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Research report

Presynaptic facilitation by tetracaine of glutamatergic spontaneous excitatory transmission in the rat spinal substantia gelatinosa – Involvement of TRPA1 channels

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ABSTRACT

The amide-type local anesthetic (LA) lidocaine activates transient receptor potential (TRP) ankyrin-1 (TRPA1) channels to facilitate spontaneous L-glutamate release onto spinal substantia gelatinosa (SG) neurons, which play a crucial role in regulating nociceptive transmission. In contrast, the ester-type LA procaine reduces the spontaneous release of L-glutamate in SG neurons. In order to determine whether TRPA1 activation by LAs is specific to amide-types, we examined the actions of tetracaine, another ester-type LA, and other amide-type LAs on glutamatergic spontaneous excitatory transmission in SG neurons by focusing on TRP activation. Whole-cell patch-clamp recordings were performed on SG neurons of adult rat spinal cord slices at a holding potential of −70 mV. Bath-applied tetracaine increased spontaneous excitatory postsynaptic current (sEPSC) frequency in a concentration-dependent manner. Tetracaine activity was resistant to the voltage-gated Na^+-channel blocker tetrodotoxin, the TRP vanilloid-1 antagonist capsazepine, and the TRP melastatin-8 antagonist BCTC, but was inhibited by the non-selective TRP antagonist ruthenium red and the TRPA1 antagonist HC-030031. With respect to amide-type LAs, prilocaine had a tendency to increase sEPSC frequency, while ropivacaine and levobupivacaine reduced the frequency. In conclusion, tetracaine facilitated spontaneous L-glutamate release from nerve terminals by activating TRPA1 channels in the SG, resulting in an increase in the excitability of SG neurons. TRPA1 activation was not specific to amide-type or ester-type LAs. The facilitatory action of LAs may be involved in pain occurring after recovery from spinal anesthesia.

1. Introduction

Transient receptor potentials (TRPs), which are non-selective cation channels, play a role in conveying nociceptive information to the central nervous system through dorsal root ganglion (DRG) neuronal fibers from the periphery (Patapoutian et al., 2009). For instance, in the peripheral terminals of DRG neurons, TRP vanilloid-1 (TRPV1) is activated by noxious hot temperature, protons, and capsaicin (a component of red peppers); TRP ankyrin-1 (TRPA1) is activated by noxious cold temperature, allyl isothiocyanate (AITC; a pungent ingredient of wasabi), and cinnamaldehyde (CA; the main component of cinnamon); and TRP melastatin-8 (TRPM8) is activated by mild cool temperature and menthol (the chief constituent of peppermint). Such channel activation depolarizes membranes of the terminals, resulting in the production of action potentials (APs) in neuronal fibers. In contrast, the activation of TRPV1, TRPA1, and TRPM8, which are expressed in the central terminals of DRG neurons, leads to the enhancement of the spontaneous release of L-glutamate in the spinal substantia gelatinosa (SG; lamina II of Rexed) neurons (Jiang et al., 2016; Kosugi et al., 2007; Kumamoto et al., 2014; Yang et al., 1998; Yue et al., 2013) which plays a crucial role in the regulation of nociceptive transmission from the periphery (Willis and Coggeshall, 1991).

TRPs are activated not only by plant-derived chemicals, but also a variety of chemical substances including general anesthetics and local anesthetics (LAs). General anesthetics, such as isoflurane and propofol, activate TRPA1 expressed in heterologous cells and DRG neurons (Matta et al., 2008). Isoflurane does not activate TRPV1,

Abbreviations: AITC, allyl isothiocyanate; AP, action potential; CA, cinnamaldehyde; DMSO, dimethyl sulfoxide; DRG, dorsal root ganglion; LA, local anesthetic; sEPSC, spontaneous excitatory postsynaptic current; SG, substantia gelatinosa; TNS, transient neurologic symptom; TRP, transient receptor potential; TRPA1, TRP ankyrin-1; TRPM8, TRP melastatin-8; TRPV1, TRP vanilloid-1; TTX, tetrodotoxin; V_H, holding potential.

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but sensitizes its activation by protons and capsaicin (Cornett et al., 2008). The LA lidocaine activates TRPV1 (Leffler et al., 2008) and also, to a lesser extent, TRPA1 (Leffler et al., 2011) in DRG neurons. In contrast, TRPA1, but not TRPV1, in the SG is activated by lidocaine; however, procaine, another LA, had no effect on TRPs (Piao et al., 2009).

LAs are classified into two types, i.e., amide-type LAs such as lidocaine, and ester-type LAs such as procaine (Catterall and Mackie, 2011). LAs not only have a local anesthetic action, but also have neurotoxic properties. There was a difference among LAs in the extent of neurotoxicity following spinal anesthesia in humans (Hampl et al., 1998; Rigler et al., 1991; Zaric et al., 2005) and animals (Johnson, 2000; Kishimoto et al., 2002; Ready et al., 1985; Takenami et al., 2009), increase in L-glutamate concentration in cerebrospinal fluid microdialysate following intrathecal administration in rabbits (Yamashita et al., 2003), apoptosis in rat cortical astrocytes and human neuroblastoma cell lines (Lee et al., 2009; Werdehausen et al., 2009), and inhibition of AP conduction in frog sciatic nerve fibers (Uemura et al., 2014). In order to reveal what kinds of LA mimic lidocaine activity in the SG and whether there is a difference in activating TRPs among LAs, we examined the effect of the ester-type LA (tetracaine) and amide-type LAs (ropivacaine, levobupivacaine, and prilocaine; see Fig. 1 for their chemical structures) on glutamatergic spontaneous excitatory transmission by focusing on TRP activation, where whole-cell patch-clamp recordings were performed from SG neurons of adult rat spinal cord slices.

