Role of Protein Kinase in the Substance P-Induced Inhibition of the GABA Response in Neurons of the Bullfrog Dorsal Root Ganglia

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The GABA<sub>α</sub> receptor is known to be the site of action for a variety of drugs with hypnotic, anaesthetic and anticonvulsant properties (see Reviews, Bormann, 1988; Sieghart, 1992; Macdonald and Olsen, 1994; Smith and Olsen, 1995). The function of the GABA<sub>α</sub> receptor is also modulated by intracellular substances, such as Ca<sup>2+</sup> in DRG neurons (Inoue et al. 1986; Taleb et al. 1987; Behrends et al. 1988; Llano et al. 1991; Kano et al. 1992; Mulle et al. 1992) and ATP in guinea pig hippocampal neurons and chick spinal cord neurons (Gyenes et al. 1988, 1994; Stelzer et al. 1988; Shirasaki et al. 1992). Phosphorylation and dephosphorylation of receptor-associated chloride channels have been considered as an important step in the modulation of GABA<sub>α</sub> receptor function (Nestler and Greengard, 1984; Stelzer et al. 1988; Chen et al. 1990; Chen and Wong, 1995). Recently, it was reported that substance P (SP) inhibited a GABA<sub>α</sub>-induced inward current in primary afferent neurons of the bullfrog dorsal root ganglia (DRG) (Yamada and Akasu, 1996). In the present study, a role of a protein kinase in the SP-induced inhibition of the GABA<sub>α</sub> response in DRG neurons was observed.

Neurons were dissociated from the bullfrog DRG (Rana catesbeiana) in a Ringer solution containing trypsin (Sigma type XI, 2.5 mg/ml) and collagenase (Sigma type A, 0.5 mg/ml) (Tokimasa and Akasu, 1990 a, b). The Ringer solution had the following composition (mM): NaCl, 112; KCl, 2; CaCl<sub>2</sub>, 1.8; N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), 4 and tris (hydroxymethyl) aminomethane (Tris), 1. Cells with diameters greater than 50 μm (A-type cells) were used for whole-cell patch-clamp recordings. Pipettes for the whole-cell clamp had tip resistances of 3-6 MΩ, when filled with a pipette solution having the following composition (mM): KCl, 100; MgCl<sub>2</sub>, 4; ATP, 5; BAPTA, ethylene glycol-bis (β-aminoethyl ether) N, N’, N’-tetraacetic acid (EGTA), 1; and HEPES (sodium salt), 2.5 (pH adjusted with KOH to 7.0). All experiments were carried out at 22-24 ºC. The
statistics are expressed as mean±S.E.M. for the cells tested. The pH of the superfusate was adjusted to 7.2.

DRG neurons were voltage-clamped at −60 mV in a whole-cell configuration. The addition of GABA (1 μM-1 mM) to the superfusing solution for 5 s induced an inward current (IGABA) associated with an increased membrane conductance. The IGABA was blocked by the bath-application of picrotoxin (100 μM) or bicuculline (100 μM). Repeated applications of GABA at intervals of 5-10 min showed no significant ‘run down’ of the inward current for 1 h. The bath-application of SP (100 nM) induced an inward current associated with a decreased membrane conductance in Ringer solution containing TTX (1 μM) (Ishimatsu, 1994). The IGABA was depressed during the application of SP (Fig. 1). At a concentration of 1 μM, SP reduced the IGABA by 38±6% (n=8). When GABA (1 mM) was continuously applied for more than 1 min, the response rapidly reached a peak amplitude and then slowly declined.

