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A G Protein Couples Serotonin and $GABA_B$ Receptors to the Same Channels in Hippocampus

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Both serotonin and the selective γ-aminobutyric acid$_B$ ($GABA_B$) agonist, baclofen, increase potassium (K^+) conductance in hippocampal pyramidal cells. Although these agonists act on separate receptors, the potassium currents evoked by the agonists are not additive, indicating that the two receptors share the same potassium channels. Experiments with hydrolysis-resistant guanosine triphosphate (GTP) and guanosine diphosphate analogs and pertussis toxin indicate that the opening of the potassium channels by serotonin and $GABA_B$ receptors involves a pertussis toxin–sensitive GTP-binding (G) protein, which may directly couple the two receptors to the potassium channel.

MEMBRANE RECEPTORS OFTEN signal cellular responses via second messenger systems. One ubiquitous feature of such indirect coupling systems is their dependence on guanosine triphosphate (GTP)–binding proteins (G proteins) for the transmembrane signaling process. A number of distinct G proteins have been identified and some of the intracellular second messengers and effectors associated with their actions elucidated [for review, see (*1*)]. A great deal is now known about the role of these G proteins and assorted second messengers in controlling metabolic and secretory processes in a variety of cells. G proteins are present in extraordinarily high concentrations in the mammalian central nervous system (CNS) (*2*); for example, G_O accounts for 1% of membrane protein. Moreover, on the basis of the GTP sensitivity of the receptor binding of neurotransmitter agonists (*3, 4*) it is believed that G proteins are associated with many types of brain neurotransmitter receptors. However, little is known about the functional roles these G proteins play in controlling neuronal excitability and, hence neuronal communication in the CNS. Here we demonstrate that serotonin (5-HT) and γ-aminobutyric acid$_B$ ($GABA_B$) receptors modulate the same potassium channels through a mechanism that involves a pertussis toxin–sensitive G protein. We suggest, as has been proposed for muscarinic inhibition of the heart (*5*), that G proteins may directly couple receptors to the K^+ channels in mammalian brain.

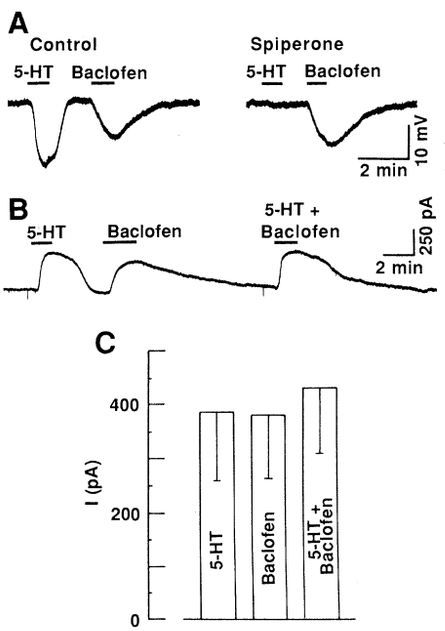

Fig. 1. 5-HT and baclofen hyperpolarize pyramidal cells by separate receptors but through a common conductance mechanism. (A) The hyperpolarization to 5-HT (10 μ*M*), but not to baclofen (10 μ*M*), is abolished by application of spiperone (5 μ*M*) for 20 minutes. The drugs were added to the superfusion medium and were applied by switching the inflow to the drug-containing medium. The resting membrane potential was −58 mV. (B) Continuous voltage clamp record of the outward currents evoked by 5-HT (30 μ*M*) and baclofen (30 μ*M*) applied in the bath alone or together. Note that the response evoked by the combined application of the two drugs is no larger than that evoked by 5-HT alone. (C) Bar graph of responses, such as those in (B), recorded from five cells. The bracket represents the standard deviation for the responses.

