*Proc. Natl. Acad. Sci. USA*
Vol. 87, pp. 1315–1318, February 1990
Neurobiology

# Protein kinase C and cAMP-dependent protein kinase phosphorylate the β subunit of the purified γ-aminobutyric acid A receptor

(protein phosphorylation)

MICHAEL D. BROWNING*†, MICHEL BUREAU‡§, ELLEN M. DUDEK*, AND RICHARD W. OLSEN‡

*Department of Pharmacology, University of Colorado Health Sciences Center, Denver, CO 80262; and ‡Department of Pharmacology, University of California, Los Angeles School of Medicine, Los Angeles, CA 90024

*Communicated by James L. McGaugh, November 17, 1989 (received for review August 3, 1989)*

**ABSTRACT** **A number of recent studies have suggested that phosphorylation of the γ-aminobutyric acid A ($GABA_A$) receptor could modulate receptor function. Activators of protein kinase C and cAMP-dependent protein kinase have been shown to influence $GABA_A$ receptor function. In addition, Sweetnam *et al.* [Sweetnam, P. M., Lloyd, J., Gallombardo, P., Malison, R. T., Gallager, D. W., Tallman, J. F. & Nestler, E. J. (1988) *J. Neurochem.* 51, 1274–1284] have reported that a kinase associated with a partially purified preparation of the receptor could phosphorylate the α subunit of the receptor. Moreover, Kirkness *et al.* [Kirkness, E. F., Bovenkerk, C. F., Ueda, T. & Turner, A. J. (1989) *Biochem. J.* 259, 613–616] have recently shown that cAMP-dependent protein kinase could phosphorylate a muscimol binding polypeptide of the $GABA_A$ receptor. To explore the issue further, we have examined the ability of specific kinases to catalyze significant phosphorylation of the $GABA_A$ receptor that has been purified to near homogeneity. The $GABA_A$ receptor was purified as previously described using benzodiazepine affinity chromatography. The purified receptor possessed no detectable kinase activity. Protein kinase C and cAMP-dependent protein kinase catalyzed the phosphorylation of the β and α subunits of the receptor. However, most of the phosphate incorporation was associated with the β subunit. Two muscimol binding polypeptides designated $\beta_{58}$ ($M_r$ 58,000) and $\beta_{56}$ ($M_r$ 56,000) were present in the preparation. The higher molecular weight polypeptide, $\beta_{58}$, was phosphorylated specifically by cAMP-dependent protein kinase. $\beta_{56}$ was phosphorylated specifically by protein kinase C. $\beta_{58}$ and $\beta_{56}$ gave distinct patterns in a one-dimensional phosphopeptide analysis. The stoichiometry of phosphorylation (mol of phosphate/mol of muscimol binding) catalyzed by cAMP-dependent protein kinase was 0.52 and that catalyzed by protein kinase C was 0.38. Taken together these data confirm that there are two forms of the β subunit of the $GABA_A$ receptor and suggest that these two forms of the β subunit are phosphorylated by distinct kinases.**

Various ligand-gated ion channels [nicotinic acetylcholine receptor, glycine receptor, and γ-aminobutyric acid A ($GABA_A$) receptor] have been cloned and their primary sequences have been deduced. Significant homologies exist among all of these proteins (1, 2). A number of recent reports have shown that the nicotinic acetylcholine receptor (AcChoR) is phosphorylated and that this phosphorylation regulates the rate of desensitization of the receptor (3–5). The AcChoR has been shown to be phosphorylated by the cAMP-dependent protein kinase (PKA) and by the $Ca^{2+}$/phosphatidylserine-dependent protein kinase (PKC) (6–8).

