Br. J. Pharmac. (1985), **84**, 249–257

$GABA_B$ receptor modulation of adenylate cyclase activity in rat brain slices

D.R. Hill

Department of Pharmacology, The School of Pharmacy, University of London, 29–39 Brunswick Square, London WC1N 1AX

1 An investigation of the effects of γ-aminobutyric acid (GABA) and the selective $GABA_B$ receptor agonist, baclofen, on basal and stimulated adenosine 3′:5′-cyclic monophosphate (cyclic AMP) levels in slices of rat cerebral cortex has been carried out.

2 Neither GABA nor baclofen produced any significant change in basal cyclic AMP levels. By contrast noradrenaline and forskolin both produced dose-dependent increases in cellular cyclic AMP accumulation.

3 GABA (in the presence of nipecotic acid) and baclofen both potentiated the maximal response to noradrenaline with baclofen (100 μM) increasing the level of cyclic AMP produced by noradrenaline (100 μM) by 133%. GABA (0.3–100 μM) was rather less effective than baclofen, increasing the response to noradrenaline by 70% at 100 μM. (–)-Baclofen was the active isomer with (+)-baclofen failing to potentiate noradrenaline responses. Bicuculline-methobromide (100 μM) failed to block the action of either GABA or baclofen.

4 The enhancement of adrenoceptor-stimulated cyclic AMP accumulation persisted in the presence of a phosphodiesterase inhibitor (1 mM 3-isobutyl-1-methylxanthine) and also in Ca^{2+}-free solution.

5 When forskolin was used to stimulate adenylate cyclase, the effect of baclofen was to inhibit the rise in cyclic AMP levels. Thus (–)-baclofen (100 μM) shifted the dose-response curve to forskolin to the right 5 fold in an apparently parallel fashion. The effect was again stereospecific for the (–)-isomer of baclofen.

6 When GABA uptake was reduced using low sodium (40 mM) incubation medium, GABA also attenuated the rise in cyclic AMP induced by 10 μM forskolin. GABA produced little effect in normal Krebs solution.

7 It is concluded that $GABA_B$ receptor activation may influence cellular cyclic AMP accumulation. But the nature of $GABA_B$ receptor modulation depends upon the initial stimulus to adenylate cyclase.

Introduction

γ-Aminobutyric acid (GABA) receptors in the mammalian CNS have been divided into two categories: $GABA_A$ and $GABA_B$ receptors. This classification has been based upon the results from a variety of experiments which have shown there to be major differences in the sensitivity of the receptors to a wide range of pharmacological agents. For instance, the effects of $GABA_A$ receptor stimulation may be antagonized by bicuculline (Curtis *et al.*, 1970; 1971) whilst $GABA_B$ receptors are insensitive to this antagonist (Bowery *et al.*, 1980; Hill & Bowery, 1981). Furthermore, muscimol, isoguvacine and piperidine-4-sulphonic acid are potent agonists at $GABA_A$ receptors (Krogsgaard-Larsen *et al.*, 1977; 1980) but, are at best only weakly active at $GABA_B$ sites whereas baclofen is a potent and stereoselective $GABA_B$ agonist (Hill & Bowery, 1981; Bowery *et al.*, 1983).

Marked differences also exist between $GABA_A$ and $GABA_B$ receptors in the post-receptor coupling mechanisms. $GABA_A$ receptors are linked directly to a chloride channel (Obata *et al.*, 1967; Gold & Martin, 1984; McBurney, 1984) and activation leads to an immediate increase in chloride conductance. $GABA_B$ receptors show no such association with chloride ions but may influence cellular calcium influx (Dunlap, 1981; Desarmenien *et al.*, 1984;

Cherubini & North, 1984). This may be a primary effect at the level of the calcium channel (although baclofen does not directly affect guinea-pig hippocampal somatic calcium currents, J.V. Halliwell, unpublished) or it may be secondary to a change in K^+ conductance (see, for example, Werz & Macdonald, 1983). Indeed, baclofen has been shown to hyperpolarize rat hippocampal neurones by increasing K^+ conductance (Newberry & Nicoll, 1984a).

Another major difference between $GABA_A$ and $GABA_B$ post-receptor mechanisms is reflected in their sensitivity to guanyl nucleotides. Radioligand binding experiments have shown that, in contrast to $GABA_A$ sites, ligand binding to $GABA_B$ receptors can be depressed by guanyl nucleotides such as GTP (Bowery *et al.*, 1982; Hill *et al.*, 1984). As Rodbell and his colleagues have shown (Rodbell, 1980) guanosine triphosphate (GTP) is an essential co-factor in the activation or inhibition of adenylate cyclase. Indeed a variety of receptors which are sensitive to *in vitro* modulation by GTP can also influence basal or stimulated adenylate cyclase activity (see Rodbell, 1980).

In order to investigate whether the sensitivity of $GABA_B$ receptors reflects a coupling with adenylate cyclase, a series of experiments was performed in which the effects of $GABA_B$ receptor activation on basal and stimulated cyclic AMP production were measured in slices of rat cerebral cortex. Noradrenaline and forskolin were chosen as stimulating agents as both compounds have been shown to produce large increases in cyclic AMP production in rat cortical slices (Nathanson, 1977; Saemon & Daly, 1983).