The methods we have used here are similar to those used in other studies from our laboratory (*6*). Rat hippocampal slices, 400-μm thick, were cut and placed in a holding chamber for at least 1 hour. A single slice was then transferred to the recording chamber and held between two nylon nets, submerged beneath a continuously superfusing medium that had been gassed with 95% O_2 and 5% CO_2. The composition of this medium was 119 m*M* NaCl, 2.5 m*M* KCl, 1.3 m*M* $MgSO_4$, 2.5 m*M* $CaCl_2$, 1.0 m*M* NaH_2PO_4, 26.2 m*M* $NaHCO_3$, and 11.0 m*M* glucose. The temperature of the medium was maintained between 29 and 31°C.

Application of either 5-HT (*7*) or baclofen (*8*), a $GABA_B$ agonist (*9*), to CA1 pyramidal cells, causes large hyperpolarizations, which are due to a selective increase in K^+ conductance; neither of the responses desensitizes with repeated applications. Although both compounds increase K^+ conductance, they do so via distinct receptors. There are no antagonists for $GABA_B$ receptors, but spiperone blocks responses to 5-HT, which are mediated by a 5-HT1a receptor (*10*). Concentrations of spiperone that abolish responses to 5-HT have no effect on responses to baclofen (Fig. 1A), indicating that these two agonists act through different receptors. If the increase in K^+ conductance evoked by these two receptors resulted from entirely separate mechanisms one would expect that the maximal currents generated by the two agonists would summate. This is not the case (Fig. 1, B and C). When near maximal concentrations of 5-HT and baclofen are applied together, the current is no larger than that produced by 5-HT alone. The results of five experiments are summarized in Fig. 1C, and indicate that the size of the maximal responses to the two agonists are similar and that there is no additivity between the currents activated by the two receptors. These findings indicate that 5-HT and baclofen act on different receptors, but control the same conductance.

5-HT1a agonists (*11*) and baclofen (*12*) inhibit adenylate cyclase. It is therefore pos-

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Table 1. Effect of blocking and activating G proteins on hippocampal pyramidal cells. All values are mean ± SD. The number of cells for each observation is in parentheses. The responses to 30 μ*M* serotonin (5-HT) or baclofen (BACLO) were measured from the resting membrane potential (E_m) to the peak of the response. The input resistance (R_{in}) was measured with a 60- to 100-msec current pulse that hyperpolarized the membrane by 5 to 10 mV. The action potential height (AP) was measured from the resting membrane potential to the peak of the action potential. The values obtained from the sham operated animals were no different from control. The *P* values for the results obtained in pertussis toxin (PT)–treated animals are derived by comparison to sham-operated animals. The *P* values for results with GTPγS and GDPβS are derived by comparison to control results.

Parameter	Treatment				
	Control	Sham	PT	GTPγS	GDPβS
5-HT (mV)	−13.0 ± 3.4 (14)	−12.7 ± 3.0 (15)	−0.5 ± 3.1* (27)	−0.1 ± 0.9* (6)	−6.6 ± 2.0* (7)
BACLO (mV)	−12.8 ± 1.3 (12)	−12.5 ± 2.8 (13)	−1.1 ± 2.5* (23)	0.0 ± 0.0* (6)	−7.2 ± 3.4* (7)
E_m (mV)	−61.2 ± 4.2 (17)	−60.3 ± 2.6 (13)	−61.1 ± 3.5 (29)	−78.2 ± 4.4* (6)	−62.0 ± 3.6 (14)
R_{in} (Mohm)	64 ± 14 (14)	62 ± 13 (14)	69 ± 14 (29)	36 ± 9* (14)	62 ± 14 (14)
AP (mV)	88 ± 5 (13)	92 ± 6 (14)	87 ± 7 (27)	103 ± 2* (6)	91 ± 5 (14)

*$P < 0.001$.