The $GABA_A$ receptor mediates the majority of inhibitory synaptic transmission in the central nervous system. Given the significant import of this receptor to central nervous system function, we have been particularly interested in the possibility that the $GABA_A$ receptor might exhibit posttranslational modification that resembles that seen in the AcChoR. A number of recent reports have suggested that phosphorylation of the $GABA_A$ receptor could modulate receptor function. Sigel and Baur (9) reported that activators of PKC led to decreased activity of the $GABA_A$ receptor that had been expressed in oocytes. In addition, activators of PKA decreased $GABA_A$ receptor-mediated $Cl^-$ flux in rat brain synaptoneurosomes (10) and induced a decline in GABA-gated chloride currents in cultured chick cortical neurons (11). Conversely, phosphorylation has also been reported to maintain GABA receptor function in dissociated hippocampal neurons and in cultured chick spinal neurons (12, 13). A consensus sequence preferred by PKA has also been identified in the sequence of the β subunit of the bovine $GABA_A$ receptor (2). There have been two reports of phosphorylation of the $GABA_A$ receptor. Sweetnam *et al.* (14) have shown that a kinase associated with the partially purified receptor could phosphorylate the α subunit of the bovine receptor. In addition, Kirkness *et al.* (15) have recently shown that PKA could phosphorylate the β subunit of the receptor that had been purified from pig cerebral cortex.

The $GABA_A$ receptor from bovine brain has been purified and two subunits have been cloned (α, $M_r$ 53,000; β, $M_r$ 57,000). However, a family of genes appears to exist for α (16) and β (17) as well as other possible subunits called γ (18). We have recently purified the $GABA_A$ receptor to near homogeneity from rat cortex using benzodiazepine affinity chromatography (19). The purified receptor contains two muscimol binding polypeptides with approximate $M_r$ of 58,000 and 56,000 (20–22). These have been designated $\beta_{58}$ ($M_r$ 58,000) and $\beta_{56}$ ($M_r$ 56,000). The receptor preparation also contains flunitrazepam binding polypeptides of $M_r$ 51,000–53,000 (19, 21, 22) and what appears to be a breakdown product at $M_r$ 45,000 (19, 20). We have examined the ability of PKC, PKA, and $Ca^{2+}$/calmodulin kinase II (CaM kinase II) to phosphorylate this receptor. We have found that PKA phosphorylates the $\beta_{58}$ subunit of the receptor and that PKC phosphorylates the $\beta_{56}$ subunit of the receptor.

## MATERIALS AND METHODS

**Materials.** The catalytic subunit of PKA was purified from bovine heart as described (23). PKC was purified by the

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: AcChoR, nicotinic acetylcholine receptor; GABA, γ-aminobutyric acid; PKA, cAMP-dependent protein kinase; PKC, protein kinase C; Cam kinase II, $Ca^{2+}$/calmodulin kinase II.

†To whom reprint requests should be addressed.
§Present address: Laboratoire de Biochemie Générale et Comparee, Universite de Liege, 17 Place Delcour, 4020 Liege, Belgium.

method of Woodgett and Hunter (24). CaM kinase II was purified by the method of McGuinness *et al.* (25). The $GABA_A$ receptor was purified as described (19). $^{125}$I-labeled protein A ($^{125}$I-protein A) and [*methylene*-$^3$H(N)]muscimol (15–31 Ci/mmol; 1 Ci = 37 GBq) (N = nominal) were obtained from New England Nuclear. [$\gamma$-$^{32}$P]ATP was obtained from either Amersham or ICN. Phosphatidylserine was obtained from Avanti Polar Lipids. *Staphylococcus aureus* V8 protease was from Miles. All other chemicals were reagent grade or better.

