Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 1590–1594, February 1995
Neurobiology

Opposing mechanisms of regulation of a G-protein-coupled inward rectifier K^+ channel in rat brain neurons

(substance P/somatostatin/[Met]enkephalin/locus coeruleus)

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Communicated by Clay M. Armstrong, University of Pennsylvania, Philadelphia, PA, October 21, 1994 (received for review January 25, 1994)

ABSTRACT **In locus coeruleus neurons, substance P (SP) suppresses an inwardly rectifying K^+ current via a pertussis toxin-insensitive guanine nucleotide binding protein (G protein; G_{nonPTX}), whereas somatostatin (SOM) or [Met]enkephalin (MENK) enhances it via a pertussis toxin-sensitive G protein (G_{PTX}). The interaction of the SP and the SOM (or MENK) effects was studied in cultured locus coeruleus neurons. In neurons loaded with guanosine 5′-[γ-thio]triphosphate (GTP[γS]), application of SOM (or MENK) evoked a persistent increase in the inward rectifier K^+ conductance. A subsequent application of SP suppressed this conductance to a level less than that before the SOM (or MENK) application; the final conductance level was independent of the magnitude of the SOM (or MENK) response. This suppression by SP was persistent, and a subsequent SOM (or MENK) application did not reverse it. When SP was applied to GTP[γS]-loaded cells first, subsequent SOM elicited only a small response. In GTP-loaded neurons, application of SP temporarily suppressed the subsequent SOM- (or MENK)-induced conductance increase. These results suggest that the same inward rectifier molecule that responds to an opening signal from G_{PTX} also responds to a closing signal from G_{nonPTX}. The closing signal is stronger than the opening signal.**

Inward rectifier K^+ channels exist in various cell types and determine the resting conductance and potential (1–4). In addition to these ordinary inward rectifiers, there is another class of inward rectifiers, the guanine nucleotide binding protein (G protein)-coupled inward rectifiers. In atrial cells G-protein regulation of inward rectifiers is responsible for the hyperpolarization caused by stimulation of the vagal nerve (5–8).

G-protein-coupled inward rectifier K^+ channels also exist in various types of vertebrate neurons, and the modulation of these channels generates slow synaptic potentials (9–16). In cholinergic neurons from the nucleus basalis of Meynert, substance P (SP) excites neurons by reducing an inward rectifier current (9), and this effect is mediated by a pertussis toxin-insensitive G protein (G_{nonPTX}) (14). In contrast, in locus coeruleus neurons somatostatin (SOM) and [Met]enkephalin (MENK) inhibit neurons by enhancing an inward rectifier current via a pertussis toxin-sensitive G protein (G_{PTX}) (10, 13).

In the present experiments, we have studied the interaction of SP effects and SOM (and MENK) effects on the inward rectifier in locus coeruleus neurons. The results strongly suggest that the same inward rectifier molecule responds to two opposing signals: an opening signal from SOM or MENK (via G_{PTX}) and a closing signal from SP (via G_{nonPTX}). More strikingly, in the presence of guanosine 5′-[γ-thio]triphosphate (GTP[γS]), the sustained enhancement of inward rectifier current induced by SOM or MENK was suppressed by SP, while channels shut by SP could not be reopened by SOM or MENK. Preliminary data were reported (17).

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MATERIALS AND METHODS

A detailed description of the methods has been given (18). Neuronal cultures from the locus coeruleus were made as described (18, 19). For electrophysiology, the tight seal whole-cell patch clamp technique was used. The external solution (10 mM K^+ solution) contained 141 mM NaCl, 10 mM KCl, 2.4 mM $CaCl_2$, 1.3 mM $MgCl_2$, 11 mM D-glucose, 0.0005–0.001 mM tetrodotoxin, and ≈5 mM Hepes·NaOH (pH 7.4). The patch pipette solution contained 120 mM potassium aspartate, 40 mM NaCl, 3 mM $MgCl_2$, 0.25 mM $CaCl_2$, 0.5 mM EGTA·KOH, 2 mM Na_2ATP, 0.1 mM Na_3GTP (or 0.1–0.2 mM GTP[γS]), 5 mM Hepes·KOH, and ≈5 mM KOH (pH 7.2). Temperatures of the experimental bath were ≈31°C. Unless otherwise stated, average values are means ± SEM.

