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Na^+ and high-voltage-activated Ca^{2+} channel blocking actions of NS-7, a novel neuroprotective agent, in NG108-15 cells

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Abstract

The actions of a novel neuroprotective compound, 4-(4-fluorophenyl)-2-methyl-6-(5-piperidinopentyloxy)pyrimidine hydrochloride (NS-7), on voltage-gated Na^+, Ca^{2+} and K^+ channels were investigated in a mouse neuroblastoma and rat glioma hybrid cell line, NG108-15, using a whole-cell voltage clamp technique. NG108-15 cells have a tetrodotoxin-sensitive Na^+ channel, three types of Ca^{2+} channel (L, N and T) and voltage-gated K^+ channels, all of which were inhibited by NS-7 in a concentration-dependent manner. However, there was a considerable difference in its potency: the IC_{50} values for the tetrodotoxin-sensitive Na^+ channel, L-type Ca^{2+} channel and N-type Ca^{2+} channel were similar (7.8, 4.5 and 7.3 μM, respectively), lower than the IC_{50} value for the T-type Ca^{2+} channel (17.1 μM), and much lower than the IC_{50} value for the voltage-gated K^+ channel (160.5 μM). NS-7 altered neither the shape nor the reversal potential of the current–voltage curves for Na^+, L-type or N-type Ca^{2+} channels, although the currents were reduced at every potential tested. These results indicate that NS-7 is a Na^+ and high-voltage-activated (L- and N-type) Ca^{2+} channel blocker, and its channel-blocking properties may contribute to its neuroprotective action. © 1997 Elsevier Science B.V.

Keywords: Neuroprotection; Ischemia; Inward current; Outward current; Ion channel; Patch clamp

1. Introduction

NS-7 [4-(4-fluorophenyl)-2-methyl-6-(5-piperidinopentyloxy)pyrimidine hydrochloride] is a novel neuroprotective compound (Fig. 1) that markedly reduces the infarct size in the cerebral cortex after middle cerebral artery occlusion in rats (Aoki et al., 1997; Takagaki et al., 1997). Although the precise mechanism underlying the neuroprotective action of NS-7 is unknown, several findings suggest that the drug may interact with both voltage-gated Na^+ channels and voltage-gated Ca^{2+} channels. First, NS-7 displaces [^{3}H]batrachotoxin binding to membrane preparations from rat brain (Shimidzu et al., 1997). Second, it inhibits veratridine- and K^+-stimulated nitric oxide synthase activity in rat primary cultured neurons (Tatsumi et al., 1997). Finally, it produces a concentration-dependent inhibition of ischemia-induced fodrin breakdown, and its inhibitory effect is thought to be due to the inhibition of the activation of the Ca^{2+}-stimulated neutral protease calpain (Takagaki et al., 1997).

Na^+ channel blockers, including tetrodotoxin (Lysko et al., 1994; Weber and Taylor, 1994), riluzole (Pratt et al., 1992) and lamotrigine (Rataud et al., 1994; Smith and Meldrum, 1995) provide neuroprotection against both focal and global cerebral ischemia. Furthermore, lifarizine, a Na^+ and Ca^{2+} channel blocker, has been shown to be highly effective in a wide range of animal models (Brown et al., 1993; Taylor and Meldrum, 1995). The aim of this study was to determine whether NS-7 affects voltage-gated Na^+, Ca^{2+} or K^+ channels in a mouse neuroblastoma × rat glioma hybrid cell line, NG108-15, which expresses a Na^+ channel, three types of Ca^{2+} channel and Ca^{2+}-activated K^+ channels (Brown and Higashida, 1988; Buisson et al., 1992; Kasai and Neher, 1992; Lamas et al., 1995). The effects of NS-7 on each ion channel were investigated

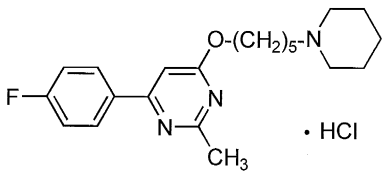

Fig. 1. Chemical structure of NS-7.

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using a whole-cell voltage-clamp technique after each channel was functionally isolated by its pharmacological and kinetic properties. A preliminary account of some of these data has been presented in abstract form (Suma et al., 1997).

