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Antinociceptive Effects of Sodium Channel-Blocking Agents on Acute Pain in Mice

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Abstract. The effects of various sodium channel blocking agents on acute thermal and mechanical nociception, as assessed using the plantar and tail pressure tests, respectively, were compared with the effects of morphine. The drugs used were mexiletine, lidocaine, carbamazepine, phenytoin, eperisone, tolperisone, and zonisamide. The sodium channel blocking agents exhibited a rather preferential elevation of the threshold for thermal nociception. By contrast, morphine produced similar analgesic effects on thermal and mechanical nociception. In the sciatic nerve isolated from mice, mexiletine, lidocaine, eperisone, and tolperisone impaired the propagation of low frequency action potentials (evoked at 0.2 Hz). Carbamazepine, phenytoin, and zonisamide generated a more frequency-dependent local anesthetic action with their obvious effects on higher frequency action potentials (evoked at 5 and/or 10 Hz). Our results show that sodium channel blocking agents have a preferential antinociceptive action against thermal stimulation that is likely to be attributed to their local anesthetic action.

Keywords: sodium channel blocker, plantar test, tail pressure test, local anesthetic action

Introduction

Some local anesthetics and antiepileptic agents are used clinically in the treatment of neuropathic pain (1 – 4). These drugs generally possess sodium channel blocking properties, and experiments involving animal models of neuropathic pain (rat) have in fact revealed that the sodium channel blocking agents exhibit analgesic effects (5 – 7). However, little is known about the role of sodium channels in the generation or conduction of acute or chronic pain signals. So far, there has been no study comparing the antinociceptive effects of different sodium channel blocking agents on acute pain.

In the study presented here, we investigated the effects of lidocaine, mexiletine, carbamazepine, phenytoin, zonisamide, eperisone, and tolperisone on the acute pain. Lidocaine and its structural analog mexiletine are class 1b antiarrhythmic drugs; carbamazepine, phenytoin, and zonisamide are used to treat epilepsy; eperisone and tolperisone are centrally acting muscle relaxants. Thus, although the principal therapeutic applications of these drugs are different, their common and major pharmacological target is thought to be sodium channels.

The goal of this study was to assess whether these sodium channel blocking agents generate qualitatively similar analgesic effects on the acute pain induced by thermal and mechanical stimulation.

Materials and Methods

All experimental protocols used were approved by the Animal Care and Use Committee of Nagoya City University and were conducted according to the guidelines of the National Institutes of Health and the Japanese Pharmacological Society.

Animals

Adult male ddY mice (5-week-old) were used in all experiments. Animals were allowed free access to food and water on a 12 h light/dark cycle in rooms where temperature and humidity were controlled.

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Effects on acute nociception

The degree of antinociception was determined using the plantar test and the tail pressure test.

Plantar test (nociceptive thermal stimulation): The degree of acute thermal nociception was assessed using the plantar test (Ugo Basile, Comerio, Italy) following a modification of the method of Hargreaves et al. (8). Mice were placed in a clear plastic chamber with a glass floor and allowed to acclimate to their environment before testing. During this time, mice initially demonstrated exploratory behavior, but subsequently stopped exploring and stood quietly with occasional bouts of grooming. A mobile radiant heat source, located under the glass floor, was focused onto the plantar surface of the left hindpaw, and paw withdrawal latencies (PWLs) were recorded. PWLs were measured twice for the left hindpaw of each animal, and the mean of the two values was used for calculations. The intensity of the heat stimulus was adjusted so that the baseline latency was 6 s and a 15-s cut-off time was imposed to avoid tissue damage.

Tail pressure test (nociceptive mechanical stimulation): Following the plantar test, mice were subjected to the tail pressure test (Pressure Analgesy-Meter; Muromachi Kikai, Tokyo) to assess their threshold for acute mechanical nociception. Pressure was applied about 1.5 cm from the base of the tail via a blunt probe. The pressure level was increased at a rate of 10 mmHg/s, and the pressure (mmHg) required to elicit a response was determined for each mouse; that pressure was defined as the nociceptive threshold. Tail pressure measurements were taken twice, and the mean of the two values was used for calculations. The cut-off pressure was 100 mmHg.

