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Characterization of the Relationship between γ-Aminobutyric Acid B Agonists and Transmitter-Coupled Cyclic Nucleotide-Generating Systems in Rat Brain[1]

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SUMMARY

Baclofen and other γ-aminobutyric acid B ($GABA_B$) agonists potentiate the cyclic AMP response in rat brain slices that occurs during exposure to norepinephrine, isoproterenol, adenosine, vasoactive intestinal peptide, and histamine. By themselves the $GABA_B$ agonists have only a slight effect on basal cyclic AMP levels. Dose-response and time-course studies revealed that baclofen has little influence on neurotransmitter recognition site affinity, but rather enhances the synthesis or accumulation of second messenger that occurs in response to these agents. Baclofen appears to be neither an inhibitor of phosphodiesterases nor does it require adenosine to promote the response to other transmitters. The synergistic interaction between baclofen and catecholamines is a calcium-dependent process and is evident only in the rat brain cerebral cortex, hippocampus and corpus striatum, being undetectable in the pons-midbrain, cerebellum, and spinal cord. In contrast to the findings with neurotransmitter receptor stimulants, $GABA_B$ agonists inhibited the cyclic AMP response to forskolin. It remains unclear whether this action is related to the neurotransmitter potentiating effect of baclofen. These data suggest that $GABA_B$ agonists may modulate neurotransmitter receptor function by influencing a component of the cyclic nucleotide-generating system beyond the level of the hormone recognition site.

INTRODUCTION

Many central nervous system neurotransmitter receptors are coupled to a second messenger system making cyclic nucleotide production a useful measure of receptor function (1, 2). It appears that neurotransmitter or hormone-stimulated cyclic AMP formation results from the interaction of multiple membrane components (3, 4). These include the neurotransmitter receptor recognition site, the guanine nucleotide-binding protein (N_s) and a catalytic unit (C). In addition, cyclic nucleotide levels are regulated by the action of phosphodiesterases which convert the second messengers to their corresponding 5′-nucleotides (5). Thus, cyclic nucleotide levels can be increased by directly activating the receptor recognition site, N_s, C, or by inhibiting phosphodiesterase. It is possible that modifications in the postrecognition site components may be a mechanism for regulating receptor activity. This may explain how simultaneous stimulation of different receptors alters the response obtained with either neurotransmitter alone. For example, both adenosine and norepinephrine stimulate cyclic AMP accumulation in brain slices, but a synergistic response is observed when the tissue is exposed to both substances (6). Also, α-adrenergic agonists are known to greatly potentiate the cyclic AMP responses to neurotransmitters in brain slices, while having only a minor effect on second messenger production themselves (7).

It has been suggested that there are at least two pharmacologically and functionally distinct receptor systems for GABA[2] in the central nervous system. While $GABA_A$ receptors appear to be coupled to chloride ion channels and benzodiazepine-binding sites, the effector mechanism for the $GABA_B$ system is less well defined, though it would appear to be associated with divalent cations. Recently, it has been reported that $GABA_B$ receptor agonists potentiate the cyclic AMP response to norepinephrine in rat brain cerebral cortical slices (8). Evidence that this may be a $GABA_B$-mediated phenomenon was provided by the discovery that only $GABA_B$ agonists (e.g., baclofen) elicit this response, whereas $GABA_A$ agonists such as THIP are inactive. While there are no known antagonists for the $GABA_B$ system, the interaction between baclofen and norepinephrine was not

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[2] The abbreviations used are: GABA, γ-aminobutyric acid; THIP, 4,5,6,7-tetrahydroisoxazolo(5,4-c)pyridin-3-ol; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; VIP, vasoactive intestinal peptide.

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influenced by bicuculline, a $GABA_A$ receptor antagonist. These data suggest that $GABA_B$ agonists modify the responsiveness of the norepinephrine-coupled cyclic nucleotide system in brain.

The present study was undertaken to further characterize this phenomenon. The data indicate that $GABA_B$ agonists potentiate the cyclic AMP response to a variety of agents in a calcium-dependent manner, that the interaction is region specific in rat brain, and that these substances inhibit forskolin-stimulated cyclic AMP production in brain slices. These results suggest that $GABA_B$ agonists influence brain second messenger responses to a variety of agents by modifying a component of the cyclic nucleotide system beyond the neurotransmitter/hormone recognition site.

