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Inhibition by non-steroidal anti-inflammatory drugs of compound action potentials in frog sciatic nerve fibers

Rika Suzuki, Tsugumi Fujita, Kotaro Mizuta, Eiichi Kumamoto*

Department of Physiology, Saga Medical School, Nabeshima 5-1-1, Saga 849-8501, Japan

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ABSTRACT

Aims: Although antinociception produced by non-steroidal anti-inflammatory drugs (NSAIDs) is partly attributed to nerve conduction inhibition, this has not been thoroughly examined yet. The aim of the present study was to reveal quantitatively how various types of NSAIDs affect compound action potentials (CAPs), a measure of nerve conduction.

Main methods: CAPs were recorded from the frog sciatic nerve by using the air-gap method.

Key findings: Soaking the sciatic nerve with acetic acid-based NSAIDs (diclofenac and aceclofenac) reduced the peak amplitude of CAP in a concentration-dependent manner; their IC_{50} values were 0.94 and 0.47 mM, respectively. Other acetic acid-based NSAIDs (indomethacin, acemetacin and etodolac) also inhibited CAPs [the extent of inhibition: some 40% (1 mM), 40% (0.5 mM) and 15% (1 mM), respectively], except for sulindac and felbinac at 1 mM that had no effects on CAP peak amplitudes. A similar inhibition was produced by fenamic acid-based NSAIDs [tolfenamic acid (IC_{50} = 0.29 mM), meclofenamic acid (0.19 mM), flufenamic acid (0.22 mM) and mefenamic acid] which are similar in chemical structure to diclofenac and aceclofenac; their derivatives (2,6-dichlorodiphenylamine and *N*-phenylanthranilic acid) also inhibited. On the other hand, salicylic acid-based (aspirin), propionic acid-based (ketoprofen, naproxen, ibuprofen, loxoprofen and flurbiprofen) and enolic acid-based (meloxicam and piroxicam) NSAIDs had no effects on CAP peak amplitudes.

Significance: At least a part of antinociception produced by NSAIDs used as a dermatological drug to alleviate pain may be attributed to their inhibitory effects on nerve conduction, which depend on the chemical structures of NSAIDs.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit the synthesis of prostaglandins from arachidonic acid by inhibiting the cyclooxygenase enzyme [1], resulting in anti-inflammation, pyretolysis and antinociception [2–5]. For antinociception produced by NSAIDs, there are possibly additional mechanisms, such as inhibition of acid-sensitive ion channels [6] and transient receptor potential (TRP) channels [7,8], activation of several K^+ channels ([9–13]; for review see [14,15]), substance P depletion [16], an interaction with the adrenergic system [17] and an involvement of opioids [18,19] and endocannabinoids [20]. This idea about an involvement of mechanisms other than cyclooxygenase inhibition in antinociception is supported by the observation that there is a dissociation between anti-inflammation and antinociception produced by NSAIDs [21].

Nerve conduction inhibition contributes to at least a part of antinociception produced by analgesic and its adjuvants [22]. We previously reported the inhibitory effects of a variety of drugs involved in antinociception on fast-conducting and Na^+-channel blocker tetrodotoxin (TTX)-sensitive compound action potentials (CAPs; a measure of nerve conduction) recorded from the frog sciatic nerve. For instance, CAPs were inhibited by opioids, local anesthetics, α_2-adrenoceptor agonists, antiepileptics and antidepressants [23–27].