RESULTS

MENK-Induced Conductance. Inoue *et al.* (13) reported that SOM induced an inward rectifier K^+ conductance in locus coeruleus neurons. Here, we describe the effects of MENK on K^+ conductance. As shown in Fig. 1*A*, application of MENK (1 μM) increased the membrane conductance. The average conductance increase was 14.7 ± 2 nS (n = 25). Fig. 1*B* shows MENK-induced currents, which were obtained by subtracting the control currents from the current during the effect of MENK. As shown in Fig. 1*C*, the MENK-induced currents exhibited an inward rectification with a reversal potential near the K^+ equilibrium potential (E_K = −66 mV) in both GTP-loaded cells (open circles) and GTP[γS]-loaded cells (solid circles). Thus, the properties of the MENK-induced conductance are essentially the same as those of the SOM-induced current (13).

SP Effects. SP reduces a resting K^+ conductance in nucleus basalis neurons, and the SP-suppressed conductance rectifies to the inward direction (9). Thus, the SP effect was an approximate mirror image of the SOM or MENK effects.

The effects of SP on locus coeruleus neurons are more complicated than those on nucleus basalis neurons (18, 20). As shown in Fig. 2*A*, SP produced two different responses in some locus coeruleus neurons: an initial inward current (arrow) and a late conductance decrease (double-headed arrow). The initial inward current represents the activation of a nonselective ion conductance, which may not be related to a G protein (18). The second phase, the conductance decrease, represents sup-

Abbreviations: GTP[γS], guanosine 5′-[γ-thio]triphosphate; G protein, guanine nucleotide binding protein; SP, substance P; SOM, somatostatin; MENK, [Met]enkephalin; G_{PTX}, pertussis toxin-sensitive G protein; G_{nonPTX}, pertussis toxin-insensitive G protein.

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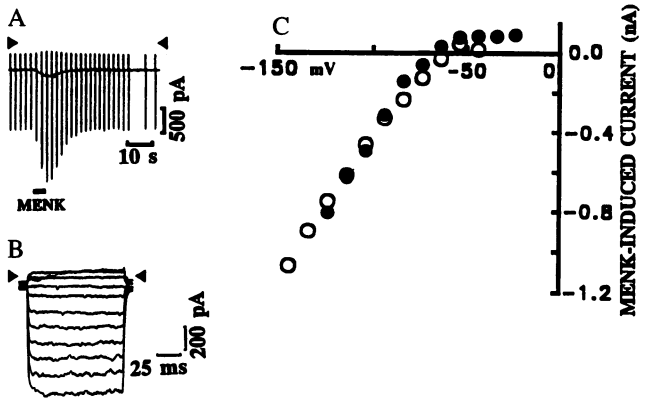

FIG. 1. MENK (1 μM) effects on locus coeruleus neurons. External solution, 10 mM K^+; holding potential, −74 mV. (*A*) Patch pipette contained GTP. Each command pulse sequence consisted of a square-wave depolarization (20 mV; 100 ms), an interval (100 ms), and a hyperpolarization (50 mV; 100 ms); in this record, each sequence appears to be a single vertical line. Arrowheads indicate zero current level. (*B*) MENK-induced currents, which were obtained by subtracting control currents from currents during the MENK effect. GTP-containing internal solution was used. Potential ranged from −144 to −44 mV in 10-mV increments. (*C*) Current–voltage relation of the MENK-induced currents plotted at 15–45 ms from the start of pulses. Open circles, from record in *B* (with GTP-containing internal solution); solid circles, data from another cell with an internal solution containing GTP[γS] (150 μM). Errors originated from the residual series resistance and from the fact that the MENK effect was declining during measurements of the current–voltage relation and were corrected as described (13, 18). MENK was applied through a thoroughly washed glass capillary by pressure ejection. When not in use, the capillary was kept in the air to prevent peptide leakage.

pression of an inward rectifier mediated by a G protein (18). Previously, we showed that in nucleus basalis neurons the SP effect on the inward rectifier was not affected by pertussis toxin (14). We repeated this experiment on locus coeruleus neurons and found that the SP effect was again pertussis toxin insensitive. In cells pretreated with pertussis toxin (1 μg/ml for 20–24 hr), the SP-induced reduction of conductance was 21% ± 5.8% (n = 4) of the total conductance, while in control cells it was 24% ± 4.9% (n = 5). Cells loaded with 100 μM GTP[γS] were used for this experiment. Thus, the reduction of the inward rectifier by SP in locus coeruleus neurons, as in the case of nucleus basalis neurons, is mediated by G_{nonPTX}.

