$GABA_B$ receptors couple to G proteins G_o, G_o^* and G_{i1} but not to G_{i2}

Rika Morishita, Kanefusa Kato and Tomiko Asano

Department of Biochemistry, Institute for Develomental Research, Aichi Prefectural Colony, Kasugai, Aichi 480-03, Japan

Received 2 July 1990; revised version received 13 August 1990

We studied the selectivity of $GABA_B$ receptors for coupling to G proteins by testing the ability of various purified G proteins to increase GABA binding to *N*-ethylmaleimide (NEM)-treated membranes of bovine brain. The addition of G_o, G_o^* or G_{i1} to NEM-treated membranes increased GABA binding in a dose-dependent manner. However, the addition of G_{i2} did not elicit a marked increase in GABA binding. When α subunits of G proteins were mixed with various brain $\beta\gamma$ subunit complexes composed of different γ subunits, and they were added to the NEM-treated membranes, G_{i2} with any $\beta\gamma$ subunits hardly increased GABA binding. On the other hand, G_o with any $\beta\gamma$ subunits caused a marked increase, though G_o with a small γ subunit was more effective than that with a large γ subunit. These data suggest that the selective coupling of the G proteins to $GABA_B$ receptors is determined by the α subunit.

$GABA_B$ receptor; GTP-binding protein; *N*-Ethylmaleimide; Reconstitution

1. INTRODUCTION

The GTP-binding proteins (G proteins) comprise a family of structurally homologous regulatory proteins serving as intermediaries in transmembrane signal transduction [1]. G proteins couple cell-surface receptors to various effectors, such as enzymes generating second messengers and ion channels. Among G proteins, there are several proteins sensitive to pertussis toxin, such as G_i, G_o and transducin. Molecular cloning has revealed the presence of three forms of G_i subspecies, G_{i1}, G_{i2} and G_{i3} [1], and proteins corresponding to these genes have been identified [2–7]. Recently, G_o^*, which may represent a novel form of G_o, has been purified from brain [8].

To elucidate the functional difference among G proteins, the selectivity of receptor-G protein coupling and of G protein-effector coupling was studied by the use of various systems [9]. Interactions between G proteins and receptors have been examined using reconstitution techniques. Rhodopsin [10], α_2-adrenergic [10,11], μ-opioid [12], muscarinic cholinergic [13], D_2 dopamine [14] and chemotactic peptide [15] receptors did not appear to distinguish particularly well between 'G_o' and 'G_i' in most cases. In early experiments, however, purified 'G_o' and 'G_i' were used which were not strictly identified and might be a mixture of multiple subspecies. Recently, Haga et al. [16] have shown that muscarinic acetylcholinc receptors similarly interacted with G_{i1}, G_{i2} and G_o. On the other hand, Senogles et al. [17] have shown that D_2 dopamine receptors selectively coupled to G_{i2} with about 10-fold higher affinity than G_{i1} or G_{i3} and did not couple to G_o, though the results conflicted with the report by Ohara et al. [14]. Furthermore, insulin-like growth factor-II receptors interacted only with G_{i2}, but not with G_{i1} or G_o [18].

In our previous studies, we demonstrated the coupling between $GABA_B$ receptors and G proteins using reconstitution techniques, but there was no selectivity for the coupling of 'G_o' and 'G_i' to receptors [19,20]. Because G_o and G_i subspecies could recently be purified and identified as mentioned above, we could study the selectivity of G proteins for coupling to $GABA_B$ receptors more precisely. In the present study, we compared the ability of four G proteins including G_o, G_o^*, G_{i1} and G_{i2} to couple to $GABA_B$ receptors. We also examined the effect of two $\beta\gamma$ subunit complexes, which were composed of distinct γ subunits and recently isolated from bovine brain [21], on the coupling to $GABA_B$ receptors.

