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Anti- and proconvulsive actions of levcromakalim, an opener of ATP-sensitive K^+ channel, in the model of hippocampus-generating partial seizures in rats

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Abstract

We assessed the effect of an opener of ATP-sensitive K^+ channel, levcromakalim (BRL 38227, (−)6-cyano-3,4-dihydro-2,2-dimethyl-*trans*-4-(2-oxo-1-pyrrolidyl)-2*H*-1-benzopyran-3-ol) on seizure threshold and severity of the hippocampus-generating partial seizures in rats. For comparison, an ATP-sensitive K^+ channel blocker, glibenclamide; K^+ channel blocker, tetraethylammonium; Ca^{2+} channel antagonist, nimodipine and Ca^{2+} channel agonist, (±)-BAY K 8644 (1,4-dihydro-2,6-dimethyl-5-nitro-4-[2-(trifluoromethyl)-phenyl]-3-pyridinecarboxylic acid methyl ester) were also examined. Seizure threshold was determined using pulse number threshold and seizure severity was ascertained using afterdischarge duration. Levcromakalim decreased afterdischarge duration at 10 nmol i.c.v. and decreased pulse number threshold at 100 nmol i.c.v. Tetraethylammonium at 10 nmol i.c.v. increased afterdischarge duration selectively and at 100 nmol i.c.v. induced spontaneous seizures. Glibenclamide (1–100 nmol i.c.v.) failed to change pulse number threshold and afterdischarge duration. Nimodipine (40 mg/kg i.p.) decreased afterdischarge duration and pulse number threshold. BAY K 8644 (1 mg/kg i.p.) decreased pulse number threshold and increased afterdischarge duration. In addition, voltage-clamp recording from neuroblastoma × glioma hybrid cells indicates that levcromakalim inhibited the fast component of Ca^{2+}-dependent K^+ currents, in addition to the inhibition of T- and L-types of voltage-dependent Ca^{2+} currents reported (Ito et al., FEBS Lett. 262, 313, 1990). These results suggest that levcromakalim shows anti- and proconvulsive actions in the hippocampus-generating partial seizures in rats and these effects might be, at least partly, caused by inhibiting Ca^{2+} channel and Ca^{2+}-dependent K^+ channel, respectively.

Keywords: K^+ channel; Seizure; Ca^{2+} channel; Hippocampus; Levcromakalim; Patch clamp

1. Introduction

Neuronal excitability is controlled or modulated by increase of Na^+, K^+ and Ca^{2+} conductances. Inhibitors of K^+ conductances, such as aminopyridines or guanidine, increase action potentials firing, which leads to convulsions in some experimental animal models (Löscher and Schmidt, 1994). One of the approaches to diminish neuronal excitability is thus to stimulate the opening of K^+ channels, which could be one of the treatments of epilepsy. Activation of K^+ channels would be expected either to hyperpolarize neurons and inhibit them or to limit action potential firing by increasing the opposing influence that K^+ current normally have on depolarizing Na^+ currents (Porter and Rogawski, 1992). In this respect, recent studies have shown that openers of ATP-sensitive K^+ channel inhibit seizure activities in in vitro studies (Alzheimer and Ten Bruggencate, 1988; Abele and Miller, 1990; Popoli et al., 1991; Mattia et al., 1994) and in in vivo epilepsy models (Gandolfo et al., 1989a,b). In order to evaluate the effects of the openers of K^+ channel on seizure activities more critically, it will be important to test the drugs in animal models of the most frequent type of epilepsy in adult human, i.e. complex partial seizures derived from

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temporal lobe cortex, and to select an epileptic focus of brain regions where ATP-sensitive K^+ channels are enriched.