sible that negative coupling to adenylate cyclase might be the common step in the signaling process at these two receptors. Because an elevation of intracellular adenosine 3′,5′-monophosphate (cAMP) is known to block the Ca^{2+}-activated K^+ current (I_{AHP}) that underlies the slow afterhyperpolarization (AHP) in these cells (*13*), one might expect that an inhibition of adenylate cyclase would increase I_{AHP} and therefore hyperpolarize the cell if the basal level of cAMP were high enough to block a persistent I_{AHP}. If such a mechanism existed, exogenous administration of cAMP should prevent the action of the agonists because inhibition of adenylate cyclase would then be unable to reduce intracellular levels of cAMP. Neither bath application of the membrane-soluble analog of cAMP, 8-bromo-cAMP (8-Br-cAMP) (Fig. 2), nor the intracellular injection of cAMP from electrodes containing 100 m*M* cAMP reduced the response to 5-HT or to baclofen, although the Ca^{2+}-dependent slow AHP was rapidly blocked in these experiments. These results exclude the possibility that a decrease in cAMP could signal the responses to 5-HT or baclofen.

We next considered the possibility that the responses might be mediated by the release of intracellular Ca^{2+} and subsequent activation of K^+ channels, as has been shown in exocrine cells for a number of transmitters (*14*). Although the results shown in Fig. 2 exclude a role for Ca^{2+} activation of I_{AHP} in the responses, in these cells intracellular Ca^{2+} can activate another K^+ current, I_C (*15*). To test the involvement of I_C, we examined the sensitivity of the 5-HT and baclofen responses to bath applied tetraethylammonium chloride (TEA). I_C is very sensitive to TEA (*15–17*); 1 m*M* TEA produces a 50% block in sympathetic ganglion cells (*16*). Application of 5 m*M* TEA, which is known to block I_C in hippocampal pyramidal neurons (*15*), caused marked broadening of the action potential (Fig. $3A_2$). However, TEA at this concentration did not reduce the 5-HT or baclofen response (Fig. $3A_1$). Another approach to determine whether Ca^{2+} is involved in the 5-HT and baclofen response was to inject the Ca^{2+} chelator, EGTA, into the cells. Under this condition, 5-HT (30 μ*M*) or baclofen (30 μ*M*) produced responses that were of equal amplitude to those recorded from control cells (Fig. $3B_1$, Table 1), even though the Ca^{2+}-dependent AHP (Fig. $3B_2$) and spike frequency adaptation (Fig. $3B_3$) were blocked 8 minutes before the application of agonists. In the four cells tested, the average size of the 5-HT response was 11.1 ± 2.9 mV and that of baclofen was 11.0 ± 2.9 mV (mean ± SD used throughout). These values, as well as the values for membrane potential, input resistance, and spike height, did not differ from control values (Table 1). These results make it most unlikely that the increase in K^+ current evoked by 5-HT and baclofen is mediated by an increase in intracellular Ca^{2+} (*18*).

Although we have been unable to find a second messenger system that could account for the responses to 5-HT and baclofen, a number of neurochemical studies indicate that the binding of agonists to both receptor types is sensitive to GTP (*4*), suggesting that a G protein may be involved in their actions. To address this issue, we examined the effects of pertussis toxin, which adenine diphosphate ribosylates and inactivates some G proteins, on the 5-HT and baclofen response (*19*). Intraventricular injection of pertussis toxin 3 days before the acute experiment had dramatic effects, either greatly attenuating or eliminating the responses (*20*). Typical responses from an animal that had been injected with vehicle plus 1.5 μg of bovine serum albumin are shown in Fig. 4A. The responses were no different than those in slices obtained from control rats (Table 1). In contrast, neither 5-HT nor baclofen had any effect on the membrane potential or input resistance of a cell recorded in a slice