2. MATERIALS AND METHODS

2.1. *Purification of G proteins*

G_o, G_{i1} and G_{i2} were purified from bovine brain or lung by the method of Katada et al. [22]. G_o^* was purified from bovine brain as described by Goldsmith et al. [8]. The α subunits of G_o and G_{i2} were purified from bovine brain and lung, respectively, as described previously [5,23]. Analysis of the purified G proteins by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is shown in Fig. 1. The $\beta\gamma$ subunit complexes composed of different γ subunits were purified from bovine brain by the method of Asano et al. [21]. Final preparations of all G proteins were in the medium containing 20 mM Tris-HCl (pH 8.0), 0.1 mM EDTA, 0.5 mM dithiothreitol (DTT), 0.1% Lubrol PX and 0.1 M potassium phosphate. Protein in the G protein preparations was assayed by the

Correspondence address: T. Asano, Department of Biochemistry, Institute for Developmental Research, Aichi Prefectural Colony, Kasugai, Aichi 480-03, Japan

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

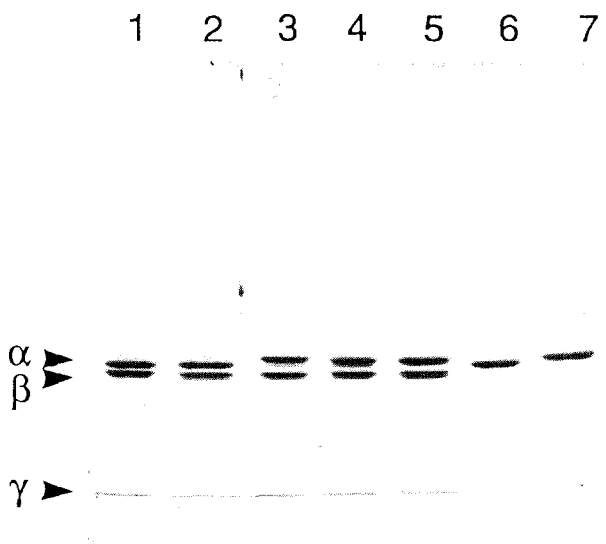

Fig. 1. SDS-PAGE patterns of purified G proteins and α subunits. Purified G proteins (1 μg) and α subunits (0.5 μg) were subjected to SDS-PAGE. The gel was stained with Coomassie blue. Lane 1, G_o; lane 2, G_o^*; lane 3, G_{i1}; lane 4, brain G_{i2}; lane 5, lung G_{i2}; lane 6, $G_o\alpha$; lane 7, lung $G_{i2}\alpha$.

method of Schaffner and Weissman [24]. The binding of GTPγS and incorporation of ADP-ribose to purified G proteins were in a range from 0.5 to 0.9 mol/mol protein. The amounts of G protein in this paper were shown in mols quantified by their ability to bind GTPγS.

2.2. *Preparation of N-ethylmaleimide-treated membranes and reconstitution with purified G proteins*

The agonist binding to the G protein-coupled receptors shows the high affinity only when G proteins bind to receptors. We previously showed that treatment of bovine brain membranes with pertussis toxin [19] or *N*-ethylmaleimide (NEM) [20] caused a loss of the high-affinity binding of GABA to $GABA_B$ receptors because the ADP-ribosylation or alkylation of endogenous G proteins in the membranes caused uncoupling of G proteins from receptors. The addition of the purified 'G_o' or 'G_i' to pertussis toxin- or NEM-treated membranes restored the high-affinity GABA binding. In the present study, we used the reconstitution technique as described below. The membranes from bovine cerebral cortex were treated with 0.2 mM NEM at 0°C for 30 min as described previously [20], and then were centrifuged at 20000 × *g* for 10 min. After washing three times with 20 mM Tris-HCl (pH 8.0), 1 mM EDTA, and 1 mM DTT (TED), the membrane preparations were stored at −80°C. Before use, NEM-treated membranes were thawed, washed twice with 50 mM Tris-HCl (pH 8.0), and resuspended in TED (10 mg of protein/ml). For non-treated membranes, the membranes were prepared by the same procedure except that 1 mM DTT was added to the membranes before the addition of 0.2 mM NEM. Protein in the membrane preparations was determined by the method of Lowry et al. [25].

Purified G proteins were incubated with NEM-treated membranes (about 700 μg of protein) in TED at 0°C for 1 h in the presence of 5 mM $MgCl_2$ and 0.02% Lubrol PX in a total volume of 150 μl. After incubation the mixture was diluted with TED (2.5 mg of protein/ml) and used for the GABA binding assay. The α subunits of G proteins were preincubated with $\beta\gamma$ subunits to form a trimer at 0°C for 15 min and then reconstituted with the membranes.

