Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 7810–7814, October 1988
Neurobiology

Role of two different guanine nucleotide-binding proteins in the antagonistic modulation of the S-type K^+ channel by cAMP and arachidonic acid metabolites in *Aplysia* sensory neurons

(signal transduction/second messengers/channel modulation)

A. VOLTERRA AND S. A. SIEGELBAUM

Department of Pharmacology, Center for Neurobiology and Behavior, Howard Hughes Medical Institute, Columbia University, 630 West 168 Street, New York, NY 10032

Communicated by Eric R. Kandel, June 29, 1988

ABSTRACT **The role of guanine nucleotide-binding proteins (G proteins) in the cAMP-dependent action of serotonin (5-HT) and the antagonistic action of the neuropeptide Phe-Met-Arg-Phe-NH_2 (FMRF-amide), mediated by the lipoxygenase metabolites of arachidonic acid, was investigated in *Aplysia* sensory neurons. Intracellular injection of guanosine 5′-[γ-thio]triphosphate (GTP[γ-S]) mimics the hyperpolarizing action of FMRF-amide due to activation of the S K^+ current and alters the transient response to FMRF-amide into an irreversible (or only partially reversible) response. At higher concentrations, GTP[γ-S] occludes the response to FMRF-amide. Injection of activated pertussis toxin inhibits the response to FMRF-amide but not to 5-HT. Injection of guanosine 5′-[β-thio]diphosphate inhibits the response to FMRF-amide by ≈50% and completely blocks the response to 5-HT. Three lines of evidence suggest that the FMRF-amide-activated G protein is involved at an early stage of the arachidonic acid cascade, prior to the release of arachidonate. (*i*) Pertussis toxin injection blocks the hyperpolarizing response to FMRF-amide but not to exogenously applied arachidonic acid. (*ii*) Two blockers of the arachidonic acid cascade inhibit the hyperpolarizing responses to both FMRF-amide and GTP[γ-S] (and unmask a 5-HT-like depolarizing response to the nucleotide). (*iii*) Concentrations of GTP[γ-S] that alter the kinetics of the FMRF-amide response have no effect on the hyperpolarizing response to arachidonic acid. We conclude that a pertussis toxin-sensitive G protein most likely acts to couple the FMRF-amide receptor to phospholipase activation and arachidonic acid release, whereas a pertussis toxin-insensitive G protein couples the 5-HT receptor to adenylate cyclase.**

Many neurotransmitter actions are mediated through a family of guanine nucleotide-binding proteins (G proteins; see refs. 1 and 2 for review). So far, transmitters have been shown to stimulate or inhibit adenylate cyclase activity through the G proteins G_s and G_i, respectively, and to activate phospholipase C and control K^+ channel activity through unidentified G proteins. Are other neurotransmitter actions regulated by G proteins coupled to different effector systems? Can one transmitter antagonize the action of a second transmitter other than through an inhibitory G_i-like mechanism? To address these questions, we have studied the role of G proteins in the antagonistic responses to serotonin (5-HT) and Phe-Met-Arg-Phe-NH_2 (FMRF-amide) in *Aplysia* sensory neurons.

Recently, we have shown that a single K^+ channel in *Aplysia* sensory neurons (the S channel) is modulated in opposing directions by 5-HT, acting through cAMP-dependent protein phosphorylation, and FMRF-amide, acting through lipoxygenase metabolites of arachidonic acid (3, 4). 5-HT produces prolonged all-or-none closures of the S channel, which result in a slow depolarization, an increase in action potential duration, and an increase in transmitter release from the sensory neuron terminals. FMRF-amide, by contrast, increases the probability that an S channel is open, which leads to a hyperpolarization, a decrease in action potential duration, and a decrease in transmitter release. FMRF-amide also antagonizes the effects of 5-HT or cAMP by reopening S channels closed by these agents.

How is the binding of FMRF-amide to its membrane receptor coupled to the activation of phospholipase and release of arachidonic acid? How do lipoxygenase metabolites lead to the increase in S channel opening? One interesting possibility is that separate G proteins may be involved at both an early stage in the arachidonate cascade, coupling the receptor to phospholipase activation (5), and a late stage in the cascade, regulating K^+ channel opening (6–11). Here we report evidence that two different G proteins are involved in the cAMP and arachidonic acid cascades. There is only a single role for G proteins in the FMRF-amide response, coupling the receptor to the release of arachidonic acid. Some of these results have been presented in an abstract (12).

MATERIALS AND METHODS

Abdominal ganglia were dissected from *Aplysia californica* (80–120 g), and the sensory neuron clusters were exposed by desheathing and then were superfused with artificial sea water containing 460 mM NaCl, 10 mM KCl, 55 mM $MgCl_2$, 11 mM $CaCl_2$, and 10 mM Hepes (pH 7.6).

