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Biochemical Pharmacology 62 (2001) 183–190

Biochemical Pharmacology

$\beta\gamma$-mediated enhancement of corticotropin-releasing hormone-stimulated adenylyl cyclase activity by activation of γ-aminobutyric acid$_B$ receptors in membranes of rat frontal cortex

Pierluigi Onali*, Maria C. Olianas

Section of Biochemical Pharmacology, Department of Neuroscience, University of Cagliari, 09124 Cagliari, Italy

Received 12 October 2000; accepted 6 February 2001

Abstract

A number of studies have shown that activation of γ-aminobutyric acid$_B$ (GABA$_B$) receptors potentiates neurotransmitter-induced accumulation of cyclic AMP in brain slices, but the mechanisms involved in the facilitatory effect have not been fully elucidated. In the present study, we showed that in membranes of rat frontal cortex the GABA$_B$ receptor agonist (−)baclofen increased basal adenylyl cyclase activity and potentiated the maximal enzyme stimulation elicited by corticotropin-releasing hormone (CRH). The less active enantiomer (+)baclofen had no effect on cyclic AMP formation, whereas the natural agonist GABA mimicked the stimulatory action of (−)baclofen. In radioligand-binding experiments, the affinity and maximal binding capacity of ^{125}I-Tyr-CRH was not affected by (−)baclofen. The GABA$_B$ receptor antagonist CGP 55845A competitively counteracted the (−)baclofen potentiation of CRH-stimulated adenylyl cyclase activity with a pA$_2$ value of 6.70. Moreover, both (−)baclofen and GABA, but not (+)baclofen, caused a concentration-dependent stimulation of [^{35}S]GTPγS binding to membrane G-proteins. The intracerebral injection of pertussis toxin significantly reduced the facilitatory effects of (−)baclofen on both basal and CRH-stimulated adenylyl cyclase activities. Moreover, membrane incubation with the GDP-bound form of the α subunit of transducin, a scavenger of G protein $\beta\gamma$ subunits, blocked the stimulatory effects of (−)baclofen. The data indicate that in rat frontal cortex activation of GABA$_B$ receptors potentiates the CRH stimulation of adenylyl cyclase activity through a mechanism involving the $\beta\gamma$ subunits of the pertussis toxin-sensitive G protein G$_i$/G$_o$.

Keywords: γ-Aminobutyric acid$_B$ receptors; Adenylyl cyclase; [^{35}S]GTPγS binding; G-protein $\beta\gamma$ subunits; Corticotropin-releasing hormone; Rat frontal cortex

1. Introduction

The metabotropic GABA$_B$ receptors are known to interact with and activate pertussis toxin-sensitive G proteins of the G$_i$/G$_o$ family and, by this route, to regulate different signalling pathways, including the inhibition of Ca^{2+} channels, stimulation of K^+ conductance, and inhibition of adenylyl cyclase activity [1]. The inhibitory coupling of GABA$_B$ receptors to adenylyl cyclase has been observed in tissue preparations of different brain regions [2], in a reconstituted system [3], and in cell lines transfected with the cloned receptors [4–6]. However, activation of GABA$_B$ receptors has also been found to exert positive effects on adenylyl cyclase. Thus, in brain slices the GABA$_B$ receptor agonist baclofen not only inhibited forskolin-stimulated adenylyl cyclase activity but also enhanced the cyclic AMP formation elicited by neurotransmitters, such as noradrenaline, vasoactive intestinal peptide, adenosine, and prostaglandins [7–12]. In addition, *in vivo* evidence that GABA$_B$ receptor can exert a dual control on cyclic AMP formation has recently been provided by using a microdialysis technique [13]. On the basis of the different sensitivity to some antagonists, it has been proposed that the GABA$_B$ receptors mediating stimulation and inhibition of cyclic AMP are pharmacologically distinct, thus raising the possibility of the involvement of multiple receptor subtypes [14].

Early studies conducted in brain slices and addressed to identify the molecular mechanisms mediating the GABA$_B$ receptor-induced facilitation of cyclic AMP formation have

* Corresponding author. Tel.: +39-070-675-8402; fax: +39-070-657-237.

E-mail address: onali@unica.it (P. Onali).

Abbreviations: α_{tGDP}, GDP-bound form of the α-subunit of transducin; CRH, corticotropin-releasing hormone; DTT, dithiothreitol; and GABA$_B$, γ-aminobutyric acid$_B$.