In the present studies, to ascertain the action of an opener of ATP-sensitive K^+ channel (levcromakalim, BRL 38227: (−)6-cyano-3,4-dihydro-2,2-dimethyl-*trans*-4-(2-oxo-1-pyrrolidyl)-2*H*-1-benzopyran-3-ol, which has twice as strong pharmacological activity against ATP-sensitive K^+ channel as cromakalim, BRL 34915), we took two approaches. First approach is to examine the effects of levcromakalim on the hippocampus-generating partial seizures elicited by low-frequency electrical stimulation in the freely moving rats. Using this method, we previously reported several pharmacological studies (Emori and Minabe, 1990; Minabe and Emori, 1992; Minabe et al., 1992, 1993). The advantage of the low-frequency stimulating procedure is that we can quantitatively measure afterdischarge threshold through a single stimulation train, whereas the conventional train-intensity procedure requires the delivery of at least several trains and cannot neglect the influence of repetitive sub-threshold testing stimuli. In this study, we chose the dentate gyrus as the stimulatory site in the following reasons. First, clinical data suggests that this area of the hippocampus is believed to play a critical role in generating temporal lobe seizures (Babb and Brown, 1987). Second, seizures can be triggered by 2-Hz electrical stimulation from hippocampus more easily and reliably than from other sites, such as amygdala in the rat (Minabe et al., 1992). More critically, since recent studies utilizing electrophysiolgical and radio-ligand binding techniques have provided evidences for ATP-sensitive K^+ channels in brain with high densities in the hippocampus, particularly in granular cells of the dentate gyrus, in pyramidal cells of CA1 and CA3 field (Ashford et al., 1988; Mourre et al., 1989; Tremblay et al., 1991), it is of great value to test the openers of K^+ channel on hippocampus-generating seizures. In this time, pulse number threshold, the number of stimulating pulses required for the triggering of epileptic afterdischarge and afterdischarge duration were used as two major seizure parameters. For comparison, a ATP-sensitive K^+ channel blocker (glibenclamide) and non-specific K^+ channel blocker (tetraethylammonium) were also examined. In addition, since it has been shown that cromakalim selectively inhibit the activity of inward Ca^{2+} currents (Ito et al., 1990), we hypothesized that actions via Ca^{2+} channels play a role in the effects of levcromakalim on the seizure. Therefore, Ca^{2+} channel antagonist (nimodipine) and Ca^{2+} channel agonist ((±)-BAY K 8644: 1,4-dihydro-2,6-dimethyl-5-nitro-4-[2-(trifluoromethyl)-phenyl]-3-pyridinecarboxylic acid methyl ester) also examined.

And for better understanding of these in vivo results, the second approach is to measure membrane currents of NG108-15 cells, which are established neuronal model cells (Brown and Higashida, 1988a,b), in in vitro patch-clamp experiments. This cell line is suitable to examine the effect of the openers of K^+ channel on Ca^{2+} channels and Ca^{2+}-dependent K^+ channels under no influence with ATP-sensitive K^+ channel activity, because it lacks ATP-sensitive K^+ channel (Ito et al., 1990).

Here, we demonstrate that an opener of K^+ channel inhibits Ca^{2+}-dependent K^+ channels indirectly through direct inhibition of Ca^{2+} currents.

2. Materials and methods

2.1. In vivo experiment

2.1.1. Animals

Male Wistar rats ($n = 43$), weighing 250 g at the time of surgery, were used. The rats were housed in plastic cages with wood chip bedding under controlled environmental conditions (ambient temperature 23–25°C, humidity 50–60%, 12/12 h light/dark cycle, light on at 08:00 h) and permitted free access to food and water at all times. All the pharmacological experiments were done in the morning (09:00–11:00 h) to minimize the bias of circadian rhythms.

2.1.2. Preparation of animals

The rats were anesthetized with pentobarbital (50 mg/kg i.p.) and mounted in a stereotaxic instrument. Tripolar electrodes (3 twisted 0.2 mm diameter polyurethane-coated stainless steel lines) and i.c.v. cannulae were implanted bilaterally. Tip of the electrodes or guide cannulae was placed in the dentate gyrus of dorsal hippocampus (AP −3.5 mm from bregma, L ± 2.0 mm, V +4.0 mm) and separated vertically by 0.5 mm. I.c.v. guide cannulae were placed in the lateral ventricles (AP −0.8 mm, L ± 1.5 mm, V +2.5 mm). Stainless steel screws served as anchors and the reference electrodes. The electrodes and guide cannulae were attached to the skull by dental acrylic cement and connected with a socket.

2.1.3. Stimulation procedure

After a post-operative period of 2 weeks, freely moving rats received electrical stimulations. 2-Hz stimulation pulses for 12.5 s (500–800 μA base-to-peak, 1 ms duration, biphasic square-wave pulses) were delivered to trigger seizures, using computer-assisted automatic stimulating systems (NIHON KODEN, SEN-7103) and constant current units. If the frequency of the stimulation is more than 3 Hz, we sometimes could not determine the onset of AD definitely due to the stimulation artifact on EEG recording. Therefore, we usually use 2-Hz stimulation to trigger seizures. It is known that dentate gyrus kindling takes more stimulations to progress than the conventional amygdala kindling and shows instability of the behavioral seizure stage (Grace et al., 1990). However, in our preliminary trials, the seizure parameters, such as afterdischarge duration and pulse number threshold, are rather stable during