MATERIALS AND METHODS

Male Sprague-Dawley rats (Timco, Houston, TX) weighing 150–200 g were housed five to a cage with free access to food and water. The animals were maintained on a 12-hr light/dark cycle. Cyclic AMP production in brain slices was measured using the technique of Shimizu *et al.* (9). Briefly, the rats were decapitated and the brains rapidly were removed and placed into ice-cold Rall's buffer (118 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, 2.0 mM KH_2PO_4, 0.02 mM NaEDTA, 2.0 mM $MgSO_4$, 25 mM $NaHCO_3$, 11.1 mM glucose) and slices (260 μm) were prepared with a McIlwain chopper. The slices were preincubated for 15 min in oxygenated (95% O_2/5% CO_2) buffer at 37°. After changing the medium, the tissue was incubated for 40 min at 37° in buffer containing 0.1 μM [^{3}H]adenine. Following two rinses with fresh medium, 50–75-mg portions of tissue were placed into tubes containing 5 ml of buffer and then incubated for 5 min at 37° before adding drugs and/or neurotransmitters, after which the samples were incubated for a further 10 min. In some cases, substances (adenosine deaminase or EGTA) were added 2 min prior to the addition of neurotransmitter. The reaction was terminated by decanting the supernatant and adding 1 ml of 5% trichloroacetic acid. Following homogenization and a 20-min centrifugation (13,000 × *g* at 4°), total radioactivity was quantified in 50-μl portions of the acid supernatant. The remaining supernatant was monitored for [^{3}H]cyclic AMP content using the double column method of Salomon *et al.* (10). The results are expressed as the percentage of total radioactivity present as cyclic AMP (per cent conversion). The level of significance for differences between means was calculated using a two-tailed Student's *t* test.

High affinity cyclic AMP and cyclic GMP phosphodiesterase activities were assayed by the method of Thompson *et al.* (11).

Materials. [^{3}H]Adenosine (29 Ci/mmol) and [^{14}C]cyclic AMP (44 mCi/mmol) were purchased from ICN Pharmaceuticals (Irvine, CA). Unlabeled cyclic AMP, (−)norepinephrine bitartrate, phenylephrine, adenosine, 2-chloroadenosine, adenosine deaminase, VIP, *l*-isoproterenol, and histamine dihydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO). GABA and forskolin were obtained from Calbiochem (San Diego, CA). (±)Baclofen was a gift from Ciba-Geigy (Summit, NJ), isoguvacine was provided by Dr. Povl Krogsgaard-Larsen (Copenhagen, Denmark), and THIP was donated by Dr. A. V. Christensen of H. Lundbeck Co. (Copenhagen, Denmark). Kojic amine was provided by Dr. George Yarbrough of Merck, Sharp & Dohme (West Point, PA) and RO 20-1724 was donated by Dr. W. Burkhardt of F. Hoffmann-LaRoche (Basel, Switzerland).

RESULTS

Baclofen induced a 3-fold increase in the response to saturating (100 μM) concentrations of norepinephrine or isoproterenol but had no effect on phenylephrine-stimulated cyclic AMP accumulation in rat brain cortical slices (Table 1). Baclofen was also found to potentiate the cyclic AMP response to noncatecholamines (Table 1). Thus, cyclic AMP accumulation in response to histamine or VIP was increased 3-fold in the presence of baclofen and the $GABA_B$ agonist doubled the response to adenosine and 2-chloroadenosine. Baclofen enhanced the synergistic response observed when tissue slices were exposed simultaneously to saturating concentrations of adenosine and norepinephrine. While adenosine deaminase lowered the response to isoproterenol alone, the enzyme had no effect on the ability of baclofen to poten-

TABLE 1

Effect of baclofen on neurotransmitter- and drug-stimulated cyclic AMP accumulation in rat brain cortical slices

The influence of baclofen (100 μM) on cyclic AMP accumulation was examined in the presence and absence of a variety of receptor agonists. Except for VIP, which was tested at 0.25 μM, all of the receptor agonists were present at a concentration of 100 μM. Adenosine deaminase (10 μg/ml) was added 2 min prior to the addition of isoproterenol or isoproterenol + baclofen. Preliminary experiments demonstrated that at this concentration adenosine deaminase completely abolished the cyclic AMP response to 100 μM adenosine. For all experiments, cyclic AMP accumulation was studied using a prelabeling technique. Values represent the mean ± SE of three or four separate experiments, each of which was performed in duplicate.

