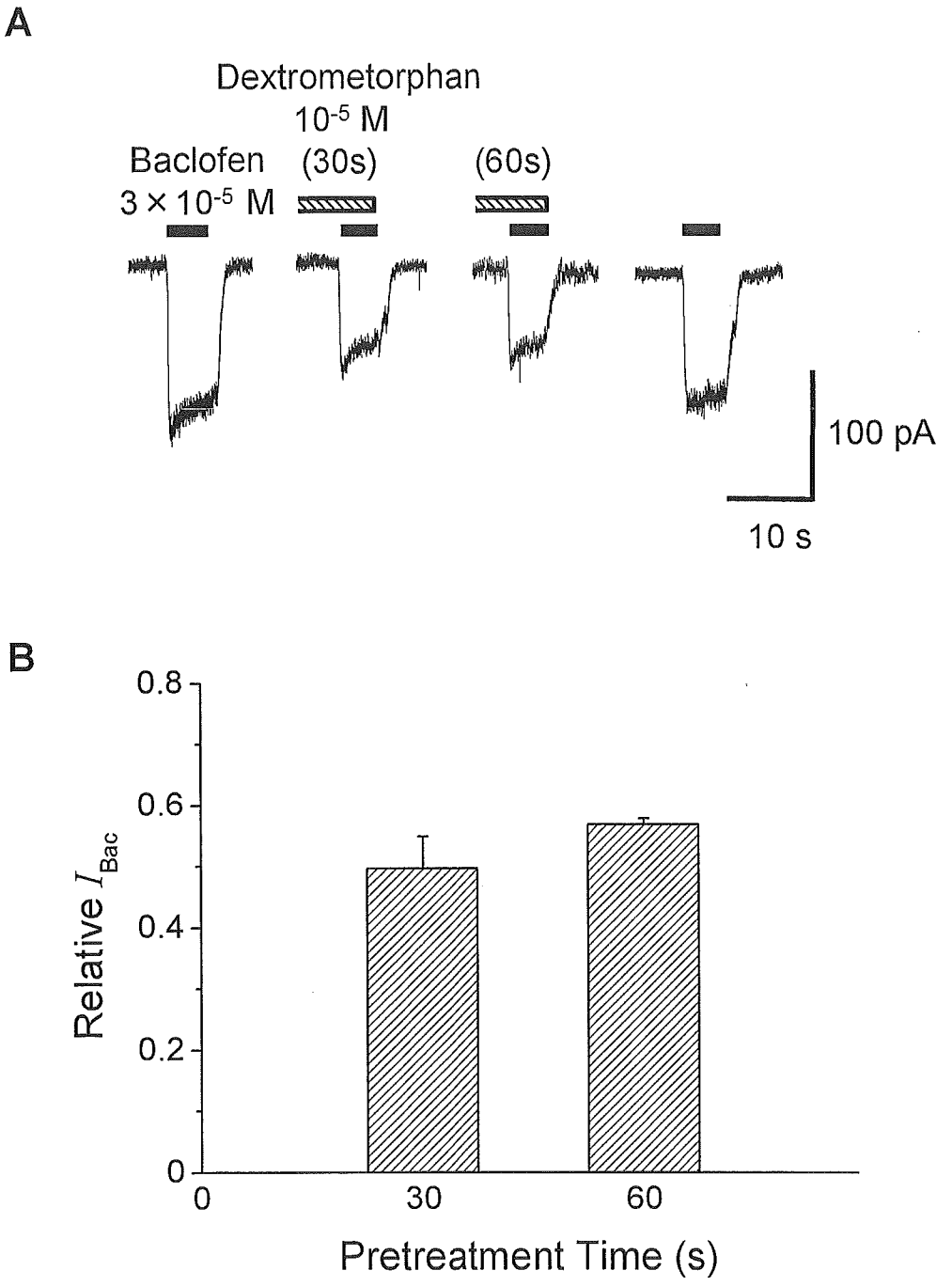
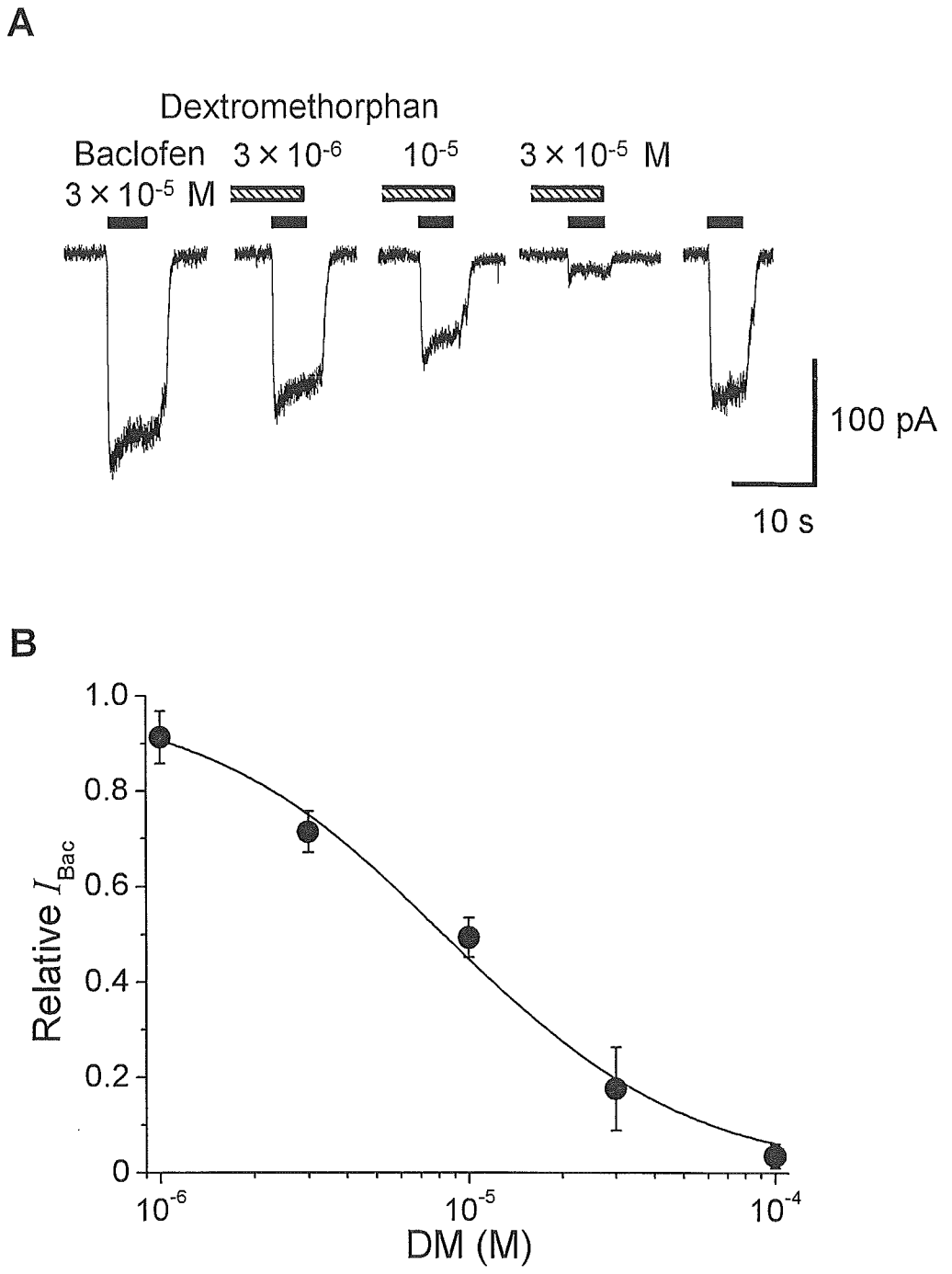
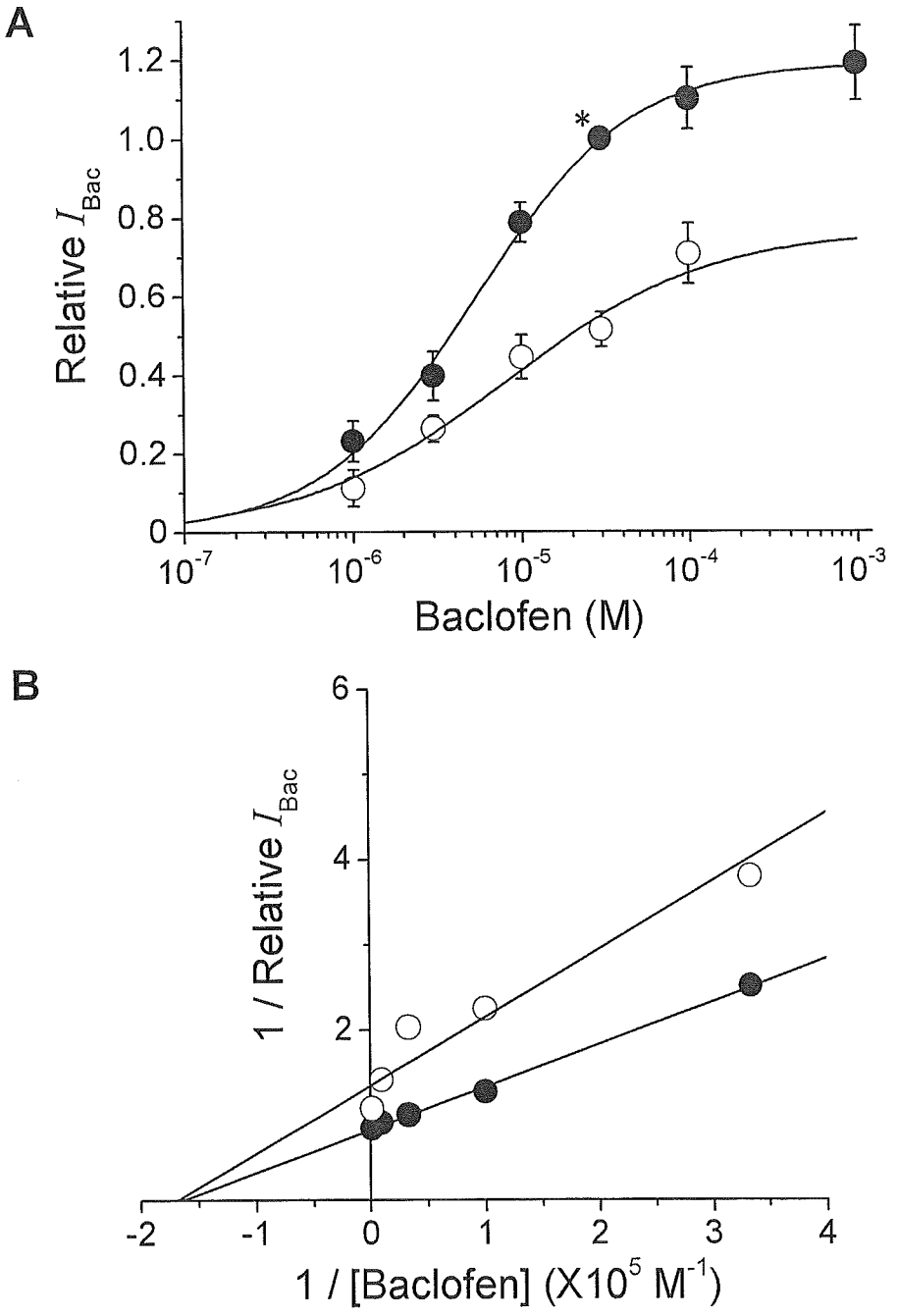
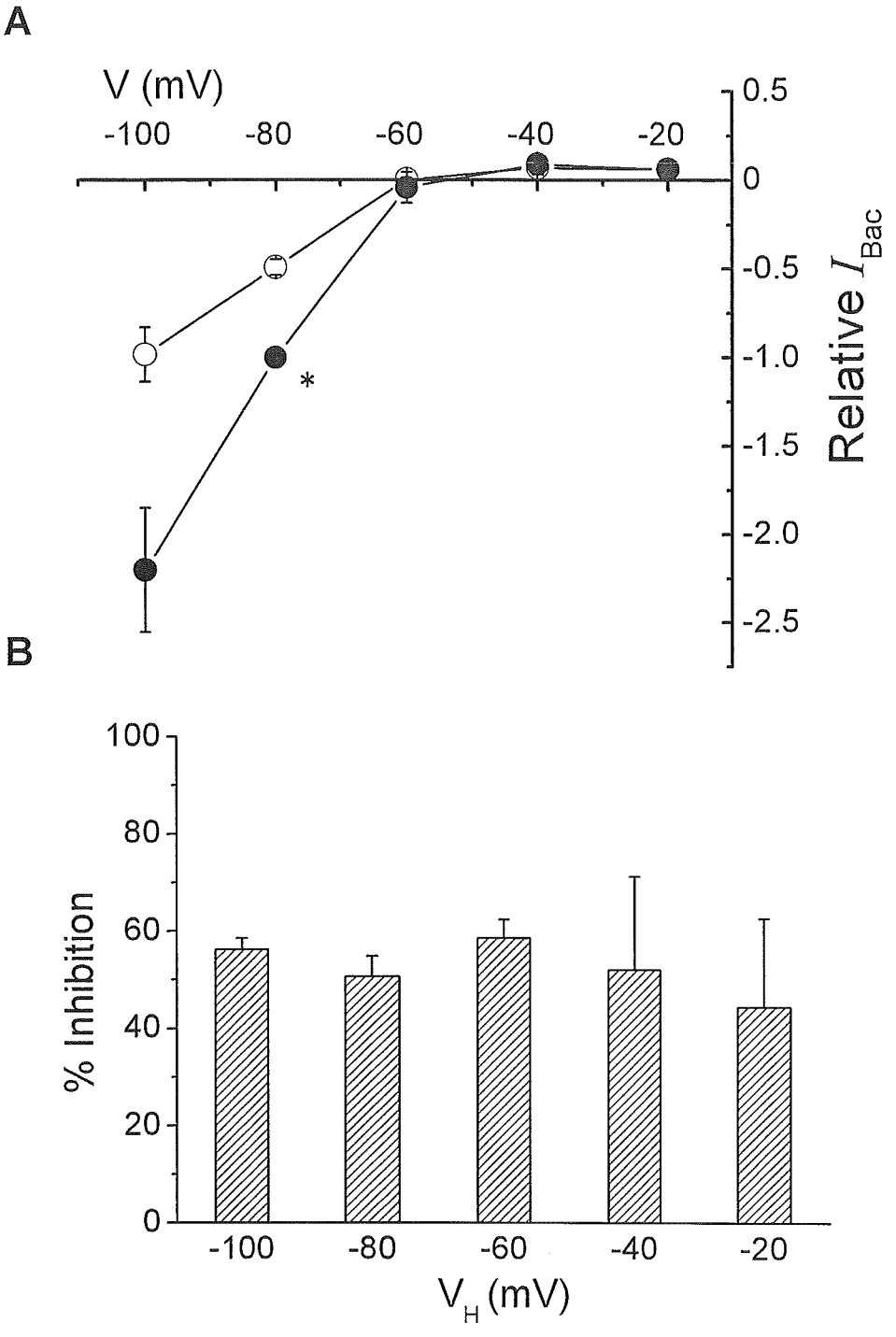
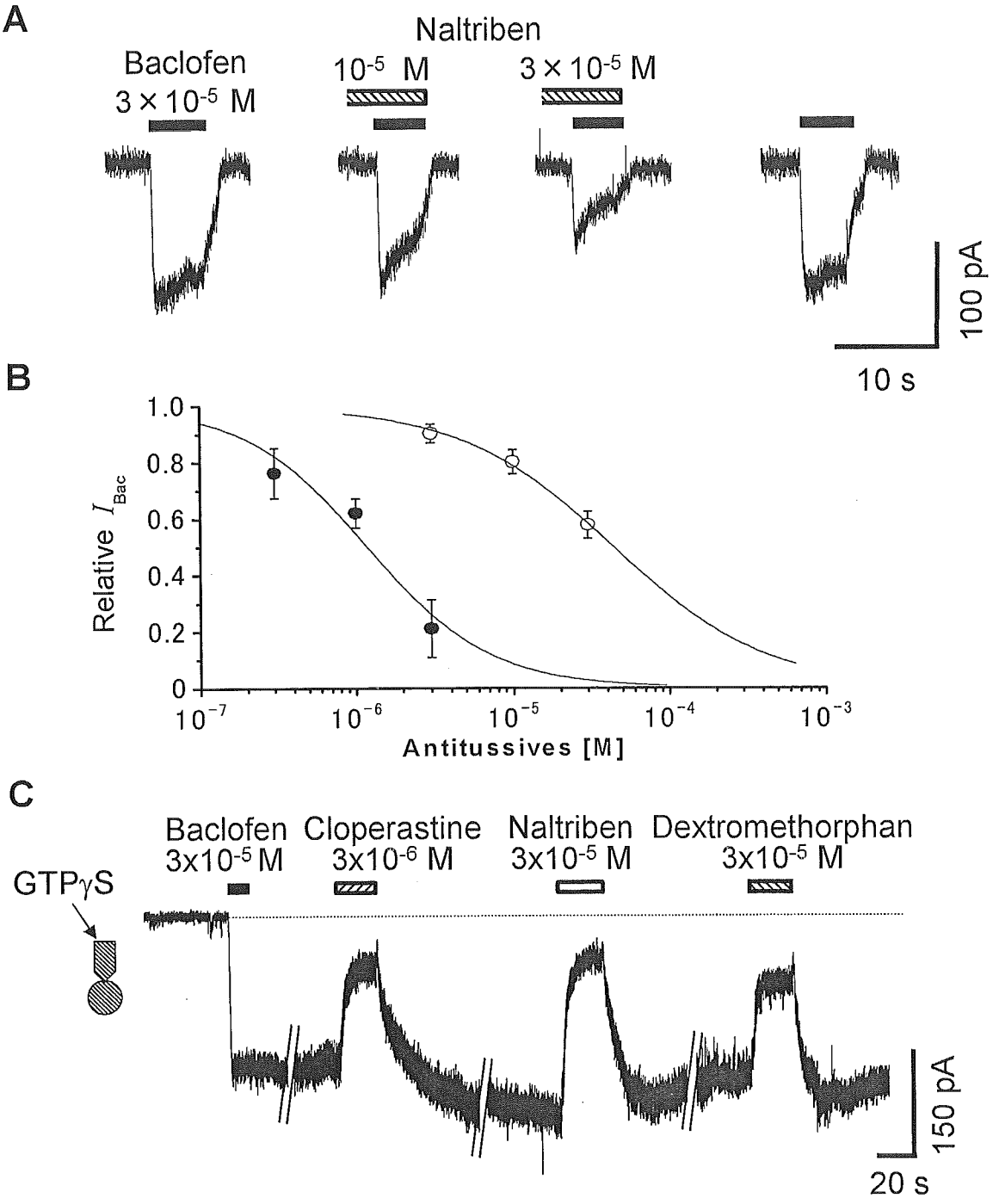
Fig. 5. Inhibitory effects of antitussives on I_{Bac}. A: Representative current traces illustrating the concentration-dependent inhibition of I_{Bac} by naltriben. Neurons were pretreated with naltriben for 30 sec before simultaneous application with 3×10^{-5} M baclofen. Neurons were held at a V_H of - 80 mV. B: Concentration-inhibition relationship for cloperastine (●) and naltriben (○). All current amplitudes were normalized to the current amplitude induced by 3×10^{-5} M baclofen alone. Data are represented as the means ± S.E.M. (n = 3). C: Effect of antitussives on the irreversibly activated current by baclofen in the presence of intracellular GTPγS. The neurones were intracellularly perfused with an internal solution containing 0.1 mM GTPγS by using the conventional whole-cell patch clamp recording configuration. Other recording condition was the same as that for the nystatin perforated mode of patch clamp.