2.3. *GABA binding assay*

The binding of [^{3}H]GABA was measured essentially as described previously [19]. In brief, 80 μl (200 μg of protein) of the membrane suspension was incubated in 200 μl of 50 mM Tris-HCl (pH 7.5) containing 2.5 mM $CaCl_2$, 5 mM $MgCl_2$, 1 mM DTT, 50 μM isoguvacine, and 10 nM [^{3}H]GABA in the presence or absence of 100 μM (±)-baclofen for 10 min at 25°C. The final concentration of Lubrol PX was 0.0043% in assay mixture. The reaction mixture was then centrifuged at 20000 × *g* for 10 min and the pellet was rapidly and superficially rinsed with cold 50 mM Tris-HCl (pH 8.0) and solubilized in Protosol (New England Nuclear) to be measured for radioactivity. Specific binding is defined as the difference between the total binding and the binding in the presence of 100 μM baclofen (the nonspecific binding). The nonspecific binding to NEM-treated membranes was about 120 fmol/mg protein either with or without G proteins. The difference obtained with G proteins was due to an increase in the total binding per mg protein.

2.4. *Other methods*

SDS-PAGE was carried out by the method of Laemmli [26]. 8 M urea/SDS-PAGE was performed by the method of Swank and Munkres [27].

3. RESULTS

NEM-treated membranes were incubated with various amounts of the purified G proteins for 1 h at 0°C and analyzed for GABA binding (Fig. 2). The reconstitution of G_o, G_o^* or G_{i1} markedly increased GABA binding to NEM-treated membranes in a dose-dependent manner. Because these three G proteins showed similar dose-dependent curves, they seemed to have similar ability to couple to $GABA_B$ receptors. The final protein concentration of membranes was about 1 mg/ml in these experiments, and the concentration of endogenous G_o determined by immunoassay [28] was about 75 pmol/mg protein, indicating that the effective concentrations of added G_o were almost equivalent to its endogenous concentration.

In contrast, the addition of the G_{i2} purified from brain or lung slightly increased GABA binding to NEM-treated membranes (Fig. 2). When the effects by two G_{i2} preparations were compared, brain G_{i2} was apparently more effective than lung G_{i2} in restoring GABA binding. However, this appeared to be due to the contamination of the brain G_{i2} preparation with other G proteins including G_o, because SDS-PAGE analysis of G proteins revealed the presence of a small amount of the protein with about 39 kDa in brain G_{i2} preparation (Fig. 1). In addition, when the immunoreactivity of G_o was measured in G_{i2} preparations, it was equivalent to a 10% amount of G_{i2} in the brain preparation, while it was below 0.1% in the lung preparation. The increase of $GABA_B$ binding with 500 pmol/ml of lung G_{i2} was almost equal to that with 15 pmol/ml of G_o, G_o^* or G_{i1}, indicating that the effect of G_{i2} was 30-times less potent than the effects of the other G proteins.

The G proteins used here were separated by DEAE-chromatography and were identified by their α subunits. The $\beta\gamma$ complexes had been usually considered to be identical or very similar among these G proteins and were not well analyzed. The SDS-PAGE