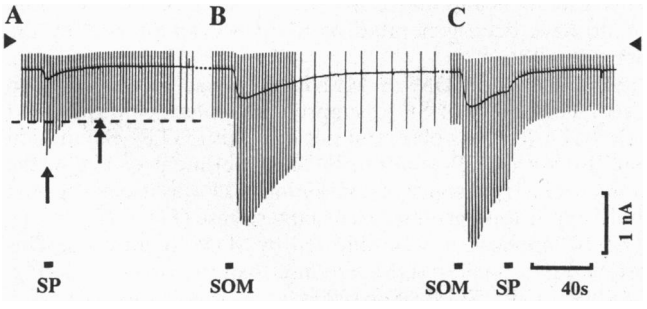

FIG. 2. Interaction of SP (0.3 μM) and SOM (0.2 μM) effects with the standard internal solution containing GTP. External solution, 10 mM K^+; holding potential, −74 mV. (*A*) SP produced an inward current (nonselective ion conductance) (arrow) followed by a slow decrease in conductance (double-headed arrow). (*B*) SOM was applied 385 s after the SP in *A*. (*C*) Another application of SOM followed by SP. SOM in *C* was applied 262 s after SOM in *B*. Note that in *B* and *C*, SOM did not produce an outward current shift at 20-mV depolarizing pulses (−54-mV level); this is different from Fig. 1*A*. Near −54 mV, where the SOM- or MENK-sensitive inward rectifier current becomes miniscule (open circles of Fig. 1*C*), contamination from even small currents originating from other unknown channels that are also sensitive to SOM would distort the SOM-sensitive inward rectifier current.

SOM or MENK Effect on SP Response in GTP-Loaded Cells. In the experiment of Fig. 2, the standard patch pipette solution containing GTP was used. Application of SP (Fig. 2*A*) produced a sequence of two responses as described above: activation of a nonselective ion conductance (arrow), followed by a slower phase of reduction of an inward rectifier current (double-headed arrow). In Fig. 2*B*, SOM produced a large conductance increase. This response represents an enhancement of an inward rectifier K^+ current (13). In Fig. 2*C*, another SOM application produced a similar conductance increase, and SP was applied while the SOM effect still persisted; SP caused a relatively quick termination of the SOM effect. The conductance decrease produced by the second SP application (Fig. 2*C*) appears to be much larger than that produced by the first SP application (Fig. 2*A*, double-headed arrow). Similar results were obtained in four additional cells (using SOM or MENK). The results suggest that the conductance enhanced by SOM or MENK was reduced by SP. (In Fig. 2*B*, unlike the MENK effect in Fig. 1*A*, SOM did not produce an outward current shift at 20-mV depolarizing pulses. See Fig. 2 legend.)

SOM or MENK Effect on SP Response in GTP[γS]-Loaded Cells. The experiments described here were done by using a patch pipette solution containing GTP[γS] (100–200 μM), a nonhydrolyzable GTP analogue. The SP-induced reduction of K^+ conductance in locus coeruleus neurons is mediated by a G_{nonPTX}, while the SOM- or MENK-induced increase of K^+ conductance is mediated by a G_{PTX} (10, 13). When the cells were loaded with GTP[γS], SP produced a persistent reduction of conductance, and a second application of SP did not produce further reduction, suggesting that the first response was maximal (18). Similarly, SOM applied to GTP[γS]-loaded cells caused a persistent and maximum increase in conductance (two cells). Thus, by using GTP[γS]-loaded cells, we can observe how the persistent presence of activated G_{PTX} and G_{nonPTX} influences the activity of the inward rectifier, and we can bypass consideration of events taking place at the level of the receptors.