Local anesthetic action

The sciatic nerves were isolated from male ddY mice anesthetized with 80 mg/kg of intraperitoneally applied thiopental, and the desheathed peroneal nerve bundle was placed on the chamber in vitro. One end of the nerve was stimulated (0.2 – 10-Hz rectangular pulses, 0.05 ms in duration, 5 V), and action potentials were recorded from the other end of the nerve. Bipolar Ag-AgCl wire electrodes were used for stimulation and recording. Action potentials were displayed on an oscilloscope (Nihon Kohden, Tokyo), and eight consecutive responses were averaged by an averager (Nihon Kohden). Drugs were applied to the bath, which was filled with Locke-Ringer solution (see below, 21 – 24°C) and situated between the stimulating and recording electrodes. The concentration of drug in the bath was increased every 5 min by exchanging the drug solution (9).

Drugs

Mexiletine HCl was obtained from Sigma Chemical (St. Louis, MO, USA). Lidocaine HCl was obtained from Iwaki Seiyaku (Tokyo), carbamazepine and phenytoin from Wako Chemical (Tokyo), tolperisone HCl from Nippon Kayaku (Tokyo), eperisone HCl from Eisai (Tokyo), and morphine HCl from Shionogi (Osaka). Zonisamide was donated by Dainippon Pharmaceutical (Osaka). For measurements of the effects on acute pain, carbamazepine and phenytoin were suspended in 8 ml of propylene glycol and 2 ml of 3% Tween 80 solution (10), and the other drugs were dissolved in 0.9% w/v physiological saline. Drugs (or saline or propylene glycol and Tween 80 for control animals) were administered subcutaneously (s.c.) at 10 ml/kg.

For the measurements of local anesthetic properties, with the exception of carbamazepine and phenytoin, the drugs were dissolved in Locke-Ringer solution containing 154 mM NaCl, 5.6 mM KCl, 2.4 mM $NaHCO_3$, 1.6 mM $CaCl_2$, 1 mM $MgCl_2$, and 2.8 mM D-glucose, adjusted to pH 7.4 with HCl. Carbamazepine and phenytoin was dissolved in dimethyl sulfoxide (DMSO), and was then diluted to the final concentration (0.3 mM) with Locke-Ringer solution (0.1% DMSO).

Statistical analyses

All data are expressed as means ± S.E.M. The effects of drugs on the nociceptive threshold in both tests were evaluated in a time-course study, where each drug was administered at time zero. The nociceptive threshold of each time point was normalized to the pre-drug value. The dose-dependent analgesic actions of the drugs were assessed using the area under the time-course curve (AUC) between time zero and 45 min.

The amplitudes of action potentials were expressed as percentages of the corresponding values obtained before drug application.

One-way analysis of variance (ANOVA) followed by the two-tailed multiple *t*-test with Bonferroni correction (11) was used for multiple comparisons of control and treated groups. In the study of the local anesthetic action, the paired *t*-test was used to compare the action potential amplitudes between before and during drug application. Differences at $P<0.05$ were considered to be significant.

Results

The effects of test drugs on the nociceptive threshold were evaluated in a time-course study (Fig. 1 and 2) as well as using the area under the time-course curve (AUC, Fig. 3).