0006-2952/01/$ – see front matter
PII: S0006-2952(01)00659-1

suggested the occurrence of an indirect coupling mediated by activation of phospholipase A_2 [15,16] and 5-lipoxygenase [11]. Moreover, a modulatory effect of baclofen on β-adrenergic receptors has been described, indicating that the $GABA_B$ receptor facilitation of β-adrenergic stimulation of cyclic AMP may be partially due to an enhanced coupling of β-adrenergic receptor to the adenylyl cyclase stimulatory G-protein G_S [17].

With the identification of adenylyl cyclase isoforms II and IV, which can be activated by G-protein $\beta\gamma$ subunits synergistically with G_S [18], a novel mechanism by which $GABA_B$ receptors may positively regulate cyclic AMP formation has emerged. Thus, it has been demonstrated that in Xenopus oocytes injected with rat brain cortex poly (A)+ RNA and cRNA for type II adenylyl cyclase, baclofen induced a $\beta\gamma$-mediated stimulation of cyclic AMP accumulation [19]. Moreover, we have recently reported that in membranes of specific layers of the rat olfactory bulb, activation of $GABA_B$ receptors stimulated adenylyl cyclase activity through the action of $\beta\gamma$ subunits of G_i/G_o, indicating that this coupling mechanism occurs in brain membranes expressing receptors, G-proteins, and effector molecules under native conditions [20]. However, if $\beta\gamma$-mediated stimulation of adenylyl cyclase were a common mechanism involved in the positive coupling of $GABA_B$ receptors to cyclic AMP, then it should be possible to demonstrate its occurrence in brain areas other than the olfactory bulb.

In the present study, we report that in membranes of rat frontal cortex activation of $GABA_B$ receptors enhances basal adenylyl cyclase activity and markedly potentiates the enzyme stimulation by CRH, and provide evidence that this facilitatory effect is mediated by $\beta\gamma$ subunits of G_i/G_o. Part of this study has previously been presented in an abstract form [21].

2. Materials and methods

2.1. Materials

[α-^{32}P]ATP (30–40 Ci/mmol), [2,8-^{3}H]cyclic AMP (25 Ci/mmol), ^{125}I-Tyr-CRH (human, rat) (2200 Ci/mmol), and [^{35}S]GTPγS (1306 Ci/mmol) were obtained from New England Nuclear. (−)Baclofen, (+)baclofen, and CGP 55845A (*β-N* [1-(*S*)-(3,4-dichlorophenyl)ethyl]amino-2-(*S*)hydroxy-propyl-*P*-benzyl-phosphinic acid) were obtained from Ciba-Geigy. CRH (human, rat) was from Neosystem. α_{tGDP}, purified from bovine retina, was kindly provided by Prof. Heidi E. Hamm, University of Illinois at Chicago. Stock solutions were made in a storing buffer containing 50% glycerol, 75 mM Tris–HCl, 3.75 mM dithiothreitol, and 75 μM phenylmethylsulfonyl fluoride (pH 7.3). Dilutions were made in tenfold-diluted storing buffer containing 0.1% BSA immediately before the experiments. Pertussis toxin was from Calbiochem. GABA and the other reagents were from Sigma Chemical Co.

2.2. Membrane preparation

Male Sprague–Dawley rats (200–250 g) were killed by decapitation and the frontal cortex was rapidly dissected from the brain. The frontal lobes were homogenized using a Teflon glass tissue grinder (12 up-and-down strokes by hand) in 10 volumes (wt/vol) of ice-cold buffer containing 10 mM HEPES–NaOH, 1 mM EGTA, 1 mM dithiothreitol, and 1 mM $MgCl_2$ (pH 7.4). The homogenate was diluted fivefold with the same medium and centrifuged at 27,000 *g* for 20 min at 4°. The pellet was resuspended and centrifuged as above. The final pellet was resuspended in the same buffer to a protein concentration of 1.0–1.5 mg/mL and used immediately for the adenylyl cyclase assay.

2.3. Adenylyl cyclase assay

The enzyme activity was assayed by monitoring the conversion of [α-^{32}P]ATP to [^{32}P]cyclic AMP as previously reported [22]. The reaction mixture (final volume 100 μL) contained 50 mM HEPES/NaOH (pH 7.4), 2.3 mM $MgCl_2$, 0.1 mM [α-^{32}P]ATP (100–130 cpm/pmol), 0.5 mM [^{3}H]cyclic AMP (80 cpm/nmol), 1.3 mM EGTA, 1.3 mM DTT, 1 mM 3-isobutyl-1-methylxanthine, 5 mM phosphocreatine, 50 U/mL of creatine kinase, 10 μM GTP, 50 μg of BSA, 10 μg of bacitracin, and 10 kallikrein inhibitor units of aprotinin. The reaction was started by adding the tissue preparation (30–40 μg of protein) and carried out at 30° for 10 min. When the effects of α_{tGDP} were examined, 10 μL of the membrane preparation (20–25 μg of protein) were preincubated with an equal volume of a solution containing either vehicle or α_{tGDP} for 60 min at ice-bath temperature. Thereafter, the receptor agonists were added immediately followed by the addition of the reaction mixture to yield a final volume of 50 μL. The final concentration of α_{tGDP} was 0.5 μM. Assays were performed in duplicate.

