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Enhancing effect of dimethyl sulfoxide on nociceptive transmission in isolated spinal cord of newborn rat

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Abstract

The effect of dimethyl sulfoxide (DMSO) on the slow ventral root potential, which is related to nociceptive transmission, was investigated in the isolated spinal cord of a newborn rat. DMSO at 0.3–1% (v/v) enhanced the slow ventral root potential, but not mono- and polysynaptic reflex discharges. DMSO at 1% also enhanced the depolarization induced by substance P or capsaicin. In the presence of tetrodotoxin (0.3 μM), DMSO at 1% did not influence the substance P-induced depolarization but enhanced the acetylcholine-induced depolarization. Edrophonium at 10 μM also enhanced the slow ventral root potential, and the magnitude of the effect was comparable to that of 1% DMSO. In the presence of atropine (0.3 μM) and hexamethonium (30 μM), the effect of edrophonium disappeared, but half of the effect of DMSO remained. Artificial cerebrospinal fluid containing either 0.87% (w/v) urea or 4.6% (w/v) sucrose, which has the same osmotic pressure as that containing 1% DMSO, did not have the same effect as DMSO on the slow ventral root potential. In the saphenous nerve–dorsal root preparation, the compound action potential was enhanced by 4-aminopyridine (10 μM), but was not affected by DMSO up to 3%. The results suggest that DMSO enhances the slow ventral root potential through mechanisms based on the inhibition of cholinesterase activity and other action(s) involved in increasing transmitter release from nerve endings in nociceptive transmission pathways in the isolated spinal cord of the newborn rat. Neither the blockade of K^+ channels nor hyperosmotic effects are likely mechanisms of DMSO action.

Keywords: Dimethyl sulfoxide; Nociceptive transmission; Spinal cord, newborn rat

1. Introduction

Dimethyl sulfoxide (DMSO) is widely used as a solvent for water-insoluble chemicals. Formerly, it was used in the medical field as an analgesic by being applied directly to the skin over the painful area (Brown, 1966; Demos et al., 1967; Lockie and Norcross, 1967; Matsumoto, 1967). There are several studies that suggest the analgesic effect of DMSO. Shealy (1966) observed in cat spinal cord, medulla and tegmentum that a prolonged discharge, which was related to nonmyelinated C fiber activity following stimulation of superficial radial and sural nerves, quickly disappeared after exposure of the nerves to 5–10% (v/v) DMSO. In the isolated sural nerve of the cat, DMSO at 5–7% was shown to decrease the amplitude of compound action potentials and the conduction velocity of C fibers (Evans et al., 1993). A much higher concentration of DMSO was needed to block myelinated A fibers, i.e., 50% DMSO was necessary to block muscle twitching in a frog motor nerve-gastrocnemius muscle preparation (Davis et al., 1967), and 75% DMSO was needed to decrease the amplitude of compound action potentials of Aβ, Aδ and C fibers in the cat superficial radial nerves (Becker et al., 1969). This, therefore, suggests that DMSO at low concentrations inhibits pain selectively and at higher concentrations other sensations.

The studies mentioned above dealt with the peripheral action of DMSO. In the present study, we examined the effect of DMSO on nociceptive transmission in the spinal cord in which nociceptive information from primary afferent neurons is processed first in the central nervous system. When the dorsal root of the isolated spinal cord of the newborn rat is electrically stimulated, the slow ventral root potential can be recorded following mono- and polysynaptic reflex discharges. The slow ventral root potential is considered to be related to pain, since it is evoked by activation of C fibers (Akagi et al., 1985) and is inhibited

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by morphine (Yanagisawa et al., 1985). The slow ventral root potential is inhibited also by substance P antagonists (Yanagisawa et al., 1982; Akagi et al., 1985; Otsuka and Yanagisawa, 1988; Hosoki et al., 1994; Guo et al., 1995). It is thought that substance P is released from C fiber terminals and induces the slow excitatory postsynaptic potentials in the dorsal horn neuron of the spinal cord, which in turn generate the slow ventral root potential in the motoneuron via polysynaptic pathways (Yanagisawa et al., 1982).

2. Materials and methods

All experiments were carried out according to the guidelines provided by the Institutional Animal Care and Use Committee of Sankyo (Tokyo, Japan).