**Phosphorylation Assays.** The PKA phosphorylation assay (final volume, 100 $\mu$l) mixture contained 50 mM Hepes (pH 7.4), 10 mM $MgCl_2$, PKA at 3 $\mu$g/ml, and 50 $\mu$M ATP (specific activity, $10^7$ cpm/nmol). The activity of PKC and CaM kinase II appeared to be inhibited by a factor in the receptor preparation as the autophosphorylation of both kinases was dramatically inhibited in the presence of the receptor. This inhibition was likely due to the presence of detergent and could be diminished by exhaustive dialysis. In addition for PKC phosphorylation, use of the mixed micelle procedure of Hannun *et al.* (26) was optimal. Briefly, phosphatidylserine and diolein equivalent to 8.0 mol % and 2.5 mol %, respectively, of the Triton X-100 were dried under a stream of nitrogen in a glass tube. The dialyzed receptor in 0.2% Triton X-100 was then added to the tube and spun vigorously in a Vortex for 1 min. Appropriate amounts of the receptor were then added to the PKC reaction mixture, which was identical to the PKA reaction except for the following additions (final concentrations): 300 $\mu$M $CaCl_2$ and PKC at 3 $\mu$g/ml; PKA was omitted. The CaM kinase II assay mixture was the same as the PKA assay except that 30 $\mu$g of calmodulin per ml and 3 $\mu$g of CaM kinase II per ml were included and PKA was omitted. The concentration of the receptor in the assay was 8–10 pmol/ml. All reactions were carried out at 30°C.

**Photoaffinity Labeling with [$^3$H]Muscimol.** The $GABA_A$ receptor was affinity labeled with [$^3$H]muscimol essentially as described (19, 27). Briefly, the purified receptor was incubated with 20 nM [$^3$H]muscimol for 30 min at 4°C followed by irradiation with short-wave ultraviolet light at a distance of 10 cm for 10 min.

**Immunoblot.** The purified receptor was phosphorylated as described above and then subjected to sodium dodecyl sulfate (SDS)/polyacrylamide gel electrophoresis (PAGE) on 10% polyacrylamide gels (28). In some samples, radiolabeled ATP was excluded from the reaction. The proteins in the gel were then transferred to nitrocellulose sheets [BA 83 (0.2 mm); Schleicher & Schuell] for 8 hr at 20 V. After transfer, the lane that contained the receptor that had been phosphorylated without labeled ATP was cut from the nitrocellulose sheet and incubated in buffer A [final concentrations: 150 mM NaCl, 25 mM Tris·HCl (pH 7.4), 10 mM EDTA, 0.05% (wt/vol) sodium azide, and 0.05% (vol/vol) Tween-20 (to block nonspecific absorption)]. The strip was then exposed to antibody specific for the $\alpha$ subunit of the $GABA_A$ receptor (19) in buffer A for 2 hr. After three 20-min washes in buffer A, the strip was incubated with $^{125}$I-protein A (200,000 cpm/ml) in buffer A for 2 hr. The mobility of the polypeptide bands labeled with $^{125}$I-protein A was compared with that of the polypeptides that had been labeled with $^{32}$P.

**One-Dimensional Phosphopeptide Maps.** One-dimensional phosphopeptide analysis was performed in a slight modification of the procedure of Cleveland *et al.* (29). Phosphorylated bands were localized by autoradiography, cut from the dried gel, and subjected to liquid scintillation spectroscopy. The bands were then removed from liquid scintillation cocktail, rinsed in ether, agitated for 60 min in SDS/PAGE sample buffer, and resubjected to SDS/PAGE in a 15% polyacrylamide gel in the presence of *S. aureus* V8 protease (5 $\mu$g per lane).

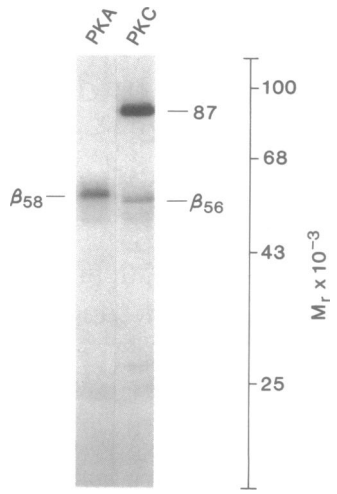


FIG. 1. Phosphorylation of the $GABA_A$ receptor by PKA and PKC. Autoradiograph depicting the pattern of incorporation of $^{32}$P into polypeptides. PKA catalyzed the phosphorylation of a polypeptide with a $M_r$ of ≈58,000 (designated $\beta_{58}$). Some incorporation of label into faster migrating polypeptides was also noted. PKC catalyzed the phosphorylation of a polypeptide with a $M_r$ of 56,000 (designated $\beta_{56}$). Some incorporation of label in polypeptides with faster mobilities was also noted. In addition, the labeling of the polypeptide labeled "87" reflects the autophosphorylation of PKC.