2.4. [^{35}S]GTPγS binding assay

Membranes of rat frontal cortex were prepared as described above with the exception that the homogenization buffer was 10 mM HEPES–NaOH plus 1 mM EDTA. The binding of [^{35}S]GTPγS was assayed at 30° in a reaction mixture (final volume 100 μL) containing 25 mM HEPES–NaOH (pH 7.4), 5 mM $MgCl_2$, 1 mM EDTA, 150 mM NaCl, 50 μM GDP, and 2.0–2.5 μg of membrane protein. The concentration of [^{35}S]GTPγS was 1.0 nM. The incubation was terminated by adding 5 mL of ice-cold buffer containing 10 mM HEPES–NaOH (pH 7.4) and 1 mM $MgCl_2$, immediately followed by rapid filtration on glass fiber filters presoaked in the same buffer. The filters were washed twice with 5 mL of buffer and the radioactivity trapped was determined by liquid scintillation spectrometry.

Non-specific binding was determined in the presence of 100 μM GTPγS. Assays were performed in duplicate.

2.5. ^{125}I-Tyr-CRH binding assay

The frontal lobes were homogenized in 10 volumes of ice-cold buffer containing 50 mM Tris–HCl (pH 7.1), 1 mM EGTA, and 0.1 mM phenylmethylsulfonyl fluoride. The homogenate was centrifuged at 27,000 *g* for 20 min at 4° and the pellet was resuspended and centrifuged as above. The final pellet was resuspended in the same buffer at the final protein concentration of 2.0–3.0 mg/mL. The incubation buffer (final volume 300 μL) contained 50 mM Tris–HCl (pH 7.0), 1 mM EGTA, 1 mM DTT, 10 mM $MgCl_2$, 0.2% BSA, 100 μM bacitracin, 30 kallikrein inhibitor units of aprotinin, 0.1 mM PMSF, and 200–300 μg of membrane protein. In saturation binding assays, the concentration of ^{125}I-Tyr-CRH ranged from 0.01 to 0.80 nM, whereas in competition experiments it was 60 pM. The incubation was carried out at 20° for 2 hr and was terminated by adding 5 mL of ice-cold buffer containing 50 mM Tris–HCl and 0.1% BSA (pH 7.0), immediately followed by rapid filtration through glass fiber filters (Whatman GF/C) presoaked in 1% BSA for at least 24 hr. The filters were washed three times with 5 mL of buffer and the radioactivity trapped was counted by liquid scintillation spectrometry. Non-specific binding was determined in the presence of 2 μM unlabeled CRH. Assays were performed in duplicate.

2.6. Intracerebral injection of pertussis toxin

The injection of pertussis toxin into frontal cortex was performed as previously described [22]. Control animals received an equal volume of vehicle containing 3 μg of BSA. The animals were sacrificed 5 days after surgery and membranes were prepared from vehicle and toxin-treated frontal cortex. Three tissue preparations were investigated. Protein was determined by the method of Bradford [23], using BSA as a standard.

2.7. Statistical analysis

Results are reported as means ± SEM values. Data from concentration–response curves were analyzed by a least-squares curve-fitting computer program (GraphPad Prism). Antagonist pA_2 values were calculated from Arunlakshana–Schild regressions, in which the log of dose ratios-1 is plotted as a function of the antagonist concentration. ^{125}I-Tyr-CRH binding data were analysed by the combined EBDA-LIGAND computer programs (Biosoft), which provided the IC_{50}, K_d, and B_{max} values. The statistical significance of the difference between means was determined by Student's *t*-test.