The tetralithium salt of guanosine 5′-[γ-thio]triphosphate (GTP[γ-S]) at 2.5–25 mM and the trilithium salt of guanosine 5′-[β-thio]diphosphate (GDP[β-S]) at 0.25–250 mM (Boehringer Mannheim) were dissolved in 0.5 or 2 M KCl solutions. Conventional intracellular electrodes were filled with these solutions and beveled to resistances of 30–50 MΩ (in 0.5 M KCl) or 10–15 MΩ (in 2 M KCl). The nucleotides were iontophoresed into sensory neurons by using negative current pulses (0.1–0.3 nA).

Pertussis toxin (PTX) (List Biological Laboratories, Campbell, CA) was prepared at a final concentration of 0.1 μg/μl in a solution containing 50 mM NaCl, 500 mM KCl, 10 mM dithiothreitol (to reduce disulfide bonds and activate the toxin; ref. 13), 10 mM NAD^+, and 10 mM sodium phosphate at pH 7.6. Toxin or toxin vehicle were injected by using pressure pulses of about 65 psi (1 psi = 6.89 kPa), lasting 20–200 msec, applied from a Picospritzer II (General Valve, Fairfield, NJ).

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: FMRF-amide, Phe-Met-Arg-Phe-NH_2; GTP[γ-S], guanosine 5′-[γ-thio]triphosphate; GDP[β-S], guanosine 5′-[β-thio]diphosphate; PTX, pertussis toxin; 5-HT, serotonin; G protein, guanine nucleotide-binding protein; G_s and G_i, G proteins that stimulate and inhibit adenylate cyclase, respectively; G_o, a G protein of unknown function.

FMRF-amide (10–20 μM) (Peninsula Laboratories, San Carlos, CA), 5-HT (10–20 μM) (creatine sulfate complex, Sigma), and arachidonic acid (Nu-Chek-Prep) were applied by brief pressure ejection from a wide-mouth pipette. FMRF-amide and 5-HT were dissolved in artificial sea water. Arachidonic acid was prepared as a 10 mM stock solution in dimethyl sulfoxide (kept in the dark). Just before application, this solution was diluted in artificial sea water to a final arachidonate concentration of 50 μm (0.5% dimethyl sulfoxide) and was briefly sonicated.

p-Bromophenacyl bromide (Sigma) and nordihydroguaiaretic acid (Biomol, Plymouth Meeting, PA) were dissolved in dimethyl sulfoxide and diluted in artificial sea water to a final concentration of 50 μM (0.1% dimethyl sulfoxide). Drugs were slowly superfused onto the ganglion for at least 2 hr. Shorter perfusion resulted in incomplete blockade of the FMRF-amide effects. The entire experiment was performed in the dark.

Membrane potential was recorded with an Axoclamp amplifier (Axon Instruments, Burlingame, CA) in the bridge mode. Voltage-clamp experiments were carried out by using the Axoclamp amplifier in the single-microelectrode discontinuous voltage-clamp mode. Switching frequencies of 6–8 kHz were employed.

RESULTS

GTP[γ-S] Effects on Membrane Potential and Ionic Current. Intracellular injection of GTP[γ-S], an irreversible activator of G proteins (14), simulates the action of FMRF-amide (but not 5-HT) on membrane potential and ionic current as shown in Fig. 1. A brief (10-sec) application of FMRF-amide onto a sensory neuron results in a transient hyperpolarization under current clamp (Fig. $1A_1$; mean ± SEM = −12.4 ± 4.6 mV, n = 7) and a transient increase in outward current under voltage clamp (Fig. $1A_2$; mean current = +0.78 ± 0.2 nA at −40 mV, n = 5). By comparison, iontophoretic injection of GTP[γ-S] into a sensory neuron produces a slow, irreversible hyperpolarization associated with an increase in input conductance (Fig. $1B_1$; mean hyperpolarization = −21 ± 3.3 mV, n = 5). With 25 mM GTP[γ-S] in the pipette, the membrane reaches a maximally negative potential after 15–20 min of iontophoretic pulses. Identical injections with 0.5 M KCl or 0.5 M KCl plus 100 mM LiCl in the microelectrodes have no effect on membrane potential or input conductance. The GTP[γ-S]-induced hyperpolarization is not blocked by 30 mM tetraethylammonium chloride, a concentration that blocks most K^+ currents but has little effect on S current (15).