As shown in Fig. 3 (*A* and *B*), when a pipette solution containing GTP[γS] (100–200 μM) was used, a spontaneous slow increase in conductance often occurred, possibly reflecting spontaneous G-protein activation. The first application of SOM or MENK produced a prolonged increase in conductance (Fig. 3*A* and *B*). A subsequent application of SP induced a large reduction of the K^+ conductance to less than the level before the application of SOM or MENK (Fig. 3*A* and *B*); the half-time of the reduction was 12 ± 1.8 s (n = 18; 10 mM K^+ solution). The result indicates that the sustained enhancement of activity of the inward rectifier produced by the continuous presence of activated G_{PTX} is readily reversed by activated G_{nonPTX}. After the conductance was suppressed by SP, a second SOM or MENK application could not increase the K conductance (14 cells) (Fig. 3 *A* and *B*).

In Fig. 3*C*, the conductance enhanced by SOM or MENK ($\Delta G_{S,M}$) was compared to that suppressed by the subsequent SP application (ΔG_{SP}); these quantities ($\Delta G_{S,M}$ and ΔG_{SP}) are expressed in reference to the control conductance before the drug application (G_1) (diagrammed in Fig. 3*D*). It can be seen that in all but two cells, $\Delta G_{SP}/G_1$ was larger than $\Delta G_{S,M}/G_1$, suggesting that SP eliminated both the SOM- and MENK-induced conductance and part of the control conductance (G_1). The figure also shows that as $\Delta G_{S,M}$ (in reference to G_1) became larger, ΔG_{SP} (in reference to G_1) became larger, with a strong correlation (r = 0.79 in 10 mM external K^+ solution), indicating that SP suppressed the SOM- or MENK-induced conductance effectively no matter how large the increase.

As shown in Fig. 3*D*, the quantity G_3 represents the residual conductance after the SP effect. This quantity (normalized to the value of G_1 for each cell) was independent of the magni-