2.1. Isolated spinal cord

Newborn (1–6 day-old) Wistar-Imamichi rats (the Imamichi Institute of Animal Reproduction, Ibaraki, Japan) were used. The isolated spinal cord preparation was made according to the method reported by Otsuka and Konishi (1974). The rats were decapitated under ether anesthesia and the vertebral column below the thoracic region was isolated and placed in a dissecting bath filled with artificial cerebrospinal fluid (CSF), which was saturated with 95% O_2 and 5% CO_2 and kept at room temperature. The vertebral column was opened from the ventral side and the spinal cord was isolated together with the lumbar dorsal and ventral roots. The spinal cord was hemisected, placed in a recording bath of 0.2 ml, and perfused with artificial CSF saturated with 95% O_2 and 5% CO_2 at a rate of 2 ml/min and kept at 27 ± 1°C. The composition of the artificial CSF was as follows (mM): NaCl 138.6, KCl 3.35, $CaCl_2$ 1.26, $MgCl_2$ 2.0, $NaHCO_3$ 21.0, NaH_2PO_4 0.58, glucose 10.

2.2. Isolated dorsal root–peripheral nerve

The isolated dorsal root–peripheral nerve preparation from the newborn rat (3–5 day-old) was made according to the method reported by Nussbaumer et al. (1989). The saphenous nerve was sectioned where it runs superficially over the medial aspect of the knee joint and was dissected free up to its origin from the femoral nerve. The dorsal root–dorsal root ganglion (L3)–femoral or saphenous nerve preparation was removed and placed in a recording bath.

2.3. Extracellular recordings

Membrane potential changes were recorded extracellularly from the ventral root (L3) in the isolated spinal cord preparation (Otsuka and Konishi, 1974; Saito, 1979), and from the dorsal root (L3) in the isolated dorsal root-peripheral nerve preparation (Nussbaumer et al., 1989) with an extracellular suction electrode. The tip of the suction electrode was connected through silicon rubber tubing to the main part of the suction electrode containing an Ag–AgCl wire and the artificial CSF. This tip could be exchanged depending on the size of the root. The potential changes were led through a preamplifier to a pen recorder and an oscilloscope. The dorsal root of the same segment (L3) in the former preparation and the saphenous nerve in the latter preparation were stimulated through another suction electrode with square pulses of 100–300 μs duration and 100 V intensity. Mono- and polysynaptic reflex discharges and compound action potentials were stored in a transient memory device and displayed on a pen recorder. Depolarizations induced by DMSO, substance P, capsaicin and acetylcholine were also recorded from the ventral root.

2.4. Drug application to isolated preparation

The drugs were dissolved in the artificial CSF and applied to the spinal cord by changing the perfusion solution to the artificial CSF containing the following drugs: capsaicin, acetylcholine chloride, edrophonium chloride, atropine sulfate, hexamethonium bromide, 4-aminopyridine (Sigma, St. Louis, MO, USA), substance P (Peptide Institute, Osaka, Japan), DMSO, sucrose, urea (Wako, Japan), tetrodotoxin (Sankyo, Tokyo, Japan).

3. Results

3.1. Effects on spinal reflexes

The effect of DMSO on the slow ventral root potential was examined in the isolated spinal cord of the newborn rat. As shown in Fig. 1A, DMSO at 1% (v/v) increased the magnitude of the slow ventral root potential. In contrast to this, mono- and polysynaptic reflex discharges were unaffected by DMSO (Fig. 1B). The magnitude of slow ventral root potential, estimated by area in mV · s, was increased to 129 ± 3 ($n = 3$) and 189 ± 9% ($n = 12$) by DMSO at concentrations of 0.3 and 1%, respectively (Fig. 2A). The time-course of the enhancing effect of 1% DMSO on the slow ventral root potential is shown in Fig. 2B. The maximum effect was exhibited within 17 min and, after the removal of DMSO, the potentiated slow ventral root potential recovered within 12 min.

The effect of DMSO was not observed below 0.3%. The occurrence of spontaneous discharges, or the increase in their frequency and amplitude was observed during exposure to 1% DMSO in 32 out of 49 preparations. In the rest of the preparations, DMSO did not affect the direct current level per se. At higher concentrations of 3–9%, DMSO induced concentration-dependent sustained depolarizations (Fig. 1C). The depolarization induced by 9% DMSO was blocked by 0.3 μM tetrodotoxin (data not shown).