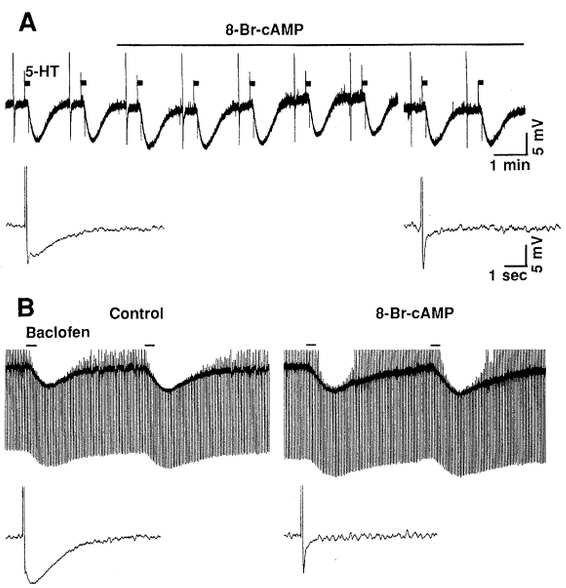

Fig. 2. The 5-HT and baclofen responses do not involve negative coupling to adenylate cyclase. (A) 5-HT was iontophoresed (squares) at regular intervals as 8-Br-cAMP (1 m*M*) was applied via the bath. The cell depolarized slightly, but there was little change in the 5-HT responses, even when the membrane potential was returned to its control value with steady hyperpolarizing current. The lower traces show that the slow AHP after a series of action potentials is blocked by 8-Br-cAMP. For iontophoresis, broken pipettes (tip diameter, 2 to 6 μm), filled with 5-HT (40 m*M*, *p*H 4), were positioned immediately above the surface of the slice approximately 50 μm from the pyramidal cell layer in stratum radiatum. The resting membrane potential was −60 mV. (B) The responses to iontophoretically applied baclofen are not decreased by bath-applied (15 minutes) 8-Br-cAMP. The iontophoretic electrode was filled with 20 m*M* baclofen at *p*H 3 and was positioned in a manner identical to the 5-HT–containing electrode in (A). The resting membrane potential was −56 mV. The calibration in (A) for the drug responses and AHP's also apply to (B).

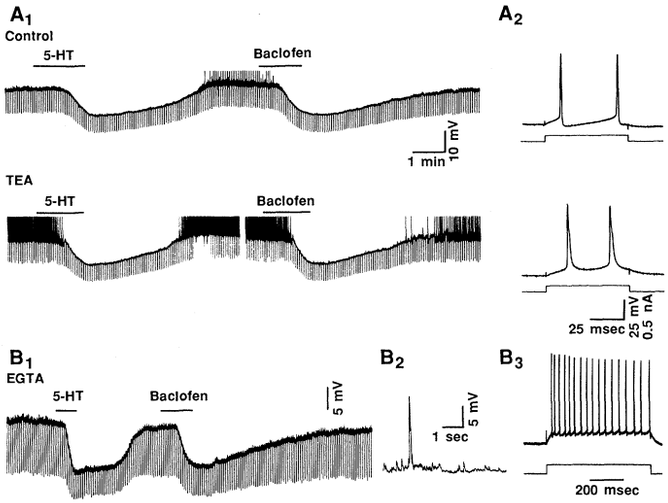

Fig. 3 (left). The 5-HT and baclofen responses do not involve a calcium-activated potassium conductance. (A) 5-HT (30 μM) and baclofen (30 μM) were applied in the superfusion medium. Application of TEA for 15 minutes caused distinct broadening of the action potential evoked by a depolarizing current pulse (A_2) but did not reduce the drug responses ($n = 5$). The resting membrane potential was −57 mV. (B) Bath application of 5-HT (30 μM) and baclofen (30 μM) produced normal sized responses in a neuron injected with the Ca^{2+} chelator EGTA. (B_2) shows that the EGTA blocked the slow AHP and (B_3) shows that spike frequency adaptation is severely reduced in this cell. The resting membrane potential was −64 mV. To load cells with EGTA the recording electrode was filled with 0.2M EGTA in 2M $KMeSO_4$. The EGTA diffuses from these electrodes and quickly blocks the Ca^{2+}-dependent slow I_{AHP}'s. The time calibration for the drug responses in (A_1) also applies to the drug responses in (B_1). The gain for the responses in (B_1) is twice that for (A_1). The current and voltage calibration in (A_2) also applies to (B_3).