There are many reports showing that NSAIDs inhibit voltage-gated Na^+ channels involved in action potential conduction. For example, diclofenac reduced the peak amplitudes of TTX-sensitive Na^+-channel currents in rat dorsal root [28] and mouse trigeminal ganglion neurons [29]. Similar Na^+-channel current amplitude reductions produced by diclofenac have been shown in rat myoblasts [30] and ventricular cardiomyocytes [31]. Diclofenac and also flufenamic acid reduced Na^+-channel current amplitudes in rat hippocampal CA1 neurons [32–34]. Activity increases of cat corneal sensory nerve fibers in response to chemical irritation were inhibited by NSAIDs [29,35]. Thus, it is possible that an inhibition of voltage-gated Na^+ channel, i.e., nerve conduction, produced by NSAIDs is involved in their antinociceptive effects. Although an inhibition of neuronal activity or Na^+ channels,

* Corresponding author.
E-mail address: kumamote@cc.saga-u.ac.jp (E. Kumamoto).

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produced by NSAIDs, appears to be distinct in extent among different types of NSAIDs [28,29,35], this has not been fully examined.

The present study examined quantitatively how various types of NSAIDs affect frog sciatic nerve CAPs and compared their activities among different types of NSAIDs. The NSAIDs used were (1) acetic acid-based (diclofenac, aceclofenac, indomethacin, acemetacin, etodolac, sulindac and felbinac), (2) fenamic acid-based (tolfenamic acid, meclofenamic acid, mefenamic acid and flufenamic acid), (3) salicylic acid-based (aspirin), (4) propionic acid-based (ketoprofen, naproxen, ibuprofen, loxoprofen and flurbiprofen) and (5) enolic acid-based (meloxicam and piroxicam) NSAIDs (for review see [3]). Their analogs (2,6-dichlorodiphenylamine and *N*-phenylanthranilic acid) were also tested, albeit being not NSAIDs. The experiments were performed by applying the air-gap method to the frog sciatic nerve.

2. Materials and methods

This study was approved by the Animal Care and Use Committee of Saga University.

2.1. Animals

The method used for obtaining frog sciatic nerve preparations has been described previously [25,36,37]. In brief, either sex of frogs (*Rana nigromaculata*) was decapitated and then pithed. Thereafter, the sciatic nerve (length and diameter: 4–5 cm and ca. 0.05 cm, respectively) was dissected from the lumbar plexus to the knee in Ringer's solution. The isolated sciatic nerve was carefully desheathed under a binocular microscope and then loosely placed on five platinum wires that were glued to a Lucite plate, and the two ends of the nerve were tied to the wires with threads. The plate was put on a beaker having Ringer's solution, immersing the sciatic nerve. The composition of Ringer's solution used was (mM): NaCl, 115.5; KCl, 2.0; $CaCl_2$, 1.8; Na_2HPO_4, 1.3; and NaH_2PO_4, 0.7 (pH = 7.0). This composition was the same as that used in our previous studies that examined the action of adrenaline on synaptic transmission in frog sympathetic ganglion neurons (for example see [38]). Although pH of this Ringer's solution is somewhat different from 7.4, a value typical for clinical studies, Ringer's solution having pH of 7.0 is frequently used in studies using frog nerve preparations (for instance see [39]).

2.2. Recordings of CAPs

As mentioned previously [25,36,37], the Lucite plate with the platinum wires attached to the sciatic nerve was moved from the beaker containing Ringer's solution to a vacant one, and CAPs were recorded in air using a preamplifier (Model LI-75A, NF Electronics Instruments, Yokohama, Japan; frequency response: DC-1 MHz; gain: 100). Two of the platinum wires were used to record CAPs, and another two were used to stimulate the sciatic nerve at 1 Hz with a stimulator, where rectangular pulses having 0.1 ms duration and various strengths were used. The air-gap method used in the present study measures the potential difference established by a flow of action potential current through the high resistance external to the sciatic nerve that is excited by electrical stimuli. CAPs recorded by this method remained unchanged over at least 1 h [23].