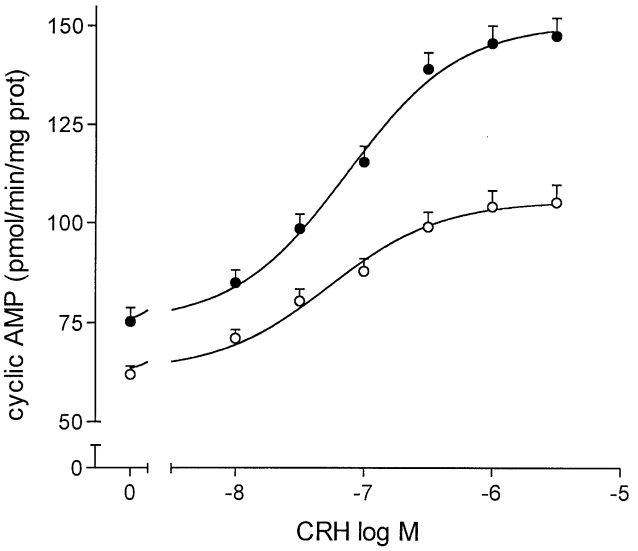

Fig. 1. Enhancement of CRH stimulation of adenylyl cyclase activity by (−)baclofen in membranes of rat frontal cortex. The enzyme activity was assayed at the indicated concentrations of CRH in the absence (○) and in the presence (●) of 1 mM (−)baclofen. Values are the means ± SEM of four experiments.

3. Results

3.1. Agonist effects on adenylyl cyclase activity

In membranes of rat frontal cortex, the addition of (−)baclofen markedly enhanced the stimulation of cyclic AMP formation elicited by CRH (Fig. 1). In the absence of (−)baclofen, CRH maximally increased the basal adenylyl cyclase activity by 70.3 ± 4.5% ($P < 0.001$) with an EC_{50} value of 53.8 ± 4.9 nM. (−)Baclofen (1 mM) increased the maximal enzyme stimulation elicited by the neuropeptide to 110.8 ± 5.8% ($P < 0.05$, N = 4) without significantly changing the EC_{50} value of CRH (79.0 ± 7.5 nM, $P > 0.05$, N = 4). Thus, the CRH-stimulated enzyme activity (that is, the net increase produced by 1 μM CRH above basal value) corresponded to 41.2 ± 3.2 and 75.4 ± 5.8 pmol of cyclic AMP/min/mg protein in the absence and in the presence of 1 mM (−)baclofen, respectively ($P < 0.001$, N = 4). (−)Baclofen (1 mM) also caused a modest (22.5 ± 3.5% increase) but significant ($P < 0.05$, N = 4) stimulation of basal adenylyl cyclase activity (Fig. 1).

The stimulatory effect of (−)baclofen was concentration-dependent with EC_{50} values of 166 ± 10 and 220 ± 20 μM for the enhancement of CRH-stimulated and basal adenylyl cyclase activities, respectively (Fig. 2). Conversely, the addition of the less active enantiomer (+)baclofen at the same concentration range failed to affect either basal or CRH-stimulated cyclic AMP formation. Like (−)baclofen, the naturally occurring agonist GABA induced a concentration-dependent potentiation of CRH receptor activity with an EC_{50} value of 800 ± 40 μM (Fig. 2).

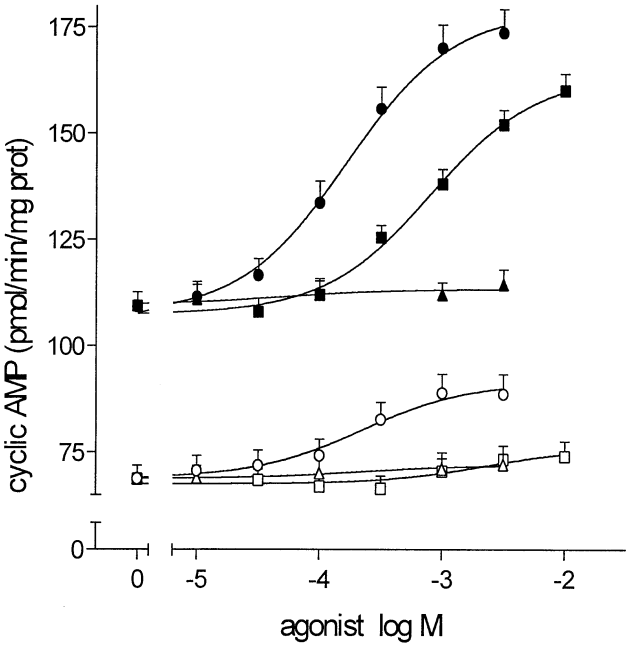

Fig. 2. Effects of baclofen enantiomers and GABA on adenylyl cyclase activity of rat frontal cortex. The enzyme activity was assayed in the absence (open symbols) and in the presence (closed symbols) of 1 μM CRH at the indicated concentrations of (−)baclofen (○, ●), (+)baclofen (△, ▲), and GABA (□, ■). Values are the means ± SEM of four experiments.

