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Halothane attenuates the cerebroprotective action of several Na^+ and Ca^{2+} channel blockers via reversal of their ion channel blockade

Michiko Oka [a], Yoshinori Itoh [b], Takuya Fujita [a,*]

[a] *Department of Biochemical Pharmacology, Kyoto Pharmaceutical University, Misasagi, Yamashina, Kyoto 607-8414, Japan*
[b] *Department of Hospital Pharmacy, Faculty of Medicine, Kyushu University, 3-1-1 Maidashi, Higashi, Fukuoka 812-8582, Japan*

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Abstract

We have previously shown the involvement of Na^+ channel as well as N-type and P/Q-type Ca^{2+} channels in the oxygen and glucose deprivation-induced injury in rat cerebrocortical slices. In the present study, we investigated the influence of halothane on the cerebroprotective effects of a variety of Na^+ and Ca^{2+} channel blockers in rat cerebrocortical slices. The hypoxic injury was attenuated by Na^+ channel blockers including tetrodotoxin, lidocaine and dibucaine, and Ca^{2+} channel blockers, such as verapamil, ω-agatoxin IVA and ω-conotoxin GVIA. Halothane abolished the protective effects of lidocaine, dibucaine and verapamil, all of which block the respective cation channels in a voltage-dependent manner, without affecting the actions of tetrodotoxin, ω-agatoxin IVA and ω-conotoxin GVIA, which reveal voltage-independent blockade. On the other hand, the nitric oxide synthesis estimated from the extracellular cyclic GMP formation was elevated during exposure to hypoxia. All channel blockers tested here attenuated hypoxia-evoked nitric oxide synthesis. Halothane blocked almost completely these actions of lidocaine and verapamil. Moreover, the Na^+ and Ca^{2+} channel blockade by these compounds, as determined by veratridine- and KCl-stimulated nitric oxide synthesis, respectively, was also reversed by halothane. These findings suggest that an anesthetic agent halothane reversed the Na^+ and Ca^{2+} channel blockade of several voltage-dependent ion channel blockers, leading to the attenuation of their cerebroprotective actions. Therefore, the influence of halothane anesthesia should be taken into consideration for the evaluation of neuroprotective action of Na^+ and Ca^{2+} channel blockers.

Keywords: Halothane; Ca^{2+} channel blocker; Na^+ channel blocker; Hypoxia; Nitric oxide (NO) synthesis; Cerebral cortex

1. Introduction

Although the cellular mechanisms underlying the ischemic or hypoxic brain damage are not fully understood, the breakdown of intracellular Ca^{2+} homeostasis and the resultant aberrant rise in intracellular Ca^{2+} is implicated in the etiology of cerebral ischemia (Orrenius et al., 1989; Choi, 1992; Kristián and Siesjö, 1998). Several lines of evidence have demonstrated that voltage-gated Na^+ and Ca^{2+} channels are involved in the elevation of intracellular Ca^{2+} induced by cerebral ischemia (Pratt et al., 1992; Leach et al., 1993; Buchan et al., 1994; Lysko et al., 1994; Rataud et al., 1994; Barone et al., 1995; Smith and Meldrum, 1995). During cerebral ischemia, the dysfunction of Na^+/K^+ ATPase occurs due to the depletion of ATP, which, in turn, causes an elevation of intracellular Na^+ concentration and the extracellular K^+, thereby causing membrane depolarization and subsequent activation of voltage-gated Na^+ channel and Ca^{2+} channels. Several Na^+ channel blockers including tetrodotoxin and antiepileptic agents have been reported to produce a cerebroprotective action both in vivo (McDonald and Johnston, 1990; Leach et al., 1993; Lysko et al., 1994) and in vitro (Fern et al., 1993; Lynch et al., 1995). In addition, N-type Ca^{2+} channel blockers such as ω-conopeptide SNX-111 (Valentino et al., 1993; Buchan et al., 1994) and SB 201823-A (Barone et al., 1995) have been shown to protect neurons against focal as well as global cerebral ischemia in rodents.