the long period showing stage-1 seizures (by the modified Racine classification; Racine, 1972). Thus, we used the rats showing only hippocampus-generating stage-1 partial seizures, i.e. immobility, facial grooming, wet-dog-shake behavior or locomotion without any clonic component, not using hippocampal-fully-kindled rats showing secondarily generalized convulsive seizures. All rats were stimulated twice daily for 10 consecutive days without drug treatment. After the stability of the seizure parameters was examined, the pharmacological experiments were conducted. Afterdischarge duration was measured as the total time of epileptic discharges (with an amplitude of at least twice the height of the pre-stimulus background activity) on the EEG and did not include secondary afterdischarge, which appeared after the cessation of the initial primary afterdischarge (see Fig. 1). Pulse number threshold, the number of stimulating pulses required for provocation of afterdischarge was also measured (see Fig. 1 Fig. 2). The EEG recording was digitized at a sampling rate of 5 kHz per each electrode by using Analog-Digital converter (National Instruments, NB-MIO-16X) and was acquired into personal computer system (Apple, Macintosh Quadra 950). The data were saved to magneto-optical disk and analyzed. The behavior of the rat was videotaped using EEG-VTR system (NIHON KODEN, VY-440A). These series of procedures were controlled automatically using a computer software program (National Instruments, Lab VIEW 2).

2.1.4. Evaluation of anticonvulsant effects

All the drugs and vehicles, served as controls, were injected 1 h before each stimulation. The total volume of bilateral i.c.v. injection was 10 μl (i.e. 5 μl per site) over a period of 2 min by using Hamilton microliter syringes located on an infusion pump (Harvard Apparatus, Pump 22) and after the injection the injection needle remaining in the cannulae 1 min to avoid back flow of the solution. Control recordings were done 2 days before (pre-drug control) and 3 days after (post-drug control) each drug treatment. The drug effects were evaluated by using two parameters; pulse number threshold which was used as an indicator for seizure-generating threshold and afterdischarge duration as an indicator for the severity of the induced seizures. If afterdischarge was not identified after low-frequency (2 Hz) stimulation, 25 10-Hz stimulating pulses were delivered after a 10-min rest period to trigger afterdischarge. In this case, only afterdischarge duration was measured and pulse number threshold was recorded as

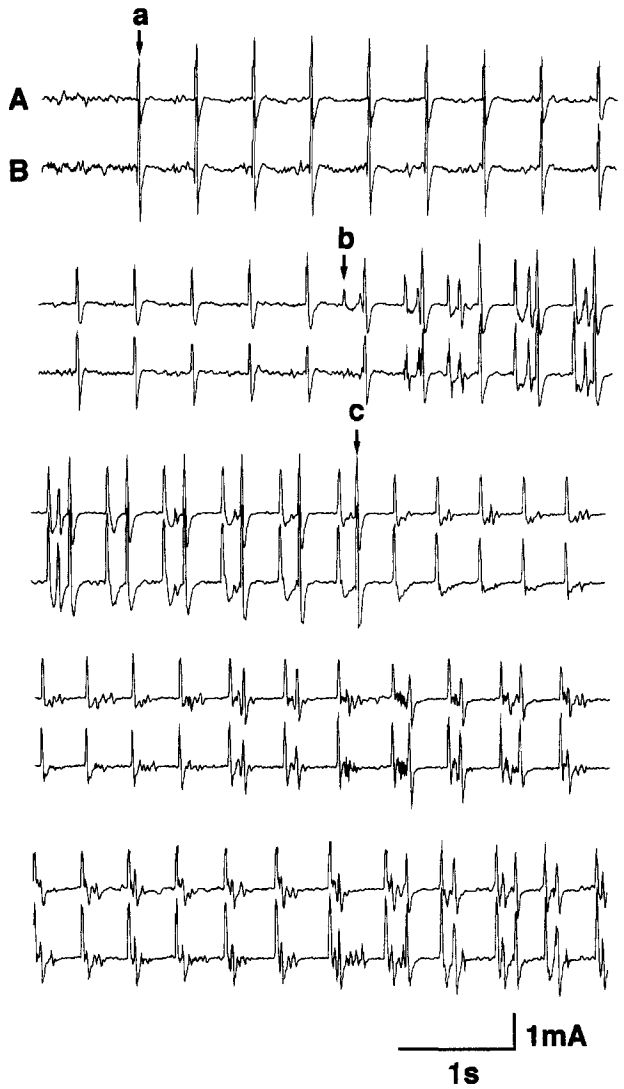

Fig. 2. Intracranial EEG recording from the bilateral sides of dentate gyrus during 2-Hz electrical stimulation. Consecutive tracings are shown from top to bottom. (A) Ipsilateral recording of the stimulation site; (B) contralateral recording; (a) the beginning of stimulation; (b) triggering of afterdischarge; (c) the end of stimulation. In this case, pulse number threshold is 14 (i.e. the count number of stimulating pulses from a to b).