2. Results

Whole-cell patch-clamp recordings were performed from a total of 252 SG neurons. Stable recordings could be obtained from slices maintained *in vitro* for more than 10 h, and recordings were obtained from single SG neurons for up to 3 h. All SG neurons examined had resting membrane potentials lower than −60 mV and exhibited spontaneous excitatory postsynaptic currents (sEPSCs) at a holding potential (V_H) of −70 mV, which was close to the reversal potential for inhibitory postsynaptic currents (Piao et al., 2009).

2.1. Effect of tetracaine on spontaneous excitatory transmission in SG neurons

Bath-applied tetracaine for 2 min facilitated spontaneous excitatory transmission in SG neurons. This facilitatory action was concentration-dependent in a range of 1–5 mM. Fig. 2Aa, Ab, and Ba show recordings of sEPSCs under the actions of tetracaine at 1, 2, and 5 mM, respectively. Tetracaine activity was because of an increase in the frequency of sEPSC, which was accompanied by a small increase in its amplitude (Fig. 2C).

We next examined a detail of the effect of tetracaine at 5 mM on spontaneous excitatory transmission. As shown in Fig. 2Bb, sEPSC frequency augmented gradually over time, peaking around 4 min after tetracaine addition; this increase disappeared within 10 min after tetracaine removal. Tetracaine increased the proportion of sEPSCs having a shorter inter-event interval and a larger amplitude (Fig. 2Bc); this action was confirmed in three other neurons.

In 92% of the neurons tested ($n = 86$), sEPSC increased in frequency >5% after the end of tetracaine (5 mM) superfusion. The average frequency increased by 307 ± 28% ($P < 0.05$) from 10.1 ± 0.8 Hz (control; before the application of tetracaine) to 30.8 ± 1.4 Hz ($n = 79$) approximately 4 min after its addition (Fig. 2C). In some neurons, the sEPSC frequency increase occurred together with an inward current or a current having both an inward and outward component (Fig. 2Ba), as seen with lidocaine

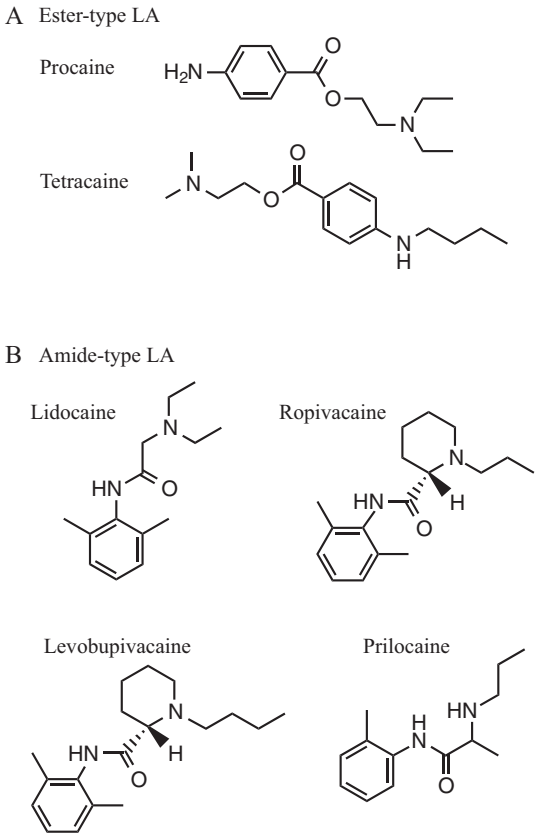

Fig. 1. Chemical structures of the local anesthetics (LAs) used. (A, B) Ester-type LAs (A: procaine and tetracaine) and amide-type LAs (B: lidocaine, ropivacaine, levobupivacaine, and prilocaine).

(5 mM; Piao et al., 2009). The inward and outward currents had average peak amplitudes of 30.1 ± 2.5 pA ($n = 73$) and 17.3 ± 1.3 pA ($n = 39$), respectively.

Spontaneous excitatory transmission enhancement following exposure to the TRPV1 agonist capsaicin did not recover from desensitization 1 h after its washout (Yang et al., 1998) while that by the TRPA1 agonist AITC occurred repeatedly at a time interval of 20 min (Kosugi et al., 2007). We therefore investigated whether tetracaine (5 mM) repeatedly facilitates spontaneous excitatory transmission. As seen in Fig. 3Aa, the response to tetracaine was repeated at 20 min intervals. Relative to the control, sEPSC frequency and amplitude under the action of tetracaine following initial application were almost comparable to the second application (Fig. 3Ab), indicating that tetracaine activity was consistent with the recovery from desensitization following TRPA1, but not TRPV1 activation.

Because tetracaine (5 mM) repeatedly increased sEPSC frequency, we next investigated how various drugs affect this increase in the same neuron to reliably know the effect of the drug. Tetracaine activity was not affected by pretreatment with the voltage-gated Na^+-channel blocker tetrodotoxin (TTX; 0.5 μM) for 3–4 min, as seen in Fig. 3Ba. Relative to the control, sEPSC frequency and amplitude under the action of tetracaine in the presence of TTX did not differ from those in the absence of TTX (Fig. 3Bb), indicating that the effect of tetracaine occurred without an increase in neuronal activity.

2.2. TRPA1, but not TRPV1 and TRPM8, activation mediates the presynaptic activity of tetracaine

In order to reveal whether the presynaptic effect of tetracaine (5 mM) is mediated by TRPs, as seen with lidocaine (Piao et al.,