Methods

Tissue preparation

Wistar rats were killed by decapitation, the brains rapidly removed and the cerebral cortex dissected free of the underlying tissue. Transverse slices of tissue (0.3 mm thick) were cut with a McIlwain chopper and the slices were preincubated in Krebs-Henseleit solution containing 0.005% ascorbic acid for 30 min at 37°C to allow cyclic AMP levels to equilibrate. The slices were then transferred to small test tubes (2 slices per tube, ~ 1.5 mg protein/tube) containing fresh Krebs solution with or without the phosphodiesterase inhibitor, isobutyl methylxanthine (IBMX) (1 mM) and maintained at 37°C for 5 min before the addition of test drugs. Test drugs such as GABA or baclofen were added separately in 50 µl aliquots immediately before addition of noradrenaline or forskolin. Nipecotic acid was also added separately in a 50 µl aliquot just before the other drugs. The tissue was then incubated in a final volume of 0.5 ml for 10 min at 37°C before terminating the reaction in a boiling water bath. Cyclic AMP was measured in the supernatant using the saturation method of Brown *et al.* (1971) and a cyclic AMP binding protein prepared from dog heart.

The tissue was then digested in 0.25 M NaOH and the protein concentration measured by the method of Lowry *et al.* (1951). The cyclic AMP content of each tube was then calculated in terms of protein concentration.

Solutions

Krebs-Henseleit solution was of the composition (mM): NaCl 115, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11.0.

The composition of Tris-Krebs was (mM): Tris-HCl pH 7.4 85, NaCl 15, KCL 4.7, $MgSO_4$ 1.2, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11.0.

Calcium-free solution was as for normal Krebs-Henseleit with removal of calcium and further addition of 2.8 mM $MgSO_4$ to give 4.0 mM final concentration.

Drugs and chemicals

GABA (BDH), (±)-baclofen (+)- and (−)-baclofen (Ciba-Geigy Ltd.); noradrenaline bitartrate (Sigma Ltd.); forskolin (Calbiochem-Behring); nipecotic acid (Dr J. Collins, London); bicuculline methodbromide (Cambridge Research Biochemicals Ltd.); Tris-base (Aristar, BDH); IBMX (3-isobutyl-1-methylxanthine) (Sigma Ltd.).

All drugs except forskolin were dissolved in buffer. Forskolin was dissolved in polyethylene glycol 400.

Results

Basal cyclic AMP accumulation

Throughout the entire series of experiments and in the absence of any phosphodiesterase inhibitor, basal cyclic AMP levels varied between experiments. Over the course of the first series of experiments, basal levels equalled 1.91 ± 0.29 pmol mg^{-1} protein ($n = 13$). In the presence of the phosphodiesterase inhibitor, isobutyl methylxanthine (IBMX) (1 mM), basal levels were increased to 16.24 ± 1.5 pmol mg^{-1} protein ($n = 5$). Neither GABA (100 µM) nor (−)-baclofen (100 µM) had any significant effect on basal levels, even in the presence of IBMX.

A drenoceptor-stimulated cyclic AMP accumulation

Noradrenaline (1–100 µM) produced a dose-

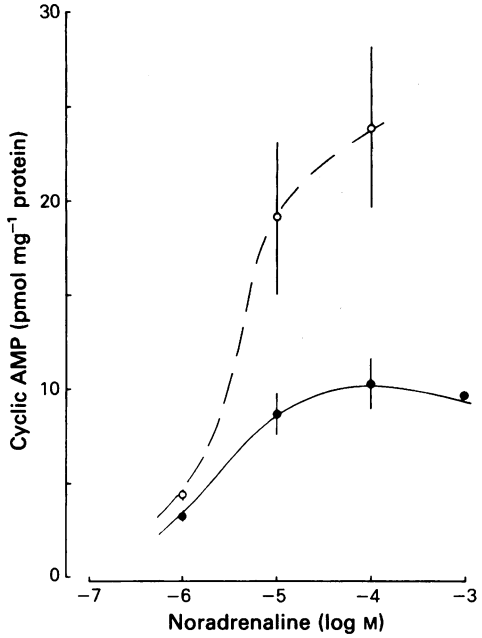

Figure 1 Potentiation by (±)-baclofen of the increase in cellular cyclic AMP accumulation produced by noradrenaline. In the absence of baclofen (●), noradrenaline produced a dose-dependent rise in the cyclic AMP level of the brain slices. A maximum increase of approximately 5 fold was obtained at 100 μM noradrenaline. Inclusion of 100 μM (±)-baclofen (○) in the incubation medium potentiated the maximum response to noradrenaline. Each point is the mean of between 3 and 7 separate experiments, except for 1 mM noradrenaline, which is the result of a single experiment; s.e.mean shown by vertical lines (where appropriate). Phosphodiesterase inhibitors were not present.