Condition	Cyclic AMP accumulation	
	Control	+Baclofen
	% conversion	
Basal	0.10 ± 0.003	0.19 ± 0.04
Norepinephrine	1.03 ± 0.03	2.99 ± 0.26
Isoproterenol	0.61 ± 0.01	1.82 ± 0.14
Phenylephrine	0.26 ± 0.06	0.26 ± 0.04
VIP	1.29 ± 0.05	3.83 ± 0.20
Histamine	0.21 ± 0.03	0.67 ± 0.12
Adenosine	1.76 ± 0.05	3.83 ± 0.14
2-Chloroadenosine	2.82 ± 0.21	5.37 ± 0.36
Adenosine + norepinephrine	4.92 ± 0.41	6.76 ± 0.43
Isoproterenol + adenosine deaminase	0.41 ± 0.02	1.51 ± 0.13

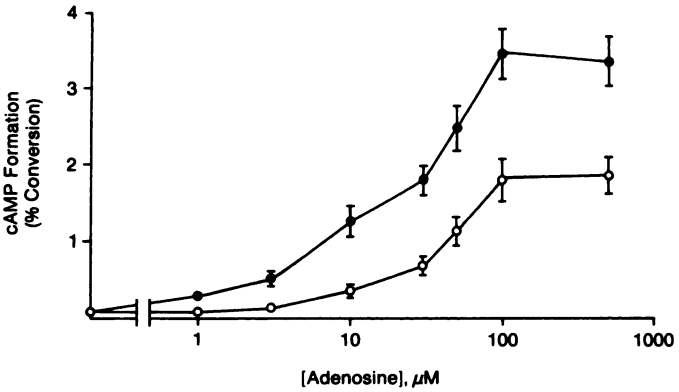

FIG. 1. *Effect of baclofen on the dose-response characteristics of adenosine-stimulated cyclic AMP accumulation in rat brain cortical slices*

Cyclic AMP accumulation was studied using a prelabeling technique. Basal per cent conversion was 0.08 ± 0.006 and 0.09 ± 0.006 in the absence (○) and presence (●) of baclofen (100 μM), respectively. Each *point* is the mean ± SE of five separate experiments, each of which was performed in duplicate. In some cases, this was too small to accurately depict on the figure.

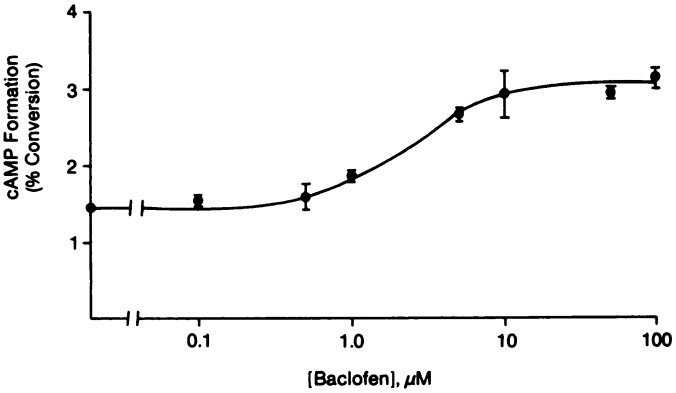

FIG. 2. *Adenosine-stimulated cyclic AMP accumulation in rat brain cortical slices in the presence of varying concentrations of baclofen*

Cyclic AMP accumulation was studied using a prelabeling technique. Basal per cent conversion was 0.08 ± 0.006 and 0.09 ± 0.007 in the absence and presence of 100 μM baclofen, respectively. In all cases, the assay was conducted in the presence of 100 μM adenosine which alone yielded a per cent conversion of 1.46 ± 0.05. Each *point* is the mean ± SE of three separate experiments, each of which was performed in duplicate. In one case (adenosine alone), this was too small to depict on the figure.