Under voltage clamp, GTP[γ-S] injection causes a slow irreversible activation of an outward current (Fig. $1B_2$, mean = 1.8 ± 1.1 nA at −40 mV, n = 6). Figs. $1A_3$ and $1B_3$ compare the FMRF-amide and GTP[γ-S]-induced outward currents over a wide range of potentials by using a staircase voltage command to rapidly step the membrane potential between −80 and −5 mV. The net currents show an identical outwardly rectifying current–voltage curve characteristic of the S current (16). With normal external K^+ (10 mM), no clear reversal potential is observed due to the outward rectification. However, when external K^+ is increased to 50 mM, the GTP[γ-S]-induced current reverses at around −40 mV, which is consistent with this being primarily a K^+ current.

Further evidence that the action of FMRF-amide is mediated by a G protein comes from a study of the interaction between FMRF-amide and GTP[γ-S]. Normally, a brief application of FMRF-amide produces a transient hyperpolarization or increase in outward current (e.g., Fig. 1 A_1 and A_2). However, when FMRF-amide is applied to a sensory neuron soon after its impalement with a GTP[γ-S]-containing electrode (before GTP[γ-S] has produced a significant hyperpolarization), the response to the peptide becomes partly or completely irreversible (Fig. 2). Under current clamp, the membrane potential now only recovers by 33 ± 31% (n = 7) from the peak FMRF-amide-induced hyperpolarization

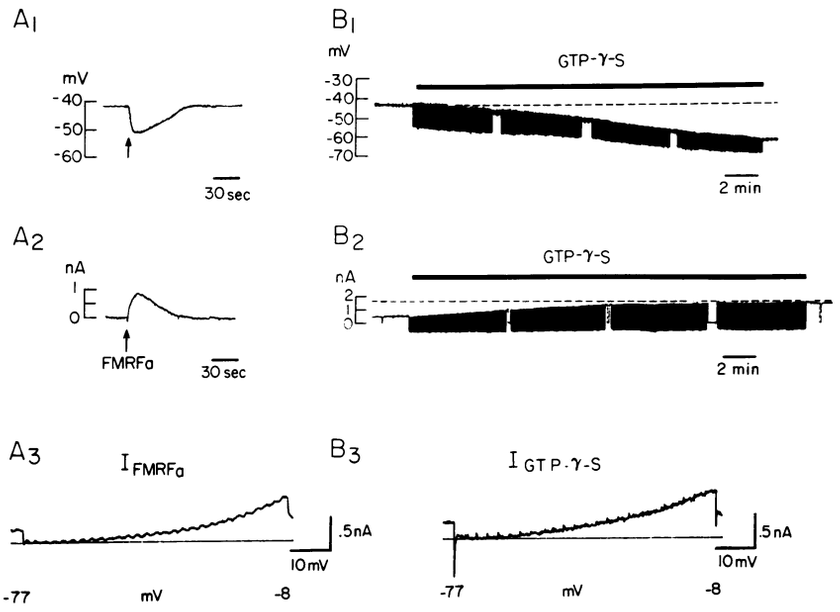

FIG. 1. Effects of FMRF-amide (FMRFa) and GTP[γ-S] on sensory neuron membrane potential and current. FMRF-amide (10 μM) was applied to a sensory cell in current clamp (A_1) or voltage clamp (A_2) at −40 mV. (A_3) FMRF-amide-sensitive current–voltage relation (i.e., current during FMRF-amide application minus control current before FMRF-amide application) obtained by using a 500-msec "staircase" voltage command from −80 to −5 mV. (B_1) Effect of GTP[γ-S] on membrane potential. The nucleotide was injected with 5-min periods of −0.2 nA current pulses (500 msec long at a frequency of 1 Hz) from a microelectrode containing 25 mM GTP[γ-S]. Input resistance is given by the amplitude of the hyperpolarizing response to current pulses (regions with a thickened trace). (B_2) Effects of GTP[γ-S] on membrane current under voltage clamp at −40 mV. The nucleotide was injected with negative voltage steps (500 msec at 1 Hz from −40 mV to −60 mV). (B_3) GTP[γ-S]-sensitive outward current in response to a staircase voltage step command as in A_3. Control current was obtained immediately after impalement. GTP[γ-S] (8 mM) record obtained after 15 min injection. In A_3 and B_3, 50 μM tetrodotoxin was added to block inward Na^{2+} currents. I, current.

