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# The reduction of neuronal calcium currents by ATP- $\gamma$ -S is mediated by a G protein and occurs independently of cyclic AMP-dependent protein kinase

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We studied the effects of ATP- $\gamma$ -S on the T, N and L calcium current components of nodose ganglion neurons using the whole cell variation of the patch clamp technique. ATP- $\gamma$ -S can serve as a phosphate donor in kinase-mediated reactions, the donated phosphate group being resistant to the action of phosphatases. We therefore compared the effect of ATP- $\gamma$ -S to that of the catalytic subunit of the cyclic AMP-dependent protein kinase (AK-C), included in the recording pipette with 5 mM ATP. AK-C (50  $\mu$ g/ml) had no effect on the T current, and caused a ~30% increase in currents containing the N and L components during a 20-min recording, as compared to a ~45% decrease in control currents. In contrast, in the presence of 2.5 mM ATP- $\gamma$ -S, T currents declined ~30%, and currents containing the N and L components declined to a greater extent than control currents, about 65%. In addition, the time to peak current was increased from ~14 ms to ~40 ms. This effect of ATP- $\gamma$ -S on calcium currents was similar to that of certain neurotransmitters or GTP- $\gamma$ -S, an activator of G proteins, except that the effects of ATP- $\gamma$ -S were delayed 5–7 min relative to GTP- $\gamma$ -S. The effects of both ATP- $\gamma$ -S and GTP- $\gamma$ -S were reduced or abolished in neurons treated with pertussis toxin. We conclude that AK-C regulates neuronal calcium currents, presumably by phosphorylation of channels or associated proteins, and that the ATP- $\gamma$ -S-induced reduction of calcium currents cannot be due to its serving as a phosphate donor for endogenous AK. Instead, ATP- $\gamma$ -S reduces calcium currents via a G protein, perhaps by its conversion to GTP- $\gamma$ -S.

## INTRODUCTION

Many neurotransmitters regulate the activity of voltage-sensitive neuronal calcium channels, but the exact mechanisms by which channel activity is affected are not yet fully defined. For example, norepinephrine, adenosine, neuropeptide Y, bradykinin, dynorphin A and acetylcholine<sup>2,8,10,11,15,29,35,36</sup> reduce calcium currents in a variety of neuronal cell types. In most cases, the neurotransmitter receptors are coupled to calcium channels by GTP binding (G) proteins<sup>10,11,17,24,35,36</sup> sensitive to pertussis toxin (PTX). GTP- $\gamma$ -S, a stable thiol derivative of GTP which activates G proteins<sup>14,30</sup>, has effects similar to some of these neurotransmitters, e.g., calcium currents are reduced and current activation is slowed<sup>7,19,35</sup>. Furthermore, in neurons treated with PTX, which inactivates G<sub>i</sub>- and G<sub>o</sub>-type G proteins, purified  $\alpha_o$  or  $\alpha_i$  subunits can 'reconstitute' the neurotransmitter response<sup>10,11,21</sup>. These findings demonstrate the central role of G proteins in regulating calcium channel activity.

It is not clear from these studies whether G proteins influence neuronal calcium channel activity directly, as occurs for cardiac calcium channels gated by G<sub>s</sub><sup>37</sup> or

whether they influence channels indirectly by coupling neurotransmitter receptors to second messenger systems, which also are important regulators of calcium channel activity. Activation of protein kinase C, for example, reduces vertebrate neuronal calcium currents<sup>16,33</sup> and increases invertebrate calcium currents<sup>6,34</sup>. Cyclic AMP and its analogs increase invertebrate neuronal calcium currents as well as those in vertebrate heart cells<sup>3,20,22</sup>. The cyclic AMP-dependent protein kinase (AK) increases calcium channel activity in a clonal pituitary cell line<sup>1</sup>. Furthermore, ATP- $\gamma$ -S prevents run-down of calcium currents in invertebrate neurons and vertebrate cardiac myocytes, an effect similar to that of cyclic AMP or AK<sup>9,27</sup>.

These observations prompted us to examine the effect of ATP- $\gamma$ -S on the calcium current components of nodose ganglion neurons. ATP- $\gamma$ -S can serve as a phosphate donor in kinase-mediated reactions, and although it is a relatively poor substrate compared to ATP, the donated phosphate group is virtually immune to phosphatases<sup>4,5</sup>. We have previously shown that the catalytic subunit of AK (AK-C) selectively increased neuronal N- and L-type calcium currents<sup>18</sup>, and we therefore compared the effect

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of ATP- $\gamma$ -S to that of AK-C. We anticipated that ATP- $\gamma$ -S would serve as a phosphate donor to endogenous AK and that its effects would be similar to those of exogenous AK-C. Currents evoked in the presence of ATP- $\gamma$ -S were unlike those of AK-C, however, and were similar to those of GTP- $\gamma$ -S<sup>19</sup>. We therefore performed additional experiments comparing the effects of ATP- $\gamma$ -S and GTP- $\gamma$ -S, which showed that the actions of both compounds were mediated by a G protein.