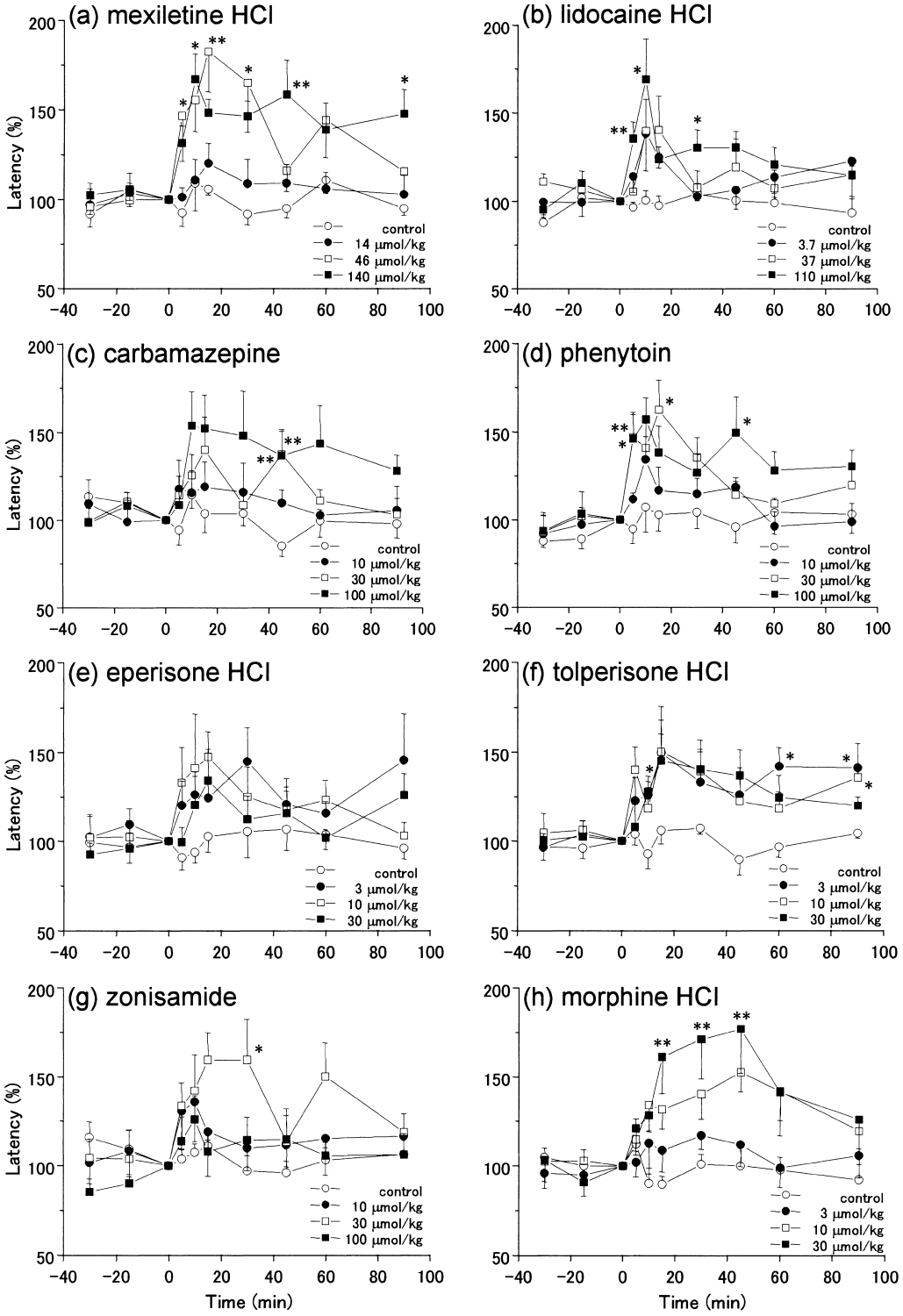

Fig. 1. The antinociceptive effects of sodium channel blocking agents on the paw-withdrawal (noxious heat-induced) threshold, as measured by the plantar test, in mice. a: mexiletine HCl (14, 46, and 140 μmol/kg, s.c.), b: lidocaine HCl (3.7, 37, and 110 μmol/kg, s.c.), c: carbamazepine (10, 30, and 100 μmol/kg, s.c.), d: phenytoin (10, 30, and 100 μmol/kg, s.c.), e: eperisone HCl (3, 10, and 30 μmol/kg, s.c.), f: tolperisone HCl (3, 10, and 30 μmol/kg, s.c.), g: zonisamide (10, 30, and 100 μmol/kg, s.c.), and h: morphine HCl (3, 10, and 30 μmol/kg, s.c.). Each data point represents the mean ± S.E.M. of six mice per group. Ordinates: mean latency expressed as a percentage of the corresponding value at time 0. Abscissae: time in minutes after drug administration. The significance of differences between the test and control values was determined by analysis of variance (ANOVA) followed by the two-tailed multiple *t*-test with Bonferroni correction (3 comparisons in 4 groups). **P*<0.05 and ***P*<0.01 *vs* control in respective time.