3.2. Effects on ^{125}I-Tyr-CRH binding

The binding of ^{125}I-Tyr-CRH to membranes of rat frontal cortex was saturable and yielded a linear Scatchard plot, indicating the presence of a high-affinity binding site with a K_d value of 0.45 ± 0.05 nM and B_{max} of 47 ± 3.0 fmol/mg protein (N = 3) (Fig. 3). The addition of (−)baclofen (0.2 mM) failed to affect the ^{125}I-Tyr-CRH binding properties (K_d = 0.47 ± 0.06 nM; B_{max} = 45 ± 4 fmol/mg protein) (Fig. 3). In competition experiments, unlabeled CRH (from 0.1 nM to 2 μM) displaced the binding of ^{125}I-Tyr-CRH with an IC_{50} value of 6.2 ± 1.7 nM. The addition of (−)baclofen (0.2 mM) did not change the IC_{50} value of CRH (6.4 ± 1.8 nM, N = 3) (results not shown).

3.3. Antagonism by CGP 55845A

The concentration–response curve of (−)baclofen in potentiating the CRH-stimulated adenylyl cyclase activity was progressively shifted to the right by increasing concentrations of the $GABA_B$ receptor antagonist CGP 55845A (Fig. 4). Schild plot analysis of CGP 55845A antagonism yielded a pA_2 value of 6.70 ± 0.03 and a slope value of 0.89 ± 0.07.

3.4. Effects on [^{35}S]GTPγS binding

(−)Baclofen stimulated the binding of [^{35}S]GTPγS to membranes of rat frontal cortex with an EC_{50} value of

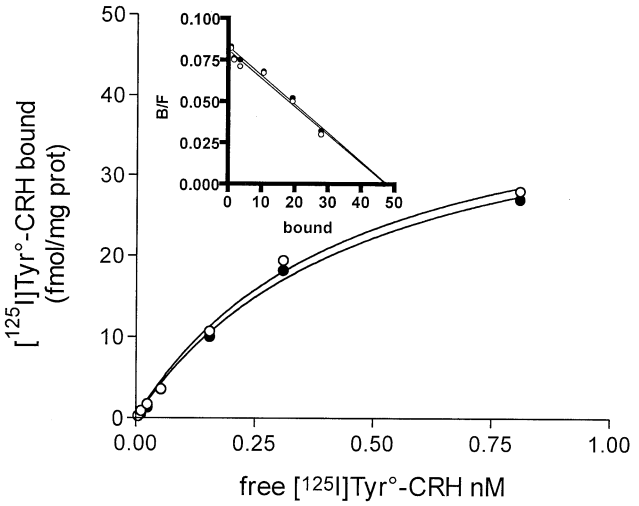

Fig. 3. Effect of (−)baclofen on ^{125}I-Tyr-CRH binding. Membranes of rat frontal cortex were incubated with increasing concentrations of ^{125}I-Tyr-CRH in the absence (○) and in the presence (●) of 0.2 mM (−)baclofen for 120 min at 20°. Non-specific binding was determined in the presence of 2 μM CRH. Data are from one experiment representative of three experiments. Inset: Scatchard plot of ^{125}I-Tyr-CRH binding in the absence (○) and the presence (●) of (−)baclofen.

18.2 ± 0.5 μM and a maximal effect corresponding to a 250 ± 20% increase in the basal value ($P < 0.001$, N = 3) (Fig. 5). (+)Baclofen was without effect. GABA stimulated [^{35}S]GTPγS binding as effectively as (−)baclofen and with an EC_{50} value of 150 ± 10 μM (Fig. 5).

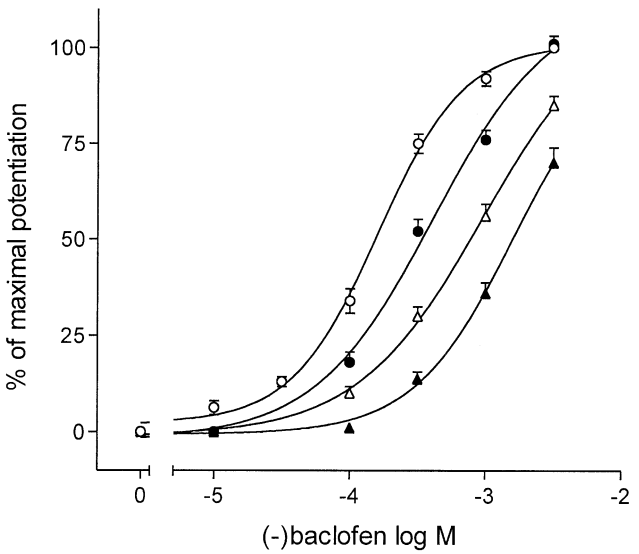

Fig. 4. Antagonism of (−)baclofen-induced potentiation of CRH-stimulated adenylyl cyclase activity by CGP 55845A. The (−)baclofen potentiation of the enzyme activity stimulated by 1 μM CRH was determined in the absence (○) and in the presence of 0.3 (●), 1.0 (△), and 3.0 (▲) μM CGP 55845A. The data are expressed as percent of the maximal potentiation measured in the absence of the antagonist and represent the means ± SEM of three experiments.

