Volume 278, number 2, 222–224 FEBS 09361 January 1991

ADONIS 001457939100072J

Expression of the $GABA_B$ receptor in *Xenopus* oocytes and inhibition of the response by activation of protein kinase C

Kohtaro Taniyama[1,*], Koichiro Takeda[1], Hiroshi Ando[2], Takayosi Kuno[1] and Chikako Tanaka[1]

Departments of [1]Pharmacology and [2]Physiology, Kobe University School of Medicine, Kobe 650, Japan

Received 28 October 1990; revised version received 6 December 1990

The functional $GABA_B$ receptor was expressed in *Xenopus* oocytes by injecting mRNA obtained from the cerebellum of the rat. Application o GABA in the presence of bicuculline induced a hyperpolarization under current-clamp conditions and an outward current under voltage-clamp conditions. Baclofen mimicked the effect of GABA in the presence of bicuculline, and the effect of baclofen was antagonized by phaclofen. Th GABA-induced outward current was slightly inhibited by treatment with GDP-β-S and was completely inhibited by treatment with GTP-γ-S. Th activation of protein kinase C by 12-*O*-tetradecanoylphorbol-13-acetate (TPA), but not 4α-phorbol-12,13-didecanoate, suppressed the $GABA_B$ re ceptor-mediated hyperpolarization, and the effect of TPA was antagonized by sphingosine. Thus, activation of protein kinase C inhibits the ex pressed $GABA_B$ receptor-mediated response

$GABA_B$ receptor, mRNA injected oocyte, Protein kinase C

1. INTRODUCTION

The γ-aminobutyric acid (GABA) receptors have been classified into two subtypes, termed $GABA_A$ and $GABA_B$ receptors, on the basis of their pharmacological properties [3,4]. The $GABA_A$ receptor with its integrated Cl^- channel is now well characterized and recent studies revealed the amino acid sequence of this receptor [16,22]. $GABA_B$ receptors have been proposed to be coupled to Ca^{2+} channels in dorsal root ganglia [8,11] and to K^+ channels in hippocampal pyramidal cells [2,9]. The *Xenopus* oocyte is a useful model to examine the molecular mechanisms of the modulation of neurotransmitter responsed and ion channels. The $GABA_A$ receptor has been expressed in *Xenopus* oocytes by injecting RNA from the rat brain [12] and the chick brain [17,25] and retina [1], and the $GABA_A$ receptor-mediated response was found to be suppressed by the activation of protein kinase C [18]. On the other hand, the expression of the $GABA_B$ receptors in the *Xenopus* oocytes by injecting RNA has not been reported. The concentration of $GABA_B$ sites in the cerebellum has been shown to be higher than elsewhere in the rat brain, as determined by receptor autoradiography [27]. We injected mRNA from the rat cerebellum and examined the properties of the $GABA_B$ receptor in relation to intracellular signal transduction in *Xenopus* oocytes.

Correspondence address C Tanaka, Department of Pharmacology, Kobe University School of Medicine, Kobe 650, Japan