2. Materials and methods

2.1. Cell culture

NG108-15 cells were grown in culture dishes with the culture medium in humidified 95% air + 5% CO_2 at 37°C in an incubator. The culture medium was composed of DMEM (Dulbecco's modified Eagle's medium), 10% fetal calf serum, hypoxanthine–aminopterin–thymidine supplement, 100 U/ml penicillin and 0.1 mg/ml streptomycin. Two days prior to differentiation, the cells were transferred to 35 mm dishes containing poly-L-lysine coated glass cover-slips (3 mm × 10 mm). Cells were allowed to differentiate for 3–10 days in a low-serum growth medium composed of DMEM, 2% fetal calf serum, hypoxanthine–aminopterin–thymidine, penicillin, streptomycin and 1 mM dibutyryl cyclic AMP.

2.2. Solutions and drugs

The normal external solution was a Na^+-rich solution (145 mM NaCl, 5.5 mM KCl, 1 mM $MgCl_2$, 10 mM HEPES and 5.5 mM D-glucose, adjusted to pH 7.2 with NaOH). This solution was used to record K^+ currents. For measuring Na^+ currents, experiments were performed in the Na^+-rich solution in which KCl was replaced with CsCl (Cs^+-containing solution) in addition to 0.5 mM $CdCl_2$ to block K^+ and Ca^{2+} channels. The external solution used to record Ca^{2+} currents was similar to the Cs^+-containing solution except it contained 10 mM $BaCl_2$ instead of $CdCl_2$ (Ba^{2+}-rich solution). The pipette (internal) solution used to record Na^+ and Ca^{2+} currents was 125 mM CsCl, 20 mM tetraethylammonium chloride, 1 mM $MgCl_2$, 5 mM Na_2ATP, 0.3 mM Na_3GTP, 5 mM EGTA, 10 mM HEPES and 5 mM 1,2-bis(*o*-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′,-tetraacetic acid (BAPTA), adjusted to pH 7.2 with CsOH. The pipette solution used to record K^+ currents was 140 mM KCl, 5 mM $MgCl_2$, 5 mM K_2ATP, 0.3 mM Na_3GTP, 5 mM EGTA and 5 mM HEPES, adjusted to pH 7.2 with KOH, and the concentration of free Ca^{2+} was buffered to 1 μM according to the calculation described by Oiki et al. (1994).

NS-7 was synthesized in our laboratory. It was dissolved in distilled, deionized water to give a 100 mM stock solution. Before experiments, the stock solution was diluted with external solution. Tetrodotoxin, Na_2ATP, K_2ATP and Na_3GTP were purchased from Sigma (St. Louis, MO, USA), CsCl, tetraethylammonium chloride, EGTA and HEPES were from Nacalai Tesque (Kyoto, Japan) and *ω*-conotoxin GVIA was from Peptide Institute (Osaka, Japan). All other chemicals were of reagent grade.

2.3. Electrophysiology

A glass cover-slip with cultured cells was transferred to a 0.5 ml chamber on the stage of an inverted microscope (Diaphot-TMD, Nikon, Tokyo, Japan). Transmembrane currents were recorded with a patch-clamp amplifier (Axopatch 200 or Axopatch 1D, Axon Instruments, Foster City, CA, USA) using the conventional whole-cell voltage-clamp technique (Hamill et al., 1981). Patch pipettes were fabricated from borosilicate glass capillaries (Hilgenberg, Malsfeld, Germany) with a micropipette puller (model P-87, Sutter Instruments, San Rafael, CA, USA), and they had a tip resistance of 3-4 MΩ when filled with the pipette solution. Series resistance and capacitance were maximally compensated before recording. The shanks of the pipettes were coated with Sylgard (184W/C, Dow Corning Japan, Yokohama, Japan) and the tips were fire-polished immediately before use.

For measuring Na^+ currents, cells were allowed to differentiate for 7–10 days and were then perfused with a Cs^+-containing external solution. During the experiments the cells were clamped at a holding potential of −80 mV, then 25 ms depolarizing test pulses were applied at a frequency of 0.2 Hz. High-voltage-activated Ca^{2+} currents were recorded in cells that had been differentiated for 3–6 days, and were elicited by 200 ms depolarizing pulses to 0 mV from a holding potential of −40 mV at 0.1 Hz. In NG108-15 cells, high-voltage-activated Ca^{2+} current consists of two components (L- and N-type Ca^{2+} currents), which can be separately measured by taking advantage of their different pharmacological properties: L-type Ca^{2+} currents can be measured after a short application of *ω*-conotoxin GVIA, and N-type Ca^{2+} currents can be measured in the presence of nisoldipine, a dihydropyridine compound (Kasai and Neher, 1992). Low-voltage-activated Ca^{2+} currents were recorded in non-differentiated cells, and were elicited by 50 ms depolarizing pulses to −20 mV from a holding potential of −80 mV at 0.1 Hz in the presence of tetrodotoxin to eliminate the Na^+ current (Buisson et al., 1992). For measuring K^+ currents, cells were allowed to differentiate for 7–10 days and were then perfused with a Na^+-rich external solution. During the experiments the cells were clamped at a holding potential of −50 mV, then 100 ms depolarizing test pulses to +40 mV were applied at a frequency of 0.1 Hz. Na^+, low-voltage-activated Ca^{2+} and K^+ currents were measured at 22–24°C and high-voltage-activated Ca^{2+} currents at 35–36°C.