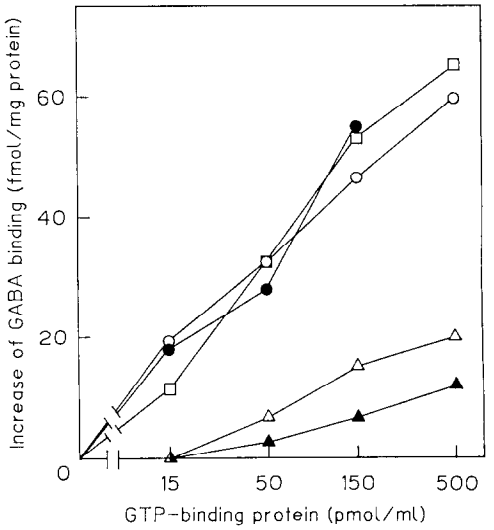

Fig. 2. Effect of purified G proteins on [^{3}H]GABA binding to NEM-treated membranes. NEM-treated membranes were incubated with various concentrations of the purified GTP-binding proteins as described in section 2. Binding assays were carried out with 10 nM [^{3}H]GABA. Concentrations of G proteins are shown as the final concentrations in binding assay with the values quantified by [^{35}S]GTPγS binding. Protein concentration of membranes was 1 mg/ml. Basal GABA binding (without G proteins) to NEM-treated membranes was 34 fmol/mg protein. The increase of GABA binding is shown as the binding with G proteins minus basal binding. Specific binding to nontreated membranes was 170 fmol/mg protein. G proteins were as follows: G_o (○), G_o^* (●), G_{i1} (□), brain G_{i2} (△) and lung G_{i2} (▲).

analysis (Fig. 1) revealed that each G protein contained both 36 kDa and 35 kDa β (36 kDa > 35 kDa), but G_o and G_{i2} contained more 35 kDa β than G_o^* and G_{i1}, as shown by Goldsmith et al. [8]. Further analysis of their γ subunits by urea/SDS-PAGE showed that brain G proteins had various amounts of a large γ (γ-1) in addition to a major small γ (γ-2) [21] (Fig. 3). With regard to G_{i2}, which hardly couple to $GABA_B$ receptors, both brain and lung G_{i2} lacked γ-1 but lung G_{i2} had another large γ (Fig. 3). These results raised the question as to which subunit was crucial for the selective coupling to receptors. The α subunits of G_o and G_{i2} were purified from bovine brain and lung, respectively, and two βγ subunit complexes, βγ-1 and βγ-2, were isolated from bovine brain [21]. Both α subunits were homogeneous on SDS-PAGE, as shown in Fig. 1. The βγ-1 was composed of 36 kDa β and 6 kDa γ and βγ-2 was composed of 36 kDa and 35 kDa β and 4.5 kDa γ [21], and two γ subunits probably had different primary structures [29]. Each α and βγ subunit was mixed with a ratio of α to βγ of 1:1 to form G_o or G_{i2}, and their abilities to increase GABA binding were determined by reconstitution with NEM-treated membranes. As shown in Table I, the α subunit alone of either G_o and G_{i2} caused a small or no effect on GABA binding. The βγ alone gave no effect (not shown). When the trimeric G proteins were reconstituted to NEM-treated membranes, GABA binding was markedly increased with G_o composed of either βγ subunits, but not with G_{i2} composed with either βγ (Table I). These results indicated that the α subunit was crucial for the selective coupling to receptors. However, Table I shows that the G_o with βγ-2 was more effective than that with βγ-1 in restoring GABA binding. The G_o composed of the mixture of βγ-1 and βγ-2 was also very effective, and the increase in GABA binding was equal to that by the G_o purified as a trimer form (Fig. 2).

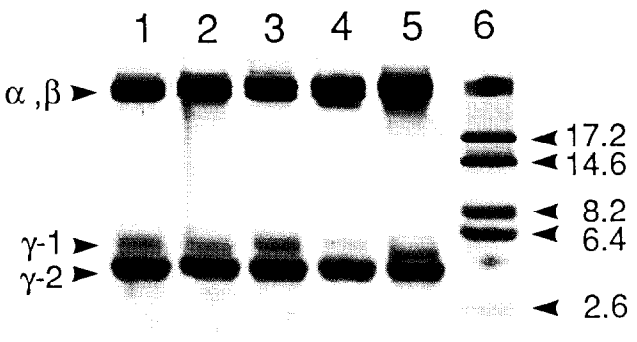

Fig. 3. 8 M urea/SDS-PAGE patterns of purified G proteins. Purified G proteins (1 μg) were subjected to urea/SDS-PAGE. The gel was stained with silver. Lane 1, G_o; lane 2, G_o^*; lane 3, G_{i1}; lane 4, brain G_{i2}; lane 5, lung G_{i2}; lane 6, M_r standards. Numbers on the right indicate molecular mass in kDa.