TABLE 2

Effect of GABA agonists on adenosine-stimulated cyclic AMP accumulation in rat brain cortical slices

The influence of GABA agonists on adenosine-stimulated cyclic AMP accumulation was examined by incubating the brain slices with adenosine (100 μM) in the presence and absence of a variety of GABA receptor stimulants (1 mM). Cyclic AMP accumulation was studied using a prelabeling technique. Values represent the mean ± SE of four separate experiments, each of which was performed in duplicate.

Condition	Cyclic AMP accumulation
	% conversion
Basal	0.08 ± 0.007
Adenosine	2.03 ± 0.15
Adenosine + kojic amine	3.32 ± 0.28
Adenosine + GABA	3.33 ± 0.20
Adenosine + isoguvacine	2.08 ± 0.07
Adenosine + THIP	2.02 ± 0.16

tiate second messenger production during exposure to the β-agonist.

At all concentrations of adenosine examined (1–500 μM), baclofen (100 μM) potentiated the cyclic AMP response to this agent (Fig. 1). While baclofen slightly increased the potency of adenosine, the effect on cyclic AMP production appeared to be due primarily to an increased synthesis or accumulation of second messenger. When cyclic AMP production was examined in the presence of varying concentrations of baclofen (0.1–100 μM) and a fixed concentration (100 μM) of adenosine, it was found that the EC_{50} for baclofen was approximately 2.5 μM (Fig. 2). Both kojic amine and GABA, nonselective GABA agonists, were also capable of enhancing the response to adenosine (Table 2). In contrast, neither isoguvacine nor THIP, GABA$_A$ agonists, had any effect on adenosine-stimulated cyclic AMP production.

The interaction between baclofen and norepinephrine was found to be regionally selective in the rat central nervous system (Table 3). Baclofen enhanced the response to norepinephrine to a similar extent in the cerebral cortex, corpus striatum, and hippocampus. On the other hand, baclofen had no significant effect on norepinephrine-stimulated cyclic AMP accumulation in the spinal cord, cerebellum, and pons-midbrain.

A time-course study revealed that cerebral cortical cyclic AMP levels were maximal within 10 min following exposure to 100 μM norepinephrine and remained constant for up to 45 min (Fig. 3). When the experiment was repeated in the presence of 100 μM baclofen, cyclic AMP accumulation was greater at all time points. As with the control samples, equilibrium was attained within the first 10 min and cyclic AMP levels remained constant for up to 45 min. The equilibrium value in the presence of baclofen was approximately 3-fold greater than in the absence of the GABA$_B$ agonist.

Preliminary experiments revealed that the maximal accumulation of cyclic AMP occurs in the presence of 25 μM RO 20-1724, a selective inhibitor of phosphodiesterase (12). At this concentration, basal cyclic AMP levels were four times greater than in the absence of RO 20-1724 (Table 4). Baclofen alone had little effect on cyclic AMP production during exposure to RO 20-1724, but increased further the amount of cyclic AMP accumulated in response to norepinephrine. Thus, the per cent conversion during exposure to the norepinephrine-RO 20-1724 combination was 3.4, whereas almost a 6% conversion was noted when 100 μM baclofen was added to the incubation medium (Table 4). Moreover, at concentrations up to 100 μM, baclofen had no effect on high affinity cyclic AMP or cyclic GMP phosphodiesterase activity (data not shown).

Forskolin dramatically increases cyclic AMP levels in rat brain slices, apparently by influencing some component of the cyclic nucleotide-generating system beyond the level of the neurotransmitter recognition site (13, 14). In the present study, it was found that forskolin increased cyclic AMP production some 120-fold in a concentration-dependent manner (Fig. 4). However, baclofen did not enhance the response to forskolin, but rather significantly reduced the response to the diterpene. In the presence of a fixed concentration (5 μM) of forskolin, baclofen inhibited cyclic AMP production nearly 50% at a concentration of 100 μM (Fig. 5). Both GABA and kojic amine were also capable of inhibiting the second messenger response to forskolin (Table 5). In contrast, neither isoguvacine nor THIP had any effect on forskolin-stimulated cyclic AMP accumulation at concentrations up to 1 mM.