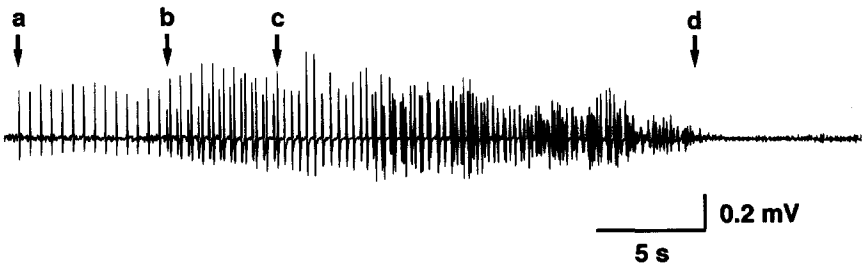

Fig. 1. Intracranial EEG recording from the dentate gyrus of the stimulation side during 2-Hz electrical stimulation. (a) The beginning of the stimulation; (b) triggering of afterdischarge; (c) the end of stimulation; (d) the end of afterdischarge (primary afterdischarge). In this case, afterdischarge duration is the duration from b to d.

25 (i.e. the number of maximal stimulating pulses). If the same rat was used in this experiment repeatedly, at least 7 days elapsed between the injections of different drugs in order to avoid alteration due to drug accumulation or interaction.

2.1.5. Statistics

All data of seizure parameters were given as means ± S.E.M. In this study, significant differences to pre-drug control on pulse number threshold and afterdischarge duration were assessed by Wilcoxon signed-rank test for paired replicates.

2.1.6. Drugs

Levcromakalim (BRL 38227) was gifted from Smith-Kline Beecham Pharmaceuticals (Tokyo, Japan). Tetraethylammonium, glibenclamide, nimodipine and BAY K 8644 were purchased from Research Biochemicals International (Natick, MA, USA). Levcromakalim, tetraethylammonium and glibenclamide were injected i.c.v. bilaterally in a volume of 10 μl (i.e. 5 μl per site). Nimodipine and BAY K 8644 were injected i.p. in a volume of 2–3 ml/kg. Levcromakalim and BAY K 8644 were freshly dissolved in ethanol completely and then adjusted in 0.7–7% v/v ethanol/saline before each experiment. Glibenclamide and nimodipine were dissolved in the 5% dimethyl sulfoxide (DMSO) in isotonic saline solution. Tetraethylammonium was dissolved in saline. The same solution as each dissolved drug was used for the vehicle.

2.2. Membrane current measurements

Cell used in these experiments were NG108-15 mouse neuroblastoma × rat glioma hybrid cells. Hybrid cells were cultured in polyornithine-coated dishes and induced to differentiate by growing in Dulbeco's modified Eagle's medium containing 1% fetal bovine serum, 100 μM hypoxanthine, 16 μM thyamidine and 0.25 mM dibutyryl cyclic AMP for 2–3 weeks as described previously (Brown and Higashida, 1988a). Membrane currents were recorded via a discontinuous single electrode voltage-clamp amplifier (model 2A, Axon Instruments). Sample rate (2–6 kHz), capacitance neutralization, phase and gain were adjusted for optimum clamp fidelity as described previously (Brown and Higashida, 1988a). Ca^{2+}-dependent K^+ currents were measured with sharp microelectrodes (filled with 1 M K^+ citrate, 10 MΩ) by impaling cells soaked in Tris-HCl-buffered saline (TBS) (in mM): NaCl 140, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1, CsCl 5, glucose 20, Hepes 10; pH 7.4. Levcromakalim was dissolved to make a stock solution (50 mM) in 100% dimethyl sulfoxide (DMSO). Cells were superfused at 33–35°C with the buffer solution plus 1% DMSO with or without levcromakalim. In order to exclude artifacts of the solvent, control experiments were carried out with levcromakalim in 0.1% ethanol

