cAMP and forskolin decrease γ -aminobutyric acid-gated chloride flux in rat brain synaptoneurosomes

(γ -aminobutyric acid receptor/chloride uptake/ion channels/cyclic nucleotides/1,9-dideoxyforskolin)

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ABSTRACT The effects of the cyclic nucleotide cAMP on γ -aminobutyric acid-gated chloride channel function were investigated. The membrane-permeant cAMP analog N^6, O^2' -dibutyryl-adenosine 3',5'-cyclic monophosphate inhibited muscimol-induced $^{36}\text{Cl}^-$ uptake into rat cerebral cortical synaptoneurosomes in a concentration-dependent manner ($\text{IC}_{50} = 1.3$ mM). The inhibition was due to a decrease in the maximal effect of muscimol, with no change in potency. Similar effects were observed with 8-(4-chlorophenylthio)adenosine 3',5'-cyclic monophosphate, 8-bromoadenosine 3',5'-cyclic monophosphate, and the phosphodiesterase inhibitor isobutylmethylxanthine. The effect of endogenous cAMP accumulation on the γ -aminobutyric acid-gated Cl^- channel was studied with forskolin, an activator of adenylate cyclase. Under identical conditions, in the intact synaptoneurosomes, forskolin inhibited muscimol-induced $^{36}\text{Cl}^-$ uptake and generated cAMP with similar potencies ($\text{IC}_{50} = 14.3$ μM ; $\text{EC}_{50} = 6.2$ μM , respectively). Surprisingly, 1,9-dideoxyforskolin, which does not activate adenylate cyclase, also inhibited the muscimol response, suggesting that forskolin and its lipophilic derivatives may interact with the Cl^- channel directly. Indeed, forskolin inhibition of muscimol-induced $^{36}\text{Cl}^-$ uptake was extremely rapid (within 5 sec), preceding the accumulation of sufficient levels of cAMP. After 5 min, a slower phase of inhibition was seen, similar to the time course for cAMP accumulation. The data suggest that γ -aminobutyric acid (GABA_A) receptor function in brain can be regulated by cAMP-dependent phosphorylation.

γ -Aminobutyric acid (GABA), the major inhibitory neurotransmitter in the vertebrate central nervous system, produces neuronal inhibition by binding to a supramolecular receptor complex and opening its associated chloride (Cl^-) channel (ref. 1; for review, see ref. 2). The GABA_A receptor is activated by GABA and muscimol and is competitively blocked by bicuculline, while the GABA_B receptor, which is insensitive to muscimol and bicuculline (3), is not linked to a Cl^- channel. The action of GABA can be modulated by a variety of compounds (benzodiazepines, barbiturates, and "cage" convulsants) that bind to distinct recognition sites on the GABA_A receptor/ Cl^- channel complex (for review, see ref. 2). These compounds are useful experimental and clinical tools to study the relationship between GABA-regulated neuronal excitability and anxiety, sedation, and seizure disorders.

Neurotransmitter- and voltage-gated ion channels have been shown to be regulated by intracellular second messengers, such as cyclic nucleotides (4–6). Regulation of ion channels by cAMP and/or cGMP primarily involves cyclic nucleotide-dependent phosphorylation of receptor and/or ion-channel proteins (4–6). Currently, little is known about

regulation of the GABA-gated Cl^- channel by second messenger systems and protein phosphorylation. Structural studies suggest that the β subunit of the GABA_A receptor contains a consensus sequence for a cAMP-dependent phosphorylation site (7). Sweetnam *et al.* (8) reported phosphorylation of the α subunit by an endogenous kinase with a substrate specificity similar to calcium/diacylglycerol-dependent protein kinase. Cyclic nucleotide-dependent phosphorylation of the α subunit was not observed (8). Two electrophysiological studies showed a progressive loss of GABA-evoked currents in cultured neurons that could be prevented by intracellular hydrolysis of ATP (9, 10). Although both studies (9, 10) suggested that an ATP-dependent process, possibly phosphorylation, is involved in the maintenance of GABA_A receptor function, no evidence for the role of cAMP or cGMP in that regulatory mechanism was provided. Indirect evidence for the regulation of the GABA_A receptor complex by phosphorylation was obtained in studies in which the activation of protein kinase C decreased GABA-induced Cl^- conductance in *Xenopus* oocytes injected with chicken fore-brain mRNA (11).