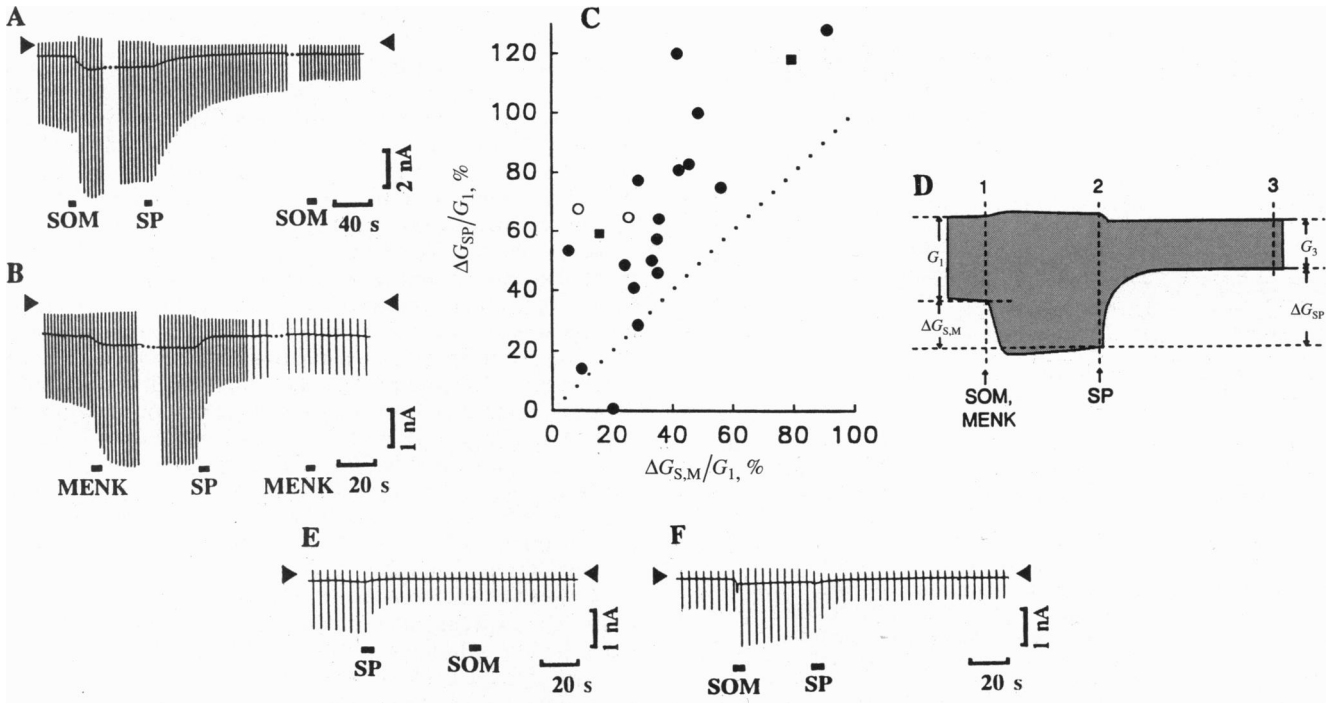

FIG. 3. Interaction of SP (0.3 μM) and SOM (0.2 μM) or MENK (1 μM) effects. Internal solution contained 100–200 μM GTP[γS]. (*A*) Interval between the first SOM and SP applications was 86 s, and that between the SP application and the last SOM application was 360 s. External solution contained 10 mM K^+. Soma diameter, ≈44 μm. (*B*) Interval between the first MENK and SP applications was 102 s, and that between SP and the last MENK applications was 120 s. External solution, 10 mM K^+; soma diameter, ≈40 μm. (*C*) Relationship between the SOM (or MENK) and SP responses in cells loaded with GTP[γS] (100–200 μM). Abscissa is SOM- or MENK-induced conductance ($\Delta G_{S,M}$) in reference to control conductance (G_1). Ordinate is the conductance suppressed by SP (ΔG_{SP}) in reference to G_1. Average value of G_1 was 39.5 ± 4.9 nS (n = 19; 10 mM K^+ experiments). Each point represents one cell. Solid circles, SOM effect in 10 mM K^+ external solution; solid squares, MENK effect in 10 mM K^+ external solution; open circles, SOM effect in 5 mM K^+ external solution. Only cells that responded to SOM or MENK with >1.5 nS are listed. (*D*) Definition of various conductances. (*E* and *F*) SP suppressed a subsequent SOM response. Internal solution contained 100 μM GTP[γS]. (*E*) Test experiment. SP was applied for the first time, followed by SOM. Time between patch break and first application of SP was 193 ± 6.6 (SD) s (n = 9), and time between patch break and SOM application was 246 ± 3.3 (SD) s (n = 9). (*F*) Control. SOM was applied without a precedent SP application, producing a large SOM response. Time between patch break and SOM application was 239 ± 15.6 (SD) s (n = 10). This timing of SOM application was matched with the test experiment in *E*. The same culture batches as in the test experiment were used. Note that in the past we performed experiments similar to those in *E* under slightly different conditions from those described in the text. Residual conductance after SP, in reference to G_1, was 70% ± 4.9%, n = 9, t_1 = 354 ± 35 (SD) s in 260 μM GTP[γS] and 60% ± 5.1%, n = 6, t_1 = 135 ± 45 (SD) s in 50–75 μM GTP[γS], indicating that the residual conductance is approximately the same over those ranges of t_1 and GTP[γS] concentration.

tude of the SOM or MENK effect ($\Delta G_{S,M}/G_1$) (r = −0.26), indicating that the final conductance level was approximately the same regardless of the size of the SOM (or MENK) response.

The average value of the residual conductance after the SP effect (G_3/G_1) (Fig. 3*C*; 10 mM K^+) was 72% ± 5% (n = 19) [the time between the break of the patch and the first SOM (or MENK) application, t_1, was 195 ± 49 (SD) s]. This was not significantly different from the residual conductance after simply applying SP without a preceding SOM or MENK application [71% ± 6.3%; n = 14; the time between the patch break and the SP application, t_1 = 191 ± 21 (SD) s, in 100–150 μM GTP[γS]; see Fig. 3 legend]. The result again suggests that the final conductance level after SP in GTP[γS]-loaded cells is approximately the same with or without a preceding SOM or MENK application. Thus, most (if not all) of the conductance generated by SOM or MENK was effectively eliminated by SP.