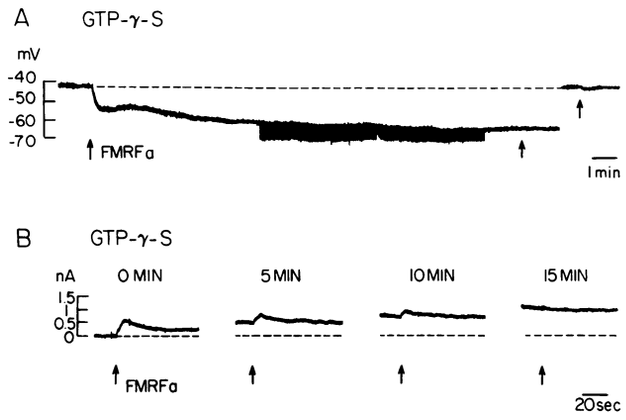

FIG. 2. Interaction between FMRF-amide (FMRFa) and GTP[γ-S]. (*A*) FMRF-amide (10 μM) applied under current clamp soon after the neuron was impaled with the 25 mM GTP[γ-S] electrode. FMRF-amide induces a rapid irreversible hyperpolarization followed by a slower phase of hyperpolarization. After 10 min of GTP[γ-S] injection (thickened trace), a maximal hyperpolarization was reached, which occluded subsequent responses to FMRF-amide at both the hyperpolarized level (second arrow) and after depolarizing the membrane back to its original resting potential by an outward current injection (third arrow). (*B*) FMRF-amide applied under voltage clamp (−40 mV) to a cell impaled with the GTP[γ-S] electrode. The response is only partially reversible. After repeated 5-min periods of GTP[γ-S] injections (breaks between traces), the holding current is shifted outward, and the FMRF-amide response is occluded.

(where 100% represents the normal complete recovery; Fig. 2*A*). In some experiments, the membrane potential remains stable at this new level. However, in other experiments (e.g., Fig. 2*A*), there is a second phase of hyperpolarization. After extensive injection of GTP[γ-S], when the hyperpolarization has reached its maximal level, the response to FMRF-amide is largely occluded [reduced to 12 ± 7% of its initial value (n = 7)]. A similar effect of GTP[γ-S] on the membrane current response to FMRF-amide is observed under voltage clamp (Fig. 2*B*).

Effects of G-Protein Inhibitors on Responses to FMRF-amide and 5-HT. Independent evidence that G-protein activation is a necessary step in the action of FMRF-amide comes from experiments that test the effects of two G-protein inhibitors. PTX, a bacterial toxin that blocks the activation of several distinct G proteins through ADP-ribosylation (17), reduces the FMRF-amide-induced hyperpolarization within 10–20 min after pressure injection into the sensory neurons (Fig. 3 A_2 and *C*) but does not block the response to 5-HT (Fig. 3*C*). The blockade persists with the response to FMRF-amide is tested 40 min after toxin injection. Injection of the toxin vehicle (Fig. 3 A_1 and *C*) has little effect on the response to FMRF-amide.

In other experiments, we compared the effects of iontophoretic injection of the nonhydrolyzable GDP analog GDP[β-S], another G-protein inhibitor (18), on the responses to FMRF-amide and 5-HT. With 25 mM GDP[β-S] in the pipette, prolonged injection (15–30 min) produces a maximal inhibition in the response to FMRF-amide of 46.3 ± 9.6% (n=9) (Fig. 4*A*). At higher concentrations (e.g., 250 mM), GDP[β-S] behaves as a partial agonist and blocks the response to FMRF-amide by ≈80% but also induces a slow increase conductance hyperpolarization and a prolongation of the recovery phase of the FMRF-amide response (data not shown). Interestingly, the response to 5-HT is two orders of magnitude more sensitive to blockade with GDP[β-S]. With 25 mM GDP[β-S]-filled pipettes, the response to 5-HT is completely blocked when tested immediately after impalement. With 0.25 mM GDP[β-S], the response to 5-HT is reduced by >50% after a 15- to 30-min period of injection (Fig. 4*B*), which is comparable to the blockade of the FMRF-amide response with 25 mM GDP[β-S].

The results with PTX and GDP[β-S] indicate that 5-HT and FMRF-amide activate their respective cascades through pharmacologically distinct G proteins. However, if 5-HT does indeed act through a G protein, then why does GTP[γ-S] injection mimic the response to FMRF-amide but not to 5-HT? This question will be considered below.

Role for a G Protein at an Early Stage of the Arachidonic Acid Cascade. At which stages of the arachidonate cascade are G proteins involved? As mentioned above, G proteins might be involved at an early stage of the cascade, before the release of arachidonic acid from the membrane, and/or at a late stage of the cascade, perhaps directly coupled to the S channel.

To investigate this question, we first studied the effects of PTX injection on the response to exogenously applied arachidonic acid. Although PTX consistently inhibits the response to FMRF-amide, it has little effect on the hyperpolarizing response to arachidonic acid (Fig. 3 *B* and *C*). This suggests that a PTX-sensitive G protein is involved in the arachidonic acid cascade at some stage before arachidonic acid is released.