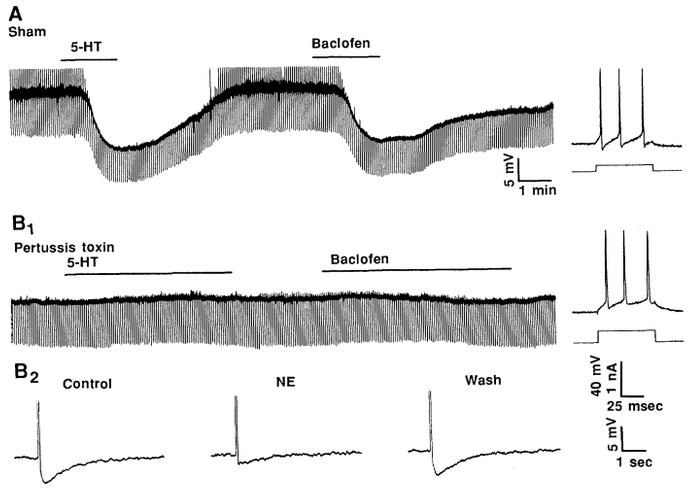

Fig. 4 (right). Pertussis toxin blocks the actions of 5-HT and baclofen. (A) Pyramidal cell responses to bath applied 5-HT (30 μM) and baclofen (30 μM) are shown in a cell from a hippocampal slice taken from an animal sham injected with bovine serum albumin (1.5 μg) (Sham). Downward deflections are hyperpolarizing responses to constant current pulses. The resting membrane potential was −60 mV. (B_1) 5-HT and baclofen responses from a slice taken from an animal injected with pertussis toxin (1.5 μg). The action potentials recorded from the two cells are shown to the right. (B_2) shows that the same cell, which did not respond to 5-HT or baclofen, responds normally to 5 μM norepinephrine (NE) with a decrease in the size of the AHP (*13*). The resting membrane potential was −60 mV. Calibrations below (A) apply to the 5-HT and baclofen responses in (A) and (B_1). Calibrations for the action potentials are below the spikes in (B_1). Calibrations for the AHP's are to the right of (B_2).

from a rat that had been injected with 1.5 μg of pertussis toxin 3 days before the acute experiment (Fig. 3B), even though the agonists were applied for a much longer time. In every other respect [resting membrane potential, input resistance, and action potential height (Fig. 4 and Table 1)] the cells were normal. In addition, the cells exhibited a normal AHP, which results from an activation of K^+ conductance triggered by the influx of Ca^{2+} (Fig. 4B_2) and a normal response to norepinephrine (Fig. 4B_2), which reduces the AHP by increasing cAMP via G_S (*13*).

Further support for the involvement of a G protein came from experiments in which hydrolysis-resistant guanine nucleotides were injected into pyramidal cells. Guanosine 5′-*O*-(2-thiophosphate) (GDPβS) is known to bind to G proteins and inhibit the binding of GTP, thus blocking GTP-dependent activation of G proteins (*21, 22*). When cells were recorded with electrodes containing GDPβS (33 mM), responses to 5-HT and baclofen were clearly reduced (Fig. 5A and Table 1) (*23*). Cells in the immediate vicinity from the same slice subsequently penetrated with a normal electrode gave normal sized responses (Fig. 5A). In addition, the effect of the hydrolysis-resistant analog of GTP guanosine 5′-*O*-13-thiotriphosphate (GTPγS), which binds to and activates G proteins was also examined. When cells were impaled with electrodes containing GTPγS (25 mM) they had resting membrane potentials more negative than control cells and their input resistances were about half that of control cells (Table 1). Because these membrane potentials and input resistances were similar to those obtained during a maximal response to 5-HT or baclofen, these results suggested that GTPγS fully activates the K^+ channels normally opened by these agonists. It was consistent with this possibility that application of 5-HT or baclofen had no additional effect on membrane potential or input resistance in these cells (Fig. 5B_1), even if the membrane potential was held at a more depolarized level (Fig. 5B_2). Responses from a cell in the immediate vicinity in the same slice subsequently recorded with a normal electrode gave normal sized responses at the two membrane potentials (Fig. 5, B_1 and B_2). Table 1 summarizes the results of all the experiments.