SP Effects on SOM Response in GTP[γS]-Loaded Cells. In Fig. 3*E*, SP was applied ≈3 min after the break of the patch membrane in a GTP[γS]-loaded cell, and SOM was applied ≈4 min after the break of the patch. SOM hardly produced a response. The average SOM response in this set of experiments was only 8% ± 2.3% (n = 9; with respect to G_1). Another SOM application 3 min later elicited no response (n = 7; data not shown). As a control (Fig. 3*F*), by using the same culture batches as in the test experiment, SOM was applied ≈4 min after the patch break without a preceding SP application. SOM elicited a large response that averaged 48% ± 7.9% (n = 10). This result indicates that most (≈80%) of the conductance that would have been generated by SOM was suppressed by the preceding SP.

SP Effects on SOM or MENK Response in GTP-Loaded Cells. The ability of SP to suppress the subsequent SOM (or MENK) effect was observed not only with GTP[γS] solution but also with GTP solution. In the experiment of Fig. 4, the standard internal solution containing GTP was used. The first SOM application produced a usual response (Fig. 4*A1*). In Fig. 4*A2*, SP application was followed by SOM application. This resulted in a smaller SOM response than the first one. In Fig. 4*A3*, we applied SOM again (220 s later); this produced a much larger response than that in Fig. 4*A2*. The result suggests that the second SOM response (Fig. 4*A2*) was suppressed by the preceding SP application. Fig. 4*B* shows a control experiment in which SOM alone was applied repeatedly at intervals of 3–4 min without SP application. The response became monotonically smaller; this represents gradual desensitization of the SOM effect. The same suppressing effect of SP occurred when a combination of SP and MENK was used (Fig. 4 *C* and *D*).

The SOM data are summarized in Fig. 4*E1*. Ordinate represents amplitudes of SOM responses obtained on the first, second, and third applications. Abscissa shows time of SOM application. Solid circles (test experiments) indicate that the second SOM response, which was preceded by SP (arrow), was