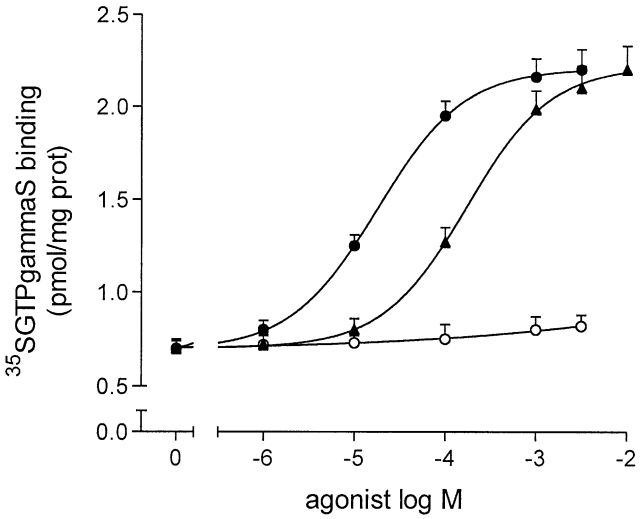

Fig. 5. Stimulation of [^{35}S]GTPγS binding by (−)baclofen and GABA and lack of effect of (+)baclofen. Rat frontal cortex membranes were incubated in the presence of the indicated concentrations of (−)baclofen (●), (+)baclofen (○), and GABA (▲). Values are the means ± SEM of three experiments.

3.5. Effects of pertussis toxin

The intracerebral injection of pertussis toxin was particularly effective in preventing the facilitatory effects of (−)baclofen on either basal or CRH-stimulated adenylyl cyclase activity (Fig. 6). On the other hand, the toxin treatment failed to significantly affect either basal or CRH-stimulated adenylyl cyclase activity.

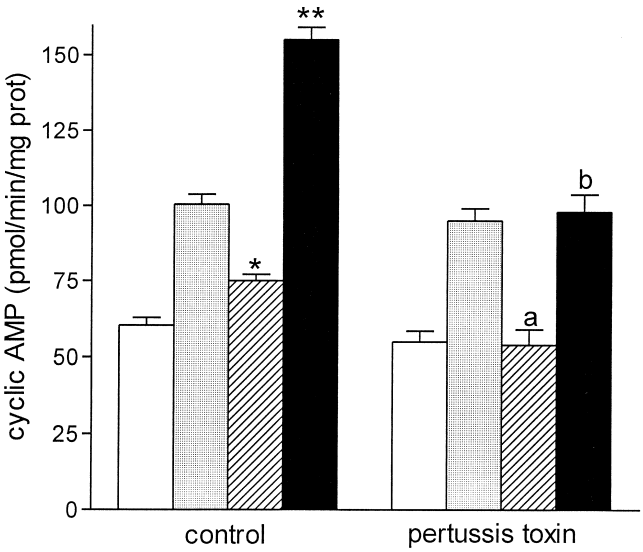

Fig. 6. Effects of the intracerebral injection of pertussis toxin on (−)baclofen stimulation of adenylyl cyclase activity. Membranes were prepared from control and pertussis toxin-injected rats and the adenylyl cyclase activity was determined in the absence (empty columns) and in the presence of 1 μM CRH (dotted columns), 1 mM (−)baclofen (striped columns), and CRH plus baclofen (filled columns). Values are the means ± SEM of three experiments performed on three separate membrane preparations. *$P < 0.05$ vs basal; **$P < 0.05$ vs CRH alone; a, not significantly different vs basal; b, not significantly different vs CRH alone.