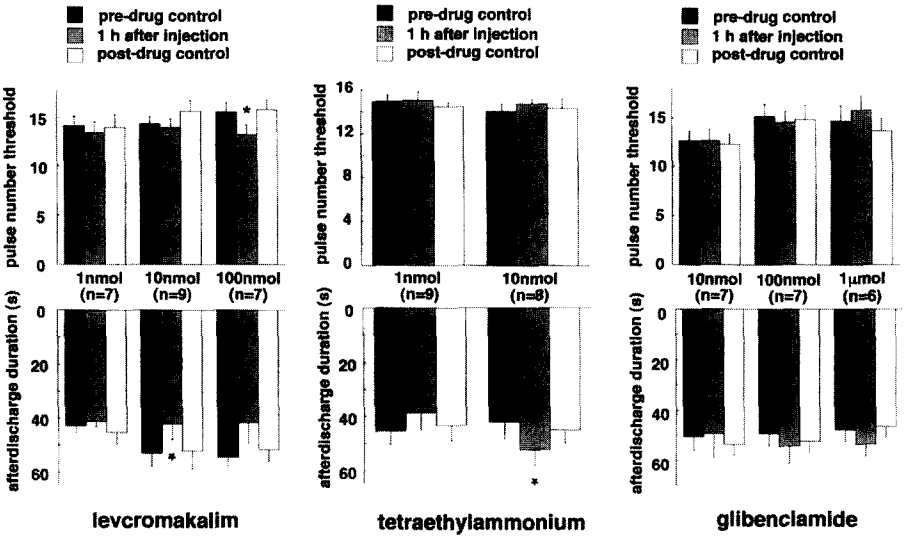

Fig. 3. Effects of levcromakalim, tetraethylammonium and glibenclamide on hippocampal-generating partial seizures in rats. In all figures, the upper columns show pulse number threshold and lower columns show afterdischarge duration. All drugs and vehicle controls were injected 1 h prior to each stimulation. Control recordings were done 2 days before (pre-drug control) and 3 days after (post-drug control) each drug administration. There are no significant differences between pre-drug controls and post-drug controls in all experiments. Significant differences compared with pre-drug control are indicated by asterisks (* $P < 0.05$, Wilcoxon signed-rank test for paired replicates). Doses of drugs are indicated between columns. The numbers in parenthesis represent the number of animals examined. All data are shown as mean ± S.E.M. The injection of levcromakalim, 10 nmol i.c.v., decreased afterdischarge duration and 100 nmol decreased pulse number threshold. The injection of tetraethylammonium (10 nmol i.c.v.) increased afterdischarge duration. 100 nmol i.c.v. tetraethylammonium induced spontaneous seizures (data not shown). The injection of glibenclamide (1, 10 and 100 nmol i.c.v.) did not alter pulse number threshold or afterdischarge duration.

solution. Results obtained with the two solvents were identical and DMSO alone did not mimic the effect of levcromakalim.

3. Results

3.1. In vivo experiments

I.c.v. injection of levcromakalim, an opener of ATP-sensitive K^+ channel, significantly decreased afterdischarge duration at 10 nmol ($n = 9$) from 52.9 ± 4.7 s (mean ± S.E.M.) to 42.0 ± 5.9 s ($P < 0.05$), while little or no change at 1 nmol (Fig. 3). At 100 nmol levcromakalim, the inhibition of afterdischarge duration was observed with no statistical significance. The decrease of pulse number threshold was significant only at 100 nmol ($n = 7$): from 15.6 ± 1.0 to 13.3 ± 1.0 ($P < 0.05$). I.c.v. injection of glibenclamide, an ATP-sensitive K^+ channels blocker, at 10, 100 and 100 nmol had little or no effect on both pulse number threshold and afterdischarge duration. The similar application of tetraethylammonium, a K^+ channels blocker, at 1 nmol had no effect on both pulse number threshold and afterdischarge duration. 10 nmol of tetraethylammonium ($n = 8$) increased afterdischarge duration significantly from 42.0 ± 6.3 s before application to 52.3 ± 5.7 s ($P < 0.05$).

I.p. injection of Ca^{2+} channel modifiers showed also significant effects (Fig. 4). I.p. injection of nimodipine, a Ca^{2+} channel antagonist, at 40 mg/kg ($n = 8$) but not at

