Effects of CP-99,994, an NK₁-Receptor Antagonist, on the Inward Current Produced by Substance P

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Immunoreactivities of mammalian tachykinins have been widely demonstrated in the peripheral and central nervous systems (Ogawa et al. 1985; Leeman et al. 1991; Otsuka and Yoshioka, 1993). Dalsgaard et al. (1985) reported the co-existence of substance P(SP) and neurokinin A (NKA)-like immunoreactivities in neurons of rat dorsal root ganglia (DRG) and the dorsal horn of the spinal cord. Recently, molecular cloning of cDNAs revealed three distinct types of tachykinin receptors, NK₁, NK₂ and NK₃, that belong to the GTP binding protein-coupled receptor superfamily (Masu et al. 1987; Hershey and Krause, 1990; Shigemoto et al. 1990). Electrophysiological studies demonstrated that NKA and neurokinin B (NKB) depolared the neuronal membrane of sympathetic ganglia (Saria et al. 1985; Konishii et al. 1992). Under voltage-clamp conditions, application of SP and NKA produced inward currents that underlie the tachykinin-induced depolarizations in neurons of the bullfrog DRG (Ishimatsu, 1994). The purpose of the present study is to examine the receptor type for SP that produces the inward current in bullfrog DRG cells by using newly identified non-peptide antagonists (Snider et al. 1991; McLean et al. 1993; Watson and Girdlestone, 1994).

The methods for the isolation of single neurons of the DRG are essentially the same as those described previously (Tokimasa and Akasu, 1990). DRG cells were dispersed in a Ringer solution containing trypsin (Sigma type XI; 2.5 mg/ml) and collagenase (Sigma type A; 0.5 mg/ml) at 36°C for 15-30 min. Ringer solution had the following composition (mM): NaCl, 112; KCl, 2; CaCl₂, 1.8; and NaHCO₃, 2.4. The pH of the superfusate was adjusted to 7.2. The dissociated cells were stored for 2-4 days at 4°C in Leibovitz’s L-15 medium (GIBCO 320-1415); 10-20% fetal bovine serum (GIBCO 200-6140 AG) was added and the medium was diluted to 80% with water in 35 mm culture dishes (Falcon 3001). Pipettes for the whole-cell clamp

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had a tip resistance of 3-6 MΩ, when filled with a pipette solution of the following composition (mM): KCl, 100; MgCl₂, 4; adenosine 5'-triphosphate sodium (Na₂-ATP), 5; 1,2-bis (o-aminophenoxy) ethane-N,N',N'-tetraacetic acid tetraacetoxymethyl salt (BAPTA), 1; and HEPES (sodium salt), 2.5 (pH adjusted with KOH to 7.0). A gigaohm-seal (≥1 GΩ) contact between the recording pipette and cell membrane was established by adding a gentle negative pressure to the recording pipette. A sample-and-hold voltage-clamp amplifier (Axoclamp 2A: Axon Instruments) was used at a switching frequency of 10-17 kHz. All experiments were carried out at 22-24°C. The statistics are expressed as mean ± S.E. of the mean for the cells tested.