Activation of protein kinase C in human platelets phosphorylates G_i and largely blocks the action of G_i on adenylate cyclase in human platelets and cyc^-S49 lymphoma cells (*24*). As a final test for the involvement of a GTP-binding protein, we applied phorbol-12,13-dibutyrate (PDBu), an activator of protein kinase C (*25*), to determine whether it alters the responses to 5-HT and baclofen. PDBu greatly reduced the response to 5-HT and blocked the AHP (Fig. 6A_1) (*26*). No recovery was detected after returning to the control medium for 30 minutes. On the other hand, a phorbol analog that does not activate protein kinase C (*25*) had no effect on the 5-HT response or AHP (Fig. 6A_2). PDBu also greatly reduced responses to baclofen (Fig. 6B). The average reduction in the 5-HT response was 63 + 14.8% ($n = 5$) and in the baclofen response was 71.2 ± 12.4% ($n = 6$). Although these results do not exclude an action of PDBu either on the two receptors or on the K^+ channel, the results are consistent with results indicating an action on G_i.

In summary, we have determined the way in which 5-HT1a and $GABA_B$ receptors may be coupled to K^+ channels in hippocampal pyramidal cells. We have found that these two receptors share a common saturable step in their transduction mechanism. The responses in the hippocampus do not involve a change in the level of cAMP because high concentrations of exogenous cAMP do not alter the response. In addition the responses are unlikely to involve a rise in intracellular Ca^{2+} and subsequent activation of K^+ channels because neither pharmacological blockade of the two Ca^{2+}-activated K^+ currents in these cells nor intracellular

injection of the Ca^{2+} chelator, EGTA, reduced these responses. Our experiments also suggest that it is unlikely that the breakdown of inositol phospholipids (*27, 28*) mediates the transmembrane signaling of these receptors. First, activation of protein kinase C fails to mimic the effects of 5-HT and baclofen (Fig. 6) (*26*). Second, inositol trisphosphate appears to act in a number of systems by releasing Ca^{2+} from intracellular storage sites (*28*), but we have ruled out a role for Ca^{2+} in signaling these responses. These results are in agreement with neurochemical studies that have failed to link 5-HT1a or $GABA_B$ receptors to inositol phospholipid turnover.

Although our experiments rule out these second messenger systems, they clearly indicate that a G protein is required for the receptor-mediated increase in K^+ conductance. Thus the responses were reduced by the intracellular injection of GDPβS, which is known to block the action of G proteins, and by the intraventricular injection of pertussis toxin 3 days before the acute experiment. In contrast to these manipulations, intracellular injection of the hydrolysis-resistant GTP analog, GTPγS, which activates G proteins, mimicked the effects of 5-HT and baclofen and appeared to occlude their actions. Finally, a phorbol ester, which has been shown to phosphorylate and suppress G_i, markedly reduced the responses. Because the opening of K^+ channels by 5-HT1a and $GABA_B$ receptor activation involves a pertussis toxin–sensitive G protein, but not any of the known second messenger systems associated with this family of proteins, we favor the mechanism recently proposed for muscarinic inhibition of heart cells (*5*) in which the G protein may directly couple the receptor to the K^+ channel.