**Present address* The 2nd Department of Pharmacology, Nagasaki University School of Medicine, Nagasaki 852, Japan

2. MATERIALS AND METHODS

Total RNA was extracted from the cerebellum of 28 day old ra using a guanidine thiocyanate/phenol/chloroform procedure [5] Poly-A$^+$ RNA (mRNA) was purified by passage through an oligo (d cellulose affinity column. The oocytes from *Xenopus laevis* wer defolliculated by treatment with collagenase. Each defolliculate oocyte was injected with 50 nl of mRNA solution (1 mg/ml in water) and incubated for 2–7 days at 18°C in sterile Barth's solution (NaC 90.7 mM, KCl 1 mM, $NaHCO_3$ 2.38 mM, $MgSO_4$ 0.81 mM, $Ca(NO_3)$ 0.34 mM, $CaCl_2$ 0.41 mM, Hepes/NaOH 9.1 mM, pH 7.6) with 0 mg/ml gentamycin and 50 U/ml nystatin. A single oocyte was place in a chamber and perfused with medium composed of NaCl 90.7 mM KCl 1 mM, $NaHCO_3$ 2.38 mM, $MgSO_4$ 0.81 mM, $CaCl_2$ 0.82 mM Hepes/NaOH 10 mM, pH 7.6, at room temperature. The oocyte wa impaled with two microelectrodes, and responses to substances wer recorded using current-clamp or voltage-clamp circuits. The follow ing chemicals were obtained from the indicated firms: γ-aminobutyri acid (GABA), bicuculline methiodide, GDP-β-S, GTP-γ-S, 12-*O*- tetradecanoylphorbol-13-acetate (TPA) and 4α-phorbol-12,13-d decanoate (4α-PDD) (Sigma), baclofen (Ciba Geigy), phaclofe (Tocris) and sphingosine (Serdary Research Laboratories).

3. RESULTS AND DISCUSSION

The effect of GABA was usually tested in th presence of bicuculline, to avoid the $GABA_A$ receptor mediated response. Under current-clamp conditions the application of GABA in the presence of bicucullin induced a hyperpolarization in the oocytes injected wit RNA derived from the rat cerebellum (Fig. 1A), but nc in the uninjected oocytes or in oocytes injected wit medium containing no RNA. The GABA-induce hyperpolarization occurred in 5 of 88 oocytes, 7 of 6 oocytes, 7 of 71 oocytes and 0 of 41 oocytes 3 days, days, 5 days and 7 days after injecting RNA, respective

Published by Elsevier Science Publishers B V (Biomedical Divisio

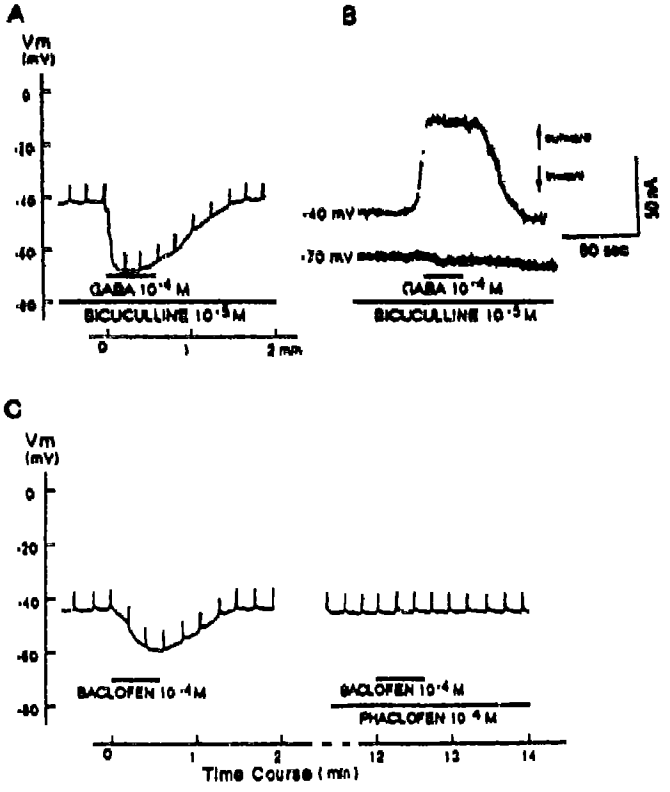

Fig 1 GABA-agonist-induced hyperpolarization and outward current in the membrane of oocytes injected with mRNA Bicuculline at 10^{-5} M was present in the perfusion medium during experiments. Oocytes injected with mRNA were incubated for 4 days. (A) GABA-induced hyperpolarization under current-clamp conditions, (B) GABA-induced outward current under voltage-clamp conditions and (C) antagonism by phaclofen of baclofen-induced hyperpolarization under current clamp conditions