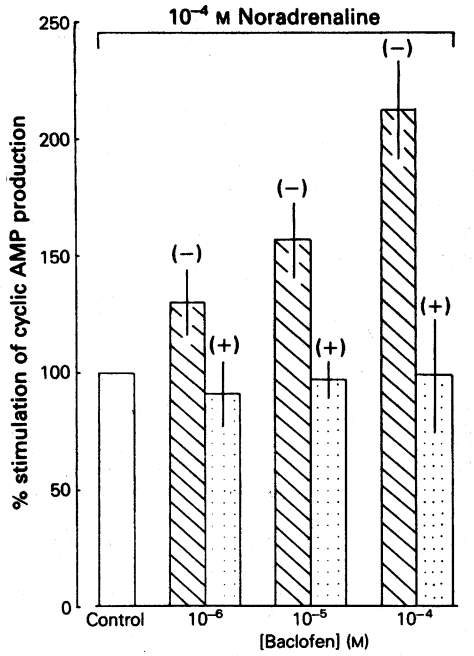

Figure 2 The effect of (+)- and (−)-baclofen on the rise of cellular cyclic AMP produced by 100 μM noradrenaline. In these experiments 1 mM isobutylmethylxanthine (IBMX) was present throughout the final incubation with drugs. The response to noradrenaline which was present throughout is represented as 100%. (−)-Baclofen produced a dose-dependent potentiation of the response to a fixed concentration of noradrenaline, such that cyclic AMP levels were more than doubled at 100 μM (−)-baclofen. By contrast (+)-baclofen produced no significant change in cyclic AMP levels when compared with the noradrenaline control. Each point is the mean of 3 or 4 experiments; s.e.mean shown by vertical lines.

dependent stimulation of cyclic AMP production from basal levels to a maximum of 10.23 ± 1.35 pmol mg^{-1} protein ($n = 7$) (Figure 1). When (±)-baclofen (100 μM) was included with noradrenaline a large potentiation in the response to noradrenaline was observed (Figure 1). Thus, in the presence of 100 μM baclofen, the maximum response to noradrenaline was raised to 23.90 ± 4.30 ($n = 6$) pmol mg^{-1} protein. The response to 1 μM noradrenaline was potentiated to a much smaller degree.

To determine whether this action of baclofen displayed the same degree of stereoselectivity seen in other $GABA_B$ receptor-mediated systems, the enantiomers of baclofen were tested for their ability to potentiate the response to noradrenaline. Figure 2 shows the combined results from 3–4 experiments performed in the presence of IBMX (1 mM). The data have been normalized with respect to the effect of noradrenaline 100 μM included in each experiment.

(−)-Baclofen (1–100 μM) produced a dose-dependent increase in the response to noradrenaline, whilst (+)-baclofen, even at 100 μM did not significantly alter the response to the catecholamine.

GABA mimicked this action of baclofen although to a lesser extent, both in the absence and presence of the $GABA_A$ receptor antagonist (+)-bicuculline methobromide (100 μM) (Figure 3). Under the incubation conditions employed, uptake of GABA into both neuronal and glial elements of the slice would be considerable (Iversen & Neal, 1968; Iversen & Kelly, 1975). To prevent uptake, nipecotic acid 1 mM was included in the incubation medium. In all experiments, the inclusion of nipecotic acid itself produced a small potentiation in the response to noradrenaline, perhaps either by preventing GABA reuptake or by displacing endogenous GABA. A direct effect seems unlikely as nipecotic acid is inactive in other $GABA_A$

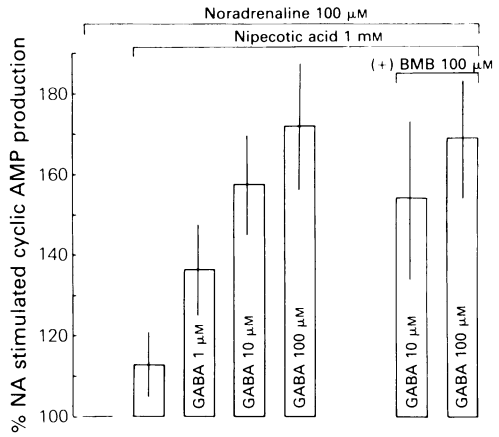

Figure 3 Potentiation by GABA of noradrenaline (NA)-induced accumulation of cyclic AMP. To prevent uptake of GABA into the brain slices, nipecotic acid (1 mM) was included in the final incubation medium which also contained 100 μM noradrenaline. Nipecotic acid alone induced a small potentiation of the noradrenaline response (represented as 100%); GABA 1–100 μM produced a further stimulation of this. Inclusion of the GABA antagonist (+)-bicuculline methobromide did not block the GABA response. Isobutylmethylxanthine was not present. Results are expressed as means of 4 experiments; s.e.mean shown by vertical lines.

and $GABA_B$ receptor systems (Bowery *et al.*, 1976; Bowery *et al.*, 1983). The maximum potentiation of the noradrenaline response produced by GABA equalled $72.1 \pm 15.8\%$ ($n = 4$) in the absence of 100 μM (+)-bicuculline methobromide, $68.8 \pm 14.8\%$ ($n = 4$) in the presence of the antagonist. These values were equivalent to 13.61 ± 2.7 ($n = 4$) pmol cyclic AMP mg^{-1} protein in the presence of noradrenaline (100 μM) alone and 24.03 ± 3.4 ($n = 5$) pmol mg^{-1} protein for noradrenaline plus GABA (100 μM) and no (+)-bicuculline methobromide.