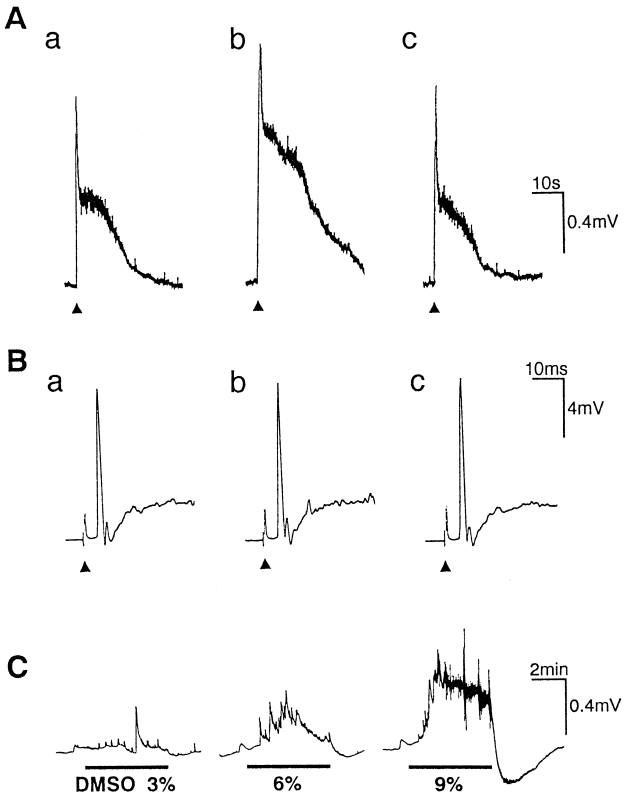

Fig. 1. Effect of DMSO on electrical potential changes recorded from the ventral root. Slow ventral root potential (A) and mono- and polysynaptic reflex discharges (B) were recorded following a single-shock stimulation to the ipsilateral dorsal root every 5 min. Stimulation was given at the time indicated by ▲. (a) Controls, (b) after a 7-min exposure to DMSO (1%), (c) 7 min after discontinuation of the drug. (C) DMSO was applied to the spinal cord as indicated by the horizontal bar showing the concentration applied.

3.2. Effect on capsaicin-induced depolarization

Capsaicin is known to stimulate transmitter release selectively from C fibers (Holzer, 1991). As shown in Fig. 3a, a depolarization was recorded from the ventral roots after application of capsaicin to the spinal cord. The depolarization was reproducible for at least 150 min with an appropriate amount of capsaicin (application of 5 μM solution to the perfusing medium for 3 or 5 s) and with an application interval of more than 40–50 min. DMSO at 1% potentiated the amplitude and duration of the capsaicin-induced depolarization and the effect was reversible (Fig. 3). This result also indicates DMSO enhances the nociceptive transmission mediated by C fibers in the spinal cord.

3.3. Effect on substance P-induced depolarization

It was investigated whether DMSO acts presynaptically or postsynaptically to enhance the slow ventral root potential. It is known that substance P, released from C fibers, is involved in the generation of the slow ventral root potential (Yanagisawa et al., 1982; Akagi et al., 1985; Otsuka and Yanagisawa, 1988; Hosoki et al., 1994; Guo et al., 1995). In normal artificial CSF, application of substance P (30 nM) to the spinal cord induced depolarization of the ventral root. This response reflects a depolarization of spinal motoneurons evoked by an indirect action of substance P through interneurons and a direct action on motoneurons (Otsuka and Yanagisawa, 1980). When DMSO at a concentration of 1% was added to the normal artificial CSF, the substance P-induced depolarization was markedly potentiated, and after the removal of DMSO, the response immediately returned to the control level