2.4. Data acquisition and analysis

The whole cell current signal was filtered at 2 to 5 kHz through a Bessel-type filter (FV-664, NF Electronic Instruments, Yokohama, Japan), digitized at 5 to 20 kHz and stored on a IBM PC/AT compatible computer. Data acquisition and analysis were performed with pCLAMP software (Ver. 6.0.3; Axon Instruments). Leak and capacitance currents were subtracted using a P/4 procedure with hy-

perpolarizing pulses from the holding potential. The inhibition constant for NS-7 was determined by fitting the data to the Hill equation with the Marquardt–Levenberg algorithm: $I_{test}/I_{control}(\%) = 1/[1 + (IC_{50}/C)^{n_H}]$, where I_{test} and $I_{control}$ are the current amplitudes obtained during the test pulse in the presence and absence of the drugs, respectively, IC_{50} is the concentration which produces 50% inhibition, C the drug concentration and n_H the Hill coefficient.

3. Results

3.1. Effect of NS-7 on the tetrodotoxin-sensitive Na^+ current

In differentiated cells perfused with the Cs^+-containing external solution, a command pulse applied from a holding potential of −80 mV to a test pulse of 0 mV elicited the inward current, which was activated rapidly and then inactivated. The current was completely inhibited by 1 μM tetrodotoxin (data not shown), indicating that the inward current is almost exclusively tetrodotoxin-sensitive Na^+ current, as reported by Lamas et al. (1995). NS-7 (0.1–100 μM) inhibited the Na^+ current in a concentration-dependent manner. Fig. 2A shows representative current traces (left panel) and the time course (right panel) showing the inhibitory action of NS-7 (3, 10 and 100 μM) on the Na^+ current. Almost 80% of the Na^+ current was restored after washout of NS-7. Fig. 2B shows the concentration–response curve for the inhibitory effect of NS-7 on the Na^+ current, which yields an IC_{50} value of 7.8 μM ($n_H = 0.7$).

3.2. Effect of NS-7 on Ca^{2+} currents

In differentiated cells perfused with the Ba^{2+}-rich external solution, a command pulse applied from a holding potential of −40 mV to a test pulse of 0 mV elicited the non-inactivating inward current. Although the current was sensitive to both an N-type Ca^{2+} channel blocker ω-con-