Table I

Increase of GABA binding to NEM-treated membranes by G_o or G_{i2} composed of different βγ complexes

Addition	Increase of GABA binding (fmol/mg protein)	
	$G_o\alpha$	$G_{i2}\alpha$
None	5.2	0.0
βγ-1	14.1	0.0
βγ-2	41.6	0.6
βγ-1/2[a]	46.8	0.5

[a] A mixture of βγ-1 and βγ-2

150 pmol/ml of α subunits with or without equal molar of various βγ subunit complexes were added to NEM-treated membranes and the increase of [^{3}H]GABA binding in the membranes was measured. Basal GABA binding (without G proteins) to NEM-treated membranes was 33 fmol/mg protein. Data are mean values from 3 experiments

4. DISCUSSION

In our previous paper, we showed that there was no selectivity for the coupling of 'G_o' and 'G_i' to $GABA_B$ receptors [19,20]. In these early studies, however,

purified 'G_o' and 'G_i' were not strictly identified and might be a mixture of the G_o or G_i family. Three subspecies of G_i were recently purified from brain or other tissues or cells [2–7], though G_{i3} was not yet purified in an active form with enough amounts to use for experiments such as the reconstitution with receptors [6]. A novel form of G_o, G_o^*, was also identified [8]. In order to clarify the specificity of G proteins, we compared the ability of four G proteins, including G_o, G_o^*, G_{i1} and G_{i2}, to couple to $GABA_B$ receptors in this study. The reconstitution study showed that $GABA_B$ receptors coupled to purified G_o, G_o^* and G_{i1}, but hardly to G_{i2}. The inability of G_{i2} to interact with receptors did not appear to be due to the inactivation, because the amounts of G proteins reconstituted were quantified with their ability to bind GTPγS, and also because mastoparan stimulated the rate of the GTPγS binding to G_{i2} as well as that to other G proteins (not shown).

The selectivity of $GABA_B$ receptors for coupling to G proteins is quite different from that of D_2 dopamine receptors [17], which coupled most efficiently to G_{i2} but not to G_o. However, G_o is located predominantly in the nervous tissues and neuroendocrine cells, while G_{i2} is located in all tissues [30,31]. In the brain, the level of G_o was about 15-fold higher than that of G_{i2}, and the concentrations of G_{i2} were constant throughout ontogenic development, while the G_o levels markedly increased coincidently with neural development [31]. These facts suggest that G_o is involved in the neurotransmission and G_{i2} in the fundamental process common to the various cellular functions rather than in neurotransmission. Our present results are in line with the aspect described above, and suggest that $GABA_B$ receptors selectively coupled to G_o (and G_i).

The G_{i2} preparation purified from bovine brain coupled to $GABA_B$ receptors better than the G_{i2} purified from bovine lung. However, this apparent inconsistency seemed to be due to the coupling of the contaminated G_o to receptors. We obtained brain G_{i2} preparation by repeating rechromatography on Mono Q column, but it still contained G_o. These observations suggest that the apparently low specificity of brain G proteins for coupling to receptors reported previously might be due to the contaminant of the preparation used with other G proteins. The lung G_{i2} preparation used in the present study contained little other G proteins, because the G_{i2} is a major G protein in the lung [5].

In the present study, G_o and G_{i1} displayed a similar efficacy to couple to $GABA_B$ receptors. However, it is likely that $GABA_B$ receptors separately regulate several effectors via G_o and G_{i1}, because the selectivity of G protein-effector coupling was also observed in various systems. It was reported that $GABA_B$ receptor agonist caused: (1) inhibition of Ca^{2+} channel [32,33]; (2) inhibition of adenylyl cyclase [34]; (3) stimulation of K^+ channel [35–37]. First, with respect to Ca^{2+} channel, Hescheler et al. [38] reported that G_o was clearly more effective than G_i for restoration of opioid inhibition of Ca^{2+} currents in NG 108-15 cells. In addition, antibodies to G_o, but not those to G_i, antagonized noradrenalin-induced Ca^{2+} current inhibition in NG 108-15 cells [39]. Therefore, $GABA_B$ receptors maybe regulate Ca^{2+} channel more efficiently via G_o than via G_{i1}-like neuropeptide Y receptors [40]. Second, adenylyl cyclase can be inhibited with activated $G_{i1}\alpha$ but not with $G_o\alpha$ and $G_{i2}\alpha$ [22]. Therefore, $GABA_B$ receptors may inhibit adenylyl cyclase via G_i, but it is still possible that G_o inhibits this enzyme by its $\beta\gamma$ subunits [41,42]. However, the third effector, the K^+ channel, did not reveal selectivity for G protein when various α subunits were reconstituted with the K^+ channel from cardiac atrial cells [1,43]. These results suggest that G_{i1} and/or G_o mediate(s) the stimulation of K^+ channel by $GABA_B$ receptors.