A
Dextrometorphan
10-5 M
Baclofen
(30s)
(60s)
3 × 10-5 M
100 pA
10 s
B
0.8
0.6
0.4
0.2
0
Relative IBac
0
30
60
Pretreatment Time (s)


A
Dextromethorphan
Baclofen 3 × 10-6 10-5 3 × 10-5 M
3 × 10-5 M
100 pA
10 s
B
Relative IBac
1.0
0.8
0.6
0.4
0.2
0
10-6
10-5
10-4
DM (M)


A
Relative I_{Bac}
1.2
1.0
0.8
0.6
0.4
0.2
0
*
10^{-7}
10^{-6}
10^{-5}
10^{-4}
10^{-3}
Baclofen (M)
B
1 / Relative I_{Bac}
6
4
2
-2
-1
0
1
2
3
4
1 / [Baclofen] (X10^{5} M^{-1})


A
V (mV)
-100
-80
-60
-40
-20
0.5
0
-0.5
-1.0
-1.5
-2.0
-2.5
Relative I_{Bac}
*
B
100
80
60
40
20
0
% Inhibition
-100
-80
-60
-40
-20
V_H (mV)


A
Naltriben
Baclofen
3 × 10-5 M
10-5 M
3 × 10-5 M
100 pA
10 s
B
Relative IBac
1.0
0.8
0.6
0.4
0.2
0
10-7
10-6
10-5
10-4
10-3
Antitussives [M]
C
Baclofen
3x10-5 M
Cloperastine
3x10-6 M
Naltriben
3x10-5 M
Dextromethorphan
3x10-5 M
GTPγS
150 pA
20 s

Inhibition of Na^+ and K^+ currents by cloperastine in rat acutely dissociated dorsal raphe neurons

Sokichi Honda, Tetsuya Shirasaki, Fumio Soeda and Kazuo Takahama*

Department of Environtal & Molecular Health Sciences, Graduate School of Pharmaceutical Sciences, Kumamoto University.

5-1 Oe-honmachi, Kumamoto 862-0973, Japan

9 text pages and 2 figures

* Corresponding author

Kazuo Takahama, Ph.D. Professor
Department of Environmental & Molecular Health Sciences,
Graduate School of Pharmaceutical Sciences,
Kumamoto University
5-1 Oe-honmachi, Kumamoto 862-0973, Japan
Tel +81-96-371-4334
Fax +81-96-371-4334
E-mail takahama@gpo.kumamoto-u.ac.jp

Abstract

We investigated the effects of cloperastine on voltage dependent Na^+ and K^+ ~~ion~~ currents in rat acutely dissociated dorsal raphe neurons. Cloperastine did not affect the kinetics of sodium current (I_{Na}), transient potassium current (I_A) and delayed rectifier K^+ current (I_{KD}). However, cloperastine inhibited these currents in a concentration-dependent manner. In particular, cloperastine more potently inhibited the late component than the peak of I_A and I_{KD}. IC_{50} values for I_{Na}, $I_{A(peak)}$, $I_{A(late)}$, $I_{KD(peak)}$ and $I_{KD(late)}$ were $1.86x10^{-5}$ M, $2.60x10^{-6}$ M, $2.45x10^{-5}$ M, $5.20x10^{-5}$ M and $9.68x10^{-5}$ M, respectively. There results suggest that cloperastine has weak blocking actions on voltage dependent Na^+ and K^+ channels at higher concentrations.

Key words

cloperastine, voltage-gated channel, patch clamp, raphe neurons

Introduction

G-protein coupled inwardly rectifying K^+ (GIRK) channels widely distribute in the nervous system.[1] These channels couple with many $G_{i/o}$ -coupled receptors such as 5-HT_{1A} serotonergic, D_2 dopaminergic, and κ -, δ -, μ - opioid receptors.[2)-6)] Therefore, drugs that act on the GIRK channel should affect brain functions and cause some pharmacological effects. Only a few drugs, however, are known as those affecting the GIRK channel activities. We have recently found that dextromethorphan (DM), a non-narcotic centrally acting antitussives, inhibited GIRK channel activated current (I_{GIRK}) mediated by 5-HT_{1A} serotonergic receptors in single brain neurons.[7] In addition, DM inhibited I_{GIRK} mediated by α_2 adrenergic receptors, although it is unknown whether there is some differences in composition of subunits of GIRK channel between both receptors.[7] Furthermore, we found that cloperastine, which is a non-narcotic centrally acting antitussives and has a chemical structure quite different from that of DM, has a potent inhibitory effect on I_{GIRK} mediated by 5-HT_{1A} receptors.[8] The IC_{50} value for cloperastine of I_{GIRK} activated by 10^{-7} M 5-HT was 0.86 μ M. In general, substances that act on some channels or receptors often affect other channels and receptors. It is of interest to know whether or not cloperastine acts on other channels than the GIRK channel. In this study, we investigated the effects of cloperastine on sodium current (I_{Na}), transient potassium current (I_A) and delayed rectifier potassium current (I_{KD}), which play important roles in regulation of brain function resulted from determining the generation, transduction and frequency of action potential.