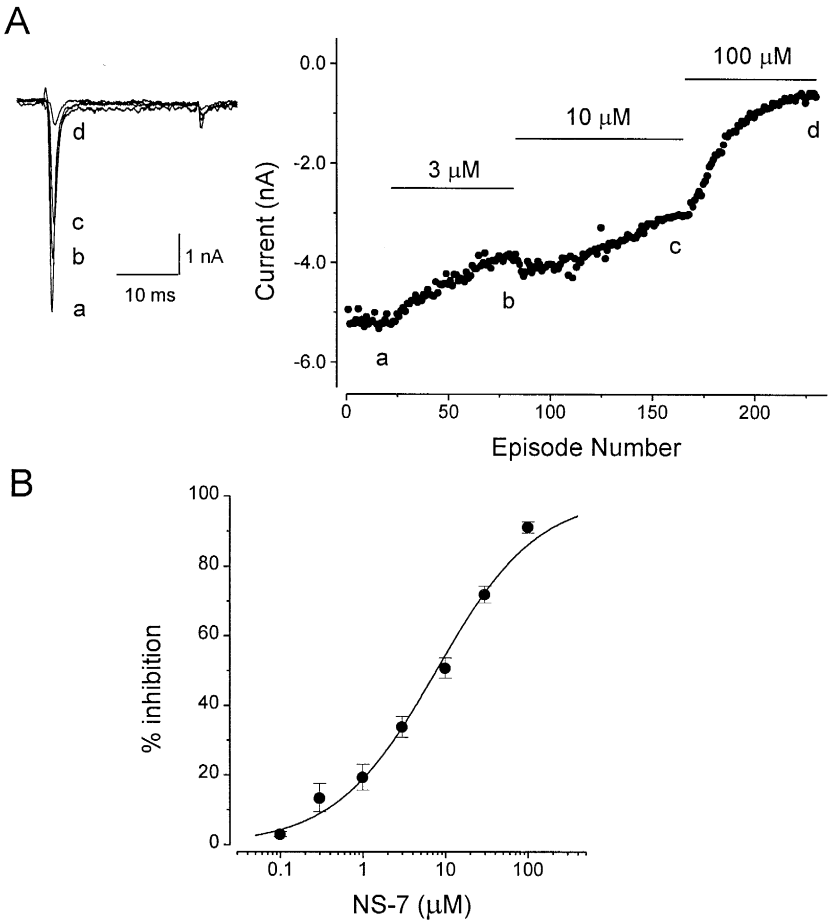

Fig. 2. Effect of NS-7 on tetrodotoxin-sensitive Na^+ currents in differentiated NG108-15 cells. (A) Superimposed representative current traces of Na^+ currents in the absence (a) and presence of 3 μM (b), 10 μM (c) and 100 μM (d) NS-7 (left) and the time course of the inhibitory action of NS-7 on Na^+ currents (right). The letters (a–d) on the time courses correspond to the letters (a–d) on the current traces on the left. The horizontal bars indicate the period for which 3, 10 and 100 μM NS-7 was applied. (B) Concentration–response curve for NS-7 on Na^+ current inhibition. Each point and vertical bar represent the mean ± S.E.M. of the percentage inhibition corresponding to 4 to 18 observations. The curves were obtained by fitting the data to the Hill equation.

otoxin GVIA (5 μM) and an L-type Ca^{2+} channel blocker nisoldipine (1 μM), each blocker alone only partially inhibited the current; but the combined application of the blockers almost completely inhibited the current (data not shown), indicating that the high-voltage-activated Ca^{2+} current consisted of L- and N-type Ca^{2+} currents, as previously reported (Kasai and Neher, 1992). In contrast, in non-differentiated cells perfused with the Ba^{2+}-rich external solution containing 1 μM tetrodotoxin to exclude the contamination of voltage-gated Na^{+} channel, the command pulse applied from a holding potential of −80 mV to a test pulse of −20 mV elicited the rapidly inactivating inward current, which was markedly inhibited by 100 μM Ni^{2+} (data not shown), indicating that the inward current represents the T-type Ca^{2+} current, as previously reported (Buisson et al., 1992).

NS-7 inhibited all components of the Ca^{2+} currents in a concentration-dependent manner (at concentrations ranging from 0.1 to 100 μM for high-voltage-activated Ca^{2+} currents and from 1 to 100 μM for low-voltage-activated Ca^{2+} currents). Fig. 3A shows representative current traces (left panels) and time courses (right panels) of the in-

