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Effect of nicardipine on abnormal excitability of CA3 pyramidal cells in hippocampal slices of spontaneously epileptic rats

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Abstract

The effects of nicardipine, a Ca^{2+} channel antagonist, on the abnormal excitability of hippocampal CA3 neurons in spontaneously epileptic rats (SER), a double mutant (*zi/zi*, *tm/tm*), were examined to elucidate whether or not the abnormality was due to that of Ca^{2+} channels. An intracellular recording study was performed using brain slice preparations of SER 12–15 weeks of age, when SER showed both tonic convulsions and absence-like seizures. Bath application of nicardipine (10 nM) completely inhibited the depolarizing shifts lasting for 60–120 ms and accompanying repetitive firings on mossy fiber stimulation in SER. However, this drug did not affect the single action potential induced by the mossy fiber stimulation in CA3 neurons of SER and normal Wistar rats. In the CA3 pyramidal neurons of SER, the Ca^{2+} spikes induced by the depolarizing pulse applied in the cell in the presence of tetrodotoxin and tetraethylammonium had a different configuration from that in normal Wistar rats. Nicardipine also inhibited the Ca^{2+} spikes in SER CA3 neurons at a concentration (1 nM) that had no effect on those in normal Wistar rats, while the Ca^{2+} spikes in Wistar rat CA3 neurons were inhibited by 10 nM nicardipine. These findings suggest that the abnormal excitability of CA3 pyramidal neurons in SER might be attributed to abnormalities of the Ca^{2+} channels, and that the Ca^{2+} channel antagonist may be effective as an antiepileptic drug.

Keywords: Spontaneously epileptic rat (SER); Hippocampus; CA3 neuron; Ca^{2+} spike; Nicardipine

1. Introduction

Spontaneously epileptic rats (SER), a double mutant (*zi/zi*, *tm/tm*) obtained by mating heterozygotes of tremor rats (*tm/+*) and homozygotes of zitter rats (*zi/zi*), show both tonic convulsions and absence-like seizures characterized by a sudden appearance of 5–7 Hz spike-wave-like complexes in the cortical and hippocampal EEG (Sasa et al., 1988; Serikawa and Yamada, 1986; Yamada et al., 1985). Our previous studies have suggested that SER are useful as a model animal for evaluation of acute and chronic effects of antiepileptic drugs (Sasa et al., 1988; Serikawa et al., 1990), since the profile of antiepileptics in this animal resembled that in human epilepsy.

We have recently reported that hippocampal CA3 pyramidal cells in SER show a long-lasting depolarization shift accompanied by repetitive firing following a single stimulation of mossy fibers (Ishihara et al., 1993). Neural hyperexcitability has been also reported in hippocampal pyramidal cells in tottering mice (Kostopoulos and Psarropoulou, 1990) and kindled rats (Bragdon et al., 1988; King et al., 1985; Stringer and Lothman, 1988). However, the mechanism underlying the hyperexcitability in such epilepsy models remains largely unknown. Recently, an enhancement of Ca^{2+} current in the hippocampal neurons was observed in the kindling rats (Vreugdenhil and Wadman, 1994). Therefore, to determine whether or not such an abnormal

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excitability of SER CA3 neurons is due to that of the Ca^{2+} channels, we examined the effect of nicardipine, a Ca^{2+} channel antagonist, on the abnormal excitability and Ca^{2+} spikes of hippocampal CA3 pyramidal cells in SER, using brain slice preparations.

2. Materials and methods

2.1. Slice preparation

Adult SER (12–15 weeks of age), which were confirmed to have the seizures, and age-matched normal Wistar rats, one of the parent strains, were used. After decapitation, the brain was quickly removed, and the cooled block containing the hippocampus was cut into slices 400–500 μm thick using a microslicer (DTK-1000, Dosaka EM, Japan).