We have recently reported that the hypoxic injury induced by a transient exposure of rat cerebrocortical slices to oxygen and glucose deprivation followed by reoxygenation is effectively inhibited by a combined treatment with Na^+ channel blockers and N-type or P/Q-type Ca^{2+} channel blockers (Tatsumi et al., 1998; Oka et al., 2000b). Moreover, these Na^+ and Ca^{2+} channel blockers attenuate the activation of

* Corresponding author. Tel.: +81-75-595-4662; fax: +81-75-595-4761.
E-mail address: fujita@mb.kyoto-phu.ac.jp (T. Fujita).

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nitric oxide synthase (NOS), a Ca^{2+}-activated enzyme, induced by the hypoxic insult (Oka et al., 2000b).

It has been demonstrated that several anaesthetic agents affect the function of Na^+ channel as well as Ca^{2+} channel function by interacting directly with such channels (Eskinder et al., 1993; Study, 1994; McDowell et al., 1996; Pancrazio, 1996; Rehberg et al., 1996). Therefore, it seems probable that the cerebroprotective efficacies of a variety of Na^+ and Ca^{2+} channel blockers are influenced by such anesthetic agents. To ascertain this idea, the effect of halothane on the cerebroprotective actions and ion channel blockade by several types of Na^+ channel and Ca^{2+} channel blockers was investigated in rat cerebrocortical slices.

2. Materials and methods

2.1. Animals

Male 8–11 weeks old Sprague–Dawley rats (Shimizu Laboratory Supplies Co., Kyoto, Japan) were used. Rats were housed in groups of five to six in a room controlled at 21–25 °C, 45–65% with humidity and maintained in an alternating 12-h light/dark cycle (lights automatically on at 8:00 A.M.). Food and water were freely given. Experiments were all carried out in accordance with the Guide for the Care and Use of Laboratory Animals written by the Japanese Pharmacological Society.

2.2. Hypoxia/reoxygenation-induced tissue injury in rat cerebrocortical slices

Hypoxic tissue injury was induced in rat cerebrocortical slices, as described previously (Tatsumi et al., 1998; Oka et al., 2000b). Briefly, after immersing the whole brain in ice-cold 5% glucose solution for 10 min, the cerebral cortex was dissected on ice and serial coronal sections of 500-μm thickness were prepared using McIlwain tissue chopper (Mickle Laboratory Engineering, Gomshall, U.K.). Then, the chopped tissue was transferred to a glass dish containing ice-cold Krebs–Ringer bicarbonate solution (KRB: 118 mM NaCl, 4.7 mM KCl, 2.54 mM $CaCl_2$, 1.2 mM $MgCl_2$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$ and 11.5 mM D-glucose, pH 7.4), and each slice was gently isolated with forceps. A piece of slices was transferred to a 24-well culture plate containing 2 ml KRB and preincubated at 37 °C for 1 h under continuous bubbling with a gas mixture of 95% O_2 and 5% CO_2. After preincubation, the slice was incubated in glucose-deprived KRB at 37 °C for 45 min under continuous bubbling with 95% N_2 and 5% CO_2 in the absence or presence of halothane, followed by reoxygenation for 5 h in normal KRB in the absence of halothane. The concentration of halothane was 300 μM, which was chosen on the basis that the aqueous halothane concentration for surgical anesthesia is between 0.21 and 0.35 mM (Smith et al., 1981). Tissue damage was assessed by the leakage of lactate dehydrogenase (LDH) into the incubation medium. LDH was determined by an enzymatic assay kit (Wako, Osaka, Japan). In normoxic group, slices were incubated for 45 min in KRB under continuous bubbling with 95% O_2 and 5% CO_2 with or without halothane, followed by reoxygenation for 5 h in normal KRB in the absence of halothane. Halothane was passed over KRB using a carrier gas of 95% N_2 and 5% CO_2 (in the case of hypoxia), or 95% O_2 and 5% CO_2 (in the case of normoxia) and a halothane vaporizer (AHV-5, Kimura Medical Instrument, Tokyo, Japan). The concentration of halothane (1% at the vaporizer) was 0.30 ± 0.02 mM (mean ± S.E.M., $N=3$), which was determined by using a gas chromatograph (GC-15A, Shimadzu, Kyoto, Japan). At the end of experiments, brain slices were dissolved in 1 ml of 1 M NaOH and protein content was determined by the method of Bradford (1976) using bovine serum albumin as the standard.