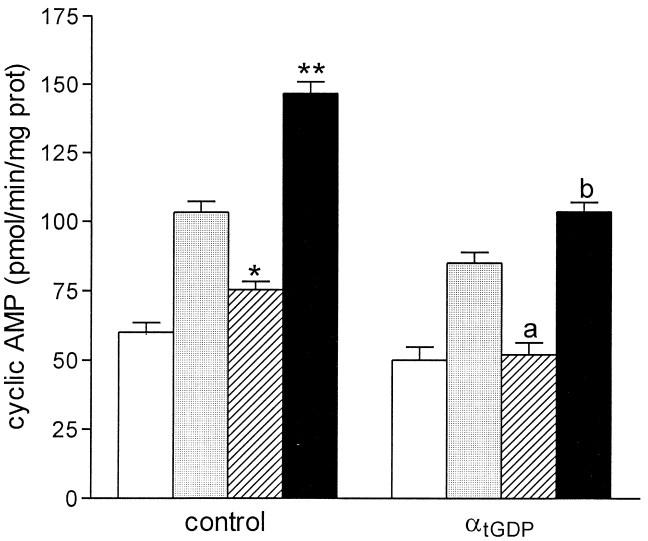

Fig. 7. Effects of α_{tGDP} on (−)baclofen stimulation of adenylyl cyclase activity. Membranes of rat frontal cortex were preincubated with either vehicle (control) or α_{tGDP} for 60 min at ice-bath temperature. Thereafter, adenylyl cyclase activity was assayed in the absence (empty columns) and in the presence of 1 μM CRH (dotted columns), 1 mM (−)baclofen (striped columns) and CRH plus baclofen (filled columns). Values are the means ± SEM. *$P < 0.05$ vs basal; **$P < 0.05$ vs CRH alone; a, not significantly different vs basal; b, not significantly different vs CRH alone.

3.6. Effects of α_{tGDP}

Preincubation of frontal cortex membranes with α_{tGDP}, a scavenger of G-protein βγ subunits [24], markedly inhibited the (−)baclofen stimulatory effects (Fig. 7). Basal and CRH-stimulated adenylyl cyclase activities were modestly decreased by α_{tGDP} treatment.

4. Discussion

In the present study, we show that (−)baclofen causes a significant enhancement of basal and CRH-stimulated adenylyl cyclase activity in membranes of rat frontal cortex. The (−)baclofen facilitation of CRH-stimulated cyclic AMP formation consists of an amplification of the maximal response without a significant change in the potency of the peptide. This suggests that (−)baclofen acts by enhancing the efficacy of CRH receptor signalling rather than changing the affinity of the receptor for CRH. Indeed, radioligand binding experiments show that (−)baclofen does not affect either the K_d or the B_{max} of ^{125}I-Tyr-CRH. A similar behaviour was displayed by activation of muscarinic M_1 receptors, which also potentiates the maximal CRH receptor activity in rat frontal cortex [22].

The specificity of the (−)baclofen facilitatory effects is demonstrated by the lack of activity of the enantiomer

(+)baclofen. The stereoselectivity to baclofen is a common property of several $GABA_B$ receptor-mediated responses, including the regulation of adenylyl cyclase activity [1,2,8, 20]. The potency of (−)baclofen in potentiating the CRH-stimulated adenylyl cyclase activity (EC_{50} = 166 μM) is similar to that reported for the stimulation of cyclic AMP formation in bovine chromaffin cells (EC_{50} = 146 μM) [25]. High concentrations (0.1–1.0 mM) of (±) baclofen were also required to modulate rat striatal cyclic AMP formation *in vivo* [13]. The potencies of (−)baclofen and GABA are, however, severalfold lower than those reported by previous studies describing $GABA_B$-mediated potentiation of neurotransmitter-induced accumulation of cyclic AMP in cerebral cortical slices [7–12]. Moreover, while GABA and baclofen are generally found to be equally potent in binding and functional assays [1], in the present study GABA was much less potent than (−)baclofen. The precise reason for these discrepances is unclear, but several factors could be responsible. For instance, brain slices were incubated in the presence of calcium, which enhances agonist affinity for $GABA_B$ receptors [26]. In contrast, in the present study EGTA was used for membrane preparation and cyclase assay because calcium chelation is required for optimal adenylyl cyclase stimulation by CRH [27]. The calcium chelation may also explain the lower potency of GABA as compared to that of (−)baclofen, as it reduces the affinity of GABA for native $GABA_B$ receptor to a greater extent than that of baclofen [26]. It is also possible that disruption of cell integrity may lead to the loss of intracellular factors, such as receptor and effector phosphorylation, which may potentiate the efficiency of $GABA_B$ receptor coupling to cyclase. It is unlikely, however, that the membrane preparation procedure caused low agonist potencies by inducing receptor–G–protein uncoupling. In fact, the results obtained in [^{35}S]GTPγS binding assays indicate that (−)baclofen and GABA are both effective in eliciting G-protein activation with a potency of 18 and 150 μM, respectively. Importantly, from the comparison of the agonist response curves in stimulating [^{35}S]GTPγS binding and in potentiating CRH receptor activity, it appears that the latter response requires maximal G-protein activation by the agonists.