2.2. Intracellular recording

After a 1–2 h incubation at room temperature, one slice was transferred into the recording chamber. Artificial cerebrospinal fluid (ACSF) was continuously perfused over the slice at approximately 2.0 ml/min at 30–33° C. ACSF was composed of (in mM): NaCl 116.4, KCl 3.0, $MgSO_4$ 1.3, NaH_2PO_4 0.92, $CaCl_2$ 2.5, $NaHCO_3$ 26.2 and glucose 11.0. In order to evoke Ca^{2+} spikes, 1 μM tetrodotoxin and 10 mM tetraethylammonium were added to ACSF, and NaCl was reduced by 10 mM. The fluid was continuously bubbled with a mixture of 95% O_2 and 5% CO_2. A bipolar stimulating electrode was placed in the granule cell layer of the dentate gyrus and a single stimulus of 0.1 ms duration was given to the mossy fibers every 5 s. Intracellular recording was made from the CA3 region of the hippocampus using a glass microelectrode containing 3 M KCl. Under blockade of Na^+ and K^+ channels by tetrodotoxin and tetraethylammonium, respectively, a Ca^{2+} spike was elicited by applying a depolarizing pulse in the cell through the recording electrode. Responses were amplified with a microelectrode amplifier (MEZ-8201, Nihon Kohden, Japan) and displayed on a digital oscilloscope (VC-11, Nihon Kohden, Japan). They were then recorded with a thermal array recorder (RTA-1000, Nihon Kohden, Japan) and simultaneously stored with a data recorder (A-65, SONY, Japan) for further analysis.

2.3. Statistical analysis

Only data from neurons with a resting membrane potential over −45 mV were analyzed. Unless otherwise stated, each value indicates the mean ± S.E.M. The statistical significance of differences of values between SER and Wistar rats was determined with Student's *t*-test.

3. Results

3.1. Effect of nicardipine on intracellularly recorded action potentials and depolarization shift on mossy fiber stimulation

Intracellular recordings were made in 30 hippocampal neurons of 17 mature SER and in 7 neurons of 5 normal Wistar rats. As previously reported (Ishihara et al., 1993), hippocampal CA3 neurons of mature SER were classified into three groups according to the duration of the depolarization shift elicited by a single stimulation of mossy fibers. Fig. 1A (left trace) shows an example of the depolarization shift. The duration of the depolarization shift was 93.1 ± 2.9 and 43.9 ± 2.8 ms for the neurons in group 1 and 2, respectively, and group 3 neurons did not show such a depolarization shift. In the present study, 15, 8 and 7 neurons recorded belonged to groups 1, 2 and 3, respectively. The electrophysiological properties of the neurons in each group were similar to those reported previously (Ishihara et al., 1993); the input impedance of CA3 neurons in group 1 (24.9 ± 1.3 MΩ) and 2 (25.4 ± 1.9 MΩ) was significantly ($P < 0.01$) lower than that of the neurons in group 3 (37.9 ± 2.1 MΩ) and normal Wistar rats (40.6 ± 2.4 MΩ), although there was no significant difference in the resting membrane potential of neurons among groups 1, 2, 3 and the Wistar rats.

The effects of nicardipine on the intracellularly recorded action potentials and/or depolarization shift elicited by the stimulation of mossy fibers were examined in six, seven, four and three SER CA3 neurons in groups 1, 2, and 3 and in the normal Wistar rats, respectively. Nicardipine applied in the bath at a concentration of 1 nM had no effect on the depolarization

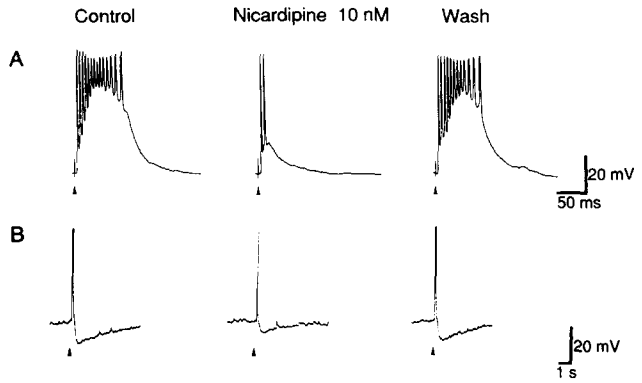

Fig. 1. Effect of nicardipine at a concentration of 10 nM on intracellularly recorded action potentials (A) and after-hyperpolarization (B) elicited by stimulation of mossy fibers in a group 1 hippocampal CA3 neuron of the mature SER. Solid triangles indicate the stimuli applied to the mossy fibers. Voltage of the stimulus to the mossy fiber was 5 V. Calibration, 20 mV and 50 ms for A, and 20 mV and 1 s for B.