2.3. Measurement of nitric oxide (NO) synthesis in the extracellular fluids and in slices of the rat cerebral cortex

The NO synthesis during exposure of rat cerebrocortical slices to the oxygen and glucose deprivation was estimated from cyclic GMP formation in the extracellular fluids, as described previously (Oka et al., 2000a). Briefly, the rat cerebellum was homogenized with 10 volumes of ice-cold buffer-A (50 mM Tris–HCl containing 1 mM EDTA, 1 mM dithiothreitol and 200 μM phenylmethylsulfonyl fluoride) using Polytron (PT-3000, Kinematica, Littau, Switzerland). After centrifugation at 30,000 × *g* for 30 min, the supernatant fraction was used for the source of guanylate cyclase. The cerebrocortical slice was exposed for 45 min to 2 ml of oxygen- and glucose-deprived KRB, in which 0.5 mM GTP, 1 mM 3-isobutyl-1-methylxanthine (IBMX) and 20 μl aliquot of the supernatant fraction of rat cerebellum were included. Halothane (300 μM) was applied as described above. The cyclic GMP produced in the incubation medium was measured by using the Amersham cyclic GMP enzyme immunoassay kit.

In another set of experiments where the NO synthesis was evoked by veratridine or KCl stimulation, the cyclic GMP accumulation in cerebrocortical tissues was measured, as described previously (Oka et al., 1999). In brief, a piece of brain slices was transferred to a 24-well culture plate containing 2 ml of KRB under continuous gassing with 95% O_2 and 5% CO_2 and preincubated at 37 °C for 1 h. Then, the slice was incubated with 10 μM veratridine or 50 mM KCl for 20 min in the presence of 1 mM IBMX, and the reaction was terminated by addition perchloric acid (final concentration was 0.2 M). Halothane (300 μM) was applied as described above. The slice was homogenized, and the homogenate was centrifuged at 10,000 × *g* for 15 min at 4 °C. The resultant supernatant was neutralized with 10% K_2CO_3, then centrifuged at 10,000 × *g* for 15 min at 4 °C. The cyclic GMP content in the supernatant was determined as described above.

2.4. Chemicals

The following chemicals and drugs were obtained from commercial sources: IBMX was obtained from Sigma (St. Louis, MO, USA), ω-conotoxin GVIA and ω-agatoxin IVA were purchased from Peptide Institute (Osaka, Japan); verapamil hydrochloride, dibucaine hydrochloride, lidocaine and tetrodotoxin were from Wako; halothane was from Takeda Chemical Ind. (Osaka, Japan). The cyclic GMP enzyme immunoassay system was obtained from Amersham (Buckinghamshire, U.K.). Other chemicals were all of guaranteed grade.

2.5. Statistical analysis

Data were all analyzed by using SAS program (SAS/STAT, 1990, SAS Institute, Cary, NC, USA). Unless otherwise indicated, data were analyzed by one-way analysis of variance, followed by Dunnett's test for multiple comparison.

3. Results

3.1. Effect of halothane on cerebroprotective actions of various Na^+ and Ca^{2+} channel blockers against hypoxic injury in rat cerebrocortical slices

When rat cerebrocortical slices were exposed to oxygen and glucose deprivation for 45 min followed by reoxygenation for 5 h, a significant increase in LDH leakage was observed. The Na^+ channel blockers including 1 μM tetrodotoxin, 30 μM lidocaine and 10 μM dibucaine reversed the hypoxic injury (Fig. 1A). Halothane (300 μM) by itself did not affect the LDH leakage, while it reversed almost completely the protective actions of lidocaine and dibucaine without affecting that of tetrodotoxin (Fig. 1B). In addition, the hypoxic injury was attenuated by 10 μM verapamil alone or the combined treatment with N-type Ca^{2+} channel blocker ω-conotoxin GVIA (5 μM) (Regan et al., 1991) and P/Q-type Ca^{2+} channel blocker ω-agatoxin IVA (0.2 μM) (Mintz et al., 1992) (Fig. 2A). Halothane markedly reversed the protective action of verapamil, whereas it failed to counteract the protective action of the combination of ω-agatoxin IVA and ω-conotoxin GVIA (Fig. 2B).