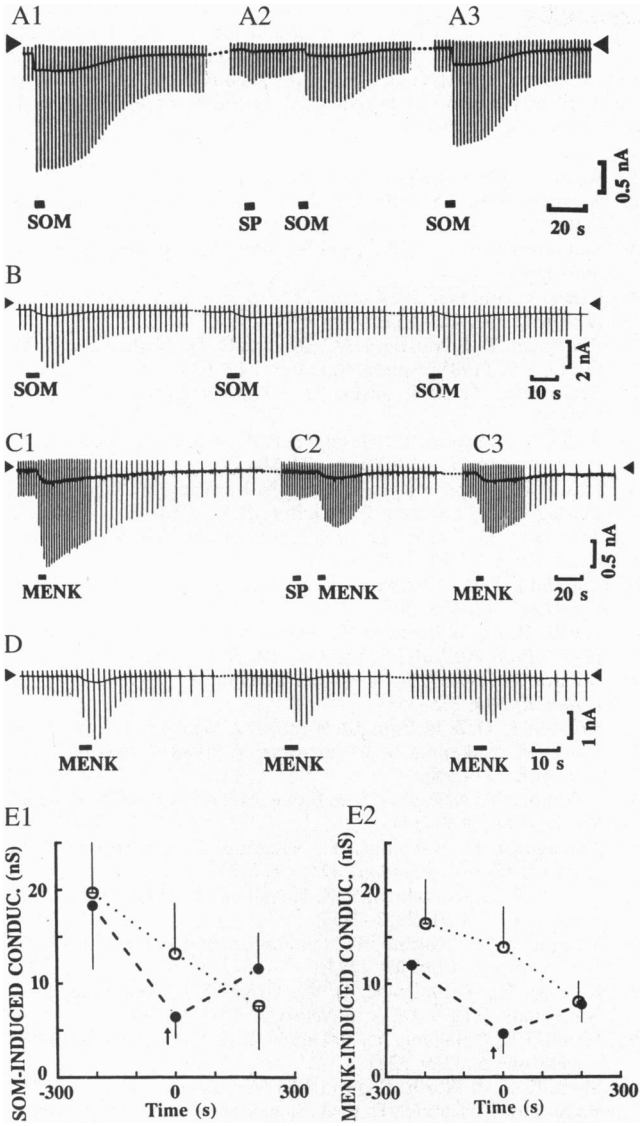

FIG. 4. SP (0.3 μM) temporarily suppressed a subsequent SOM (0.2 μM) or MENK (1 μM) effect. Patch pipette contained GTP. (*A*) First, SOM (*A1*); 187 s later SP followed by SOM (*A2*); and another 220 s later SOM (*A3*). (*B*) Control. SOM alone was applied repeatedly with an interval of 205–245 s. (*C*) Interval between the first MENK (*C1*) and SP applications (*C2*) and that between SP (*C2*) and the last MENK (*C3*) applications were 206–214 s. (*D*) Control. MENK alone was applied repeatedly with an interval of 180–185 s. (*E*) Mean SOM- (or MENK)-induced conductances vs. mean times of the three SOM (or MENK) applications. Vertical and horizontal bars are SEMs of conductances and SEMs of application times. Time of the second SOM (or MENK) application was defined as zero. In *E1*, open circles represent control experiments (records in *B*). Cell numbers were as follows: first measurement, 6; second measurement, 6; third measurement, 5. Solid circles in *E1* are interaction experiments ($n = 7$). Just before (-20 ± 1 s; $n = 7$) the second SOM application, SP was applied (arrow). In *E2*, open circles are control MENK experiments ($n = 7$). Solid circles in *E2* are interaction experiments ($n = 4$). SP was applied at -25 ± 9 sec ($n = 4$) (arrow). Note that in *E* the control responses declined monotonically. This is the average behavior of this sample. Rarely (2 of 13 cells) the second response was larger than the first one, but in all cells the third response was smaller than the second one. [In Fig. 2 the second SOM response (Fig. 2*C*) was larger than the first (Fig. 2*B*); this could have been due to a suppressing effect still remaining in Fig. 2*B* from the first SP application (Fig. 2*A*).]

smallest. In contrast, repeated applications of SOM alone (open circles) produced a monotonic decline of the response. The same result was obtained in experiments using the combination of SP and MENK (Fig. 4*E2*).

To quantitate the data, the second SOM response was compared to the third response for each experiment. In the control experiments with SOM alone, the magnitude of the second response was 189% ± 19% ($n = 5$) that of the third response (170% ± 16%; $n = 7$ in MENK experiments), whereas in the test experiment with the preceding SP application, the magnitude of the second response was 57% ± 12%, ($n = 7$) of the third response (49% ± 15%; $n = 4$ in MENK experiments). The differences between the control and test experiments were extremely significant in both the SOM ($P = 0.0001$) and MENK ($P = 0.0007$) experiments. From these data, we find that SP suppresses subsequent SOM and MENK responses, which occurs 20–25 s later, by ≈70%. The results indicate that with the standard GTP solution SP exerted an inhibitory influence on a subsequent SOM or MENK response. Unlike the situation in the GTP[γS]-loaded cells, this inhibitory influence was not permanent and dissipated with time.

DISCUSSION

Opposing Regulation of the Inward Rectifier. The main conclusion is that SP suppresses the same conductance that is enhanced by SOM or MENK and that in the presence of GTP[γS] the suppressing effect of SP is stronger than the effect of SOM or MENK. This conclusion is based on the following: (*i*) SP effectively eliminated the conductance enhanced by SOM or MENK in GTP[γS]-loaded cells (Fig. 3). The final conductance level, regardless of the presence or response size of preceding SOM or MENK application, was approximately the same, suggesting that most of the SOM- or MENK-induced conductance was suppressed by SP. (*ii*) SP inhibited the subsequent effect of SOM or MENK in both GTP-loaded cells (Fig. 4) and in GTP[γS]-loaded cells (Fig. 3 *E* and *F*), indicating that SP suppressed the same conductance that SOM or MENK would have generated. At the molecular level, this conclusion means that the same inward rectifier molecule that is opened by SOM or MENK is closed by SP. In other words, the channel molecule receives opposing influences from G_{PTX} and from G_{nonPTX}. It is noted, however, that our experiments do not necessarily show that "all" inward rectifier K^+ channels that can be suppressed by SP in locus coeruleus neurons receive the dual opposing regulations. Even in the resting neuron some inward rectifiers are constantly active without the presence of transmitters. Because of this basal activity, SP can reduce the resting inward rectifier current (18, 20). Whether this inward rectifier K^+ channel that is active in the resting state can be opened by SOM is an unanswered question.