Fig. 1. Modulation of GABA<sub>A</sub> receptor responses by tachykinins. A: Effect of SP (100 nM) on the GABA-induced inward current. GABA was applied in the bath for 5 s, as indicated by the short horizontal bars. The period of the bath-application of SP is indicated by the long horizontal bar. B: Effects of SP, NKA and NKB on the GABA-induced current. The ordinate indicates % inhibition of the GABA current. The vertical line on each column indicates the SE of the mean. The number of experiments is shown in parentheses. Asterisks indicate statistical significance (P<0.05).
Such a transient response during the prolonged application of GABA probably results from either a desensitization of the GABA<sub>A</sub> receptors and/or a re-distribution of Cl<sup>-</sup> across the plasma membranes of the neurons (Akaike et al. 1987). In DRG neurons, the decay of the current observed during the application of GABA can be described by a single exponential with a time-constant of 38±5 s (n=5). The time-constant of the decay phase of the I<sub>GABA</sub> was not significantly changed by SP. Neurokinin A (NKA, 100 nM), a neurokinin-1 (NK<sub>1</sub>) receptor agonist, also induced inward currents with amplitudes similar to those produced by SP (100 nM) (n=3). NKA (100 nM) decreased the amplitude of the I<sub>GABA</sub> by 28±3% (n=6). In neurons treated with L-703,606, a potent NK<sub>1</sub> receptor antagonist, SP decreased the I<sub>GABA</sub> by only 10±3% (n=4). These results indicate that SP depresses the GABA current through the activation of an NK<sub>1</sub> tachykinin receptor. Bath-application of forskolin (10 μM), an activator of adenylyl cyclase, for 10 min reduced the I<sub>GABA</sub> in DRG neurons by 41±5% (n=5). Db-cyclic AMP (200 μM), a membrane permeable analogue of cyclic AMP, also depressed the I<sub>GABA</sub> by 38±7% (n=10). H-9 (40 μM), a protein kinase A (PKA) inhibitor, was applied to the internal solution through the recording electrode. H-9 did not consistently affect the inhibitory action of SP on the I<sub>GABA</sub> (Fig. 2). Similarly, another PKA inhibitor, HA-1004, had no effect on the SP response. To test whether protein kinase C mediated the SP-induced inhibition of the I<sub>GABA</sub>, H-7 (200 μM), a protein kinase C inhibitor, was applied to the ganglion cells by diffusion through a whole-cell patch-pipette. In neurons treated with H-7, SP (100 nM) decreased the I<sub>GABA</sub> by only 7±5% (n=4) (Fig. 2). In DRG neurons treated with W-7 (100 μM), a Ca<sup>2+</sup>/calmodulin inhibitor, SP (100 nM) depressed the I<sub>GABA</sub> by 27±3% (n=5).

The present study has shown that SP suppresses the GABA<sub>A</sub> response in acutely dissociated neurons of the bullfrog DRG. The SP-induced depression of the I<sub>GABA</sub> does not result from either an enhanced desensitization of the GABA<sub>A</sub> receptors or a re-distribution of chloride across the cell membrane (Akaike et al. 1987). L-703,606, a non-peptide antagonist at the NK<sub>1</sub> receptor, also reduced the inhibition of the I<sub>GABA</sub> by SP. A

![Fig. 2. Role of protein kinase C in the SP-induced inhibition of the I<sub>GABA</sub>. The ordinate indicates % inhibition of the I<sub>GABA</sub> produced by SP (100 nM). H-9 (40 μM) and H-7 (200 μM) were applied, intracellularly, through a recording patch-pipette. The vertical line in each column indicates the SE of the mean. The asterisk indicates statistical significance (P<0.05).](image-url)
related agonist for tachykinin receptors, NKA, also produced an inhibition of the $I_{\text{GABA}}$. These results indicate that the SP receptors responsible for the inhibition of the $I_{\text{GABA}}$ are similar to the NK$_1$ type (Watson and Girdlestone, 1995). It has been shown that SP produces an inward current by suppressing the M-current through an activation of a G-protein in frog sympathetic ganglia (Pfaffinger, 1988; Lopez and Adams, 1989) and bullfrog DRG neurons (Ishimatsu, 1994). Pfaffinger (1988) has demonstrated that the SP-induced inhibition of $I_M$ occurs independent of PKC but is mediated directly by a G-protein. The present study demonstrates that the SP-induced inhibition of $I_{\text{GABA}}$ was blocked by a PKC inhibitor, H-7. Although db-cyclic AMP and forskolin, an adenylyl cyclase activator, depressed the $I_{\text{GABA}}$, a PKA inhibitor, H-9, did not prevent the SP-induced inhibition of the $I_{\text{GABA}}$. It can be concluded that the SP-induced inhibition of the $I_{\text{GABA}}$ is mediated by a diffusible messenger, probably PKC. There are several reports relating to the signal transduction pathway for the SP effect. SP accelerates the turnover of inositol polyphosphate metabolism by activating phospholipase C (PLC) (Hanley et al. 1980; Watson and Downes, 1983; Pfaffinger, 1988). A G-protein may activate PLC, and an increase in PLC activity can lead to the activation of PKC via diacylglycerol (Nishizuka et al. 1991). Further experiments using more specific PKC inhibitors are necessary to elucidate this hypothesis.

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