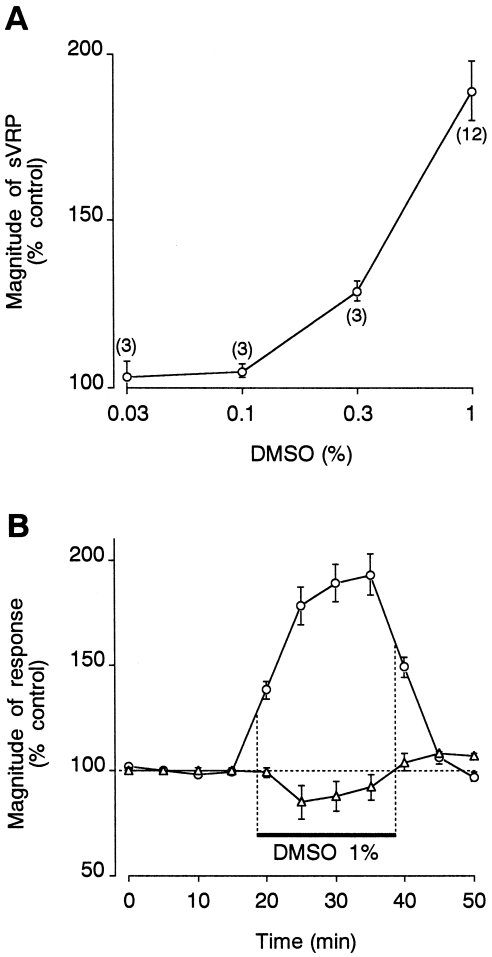

Fig. 2. Concentration–response curve of DMSO on slow ventral root potential (sVRP) (A) and time courses of DMSO effect on slow ventral root potential and monosynaptic reflex discharge (B). (A) The magnitude of the slow ventral root potential, estimated by area in mV·s, after a 12-min exposure to a given concentration of DMSO is expressed as a percentage of the average of 4 control responses recorded immediately before drug application. (B) The magnitude of the slow ventral root potential (○) and the amplitude of the monosynaptic reflex discharge (△) are expressed as percentages of the average of 4 control responses and plotted against time. DMSO at 1% was added to the perfusing medium as indicated by the horizontal bar. Each point and vertical bar represent the mean ± S.E.M. of the number of experiments shown in parenthesis in (A), of 13 (○) and 12 (△) experiments in (B).

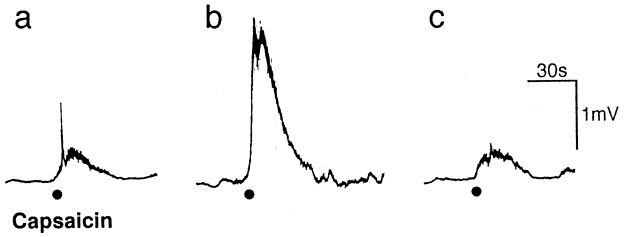

Fig. 3. Effect of DMSO on the capsaicin-induced depolarization. Capcaisin (5 μm) was applied for 3 s at the time indicated by ●. (a) Control, (b) after a 18-min exposure to DMSO (1%), (c) 90 min after removal of DMSO. Similar results were obtained in 2 other experiments.

(Fig. 4A,C). In order to eliminate a possible presynaptic component of DMSO action, the effect of DMSO on the depolarization was examined in the presence of tetrodotoxin. The mono- and polysynaptic reflex discharges and the slow ventral root potential were abolished in the presence of 0.3 μM tetrodotoxin; under these conditions, the substance P-induced depolarization was smaller in size than in the absence of tetrodotoxin. The concentration–response curve of substance P-induced depolarization was shifted to the right as shown in Fig. 4C. In the presence of tetrodotoxin, DMSO at 1% did not potentiate the substance P-induced depolarization and did not affect the concentration–response curve of substance P-induced depolarization (Fig. 4B,C). In a calcium-free and high magnesium (3 mM) medium, DMSO also did not potentiate the substance P-induced depolarization (data not shown). These results suggest that DMSO at 1% enhanced the slow ventral root potential through a presynaptic rather than a postsynaptic action.

3.4. Effect on cholinesterase activity

It is reported that DMSO inhibits cholinesterase activity in bovine erythrocytes (Sams and Carroll, 1966), chick biventer cervicis muscle (Gandiha and Marshall, 1972) and bullfrog sympathetic ganglion (Matsumoto et al., 1985). In the newborn rat spinal cord-cutaneous nerve preparation, the saphenous nerve-evoked slow ventral root potential was enhanced by the cholinesterase inhibitor, edrophonium (Kurihara et al., 1993). It was, therefore, investigated whether the slow ventral root potential was augmented by the inhibitory effect of DMSO on cholinesterase activity.

