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

KEI YAMADA^{*†}, ZHENG-XIONG XI^{*} AND TAKASHI AKASU^{*}

^{*}*Departments of Physiology and* [†]*Orthopaedics, Kurume University
School of Medicine, Kurume 830, Japan*

Received for publication December 8, 1995

Key words: substance P, dorsal root ganglia, whole-cell recordings, GABA_A receptor, PKA, PKC

The GABA_A 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_A receptor is also modulated by intracellular substances, such as Ca²⁺ 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_A 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-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_A 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; CaCl₂, 1.8; N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), 4 and tris (hydroxymethyl) aminomethane (Tris), 1. Cells with diameters greater than 50 μ m (A-type cells) were used for whole-cell patch-clamp recordings. Pipettes for the whole-cell clamp had tip resistances of 3–6 M Ω , when filled with a pipette solution having the following composition (mM): KCl, 100; MgCl₂, 4; ATP, 5; BAPTA, ethylene glycol-bis (β -aminoethyl ether) N, 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 °C. The

Reprint requests to: Dr. T. Akasu, Department of Physiology, Kurume University School of Medicine, 67 Asahi-machi, Kurume 830, Japan. Tel: +81-942-31-7543 Fax: +81-942-31-7728

statistics are expressed as mean $\pm$ 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 ($1\text{ }\mu\text{M}$ – 1 mM) to the superfusing solution for 5 s induced an inward current (I_{GABA}) associated with an increased membrane conductance. The I_{GABA} was blocked by the bath-application of picrotoxin ($100\text{ }\mu\text{M}$) or bicuculline ($100\text{ }\mu\text{M}$). 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 ($1\text{ }\mu\text{M}$) (Ishimatsu, 1994). The I_{GABA} was depressed during the application of SP (Fig. 1). At a concentration of $1\text{ }\mu\text{M}$, SP reduced the I_{GABA} by $38\pm 6\%$ ($n=8$). 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_A 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_A receptors and/or a re-distribution of Cl⁻ 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_{GABA} was not significantly changed by SP. Neurokinin A (NKA, 100 nM), a neurokinin-1 (NK₁) 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_{GABA} by $28 \pm 3\%$ ($n=6$). In neurons treated with L-703,606, a potent NK₁ receptor antagonist, SP decreased the I_{GABA} by only $10 \pm 3\%$ ($n=4$). These results indicate that SP depresses the GABA current through the activation of an NK₁ tachykinin receptor. Bath-application of forskolin (10 μM), an activator of adenylyl cyclase, for 10 min reduced the I_{GABA} in DRG neurons by $41 \pm 5\%$ ($n=5$). Db-cyclic AMP (200 μM), a membrane permeable analogue of cyclic AMP, also depressed the I_{GABA} by $38 \pm 7\%$ ($n=10$). 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_{GABA} (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_{GABA} , 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_{GABA} by only $7 \pm 5\%$ ($n=4$) (Fig. 2). In DRG neurons treated with W-7 (100 μM), a Ca²⁺/calmodulin inhibitor, SP (100 nM) depressed the I_{GABA} by $27 \pm 3\%$ ($n=5$).

The present study has shown that SP suppresses the GABA_A response in acutely dissociated neurons of the bullfrog DRG. The SP-induced depression of the I_{GABA} does not result from either an enhanced desensitization of the GABA_A receptors or a re-distribution of chloride across the cell membrane (Akaike et al. 1987). L-703,606, a non-peptide antagonist at the NK₁ receptor, also reduced the inhibition of the I_{GABA} by SP. A

Fig. 2. Role of protein kinase C in the SP-induced inhibition of the I_{GABA} . The ordinate indicates % inhibition of the I_{GABA} 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 ($P < 0.05$).

related agonist for tachykinin receptors, NKA, also produced an inhibition of the I_{GABA} . These results indicate that the SP receptors responsible for the inhibition of the I_{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 (Pffaffinger, 1988; Lopez and Adams, 1989) and bullfrog DRG neurons (Ishimatsu, 1994). Pffaffinger (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_{GABA} was blocked by a PKC inhibitor, H-7. Although db-cyclic AMP and forskolin, an adenylyl cyclase activator, depressed the I_{GABA} ; a PKA inhibitor, H-9, did not prevent the SP-induced inhibition of the I_{GABA} . It can be concluded that the SP-induced inhibition of the I_{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; Pffaffinger, 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.