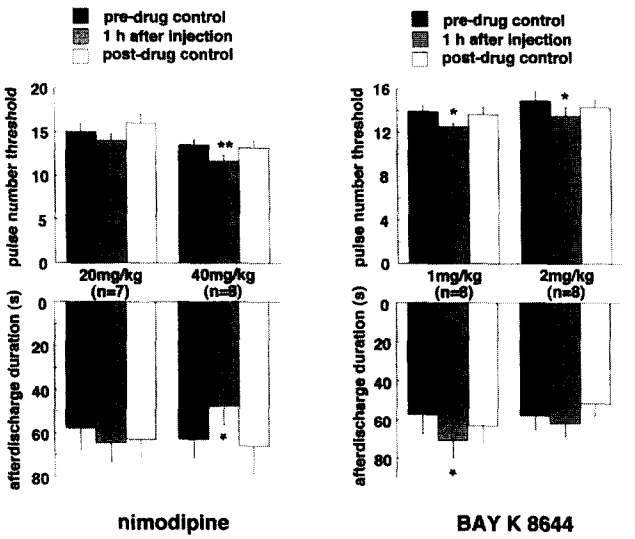

Fig. 4. Effects of nimodipine and BAY K 8644 on hippocampal-generating partial seizures in rats. All drugs and vehicles were injected 1 h prior to each stimulation. Significant differences compared with pre-drug control are indicated by asterisks (* $P < 0.05$, ** $P < 0.01$, Wilcoxon signed-rank test for paired replicates). Abbreviations as Fig. 3. Systemic administration of nimodipine (40 mg/kg i.p.) decreased pulse number threshold and afterdischarge duration. Systemic administration of BAY K 8644, 1 mg/kg i.p., increased afterdischarge duration and decreased pulse number threshold. 2 mg/kg i.p. decreased pulse number threshold.

20 mg/kg, decreased pulse number threshold from 13.5 ± 0.65 to 11.6 ± 0.65 ($P < 0.01$) and afterdischarge duration from 63.0 ± 8.2 s to 47.6 ± 8.5 s ($P < 0.05$). I.p. injection of BAY K 8644, a Ca^{2+} channel agonist, at 1 and 2 mg/kg ($n = 8$) significantly decreased pulse number threshold from 13.9 ± 0.52 and 14.9 ± 0.90 to 12.5 ± 0.33 and 13.5 ± 0.82, respectively. BAY K 8644 showed significant increase in afterdischarge duration from 57.3 ± 10.0 s to 70.9 ± 6.5 s at 1 mg/kg ($n = 8$).

In all cases, post-drug control values did not show any significant differences from pre-drug control. Furthermore, the vehicles used in this experiment did not show any significant effects on seizure parameters (data not shown). None of drugs at any tested doses, other than tetraethylammonium, appeared to produce any overt behavioral changes of the rats. The administration of tetraethylammonium induced spontaneous generalized seizures in 4 of 8 rats examined soon after the injection of 100 nmol i.c.v.

3.2. Patch-clamp (in vitro) experiments

We examined the effect of levcromakalim on 2 classes of Ca^{2+}-dependent K^+ currents in NG108-15 cells. NG108-15 cells possess two types of Ca^{2+}-activated tail currents: the fast component which is inhibited selectively by tetraethylammonium and the slow component which is inhibited dTC or apamin (Brown and Higashida, 1988a,b). In a typical voltage-clamp experiments, the cells were subjected to 0.25 s depolarizing voltage steps to 0 mV from a holding potential of −50 mV and then stopped. As illustrated in Fig. 5, the outward tail currents were evoked after such voltage steps with fast and slow decay time constants as described previously (Brown and Higashida, 1988b). Addition of 100–500 μM levcromakalim into the recording medium immediately and reversibly reduced the fast component of the outward current but not the slow component. The degree of such inhibition was $45 \pm 6\%$ ($n = 10$) by 500 μM levcromakalim. Levcromakalim (500 μM) did not alter the reversal potential of these outward tail currents. The reversal potential was 90 ± 5 mV and 85 ± 5 mV before and after levcromakalim treatment of 8 cells, respectively. The inhibitory effect on the fast component of the outward tail current was obtained by 20 mM tetraethylammonium in the recording medium, which was identical to levcromakalim's effect (Fig. 6).

4. Discussion

The results showed that i.c.v. application of levcromakalim decreased pulse number threshold and afterdischarge duration in hippocampal partial seizures. This results suggest that levcromakalim has paradoxical effects on epileptic seizures, i.e. both anticonvulsive and proconvulsive actions, because, if there is an antiepileptic effect by a

given drug, pulse number threshold should increase as the decrease in afterdischarge duration. This effect of levcromakalim was completely mimicked by the systemic administration of nimodipine, a Ca^{2+} channel antagonist, suggesting that the effect of levcromakalim could be mediated by the Ca^{2+} channel blocking effects as previously described (Ito et al., 1990). As expected, administration of a Ca^{2+} channel agonist, BAY K 8644, showed proconvulsant effects (the increase in afterdischarge duration and decrease in pulse number threshold) in our model. This suggests that our epilepsy model is very sensitive to Ca^{2+} channel modulation. Therefore, the assumption that there was little or no involvement of ATP-dependent K^+ channel modulation in anticonvulsant effect was further supported by the finding that glibenclamide, an ATP-sensitive K^+ channel blocker, had no appreciable effect on both afterdischarge duration and pulse number threshold.