It is possible that the observed enhancement was due to the release of an endogenous substance such as adenosine, even though basal cyclic AMP levels were unaltered. To test this possibility, experiments were performed using modified Krebs solution which contained zero Ca^{2+} and 4 mM Mg^{2+} to suppress synaptic release. The result of one such experiment which was repeated yielding similar results a further two times is shown in Figure 4. Under these conditions both basal and stimulated cyclic AMP production was increased, presumably due to inhibition of calcium-dependent phosphodiesterase activity. The potentiation of noradrenaline by baclofen was not reduced under these conditions.

Forskolin-stimulated cyclic AMP accumulation

Forskolin (0.3–10 μM) itself produced a large dose-dependent accumulation of cyclic AMP in slices of cerebral cortex (Figure 5) with cyclic AMP levels reaching 75.0 ± 8.0 pmol mg^{-1} protein ($n = 4$) at 10 μM forskolin (even in the absence of IBMX). In direct contrast to the results obtained with catecholamines, baclofen (100 μM) inhibited the rise in cyclic AMP levels stimulated by forskolin. Indeed the forskolin dose-response curve was shifted to the right by a factor of five in an apparently parallel manner (Figure 5). Unfortunately, owing to limitations in the amount of forskolin available, concentrations in excess of 30 μM were not tested. (−)-Baclofen was approximately 100 times more potent

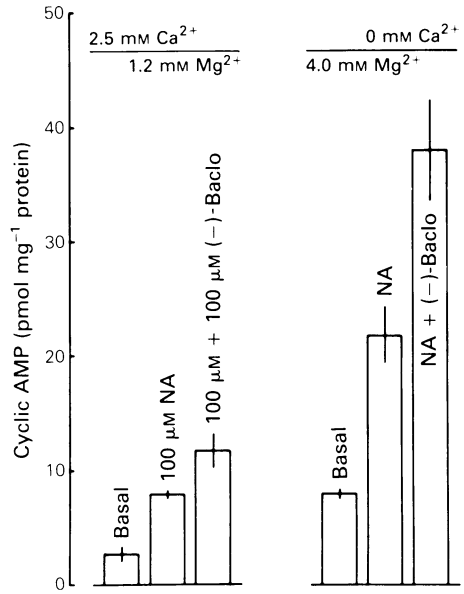

Figure 4 The effect of Ca^{2+}-free incubation solution on the ability of baclofen to enhance noradrenaline (NA)-stimulated cyclic AMP accumulation. Slices were pre-incubated in normal Krebs solution for 90 min as detailed in Methods. The tissue was then transferred to incubation chambers containing either normal Krebs solution or modified Krebs containing 0 mM Ca^{2+} and 4 mM Mg^{2+}. The experiment was then conducted as described in Methods. The omission of calcium from the incubation medium and its replacement with Mg^{2+} did not abolish the ability of baclofen to enhance the response to noradrenaline. The rise in basal and stimulated levels seen in the absence of calcium probably reflects inhibition of phosphodiesterase activity. Isobutylmethylxanthine was not present. The results shown are from a single experiment in which each treatment was performed in triplicate. Values are means; s.e.mean shown by vertical lines.

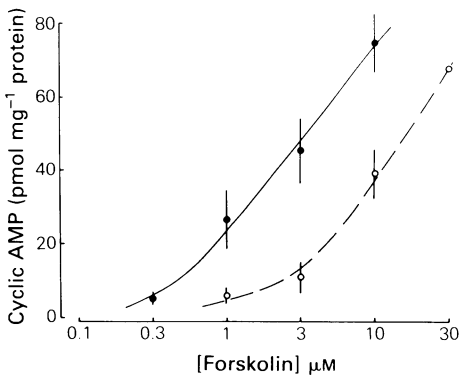

Figure 5 Inhibition by (−)-baclofen of the rise in cellular cyclic AMP induced by increasing concentrations of forskolin. Slices were incubated in normal Krebs solution in the presence of increasing concentrations of forskolin with (○) or without (●) a fixed concentration of (−)-baclofen (100 μM). Forskolin produced a dose-dependent rise in cyclic AMP production which was blocked in an apparently competitive manner by the presence of baclofen. Each point is the mean of 3 or 4 determinations (s.e.mean shown by vertical lines) except for 30 μM forskolin in the presence of baclofen. This is the mean of two experiments.

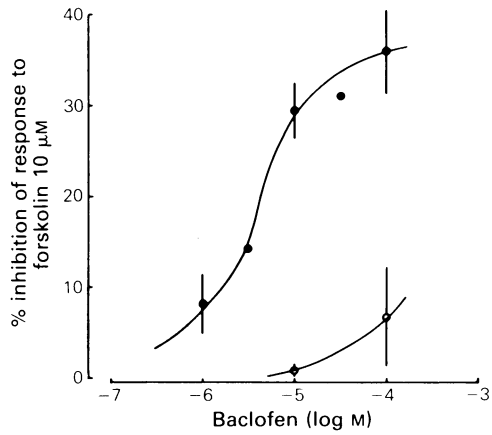

Figure 6 Stereospecificity of the inhibition of forskolin-stimulated cyclic AMP production. Slices were incubated in normal Krebs solution containing 1 mM isobutylmethylxanthine (IBMX), in the presence of a fixed concentration of forskolin (10 μM) and increasing concentrations of (+)-(○) or (−)-(●) baclofen. (−)-Baclofen at micromolar concentrations produced a dose-dependent reduction in the levels of cyclic AMP. The effect of (−)-baclofen appeared to reach a maximum at 100 μM. (+)-Baclofen was approximately 100 fold weaker than the (−)-enantiomer. The data are the means from between 3 and 5 experiments; s.e.mean shown by vertical lines. Points without vertical lines are the mean of two experiments.

than the (+)-isomer in producing inhibition of forskolin stimulated cyclic AMP production. Dose-response curves for inhibition of the response to 10 μM forskolin in the presence of 1 mM IBMX by (−)- and (+)-baclofen are shown in Figure 6.