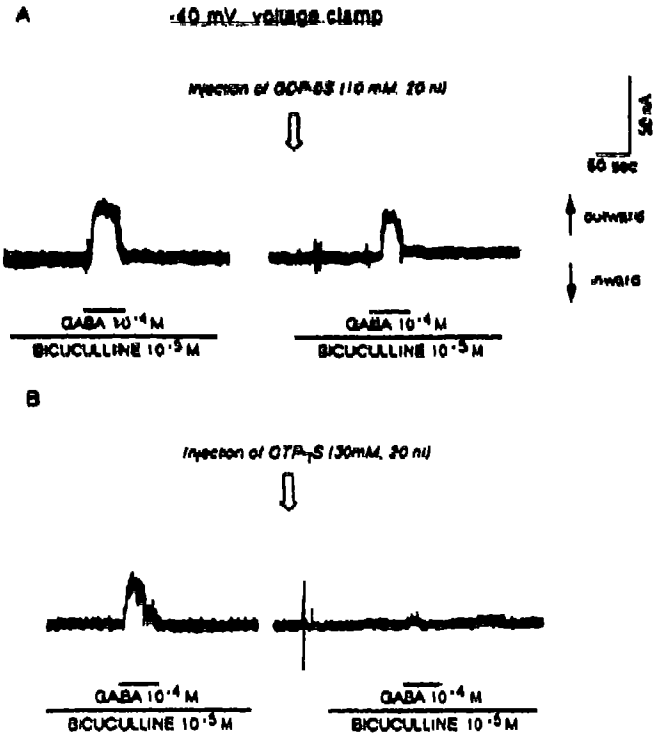

Fig. 2. Effect of GDP-β-S (A) and GTP-γ-S (B) on the GABA-induced outward current under voltage-clamp condition of -40 mV Bicuculline at 10^{-5} M was present in the perfusion medium Twenty nl of GDP-β-S at 10^{-2} M or GTP-γ-S at 3×10^{-3} M was injected 10 min before the addition of GABA. Oocytes injected with mRNA were incubated for 4 days at 18°C

ly. In the absence of bicuculline, some RNA injected oocytes from the same donor showed a depolarizing response of $GABA_A$ receptor origin. GABA generally depolarizes membranes of oocytes injected with RNA from the brain [12,17,25] and retina [1], which is mediated by the stimulation of the bicuculline-sensitive $GABA_A$ receptor. Under voltage-clamp conditions, the application of GABA in the presence of bicuculline induced an outward current at the holding potential of -40 mV, however at the holding potential of -70 mV GABA failed to induce any current (Fig. 1B), thereby suggesting that the K^+ channel was opened by stimulation of the $GABA_B$ receptor. Baclofen, a $GABA_B$ receptor agonist mimicked the effect of GABA in the presence of bicuculline, and the effect of baclofen was antagonized by phaclofen, a selective $GABA_B$ receptor antagonist (Fig. 1C). These results indicate that the functional $GABA_B$ receptor is expressed in oocytes when RNA from the rat cerebellum is injected into their tissues In the dorsal root ganglion cells, stimulation of the $GABA_B$ receptor decreases the Ca^{2+} current [7,21]. In the hippocampus, the $GABA_B$ receptors are located