Materials and Methods

Dorsal raphe neurons were acutely dissociated from 8- to 16-day-old Wistar rats as described previously. In briefly, brainstem including dorsal raphe nucleus was sliced at a thickness of 400 μm with a microslicer (DTK-1000, Dosaka, Kyoto, Japan). The brainstem

slices were treated with pronase and thermolysin for 15-30 min each at 30°C, and dorsal raphe nucleus was dissected. Then, dorsal raphe neurons were mechanically dissociated. Membrane currents were recorded with the perforated patch clamp technique at room temperature (20-27℃). Membrane potential was held at –80mV. Normal external solution contained (in mM): NaCl 131.7, KCl 5, $CaCl_2$ 2.5, $MgCl_2$ 1.2, glucose 11.5 and HEPES 10 at pH 7.4. Sodium currents were recorded in the external solution containing (in mM); NaCl 60, CsCl 5, TEA 20, $MgCl_2$ 10, 4-AP 5, glucose 5, sucrose 88 and HEPES 10. The pH was adjusted to 7.4 with HCl. Pipette solution was contained (in mM); CsCl 140, NaCl 10 and HEPES 10. The pH was adjusted to 7.2 with CsOH. For recording potassium currents, pipette solution was contained (in mM); KCl 140, choline Cl 10 and HEPES 10. The pH was adjusted to 7.2 with KOH. Delayed rectifier potassium currents were recorded in the external solution containing (in mM); choline Cl 97.5, 4-AP 30, HCl 25, KCl 5, $MgCl_2$ 10, glucose 5 and HEPES 10. The pH was adjusted to 7.4 with HCl. Transient potassium currents were recorded in the external solution containing (in mM), choline Cl 95; TEA Cl 30, KCl 5, $MgCl_2$ 10, glucose 5 and HEPES 10. The pH was adjusted to 7.4 with HCl.

Animals were treated in accordance with the Guidelines of the Japanese Pharmacological Society and Kumamoto University for the Care and Use of Laboratory Animals.

Results

Ionic current was isolated by using the external and internal solutions described above. In the solutions for I_{Na}, brief depolarization from the holding potential (V_H) of -80 mV to 0 mV induced rapidly activating and inactivating inward current in acutely dissociated dorsal raphe neurons. The current-voltage relationship for this inward current showed typical form for I_{Na} and the current amplitude was the largest at -20 mV (data not shown). Therefore, we studied the effect of cloperastine on I_{Na} at this potential.

Cloperastine inhibited the peak current amplitude of I_{Na} (Fig. 1). The potency of the inhibition did not depend on the treatment time of 30 to 90 sec. The inhibition was ~~in~~ concentration-dependent and 50 % inhibition was achieved at 1.86×10^{-5} M (Fig. 2). However, this drug had little effect on the activation and inactivation kinetics of I_{Na} (Fig. 1).

In the presence of external 4-AP and the absence of internal and external Na^+ and Ca^{2+}, very small outward current was recorded by the voltage step from the V_H of -80 to -60 mV. By the voltage step to -40 mV, the current become significant and had slow activation and very slow inactivation kinetics. The amplitude became larger, depending on the size of voltage step from the V_H. These characteristics were well agreed with those for I_{KD}. Cloperastine did not affect on the activation kinetics of I_{KD} activated at +40 mV. However, it inhibited the amplitude of I_{KD} and slightly facilitated the inactivation (Fig. 1). The potency of the inhibition did not depend on the treatment time of 30 to 90 sec. The inhibition was concentration dependent and inactivation become much faster at higher concentrations. When concentration-inhibition relationship was studied, the concentration to achieve 50 % inhibition of the peak of I_{KD} ($I_{KD\text{-peak}}$) and the late component of I_{KD} recorded at the end of voltage step for 400 msec ($I_{KD\text{-late}}$) were 9.68×10^{-5} M and 5.20×10^{-5} M, respectively.

In the presence of external TEA and the absence of internal and external Na^+ and Ca^{2+}, transient outward current was recorded by the voltage step from the V_H to -40 mV. By the step to -60 mV, this transient current was not recorded. On the other hand, the current become larger by the step to more than -20 mV. From the kinetics and the current-voltage relationship (not shown), this current was confirmed as an A type transient K^+ current. Cloperastine inhibited the amplitude and facilitated the inactivation of I_A activated by the step to +40 mV (Fig. 1). The inhibition became stable within 30 sec of treatment. The inhibition was concentration dependent and inactivation become much faster at higher concentrations. The concentration to achieve 50 % inhibition of the peak of I_A ($I_{A\text{-peak}}$) and the late component of

I_A recorded at the end of voltage step for 400 msec ($I_{A\text{-late}}$) were 2.60 x10^{-4} M and 2.40 x10^{-5} M, respectively.