Maximum inhibition of the forskolin (10 μM)-induced increase in cyclic AMP was measured at 100 μM (−)-baclofen and equalled 35.5 ± 4.3 pmol mg^{-1} protein $n = 4$: equivalent to 48.0 ± 10.2% inhibition ($n = 4$, no IBMX present). In the presence of 1 mM IBMX, (−)-baclofen produced 35.9 ± 4.6% inhibition of the forskolin response (34.8 ± 7.4 pmol mg^{-1}) protein). In terms of absolute amounts of cyclic AMP, the degree of inhibition produced by 100 μM (±)- and (−)-baclofen remained the same but represented proportionally less of the total cyclic AMP produced in the presence of the phosphodiesterase inhibitor (94.2 ± 10.2 pmol mg^{-1} protein ($n = 5$) in the presence of IBMX, 75.0 ± 8.0 pmol mg^{-1} protein ($n = 4$) in the absence of IBMX).

In experiments using noradrenaline to stimulate cyclic AMP production, nipecotic acid was used to block GABA uptake (see Figure 3). As this substance showed some undesirable intrinsic activity in these experiments a different approach was used to block GABA uptake when forskolin was used to stimulate cyclic AMP production. This was to reduce the sodium concentration from physiological concentrations to 40 mM. Tris-base was used as a substitute for sodium ions. The results from these experiments are shown in Figure 7. In normal Krebs, baclofen (100 μM) (which is not transported into cells: Bowery *et al.*, 1983) diminished the effect of forskolin by approximately 34%. This was rather less than that observed in previous experiments. Nonetheless, GABA (300 μM) failed to produce the same response. In parallel incubations using Tris-Krebs medium, baclofen again attenuated the accumulation of cyclic AMP to almost the same extent as in normal Krebs, but now GABA at 30 μM and 300 μM also reduced the forskolin response.

Discussion

The present experiments show that GABA$_B$ receptor activation can either potentiate or depress the stimulated accumulation of cyclic AMP in slices of cerebral cortex, whilst appearing to have little effect on basal cyclic AMP levels.

Previous studies (Bowery *et al.*, 1982; Hill *et al.*, 1984) have demonstrated that both GTP and its non-hydrolysable analogue guanylyl imidodiphosphate (GppNHp) inhibit the binding of [^{3}H]-GABA and [^{3}H]-baclofen to GABA$_B$ receptors on rat brain membranes. Inhibition of ligand binding to a variety

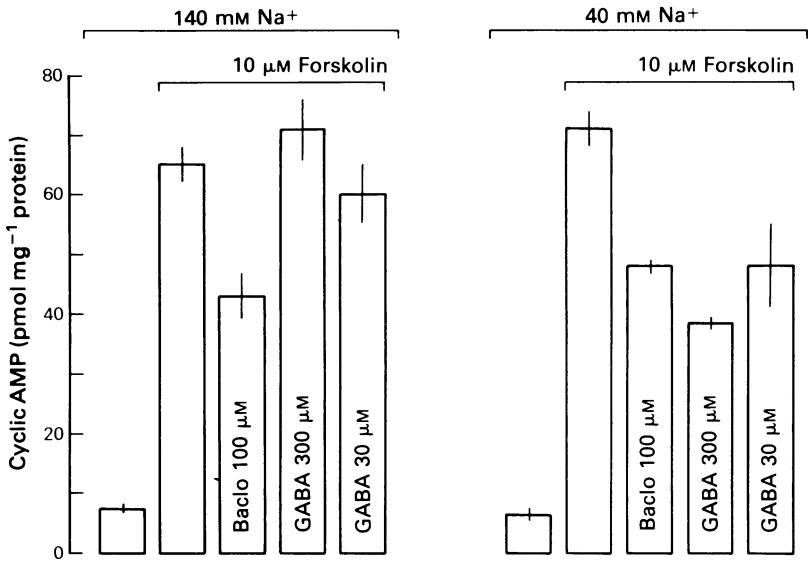

Figure 7 Inhibition by GABA of forskolin-stimulated cyclic AMP production. Slices were pre-incubated for 90 min in normal Krebs solution before being transferred to incubation chambers containing either normal Krebs containing 140 mM Na^+, or Tris-Krebs solution (40 mM Na^+). The reduced sodium concentration was employed to reduce uptake of GABA into the brain slice. In normal Krebs, (−)-baclofen (100 μM) partially blocked the rise in cyclic AMP levels produced by forskolin; under the same conditions GABA was inactive. When GABA re-uptake was attenuated in the low sodium solution, baclofen (Baclo) again inhibited the rise in cyclic AMP levels. But now GABA also mimicked baclofen in reducing the effect of forskolin. Each column is the mean of four determinations; s.e.mean shown by vertical lines.