Acknowledgments: This study was supported primarily by a Grant-in-Aid for Research from The Ishibashi Foundation and

a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

References

- Akaike N, Inomata N, and Tokutomi N. Contribution of chloride shifts to the fade of γ -aminobutyric acid-gated currents in frog dorsal root ganglion cells. *J Physiol* 1987; 391:219-234.
- Behrends JC, Maruyama T, Tokutomi N, and Akaike N. Ca^{2+} -mediated suppression of the GABA-response through modulation of chloride channel gating in frog sensory neurones. *Neurosci Lett* 1988; 86:311-316.
- Bormann J. Electrophysiology of GABA_A and GABA_B receptor subtypes. *TINS* 1988; 11:112-116.
- Chen QX, Stelzer A, Kay AR, and Wong RKS. GABA_A receptor function is regulated by phosphorylation in acutely dissociated guinea-pig hippocampal neurones. *J Physiol* 1990; 420:207-221.
- Chen QX, and Wong RKS. Suppression of GABA_A receptor responses by NMDA application in hippocampal neurones acutely isolated from the adult guinea-pig. *J Physiol* 1995; 482:353-362.
- Gyenes M, Farrant M, and Farb DH. "Run-down" of γ -aminobutyric acid_A receptor function during whole-cell recording: a possible role for phosphorylation. *Mol Pharmacol* 1988; 34:719-723.
- Gyenes M, Wang Q, Gibbs TT, and Farb DH. Phosphorylation factors control neurotransmitter and neuromodulator actions at the γ -aminobutyric acid type A receptor. *Mol Pharmacol* 1994; 46:542-549.
- Hanley MR, Lee CM, Michell RH, and Jones LM. Similar effects of substance P and related peptides on salivation and on phosphatidylinositol turnover in rat salivary glands. *Mol Pharmacol* 1980; 18:78-83.
- Inoue M, Oomura Y, Yakushiji T, and Akaike N. Intracellular calcium ions decrease the affin-

- ity of the GABA receptor. *Nature* 1986; 324: 156-158.
- Ishimatsu M. Substance P produces an inward current by suppressing voltage-dependent and -independent K^+ currents in bullfrog primary afferent neurons. *Neurosci Res* 1994; 19:9-20.
- Kano M, Rexhausen U, Dreessen J, and Konnerth A. Synaptic excitation produces a long-lasting rebound potentiation of inhibitory synaptic signals in cerebellar Purkinje cells. *Nature* 1992; 356:601-604.
- Llano I, Leresche N, and Marty A. Calcium entry increases the sensitivity of cerebellar Purkinje cells to applied GABA and decreases inhibitory synaptic currents. *Neuron* 1991; 6:565-574.
- Lopez HS, and Adams PR. A G protein mediates the inhibition of the voltage-dependent potassium M current by mescarine, LHRP, substance P and UTP in bullfrog sympathetic neurons. *Eur J Neurosci* 1989; 1:529-542.
- Macdonald RL, and Olsen RW. GABA_A receptor channels. *Annu Rev Neurosci* 1994; 17:569-602.
- Mulle C, Choquet D, Korn H, and Changeux J-P. Calcium influx through nicotinic receptor in rat central neurons: its relevance to cellular regulation. *Neuron* 1992; 8:135-143.
- Nestler EJ, and Greengard P. *Protein Phosphorylation in the Nervous System*, John Wiley & Sons, New York, 1984.
- Nishizuka Y, Shearman MS, Oda T, Berry N, Shinomura T et al. Protein kinase C family and nervous function. *Prog Brain Res* 1991; 89:125-141.
- Pfaffinger P. Muscarine and t-LHRH suppress M-current by activating an IAP-insensitive G-protein. *J Neurosci* 1988; 8:3343-3353.
- Shirasaki T, Aibara K, and Akaike N. Direct modulation of GABA_A receptor by intracellular ATP in dissociated nucleus tractus solitarii neurones of rat. *J Physiol* 1992; 449:551-572.
- Sieghart W. GABA_A receptors: ligand-gated Cl^- ion channels modulated by multiple drug-binding sites. *TIPS* 1992; 13:446-450.
- Smith GB, and Olsen RW. Functional domains of GABA_A receptors. *TIPS* 1995; 16:162-168.
- Stelzer A, Kay AR, and Wong RKS. GABA_A-receptor function in hippocampal cells is maintained by phosphorylation factors. *Science* 1988; 241:339-341.
- Taleb O, Trouslard J, Demeneix BA, Feltz P, Bossu J-L et al. Spontaneous and GABA-evoked chloride channels on pituitary intermediate lobe cells and their internal Ca requirements. *Pflügers Arch* 1987; 409:620-631.
- Tokimasa T, and Akasu T. Cyclic AMP regulates an inward rectifying sodium-potassium current in dissociated bull-frog sympathetic neurones. *J Physiol* 1990a; 420:409-429.
- Tokimasa T, and Akasu T. ATP regulates muscarine-sensitive potassium current in dissociated bull-frog primary afferent neurones. *J Physiol* 1990b; 426:241-264.
- Watson SP, and Downes CP. Substance P induced hydrolysis of inositol phospholipids in guinea-pig ileum and rat hypothalamus. *Eur J Pharmacol* 1983; 93:245-253.
- Watson S, and Girdlestone D. Receptor & ion channel nomenclature supplement. *TIPS* 1995; 6th ed.
- Yamada K, and Akasu T. Substance P modulates the GABA_A receptor function via protein kinase C in bullfrog primary afferent neurons. *Jpn J Physiol* 1996; in press.