Might a PTX-insensitive G protein be involved at a late stage of the cascade? As a test of this hypothesis, we assayed the effects of GTP[γ-S] after blockade of the arachidonic acid cascade with either the phospholipase inhibitor *para*-bromophenacyl bromide (19) or the lipoxygenase inhibitor nordihydroguaiaretic acid (20). If a G protein is directly coupled to the S channel, it should still be able to activate the channel in response to GTP[γ-S] under such conditions.

However, as Fig. 5 shows, inhibition of the arachidonic acid cascade blocks the hyperpolarizing responses to both FMRF-amide and GTP[γ-S]. Under conditions where the FMRF-amide response was abolished, GTP[γ-S] injection now results in a slow irreversible depolarization accompanied by a decrease in input conductance (Fig. 5 *A* and *B*). On average, GTP[γ-S] depolarizes the sensory neurons by 4.7 ± 4 mV (n = 6) in the presence of *para*-bromophenacyl bromide and by 7.7 ± 2.1 mV (n = 3) in the presence of nordihydroguaiaretic acid.

The depolarizing action of GTP[γ-S] revealed by the presence of the inhibitors resembles the normal decreased-conductance depolarization seen with 5-HT and probably reflects activation of the cAMP cascade due to activation of G_s. Independent evidence that GTP[γ-S] can activate the 5-HT cascade comes from the finding that, in the presence of arachidonate blockers, GTP[γ-S] makes the normal reversible response to 5-HT (Fig. $5C_1$) partially irreversible (Fig. $5C_3$). In the absence of the inhibitors, the depolarizing response to 5-HT is overwhelmed by the hyperpolarizing response to GTP[γ-S] (Fig. $5C_2$). Apparently, in the absence of blockers, activation of the FMRF-amide cascade by GTP[γ-S] masks or antagonizes the 5-HT-like action of this nucleotide, in agreement with our previous results that FMRF-amide overrides the action of 5-HT or cAMP (4).

A final piece of evidence against a role for a G protein at a late stage of the arachidonic acid cascade comes from a comparison of the effects of GTP[γ-S] on the kinetics of the FMRF-amide and arachidonic acid responses. Fig. 6 shows that, whereas the hyperpolarizing response to FMRF-amide is largely (or completely) irreversible in the presence of GTP[γ-S], the response to arachidonic acid is basically unchanged and shows its normal reversible time course. Thus, we find no evidence for a GTP[γ-S]-sensitive reaction following the release of arachidonic acid up to the final step of S channel modulation.

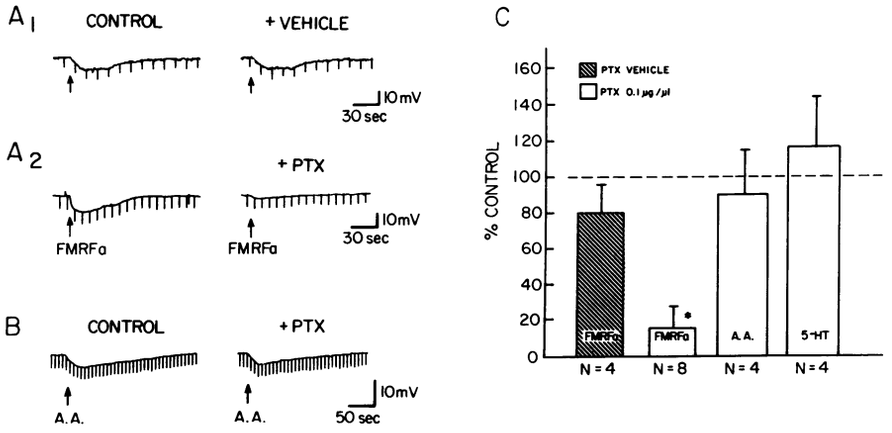

FIG. 3. Effect of PTX injection on membrane potential responses to FMRF-amide (*A* and *C*), 5-HT (*C*), and arachidonic acid (*B* and *C*). (A_1) Response to FMRF-amide (10 μM) before (*Left*) and after (*Right*) injection of PTX vehicle. The input conductance was monitored by using −0.2 nA current pulses (100 msec long at 0.1 Hz). (A_2) Response to FMRF-amide before (*Left*) and 10 min after (*Right*) injection of PTX. PTX produced no significant change in input resistance or resting potential. (*B*) Response to arachidonic acid (50 μM) before (*Left*) and 10 min after (*Right*) PTX injection. In two experiments, PTX was found to have no effect on arachidonate response in the same cells where it blocked the response to FMRF-amide. (*C*) Mean effects of PTX vehicle (hatched bars) or active PTX (open bars) on membrane potential responses to different agents. One hundred percent represents the preinjection control response. Error bars indicate the SD. *, Significant difference from the PTX vehicle response (Student's *t* test, $P < 0.005$); A.A., arachidonic acid; FMRFa, FMRF-amide.