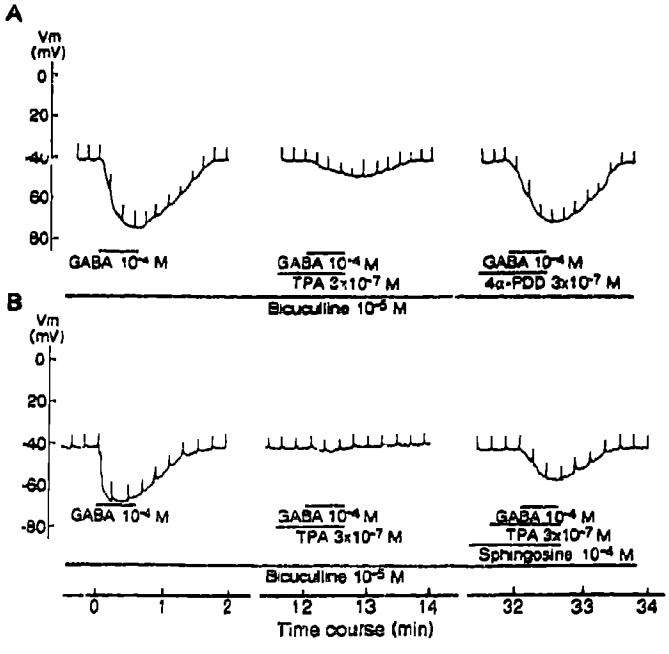

Fig 3 Suppression of GABA-induced hyperpolarization by activation of protein kinase C, under current-clamp conditions Bicuculline at 10^{-5} M was present in the perfusion medium. TPA, 4α-PDD and sphingosine were applied 10 min, 10 min and 15 min before the addition of GABA

both pre- and postsynaptically. Either baclofen or GABA in the presence of bicuculline increases K^+ conductance and induces a hyperpolarization of pyramidal cell membranes due to the stimulation of the postsynaptic $GABA_B$ receptors [9,10,14,19]. In the hippocampus, the response mediated by the activation of postsynaptic $GABA_B$, but not presynaptic $GABA_B$ receptor is phaclofen-sensitive [9]. The GABA-induced outward current was slightly inhibited by treatment with GDP-β-S, the non-hydrolysable analogue of GDP and was completely inhibited by treatment with GTP-γ-S, the non-hydrolysable analogue of GTP (Fig. 2), thereby indicating that the expressed $GABA_B$ receptor is coupled to GTP-binding proteins.

A phorbol ester, 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) suppressed the $GABA_B$ receptor-mediated hyperpolarization in the oocytes injected with RNA (Fig. 3). In contrast, 4α-phorbol-12,13-didecanoate, a non-activating analog of TPA, had no effect on the $GABA_B$ receptor-mediated response. The suppressing effect of TPA was antagonized by sphingosine, a compound which inhibits the activity of protein kinase C. These results indicate that the response mediated by stimulation of the expressed $GABA_B$ receptor is suppressed by the activation of protein kinase C, corresponding to the findings in the hippocampus, in which the activation of protein kinase C by phorbol ester suppresses the responses mediated by the stimulation of either pre- or postsynaptic $GABA_B$ receptors [12].

Protein kinase C may act through phosphorylation of specific proteins The mechanism underlying the suppression of the expressed $GABA_B$ receptor-mediated response in the oocyte requires further study. Various voltage-dependent and receptor-mediated ion channels were found to be modulated by the activation of protein kinase C [23]. It has been shown that the activation of protein kinase C modulates native ion channels of intact follicular oocytes, for example, the adenosine-induced K^+ channel [6], and the expressed ion channels of the RNA injected oocytes, such as voltage-dependent Ca^{2+} channel [15,24], Na^+ channel [24] and the $GABA_A$ receptor-mediated Cl^- channel [24].

5-Hydroxytryptamine-, GABA- and acetylcholine (ACh)-induced inward currents were shown to be suppressed by activation of protein kinase C [18]. Activation of protein kinase C was found to phosphorylate receptors such as the nicotinic ACh receptor, β-adrenoceptor, α_1-adrenoceptor, and the α-subunit of GTP-binding protein (G_i), and the Na^+ channel [13,20]. The $GABA_B$ receptor is the most plausible target protein of protein kinase C because activation of the enzyme seems to promote a form of heterogeneous desensitization [13]. It is also possible that the GTP-binding protein is a target of protein kinase C. There are multiple subspecies of protein kinase C, and at least 4 of them α, βI, βII and γ are present in the brain [20]. We could not determine which subspecies of protein kinase C participates in inhibition of the $GABA_B$ receptor-mediated response, since we did not rule out the possibility that the mRNA from the rat cerebellum used in our study may have contained mRNA encoding the multiple subspecies of protein kinase C [20,26] together with that encoding the $GABA_B$ receptor.