In the absence of evidence that cyclic nucleotides, such as cAMP, regulate the function of the GABA-gated Cl^- channel, we have investigated the effects of (i) membrane-permeant cAMP analogs and (ii) forskolin-induced endogenous cAMP accumulation on muscimol-stimulated $^{36}\text{Cl}^-$ uptake in an intact vesicular preparation (synaptoneurosomes) from rat cerebral cortex. Our findings indicate that cAMP analogs and forskolin decrease the functional activity of the GABA_A receptor complex. However, we suggest that the main actions of forskolin on the GABA-gated chloride channel are independent of its ability to generate cAMP by activation of adenylate cyclase. These findings have been reported in preliminary form (12).

MATERIALS AND METHODS

Preparation of Synaptoneurosomes. Synaptoneurosomes (13) were prepared from adult male Sprague–Dawley rats (150–225 g; Zivic Miller) as described by Schwartz *et al.* (14) with minor modifications. Brains were removed rapidly after decapitation and placed immediately in ice-cold buffer (pH 7.4) containing 10 mM glucose, 20 mM Hepes, 9 mM Tris, 118 mM NaCl, 4.7 mM KCl, 1.18 mM MgSO_4 , and 1.0 mM CaCl_2 . Cerebral cortices were dissected free of white matter and homogenized (five strokes by hand) in 7 vol (wt/vol) of buffer by using a glass–glass homogenizer. The homogenate was diluted with 30 ml of buffer and filtered by gravity through three layers of nylon mesh (160 μm , Tetko, Elmsford, NY).

Abbreviations: GABA, γ -aminobutyric acid; IBMX, 3-isobutyl-1-methylxanthine; Bt_2cAMP , N^6, O^2' -dibutyryl-adenosine 3',5'-cyclic monophosphate.

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The filtrate was then gently filtered through a 10- μ m Mitex filter (Millipore) and centrifuged at $1000 \times g$ for 15 min. The supernatant was discarded and the pellet was gently resuspended and recentrifuged ($1000 \times g$, 15 min). The final pellet was resuspended to a protein concentration of 10 mg/ml. The filtration steps described above effectively remove large cellular debris, oligodendrocytes, and intact neurons, resulting in a relatively homogenous population of both pre- and postsynaptic membrane vesicles ("synaptoneurosomes"; for details, see ref. 13).

Measurement of $^{36}\text{Cl}^-$ Uptake. $^{36}\text{Cl}^-$ uptake was measured as described by Schwartz *et al.* (15). Synaptoneurosomes (1 mg of protein) were preincubated for 20 min (unless otherwise noted) at 30°C prior to the simultaneous addition of 0.5 μCi $^{36}\text{Cl}^-$ (specific activity, 15.1 mCi/g; 1 mCi = 37 MBq, New England Nuclear) and muscimol (1–50 μM). Basal uptake of $^{36}\text{Cl}^-$ was determined in the absence of muscimol. $^{36}\text{Cl}^-$ uptake was terminated after 5 sec by dilution of the assay mixture with 5 ml of ice-cold buffer containing 100 μM picrotoxin and filtration by vacuum through a glass-fiber filter (number 30, Schleicher & Schuell). The filters were washed two times with 5 ml of ice-cold buffer (containing picrotoxin) and radioactivity was measured by conventional liquid scintillation spectrometry at an efficiency of 96%.