Discussion

In the present study, cloperastine concentration-dependently inhibited three types of voltage-gated channel currents in DRN neurons. But the inhibitory effects were much less potent. On the other hand, we have previously found that the IC_{50} value for cloperastine of the GIRK channel activated current was 8.6x10^{-7} M. Therefore, cloperastine is at least 20-fold more potent in inhibiting GIRK channel than these three channels.

Recently, a few substances have been reported to have an inhibitory effect on GIRK channel activated currents. These are fluoxetine, a selective serotonin reuptake inhibitor (SSRI)[9], bupivacaine[10], a local anesthetic, and so on. However, these non-peptide substances are less potent as inhibitor of GIRK channel, since micromolar concentrations are needed to inhibit the GIRK channel-activated currents. Further, our preliminary study revealed that cloperastine at a large concentration of 10^{-4} M had little effect on glycine-induced and NMDA-induced currents in single brain neurons[8]. Judging from the electrophysiological results obtained thus far, cloperastine should be the most potent non-peptide inhibitor of GIRK channel activated currents. In this context, it is reasonable to mention that cloperastine might be useful as a seed compound for developing a more potent inhibitor of GIRK channel activated currents or a potent GIRK channel blocker.

We have previously reported that cloperastine has ameliorating effect on urinary disturbance caused by cerebral infarction in conscious rat[11], and further that it inhibited hyperactivities caused by repetitive methamphetamine administration in mice[12]. The present results support an idea that pharmacological effects of cloperastine on urinary disturbance and methamphetamine-induced hyperactivities might be at least partly due to its GIRK channel blocking effect.

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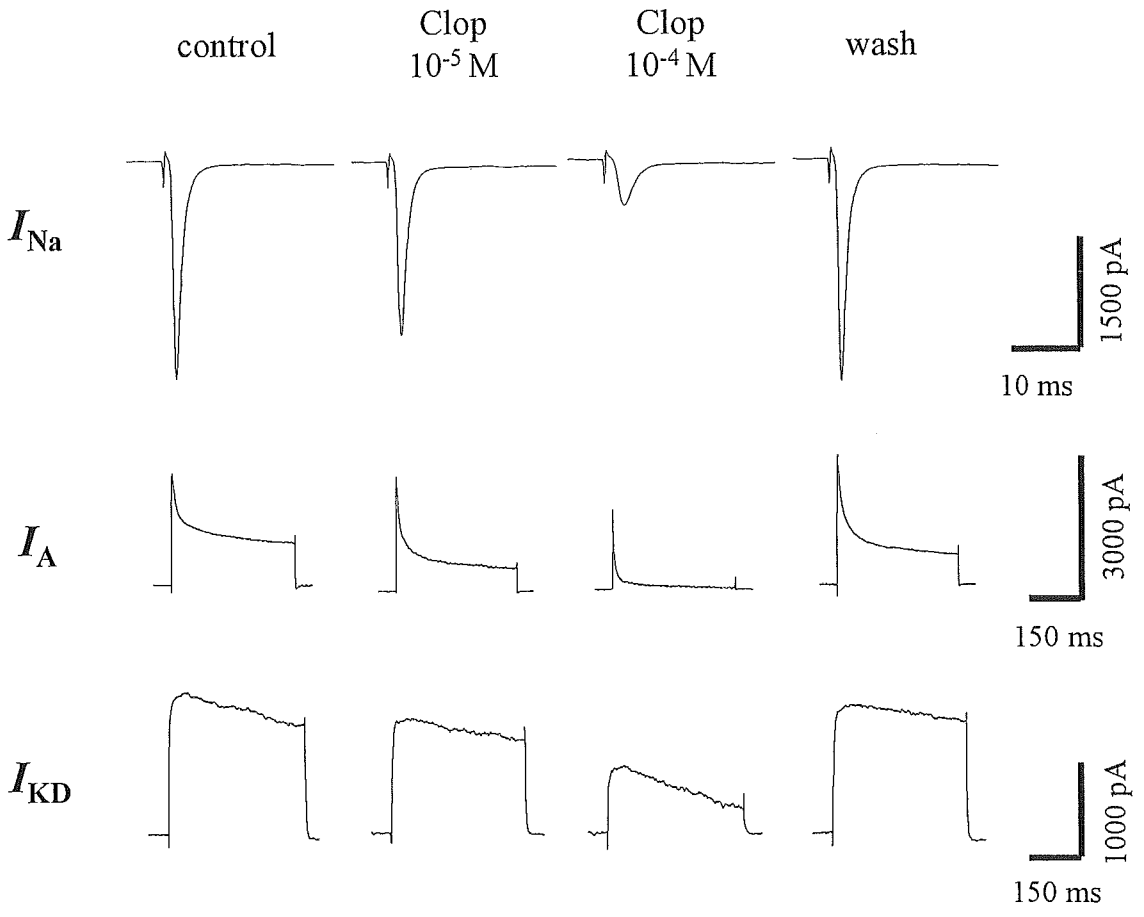
Figure Legends