## MATERIALS AND METHODS

### Preparation of acutely-dissociated neurons

Nodose ganglion neurons were prepared from 6–10-day-old rats, using a procedure<sup>18,19</sup> modified from Ikeda et al.<sup>25</sup>. Briefly, ganglia were removed and incubated in a culture dish containing 0.5 ml collagenase/ganglion (1 mg/ml in Dulbecco's Minimum Essential Medium, DMEM, GIBCO Laboratories, Grand Island, NY), for 25 min at 37 °C in a 93% air/7% CO<sub>2</sub> atmosphere. The tissue was transferred to a tube containing DMEM and 5% fetal calf serum to inhibit the enzyme, triturated gently to disperse the cells and centrifuged at ~200 rpm for 10 min. The pellet was resuspended in MEM with Earle's salts supplemented with NaHCO<sub>3</sub> (16.5 mM), glucose (28.2 mM), nerve growth factor (10 ng/ml; Boehringer Mannheim), penicillin (50 U/ml), streptomycin (50 mg/ml), CaCl<sub>2</sub> (3.6 mM) and 10% fetal calf serum. The suspension (approximately 0.5 ml/ganglion) was plated at 0.3 ml/dish on polystyrene culture dishes without a substrate and incubated at 37 °C in a 93% air/7% CO<sub>2</sub> atmosphere. An additional 1 ml of medium was added after 30 min, and the cells were used for recordings within 1–24 h.

### Preparation of solutions

The purified catalytic subunit of AK was prepared as described<sup>31</sup>, stored as a 1 mg/ml solution at 4 °C and used within 24 h of preparation. The stock solution was diluted into the pipette recording solution (see below) to a final concentration of 50  $\mu$ g/ml, and thereafter stored on ice. For all recordings in the presence of AK-C, the tip of the recording pipette was filled with standard pipette solution (1–2 mm), and the pipette was then 'back-filled' with the AK-C-containing solution.

Some neurons were pretreated with PTX before recordings were made. PTX was stored as a 50  $\mu$ g/ml solution in 0.1% bovine serum albumin and was diluted into the cultures to a final concentration of 150–200 ng/ml. Recordings were made 12–24 h later; for these recordings, the standard bath solution was used and PTX was included in the pipette solution at a final concentration of 50 or 100 ng/ml.

### Whole cell patch-clamp recordings

Whole cell voltage-clamp recordings were obtained using the whole-cell variation of the patch-clamp technique. Cells were bathed in a solution of the following (in mM): 67 choline Cl, 100 TEA, 5.6 glucose, 5.3 KCl, 5.0 CaCl<sub>2</sub>, 0.8 MgCl<sub>2</sub>, 10 Hepes (pH 7.3–7.4, 310–330 mOsm). Glass recording patch pipettes with resistances of 0.5–1.25 M $\Omega$  were filled with various recording solutions; the standard solution consisted of (in mM): 10 Hepes, 140 CsCl, 10 EGTA, 5 ATP-Mg<sup>2+</sup> and 0.1 GTP or GTP- $\gamma$ -S (all from Sigma). For experiments with ATP- $\gamma$ -S (Boehringer Mannheim), the concentrations of ATP and ATP- $\gamma$ -S (as the Mg<sup>2+</sup> salt) were 2.5 mM each. If necessary, the pH (7.3–7.4) was readjusted with 1 N CsOH after the addition of the ATP. The osmolality was 10–15% below that of the bath solution, usually 280–300 mOsm.

Recordings were made at room temperature using the Axopatch 1-B patch-clamp amplifier (Axon Instruments, Burlingame, CA). Pipette and whole-cell capacitance and series resistance were corrected by compensation circuitry on the patch clamp amplifier.

Typical initial input resistances were 500 M $\Omega$  to 1 G $\Omega$ . Series resistance was estimated by cancellation of the capacitance-charging current transient after patch rupture. Typical values for the series resistance were 2–5 M $\Omega$  and compensation of 80–90% was possible in most cases. Voltage-step commands were generated, and the evoked currents were digitized (5 kHz), stored and analyzed by a microcomputer (IBM AT or equivalent) using the program pClamp (Axon Instruments). The current traces were filtered with a Bessel filter at 10 kHz (–3 dB).

### Analysis of current components

Nodose ganglion neurons contain 3 calcium current components<sup>18,19</sup> similar to T-, N- and L-type currents previously described<sup>12,13,15</sup>. T currents were isolated by evoking currents from holding potentials ( $V_h$ ) of –80 or –90 mV at clamp potentials ( $V_c$ ) of –30 to –20 mV. Currents evoked from  $V_h = -40$  mV at  $V_c$  of +10 or +20 mV were slowly inactivating and consisted primarily of the L current component; currents evoked from  $V_h = -80$  mV at the same  $V_c$  inactivated more rapidly and also contained the N current component. These voltage-clamp protocols underestimated the L current component slightly due to steady-state inactivation at  $V_h = -40$  mV. In addition, a residual N current component was present at  $V_h = -40$  mV in some neurons.

Leak currents were estimated by using hyperpolarizing voltage commands of equal magnitude to the depolarizing commands used to evoke the inward currents. The inverse of the resulting current was digitally subtracted from the relevant inward current to obtain the calcium current.