cAMP Accumulation. Cerebral cortical synaptoneurosomes were incubated under conditions identical to those used for the $^{36}\text{Cl}^-$ uptake assay (30°C for 20 min, same buffer composition). In most experiments the same preparation was used for $^{36}\text{Cl}^-$ uptake and cAMP accumulation assays. To prevent the breakdown of cAMP generated by forskolin the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX, 1 mM) was present in all the appropriate tubes for the cAMP accumulation and $^{36}\text{Cl}^-$ uptake assays. Accumulation of cAMP was terminated by the addition of 0.5 ml of 10% (wt/vol) ice-cold trichloroacetic acid to each assay tube. After vortex mixing, the assay mixture was centrifuged for 10 min at $10,000 \times g$. An aliquot of the supernatant was diluted 1:100 with 50 mM acetate buffer (pH 5.8) and excess trichloroacetic acid was removed by repeated extraction with 2.5 ml of water-saturated diethyl ether. The measurement of cAMP was performed using a femtomole-sensitive ^{125}I -labeled cAMP radioimmunoassay (16) and cAMP accumulation was calculated as pmol/mg of protein.

Protein Determination. Protein concentrations were determined by the method of Lowry *et al.* (17) using bovine serum albumin as standard.

Materials. N^6, O^2 -Dibutyryl adenosine 3',5'-cyclic monophosphate (Bt_2 cAMP), 8-(4-chlorophenylthio)adenosine 3',5'-cyclic monophosphate, 8-bromo adenosine 3',5'-cyclic monophosphate, and IBMX were obtained from Sigma. Forskolin, 1,9-dideoxyforskolin, and a chemically modified forskolin (compound 3 from ref. 18) were obtained from Calbiochem. Stock solutions (2.5 mM) of forskolin and the 1,9-dideoxyforskolin were made in hydroxypropyl- β -cyclodextrin (250 mM) solution in water (Research Biochemicals) (18), followed by sonication for 20 min. Stock solutions were diluted with distilled water to 6 mM (or to 60 μM for forskolin). [Similar results were obtained when forskolin was dissolved in absolute ethanol (100 mM stock) and diluted to 0.03%.] Since 6 mM cyclodextrin produced stimulation (15%) of muscimol-induced $^{36}\text{Cl}^-$ uptake, it was included in all assay tubes for forskolin experiments. The ^{125}I -labeled cAMP radioimmunoassay kit was obtained from Amersham. All other drugs and reagents were obtained from standard commercial sources.

Data Analysis. $^{36}\text{Cl}^-$ uptake data are reported as net muscimol-induced $^{36}\text{Cl}^-$ uptake or percent inhibition of the maximal muscimol response (50 μM). The basal values for $^{36}\text{Cl}^-$ uptake (averaged 7 nmol/mg of protein) have been subtracted from the values determined in the presence of

muscimol. Basal cAMP levels averaged 48.8 pmol/mg of protein[†] and have been subtracted from the values determined in the presence of forskolin. Student's *t* tests were used for statistical comparisons of two groups of means. A probability level (*P*) of <0.05 was considered to be statistically significant.

RESULTS

Bt_2 cAMP, a membrane-permeant analog of cAMP, inhibited muscimol-induced $^{36}\text{Cl}^-$ uptake into cerebral cortical synaptoneurosomes in a concentration-dependent manner (IC_{50} = 1.3 mM) (Fig. 1A). Pretreatment of synaptoneurosomes with Bt_2 cAMP (1 mM) resulted in a decrease of the maximal response to muscimol (Fig. 1B). Bt_2 cAMP decreased the E_{max} from 80.1 ± 4.5 to 63.5 ± 3.5 nmol/mg of protein ($P < 0.05$) without significantly altering the $k_{0.5}$ (8.5 ± 1.1 and 9.0 ± 0.9 μM , respectively). Two other permeant analogs of cAMP, 8-(4-chlorophenylthio)adenosine 3',5'-cyclic monophosphate (1 mM) and 8-bromo adenosine 3',5'-cyclic monophosphate (2 mM), also inhibited muscimol-induced $^{36}\text{Cl}^-$ uptake (Table 1). A similar inhibition of the muscimol response resulted from preincubation of synaptoneurosomes with IBMX, the phosphodiesterase inhibitor (Table 1). No effects on basal $^{36}\text{Cl}^-$ uptake were observed.