## RESULTS

As shown in Fig. 1, PKA and PKC catalyzed the phosphorylation of polypeptide bands in the purified $GABA_A$ receptor. The polypeptide bands phosphorylated by the two kinases exhibited differences in mobilities on SDS/PAGE. PKA phosphorylated a polypeptide band with an apparent $M_r$ of 58,000 and this polypeptide has been designated $\beta_{58}$. PKC phosphorylated a polypeptide band with an apparent $M_r$ of 56,000 and this polypeptide has been designated $\beta_{56}$. Significant levels of $^{32}$P incorporation were obtained with both kinases. In a typical experiment shown in Fig. 2, PKA catalyzed the incorporation of 0.52 mol of P/mol of muscimol binding. PKC catalyzed the incorporation of 0.38 mol of P/mol of muscimol binding. CaM kinase II did not produce detectable labeling of the receptor (not shown). However, autophosphorylation of the CaM kinase II could have obscured modest phosphorylation of the $GABA_A$ receptor.

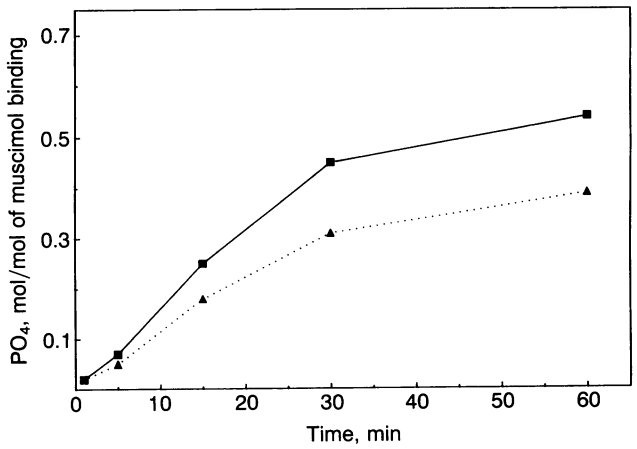


FIG. 2. Stoichiometry of phosphorylation of the $GABA_A$ receptor by PKA (■) and PKC (▲). The stoichiometry of phosphorylation obtained in the experiment depicted in Fig. 1 was calculated as the mol of P incorporation divided by the mol of muscimol binding. PKA catalyzed the incorporation of 0.52 mol of P/mol of muscimol binding. The maximal incorporation of P catalyzed by PKC was 0.38 mol/mol of muscimol binding.