Under whole-cell patch-clamp conditions, acutely dissociated neurons of the bullfrog DRG had a mean resting membrane potential of -72.2±1.6 mV (n=163) and a mean input resistance of 224±60 MΩ (n=124) in the Ringer solution. It has been reported that SP produces an inward current by suppressing a voltage-dependent, non-inactivating K⁺ current, the M-current (IM). To record the IM, DRG cells were superfused with a modified Ringer solution containing tetrodotoxin (3 μM), tetraethylammonium (30 mM), Cs⁺ (2 mM), 4-aminopyridine (2 mM) and apamin (50 nM) (Tokimasa and Akasu, 1990). Figure 1 shows examples of the effects of tachykinins on the IM at -30 mV. Application of SP (1 μM) by a series of three pressure pulses (10 psi for 30 ms) produced an inward current associated with a decreased membrane conductance in 42 of 45 neurons. The remaining cells had no significant change in the membrane current. The effect of SP in producing the inward current was compared with other mammalian tachykinins, NKA and NKB, in the same DRG cell. NKA (1 μM) induced an inward current, while no obvious current response was produced by NKB (1 μM) (Fig. 1). Analysis with concentration-response relationships for the SP-induced inward current was made at a holding voltage of -30 mV. The minimum effective concentration of SP was 3 nM, which produced an inward current with an amplitude of 0.10±0.06 nA (n=5). At a concentration of 10 nM,
SP induced a half-maximal response with a mean amplitude of 0.65±0.08 nA (n=8). The maximal response with an amplitude of 1.2±0.4 nA was produced by 1μM SP (n=8). NKA (3 nM-1 μM) also produced the inward current in a concentration-dependent manner. The amplitude of inward currents produced by 1 μM NKA was 1.3±0.4 nA (n=8). The concentration of NKA that produces the half-maximal response of the NKA-induced inward current was 9 nM (n=8). In contrast, NKB, at a concentration of 1 μM, produced a small inward current with an amplitude of less than 0.2 nA (n=4). Thus, the rank order of the agonist potency was NKA=SP>NKB. The effects of receptor antagonists on these tachykinin-induced responses were examined. Spantide, [D-Arg¹, D-Trp⁷⁹, Leu¹¹]SP (1 μM) which is a classical peptide antagonist for SP receptors, did not block the inward current produced by SP (30 nM) in five cells (Fig. 2A). Recently, (2S,3S)-3-(2-methoxybenzyl)amino-2-phenylpiperidine dihydrochloride (CP-99,994) and (2S,3S)-cis-2-(diphenylmethyl)-N-((2-methoxyphenyl)methyl)-1-azabicyclo[2.2.2]octan-3-amine (CP-96,345) have been characterized as NK₁-receptor antagonists (Snider et al. 1991; McLean et al. 1993; Watson and Girdlestone, 1994). The effects of these antagonists on the SP-induced inward current were examined in bullfrog DRG cells (Fig. 2B). Bath-application of CP-99,994 (1 μM) produced an inward shift of the holding current with an amplitude of 0.1-0.5 nA. In five DRG cells, the SP (30 nM)-induced inward current was depressed by 63±8% (n=6) by CP-99,994 (1 μM). The depression of the SP-induced inward current by 1 μM CP-99,994 was poorly reversible, even when DRG cells were rinsed with control solution for more than 30 min. On the other hand, CP-96,345 (1 μM) did not inhibit the inward current produced by SP (30 nM).

Mammalian tachykinins have been reported to produce many pharmacological effects at different tachykinin receptor types in various tissues. In contrast, the neuronal tachykinin receptor, mediating the depolarizing responses in rat autonomic neurons, does not cor-

![Fig. 2. A: the effect of spantide (1 μM) on the SP-induced inward current in a DRG neuron. The holding membrane potential was -60 mV. The upper and lower records were obtained before and 10 min after the application of spantide. B: the effect of CP-99,994 (1 μM) on the inward current produced by SP (30 nM). The upper and lower records were obtained before and 10 min after the application of CP-99,994.](image-url)
relate with a particular type of currently classified NK1, NK2 or NK3 receptor (Konishi et al. 1992). The present study demonstrated that SP and NKA (3nM-1 μM) produced inward currents with similar potencies in bullfrog DRG neurons. In contrast, NKB did not induce a significant inward current even at a concentration of 1μM. The rank order of agonist potency indicates that an NKi-receptor is responsible for the SP-induced inward current in primary afferent neurons. In support of this, CP-99,994, a newly identified NKi-receptor antagonist (Snider et al. 1991; McLean et al. 1993; Watson and Girdlestone, 1994), reduced the SP-induced inward current. However, further experiments are necessary to determine the type of tachykinin receptor in bullfrog DRG neurons, because the other NKi-receptor antagonist, CP-96, 345, did not inhibit the SP-induced inward current. Tachykinin is a putative transmitter peptide in primary sensory neurons which probably mediates the transmission of pain information (see Review Otsuka and Yoshioka, 1993). SP enhances or depresses noxious responses in the substantia gelatinosa cells of the cat dorsal horn, by modifying both the release of a sensory transmitter and the excitability of the postsynaptic membrane (Henry, 1980; Otsuka and Yanagisawa, 1987; Otsuka and Yoshioka, 1993). Activation of NKi-receptors located on pre- or post-synaptic membranes may lead to a modulation of pain transmission in the spinal cord.

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References

SHORT COMMUNICATION