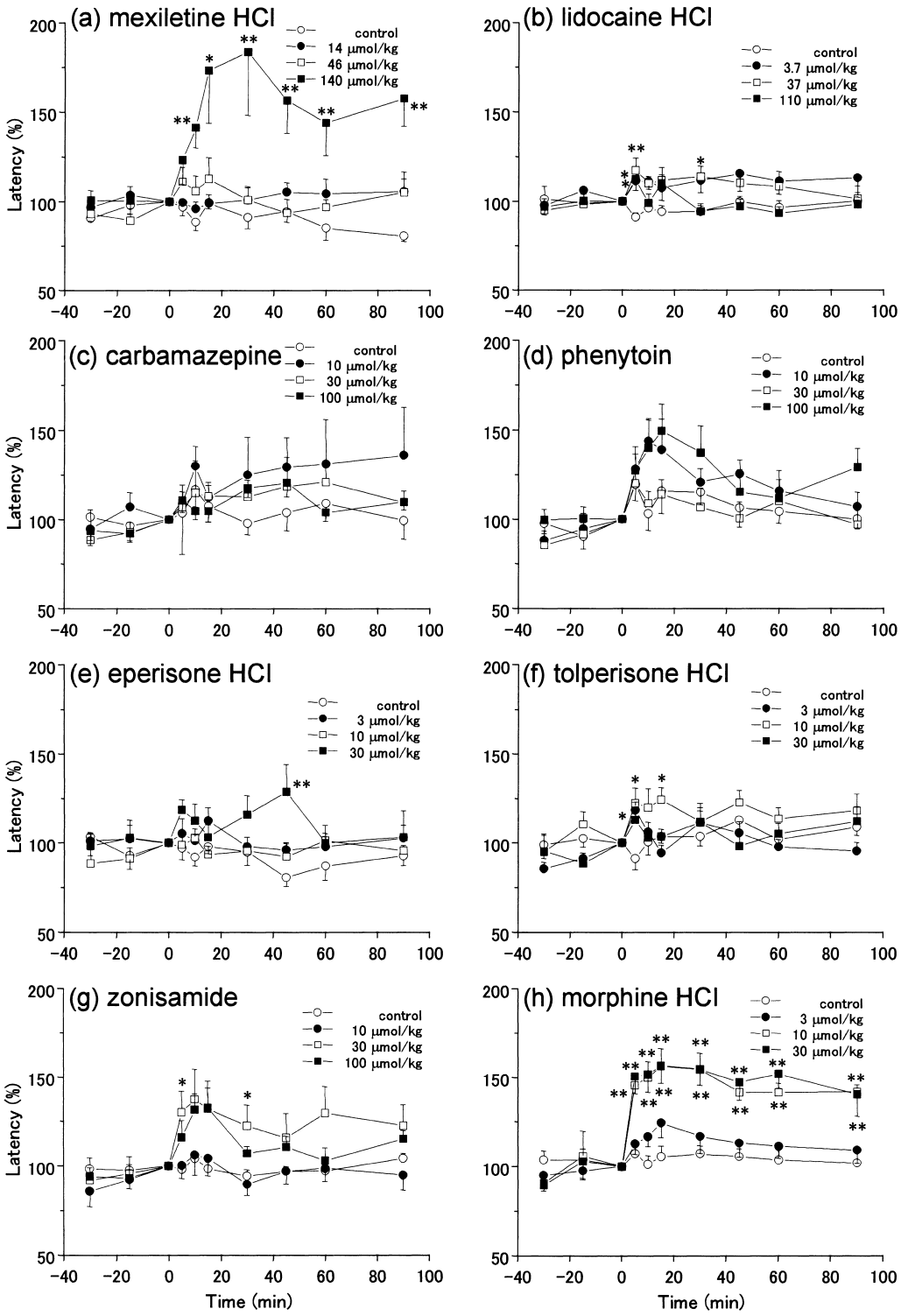

Fig. 2. The antinociceptive effects of sodium channel blocking agents on the mechanically induced pain threshold, as measured by the tail pressure test, in mice. a: mexiletine HCl (14, 46, and 140 μmol/kg, s.c.), b: lidocaine HCl (3.7, 37, and 110 μmol/kg, s.c.), c: carbamazepine (10, 30, and 100 μmol/kg, s.c.), d: phenytoin (10, 30, and 100 μmol/kg, s.c.), e: eperisone HCl (3, 10, and 30 μmol/kg, s.c.), f: tolperisone HCl (3, 10, and 30 μmol/kg, s.c.), g: zonisamide (10, 30, and 100 μmol/kg, s.c.), and h: morphine HCl (3, 10, and 30 μmol/kg, s.c.). Each data point represents the mean ± S.E.M. of six mice per group. Ordinates: mean latency expressed as a percentage of the corresponding value at time 0. Abscissae: time in minutes after drug administration. The significance of differences between the test and control values was determined by ANOVA followed by the two-tailed multiple *t*-test with Bonferroni correction (3 comparisons in 4 groups). **P*<0.05 and ***P*<0.01 *vs* control at the respective time.