To prevent the sciatic nerve from drying out in air, this procedure was quickly (20 s at the most) performed with time intervals of 2 min. When the effects of drugs on CAPs were examined, the nerve was put back into the soaking solution containing drugs. The data were monitored on a storage oscilloscope while being recorded on a thermal array recorder with a wave form storage module and stored on a USB flash memory with a Data logger (mini LOGGER GL900; GRAPHTEC, Yokohama, Japan; 10 μs/sample) for later analyses. Stimulating the sciatic nerve produced a CAP following a stimulus artifact. The peak amplitude of the CAP was measured as the difference between baseline and peak CAP level, as mentioned previously [25,36,37] (see Fig. 1 in Ref. [23]). The peak amplitude of the CAP depended on the strength of the stimulus given to the sciatic nerve, and the CAP peak amplitude increased with increasing stimulus strength, eventually attaining a maximal value. As described previously [25,36,37], we analyzed the peak amplitude of the maximal CAP. The stimulus strengths used for the maximal CAP were in a range of 2.5–7.0 V. The conduction velocity was determined using the fifth electrode as an additional stimulation site and by measuring the duration between the stimulus artifact and the peak of the CAP. All experiments were carried out at room temperature (22–27 °C) that hardly varied during an experiment.

2.3. Data analysis

The concentration-dependence curve for the reduction of the peak amplitude of the CAP in the sciatic nerve soaked with a drug for 20 min was analyzed using the following Hill equation:

$$\text{CAP amplitude (\% of control)} = 100/(1 + ([\text{Drug}]/IC_{50})^{nH}),$$

where [Drug] is drug concentration, IC_{50} is the concentration of the drug for half-maximal inhibition and n_H is the Hill coefficient.

Data were indicated as mean ± S.E.M. and statistical significance was set at $P < 0.05$ using a paired Student's *t*-test. In all cases, *n* refers to the number of sciatic nerves studied. An average of the peak amplitudes of six CAPs measured during 10 min before drug application was taken as control.

2.4. Drugs

The drugs used were aceclofenac, acemetacin, etodolac, felbinac, flufenamic acid, sodium meclofenamate hydrate, mefenamic acid, meloxicam, *N*-phenylanthranilic acid, tolfenamic acid, 2,6-dichlorodiphenylamine, piroxicam, flurbiprofen (Tokyo Chemical Industries, Co. Ltd., Tokyo, Japan), aspirin, diclofenac sodium, ibuprofen, ketoprofen, loxoprofen sodium, (S)-(+)-naproxen, sulindac (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and indomethacin (Sigma-Aldrich, St. Louis, MO, USA). All of the drugs used were first dissolved in dimethyl sulfoxide (DMSO) and then diluted to the final concentration in Ringer's solution, where the concentration of DMSO was less than 1%. DMSO at 1% did not affect CAPs. Since an alteration in osmotic pressure may affect CAPs, drugs at concentrations larger than 2 mM were not tested. The NSAIDs were tested at concentrations less than their highest dissolvable concentrations. Each of sciatic nerves was used only once to examine the effect of a drug on CAPs; if there was no significant effect of a drug on CAPs owing to low drug concentration, a nerve was re-used in another experiment. The pH of Ringer's solution containing drugs was adjusted to 7.0 with NaOH.

3. Results

The effects of various NSAIDs on fast-conducting CAPs were examined in a total of 369 sciatic nerves, and the CAPs had an average peak amplitude of 23.3 ± 0.4 mV ($n = 369$). The conduction velocity of some of the fibers averaged to be 30 ± 1 m/s ($n = 263$). These values were comparable to those reported previously [25,36,37].

3.1. Effects of acetic acid-based NSAIDs on frog sciatic nerve CAPs

First, we examined the effect of an acetic acid-based NSAID diclofenac (Fig. 1Aa) on CAPs recorded from the frog sciatic nerve. Soaking the sciatic nerve in diclofenac (1 mM)-containing Ringer's solution for 20 min reduced the peak amplitude of the CAP (Fig. 1Ab). Fig. 1Ac illustrates an averaged time course of the change in CAP peak amplitude after incubation in diclofenac solution, relative to that before its application (control), obtained from 8 sciatic nerves. The diclofenac-