DMSO at 1% augmented the acetylcholine-induced depolarization in the presence of 0.3 μM tetrodotoxin (data not shown). Edrophonium at 10 μM enhanced the slow ventral root potential, and the effect was comparable to that of 1% DMSO (see Fig. 5C). In the presence of 0.3 μM atropine and 30 μM hexamethonium, which blocked the depolarizing effect of acetylcholine, but not that of substance P (data not shown), the slow ventral root potential was decreased by $26 \pm 4\%$ ($n = 7$, data not shown), but not the monosynaptic reflex discharge. The enhancing effect of 10 μM edrophonium on the slow ventral root potential disappeared in the presence of these cholinergic antagonists (Fig. 5A,C). The enhancing effect of 1% DMSO on the slow ventral root potential was partially reduced, but did not disappear (Fig. 5B,C). This, therefore, suggests that cholinesterase inhibition was a part of the mechanism of action of DMSO on the slow ventral root potential.

3.5. Effect on K^+ channel

It is reported that in the bullfrog sympathetic ganglion, several aminopyridines, K^+ channel blockers and DMSO are able to rescue synaptic transmission from failure in a

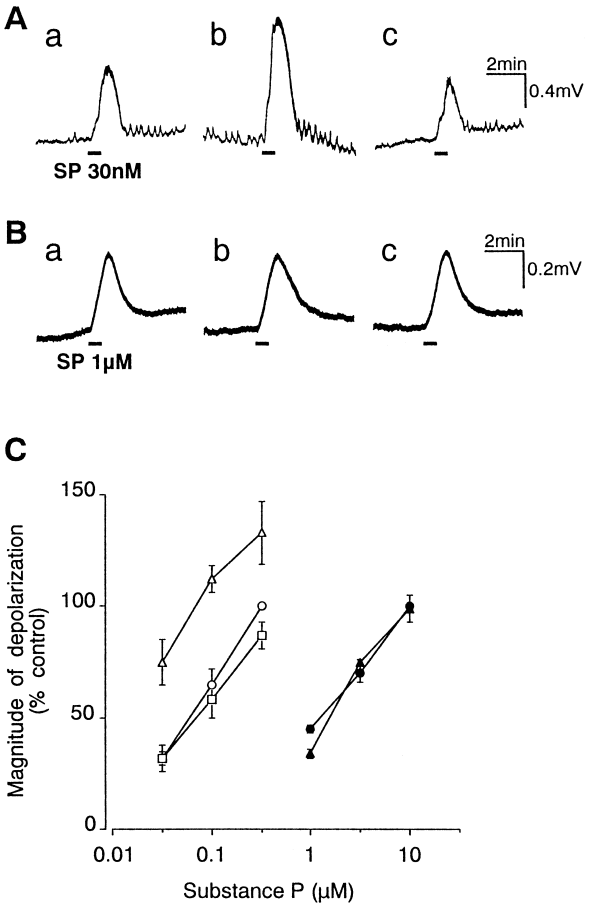

Fig. 4. Effect of DMSO on substance P (SP)-induced depolarization. Substance P was applied by perfusion for 30 s, as indicated by the horizontal bar with concentration applied at 15-min intervals in normal medium (A) and in the presence of tetrodotoxin (0.3 μM; B). (a) Controls, (b) after a 10-min exposure to DMSO (1%), (c) 10 min after removal of DMSO. (C) Effect of DMSO on the depolarization induced by various concentrations of substance P. The magnitude of the substance P-induced depolarization, estimated by area in mV·min, is expressed as a percentage of the response induced at 0.3 or 10 μM in the normal medium (open symbol) or medium containing tetrodotoxin (0.3 μM, closed symbol), respectively. The values were plotted against the concentration of substance P before (○, ●) and after (△, ▲) exposure to DMSO, and after removal of DMSO (□). Each point and vertical bar represent the mean ± S.E.M. of 4 and 3 experiments in normal medium and in the presence of tetrodotoxin, respectively.