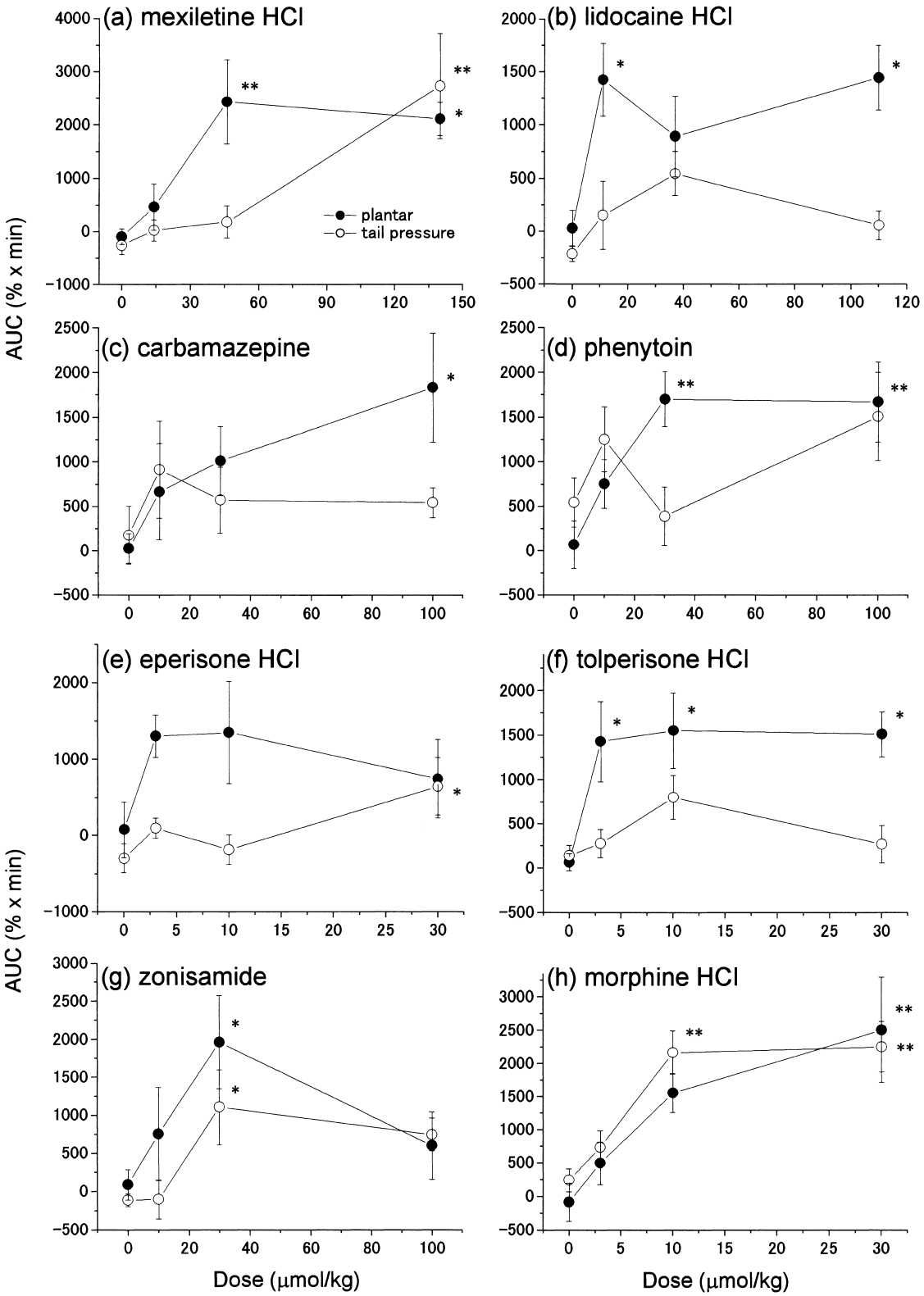

Fig. 3. Dose-response relationships of the analgesic effects of the sodium channel blockers on thermal and mechanical nociception. The effects of drugs were evaluated on the basis of the area under the time-course curve (AUC) between time zero (drug administration) and 45 min. Each value represents the mean ± S.E.M. of six mice. Ordinate: mean AUC (% × min) for the plantar and tail pressure tests (closed and open circle, respectively). The significance of differences between the test and control values was determined by ANOVA followed by the two-tailed multiple *t*-test with Bonferroni correction (3 comparisons in 4 groups). **P*<0.05 and ***P*<0.01 *vs* control.

Effects on the plantar test (nociceptive thermal stimulation)

Mexiletine HCl (14 – 140 μmol/kg equivalent to 3 – 30 mg/kg), carbamazepine (10 – 100 μmol/kg = 2.4 – 24 mg/kg), and phenytoin (10 – 100 μmol/kg = 2.5 – 25 mg/kg) elevated the withdrawal threshold for the plantar test in a dose-dependent manner (Fig. 1: a, c, and d; Fig. 3: a, c, and d). A similar elevation of the withdrawal threshold was obtained with lidocaine HCl (3.7 – 110 μmol/kg = 1 – 30 mg/kg, Fig. 1b and Fig. 3b); eperisone HCl (3 – 30 μmol/kg = 0.9 – 9 mg /kg, Fig. 1e and Fig. 3e, statistically not significant); tolperisone HCl (3 – 30 μmol/kg = 0.85 – 8.5 mg/kg, Fig. 1f and Fig. 3f); and zonisamide (3 – 100 μmol/kg = 0.6 – 21 mg/kg, Fig. 1g and Fig. 3g), while zonisamide generated antinociception with a bell-shaped dose-response relation (Fig. 3). The maximal effect of these drugs on the withdrawal threshold corresponded to that observed with 10 – 30 μmol/kg morphine HCl (= 3.2 – 9.7 mg/kg Fig. 1h and Fig. 3h). The muscle relaxant effects of eperisone and tolperisone are not thought to contribute to the elevation of the withdrawal threshold observed at the dose ranges used here (12). Thus, the sodium channel blockers examined in this study exhibit analgesic effects, as assessed using acute nociceptive thermal stimulation.

