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Activation of protein kinase C results in down-modulation of different recombinant $GABA_A$-channels

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Different combinations of cloned rat brain subunits of the $GABA_A$ receptor were expressed in *Xenopus* oocytes. The effect of the phorbol ester PMA, an activator of protein kinase C, on the expressed GABA-gated ion current was determined. Ion currents were diminished by β-PMA, but not by the control substance α-PMA, irrespective of the subunit combination studied. The mechanism of current decrease was investigated in more detail for the subunit combination $\alpha5\beta2\gamma2$. The reversal potential of the current remained unaffected, while the maximal current amplitude was decreased and the apparent K_a for GABA-dependent channel gating was shifted to higher concentrations.

Xenopus oocyte; γ-Aminobutyrate receptor; Ion channel; Expression; Phorbol ester; Protein kinase C

1. INTRODUCTION

The $GABA_A$ receptor channel is gated by the major inhibitory neurotransmitter in vertebrate brain, γ-aminobutyrate (GABA). Recently, it has been suggested that the activity of this channel may be modulated by protein kinases. It has been shown that the isolated, purified [1] or partially purified receptor protein can be phosphorylated in vitro by an endogenous, unidentified protein kinase [2], by exogenously added protein kinase A [3,4] and by protein kinase C [4]. Evidence for a functional role of protein kinase C mediated phosphorylation was first obtained in the *Xenopus* oocyte expression system, where it was shown that activation of protein kinase C by phorbol ester or diacylglycerol results in a decrease of the chloride current amplitude elicited by GABA [5,6]. cAMP analogs have been shown to decrease GABA activated chloride flux in synaptosomes [7,8] or the GABA induced current amplitude in cultured hippocampal cells [9], and to increase the rate of desensitization in cortical neurones [8]. Addition of the catalytic subunit of PKA to excised patches of mouse spinal cord neurones was found to reduce the GABA channel opening frequency [10]. ATP and ATP-γ-S have been observed to prevent run-down in guinea pig hippocampal neurones [11,12] and in chick spinal cord neurones [13]. It has been proposed that protein kinases may be involved in the stabilization of these spontaneously decreasing currents. The identity of the stabilizing protein kinase is unknown.

For our work the cloned rat GABA receptor subunits $\alpha1$, $\alpha3$, $\alpha5$, $\beta1$, $\beta2$ and $\gamma2$ were available. Primary sequence information (for review see [14]) predicts the presence of protein kinase C consensus phosphorylation sites on intracellular loops of rat subunits $\alpha1$, $\alpha3$, $\alpha5$, $\beta1$ and $\gamma2$, but none on subunit $\beta2$.

We have investigated the effect of the activator of protein kinase C, β-PMA (4β-phorbol-12-myristate-13-acetate), on GABA currents expressed in *Xenopus* oocytes. Channels formed by different subunit isoform combinations were similarly down-modulated by β-PMA. Furthermore, we studied modulation of a newly discovered splice variant of $\gamma2$ ($\gamma2$L), that differs from the the normal $\gamma2$ by an insert of an eight amino acid sequence containing an additional protein kinase C consensus phosphorylation site [15,16].

2. MATERIALS AND METHODS

The cDNAs coding for the subunits of the rat $GABA_A$ receptor channel were transcribed, capped and tailed with poly(A) as described earlier [17–19]. Combinations of the transcripts were co-precipitated in ethanol and stored at −20°C. Isolation of the follicles from the frogs, culturing of the follicles, injection with mRNA, removal of the cell layers, and electrophysiological experiments were performed at room temperature as decribed before [20,21]. GABA induced ion currents were measured using the two electrode voltage clamp technique. GABA was applied by bath perfusion. The GABA concentration used to determine the effect of PMA depended on the subunit combination under investigation. In each case, a GABA concentration was used which elicited 5–20% of the maximal current amplitude. The effect of phorbol esters was determined as follows. Control currents were measured before and 20 min after exposure to PMA. 10 nM β-PMA or 100 nM α-PMA were applied by bath perfusion for a 1 min period initially. In order to prevent both washout and accumulation of the drug, perfusion was thereafter switched to drug perfusion for a 30 s interval every 4 min.