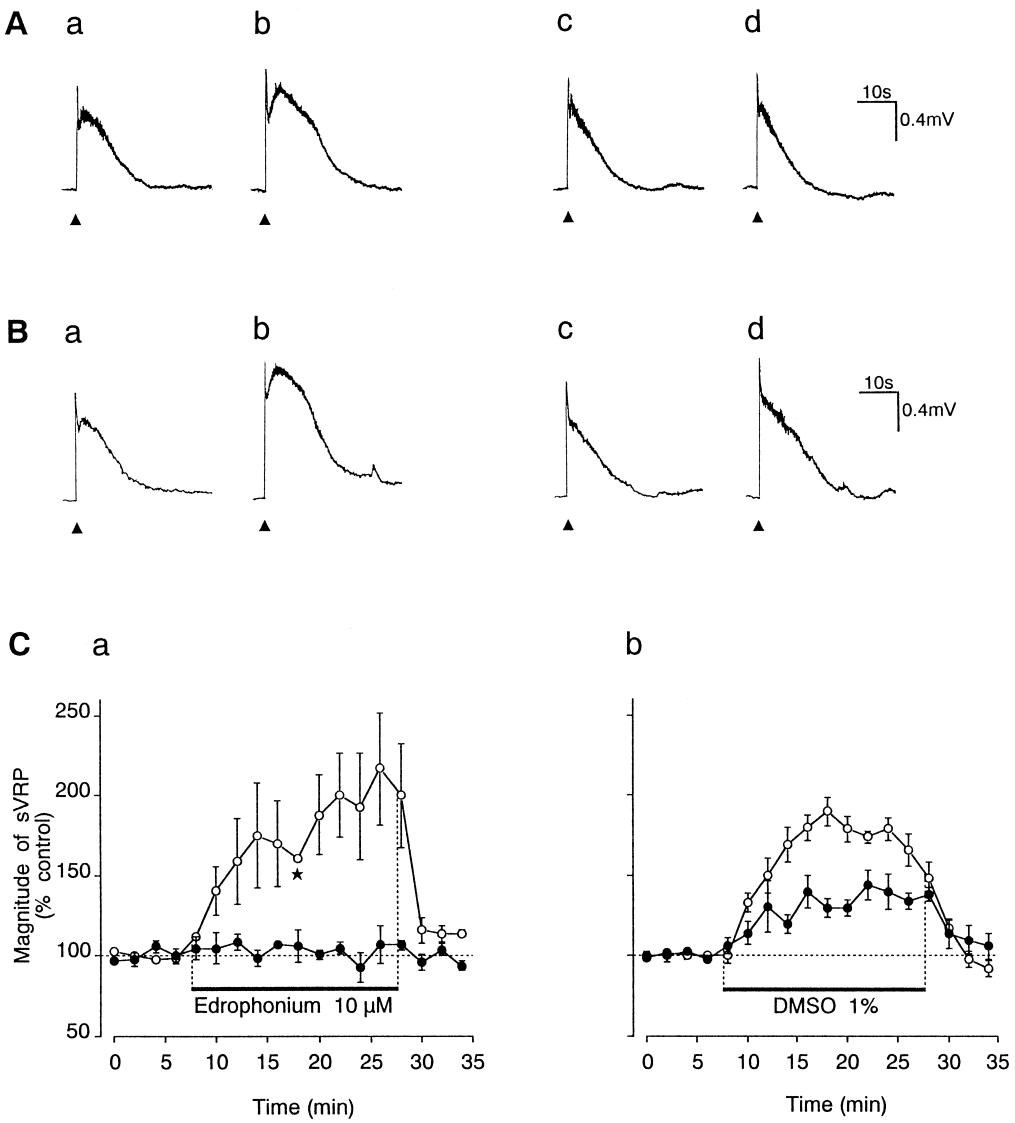

Fig. 5. Effect of cholinergic antagonists on the edrophonium- or DMSO-induced potentiation of slow ventral root potential. (A) (a) Control, (b) after a 17-min exposure to edrophonium (10 μM), (c) control in the presence of hexamethonium (30 μM) and atropine (0.3 μM), (d) after a 17-min exposure to edrophonium in the presence of hexamethonium and atrophine. (B) (a) Control, (b) after a 9-min exposure to DMSO (1%), (c) control in the presence of hexamethonium and atropine. (C) Time-courses of edrophonium (a)- or DMSO (b)-induced potentiation of slow ventral root potential (sVRP) in normal medium (○) and in the presence of hexamethonium and atropine (●). The magnitude of the slow ventral root potential, estimated by area in mV · s, is expressed as a percentage of the average of 4 control responses before exposure to edrophonium or DMSO and plotted against time. Edrophonium or DMSO was added to perfusion medium as indicated by the horizontal bar. Each point and vertical bar represent the mean ± S.E.M. of 3 and 4 experiments in (a) and (b), respectively, except for the symbol with ★ where $n = 2$.