To generate endogenous cAMP, synaptoneurosomes were preincubated with forskolin (and IBMX) to activate adenylate cyclase. Forskolin (3–60 μM) produced a concentration-dependent inhibition of muscimol-induced $^{36}\text{Cl}^-$ uptake (IC_{50} = 14.3 μM) (Fig. 2). In the same membrane preparation under identical conditions, forskolin produced a concentration-dependent increase in cAMP levels in the intact synaptoneurosomes (EC_{50} = 6.2 μM) (Fig. 2). The presence of IBMX enhanced the effect of forskolin (60 μM) on cAMP accumulation and muscimol-induced $^{36}\text{Cl}^-$ uptake 67% and 35%, respectively, compared to the effects of forskolin in the absence of IBMX (data not shown). Basal $^{36}\text{Cl}^-$ uptake was not altered by forskolin. Similar studies were performed with two other forskolin derivatives. The water-soluble modified forskolin (60 μM) (18) increased cAMP accumulation to 40% of the level induced by forskolin (60 μM) (Table 2). At this concentration, the modified forskolin did not inhibit muscimol-induced $^{36}\text{Cl}^-$ uptake (Table 2), although at higher concentrations (200–500 μM) inhibition was evident (data not shown). Conversely, 1,9-dideoxyforskolin (60 μM) failed to generate cAMP yet produced a 70% greater inhibition of muscimol-induced $^{36}\text{Cl}^-$ uptake than was produced by forskolin (Table 2). Limitations in solubility prevented the determination of an IC_{50} for 1,9-dideoxyforskolin; however, concentrations between 10 and 100 μM inhibited muscimol-induced $^{36}\text{Cl}^-$ uptake 7–50% (data not shown).

These findings suggested that forskolin inhibition of the muscimol response was not due to activation of adenylate cyclase. To examine this possibility, the time courses for forskolin-induced cAMP accumulation and inhibition of muscimol-induced $^{36}\text{Cl}^-$ uptake were compared in the same preparation. Forskolin (30 μM) significantly increased cAMP levels in synaptoneurosomes within 10 sec, while maximal accumulation of cAMP was reached only after 15 min of incubation with forskolin (Fig. 3). Under the same conditions, forskolin inhibited muscimol-induced $^{36}\text{Cl}^-$ uptake with a different time course. A rapid phase of inhibition occurred between 5 and 30 sec, reaching a plateau from 30 sec to 5 min. A slower phase of inhibition occurred between 5 and

[†]Rats were sacrificed by decapitation, accounting for the 2- to 3-fold higher basal levels of cAMP than are found in rats sacrificed by microwave irradiation or liquid nitrogen. The latter forms of sacrifice could not be used since assays of receptor function were also performed.

A

B

FIG. 1. (A) Concentration-response curve for Bt₂cAMP inhibition of muscimol-induced ³⁶Cl⁻ uptake. Synaptoneurosomes were incubated with Bt₂cAMP (0.1–5.0 mM) for 10 min (30°C) prior to the addition of muscimol (50 μM) and ³⁶Cl⁻ (0.5 μCi per assay tube). Data are the mean ± SEM from 3 to 24 experiments, performed in triplicate. The muscimol-induced ³⁶Cl⁻ uptake values were 37.4 ± 1.4 nmol/mg of protein. (B) Bt₂cAMP decreases maximal muscimol-induced ³⁶Cl⁻ uptake. Synaptoneurosomes were incubated with 1 mM Bt₂cAMP prior to addition of ³⁶Cl⁻ and muscimol (1–50 μM), as described in A. The data are the mean ± SEM of three experiments performed in triplicate. Double-reciprocal plots indicated that the *E*_{max} values were 80.1 ± 4.5 and 63.5 ± 3.5 nmol/mg of protein, for control (●) and Bt₂cAMP (Δ) conditions, respectively (*P* < 0.05, Student's *t* test). The *k*_{0.5} values were 8.5 ± 1.1 and 9.0 ± 0.9 μM, respectively (not significant). (Inset) Double-reciprocal plot for inhibition of muscimol-induced ³⁶Cl⁻ uptake by Bt₂cAMP. Curve a shows the control; curve b shows uptake in the presence of Bt₂cAMP (1 mM). U, muscimol-induced ³⁶Cl⁻ uptake expressed as nmol/mg of protein.