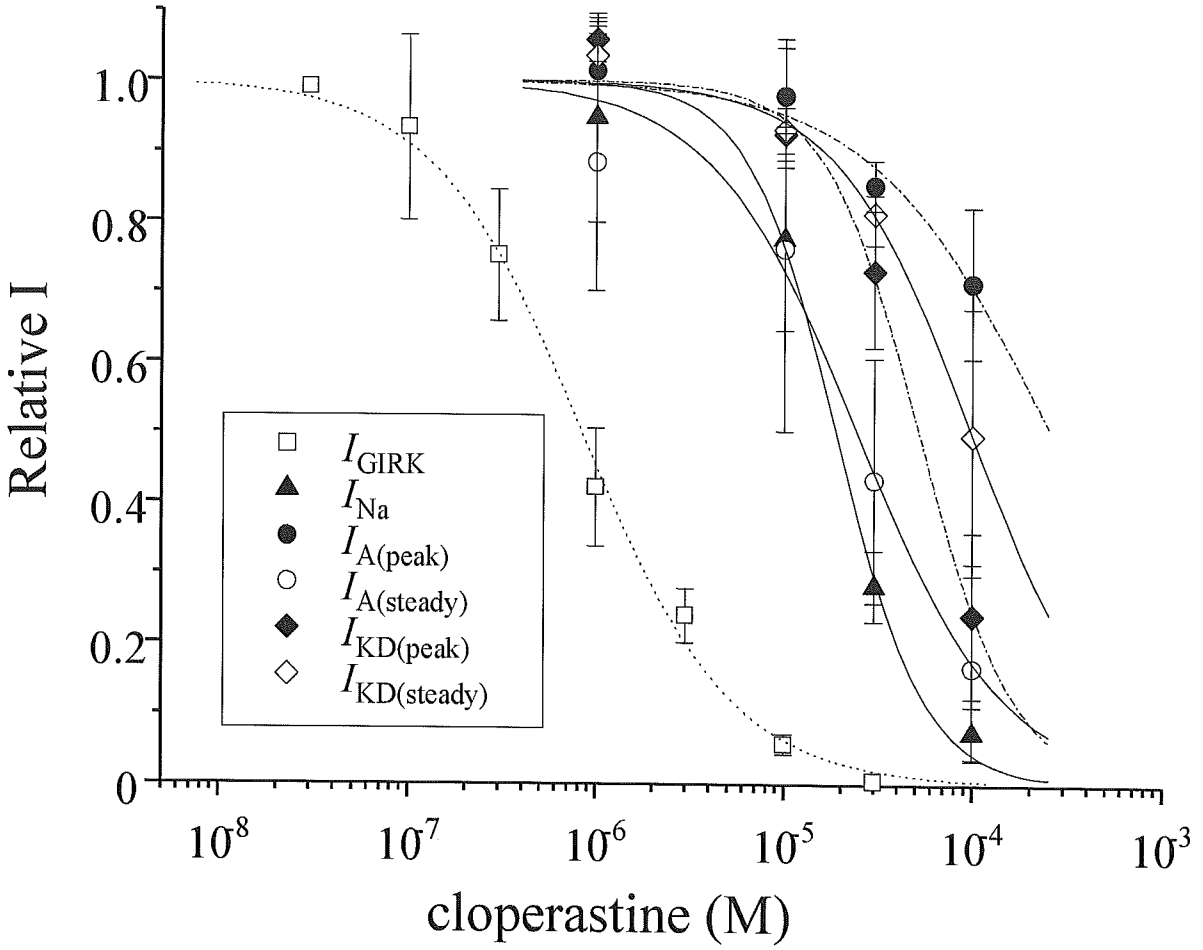
Fig. 1.

Representative current traces of I_{Na}, I_{KD} and I_A in the absence or presence of cloperastine (Clop). I_{Na} was evoked by a voltage step from a holding potential of -80 mV to -20 mV for 50 msec. I_A and I_{KD} were also evoked by a voltage step from a holding potential of -80 mV to 40 mV for 400 msec, respectively.

Fig. 2.

Dose-inhibition relationship showing the effect of cloperastine on I_{Na}, $I_{A\,(peak)}$, $I_{A\,(late)}$, $I_{KD\,(peak)}$ and $I_{KD\,(late)}$. The relationship for the inhibition of cloperastine on 5-HT-induced GIRK channel current was also indicated together for comparison. Data were shown as mean$\pm$ S.E.M. (n = 3).


control
Clop
10-5 M
Clop
10-4 M
wash
INa
1500 pA
10 ms
IA
3000 pA
150 ms
IKD
1000 pA
150 ms


Relative I
1.0
0.8
0.6
0.4
0.2
0
I_{GIRK}
I_{Na}
$I_{A(peak)}$
$I_{A(steady)}$
$I_{KD(peak)}$
$I_{KD(steady)}$
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
10^{-3}
cloperastine (M)

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発行者　主任研究者　高　濱　和　夫
熊本大学大学院医学薬学研究部
環境分子保健学分野
熊本市大江本町5番1号
Tel 096-371-4334

製本　(有)米　田　印　刷
熊本市坪井6丁目21-15
Tel　096-345-0150