Effects on the tail pressure test (nociceptive mechanical stimulation)

Mexiletine HCl (140 μmol/kg) and zonisamide (30 – 100 μmol/kg) elevated the nociceptive threshold for the tail pressure test. The maximal effects of mexiletine HCl and zonisamide corresponded to those of 10 – 30 and 3 – 10 μmol/kg of morphine HCl, respectively (Fig. 2: a, g, and h; Fig. 3: a, g, and h). However, unlike the marked antinociception against thermal stimulation, the sodium channel blockers tested here generally demonstrated weak analgesic effects against mechanical stimulation.

Local anesthetic action

A summary graph of the local anesthetic action of sodium channel blockers tested in this study is shown in Fig. 4. When the action potentials were evoked at 0.2 Hz, a concentration-dependent inhibition of action potential propagation was obtained with mexiletine, lidocaine, eperisone, and tolperisone (Fig. 4A). Carbamazepine (0.3 mM), phenytoin (0.3 mM), and zonisamide (up to 3 mM) demonstrated no or little impairment of action potential propagation evoked at this low frequency. They required rather short intervals between propagated action potentials (5 and/or 10 Hz) to generate the apparent local anesthetic action (Fig. 4B).

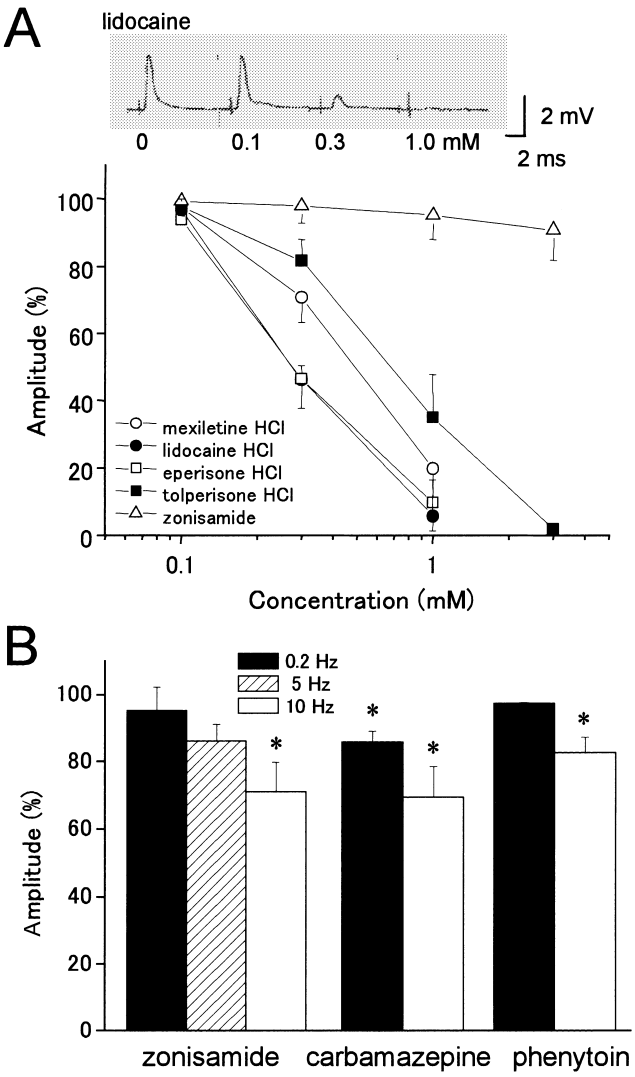

Fig. 4. Concentration-response relationships and frequency-dependency of the blocking effects of the sodium channel blockers on conductivity in the isolated mouse sciatic nerve. Each data point represents the mean ± S.E.M. of three to four preparations taken from different mice for each group. A: concentration-dependency. Action potentials were evoked at 0.2 Hz. Ordinate: mean amplitude of action potentials expressed as a percentage of the corresponding value before drug application. Abscissa: concentration of drugs. Upper traces show a representative effect of lidocaine HCl on the propagation of action potentials. B: frequency-dependency (0.2, 5, and/or 10 Hz) in the local anesthetic action of zonisamide (1 mM), carbamazepine (0.3 mM), and phenytoin (0.3 mM). *P<0.05 *vs* predrug control.