15 min (Fig. 3). Analysis of the two time courses by a two-way analysis of covariance confirmed the presence of two separable periods of forskolin's effect on ³⁶Cl⁻ uptake: up to 1 min, the time courses for inhibition of ion flux and accumulation of cAMP did not covary, but after 5 min, they did. Thus it is possible that after 5 min the level of intravesicular cAMP generated by forskolin eventually became high enough to modulate channel function (44.0 ± 5.8 pmol/mg of protein at 15 min).

In additional experiments (data not shown), receptor activation of adenylate cyclase was examined using isoproterenol and 2-chloroadenosine (100 μM). These receptor agonists generated levels of cAMP (20 and 17 pmol/mg of protein, respectively) too low to inhibit the muscimol re-

Table 1. Effect of various cAMP analogs and IBMX on muscimol-induced ³⁶Cl⁻ uptake in synaptoneurosomes

Agent	Muscimol-induced ³⁶ Cl ⁻ uptake, % inhibition
cAMP analog	
CPT-cAMP (1 mM)	50.6 ± 2.5
Bt ₂ cAMP (1 mM)	18.7 ± 1.6
8-Br-cAMP (2 mM)	19.2 ± 2.8
IBMX (1 mM)	19.2 ± 2.3

Synaptoneurosomes were incubated with cAMP analogs or IBMX for 10 min (30°C) and ³⁶Cl⁻ uptake was measured as described in Fig. 1A. Data are the mean ± SEM of 4–24 experiments, performed in triplicate. (*P* < 0.05, Student's *t* test.) CPT-cAMP, 8-(4-chlorophenylthio)adenosine 3',5'-cyclic monophosphate; 8-Br-cAMP, 8-bromo-adenosine 3',5'-cyclic monophosphate.

sponse, suggesting that the assay conditions for ³⁶Cl⁻ uptake may not be optimal for receptor-mediated cAMP accumulation.

DISCUSSION

The results of this study support the hypothesis that the GABA_A receptor/Cl⁻ channel can be regulated by the cyclic nucleotide cAMP. We have demonstrated that three membrane-permeant cAMP analogs decrease GABA-gated Cl⁻ channel function in an intact vesicular preparation from brain. In addition, we showed that forskolin, a direct activator of the catalytic subunit of adenylate cyclase (19), decreased GABA-gated ³⁶Cl⁻ flux. Forskolin has been reported to generate cAMP in broken neuronal membrane preparations and in intact cells with an EC₅₀ of 10–15 μM (20). In the present study, the potencies for forskolin-induced cAMP accumulation and for forskolin inhibition of muscimol-induced ³⁶Cl⁻ uptake were similar. This was a necessary, but not sufficient, criterion to conclude that the two forskolin actions were causally related. Surprisingly, subsequent experiments indicated that the action of forskolin to inhibit GABA receptor activity may not be entirely related to its ability to stimulate cAMP production. This was evident from two experiments. (i) 1,9-Dideoxyforskolin inhibited muscimol-induced ³⁶Cl⁻ uptake at concentrations that failed to