Acknowledgement. This work was supported by grants from the Ministry of Education, Science and Culture and the Ministry of Health and Welfare, Japan

REFERENCES

[1] Ando, H., Labarca, C. and Noell, W K. (1988) Neurosci. Res 8, S15-S25.
[2] Andrade, R., Malenka, R C and Nicoll, R A. (1986) Science 234, 1261-1265
[3] Bormann, J (1988) Trends Neurosci. 11, 112-116
[4] Bowery, N.G (1989) Trends Pharmacol. Sci 10, 401-407.
[5] Chomczynski, P. and Sacchi, N. (1987) Anal Biochem 162, 156-159.
[6] Dascal, N., Lotan, I , Gillo, B., Lester, H A. and Lass, Y. (1985) Proc Natl Acad Sci. USA 82, 6001-6005
[7] Deisz, R A. and Lux, H.D. (1985) Neurosci Lett. 56, 205-210.
[8] Dolphin, A C and Scott, R.H. (1987) J Physiol. 386, 1-17.
[9] Dutar, P and Nicoll, R A (1988) Nature 332, 156-158.
[10] Gahwiler, B H. and Brown, D A (1985) Proc. Natl. Acad Sci. USA 82, 1558-1562
[11] Holz, G.G., Rane, S G. and Dunlap, K. (1986) Nature 319, 670-672
[12] Houamed, K M , Bilbe, G , Smart, T G , Constanti, A., Brown, D.A , Barnard, E.A and Richards, B M (1984) Nature 310, 318-321.
[13] Huganir, R L. and Greengard, P (1987) Trends Pharmacol Sci 8, 472-477.
[14] Inoue, M , Matsuo, T. and Ogata, N (1985) Br J Pharmacol 84, 833-841
[15] Leonard, J P , Nargeot, J., Snutch, T P , Davidson, N and Lester, H A (1987) J Neurosci 7, 875-881
[16] Levitan, E S., Schofield, P R., Burt, D R., Rhee, L M , Wisden, W , Kohler, M , Fujita, N , Rodriguez, H F Stephenson, A , Darlison, M G , Barnard, E A and Seeburg, P H (1988) Nature 335, 76-79
[17] Miledi, R , Parker, I and Sumikawa, K (1982) Proc R Soc London B 216, 509-515
[18] Moran, O and Dascal, N (1985) Mol Brain Res 5, 193-202
[19] Newberry, N R and Nicoll, R A (1984) Nature 308, 450-452.
[20] Nishizuka, Y (1988) Nature 334, 661-665
[21] Robertson, B and Taylor, W R (1986) Br J Pharmacol 89, 661-672.
[22] Schofield, P R , Darlison, M G , Fujita, N , Burt, D R , Stephenson, F A , Rodriguez, H , Rhee, L M , Ramachandran, J , Reale, V , Glencorse, T A , Seeburg, P H and Barnard, E A (1987) Nature 328, 221-227
[23] Shearman, M.S , Sekiguchi, K and Nishizuka, Y (1990) Pharmacol Rev. 41, 211-237
[24] Sigel, E and Baur, R (1988) Proc Natl Acad Sci USA 85, 6192-6196
[25] Smart, T G , Constanti, A , Bilbe, G , Brown, D A and Barnard, E A (1983) Neurosci Lett 40, 55-59
[26] Tanaka, C., Saito, N , Kose, A , Hosoda, K , Sakaue, M , Shuntoh, H , Nishino, N and Tamyama, K (1988) in, Neuroreceptors and Signal Transduction (Kito, S , Segawa, T , Kuriyama, K , Tohyama, M and Olsen, R.W., eds), pp. 277-285, Plenum, New York
[27] Wilkin, G P., Hudson, A L , Hill, D.R and Bowery, N G (1981) Nature 294, 584-587