DISCUSSION

FMRF-amide, through the lipoxygenase metabolites of arachidonic acid, hyperpolarizes *Aplysia* sensory neurons by increasing the opening of S K^+ channels. The present study demonstrates that this effect is mediated by a PTX-sensitive G protein. A role for a G protein in mediating inhibitory responses to FMRF-amide in other *Aplysia* neurons has recently been reported (21, 22) although the nature of the second messengers in these systems is not known. Inhibitory effects of histamine on other *Aplysia* neurons that are also mediated by lipoxygenase products of arachidonic acid (23) also involve a PTX-sensitive G protein (24).

Our data further indicate that the FMRF-amide-activated G protein in the sensory neurons is involved at an early stage of the arachidonic acid cascade, coupling the FMRF-amide receptor to arachidonate release, and is not directly coupled to the S channel. This conclusion contrasts with a number of recent reports suggesting that a PTX-sensitive G protein may directly couple transmitter receptors to K^+ channel activation (6–11).

Arachidonic acid release has been associated with the action of a PTX-sensitive G protein in many vertebrate cells (see ref. 5 for a recent review). In most systems, the release of arachidonic acid is thought to be indirectly coupled to G proteins by means of the G protein-mediated activation of phospholipase C, which leads to the release of diacylglycerol and inositol 1,4,5-trisphosphate. Arachidonic acid can then be

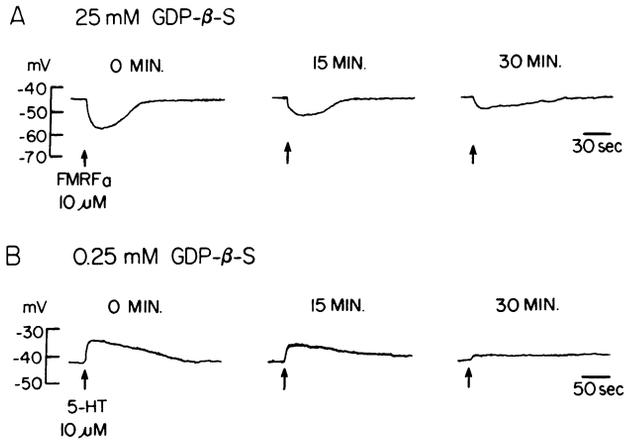

FIG. 4. GDP[β-S] blocks the response to FMRF-amide (FMRFa) and 5-HT. (*A*) FMRF-amide (10 μM) applied before and after 15 and 30 min of iontophoretic injection from an electrode containing 25 mM GDP[β-S]. (*B*) 5-HT (10 μM) responses at comparable times during injection from an electrode containing 0.25 mM GDP[β-S]. The response to 5-HT is completely blocked with 2.5 mM GDP[β-S] (data not shown).

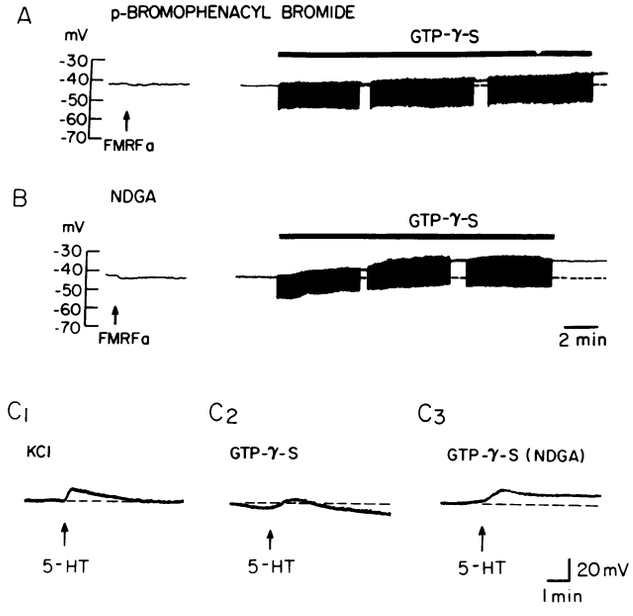

FIG. 5. Effects of GTP[γ-S] in the presence of arachidonic acid blockers. (*A* and *B*) Response to 10–20 μM FMRF-amide immediately after cell impalement with the 25 mM GTP[γ-S] electrode (left traces) in the presence of 50 μM *para*-bromophenacyl bromide (*A*) or 50 μM nordihydroguaiaretic acid (*B*). GTP[γ-S] was then iontophoretically injected for 15–20 min (right traces). Blockers had no direct effect on membrane potential, input resistance, or spike firing. (*C*) Effects of GTP[γ-S] on the response to 5-HT. (C_1) Control response to 10 μM 5-HT with the KCl electrode. (C_2) Response to 5-HT with the 25 mM GTP[γ-S] electrode. (C_3) Response to 5-HT in the presence of 50 μM nordihydroguaiaretic acid with the 25 mM GTP[γ-S] microelectrode. NDGA, nordihydroguaiaretic acid.