FIG. 2. Concentration-response curves for forskolin-induced cAMP accumulation and inhibition of muscimol-induced ³⁶Cl⁻ uptake. The two assays were performed in the same synaptoneurosomal preparations under identical conditions. Synaptoneurosomes were incubated with IBMX (1 mM, 30°C) 5 min prior to the addition of forskolin (1–60 μM). Then 15 min later either ³⁶Cl⁻ uptake (●) (using 50 μM muscimol) was measured or trichloroacetic acid was added for subsequent measurement of cAMP levels (Δ). Data are the mean ± SEM of three or four experiments, performed in duplicate (for cAMP) or triplicate (for ³⁶Cl⁻ uptake). The EC₅₀ for cAMP accumulation was 6.2 μM and the IC₅₀ for inhibition of muscimol-induced ³⁶Cl⁻ uptake was 14.3 μM. Basal cAMP levels were 58.4 ± 1.3 pmol/mg of protein. The muscimol-induced ³⁶Cl⁻ uptake values were 29.6 ± 2.1 nmol/mg of protein (in the presence of 1 mM IBMX).

Table 2. Effect of various forskolins on muscimol-induced $^{36}\text{Cl}^-$ uptake and cAMP levels in intact synaptoneurosomes

Forskolin derivative	Muscimol-induced $^{36}\text{Cl}^-$ uptake, % inhibition	cAMP accumulation, pmol/mg of protein
Forskolin	21.4 ± 1.6	66.5 ± 2.5
1,9-Dideoxyforskolin	36.6 ± 2.3	6.8 ± 0.7
Modified forskolin	0	27.2 ± 2.7

Synaptoneurosomes were incubated with forskolins (at $60 \mu\text{M}$) for 15 min, as described in Fig. 2. $^{36}\text{Cl}^-$ uptake and cAMP accumulation assays were performed in parallel. Data are the mean $\pm$ SEM of three to seven experiments, performed in triplicate (for $^{36}\text{Cl}^-$ uptake) or duplicate (for cAMP). Modified forskolin was the water-soluble forskolin analog, compound 3 from ref. 18.

generate cAMP, and (ii) the time course for forskolin inhibition of muscimol-induced $^{36}\text{Cl}^-$ uptake preceded that for generation of sufficient levels of cAMP. Similar results have been obtained for nicotinic receptor-mediated $^{86}\text{Rb}^+$ flux in PC12 cells (21); inhibition of nicotinic responses by forskolin preceded generation of cAMP, and 1,9-dideoxyforskolin, which does not activate adenylate cyclase (22), was more potent at inhibiting nicotinic responses. Those authors (21) suggested that forskolin may be acting like a general anesthetic to perturb the lipid bilayer and thus alter ion-channel function directly. A local anesthetic-like action of forskolin has also been observed in *Torpedo* nicotinic receptors expressed in *Xenopus* oocytes (23). Similarly, forskolin and 1,9-dideoxyforskolin directly altered the gating of both acetylcholine-elicited currents in skeletal muscle cells (24) and voltage-dependent K^+ channels in PC12 cells (25), independent of intracellular cAMP elevation. These studies (21, 23–25) as well as our own suggest that the direct effects of forskolin should be considered when making conclusions about its modulation of ion-channel function. This is discussed in a review (26) of the cAMP-independent effects of forskolin.

FIG. 3. Time course for forskolin-induced cAMP accumulation (○) and inhibition of muscimol-induced $^{36}\text{Cl}^-$ uptake (●). The two assays were performed in parallel. Synaptoneurosomes were incubated with IBMX (1 mM) for 20 min, and forskolin ($30 \mu\text{M}$) was added at the times indicated prior to initiation of $^{36}\text{Cl}^-$ uptake or addition of trichloroacetic acid, as described in Fig. 2. Data are the mean $\pm$ SEM (or range) of normalized values to 15 min for three or four $^{36}\text{Cl}^-$ uptake and two cAMP experiments performed in triplicate. Basal and forskolin-stimulated cAMP levels (15 min) were 48.8 ± 0.6 and 92.5 ± 6.5 pmol/mg of protein, respectively. Muscimol-induced $^{36}\text{Cl}^-$ uptake values were 25.4 ± 1.9 nmol/mg of protein (in the presence of IBMX). Mean percent inhibition of muscimol-induced $^{36}\text{Cl}^-$ uptake (15 min) was $25.4 \pm 1.9\%$. Two-way analysis of covariance indicated that the two forskolin effects did not covary up to 1 min ($F = 154.3$, $P < 0.01$) but did covary between 5 and 15 min ($F = 0.79$, not significant).