of receptors by GTP is believed to represent a coupling of a GTP binding protein, with receptor and adenylate cyclase enzyme (Rodbell, 1980). This results in a receptor enzyme complex with a diminished affinity for agonists. The GTP binding protein may be either stimulatory (designated N_s) or inhibitory (N_i) to the cyclase, resulting in either a rise or fall in cyclic AMP production. However, from binding studies alone it has not been possible to determine which GTP binding protein is responsible for the reduction in $GABA_B$ receptor agonist affinity. This is depite the fact that previous experiments (Hill *et al.*, 1984) have indicated that Na^+ ions influence the potency of GTP as an inhibitor of $GABA_B$ receptor binding. A degree of receptor sensitivity to Na^+ ions is a characteristic shared by some receptors linked to N_i (see Rodbell, 1980).

In the present experiments, activation of $GABA_B$ receptors had no discernible effect on basal cyclic AMP levels suggesting that the stimulatory binding protein (N_s) may not be involved. Both baclofen and GABA did, however, potentiate the response to noradrenaline (and isoprenaline: data not shown) in brain slices showing that $GABA_B$ receptors can exert an influence on cyclic AMP levels.

Potentiation of the rise in cyclic AMP levels produced by β-adrenoceptor activation has been shown to occur in rat striatal slices following α-adrenoceptor stimulation (see for example, Leblanc and Ciarenello, 1984). Furthermore, similar interactions between noradrenaline and adenosine (Daly *et al.*, 1980; Fredholm *et al.*, 1982) or vasoactive intestinal polypeptide (VIP) (Magistretti & Schorderet, 1984) have also been reported.

In rat striatal slices at least, the ability of α-adrenoceptor agonists to enhance β-receptor stimulation of cyclic AMP production appears to be mediated indirectly. This conclusion derives from the fact that similar effects were not observed when adenylate cyclase activity was measured directly using tissue homogenates (Leblanc & Ciarenello, 1984). It is therefore possible that the effects of GABA and baclofen seen here are due to the release of a neurohumoral agent with which noradrenaline acts synergistically. This seems unlikely as baclofen was still able to potentiate the effects of noradrenaline in an incubation medium where synaptic release would be essentially abolished. Extracellular adenosine has also been implicated as mediating at least some of the potentiating effects of α-adrenoceptor agonists on β-adrenoceptor cyclic AMP responses (Daly *et al.*, 1980). It again seems doubtful that free adenosine was responsible for the actions of baclofen and GABA seen in the present

experiments as their effects were not blocked by IBMX. At the concentration used IBMX would not only antagonize phosphodiesterase activity but also adenosine receptors (Daly, 1982). Moreover, the fact that baclofen and GABA could still enhance the effects of noradrenaline in the presence of IBMX suggests that any phosphodiesterase inactivation by $GABA_B$ agonists would not account for the potentiation.

On the basis of the present data it is rather difficult to suggest a mechanism whereby the effects of noradrenaline and isoprenaline are potentiated. An indirect action on cyclic AMP accumulation mediated by way of a change in cellular calcium levels is possible as adenylate cyclase activity and intracellular calcium levels are believed to be linked (see Fain, 1978). However, the results of the experiments using low calcium solutions suggest otherwise. Of course, intracellular calcium mobilization may not be markedly affected under such conditions. Despite these arguments, a direct action at the level of the enzyme may underlie the responses to GABA and baclofen. Indeed in experiments in which adenylate cyclase activity was measured using cell-free homogenates of whole brain, baclofen and GABA did produce a significant potentiation of the response to noradrenaline and isoprenaline (D.R. Hill and A.C. Dolphin, unpublished). Thus, a direct action between $GABA_B$ receptors and adenylate cyclase linked β-receptors, perhaps involving N_S may be involved with the disruption of cell integrity reducing the overall magnitude of the response.

Rather surprisingly, and in marked contrast to their effects on catecholamine responses, both (−)-baclofen and GABA depressed the response to forskolin. This compound has recently been shown to produce an activation of adenylate cyclase which is not blocked by receptor antagonists and appears to be mediated at the level of the catalytic subunit of the enzyme (Saemon *et al.*, 1981; Saemon & Daly, 1983). Similar results have also been obtained using cell-free homogenates of rat cerebral cortex and cerebellum (Hill & Dolphin, 1984; Wojcik & Neff (1984). It therefore seems likely that in this case, $GABA_B$ receptor activation produces a direct inhibition of the effects of adenylate cyclase activation.

Furthermore, Wojcik & Neff (1984) showed that this inhibitory action of baclofen and GABA is dependent upon the presence of GTP. Consequently the inhibitory GTP binding protein (N_i) is likely to be involved in the coupling between $GABA_B$ receptors and adenylate cyclase enzyme. Thus, it may be N_i which is responsible for mediating the GTP modulation of $GABA_B$ receptors *in vitro*.