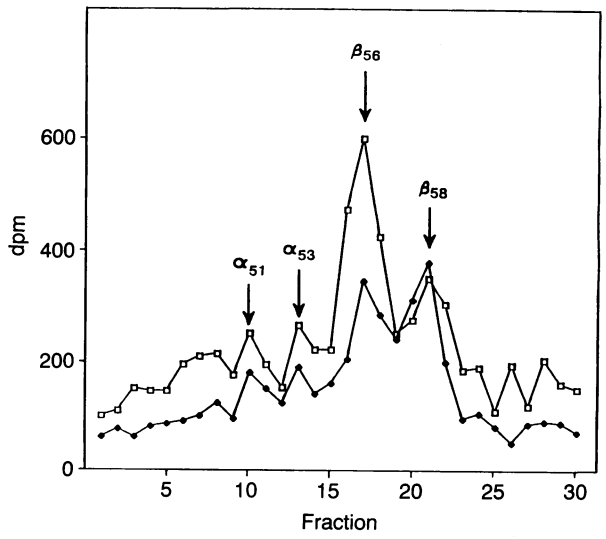


FIG. 3. Comparison of the migration in SDS/PAGE of phosphorylated and muscimol binding polypeptides. The $GABA_A$ receptor was either phosphorylated by PKC or photoaffinity labeled with [$^3$H]muscimol. The samples were then subjected to SDS/PAGE. Strips (1 mm) were then cut from the dried gel in the range of $M_r$ of 40,000–65,000. □, Incorporation of $^{32}$P in dpm. ■, Affinity labeling with [$^3$H]muscimol in dpm. Note that the peak of incorporation of PKC-catalyzed $^{32}$P incorporation comigrated with the lower molecular weight muscimol binding polypeptide $\beta_{56}$. Small amounts of $^{32}$P incorporation were found in fractions that contain the higher molecular weight binding polypeptide, $\beta_{58}$, and two lower molecular weight polypeptides, $\alpha_{53}$ and $\alpha_{51}$.

In previous studies we have shown that the rat brain $GABA_A$ receptor contains multiple muscimol binding subunits (20). These subunits have molecular weights corresponding to those for the $\beta_{58}$ and $\beta_{56}$ polypeptides described above. We compared the mobilities of the $^{32}$P-labeled polypeptides and the muscimol binding polypeptides. As shown in Fig. 3, the majority of $^{32}$P incorporation catalyzed by PKC comigrated with the lower molecular weight muscimol binding polypeptide $\beta_{56}$. The polypeptide phosphorylated by PKA comigrated with the higher molecular weight muscimol binding polypeptide (not shown).

We next compared $\beta_{58}$ and $\beta_{56}$ using the one-dimensional phosphopeptide mapping technique of Cleveland *et al.* (29).

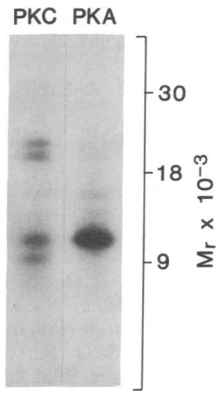


FIG. 4. One-dimensional phosphopeptide maps of the $GABA_A$ receptor $\beta$ subunits. The polypeptides phosphorylated by PKC ($\beta_{56}$) and PKA ($\beta_{58}$) were cut from a dried gel and subjected to one-dimensional phosphopeptide mapping. *S. aureus* V8 protease digestion of $\beta_{56}$ yielded four fragments with $M_r$ ranging from 24,000 to 9000. *S. aureus* V8 protease digestion of $\beta_{58}$ yielded a single prominent fragment with an $M_r$ of 11,000 and minor fragments ranging to an $M_r$ of 24,000.