low calcium medium (Matsumoto and Riker, 1984; Matsumoto et al., 1985) and that DMSO prolongs the action potential duration in *Aplysia* ganglionic neurons by decreasing K^+ conductance (Sawada and Sato, 1975). Therefore, we investigated whether DMSO increases the amplitude of the compound action potential of the dorsal root by blocking K^+ channels and augments the magnitude of stimulus inputs to spinal neurons. In a saphenous nerve–dorsal root preparation, a rapidly conducted compound action potential followed by a slow one were recorded from the dorsal root on stimulation of the saphenous nerve (Fig. 6Aa, Ba). We estimated the conduction velocity of 105 ± 6 cm/s ($n = 6$) in the former and 52 ± 2 cm/s ($n = 6$) in the latter, which were similar to the values reported for A and C fibers, respectively (Fitzgerald and Gibson, 1984; Fitzgerald, 1985). 4-Aminopyridine at 10 μM enhanced both A-fiber and C-fiber responses (Fig. 6A). In contrast, DMSO up to 3% caused neither inhibition nor augmentation of the responses (Fig. 6B). These results suggest that blockade of K^+ channels is not involved in the mechanism of the DMSO-induced enhancement of the slow ventral root potential.

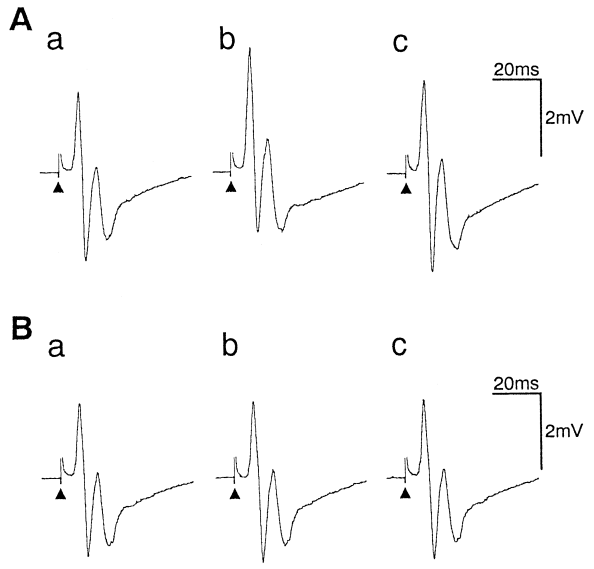

Fig. 6. Effect of DMSO or K^+ channel blocker on compound action potentials in the dorsal root. Compound action potentials were recorded following a single-shock stimulation to the saphenous nerve every 30 s. Stimulation was given at the time indicated by ▲. (A) (a) Control, (b) after a 20-min exposure to 4-aminopyridine (10 μM), (c) 60 min after removal of the drug. (B) (a) Control, (b) after a 20-min exposure to DMSO (1%), (c) 1 min after removal of the drug. Similar results were obtained in 3 other experiments.

3.6. Effect of hyperosmotic pressure on the slow ventral root potential

An aqueous solution containing about 2% DMSO is isosmotic. Therefore, the osmotic pressure of artificial CSF containing 1% DMSO is about 1.5-times higher than that of normal artificial CSF. The effect of hyperosmotic pressure on the slow ventral root potential was examined by using artificial CSF containing 0.87% (w/v) urea or 4.6% (w/v) sucrose, which was 1.5-times isotonic. The artificial CSF containing 0.87% urea did not increase, but rather depressed the slow ventral root potential ($n = 4$, data not shown). Sucrose added to artificial CSF at 4.6% either increased ($n = 3$) or decreased ($n = 1$) the slow ventral root potential (data not shown). In the former cases, the increase in the slow ventral root potential was followed by a pronounced depression for a short time after removal of sucrose, which was not observed with DMSO, suggesting that sucrose and DMSO act by different mechanisms. Therefore, hyperosmotic pressure is unlikely to be the mechanism of the enhancing action of DMSO on the slow ventral root potential.