The currents expressed from the subunit combination $\alpha5\beta2\gamma2$ are characterized by a K_a for GABA of 14±3 μM and a Hill coefficient of 1.7±0.1 [21]. In this series of experiments, we found a somewhat

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lower and more variable GABA concentration of about 3–7 μM to elicit a half-maximal current amplitude (K_a). The current elicited by 100 μM GABA was taken as an estimate of the maximal current amplitude (I_{max}). Higher concentrations of GABA did not elicit larger currents in untreated and in β-PMA treated oocytes. GABA was used at a concentration producing a current amounting to about 10% of I_{max} for the measurement of the time course of the PMA effect, the reversal potential of the GABA current and its desensitization. For the experiments on desensitization a fast perfusion system was used that allowed a rate of medium change of 70% within <0.5 s [21]. Desensitization was determined as the rate of current decline per minute expressed in % of the peak current amplitude, 30 s after application of GABA. After determination of I_{max}, the GABA concentration was established, which elicited about 40% of I_{max} ($I_{40\%}$). $I_{40\%}/I_{max}$ then represents a crude, but sensitive measure, allowing detection of shifts in the K_a.

3. RESULTS AND DISCUSSION

Fig. 1 shows the time course of the effect of 10 nM β-PMA on the current amplitude elicited by 1 μM GABA in oocytes expressing the subunit combination $\alpha5\beta2\gamma2$. The current amplitude was drastically decreased within the first 4 min of β-PMA application. In contrast, 100 nM of the stereoisomer α-PMA, which does not stimulate protein kinase C, did not affect the current amplitude in similar experiments (Fig. 1). This suggests that activation of protein kinase C results in a reduction of GABA currents.

We found that all subunit combinations tested responded stereoselectively to exposure to PMA (Table I). The fact that currents expressed from cloned subunit combinations respond to phorbol ester application indicates that protein kinase C endogenous to the oocyte is responsible for the modulation. Averaged data (Table I) indicate that there might be significant differences in the β-PMA effects between different subunit combinations. As the amplitude of the β-PMA effect varied between different oocyte batches used, this could simply

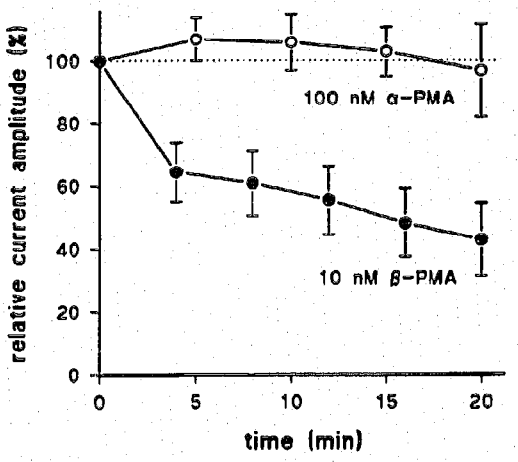

Fig. 1. Time course of the GABA current decrease after exposure to PMA. The subunit combination $\alpha5\beta2\gamma2$ was expressed in *Xenopus* oocytes. The GABA current amplitude was determined before and at different times after application of 10 nM β-PMA (●) or 100 nM α-PMA (○). Data collected from 32 and 4 oocytes, respectively, are shown as mean±SD.

be due to different batches used for the experiments. Indeed, direct comparison between the subunit combinations $\alpha1\beta1\gamma2$ and $\alpha1\beta2\gamma2$, carried out in the same batch of oocytes, failed to show a consistent difference in the time course of the β-PMA effect and in the absolute level of the decrease in current amplitude, 20 min after application of β-PMA.