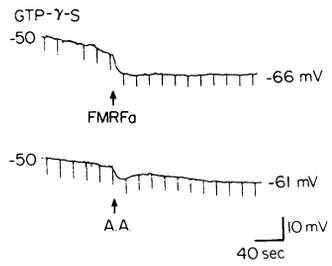

FIG. 6. Effects of GTP[γ-S] on the response to FMRF-amide (FMRFa) and arachidonic acid (A.A.). The slow hyperpolarization is due to GTP[γ-S] injected with −0.2 nA pulses at 0.1 Hz. FMRF-amide (10 μM) produces a rapid maximal and irreversible hyperpolarization (top trace). Arachidonic acid (50 μM) produces a normal transient hyperpolarization (compare with Fig. 3*B*) on top of a sloping baseline (bottom trace) due to GTP[γ-S]-induced hyperpolarization. After recovery from arachidonic acid, there is a further GTP[γ-S]-induced hyperpolarization. Reversible responses to subsequent applications of arachidonate were observed (data not shown). Data are representative of three experiments.

produced either through the action of diacylglyerol lipase or through direct activation of phospholipase A_2 as a result of inositol 1,4,5-trisphosphate-mediated Ca^{2+} release. However, there is now evidence for the direct activation of phospholipase A_2 by a G protein (5, 25–27). In *Aplysia* sensory neurons, the FMRF-amide-induced activation of the arachidonic acid cascade is unlikely to occur secondarily to Ca^{2+} mobilization since FMRF-amide produces no change in resting internal Ca^{2+} as measured by the Ca^{2+} indicator dye fura-2 (28). Moreover, in *Aplysia* neurons, neither inositol 1,4,5-trisphosphate nor phorbol esters mimic the effects of FMRF-amide or GTP[γ-S] in enhancing the S K^+ current (21). In fact, phorbol esters lead to presynaptic facilitation of transmitter release from the sensory neurons (29), an effect opposite to the presynaptic inhibition seen with FMRF-amide.

In addition to the G protein coupled to the FMRF-amide receptor, sensory neurons also contain a G protein that couples the 5-HT receptor to stimulation of adenylate cyclase (30). However, the effect of activating this G protein with GTP[γ-S] is seen only when the FMRF-amide cascade is blocked (e.g., Figs. 4 and 5). This confirms, at the level of G-protein activation, the previously observed competition between the FMRF-amide and 5-HT pathways, where the inhibitory action of FMRF-amide completely overrides the response to 5-HT or cAMP (4).

One major question that remains is the biochemical identity of the G proteins involved in the modulatory actions of 5-HT and FMRF-amide. Clearly there are functional differences between the two G proteins mediating the effects of the two transmitters. The G protein coupled to 5-HT receptors is likely to be related to G_s. It is PTX-insensitive, is involved in the activation of adenylate cyclase, and is highly sensitive to GDP[β-S] (see also refs. 30 and 31). Moreover, in *Aplysia* neural tissue, Vogel *et al.* (32) have presented evidence for a M_r 45,000 cholera toxin substrate recognized by antibodies raised against the α subunit of G_s from bovine brain. Vogel *et al.* also found, in agreement with previous results (33), a M_r 40,000 PTX substrate recognized by an antibody against the α subunit of bovine G_o (a G protein of unknown function) and reported evidence suggesting that a G_i-like protein is also present.

In view of the increasing number of G_i and G_o α subunits recently identified in the M_r 40,000–41,000 range (1), it is not yet possible to identify the G protein activated by FMRF-amide. However, from a functional view, this G protein fails to behave like G_i since FMRF-amide does not inhibit adenylate cyclase activation with 5-HT (34, 35) but exerts its inhibitory action at a later stage in the cAMP cascade (4). Future experiments using antibodies directed against different G proteins that block function could be of use in identifying the G protein involved in the arachidonic acid cascade.

We thank David Sweatt for many helpful discussions and, together with Paul Pfaffinger and James Schwartz, for a critical reading of the manuscript. We also thank Eli Shapiro for his generous help in setting up the PTX injection experiments and Kathrin Hilten and Louise Katz for preparing the figures. This work was partly supported by grant NS-19569 from the U.S. Public Health Service.