Although G proteins are fundamental components in the transduction mechanism of many receptors, their presence in CNS neurons also provides a means for the convergence of a variety of receptors onto the same ionic channel. Thus our results suggest that in the hippocampus, receptors activated by a diffuse afferent fiber system (5-HT) share the same K^+ channels as receptors activated by local inhibitory interneurons (GABA). Similarly, in locus coeruleus neurons, α_2 adrenoreceptors and opiate receptors also share a K^+ conductance (*29, 30*), although this transmembrane signaling mechanism may involve adenylate cyclase inhibition (*29, 31*). Finally, in addition to providing a mechanism for the convergence of multiple receptors onto the same ion channel, the finding that a G protein is required in the coupling of $GABA_B$ receptors to Ca^{2+} channels in dorsal root ganglion cells (*22*) suggests that G proteins might allow for coupling the same receptor to different ion channels.

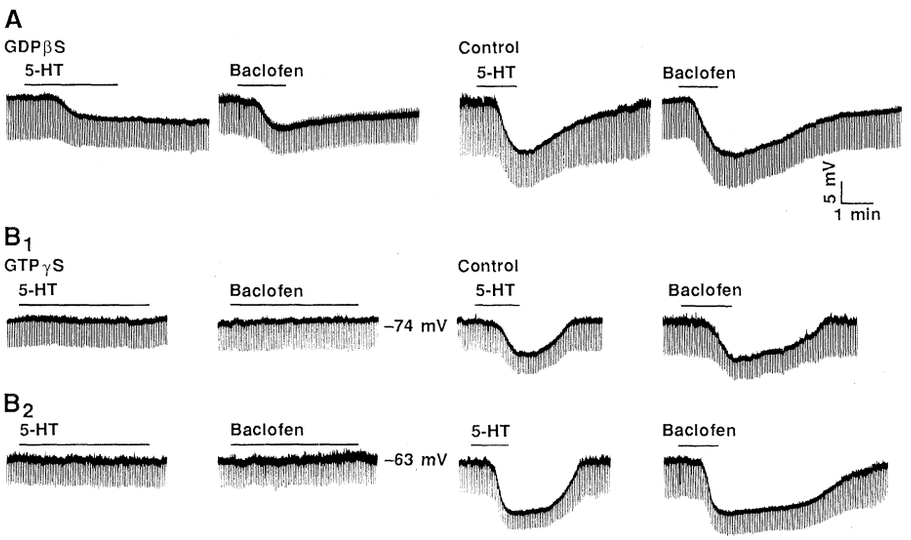

Fig. 5. Intracellular injections of hydrolysis-resistant GDP and GTP analogs reduce or mimic, respectively, the responses to 5-HT and baclofen. (A) 5-HT and baclofen responses are shown from a cell recorded with an electrode filled with 33 m*M* GDPβS. The responses are reduced and because they often did not fully recover, only one of the agonists (baclofen or 5-HT) was applied to each cell. The resting membrane potentials were −63 mV (5-HT) and −62 mV (baclofen). The control response was recorded with a normal electrode from a cell in the same slice using the same drug solutions. The resting membrane potentials were −58 mV (5-HT) and −62 mV (baclofen). (B) Drug responses are shown from a cell recorded with an electrode containing 25 m*M* GTPγS. The membrane potential was depolarized from its resting value of −74 mV (B_1) to −63 mV (B_2) with direct current injection to examine whether an increase in driving force might permit the expression of a 5-HT or baclofen response. Again the control responses are recorded with a normal electrode from a cell in the same slice. Large 5-HT and baclofen responses could be obtained from this cell even when its membrane potential was hyperpolarized from its resting value of −63 mV (B_2) to −74 mV (B_1).