The $\beta\gamma$ subunit complexes of purified G proteins were not identical and particularly those of G_{i2} from both bovine brain and lung were different from other G proteins, suggesting a possible involvement of $\beta\gamma$ to selective coupling of G proteins to $GABA_B$ receptors. Reconstitution of $GABA_B$ receptors to the $G_o\alpha$ or $G_{i2}\alpha$ with various $\beta\gamma$ complexes revealed that $G_o\alpha$ could couple to receptors with either $\beta\gamma$ complexes but $G_{i2}\alpha$ could not with any $\beta\gamma$ complexes. Thus it is the α subunit that determines the selective coupling to receptors. However, $G_o\alpha$ could couple to $GABA_B$ receptors with $\beta\gamma$-2 more efficiently than with $\beta\gamma$-1, suggesting the $\beta\gamma$ complexes may also be involved in the selective coupling of receptors to G proteins. Since we previously could not observe any difference between $\beta\gamma$-1 and $\beta\gamma$-2 except that only γ-1 was phosphorylated by protein kinase C [21], the present findings provide the first physiological difference between $\beta\gamma$-1 and $\beta\gamma$-2 complexes.

Acknowledgements: We thank Dr P. Krogsgaard-Larsen for providing isoguvacine. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan, and a research grant from the Ishida Foundation.

REFERENCES

[1] Neer, E.J. and Clapham, D.E. (1988) Nature 333, 129–134.
[2] Codina, J., Olate, J., Abramowitz, J., Mattera, R., Cook, R.G. and Birnbaumer, L. (1988) J. Biol. Chem. 63, 6746–6750.
[3] Goldsmith, P., Rossiter, K., Carter, A., Simonds, W., Unson, C.G., Vinitsky, R. and Spiegel, A.M. (1988) J. Biol. Chem. 263, 6476–6479.
[4] Itoh, H., Katada, T., Ui, M., Kawasaki, H., Suzuki, K. and Kaziro, Y. (1988) FEBS Lett. 230, 85–89.
[5] Morishita, R., Kato, K. and Asano, T. (1988) Eur. J. Biochem. 174, 87–94.
[6] Morishita, R., Asano, T., Kato, K., Itoh, H. and Kaziro, Y. (1989) Biochem. Biophys. Res. Commun. 161, 1280–1285.
[7] Nagata, K., Katada, T., Tohkin, M., Itoh, H., Kaziro, Y., Ui, M. and Nozawa, Y. (1988) FEBS Lett. 237, 113–117.