The (−)baclofen-induced facilitation of CRH-stimulated adenylyl cyclase activity is antagonized by the $GABA_B$ receptor antagonist CGP 55845A in an apparently competitive manner with a pA_2 value of 6.70. This value is close to that previously obtained for the antagonism of baclofen-induced stimulation of adenylyl cyclase in rat olfactory bulb (pA_2 7.0) [20] and potentiation of cyclic AMP accumulation in cortical slices (IC_{50} = 130 nM) [14]. A similar potency (IC_{50} = 0.11 μM) has been reported for the antagonism of $GABA_B$ receptor-induced postsynaptic hyperpolarization in the CA1 region of rat hippocampus [28]. However, CGP 55845A was also found to bind to $GABA_B$ receptors with high affinity (pK_i = 8.35) and to antagonize some baclofen-induced functional responses with high potencies (pA_2 and pEC_{50} values of 8.4 and 7.85, respectively) [29,30]. Moreover, in talamocortical neurons CGP 55845A displayed either high or low potency in blocking the effects of baclofen on different types of voltage-activated calcium channels, suggesting the involvement of different $GABA_B$ receptor types [31]. A number of studies support the idea that $GABA_B$ receptors are pharmacologically heterogeneous [1,32]. Thus, the $GABA_B$ receptors potentiating the CRH-stimulated cyclic AMP formation in rat frontal cortex may belong to a receptor subtype displaying low affinity for CGP 55845A. Recently, two distinct $GABA_B$ receptors, termed $GABA_BR1$ and $GABA_BR2$, have been cloned and shown to form heterodimers with functional and pharmacological properties similar to the native $GABA_B$ receptors [4–6,33–35]. In several brain areas, including the cerebral cortex, $GABA_BR1$ and $GABA_BR2$ appear to be coexpressed in the same neurons [36]. However, the possible existence of monomers of $GABA_BR1$ and $GABA_BR2$ has not been ruled out, and different combinations of monomers and heteromers may constitute the molecular counterparts of the distinct pharmacological subtypes [37]. Further studies are necessary to determine the molecular nature of the $GABA_B$ receptors potentiating the CRH receptor activity in rat frontal cortex.

In some cellular systems, different G_i/G_o-coupled receptors, such as opioid [38], muscarinic M_1 and M_4 [39,40], and adrenergic α_2 receptors [41] can stimulate adenylyl cyclase activity through an interaction with G_S. In the present study, tissue treatment with pertussis toxin completely blocks the (−)baclofen enhancement of either basal or CRH-stimulated adenylyl cyclase activity, thus demonstrating the involvement of G-proteins of the G_i/G_o family. Pertussis toxin has previously been reported to block $GABA_B$ receptor-induced stimulation of adenylyl cyclase activity in membranes of olfactory bulb [20] and in slices of cerebral cortex [42].

Exposure of frontal cortex membranes to α_{tGDP}, which binds to and sequesters free $\beta\gamma$ subunits [24], markedly inhibits the (−)baclofen-induced enhancement of basal and CRH-stimulated adenylyl cyclase activities. This treatment causes a marginal decrease in the CRH response, consistent with the mediation by the α subunit of G_S, rather than $\beta\gamma$ subunits, of the CRH receptor-induced stimulation of adenylyl cyclase. Collectively, the data indicate that in frontal cortex the $\beta\gamma$ subunits of G_i/G_o are involved in the positive coupling of $GABA_B$ receptors to adenylyl cyclase. The $\beta\gamma$ subunits released from G_i/G_o may stimulate types II/IV adenylyl cyclase, which are expressed in rat frontal cortex [43], and potentiate their activation by G_S-linked CRH receptors. An indication that in rat frontal cortex CRH receptors regulate types II/IV adenylyl cyclases stems from the recent observation that exogenously added $\beta\gamma$ subunits markedly enhanced the CRH stimulation of cyclic AMP formation [43].

In conclusion, the present data indicate that in rat frontal cortex $GABA_B$ and CRH receptors interact functionally by

controlling a common pool of $\beta\gamma$-sensitive adenylyl cyclase isoforms. Both receptor systems have been implicated in the development of anxiety and depression [1,44], and it is therefore possible that by enhancing the CRH receptor activity $GABA_B$ receptors may amplify neuronal responses to stress, a major determinant of anxiety and depression.

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