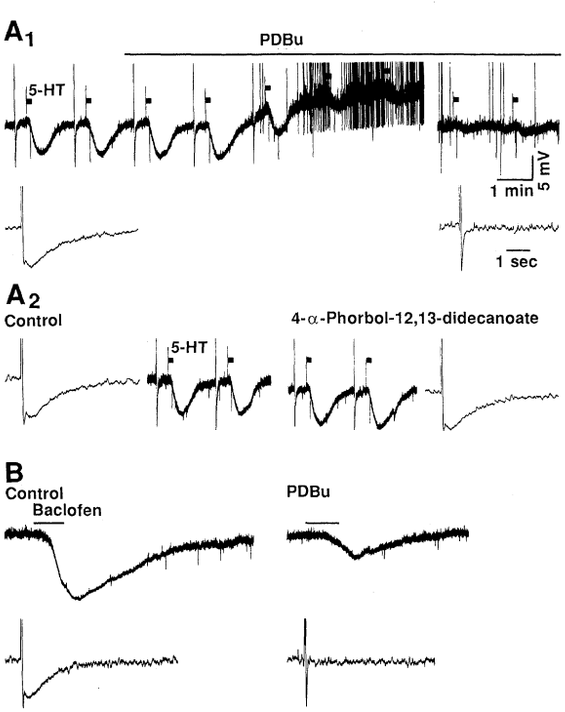

Fig. 6. Phorbol-12,13-dibutyrate (PDBu), an activator of protein kinase C, reduces the responses to 5-HT and baclofen. (A_1) 5-HT was iontophoretically applied (squares) to a pyramidal cell at regular intervals as 5 μ*M* PDBu was bath-applied. The cell depolarized slightly as the 5-HT responses were reduced. The 5-HT responses remained reduced after the membrane potential was returned to its control value. As previously reported, PDBu completely blocked the slow AHP (*26*). The resting membrane potential was −60 mV. (A_2) A phorbol analog that does not activate protein kinase C, 4α-phorbol-12,13-didecanoate, which was applied to the same cell as in (A), but prior to the application of PDBu, has no effect on either the 5-HT responses or the slow AHP. (B) Baclofen was bath-applied before and after application of 5 μ*M* PDBu. As would be expected from (A), both the responses to baclofen and the slow AHP were reduced. The resting membrane potential was −56 mV.

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Regulation of Pro-opiomelanocortin Gene Transcription in Individual Cell Nuclei

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A nonrepetitive complementary RNA probe specific for an intervening sequence of the rat pro-opiomelanocortin (POMC) gene primary transcript was used to analyze the hormonal regulation of POMC gene transcription in individual cell nuclei in the rat pituitary by in situ hybridization. This probe recognized only full-length POMC heterogeneous nuclear RNA, as verified by Northern blots of pituitary RNA. When pituitary sections were hybridized with this ^{3}H-labeled POMC intron A probe, silver grains were predominantly localized over the nuclei of cells that expressed POMC in the anterior and intermediate lobes. Adrenalectomy increased both the average grain density over corticotroph nuclei and the number of cells in the anterior pituitary with significant numbers of silver grains over their nucleus. Dexamethasone administration to intact or adrenalectomized rats results in the rapid (within 30 minutes) disappearance of silver grains over the nuclei of corticotrophs in the anterior lobe, suggesting that POMC gene transcription had been inhibited. However, adrenalectomy or dexamethasone administration did not alter the silver grain density over nuclei of intermediate lobe melanotrophs. Thus, this in situ hybridization assay utilizing an intervening sequence–specific POMC probe can measure rapid physiological changes in POMC heterogeneous nuclear RNA in individual cell nuclei.

THE PRO-OPIOMELANOCORTIN GENE and its peptide products, including adrenocorticotrophic hormone (ACTH), β-endorphin, and the melanotropins, are subject to tissue-specific differential regulation of peptide secretion and gene expression (*1*). For example, in anterior lobe corticotrophs (*2*), but not in intermediate lobe melanotrophs (*2*), ACTH is one of the primary end products of proteolytic process-

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