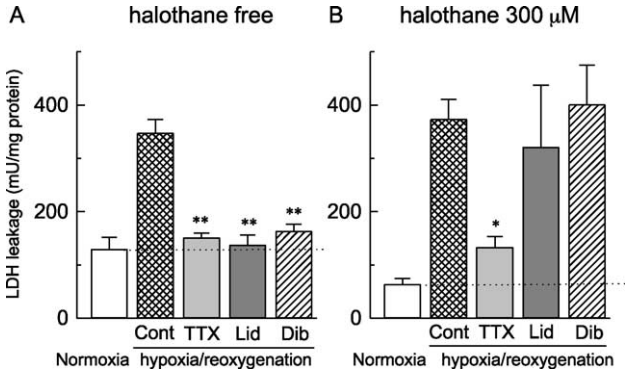

Fig. 1. Effect of halothane on the cerebroprotective action of various Na^+ channel blockers against the hypoxic injury in rat cerebrocortical slices. After preincubation for 1 h, slices were exposed to the oxygen and glucose deprivation for 45 min, followed by reoxygenation for 5 h. Tetrodotoxin (TTX; 1 μM), lidocaine (Lid; 30 μM) or dibucaine (Dib; 10 μM) was included during both the hypoxic and reoxygenation periods, while halothane (300 μM) was included during the hypoxic period. In normoxia group, slices were incubated with halothane for 45 min under the normoxic condition, followed by reoxygenation for 5 h in the absence of halothane. The tissue injury was determined by LDH leakage. Each column represents the mean ± S.E.M. of five experiments. $*p<0.05$, $**p<0.01$ as compared with respective control group (Dunnett's test).

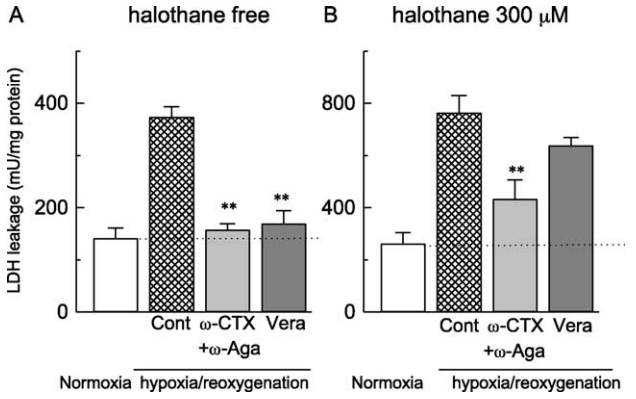

Fig. 2. Effect of halothane of the cerebroprotective action of various Ca^{2+} channel blockers against the hypoxic injury in rat cerebrocortical slices. ω-Conotoxin GVIA (ω-CTX; 5 μM) and ω-agatoxin IVA (ω-Aga; 0.2 μM) were treated in combination, while verapamil (10 μM) was added alone in the absence or presence of 300 μM halothane. In normoxia group, slices were incubated with halothane for 45 min under the normoxic condition, followed by reoxygenation for 5 h in the absence of halothane. The tissue injury was determined by LDH leakage. Each column represents the mean ± S.E.M. of five experiments. $**p<0.01$ as compared with respective control group (Dunnett's test).

3.2. Reversal by halothane of inhibitory effect of various Na^+ and Ca^{2+} channel blockers on the NO synthesis evoked by hypoxic insult

The NO synthesis during exposure of rat cerebrocortical slices to oxygen and glucose deprivation for 45 min was estimated from cyclic GMP formation in the incubation medium after addition of soluble fraction of rat cerebellum (as the source of guanylate cyclase), 0.5 mM GTP and 1 mM IBMX to the medium, since the tissue cyclic GMP content was reduced during hypoxia due to the severe depletion of GTP, a substrate for guanylate cyclase (Oka et al., 2000b). The deduced NO synthesis was markedly elevated during hypoxia (Fig. 3A). The Na^+ channel blockers and Ca^{2+} channel blockers tested in the present study all significantly attenuated the oxygen and glucose deprivation-induced enhancement of NO synthesis. As in the case of the