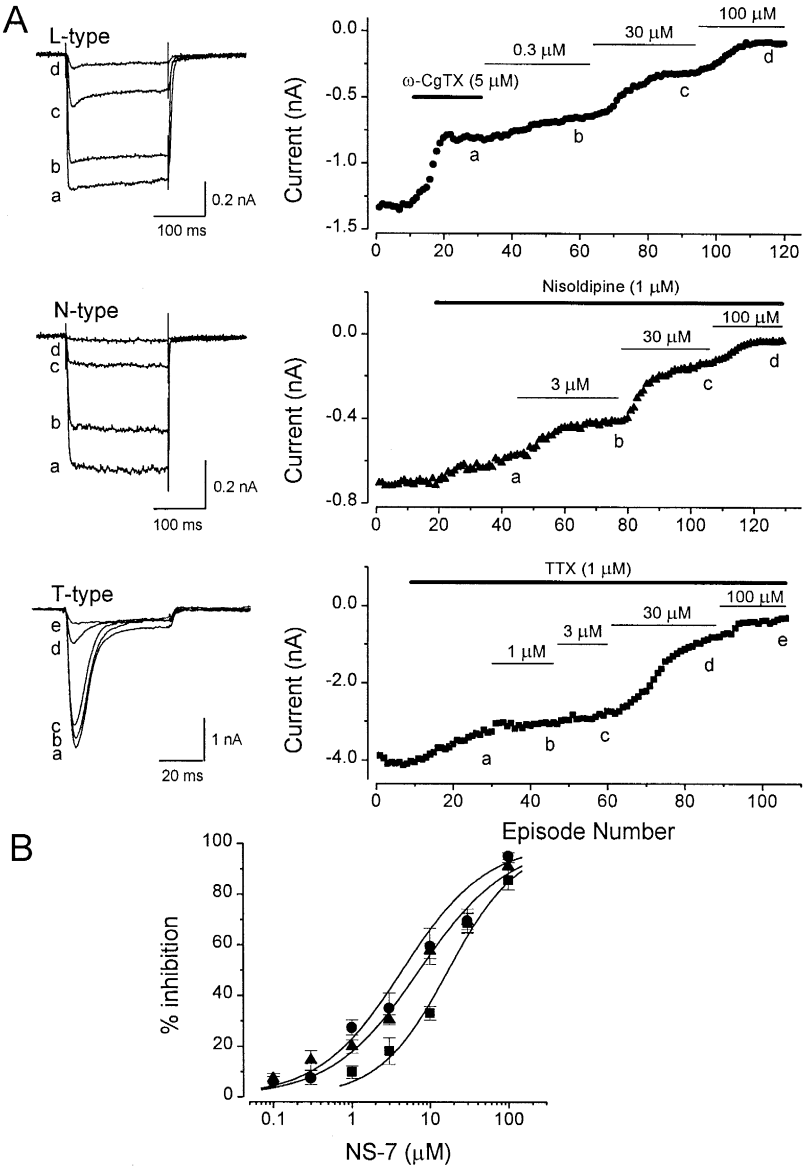

Fig. 3. Effect of NS-7 on L-, N- and T-type Ca^{2+} currents in differentiated or non-differentiated NG108-15 cells. (A) Superimposed representative current traces of L-(upper), N-(middle) and T-(lower) type Ca^{2+} currents in the absence and presence of NS-7 (left) and time courses of the inhibitory action of NS-7 on L-, N- and T-type Ca^{2+} currents (right). The letters on the time courses correspond to the letters on the current traces on the left. The thick bars indicate the period for which each channel blocker, ω-conotoxin GVIA (upper), nisoldipine (middle) or tetrodotoxin (lower), was applied and the thin bars indicate the period for which each concentration of NS-7 was applied. (B) Concentration–response curves for NS-7 on Ca^{2+} current inhibition. Each point and vertical bar represent the mean ± S.E.M. of the percentage inhibition corresponding to 4 to 8 observations. The curves were obtained by fitting the data to the Hill equation.

hibitory actions of NS-7 on L-, N- and T-type Ca^{2+} currents, respectively. The T-type Ca^{2+} current was almost completely restored after washout of NS-7, whereas L- and N-type Ca^{2+} currents were only partially. In Fig. 3B, relative Ca^{2+} currents are plotted as a function of NS-7 concentration, and the concentration–response relationships for NS-7 were best fitted by the Hill equation. The calculated IC_{50} values were 4.5 μM ($n_H = 0.8$) for L-type, 7.3 μM ($n_H = 0.8$) for N-type and 17.1 μM ($n_H = 1.0$) for T-type Ca^{2+} currents.

3.3. Effect of NS-7 on the K^+ current

In differentiated cells perfused with the Na^+-rich external solution, a command pulse applied from a holding potential of −50 mV to a test pulse of +40 mV elicited the non-inactivated outward current. The current was almost inhibited by 1 mM tetraethylammonium chloride, and most of the current inhibited by tetraethylammonium chloride was sensitive to external Ca^{2+} and was completely inhibited by 0.5 mM Cd^{2+} (data not shown), indicating that the current is dominated by Ca^{2+}-activated K^+ current, as previously reported (Brown and Higashida, 1988). To avoid the influence of Ca^{2+}-channel-blocking activity of NS-7 on the K^+ current in NG108-15 cells, the K^+ current was elicited under conditions in which the extracellular Ca^{2+} concentration was nominally free and the intracellular Ca^{2+} concentration was buffered at 1 μM (see Section 2). The K^+ current elicited under these conditions was similar to the current recorded under normal conditions without apparent rundown; however, the current was completely inhibited by tetraethylammonium chloride, although not by Cd^{2+} (data not shown), indicating that an accurate evaluation of the inhibitory effects of NS-7 on the K^+ current should be possible.