The opposing regulation of channel activity is not unique for inward rectifiers. In *Aplysia* neurons, opposing regulation acting on an outwardly rectifying K^+ channel (the S channel) was described (21). In heart muscle cells, dual regulations of the L-type Ca^{2+} channel and the hyperpolarization-activated current (I_h) by acetylcholine and norepinephrine were described. This channel modulation, however, results from opposing regulation of adenylate cyclase; the channel itself is solely regulated by the level of cyclic AMP (22, 23).

Speculation on the Interaction Mechanism. The site where the two opposing G proteins (G_{PTX} and G_{nonPTX}) interact to regulate the activity of the inward rectifier is unknown. In the presence of GTP[γS], the G proteins would be activated irreversibly by the first application of agonists, and the activated G proteins would be present persistently. Under these conditions, the interaction takes place downstream from the activation of the G proteins, not upstream, such as at the level of the receptors.

As for the identity of the G proteins, G_{PTX} is likely to belong to the G_i superfamily. On the other hand, the G_{nonPTX} that couples to the SP receptor in nucleus basalis neurons has been shown to be G_q or G_{11} (24). It is highly unlikely that the activated form of these two kinds of G proteins neutralize each other in the presence of GTP[γS].

Not much is known about the signal transduction mechanism of the SP effect on the inward rectifier. Recently, Takano *et al.* (25) have shown that protein kinase C is a second messenger of the SP effect on the inward rectifier in nucleus basalis neurons. The same mechanism may exist for the SP effect in locus coeruleus neurons.

In the case of SOM or MENK effects, there is strong evidence that G_{PTX} opens the inward rectifier directly (or via a membrane-delimited pathway) without diffusible messengers (26, 27). Therefore, the site of the interaction of the two opposing signals (regardless of the signal transduction mechanism of the SP effect) must also reside within this membrane-delimited pathway. We speculate that the most likely site of the interaction would be on the K^+-channel molecule itself.

The fact that the SP effect is stronger than the SOM or MENK effect may merely reflect the presence of two independent gating sites: when the closing gate is closed, it is impossible to observe the state of the opening gate. Interestingly, molecular structures of the cloned inward rectifiers show several consensus protein kinase phosphorylation sites (4, 7, 8); phosphorylation of such sites could be a central mechanism of channel closing.

G-Protein-Coupled Inward Rectifiers in Brain and Atrial Cells. The G-protein-coupled inward rectifier channel in atrial cells seems to respond only to an opening influence from G_{PTX}. In fact, in atrial cells we are not aware of any reports of slow excitatory synaptic actions induced by reduction of inward rectifier K^+ channel. Thus, the G-protein-coupled inward rectifiers in brain neurons differ from those in atrial cells. Alternatively, similar opposing dual regulations may exist in atrial cells that have not yet been discovered.

Interaction of Slow Synaptic Actions. The present experiments have revealed a unique interaction between slow excitatory and slow inhibitory actions. In the presence of GTP[γS] an excitatory transmitter (or G_{nonPTX}) readily suppresses the conductance enhanced by an inhibitory transmitter (or G_{PTX}), while an inhibitory transmitter cannot overcome the effect of an excitatory transmitter. In the presence of GTP, the excitatory effect of SP again overpowers the inhibitory effect of SOM or MENK: SP worked effectively in the presence of the SOM or MENK response (Fig. 2), whereas under the influence of SP the effectiveness of MENK or SOM was temporarily suppressed (Fig. 4). These kinds of interactions of synaptic actions are not well known, but there is a report demonstrating that the inhibitory action of dopamine on spike frequency is lessened in the presence of an excitatory influence by neurotensin (28). This may well be caused by the interacting effects of two different kinds of G proteins reported here.

We thank Marta Margeta-Mitrovic for participation in some of the experiments. We are indebted to Ronald H. Farkas, Tohru Kozasa, Sarah A. Shefner, and Peter R. Stanfield for critically reading the manuscript and giving valuable suggestions. Thanks are also due to Linda Johnston and Alyse G. Kondrat for technical and editorial help. The work was supported by National Institutes of Health Grants AG06093 and DA05701.

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