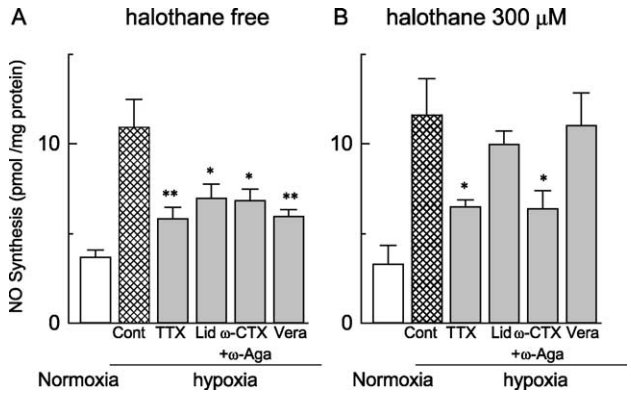

Fig. 3. Effects of halothane on the attenuation of hypoxic NO synthesis induced by tetrodotoxin, lidocaine, verapamil, ω-conotoxin GVIA and ω-agatoxin IVA in rat cerebrocortical slices. Tetrodotoxin (TTX; 1 μM), lidocaine (Lid; 30 μM), verapamil (Vera; 10 μM) and the combined treatment with ω-conotoxin GVIA (ω-CTX; 5 μM) and ω-agatoxin IVA (ω-Aga; 0.2 μM) was added to the incubation medium in the absence or presence of halothane (300 μM) during the oxygen and glucose deprivation. The NO synthesis during exposure to hypoxia was estimated from cyclic GMP formation in the extracellular fluids after addition of crude supernatant fraction of rat cerebellum (as the source of guanylate cyclase), 0.5 mM GTP and 1 mM IBMX. In normoxia group, slices were incubated with halothane for 45 min under the normoxic condition. Each column represents the mean ± S.E.M. of four experiments. * $p<0.05$, ** $p<0.01$ as compared with respective control group (Dunnett's test).

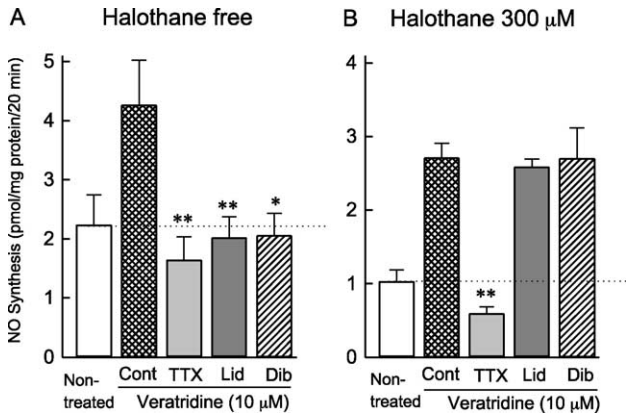

Fig. 4. Comparison of the effects of various Na^+ channel blockers on veratridine-induced NO synthesis determined in the absence (A) or presence of halothane (B) in slices of the rat cerebral cortex. Tetrodotoxin (TTX; 1 μM), lidocaine (Lid; 30 μM) or dibucaine (Dib; 10 μM) was added to the incubation medium in the absence or presence of halothane (300 μM). The NO synthesis for 20 min was estimated from cyclic GMP formation. Each column represents the mean ± S.E.M. of six experiments. * $p<0.05$, ** $p<0.01$ as compared with respective evoked values determined in the absence of ion channel blockers (Dunnett's test).

cerebroprotective action, halothane completely reversed the inhibitory effects of lidocaine and verapamil on the NO synthesis, while having no influence on the actions of tetrodotoxin or the combination of ω-agatoxin IVA with ω-conotoxin GVIA (Fig. 3B).