NS-7 (3–1000 μM) inhibited the K^+ current in a fashion similar to, but less potent than, that obtained for the Na^+ current and the Ca^{2+} currents. Fig. 4A shows representative current traces (left panel) and the time course (right panel) showing the inhibitory action of NS-7 (3, 30 and 100 μM) on the K^+ current. Fig. 4B shows the concentration–response curve for the inhibitory effect of

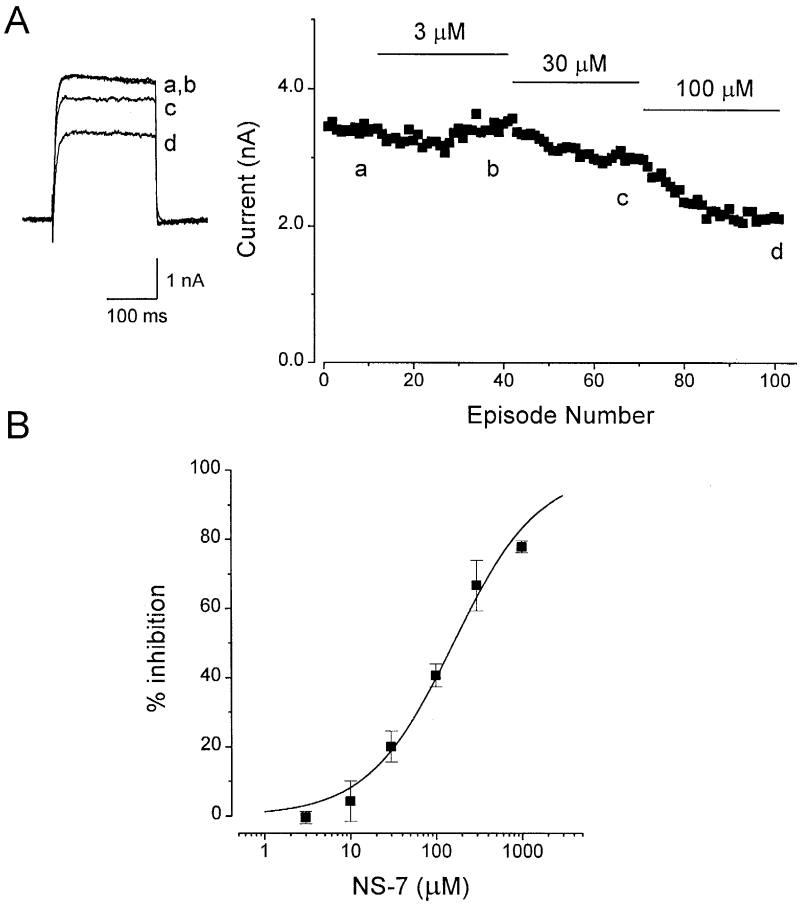

Fig. 4. Effect of NS-7 on Ca^{2+}-activated K^+ currents in differentiated NG108-15 cells. (A) Superimposed representative current traces of K^+ currents in the absence (a) and presence of 3 μM (b), 30 μM (c) and 100 μM (d) NS-7 (left) and the time course of the inhibitory action of NS-7 on K^+ currents (right). The letters (a–d) on the time courses correspond to the letters (a–d) on the current traces on the left. The horizontal bars indicate the period for which 3, 30 and 100 μM NS-7 was applied. (B) Concentration–response curve for NS-7 on K^+ current inhibition. Each point and vertical bar represent the mean ± S.E.M. of the percentage inhibition corresponding to 5 to 8 observations. The curves were obtained by fitting the data to the Hill equation.