The importance of calcium for the baclofen-isoproterenol interaction was examined by studying the influence of EGTA on the cyclic AMP response to these agents (Table 6). While EGTA had little effect on basal cyclic AMP levels, the calcium chelator decreased isoproterenol-stimulated cyclic AMP production approximately 45% at a concentration of 10 mM. However, second messenger accumulation in the presence of the baclofen-isoproterenol combination was reduced 60% by 5 mM, and 80% by 10 mM, EGTA (Table 6). Indeed, the response observed with the drug combination during ex-

TABLE 3

Effect of baclofen on norepinephrine-stimulated cyclic AMP accumulation in various regions of the rat central nervous system

The influence of baclofen (100 μM), norepinephrine (100 μM), and the combination of these agents on cyclic AMP accumulation in tissue slices obtained from various regions of the rat central nervous system was examined using a prelabeling technique. Values represent the mean ± SE of three separate experiments, each of which was performed in duplicate.

Brain region	Cyclic AMP accumulation			
	Basal	+Baclofen	+Norepinephrine	Norepinephrine + Baclofen
	% Conversion			
Cerebral cortex	0.14 ± 0.03	0.17 ± 0.03	1.70 ± 0.30	4.10 ± 0.62[a]
Hippocampus	0.16 ± 0.01	0.18 ± 0.01	2.23 ± 0.12	3.81 ± 0.17[a]
Corpus striatum	0.09 ± 0.01	0.12 ± 0.01	2.52 ± 0.18	5.18 ± 0.39[a]
Pons-midbrain	0.19 ± 0.01	0.24 ± 0.02	3.01 ± 0.50	3.51 ± 0.60
Cerebellum	0.25 ± 0.04	0.21 ± 0.03	1.10 ± 0.03	1.00 ± 0.03
Spinal cord	0.29 ± 0.02	0.24 ± 0.02	0.65 ± 0.01	0.71 ± 0.05

[a] $p < 0.05$ compared to norepinephrine alone.

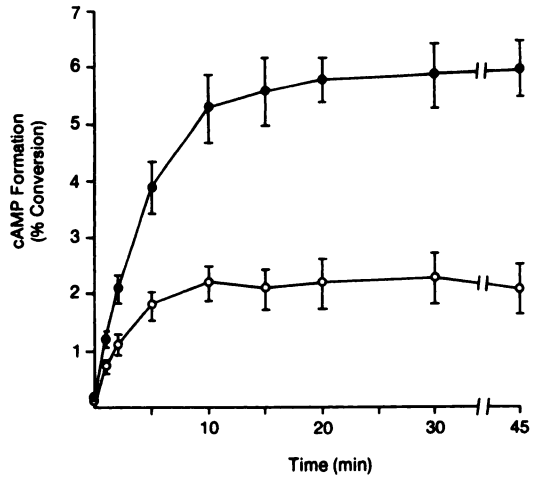

FIG. 3. *The influence of baclofen on the time course of cyclic AMP accumulation in rat brain cortical slices in response to norepinephrine*

Rat brain cortical slices were incubated for various periods of time with 100 μM norepinephrine in the absence (○) or presence (●) of 100 μM baclofen. Basal cyclic AMP levels remained constant over the 45-min test period. Cyclic AMP accumulation was studied using a prelabeling technique. Each *point* is the mean ± SE of three separate experiments, each of which was performed in triplicate.

posure to 10 mM EGTA was similar to that obtained with isoproterenol alone in the presence of the chelator.

DISCUSSION

While it has long been known that some GABA receptors are coupled to chloride ion channels (15), it has only recently been suggested that some may be related to, or influence, the cyclic nucleotide system in brain (8, 16, 17). For example, we have previously reported that $GABA_B$, but not $GABA_A$, agonists potentiate the second messenger response to norepinephrine (8, 18, 19), a finding that has been confirmed by others (20). Although the current study was conducted using a prelabeling technique to measure cyclic AMP production, experiments using a radioimmunoassay method have revealed that identical data are obtained if the endogenous levels of second messenger are analyzed (data not shown). This suggests that the $GABA_B$ agonists are not merely altering the specific activity of ATP pools. The present series of

TABLE 4

The influence of RO 20-1724 on cyclic AMP accumulation in rat brain cerebral cortical slices in response to baclofen and norepinephrine

Rat brain cortical slices were preincubated for 2 min in the presence and absence (control) of 25 μM RO 20-1724 after which baclofen (100 μM), norepinephrine (100 μM), or the combination were added and cyclic AMP accumulation was studied using a prelabing technique. Values represent the mean ± SE of four separate experiments each of which was performed in duplicate.