Table 1
Effects of nicardipine (10 nM) on after-hyperpolarization following the depolarization shift induced by mossy fiber stimulation

	No. of neurons	After-hyperpolarization (mV)	
		Before	Nicardipine (10 nM)
Group 1	6	12.0±2.0	3.3±1.0 [a]
Group 2	7	5.8±0.8	1.8±0.3 [a]

[a] $P < 0.01$, as compared with the value before drug application.

shift and/or action potentials in any of the neurons tested. Increase of the concentration of nicardipine to 10 nM resulted in a complete blockade of the depolarization shift and repetitive firings in all six group 1 neurons tested (Fig. 1A). The long-lasting hyperpolarization following the depolarization shift was also reduced by 10 nM nicardipine in group 1 and 2 neurons (Fig. 1B and Table 1). The effects of nicardipine were observed within 2 min after the start of application and eliminated by washing with the physiological solution for a few minutes. However, nicardipine at this concentration did not affect the first one or two action potentials of the group 1 neurons. Similar inhibitory effects with 10 nM nicardipine were seen in all seven neurons tested in group 2. In contrast, nicardipine at a concentration of 10 nM did not affect the action potential generation in any of four or three neurons examined in group 3 or normal Wistar rats (Fig. 2).

3.2. Effect of nicardipine on Ca^{2+} spikes

Tetrodotoxin and tetraethylammonium at a concentration of 1 μM and 10 mM, respectively, were added to the bath to block the Na^+ and K^+ channels. Under these conditions, the 0.5 nA depolarizing pulse applied for 120 ms in the cell through the recording electrode produced an action potential probably carried by Ca^{2+} (Fig. 3). The Ca^{2+} spike of the group 1 hippocampal CA3 neurons obtained from SER showed a longer duration than that of the group 3 neurons in the SER and hippocampal CA3 neurons in normal Wistar rats (Fig. 3 and Table 2).

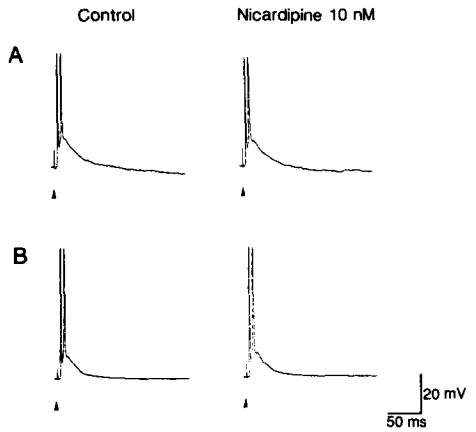

Fig. 2. Absence of effect of nicardipine on intracellularly recorded action potentials elicited by stimulation of mossy fibers in a group 3 hippocampal CA3 neuron of the mature SER (A) and in a hippocampal CA3 neuron of a normal Wistar rat. Solid triangles indicate the stimuli applied to the mossy fibers. Voltage of the stimulus to the mossy fiber was 7 V. Calibration, 20 mV and 50 ms.

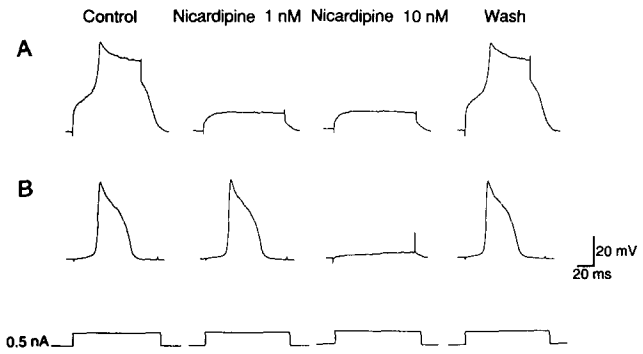

Fig. 3. Effects of nicardipine at concentrations of 1 and 10 nM on Ca^{2+} spikes induced by the intracellularly applied depolarizing pulse (0.5 nA, 120 ms) in the presence of tetrodotoxin and tetraethylammonium in a group 1 hippocampal CA3 neuron of SER (A) and in a hippocampal CA3 neuron of a normal Wistar rat (B). Calibration, 20 mV and 20 ms.

The effects of nicardipine on the Ca^{2+} spike of the hippocampal CA3 neurons elicited by an intracellularly applied depolarizing pulse were examined in four group 1 neurons and three group 3 neurons in SER, and three neurons in normal Wistar rats. The Ca^{2+} spikes in all four group 1 neurons tested were completely inhibited by nicardipine at a concentration of 1 nM (Fig. 3A). However, 1 nM nicardipine had no effect on the Ca^{2+} spike in any neurons in group 3 of SER or those of Wistar rats tested. An increase in the concentration of nicardipine to 10 nM resulted in complete blockade of the Ca^{2+} spike completely in all neurons tested in group 3 and normal Wistar rats (Fig. 3B and Table 2).