It was especially interesting to compare the newly characterized splice variant of $\gamma2$ ($\gamma2$L) containing an additional insert of eight amino acids conferring an additional consensus phosphorylation site for protein kinase C [15,16] to the normal $\gamma2$ (Table I). In a direct comparison of the subunit combinations $\alpha5\beta2\gamma2$ and $\alpha5\beta2\gamma2$L, we did not observe any difference in responsiveness between subunit combinations containing the two splice variants. If one assumes that the channel

Table I

Down-modulation of GABA currents from different expressed subunit combinations

Expressed subunit combination	Current (% of control)	
	100 nM α-PMA	10 nM β-PMA
$\alpha1\beta1$	86 ± 7 (3)	47 ± 12 (5)
$\alpha1\beta2$	107 ± 17 (4)	70 ± 6 (3)
$\alpha3\beta1$	100 ± 1 (3)	61 ± 8 (4)
$\alpha5\beta1$	98 ± 12 (7)	49 ± 9 (3)
$\beta2\gamma2$	102 ± 4 (3)	31 ± 12 (4)
$\alpha3\alpha5\gamma2$	93 ± 8 (4)	38 ± 11 (5)
$\alpha1\beta1\gamma2$	95 ± 9 (3)	41 ± 17 (10)
$\alpha1\beta2\gamma2$	94 ± 4 (4)	66 ± 12 (15)
$\alpha5\beta2\gamma2$	97 ± 15 (4)	43 ± 12 (32)
$\alpha5\beta2\gamma2$L	100 (1)	32 ± 12 (5)

The down-modulation of GABA currents was determined 20 min after exposure to 10 nM β-PMA, as described in the Methods section. In control experiments 100 nM α-PMA was used.

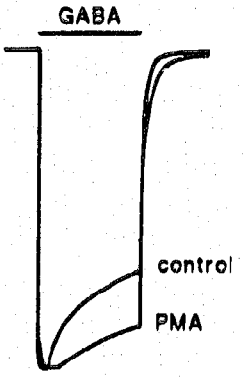

Fig. 2. Desensitization of the down-regulated GABA current. The subunit combination $\alpha5\beta2\gamma2$ was expressed in *Xenopus* oocytes. Current traces recorded in response to a 1 min application of 0.5 μM GABA (bar), before (control) and 20 min after exposure to 10 nM β-PMA (PMA), were rescaled to the same peak current amplitude and are displayed superimposed. The peak amplitude of the current was 770 nA before and 311 nA after exposure to β-PMA.

Table II

Effect of β-PMA on the properties of the current expressed by the subunit combination α5β2γ2

Parameter	Control	10 nM β-PMA
Reversal potential (mV)	−32 ± 7	−32 ± 6 (16/2)
I_{max} (rel. to control; %)	100	60 ± 19 (8/2)
$(I_{40\%}/I_{max}) \times 100$ (%)	38 ± 10[a]	26 ± 10[a] (8/2)
Desensitization (%/min)	40 ± 24[b]	22 ± 12[b] (5/2)

[a]Significant effect of β-PMA: $P < 0.001$ (Student's paired t-test)
[b]Significant effect of β-PMA: $P < 0.001$ (Student's paired t-test)
The reversal potential of the GABA current and desensitization were determined using a GABA concentration eliciting a current amounting to about 10% of I_{max}. I_{max} was determined as the current amplitude elicited by 100 μM GABA. $I_{40\%}/I_{max}$, which represents a crude indication of the K_a was determined as indicated in the Methods section. Data are given as mean ± SD (number of oocytes/number of different batches of oocytes).

subunits are directly the substrates of protein kinase C, this indicates that other phosphorylation sites present in these subunits are sufficient for the observed modulation.

In further experiments we examined whether the reversal potential of the GABA induced current was affected. In three experiments each on the subunit combinations α1β1, α3β1, α5β1, and α5β2γ2, we did not detect a significant change in the reversal potential upon exposure to β-PMA, indicating that the ion selectivity was not affected.

We tested for the subunit combination α5β2γ2 whether I_{max}, the K_a of the channel gating by GABA, and desensitization were altered upon down-modulation by PMA. Rescaled current traces obtained before and after exposure to β-PMA are shown in Fig. 2. For this experiment a GABA concentration was used that elicited a current amplitude corresponding to about 10% of I_{max}. It is evident that we would miss with the present agonist application system any possible fast phase of desensitization occurring at the subsecond time scale. The traces shown in Fig. 2 show that β-PMA slows later phases of desensitization. Therefore, the decrease in current amplitude is probably not due to increased desensitization. At 100 μM GABA, the desensitization was not affected by β-PMA (not shown).