Condition	Cyclic AMP accumulation	
	Control	+RO 20-1724
	% conversion	
Basal	0.10 ± 0.01	0.45 ± 0.05
Baclofen	0.18 ± 0.03	0.65 ± 0.06
Norepinephrine	1.50 ± 0.16	3.42 ± 0.32
Norepinephrine + baclofen	4.40 ± 0.30	5.87 ± 0.27[a]

[a] $p < 0.05$ compared to either control norepinephrine + baclofen or norepinephrine alone in the presence of RO 20-1724.

experiments revealed that this action is not limited to catecholamines since $GABA_B$ agonists were found capable of enhancing the accumulation during exposure to adenosine, VIP, or histamine. This makes it appear that $GABA_B$ agonists modify the responsiveness of the cyclic nucleotide-generating system at some point other than the hormone or neurotransmitter recognition site.

Norepinephrine stimulates cyclic AMP production in brain slices by an action at both α- and β-adrenergic receptors (7, 21). In the present study, it was found that while baclofen enhanced the response to the β-agonist isoproterenol, it had no effect on the α-agonist phenylephrine. Although phenylephrine has some limitations as an α-agonist in this system, it could be speculated that baclofen may not potentiate α-adrenergic responsiveness because the α-receptor is itself indirectly coupled to the second messenger system in brain (7, 22).

The fact that baclofen did not modify the time course of catecholamine-stimulated cyclic AMP production suggests that the rate at which the components of the cyclic AMP-generating system interact is not influenced by the $GABA_B$ agonist. Rather it would appear that the enhanced responsiveness is due to an increase in the capacity of the cell to synthesize or store second messenger.

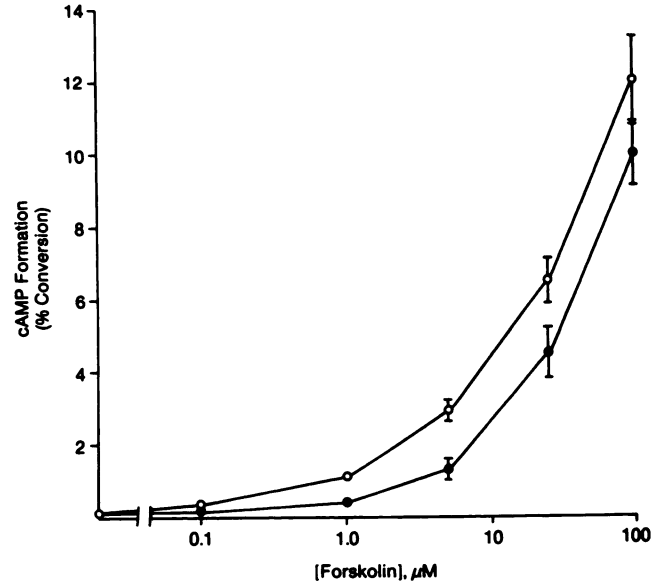

FIG. 4. *Effect of baclofen on the dose-response characteristics of forskolin-stimulated cyclic AMP accumulation in rat brain cortical slices*

Cyclic AMP accumulation was measured using a prelabeling technique. Basal per cent conversion was 0.10 ± 0.006 and 0.13 ± 0.006 in the absence (○) and presence (●) of baclofen (100 μM), respectively. Each *point* represents the mean ± SE of three separate experiments, each of which was performed in duplicate. In some cases, this was too small to accurately depict on the figure.