3.3. Influence of halothane on the inhibitory effects of various Na^+ and Ca^{2+} channel blockers on veratridine-induced and KCl-evoked NO synthesis in rat cerebrocortical slices

As shown in Fig. 4A, veratridine (10 μM) stimulated the NO synthesis estimated from the tissue cyclic GMP accumulation, which was completely blocked by 1 μM tetrodotoxin, 30 μM lidocaine or 10 μM dibucaine. Halothane (300 μM) by itself caused a moderate but not significant reduction in the veratridine-evoked NO synthesis in rat cerebrocortical slices. The inhibitory effect of tetrodotoxin was not influenced by halothane at all, whereas the inhibitory actions of lidocaine and dibucaine were completely reversed by halothane (Fig. 4B). On the other hand, the NO synthesis evoked by 50 mM KCl was significantly blocked by ω-conotoxin GVIA, ω-agatoxin IVA or verapamil (Fig. 5A). Halothane (300 μM) by itself did not affect the KCl-evoked NO synthesis in rat cerebrocortical slices, while it completely reversed the inhibitory action of verapamil without affecting those of ω-conotoxin GVIA or ω-agatoxin IVA (Fig. 5B).

3.4. Reversal by halothane of the dose–response curves for lidocaine and verapamil in blocking veratridine- and KCl-stimulated NO synthesis, respectively, in rat cerebrocortical slices

As shown in Fig. 6, in the absence of halothane, lidocaine and verapamil revealed a concentration-dependent inhibition of veratridine- and KCl-evoked NO synthesis, respectively, in rat cerebrocortical slices. However, no inhi-

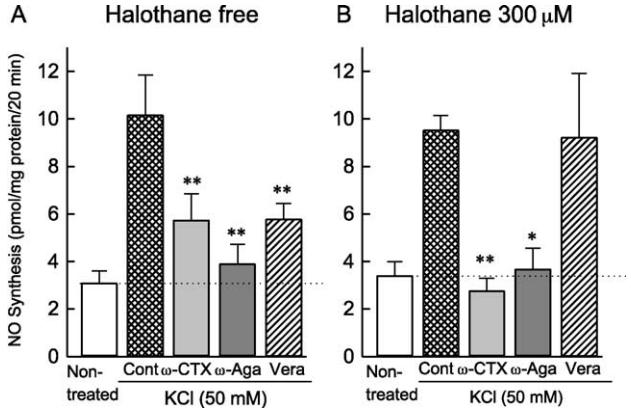

Fig. 5. Comparison of the effects of various Ca^{2+} channel blockers on KCl-induced NO synthesis determined in the absence (A) or presence of halothane (B) in slices of the rat cerebral cortex. ω-Conotoxin GVIA (ω-CTX; 5 μM), ω-agatoxin IVA (ω-Aga; 0.2 μM) or verapamil (Vera; 10 μM) was added in the absence or presence of 300 μM halothane. The NO synthesis for 20 min was estimated from cyclic GMP formation. Each column represents the mean ± S.E.M. of six experiments. * $p<0.05$, ** $p<0.01$ as compared with respective evoked values determined in the absence of ion channel blockers (Dunnett's test).