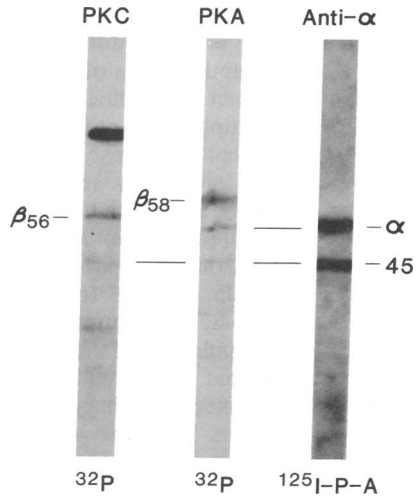


FIG. 5. Comparison of phosphorylated and immunolabeled polypeptides in the $GABA_A$ receptor. The $GABA_A$ receptor was phosphorylated by either PKC or PKA. In some cases [$\gamma$-$^{32}$P]ATP was omitted from the reaction. The phosphorylated receptor was subjected to SDS/PAGE and then transferred to nitrocellulose. A strip of nitrocellulose containing the receptor that had been phosphorylated in the absence of [$\gamma$-$^{32}$P]ATP was prepared and then subjected to immunolabeling with an antibody specific for the $\alpha$ subunit of the $GABA_A$ receptor. The strip was subsequently incubated with $^{125}$I-protein A ($^{125}$I-P-A) to reveal the pattern of antibody labeling. The left lane shows the migration pattern of polypeptides phosphorylated by PKC. The middle lane shows the pattern of migration of polypeptides phosphorylated by PKA. The right lane shows the pattern of migration of polypeptides immunolabeled by the antibody. Two polypeptides, $\alpha_{51}$ ($M_r$ 51,000) and $\alpha_{45}$ ($M_r$ 45,000), show significant immunolabeling. PKC catalyzes slight phosphorylation of a polypeptide that migrates similarly to $\alpha_{51}$. PKA catalyzes the phosphorylation of polypeptides that migrate similarly to $\alpha_{51}$ and $\alpha_{45}$.

Limited digestion of $\beta_{58}$, which had been phosphorylated by PKA, yielded a prominent phosphopeptide with an $M_r$ of ≈11,000 (Fig. 4). Minor fragments with $M_r$ ranging to 24,000 were also detected. Limited digestion of $\beta_{56}$, which had been phosphorylated by PKC, yielded four fragments ($M_r$ 24,000, 22,000, 11,000, and 9000) with approximately equal labeling with $^{32}$P.

The $GABA_A$ receptor purified from rat brain has been shown to contain one or more $\alpha$ subunits that can be photoaffinity labeled with [$^3$H]flunitrazepam (19, 21, 30, 31). We examined the ability of various kinases to phosphorylate polypeptides that comigrated with the $\alpha$ subunit in an experiment shown in Fig. 5. In this experiment, we compared the mobility of polypeptides phosphorylated by PKC and PKA with polypeptides labeled with an $\alpha$-subunit-specific antibody. The antibody labeled polypeptides with $M_r$ of 51,000 (appears as a doublet in photoaffinity labeling as in Fig. 3) and 45,000. As shown in Fig. 5, PKA phosphorylated two polypeptides that had mobilities comparable to the polypeptides labeled with the $\alpha$-subunit-specific antibody. However, the incorporation of $^{32}$P into these polypeptides was significantly less than the incorporation into $\beta_{58}$. Similarly, PKC catalyzed the incorporation of a small amount of $^{32}$P into a polypeptide that comigrated with the $M_r$ 45,000 polypeptide labeled by the $\alpha$-subunit antibody.

## DISCUSSION

We demonstrate here that PKC and PKA are capable of phosphorylating the $GABA_A$ receptor purified from rat brain. Both enzymes catalyzed significant incorporation of $^{32}$P into polypeptides that comigrated in SDS/PAGE with muscimol binding polypeptides. The polypeptides phosphorylated by PKC ($\beta_{56}$) and PKA ($\beta_{58}$) appear to be distinct. This conclu-

sion is based on two sets of data. First, the migration of polypeptides in SDS/PAGE is clearly different. Second, the one-dimensional phosphopeptide maps of the two polypeptides are also clearly distinct. A number of authors have proposed that there are multiple forms of the $GABA_A$ receptor (16–18, 30–34). Consistent with these reports of heterogeneity, we have recently shown by several techniques, including mobility on SDS/PAGE, photoaffinity labeling, immunostaining, peptide mapping, regional distribution, and pharmacological properties, that there are multiple forms of the $\alpha$ (benzodiazepine binding) and $\beta$ (muscimol binding) subunits of the receptor (20–22). The current results support the existence of different populations of the $GABA_A$ receptor and raise the possibility that they could be regulated by different protein kinases. Since phosphorylation and photoaffinity labeled polypeptides have the same mobility on SDS/PAGE, it is unlikely that phosphorylation is responsible for the different mobilities. However, caution must be exercised in hypotheses based on comigration on SDS/PAGE. Moreover, the differing phosphopeptide maps could merely reflect widely divergent sites of phosphorylation for the two kinases. It should also be noted that high concentrations of kinase were used in the *in vitro* phosphorylation reported here. Thus, it will be important to demonstrate that the receptor is phosphorylated in intact nerve cell preparations.