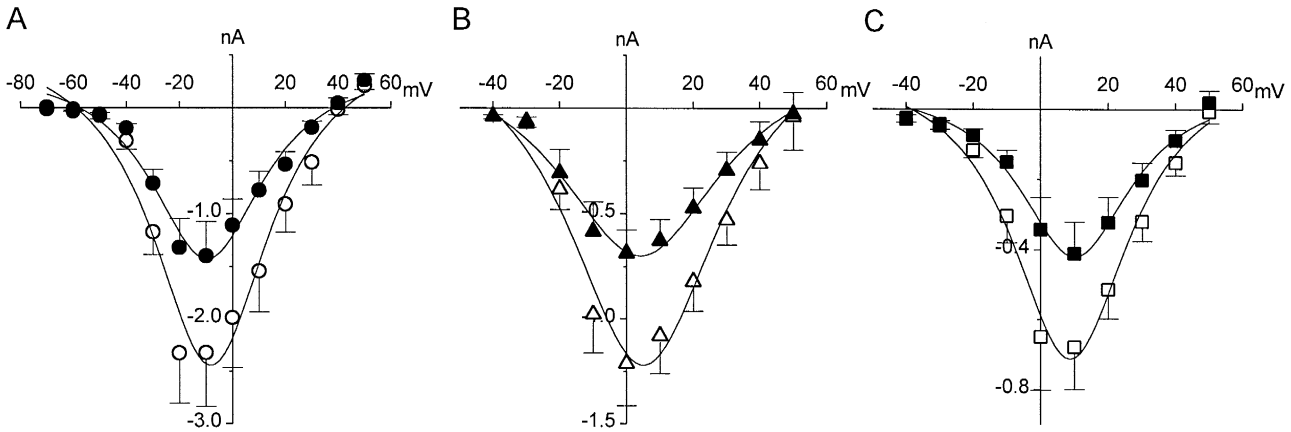

Fig. 5. Effects of NS-7 on the current–voltage relationships for Na^+ and high-voltage-activated Ca^{2+} currents in differentiated NG108-15 cells. (A) Current–voltage relationship for Na^+ currents in the absence (open symbols) or presence (closed symbols) of 10 μM NS-7 ($n = 7$). The cells were depolarized for 25 ms from a holding potential of −80 mV with a 10 mV increment to a final value of +50 mV. (B) Current–voltage relationship for L-type Ca^{2+} currents in the absence (open symbols) or presence (closed symbols) of 5 μM NS-7 after their pharmacological isolation ($n = 6$). The cells were depolarized for 200 ms from a holding potential of −40 mV with a 10 mV increment to a final value of +50 mV. (C) Current–voltage relationship for N-type Ca^{2+} currents in the absence (open symbols) or presence (closed symbols) of 10 μM NS-7 after pharmacological isolation ($n = 6$). The stimulatory pulse protocol was the same as in (B).

NS-7 on the K^+ current, with an IC_{50} value of 160.5 μM ($n_H = 0.9$).

3.4. Effect of NS-7 on the current–voltage relationships for Na^+ and high-voltage-activated Ca^{2+} currents

Current–voltage curves for the Na^+ and high-voltage-activated Ca^{2+} currents were recorded before and about 5 min after the application of NS-7, at concentrations close to the respective IC_{50} values (Fig. 5). The drug caused a marked reduction in the amplitude of the Na^+ current at every potential tested (Fig. 5A). NS-7 altered neither the shape nor the reversal potential of the current–voltage curves. The amplitude of the high-voltage-activated Ca^{2+} currents was also reduced by NS-7 at every potential tested, and no apparent change in shape or reversal potential was observed in the current–voltage curves (Fig. 5B–C).

4. Discussion

The present study demonstrated that a novel neuroprotective phenylpyrimidine derivative, NS-7, inhibited the tetrodotoxin-sensitive Na^+ current, the L-type Ca^{2+} current and the N-type Ca^{2+} current in a concentration-dependent manner with almost the same potency in differentiated NG108-15 cells, but had no effect on the shape or reversal potential of any of the current–voltage curves. These inhibitory effects of NS-7 seems to be fairly specific, since NS-7 had a weaker inhibitory action on the T-type Ca^{2+} current in non-differentiated cells, and was far less effective in inhibiting the voltage-gated K^+ current in differentiated cells.

An excessive rise in intracellular Ca^{2+} concentration plays an important role in neurodegeneration under ischemic or hypoxic conditions (Choi, 1988). The major pathways of Ca^{2+} influx are thought to be (1) reversal of the Na^+–Ca^{2+} exchanger caused by the degradation of Na^+ homeostasis resulting from the dysfunction of the Na^+/K^+ ATPase due to the energy depletion that accompanies ischemia (Stys et al., 1993; Taylor and Meldrum, 1995), (2) the postsynaptic *N*-methyl-D-aspartate receptor activated by the ischemic glutamate release mediated by the Na^+-dependent glutamate transporter operating in reverse (Choi, 1988; Taylor and Meldrum, 1995), or (3) direct activation of voltage-gated Ca^{2+} channels induced by membrane depolarization resulting from excessive intracellular Na^+ loading (Choi, 1988; Taylor and Meldrum, 1995). Thus, the inhibition of voltage-gated Ca^{2+} channels, or of voltage-gated Na^+ channels that secondarily cause Ca^{2+} channel activation, may be effective in preventing neurodegeneration under ischemic or hypoxic conditions. Indeed, there have been several reports indicating that the N-type Ca^{2+} channel blocker SNX-111 (Valentino et al., 1993), the T-type Ca^{2+} channel blocker U-92032 (Ito et al., 1994) and Na^+ channel blockers, including tetrodotoxin (Lysko et al., 1994; Weber and Taylor, 1994) and riluzole (Pratt et al., 1992), are all neuroprotective both in vitro and in vivo. Furthermore, drugs that have inhibitory actions on both Na^+ and Ca^{2+} channels, such as phenytoin and lifarizine, have more marked neuroprotective activity (Brown et al., 1993; Rataud et al., 1994; Weber and Taylor, 1994); and lamotrigine, which is one of the most effective Na^+ channel blockers for preventing neurodegeneration (Rataud et al., 1994; Smith and Meldrum, 1995), also has high-voltage-activated Ca^{2+}-channel-blocking activity (Stefani et al., 1996; Wang et al., 1996).