We observed that the current was depressed more strongly by β-PMA at a GABA concentration eliciting about 40% of I_{max} than at I_{max}. This is reflected in the lower value of $I_{40\%}/I_{max}$ that was observed after exposure to β-PMA (Table II). This could be due to a small shift of the K_a to a higher concentration. If such a shift is indeed ocurring, the observed decrease in desensitization could simply reflect the lower occupancy of the GABA binding site, because the rate of desensitization strongly depends on this parameter. In line with this interpretation is the fact that at high agonist concentration the desensitization remained unaffected.

If the events we observe at the second time scale are representative, it can be concluded that the decrease in the GABA current amplitude of recombinant channels expressed in the *Xenopus* oocyte might be a consequence of a small decrease of the affinity of channel gating by GABA and a decrease in I_{max}. As in the case of the modulation of the GABA channel by protein kinase A [10], a change in channel kinetics may be responsible for the observed phenomenon.

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REFERENCES

[1] Sigel, E., Stephenson, F.A., Mamalaki, C. and Barnard, E.A. (1983) J. Biol. Chem. 258, 6965–6971.
[2] Sweetnam, P.M., Lloyd, J., Gallombardo, P., Malison, R.T., Gallager, D.W., Tallman, J.F. and Nestler, E.J. (1988) J. Neurochem. 51, 1274–1284.
[3] Kirkness, E.F., Bovenkerk, C.F., Ueda, T. and Turner, A.J. (1989) J. Biochem. 259, 613–616.
[4] Browning, M.D., Bureau, M., Dudek, E.M. and Olsen, R.W. (1990) Proc. Natl. Acad. Sci. USA 87, 1315–1318.
[5] Sigel, E. and Baur, R. (1988) Proc. Natl. Acad. Sci. USA 85, 6192–6196.
[6] Moran, O. and Dascal, N. (1989) Mol. Brain Res. 5, 193–202.
[7] Heuschneider, G. and Schwarz, R.D. (1989) Proc. Natl. Acad. Sci. USA 86, 2938–2942.
[8] Jalilian Tehrani, M.H., Hablitz, J.J. and Barnes, E.M. (1989) Synapse 4, 126–131.
[9] Harrison, N.L. and Lambert, N.A. (1989) Neurosci. Lett. 105, 137–142.
[10] Porter, N.M., Twyman, R.E., Uhler, M.D. and Macdonald, R.L. (1990) Neuron 5, 789–796.
[11] Stelzer, A., Kay, A.R. and Wong, R.K.S. (1988) Science 241, 339–341.
[12] Chen, Q.X., Stelzer, A., Kay, A.R. and Wong, R.K.S. (1990) J. Physiol. 420, 207–221.
[13] Gyenes, M., Farrant, M. and Farb, D.H. (1989) Mol. Pharmacol. 34, 719–723.
[14] Olsen, R.W. and Tobin, A.J. (1990) FASEB J. 4, 1469–1480.
[15] Whiting, P., McKernan, R.M. and Iversen, L.L. (1990) Proc. Natl. Acad. Sci. USA 87, 9966–9970.
[16] Kofuji, P., Wang, J.B., Moss, S.J., Huganir, R.L. and Burt, D.R. (1991) J. Neurochem. 56, 713–715.
[17] Malherbe, P., Sigel, E., Baur, R., Richards, J.G. and Möhler, H. (1990) FEBS Lett. 260, 261–265.
[18] Malherbe, P., Sigel, E., Baur, R., Persohn, E., Richards, J.G. and Möhler, H. (1990) J. Neurosci. 10, 2330–2337.
[19] Malherbe, P., Draguhn, A., Multhaup, G., Beyreuther, K. and Möhler, H. (1990) Mol. Brain Res. 8, 199–208.
[20] Sigel, E. (1987) J. Physiol. 386, 73–90.
[21] Sigel, E., Baur, R., Trube, G., Möhler, H. and Malherbe, P. (1990) Neuron 5, 703–711.