Our demonstration of PKA phosphorylation of a polypeptide in the rat brain $GABA_A$ receptor that comigrates with a muscimol binding polypeptide confirms work by Kirkness *et al.* (15), who have shown PKA phosphorylation of a muscimol binding polypeptide in the $GABA_A$ receptor purified from pig brain. Our demonstration that PKC phosphorylates an apparently distinct muscimol binding polypeptide raises the possibility that both of these kinases could modulate the receptor by way of its muscimol binding subunit. However, once again, caution should be exercised in interpreting comigration on SDS/PAGE. Indeed, recent reports have suggested that some $\alpha$- and $\gamma$-subunit subtypes of the receptor may have molecular weights similar to $\beta_{58}$ and $\beta_{56}$ (18, 21). Immunoprecipitation experiments with antibodies specific for the $\beta$ subunits should help to resolve this issue.

A final question concerns the physiological consequences of phosphorylation for $GABA_A$ function. We have not addressed that question in our studies. However, we feel that the parallels between the phosphorylation we have observed for the $GABA_A$ receptor and that seen for the AcChoR are quite interesting. We have shown here that the $GABA_A$ receptor can be phosphorylated by PKA and PKC but not by the multifunctional CaM kinase II. Similarly, the AcChoR is phosphorylated by PKA and PKC but not by CaM kinase II (6, 8). This phosphorylation of the AcChoR has been shown to have the physiological consequence of increasing the rate of desensitization of the receptor (3). Given the structural similarities between the AcChoR and the $GABA_A$ receptor (1, 2), it is tempting to hypothesize that PKA and PKC phosphorylation may also lead to desensitization of the $GABA_A$ receptor. Indeed, support for this hypothesis has already appeared in studies using PKC and PKA activators (9, 10). However, it should be noted that other reports indicate that phosphorylation may be involved in the *maintenance* of $GABA_A$ receptor function (12, 13).

In sum, the data presented here raise the exciting possibility that multiple protein kinases may regulate multiple forms of the $GABA_A$ receptor. Given the critical importance of this receptor for inhibitory neurotransmission in the central nervous system such regulation could have extremely important consequences for neuronal function.

We thank R. W. Ransom for help in preparing the antibody to the $\alpha$ subunit of the $GABA_A$ receptor. This research was supported by National Institutes of Health Grants NS 26377 and DK 40483 to M.D.B. and NS 22071 to R.W.O.