The present findings showing either potentiation or inhibition of cellular cyclic AMP accumulation to be dependent upon the mode of cyclase activation, taken together with the results obtained using membrane fragments raises the question of whether different populations of adenylate cyclase may be modulated by $GABA_B$ receptors in different ways. Adenosine, for instance, has been shown to depress adenylate cyclase activity following stimulation by forskolin and isoprenaline (A.C. Dolphin, unpublished). One intriguing possibility is that potentiation of the catecholamine response represents a postsynaptic $GABA_B$ effect, whilst inhibition may be of more importance presynaptically. There is at present little evidence to support this, but inhibition of adenylate cyclase does appear to be characteristic of substances such as adenosine, clonidine or even muscarinic agonists which inhibit transmitter release (Olianas *et al.*, 1983; Wojcik & Neff, 1983).

By contrast, the bicuculline-resistant dendritic slow inhibitory postsynaptic potential (i.p.s.p.), which shows similar characteristics to the K^+-conductance activated by baclofen in the same cells is not altered by 8-Br-cyclic AMP. This suggests that this postsynaptic baclofen response may be independent of cyclic AMP (Newberry & Nicoll, 1984a, 1984b). Inhibition of adenylate cyclase activity may not of course be responsible for the inhibition of neurotransmitter release induced by $GABA_B$ receptor activation. Experiments are in progress to answer this question.

In conclusion, the sensitivity of $GABA_B$ receptors to guanyl nucleotides appears to be reflected in the ability of $GABA_B$ receptors to modulate cyclic AMP accumulation in brain slices. When adenylate cyclase was stimulated via an adrenoceptor, $GABA_B$ receptor activation resulted in a potentiation of the catecholamine response. By contrast, when the activity of the enzyme was stimulated directly, baclofen and GABA inhibited the rise in cyclic AMP production.

I am grateful to Dr N.G. Bowery and Dr K. Watling for their helpful comments made during the preparation of the manuscript. I should also like to thank Dr D. Yellon of the Rayne Insitute, St. Thomas's Hospital for his generous gift of cyclic AMP binding protein. Thanks also to Miss J. Chard for typing skills.

References

BOWERY, N.G., DOBLE, A., HILL, D.R., HUDSON, A.L., MIDDLEMISS, D.N., SHAW, J.S. & TURNBULL (1980). (−)-Baclofen decreases transmitter release in the mammalian CNS by an action at a novel GABA receptor. *Nature*, **283**, 92–94.

BOWERY, N.G., HILL, D.R. & HUDSON, A.L. (1982). Guanyl nucleotides decrease the binding affinity of $GABA_B$ receptors in the mammalian CNS. *Br. J. Pharmac.*, **75**, 86P.

BOWERY, N.G., HILL, D.R. & HUDSON, A.L. (1983). Characteristics of $GABA_B$ receptor binding sites on rat whole brain membranes. *Br. J. Pharmac.*, **78**, 191–206.

BOWERY, N.G., JONES, G.P. & NEAL, M.J. (1976). Selective inhibition of neuronal GABA uptake by cis-1,3-aminocyclohexane carboxylic acid. *Nature*, **264**, 281–284.

BROWN, B.L., ALBANO, J.D.M., EKINS, R.P., SGHERZI, A.M. & TAMPION, U. (1971). A simple and sensitive saturation assay method for the measurement of adenosine 3′:5′-cyclic monophosphate. *Biochem. J.*, **121**, 561–562.

CHERUBINI, E. & NORTH, A. (1984). Inhibition of calcium spikes and transmitter release by γ-aminobutyric acid in the guinea-pig myenteric plexus. *Br. J. Pharmac.*, **82**, 101–106.

CURTIS, D.R., DUGGAN, A.W., FELIX, A. & JOHNSTON, G.A.R. (1970). GABA, bicuculline and central inhibition. *Nature*, **226**, 1222–1224.

CURTIS, D.R., DUGGAN, A.W., FELIX, A. & JOHNSTON, G.A.R. (1971). Bicuculline, an antagonist of GABA and synaptic inhibition in the spinal cord of the cat. *Brain Res.*, **32**, 69–96.

DALY, J.W. (1982). Adenosine receptors: targets for future drugs. *J. med. Chem.*, **25**, 197–207.

DALY, J.W., PADGETT, W., NIMITKITPAISAN, Y., CREVELING, C.R., CANTACUZENE, D. & KIRK, K.L. (1980). Fluoroepinephrines: specific agonists for the activation of alpha and beta adrenergic-sensitive cyclic AMP-generating systems in brain slices. *J. Pharmac. exp. Ther.*, **212**, 382–389.

DESARMENIEN, M., FELTZ, P., OCCHIPINTI, G., SANTANGELO, F. & SCHLICHTER, R. (1984). Co-existence of $GABA_A$ and $GABA_B$ receptors on A and C primary afferents. *Br. J. Pharmac.*, **81**, 327–334.

DUNLAP, K. (1981). Two types of γ-aminobutyric acid receptor on embryonic sensory neurones. *Br. J. Pharmac.*, **74**, 579–586.

FAIN, J.N. (1978). Hormones, membranes and cyclic nucleotides. In *Receptors and Recognition*. ed. Cuatrecasas, P. & Greaves, M.F. London:Chapman and Hall.