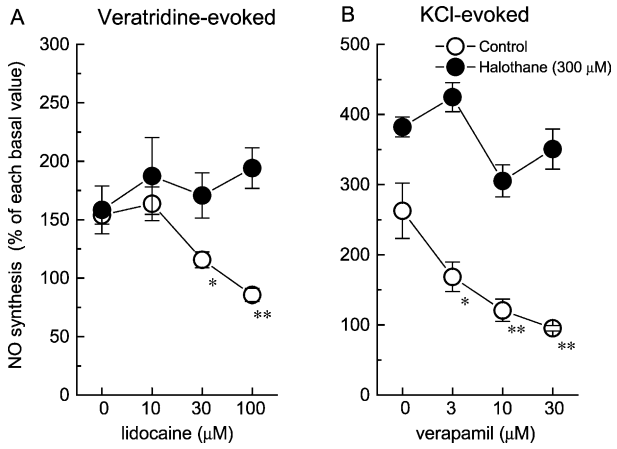

Fig. 6. Reversal by halothane of the concentration-dependent inhibitory actions of lidocaine on veratridine-stimulated NO synthesis (A) and verapamil on KCl-evoked NO synthesis (B) in rat cerebrocortical slices. Lidocaine or verapamil was added to the incubation medium in the absence or presence of halothane (300 μM). The NO synthesis was evoked by veratridine (10 μM) or KCl (50 mM) for 20 min, and estimated from tissue cyclic GMP accumulation. The ordinate represents the percentage of cyclic GMP accumulation of each basal value. In A, basal cyclic GMP levels were 1.4 ± 0.5 pmol/mg protein/20 min in the absence of halothane and 0.7 ± 0.1 pmol/mg protein/20 min in the presence of halothane, whereas in B, those values were 1.4 ± 0.1 pmol/mg protein/20 min in the absence of halothane and 0.7 ± 0.1 pmol/mg protein/20 min in the presence of halothane. Each point represents the mean ± S.E.M. of five experiments. * $p < 0.05$, ** $p < 0.01$ as compared with respective control (Dunnett's test).

bitory action of lidocaine or verapamil (up to 100 or 30 μM, respectively) was observed, when determined in the presence of 300 μM halothane.

4. Discussion

In the present study, the oxygen and glucose deprivation-induced injury was suppressed by a single treatment with Na^+ channel blockers such as tetrodotoxin, dibucaine and lidocaine, and Ca^{2+} channel blocker including verapamil, and the combined treatment with N-type Ca^{2+} channel blocker ω-conotoxin GVIA and P/Q-type Ca^{2+} channel blocker ω-agatoxin IVA. It is unlikely that the protective action of verapamil is due to the block of L-type Ca^{2+} channel, since another L-type Ca^{2+} channel blocker, nifedipine, is not effective in our hypoxic injury model (Tatsumi et al., 1998; Oka et al., 2000b). It has been demonstrated that verapamil blocks multiple types of Ca^{2+} channels including L-type, N-type and P/Q-type Ca^{2+} channels in a voltage- and use-dependent fashion (Cai et al., 1997; Furukawa et al., 1997). In our previous works (Tatsumi et al., 1998; Oka et al., 2000b), the hypoxic injury was not effectively attenuated by either ω-conotoxin GVIA or ω-agatoxin IVA, alone, but significantly inhibited by the combination of these peptides. Consistently, in the present study, the hypoxic injury was significantly reversed by the combined treatment with both peptides. Taken together, the cerebroprotective effect of verapamil may result from the blockade of both N-type and P/Q-type Ca^{2+} channels.

On the other hand, the cerebroprotective effects of dibucaine, lidocaine and verapamil were dramatically reduced by halothane, whereas the effects of tetrodotoxin, and the combination of ω-conotoxin GVIA and ω-agatoxin IVA were not affected at all by halothane.

We have recently shown that the enhanced formation of NO is implicated in the pathogenesis of the oxygen and glucose deprivation-induced injury, based on the facts that several NO synthase inhibitors N^G-nitro-L-arginine and N^G-monomethyl-L-arginine, and a NO scavenger sodium 2-(4-carboxyphenyl)-4,4,5,5- tetramethylimidazoline-1-oxyl 3-oxide (carboxy-PTIO) effectively inhibit the hypoxic injury (Oka et al., 2000a,b). Moreover, the NO synthesis, as estimated from the formation of cyclic GMP in the extracellular fluids after addition of GTP and cerebellar soluble fraction as the source of guanylate cyclase, was remarkably enhanced during hypoxia (Oka et al., 2000a,b,c). Also, in the present study, a marked elevation of NO synthesis was observed during hypoxia. Moreover, the oxygen and glucose deprivation-induced enhancement of NO synthesis was significantly reversed by a variety of Na^+ and Ca^{2+} channel blockers. Therefore, it is suggested that the cerebroprotective actions of Na^+ and Ca^{2+} channel blockers observed in the present study may be due at least in part to the inhibition of NO synthesis during hypoxia. It was noteworthy that the inhibitory effects of dibucaine, lidocaine and verapamil, but not those of tetrodotoxin, ω-conotoxin GVIA and ω-agatoxin IVA, were almost completely abolished by halothane. Consistently, halothane reversed the cation channel blocking actions of dibucaine, lidocaine and verapamil without affecting those of tetrodotoxin, ω-conotoxin GVIA and ω-agatoxin IVA, as determined by the blockade of veratridine- or KCl-stimulated NO synthesis.