1. Maelicke, A. (1988) *Trends Biochem. Sci.* **13,** 199–202.
2. Schofield, P. R., Darlison, M. G., Fujita, N., Burt, D. R., Stephenson, F. A., Rodriguez, H., Rhee, L. M., Ramachandron, J., Reale, V., Glencorse, T. A., Seeburg, P. H. & Barnard, E. A. (1987) *Nature (London)* **328,** 221–223.
3. Huganir, R. L., Delcour, A. H., Greengard, P. & Hess, G. P. (1986) *Nature (London)* **321,** 774–776.
4. Hopfield, J. F., Tank, D. W., Greengard, P. & Huganir, R. L. (1988) *Nature (London)* **336,** 677–680.
5. Eusebi, F., Molinaro, M. & Zani, B. M. (1985) *J. Cell Biol.* **100,** 1339–1342.
6. Huganir, R. L. & Greengard, P. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 1130–1134.
7. Safran, A., Sagi-Eisenberg, R., Neumann, D. & Fuchs, S. (1987) *J. Biol. Chem.* **262,** 10506–10510.
8. Huganir, R. (1987) in *Neuromodulation,* eds. Kaczmarek, L. & Levitan, I. (Oxford Univ. Press, Oxford), pp. 86–99.
9. Sigel, E. & Baur, R. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 6192–6196.
10. Heuschneider, G. & Schwartz, R. D. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 2938–2942.
11. Tehrani, M. H. J., Hablitz, J. J. & Barnes, E. M., Jr. (1989) *Synapse* **4,** 126–131.
12. Stelzer, A., Kay, A. R. & Wong, R. K. S. (1988) *Science* **241,** 339–341.
13. Gyenes, M., Farrant, M. & Farb, D. H. (1988) *Mol. Pharmacol.* **34,** 719–723.
14. Sweetnam, P. M., Lloyd, J., Gallombardo, P., Malison, R. T., Gallager, D. W., Tallman, J. F. & Nestler, E. J. (1988) *J. Neurochem.* **51,** 1274–1284.
15. Kirkness, E. F., Bovenkerk, C. F., Ueda, T. & Turner, A. J. (1989) *Biochem. J.* **259,** 613–616.
16. Stutchfield, J. & Cockcroft, S. (1988) *Biochem. Soc. Trans.* **16,** 994–995.
17. Ymer, S., Schofield, P. R., Draguhn, A., Werner, P., Kohler, M. & Seeburg, P. H. (1989) *EMBO J.* **8,** 1665–1670.
18. Pritchett, D. B., Sontheimer, H., Shivers, B. D., Yner, S., Kettenman, H., Schofield, P. R. & Seeburg, P. H. (1989) *Nature (London)* **338,** 582–585.
19. Stauber, G. B., Ransom, R. W., Dilber, A. I. & Olsen, R. W. (1987) *Eur. J. Biochem.* **167,** 125–133.
20. Olsen, R. W., Bureau, M., Ransom, R. W., Deng, L., Dilber, A., Smith, G., Khrestchatisky, M. & Tobin, A. J. (1988) in *Neuroreceptors and Signal Transduction,* eds. Kito, S., Segawa, T., Kuriyama, K., Tohyama, M. & Olsen, R. W. (Plenum, New York), pp. 1–14.
21. Bureau, M. & Olsen, R. W. (1988) *Biochem. Biophys. Res. Commun.* **153,** 1002–1011.
22. Olsen, R. W., Bureau, M., Khrestchatisky, M., MacLennan, A. J., Chiang, M. Y., Tobin, A. J., Xu, M., Jackson, M., Sternini, C. & Brecha, N. (1989) in *GABA and Benzodiazepine Receptor Subtypes,* eds. Biggio, G. & Costa, E. (Raven, New York), in press.
23. Beavo, J. A., Bechtel, P. J. & Krebs, E. G. (1974) *Methods Enzymol.* **38,** 299–308.
24. Woodgett J. R. & Hunter, T. (1987) *J. Biol. Chem.* **262,** 4836–4843.
25. McGuinness, T. L., Lai, Y. & Greengard, P. (1985) *J. Biol. Chem.* **260,** 1696–1704.
26. Hannun, Y. A., Loomis, C. R. & Bell, R. M. (1985) *J. Biol. Chem.* **260,** 10039–10043.
27. Deng, L., Ransom, R. W. & Olsen, R. W. (1986) *Biochem. Biophys. Res. Commun.* **138,** 1308–1314.
28. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
29. Cleveland, D. W., Fischer, S. G., Kirschner, M. W. & Laemmli, U. K. (1977) *J. Biol. Chem.* **252,** 1102–1106.
30. Fuchs, K. & Sieghart, W. (1989) *Neurosci. Lett.* **97,** 329–333.
31. Stephenson, F. A., Duggan, M. J. & Casalotti, S. O. (1989) *FEBS Lett.* **243,** 358–362.
32. Sato, T. N. & Neale, J. H. (1989) *J. Neurochem.* **52,** 1114–1122.
33. Braestrup, C. & Nielsen, M. J. (1981) *J. Neurochem.* **37,** 333–341.
34. Lippa, A. S., Beer, B., Sano, M. C., Vogel, R. A. & Myerson, L. R. (1981) *Life Sci.* **28,** 2343–2347.