FREDHOLM, B.B., JONZON, B., LINDGREN, E. & LINDSTROM, K. (1982). Adenosine receptors mediating cyclic AMP production in rat hippocampus. *J. Neurochem.*, **39**, 165–174.

GOLD, M.R. & MARTIN, A.R. (1984). γ-Aminobutyric acid and glycine activate Cl^- channels having different characteristics in CNS neurones. *Nature*, **308**, 639–641.

HILL, D.R. & BOWERY, N.G. (1981). ^{3}H-Baclofen and ^{3}H-GABA bind to bicuculline-insensitive $GABA_B$ sites in rat brain. *Nature*, **290**, 149–152.

HILL, D.R., BOWERY, N.G. & HUDSON, A.L. (1984). Inhibition of $GABA_B$ receptor binding by guanyl nucleotides. *J. Neurochem.*, **42**, 652–657.

HILL, D.R. & DOLPHIN, A.C. (1984). Modulation of adenylate cyclase activity by $GABA_B$ receptors. *Neuropharmacology*, **23**, 829–830.

IVERSEN, L.L. & NEAL, M.J. (1968). The uptake of ^{3}H-GABA by slices of rat cerebral cortex. *J. Neurochem.*, **15**, 1141–1149.

IVERSEN, L.L. & KELLY, J.S. (1975). Uptake and metabolism of γ-aminobutyric acid by neurones and glial cells. *Biochem. Pharmac.*, **24**, 933–938.

KROGSGAARD-LARSEN, P., JOHNSTON, G.A.R., LODGE, D. & CURTIS, D.R. (1977). A new class of GABA agonist. *Nature*, **268**, 53–55.

KROGSGAARD-LARSEN, P., FALCH, E., SCHOUSBOE, A., CURTIS, D.R. & LODGE, D. (1980). Piperidine-4-sulphonic acid, a new specific GABA agonist. *J. Neurochem.*, **34**, 756–759.

LEBLANC, G.G. & CIARANELLO, R.D. (1984). α-Noradrenergic potentiation of neurotransmitter-stimulated cAMP production in rat striatal slices. *Brain Research*, **293**, 57–65.

LIMBARD, L.E. (1983). α_2 Adrenergic systems: models for exploring hormonal inhibition of adenylate cyclase. *TIPS*, **4**, 135–138.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951). Protein measurement with the Folin phenol reagent. *J. biol. Chem.*, **193**, 265–275.

MAGISTRETTI, P.J. & SCHORDERET, M. (1984). VIP and noradrenaline act synergistically to increase cyclic AMP in cerebral cortex. *Nature*, **308**, 280–282.

McBURNEY, R.N. (1984). Membrane actions of GABA in cultured central neurones. In *Actions and Interactions of GABA and Benzodiazepines*. ed. Bowery, N.G. pp. 43–58. New York: Raven Press.

NATHANSON, J.A. (1977). Cyclic nucleotides and nervous system function. *Physiol. Rev.*, **57**, 157–256.

NEWBERRY, N.R. & NICOLL, R.A. (1984a). Direct hyperpolarizing action of baclofen on hippocampal pyramidal cells. *Nature*, **308**, 450–452.

NEWBERRY, N. & NICOLL, R. (1984b). A bicuculline-resistant inhibitory postsynaptic potential in rat hippocampal pyramidal cells *in vitro*. *J. Physiol.*, **348**, 239–254.

OBATA, K., ITO, M., OCHI, R. & SATO, N. (1967). The pharmacological properties of the postsynaptic inhibition by Purkinje cell axons and the action of γ-amino butyric acid on Dieters neurones. *Exp. Brain Res.*, **4**, 43–57.

OLIANAS, M.C., ONALI, P., NEFF, N.H. & COSTA, E. (1983). Adenylate cyclase activity of synaptic membranes from rat striatum. Inhibition by muscarinic agonists. *Mol. Pharmac.*, **23**, 393–398.

RODBELL, M. (1980). The role of hormone receptors and GTP-regulatory proteins in membrane transduction. *Nature*, **264**, 17–22.

SAEMON, K.B. & DALY, J.W. (1983). Forskolin, cyclic AMP and cellular physiology. *Trends Pharmac. Sci.*, **4**, 120–123.

SAEMON, K.B., PADGETT, W. & DALY, J.W. (1981). Forskolin; Unique diterpene activator or adenylate cyclase

in membranes and intact cells. *Proc. natn. Acad. Sci.*, **78,** 3363–3367.

WERZ, M.A. & MACDONALD, R. (1983). Opiod peptides selective for mμ and delta-opiate receptors reduce calcium-dependent action potential duration by increasing potassium conductance. *Neurosci. Letts.*, **42,** 173–178.

WOJCIK, W.J. & NEFF, N.H. (1983). Adenosine A_1 receptors are associated with cerebellar granule cells. *J. Neurochem.*, **41,** 759–763.

WOJCIK, W.J. & NEFF, N.H. (1984). γ-Aminobutyric acid-B-receptors are negatively coupled to adenylate cyclase in brain, and cerebellum – these receptors may be associated with granule cells. *Mol. Pharmac.*, **25,** 24–28.

(Received August 22, 1984.
Accepted September 24, 1984.)