Despite the lack of a direct relationship between forskolin-induced cAMP generation and its ability to inhibit GABA-gated ion flux, we did observe the modulation of GABA_A receptor activity by analogs of cAMP. Inhibition of GABA responses was also obtained when we used the phosphodiesterase inhibitor IBMX to prevent the breakdown of endogenous cAMP inside the synaptoneurosomes. The actions of IBMX and the cAMP analogs suggest that elevated intracellular cAMP levels can modify GABA_A receptor activity. cAMP, whose production is under the control of a multicomponent enzyme system (27), regulates the function of other ligand-gated ion channels, such as the nicotinic receptor (4). Na^+ , K^+ , Ca^{2+} , and Cl^- channels are also regulated by cAMP in excitable cells (6, 28, 29). In each of these systems, intracellular regulation of ion channels by cAMP involves cAMP-dependent phosphorylation (6, 28–32). 8-Bromoadenosine 3',5'-cyclic monophosphate (1–10 mM) has been shown to decrease voltage-sensitive Na^+ uptake in synaptosomes (31) and increase endogenous phosphorylation of the Na^+ channel (31, 32). Phosphorylation of the nicotinic receptor by cAMP-dependent protein kinase (4) leads to decreased $^{86}\text{Rb}^+$ transport through the nicotinic receptor-gated ion channel in reconstituted vesicles (by enhancement of desensitization) (33). Although cAMP-dependent phosphorylation of the GABA_A receptor has not yet been demonstrated, there is evidence that this may occur. Schofield *et al.* (7) have identified a consensus sequence for a cAMP-dependent phosphorylation site on the β subunit of the GABA_A receptor. Phosphorylation of the α subunit of the GABA_A receptor has been demonstrated but it appears to be cAMP-independent (8). Although we have not demonstrated cAMP-dependent phosphorylation alters GABA-gated $^{36}\text{Cl}^-$ flux, we have evidence that the three cAMP analogs used herein stimulate protein kinase A activity in the synaptoneurosomes (200–2000%) (unpublished observations).

Although cAMP regulation of ion-channel function primarily involves protein phosphorylation, there is also evidence that cAMP and its analogs can alter ion-channel function directly (34–36). The studies presented here do not discriminate between these two mechanisms for the effects of cAMP on GABA_A receptor function. The use of patch clamp techniques to measure cAMP-induced reversible changes in Cl^- conductance in the absence of exogenous ATP would provide such information (35).

The physiologic significance of cAMP regulation of the GABA_A receptor function is unknown. cAMP-dependent phosphorylation of the nicotinic receptor-gated cation channel promotes receptor desensitization (33, 37). Desensitization of the GABA_A receptor has been demonstrated in a variety of preparations (38–41), including the synaptoneurosome preparation (15, 42–45). The loss in GABA receptor function associated with desensitization may lead to increased neuronal excitability and the development of seizures (38, 40, 46, 47). Since there is substantial evidence that cAMP plays a role in seizure activity (48, 49), a relationship between GABA_A receptor desensitization and cAMP-dependent phosphorylation remains to be examined.

The studies described here suggest that the GABA-gated ion-channel activity may be regulated by biochemical processes known to regulate other ligand-gated or voltage-sensitive ion channels. The use of molecular, biochemical, and functional techniques to approach this question will undoubtedly provide a better understanding how such regulatory mechanisms lead to altered ion-channel function and abnormal GABAergic neurotransmission.

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