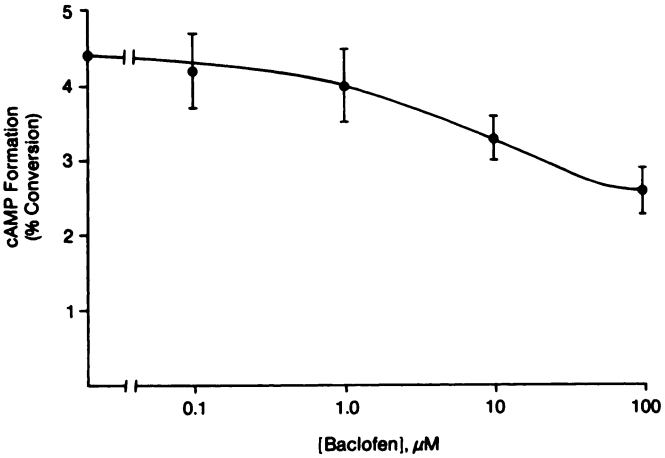

FIG. 5. *Forskolin-stimulated cyclic AMP accumulation in rat brain cortical slices in the presence of varying concentrations of baclofen*

Cyclic AMP accumulation was studied using a prelabeling technique. Basal per cent conversion was 0.11 ± 0.01 and 0.15 ± 0.02 in the absence and presence of 100 μM baclofen, respectively. In all cases, the assay was conducted in the presence of 5 μM forskolin. Each point is the mean ± SE of three separate experiments, each of which was performed in duplicate.

One possibility was that baclofen could be acting by inhibiting cyclic nucleotide degradation. However it was found that baclofen is capable of increasing norepinephrine-stimulated cyclic AMP accumulation even when phosphodiesterases are inhibited. Moreover, a direct analysis of the interaction between baclofen and two forms of phosphodiesterase indicated that the GABA$_B$ agonist has no effect on these enzymes (data not shown).

To be certain that the interaction between baclofen and noncatecholamines is characteristic of GABA$_B$ agonists, a more thorough examination of this phenomenon was undertaken. It was found that baclofen potentiated the cyclic AMP response to 2-chloroadenosine, a stable adenosine analogue that is not incorporated into nucleotide pools, as well as to adenosine itself, suggesting that the GABA$_B$ agonist facilitates adenosine receptor-mediated activation of second messenger production. Dose-response studies indicated that baclofen stimulates the response to adenosine in a manner similar to that observed with norepinephrine (8). That is, baclofen acted primarily to increase the total accumulation of cyclic AMP in response to adenosine while having little effect on the potency of adenosine. Furthermore, the EC$_{50}$ for baclofen (2.5 μM) to potentiate the adenosine response was nearly identical to that found for potentiating the response to catecholamines (8). As with norepinephrine, it was observed that GABA$_B$, but not GABA$_A$, agonists enhanced the response to adenosine, suggesting that this interaction is characteristic of the GABA$_B$ system.

Given the fact that adenosine is known to act in a synergistic manner with norepinephrine (6), it was conceivable that the effect of baclofen may be mediated indirectly through an action on the adenosine system. To examine this possibility, baclofen-potentiated cyclic AMP production was studied in the presence of adenosine deaminase to rid the system of extracellular adenosine. Because adenosine deaminase had no effect on the ability of baclofen to facilitate isoproterenol-stimulated cyclic AMP production, it would appear that adenosine is not required for the action of the GABA$_B$ agonist. Furthermore, the findings that baclofen alone has only a slight effect on basal cyclic AMP production, and that it is capable of potentiating the response to saturating concentrations of adenosine and 2-chloroadenosine, can also be taken as evidence that baclofen is not significantly modifying the release, re-uptake or metabolism of adenosine.

It could be argued that the baclofen effect is reminiscent of the α-adrenergic influence on cyclic AMP production in brain (7, 22). Like α-agonists, baclofen has little effect on basal cyclic AMP levels and yet it can greatly amplify the response to other agents. In addition, the α-adrenergic response is calcium-dependent, as is the

TABLE 5

Effect of GABA agonists on forskolin-stimulated cyclic AMP accumulation in rat brain cortical slices

Rat cerebral cortical slices were incubated with forskolin (5 μM) in the presence and absence of a variety of GABA receptor agonists (1 mM). Cyclic AMP accumulation was studied using a prelabeling technique. Values are the mean ± SE of three separate experiments, each of which was performed in duplicate.

Condition	Cyclic AMP accumulation
	% conversion
Basal	0.11 ± 0.01
Forskolin	4.40 ± 0.45
Forskolin + GABA	3.00 ± 0.29[a]
Forskolin + kojic amine	3.00 ± 0.23[a]
Forskolin + isoguvacine	4.60 ± 0.44
Forskolin + THIP	4.40 ± 0.43

[a] $p < 0.05$ compared to forskolin alone.