[8] Goldsmith, P., Backlund, Jr, P.S., Rossiter, K., Carter, A., Milligan, G., Unson, C.G. and Spiegel, A. (1988) Biochemistry 27, 7085–7090.
[9] Ross, E.M. (1989) Neuron 3, 141–152.
[10] Cerione, R.A., Regan, J.W., Nakata, H., Codina, J., Benovic, J.L., Gierschik, P., Somers, R.L., Spiegel, A.M., Birnbaumer, L., Lefkowitz, R.J. and Caron, M.G. (1986) J. Biol. Chem. 261, 3901–3909.
[11] Kim, M.H. and Neubig, R.R. (1987) Biochemistry 26, 3664–3672.
[12] Ueda, H., Harada, H., Nozaki, M., Katada, T., Ui, M., Satoh, M. and Takagi, H. (1988) Proc. Natl. Acad. Sci. USA 85, 7013–7017.
[13] Kurose, H., Katada, T., Haga, T., Haga, K., Ichiyama, A. and Ui, M. (1986) J. Biol. Chem. 261, 6423–6428.
[14] Ohara, K., Haga, K., Berstein, G., Haga, T., Ichiyama, A. and Ohara, K. (1988) Mol. Pharmacol. 33, 290–296.
[15] Kikuchi, A., Kozawa, O., Kaibuchi, K., Katada, T., Ui, M. and Takai, Y. (1986) J. Biol. Chem. 261, 11558–11562.
[16] Haga, K., Uchiyama, H., Haga, T., Ichiyama, A., Kangawa, K. and Matsuo, H. (1988) Mol. Pharmacol. 35, 286–294.
[17] Senogles, S.E., Spiegel, A.M., Padrell, E., Iyengar, R. and Caron, M.G. (1990) J. Biol. Chem. 265, 4507–4514.
[18] Nishimoto, I., Murayame, Y., Katada, T., Ui, M. and Ogata, E. (1989) J. Biol. Chem. 264, 14029–14038.
[19] Asano, T., Ui, M. and Ogasawara, N. (1985) J. Biol. Chem. 260, 12653–12658.
[20] Asano, T. and Ogasawara, N. (1986) Mol. Pharmacol. 29, 244–249.
[21] Asano, T., Morishita, R., Kobayashi, T. and Kato, K. (1990) FEBS Lett. 266, 41–44.
[22] Katada, T., Oinuma, M., Kusakabe, K. and Ui, M. (1987) FEBS Lett. 213, 353–358.
[23] Asano, T., Kamiya, N., Morishita, R. and Kato, K. (1988) J. Biochem. 103, 950–953.
[24] Shaffner, W. and Weissmann, C. (1973) Anal. Biochem. 56, 502–514.
[25] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[26] Laemmli, U.K. (1970) Nature 227, 680–685.
[27] Swank, R.T. and Munkres, K.D. (1971) Anal. Biochem. 39, 462–477.
[28] Asano, T., Semba, R., Ogasawara, N. and Kato, K. (1987) J. Neurochem. 48, 1617–1623.
[29] Robishaw, J.D., Kalman, V.K., Moomaw, C.R. and Slaughter, C.A. (1989) J. Biol. Chem. 264, 15758–15761.
[30] Asano, T., Semba, R., Kamiya, N., Ogasawara, N. and Kato, K. (1988) J. Neurochem. 50, 1164–1169.
[31] Asano, T., Morishita, R., Semba, R., Itoh, H., Kaziro, Y. and Kato, K. (1989) Biochemistry 28, 4749–4754.
[32] Holz, IV, G.G., Rane, S.G. and Dunlap, K. (1986) Nature 319, 670–672.
[33] Holz, IV, G.G., Kream, R.M., Spiegel, A. and Dunlap, K. (1989) J. Neurosci. 9, 657–666.
[34] Wojcik, W.J. and Neff, N.H. (1984) Mol. Pharmacol. 25, 24–28.
[35] Newberry, N.R. and Nicoll, R.A. (1984) Nature 308, 450–452.
[36] Gahwiler, B.H. and Brown, D.A. (1985) Proc. Natl. Acad. Sci. USA 82, 1558–1562.
[37] Andrade, R., Malenka, R.C. and Nicoll, R.A. (1986) Science 234, 1261–1265.
[38] Hescheler, J., Rosenthal, W., Trautwein, W. and Schultz, G. (1987) Nature 325, 445–447.
[39] McFadzean, I., Mullaney, I., Brown, D.A. and Milligan, G. (1989) Neuron 3, 177–182.
[40] Ewald, D.A., Pang, I.-H., Sternweis, P.C. and Miller, R.J. (1989) Neuron 2, 1185–1193.
[41] Katada, T., Oinuma, M. and Ui, M. (1986) J. Biol. Chem. 261, 5215–5221.
[42] Hildebrandt, J.D. and Kohnken, R.E. (1990) J. Biol. Chem. 265, 9825–9830.
[43] Yatani, A., Mattera, R., Codina, J., Graf, R., Okabe, K., Padrell, E., Iyenger, R., Brown, A.M. and Birnbaumer, L. (1988) Nature 336, 680–682.