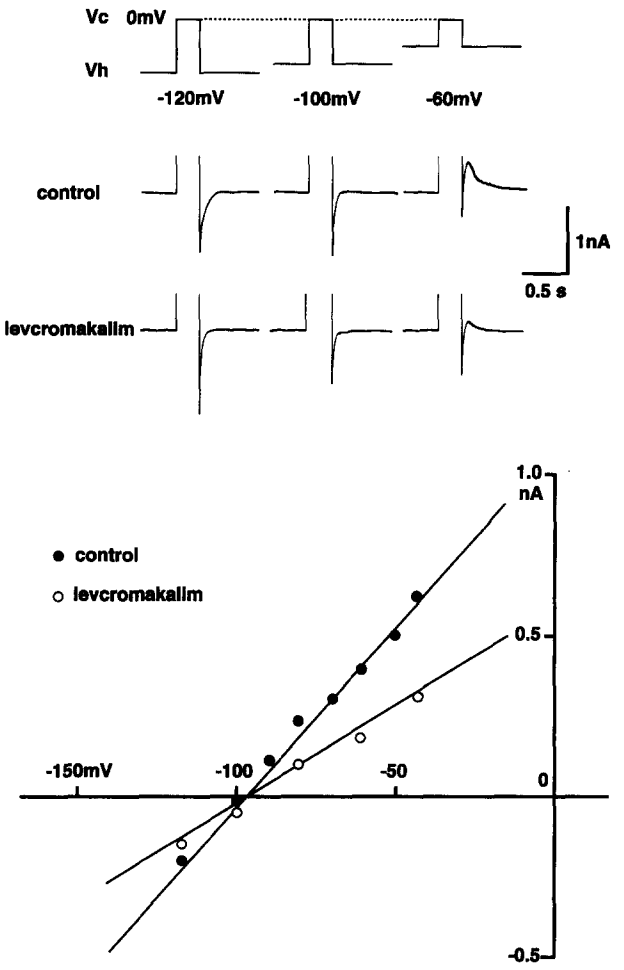

Fig. 5. Effects of levcromakalim on outwards tail currents evoked at the cessation of a 0.3-s depolarizing voltage step to 0 mV (Vc) from various holding potentials (Vh). Outward tail currents were obtained in the absence of levcromakalim (second row) and at 7–10 min after the onset 500 μM (third row). The depolarizing pre-pulse induced an inward Ca^{2+} currents followed by outward current. These currents were higher than could be measured on the amplification setting scale. Note that levcromakalim suppressed the peak amplitude of the outward tail current. The lower graph shows the relationship between the peak amplitudes of the outward tail currents and the post-pulse potentials. These currents reversed at post-pulse potentials of about −90 mV before (●) and −85 mV during (○) the addition of 500 μM levcromakalim. Note that levcromakalim did not alter the reversal potential.

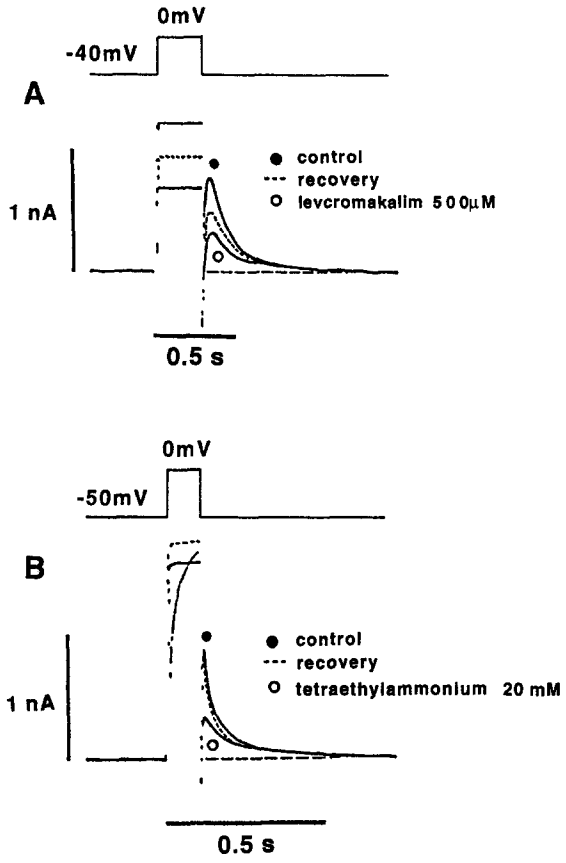

Fig. 6. Effects of levcromakalim on the two components of the outward tail current. The outward tail currents evoked at the cessation of a 0.3-s (A) or 0.1-s (B) depolarizing pre-pulse to 0 mV from holding potential of −40 (A) or −50 mV (B), as indicated by the upward rectangular deflections, before (●) and at 10 min (○) the onset of 500 μM levcromakalim (A) or 20 mM tetraethylammonium (B) and after wash (---). Cells in A and B were different. Note that both levcromakalim and tetraethylammonium inhibit the fast component.