Without these drugs, the propagated action potentials were stably recorded when evoked at 0.2, 5, or 10 Hz.

Discussion

We have compared the antinociceptive effects of various sodium channel blocking agents on acute

thermal and mechanical pain and have demonstrated that these agents have a preferential antinociceptive action against thermal stimulation. These agents generated the local anesthetic action that may play a role in the differentiation between thermal and mechanical nociception.

There exist various distinctions in the mechanisms mediating the generation and conduction of thermal and mechanical pain signals. In the conduction of nociceptive pain signals to the spinal dorsal horn neurons, Doucette et al. (13) reported that C-fibers are activated in response to noxious heat stimuli. Moreover, Yeomans et al. (14) demonstrated that the types of nociceptors (C- and/or Aδ-fibers) activated by thermal nociception depend on the rates of skin heating; low rates of heating preferentially activates C-fiber nociceptors, while high rates of skin heating preferentially activate Aδ nociceptors. By contrast, both Aδ- and C-fibers are activated in response to noxious mechanical stimuli (15). In addition, distinct receptors mediate distinct nociceptive signals. Acid-sensing ion channels are related to mechanical nociception (16), and capsaicin receptors mediate heat nociception (17). Furthermore, thermal and mechanical nociception are modulated differentially by the descending monoaminergic pathways at the spinal level. The absence of norepinephrine in the central nervous system results in thermal hyperalgesia (18). Thus, various factors contribute to the differences between the conduction and modulation of thermal and mechanical nociception. Our observation that thermal nociception was more sensitive to sodium channel blocking agents discloses a new pharmacological characteristic.

Sodium channel blocking agents block the propagation of action potentials, which in the present study may have taken place at either Aδ- or C-fibers conducting pain signals to the spinal dorsal horn neurons. The drugs used here exhibited local anesthetic actions on the isolated sections of sciatic nerve. Most of the agents used here suppressed the propagation of low frequency action potentials (0.2 Hz). By contrast, to achieve the local anesthetic action by carbamazepine, phenytoin, and zonisamide required short intervals between action potentials. Such frequency-dependency is in agreement with the studies demonstrating that those compounds specially block the sustained repetitive firing of action potentials without altering the initial firing (19 – 21). Moreover, zonisamide enhances slow inactivation of sodium channels (22), as is also described for local anesthetics (23). Since small diameter neurons are generally more susceptible to the action of local anesthetics (24), it is conceivable that the local anesthetic action of sodium channel blocking agents used here contributes to their preferential analgesic action against C-fiber-mediated thermal nociception (13, 14), leaving Aδ- and C-fiber-mediated mechanical nociception (15) little affected. However, it remains unclear whether the analgesic effects of those drugs were derived solely from the blockade of peripheral sodium channels of Aδ- or C-fibers. Further studies are needed to determine the precise sites of action, including the central nervous system.

We consider that factors other than the local anesthetic action could contribute to a bell-shaped dose-response relation observed in the analgesic effect of zonisamide against thermal nociception because other sodium channel blocking agents here generally exhibited antinociception against thermal stimulation in a dose-dependent manner. Without a bell-shaped dose-response relation, zonisamide could demonstrated much evident preferential antinociception against thermal stimulation. This also requires further studies.

In conclusion, various drugs with sodium channel blocking actions preferentially suppressed thermal nociception, as contrasted with the equal effects of morphine on both thermal and mechanical nociception. This may be partly explained by the local anesthetic action of sodium channel blocking agents and differential sensitivities to local anesthetics of the fibers activated by thermal and mechanical nociception.

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