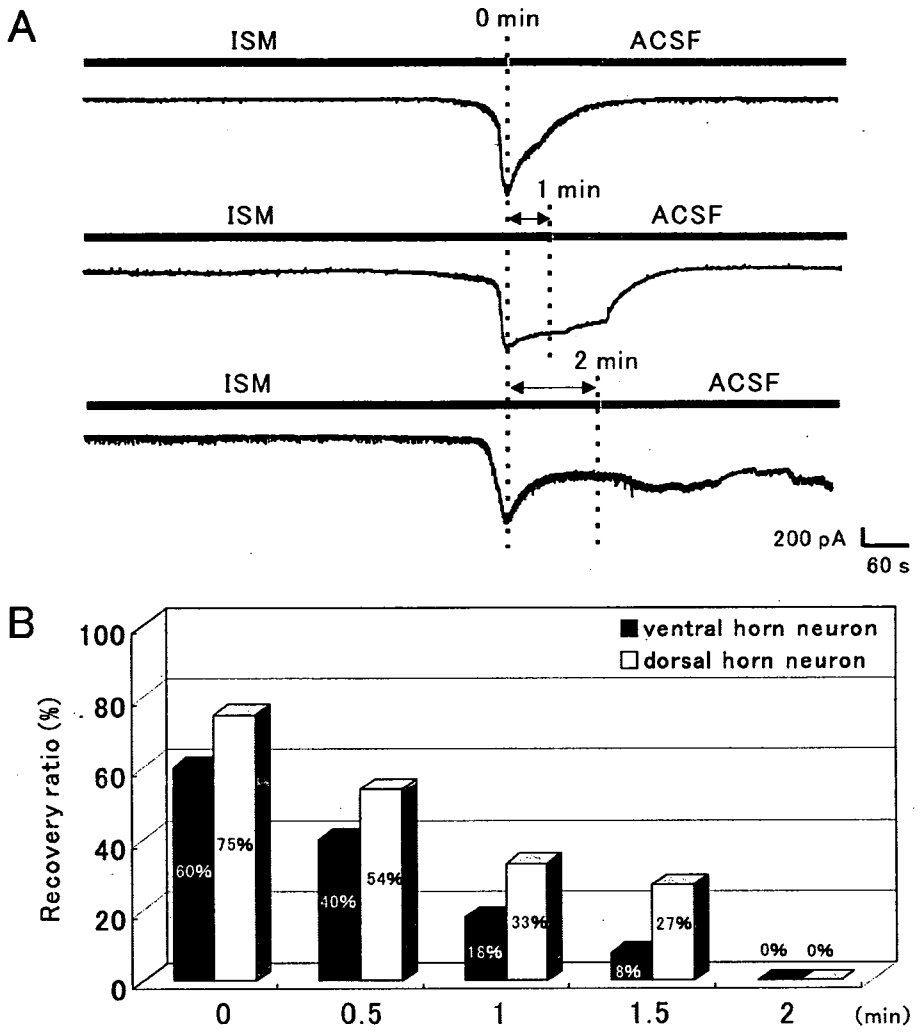

Figure 4. Comparison of the recovery rate after the reintroduction of oxygen and glucose. **A**, The changes in the holding currents by ACSF reperfusion immediately (upper trace), 1 minute (middle trace), and 2 minutes after the peak of the agonal inward current (lower trace). The DH neurons fully recovered when ACSF was readministered immediately and 1 minute after the peak of the agonal inward current (upper and middle trace). **B**, The summary of the recovery ratio by ACSF reperfusion immediately, 0.5 minute, 1 minute, 1.5 minutes, and 2 minutes after the peak of the agonal inward current in the VH and DH neurons.

bral artery occlusion.[22] As a result, the difference in the vulnerability to ischemia between spinal VH and DH neurons may not only be due to the size of the neurons, but also due to the difference in the neuronal functions such as the pumps and channels in the neuronal membrane.

Glutamate is the major excitatory neurotransmitter in the spinal cord, and most neurons as well as many oligodendrocytes and astrocytes possess glutamate receptors. Various ischemic injuries such as hypoxia, stroke, and trauma can disrupt the synaptic function leading to the accumulation of extracellular glutamate and an excessive stimulation of these receptors.[23] The activities of certain glutamate receptor/channel complexes are enhanced in the spinal cord, thereby promoting activity-dependent plasticity. The excessive stimulation of glutamate receptor/ion channel complexes triggers calcium flooding and a cascade of intracellular events that results in apoptosis and/or necrosis. The abnormal influx of Ca^{2+} through glutamate receptor channels is thought to contribute to the loss of neurons associated with a number of neuronal disorders. Recent findings have indicated that the spinal ventral horn neurons are considerably more vulnerable than the dorsal horn neurons to prolonged AMPA receptor overactivation.[24,25] In addition, the selective vulnerability of spinal motor neurons may be caused by the expression of highly Ca^{2+}-permeable AMPA receptors in the ventral horn neurons.[26] To clarify the direct role of Ca^{2+}-permeable AMPA receptors in the selective vulnerability to ischemia, further investigations will thus be required.

■ Key Points

- Whole-cell patch-clamp recordings were performed from VH and DH neurons obtained from the spinal cord slices.
- Ischemia was simulated by superfusing an oxygen- and glucose-deprived medium (ischemia simulating medium).
- The average latency of the rapid inward currents after ischemia simulating medium exposures in VH neurons was significantly shorter than that in DH neurons.
- The average amplitude of the agonal inward currents in VH neurons was significantly bigger than that of DH neurons.

• The recovery ratio by the reintroduction of oxygen and glucose in VH neurons was smaller than that in DH neurons.

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