It has been demonstrated that several local anesthetic agents including dibucaine and lidocaine block Na^+ channel in a voltage- and use-dependent manner (Catterall, 1987). On the other hand, tetrodotoxin, whose site of action is localized near the extracellular pore of the Na^+ channel moiety, blocks Na^+ channel in a voltage-independent fashion (Catterall, 1987). The site of action of verapamil in blocking voltage-dependently the Ca^{2+} channel has been shown to be located within the sixth transmembrane segment (S6) in domain IV of the Ca^{2+} channel moiety (Hering et al., 1996), which was quite similar to the site of action of the local anesthetics on Na^+ channel moiety. In contrast, the possible sites of actions of ω-agatoxin and ω-conotoxin are considered to exist on the extracellular region of S3–S4 linker of domain IV (Bourinet et al., 1999) and S5–S6 linker in domain III of the Ca^{2+} channel (Ellinor et al., 1994), respectively, and the Ca^{2+} channel block by these peptide blockers is not dependent on membrane potential. Therefore, it is notable that halothane attenuated the actions of only voltage-dependent Na^+ as well as Ca^{2+} channel blockers. It is suggested that halothane interferes with the

association or interaction of several voltage-dependent Na^+ and Ca^{2+} channel blockers with their transmembrane sites located on the S6 region in domain IV of the channel moiety.

The concentration of halothane used in the present study was 300 μM, which was chosen on the basis that the aqueous halothane concentration for surgical anesthesia is between 0.21 and 0.35 mM (Smith et al., 1981). It has been demonstrated that voltage-gated Na^+ and Ca^{2+} channels are possible candidates to be affected by halothane, although the precise mode of action is unclear. It has also recently been shown that halothane at submillimolar concentrations blocks Na^+ channels in several tissues (Eskinder et al., 1993; Study, 1994; McDowell et al., 1996; Pancrazio, 1996) and in cells transfected with rat brain Na^+ channel (Rehberg et al., 1996). In addition, halothane inhibits the veratridine-evoked $^{22}Na^+$ influx ($IC_{50}=1.1$ mM) into synaptosomes of rat cerebral cortex, and specifically antagonized [^{3}H]batrachotoxin binding to neurotoxin receptor site 2 of the Na^+ channel ($IC_{50}=0.53$ mM) without affecting [^{3}H]saxitoxin binding to neurotoxin binding site 1 of the Na^+ channel (Ratnakumari and Hemmings, 1998). Besides the action on Na^+ channel, electrophysiological studies have shown that halothane (0.59 mM) inhibits the channel function of P/Q-, L-, N- and R-types of Ca^{2+} channels expressed in *Xenopus* oocytes (Kamatchi et al., 1999). Moreover, halothane displaces the binding of dihydropyridine derivative [^{3}H]PN200-100 in membranes of rat ventricular myocytes (Kanaya et al., 1998). However, in our present study, the NO synthesis induced by 10 μM veratridine or 50 mM KCl was not affected by halothane, although the former reaction was moderately but not significantly reduced by halothane. We do not know the precise reason for such inconsistency. The differences in the concentration of halothane, indices of ion channel function or tissue preparations may result in such inconsistency between our data and those reported by others. However, halothane at a higher concentration (3 mM) indeed markedly reduced both the veratridine-stimulated and KCl-evoked NO synthesis in the present study (data not shown).

In conclusion, the oxygen and glucose deprivation-induced injury in rat cerebrocortical slices was inhibited by a variety of Na^+ and Ca^{2+} channel blockers. Halothane reversed the cerebroprotective actions of some of these ion channel blockers including local anesthetics and verapamil, all of which are known to block ion channels in a voltage-dependent manner. The inhibitory effects of such ion channel blocker as local anesthetics and verapamil on the enhancement of NO synthesis during hypoxic insult were also attenuated by halothane. Moreover, the Na^+ and Ca^{2+} channel blocking actions of these ion channel blockers, as determined by veratridine- and KCl-evoked NO synthesis, respectively, were reversed by halothane. These findings suggest that the cerebroprotective action of voltage-dependent Na^+ and Ca^{2+} channels blockers is attenuated by some anaesthetic agents such as halothane. Therefore, the influence of anesthesia should be considered in the evaluation of the neuroprotective action of such cation channel blockers using animal models of cerebral ischemia.

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