1. Gilman, A. G. (1987) *Annu. Rev. Biochem.* **56,** 615–649.
2. Dunlap, K., Holz, G. G. & Rane, S. G. (1987) *Trends Neurosci.* **10,** 241–244.
3. Piomelli, D., Volterra, A., Dale, N., Siegelbaum, S. A., Kandel, E. R., Schwartz, J. H. & Belardetti, F. (1987) *Nature (London)* **328,** 38–43.
4. Belardetti, F. & Siegelbaum, S. A. (1988) *Trends Neurosci.* **11,** 232–238.
5. Axelrod, J., Burch, R. M. & Jelsema, C. L. (1988) *Trends Neurosci.* **11,** 117–123.
6. Pfaffinger, P. J., Martin, J. M., Hunter, D. D., Nathanson, N. M. & Hille, B. (1985) *Nature (London)* **317,** 536–538.
7. Breitwieser, G. E. & Szabo, G. (1985) *Nature (London)* **317,** 538–540.
8. Yatani, A., Codina, J., Brown, A. M. & Birnbaumer, L. (1987) *Science* **235,** 207–211.
9. Logothetis, D. E., Kurachi, Y., Galper, J., Neer, E. J. & Clapham, D. E. (1987) *Nature (London)* **325,** 321–326.
10. Andrade, R., Malenka, R. C. & Nicoll, R. A. (1986) *Science* **234,** 1261–1265.
11. Sasaki, K. & Sato, M. (1987) *Nature (London)* **325,** 259–262.
12. Volterra, A., Sweatt, J. D. & Siegelbaum, S. A. (1987) *Soc. Neurosci. Abstr.* **13,** 1440.
13. Katada, T., Tamura, M. & Ui, M. (1983) *Arch. Biochem. Biophys.* **224,** 290–298.
14. Pfeuffer, T. & Helmreich, J. M. (1975) *J. Biol. Chem.* **250,** 867–876.
15. Shuster, M. J. & Siegelbaum, S. A. (1987) *J. Gen. Physiol.* **90,** 587–608.
16. Siegelbaum, S. A., Camardo, J. S. & Kandel, E. R. (1982) *Nature (London)* **299,** 413–417.
17. Ui, M. (1984) *Trends Pharmacol. Sci.* **5,** 277–279.
18. Eckstein, F., Cassel, D., Levkovitz, H., Lowe, M. & Selinger, Z. (1979) *J. Biol. Chem.* **254,** 9829–9834.
19. Dennis, E. A. (1987) *Drug Dev. Res.* **10,** 205–220.
20. Salari, H., Braquet, P. & Borgeat, P. (1984) *Prostaglandins Leukotrienes Med.* **13,** 53–60.
21. Brezina, V., Eckert, R. & Erxleben, C. (1987) *J. Physiol. (London)* **388,** 565–595.
22. Sasaki, K., Takahashi, J., Matsumoto, M., Takashima, K., Hakozaki, S. & Sato, M. (1987) *Jpn. J. Physiol.* **37,** 551–557.
23. Piomelli, D., Shapiro, E., Feinmark, S. J. & Schwartz, J. H. (1987) *J. Neurosci.* **7,** 3675–3686.
24. Vogel, S. S., Shapiro, E., Chin, G. J. & Schwartz, J. H. (1987) *Soc. Neurosci. Abstr.* **13,** 597.
25. Burch, R. M., Luini, A. & Axelrod, J. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 7201–7205.
26. Jelsema, C. L. (1987) *J. Biol. Chem.* **262,** 163–168.
27. Nakashima, S., Tohmatsu, T., Hattori, H., Suganuma, A. & Nozawa, Y. (1987) *J. Biochem.* **101,** 1055–1058.
28. Blumenfeld, H., Bug, B., Buttner, E., Sweatt, D. & Siegelbaum, S. A. (1988) *Soc. Neurosci. Abstr.* **14,** in press.
29. Hochner, B., Braha, O., Klein, M. & Kandel, E. R. (1986) *Soc. Neurosci. Abstr.* **12,** 1340.
30. Schwartz, J. H., Bernier, L., Castellucci, V. F., Palazzolo, M., Saitoh, T., Stapleton, A. & Kandel, E. R. (1983) *Cold Spring Harbor Symp. Quant. Biol.* **48,** 811–819.
31. Lemos, J. R. & Levitan, I. B. (1984) *J. Gen. Physiol.* **83,** 269–285.
32. Vogel, S., Chin, G., Mumby, S. M., Schonberg, M. & Schwartz, J. H. (1988) *Brain Res.*, in press.
33. Critz, S. D., Harper, J. F. & Byrne, J. H. (1986) *Neurosci. Lett.* **64,** 145–150.
34. Ocorr, K. A. & Byrne, J. H. (1985) *Neurosci. Lett.* **55,** 113–118.
35. Ocorr, K. A., Tabata, M. & Byrne, J. H. (1985) *Soc. Neurosci. Abstr.* **11,** 481.