TABLE 6

Effect of EGTA on baclofen-potentiated cyclic AMP responses in rat brain cerebral cortical slices

In all cases EGTA was added 2 min prior to exposing the brain slices to either isoproterenol (10 μM), baclofen (100 μM) or the drug combination. Cyclic AMP accumulation was studied using a prelabeling technique. The absence of data indicates the condition was not studied. Values represent the mean ± SE of three separate experiments, each of which was performed in duplicate.

EGTA Conc.	Condition of cyclic AMP accumulation				
	Basal	Baclofen	Isoproterenol	Baclofen + isoproterenol	Ratio[a]
mM			*% conversion*		
0	0.15 ± 0.02	0.33 ± 0.03	0.97 ± 0.09	3.30 ± 0.32	3.4
1.0			1.03 ± 0.03	3.30 ± 0.22	3.2
2.5			1.03 ± 0.02	2.60 ± 0.19	2.5
5.0	0.26 ± 0.02	0.25 ± 0.03	0.75 ± 0.05	1.40 ± 0.23	1.9[b]
10.0	0.23 ± 0.03	0.25 ± 0.04	0.54 ± 0.05	0.78 ± 0.11	1.4[b]

[a] Baclofen + isoproterenol/Isoproterenol.

[b] $p < 0.05$ as compared to corresponding condition in the absence of EGTA.

action of baclofen. Moreover, of the six central nervous system areas examined, baclofen was capable of potentiating the norepinephrine response in only the cerebral cortex, hippocampus, and corpus striatum, but not in the cerebellum, pons-midbrain, or spinal cord. This distribution is quite similar to that found for α-adrenergic potentiated responses (6). This regional distribution is also interesting inasmuch as $GABA_B$ receptor-binding sites have been found in virtually all areas of the central nervous system, with a particularly high concentration in the cerebellum (23, 24). The poor correlation between the baclofen-catecholamine interaction and $GABA_B$ binding indicates that the cyclic AMP response is either mediated by a subclass of the $GABA_B$-binding sites or involves a group of $GABA_B$ receptors not identified in the binding assay.

Because $GABA_B$ agonists potentiated second messenger responses to a variety of agents, it was surprising to find that it failed to enhance forskolin-stimulated cyclic AMP production. In fact, baclofen reduced the ability of this substance to stimulate second messenger accumulation. It is unclear whether forskolin activates cyclic nucleotide production by directly influencing N_s, C, or both components (13, 25, 26), making it difficult to predict which constituent is influenced by the $GABA_B$ agonist. Attenuation of the response to forskolin may be expected of agents which inhibit adenylate cyclase. In this regard, it is noteworthy that $GABA_B$ agonists have been reported to inhibit this enzyme in brain homogenates (19). However, because neurotransmitter-stimulated cyclic AMP production is largely abolished in brain membrane fractions, it is not possible to study the potentiating effect of baclofen under this condition. Therefore, other types of experiments will be necessary to determine which effect of baclofen (potentiation of cyclic AMP production or inhibition of adenylate cyclase) predominates *in vivo*.

While the precise mechanism of action of $GABA_B$ agonists remains unknown, these results suggest that they may regulate the responsiveness of neurotransmitter-coupled cyclic nucleotide systems in brain by way of a calcium-dependent process. Because $GABA_B$ receptor binding requires calcium ion (27), it is possible that EGTA reduces the activity of baclofen by decreasing its affinity for the $GABA_B$ receptor recognition site. Alternatively, $GABA_B$ agonists may be inducing the release of some substance which in turn mediates the observed effects on neurotransmitter-stimulated cyclic AMP production and removal of calcium ions interferes with this release process. Another possibility is that $GABA_B$ agonists may regulate the transfer of calcium ion across postsynaptic membranes, thereby influencing the sensitivity of the adenylate cyclase system. In any event, the present data indicate that activation of $GABA_B$ receptors influences the cyclic nucleotide system at a point beyond the receptor recognition site. Given the ubiquity of the brain GABAergic system (15), these findings suggest that GABA may play a crucial role in modulating responses to other neurotransmitter substances.

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