NS-7 is demonstrated to produce a potent neuroprotec-

tive action in the cerebral cortex after middle cerebral artery occlusion in rats (Aoki et al., 1997; Takagaki et al., 1997). Although the cellular mechanism of the neuroprotection produced by NS-7 has not been fully elucidated, NS-7 has been found to displace the Na^+ channel ligand, [^{3}H]batrachotoxin, in rat brain membranes without the inhibition of [^{3}H]saxitoxin binding and inhibit the glutamate release evoked by veratridine in rat cortical slices, suggesting that NS-7 has a modulatory activity on the brain Na^+ channel (Shimidzu et al., 1997). Supporting this suggestion, in the present study we demonstrated that NS-7 inhibited the TTX-sensitive Na^+ channel in NG108-15 cells. In addition, NS-7 also inhibited high-voltage-activated (L- and N-type) Ca^{2+} channels more selective than T-type Ca^{2+} channel in neuroglioma cells. The IC_{50} values for the Na^+ channel and high-voltage-activated Ca^{2+} channels were similar to the IC_{50} values of 9.3–9.6 μM previously reported by Tatsumi et al. (1997) for the inhibition by NS-7 of nitric oxide synthase activity stimulated by veratridine or KCl. Our measurements supports their suggestion that the effect of NS-7 on nitric oxide synthase is indirect, and operates through a blockade of voltage-gated Na^+ and Ca^{2+} channels. NS-7 significantly inhibits the ischemia-induced breakdown of the cytoskeletal protein fodrin breakdown in rat cerebral cortex slices at the similar concentration range (10–30 μM), and its inhibitory effect is thought to be due to the suppression of the activation of the Ca^{2+} stimulated neutral protease calpain (Takagaki et al., 1997). These findings suggest that NS-7 simultaneously blocks both the Na^+ channel and the high-voltage-activated Ca^{2+} channels in the same concentration range, and these dual channel blocking properties may contribute to the neuroprotective action of NS-7, which is similar to that of lifarizine (Spedding and Lepagnol, 1995; Taylor and Meldrum, 1995) and lamotrigine (Stefani et al., 1996; Wang et al., 1996).

NS-7 inhibited the K^+ current in NG108-15 cells; however, its IC_{50} value was about 20–30 times higher than that for the Na^+ channel and the high-voltage-activated Ca^{2+} channels. In neuronal cells, the roles of K^+ channels are related to so-called membrane stabilization because K^+ channels set the resting potentials, keep action potentials short, terminate periods of intense activity and regulate the interspike intervals during repetitive firing. Under ischemic or hypoxic conditions, the K^+ channel are also expected to play an important role in stabilizing the membrane by hyperpolarizing the membrane potential and decreasing stimulus-coupled transmitter release from presynaptic terminals. Therefore, since NS-7 inhibited the Na^+ channel and high-voltage-activated Ca^{2+} channels without the interference of K^+ currents at micromolar concentrations, it would be expected to produce superior neuroprotective action.

In the present study, NS-7 was found to inhibit L-, N- and T-type Ca^{2+} channels. However, other types of Ca^{2+} channel, such as P-/Q- and R-type Ca^{2+} channels, are also present in neurons (Spedding and Lepagnol, 1995). Among these five types of Ca^{2+} channel, N- and P-/Q-type Ca^{2+} channels contribute to synaptic transmission (Takahashi and Momiyama, 1993); we have demonstrated the inhibitory effect of NS-7 on N-type Ca^{2+} currents, but further studies are needed to determine whether NS-7 can inhibit P-/Q-type Ca^{2+} currents. Furthermore, the Na^+ channel blocking properties of NS-7, such as use- and voltage-dependency, which are associated with its influence upon ongoing neuronal activity and its selectivity for ischemic tissue, are still unclear.

In conclusion, NS-7 potently inhibited not only the tetrodotoxin-sensitive Na^+ channel but also the high-voltage-activated (L- and N-type) Ca^{2+} channels without causing any change in the shape or reversal potential of their current–voltage relationships, which may account, at least in part, for its potent neuroprotective effects.

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