4. Discussion

The present study showed that in the isolated spinal cord of the newborn rat, DMSO at 0.3–1% potentiated the slow ventral root potential, leaving mono- and polysynaptic reflex discharges unaffected. This result suggests that DMSO over this range of concentrations may facilitate synaptic transmission in the pathway from the primary afferent C fibers to the motoneurons, and that synaptic transmission from nonmyelinated C fibers is more sensitive to DMSO than that from myelinated A fibers. In this study, it was also shown that DMSO at 1% potentiated the depolarization induced by capsaicin. This result also indicates that DMSO enhances the nociceptive transmission mediated by C fibers in the spinal cord.

The sites of action of DMSO were investigated by observing the effect of DMSO on the depolarization induced by substance P. Potentiation of substance P-induced depolarization by DMSO shows that DMSO facilitates synaptic transmission between interneurons, and/or interneurons and motoneurons. The inability of DMSO to potentiate the substance P-induced depolarization in the presence of tetrodotoxin indicates that DMSO may act presynaptically but not postsynaptically. In addition, DMSO (3–9%)-induced sustained depolarizations were blocked by tetrodotoxin, suggesting that DMSO at these concentrations may also act presynaptically.

DMSO has been reported to inhibit cholinesterase activity in the bovine erythrocyte at 0.08–8% (Sams and Carroll, 1966), the chick biventer cervicis muscle at 5% (Gandiha and Marshall, 1972) and the bullfrog sympathetic ganglion at about 1% (Matsumoto et al., 1985). There are some studies to show that DMSO, in the same range of the concentrations as those described above, facilitates cholinergic transmission (Sams et al., 1966; Gandiha and Marshall, 1972; Sawada and Sato, 1975). In the present experiments, DMSO at 1% augmented the depolarization induced by acetylcholine, but not that elicited by substance P in the presence of tetrodotoxin, suggesting that DMSO has an inhibitory effect on cholinesterase activity. Cholinesterase inhibition may be one of the mechanisms of DMSO action in the enhancement of slow ventral root potential. In the newborn rat spinal cord–cutaneous nerve preparation, the saphenous nerve-evoked slow ventral root potential was enhanced by the cholinesterase inhibitor, edrophonium (Kurihara et al., 1993). In the present experiments, the slow ventral root potential was decreased in the presence of atropine and hexamethonium, suggesting that endogenous acetylcholine has an enhancing effect. The cholinergic antagonists completely blocked the enhancing effect of edrophonium on the slow ventral root potential, but partially inhibited the DMSO effect. Therefore, DMSO seemed to enhance the slow ventral root potential, not only through inhibition of cholinesterase activity, but also through other mechanisms.

The possibility has been considered that DMSO may enhance calcium entry into the nerve terminal and increase transmitter release. DMSO at 3–10% antagonized the transmission failure caused by a low extracellular calcium concentration in the bullfrog sympathetic ganglion (Matsumoto et al., 1985), and 2.5% DMSO and extracellu-

lar calcium synergistically increased the frequency of miniature endplate potentials in the frog neuromuscular junction (Geron and Meiri, 1985). It may be that DMSO, which is lipophilic, penetrates the plasma membrane and acts intracellularly. It has been reported that DMSO increases protein kinase C activity in HL-60 cells (Makowske et al., 1988). Activation of protein kinase C increases transmitter release and synaptic transmission in a variety of preparations including rat sympathetic neurons (Wakade et al., 1985), rat hippocampal slices (Malenka et al., 1987), and frog neuromuscular junction (Shapira et al., 1987).

Formerly, DMSO was used as a peripherally acting analgesic that was applied to the painful area, and was shown to suppress peripheral C fiber activity at 5–10%, and A fiber activity at higher concentrations. In contrast to these studies, the present experiment with the spinal cord showed that DMSO facilitated the slow ventral root potential selectively, suggesting a nociceptive action of DMSO in the central nervous system. The present results do not show whether DMSO affects mechanical, chemical or heat nociception differently. DMSO is used as a solvent in various experiments including neurobiological studies. The effect of DMSO on synaptic transmission, especially in the nociceptive pathway, should be taken into consideration.

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