In recent studies, cromakalim has been observed to inhibit epileptiform activities in vivo and in vitro in a number of different ways. I.c.v. injection of cromakalim increased the threshold of seizure induction by mast cell degranulating peptide (a blocker of voltage-sensitive K^+ channels) and completely prevented the epileptic crisis (Gandolfo et al., 1989a). I.c.v. injection of cromakalim decreased frequencies of spontaneous spike-wave bursts in genetically epileptic rats (Gandolfo et al., 1989b). Epileptiform activity elicited by high dose of Ca^{2+} antagonist, diltiazem, was prevented by cromakalim in the rat (Popoli et al., 1991). Cromakalim reduced seizure-like activity produced in hippocampal slice both in normal artificial cerebrospinal fluid and in Mg^{2+}-free fluid model (Alzheimer and Ten Bruggencate, 1988). However, to our knowledge, no previous studies showed the proconvulsive action of cromakalim or levcromakalim.

Epileptic seizures were rather suppressed by Ca^{2+} channel antagonist and enhanced by Ca^{2+} channel agonists in previous studies (De Sarro et al., 1988; Dolin et al., 1988). However, Van Luijtelaar et al. (1995) showed that effects of L-type Ca^{2+} channel modulation apparently differ in convulsive (tonic-clonic seizures) and in non-convulsive (showing spike-waves discharge on EEG) epilepsy because nimodipine facilitates spike-wave discharges, while it blocks tonic-clonic seizures (De Sarro et al., 1988). Interestingly, it has been reported that nifedipine, one of the L-type Ca^{2+} channel antagonists, increases cumulative duration of afterdischarge to reach the stage-4 and -5 seizures in amygdala kindling rats (Yamada and Bilkey, 1991). The discrepancy in the result is due to the difference of stimulation sites and of design of experiments. Many investigators have been revealed that amygdala-kindled seizures have a different pharmacological profile to hippocampal-kindled seizures (Sato et al., 1988; Gilbert, 1988). A possible explanation for the proconvulsive effect is that the Ca^{2+} channel antagonists, including levcromakalim, inhibit Ca^{2+}-dependent K^+ channels. In this study, we used the model of non-convulsive partial seizures generated from hippocampus, thus, we consider that it is difficult to compare our results simply with those of conventional amygdala-fully-kindled convulsive seizure models.

Another important finding of our experiments is that the tail K^+ outward current in cultured NG108-15 cells, that is due to activation of Ca^{2+}-dependent K^+ channels, was suppressed by addition of levcromakalim in recording culture dishes. The evidence for this is that the addition of tetraethylammonium or Cd^{2+} in recording medium reproduces the suppression of the fast component or both fast and slow components of the tail currents (Brown and Higashida, 1988a). The mechanism for suppression of Ca^{2+}-dependent K^+ current by levcromakalim is probably indirect. Previous reports by us (Ito et al., 1990) and others (Okabe et al., 1990) show that levcromakalim can inhibit some but not all types of voltage-dependent Ca^{2+} channels. In addition, previous study shows nimodipine may suppress Ca^{2+}-dependent K^+ channels in a dose range that modulates epilepsy (Jones and Heinemann, 1987).

In summary, what this study really shows, by analogy to the effects of nimodipine, is that the effects of levcromakalim look more to a L- or T-type Ca^{2+} channel blocker than to an opener of ATP-sensitive K^+ channel, given the absence of an effect of glibenclamide. The results obtained with NG108-15 cells suggest that the consequence of (partial) blockade of Ca^{2+} currents is that there is less activation of Ca^{2+}-dependent K^+ current. Altogether, this could be circumstantial evidence for a proconvulsant action of levcromakalim. More studies are anticipated about the openers of ATP-sensitive K^+ channel using various seizure models, particularly in vivo. In addition, to understand the effects of Ca^{2+} and K^+ channel modulation on epileptic seizures, is important for clarifying the pathophysiology of epilepsy and for developing new antiepileptic drugs.

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