Table 2
Effects of nicardipine on Ca^{2+} spikes induced by depolarizing current (0.5 nA, 120 ms) applied to the cell in the presence of tetrodotoxin and tetraethylammonium

		No. of neurons	Duration of Ca^{2+} spike (ms)		
			Before	Nicardipine	
				1 nM	10 nM
SER	Group 1	4	97.8± 3.4 [a]	0 [b]	0
	Group 3	3	55.3±12.0	52.2±9.0	0
Normal Wistar rat		3	58.7± 5.3	62.7±3.5	0

[a] $P < 0.01$, as compared with the value in group 3 of SER and normal Wistar rat.
[b] Ca^{2+} spikes were completely blocked by nicardipine at the concentration shown.

4. Discussion

The present study confirmed our previous finding that most of the CA3 pyramidal neurons in the hippocampal slices prepared from mature SER, which had shown convulsions and absence-like seizures, had abnormal excitability; that is, a long-lasting depolarization shift accompanying the repetitive action potentials upon single stimulation of mossy fibers. The electrophysiological properties of the CA3 pyramidal neurons in SER demonstrated here were similar to those reported previously (Ishihara et al., 1993).

The present study has demonstrated that 10 nM nicardipine, a Ca^{2+} channel antagonist, inhibited the long-lasting depolarizing shifts as well as accompanying repetitive firings on mossy fiber stimulation but did not affect the first one or two action potentials which fired on the normal Na^+ carried excitatory postsynaptic potential. Thus, nicardipine is suggested to have no effects on normal synaptic transmission and Na^+ channels of CA3 neurons of the SER. The concentration needed to block the abnormal excitability of SER CA3 neurons was almost equivalent to that needed to inhibit the contraction of blood vessels (Toda et al., 1991; Yamamoto et al., 1983). These findings suggest that the depolarization shift induced by mossy fiber stimulation resulted from the excessive Ca^{2+} influx due to abnormal function of Ca^{2+} channels. Blockade by Ca^{2+} channel antagonists of the abnormal excitability induced in neurons by bicuculline has been reported for the hippocampal slice preparation (Aicardi and Schwartzkroin, 1990; Straub et al., 1990). Hyperpolarizaton following the depolarization shift in SER CA3 neurons was also suppressed by nicardipine, suggesting that the hyperpolarization was due to activation of Ca^{2+}-activated K^+ channels resulting from the excessive Ca^{2+} influx.

The Ca^{2+} spike now recorded in the CA3 pyramidal neurons of SER had a longer duration than those recorded from normal Wistar rats. This difference is considered to have resulted from a difference in the Ca^{2+} channels between SER and normal Wistar rats. The Ca^{2+} channels in CA3 neurons of SER may have a longer open time or less desensitization than those in normal Wistar rats. This finding is in agreement with those reported by others, suggesting that Ca^{2+} currents are responsible for epileptiform activity in the central nervous system (Brown and Griffith, 1983; Heinemann and Hamon, 1986; Vreugdenhil and Wadman, 1994). Therefore, our findings suggest that abnormal excitability of CA3 pyramidal neurons in SER may be attributed mainly to abnormalities of Ca^{2+} channels. Although details of the Ca^{2+} channel subtype responsible for the abnormal excitability of SER still remain unknown after the present study, nicardipine had potential anti-excitatory properties, and the Ca^{2+} spike in the CA3 neurons was 10 times more sensitive to nicardipine in the SER than in normal Wistar rats. In behavioral studies, a new dihydrothienopyridine derivative Ca^{2+} channel antagonist, S-312-d (methyl-4,7-dihydro-3-isobutyl-6-methyl-4-(3-nitrophenyl)-thieno-[2,3-*b*]pyridine-5-carboxylate), which can easily pass the blood-brain barrier to the brain, was actually effective against the seizure in SER (Amano et al., 1993; Sasa et al., 1994). This suggests that Ca^{2+} channel antagonists, which will be able to pass the blood-brain barrier, will be effective as antiepileptic drugs at a dose that does not affect normal Ca^{2+} channels.

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