### Statistical comparisons

Statistical comparisons were made using Student's *t*-test.

## RESULTS

In the first series of experiments, we compared the effects of ATP- $\gamma$ -S and AK-C on calcium currents by evoking currents at 1-min intervals after patch rupture, alternating between  $V_h$  of –80 mV and –40 mV (Fig. 1). In control neurons, calcium currents stabilized within the first 5 min; thereafter, T currents were stable throughout the duration of a typical recording (20–30 min; data not shown)<sup>19</sup>, but currents containing primarily the N and L components declined steadily in magnitude (Fig. 1,A1). To compare the extent of current decline occurring in control and experimental neurons, we normalized peak current ( $I_p$ ) values as a percent of the maximal  $I_p$  value obtained from  $V_h = -80$  mV ( $I_{pmax}$ ) in that neuron. In control neurons,  $I_p$  values of currents evoked from  $V_h = -80$  mV declined to  $65 \pm 4\%$  of  $I_{pmax}$  by 15 min and  $I_p$  values of currents evoked from  $V_h = -40$  mV declined from  $60 \pm 5\%$  to  $29 \pm 4\%$  of  $I_{pmax}$  ( $n = 17$  neurons, mean  $\pm$  S.E.M.).

$I_p$ -voltage relation plots derived from currents evoked in control neurons showed that the T current was evoked at  $V_c$  at or positive to –50 mV, reaching a maximal value at about –20 mV (Fig. 1,A2). At more positive  $V_c$ , the N and L current components were evoked, the  $I_{pmax}$  occurring at ~0 mV.

In other experiments, currents were recorded in the presence of 50  $\mu$ g/ml AK-C, a concentration equivalent

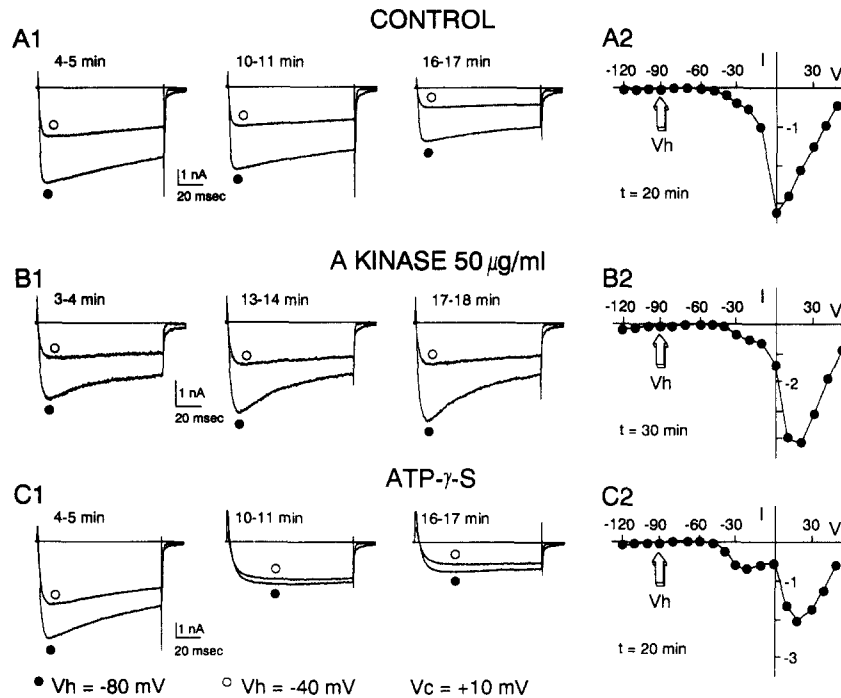


Fig. 1. AK-C increased and ATP- $\gamma$ -S reduced calcium currents in nodose ganglion neurons. In this and other figures, all currents are leak-subtracted. Inward currents are downward. In this figure, currents from 3 neurons are shown, recorded in the absence (A1) and presence of AK-C (B1) or ATP- $\gamma$ -S (C1), included in the recording pipette. Currents were evoked at  $V_c = +10$  mV from  $V_h$  of  $-80$  mV (●) and  $-40$  mV (○). The position of the circles indicates the time at which  $I_p$  occurred. The current-voltage plots were constructed from inward currents recorded in the absence (A1) or presence of AK-C (B2) or ATP- $\gamma$ -S (C2).

to that found intracellularly<sup>23</sup>. There was no effect of AK-C on T currents (not shown)<sup>18</sup>, but currents containing the N and L components increased in magnitude during the recording (Fig. 1,B1). Furthermore, the effect was greater on currents evoked from  $V_h = -80$  mV than those evoked from  $-40$  mV. After 15 min, currents evoked from  $V_h = -80$  mV had increased to  $130 \pm 6\%$  of  $I_{pmax}$  ( $n = 9$  neurons,  $P < 0.05$  compared to control), and those evoked from  $V_h = -40$  mV had increased from  $57 \pm 7\%$  to  $66 \pm 7\%$  of  $I_{pmax}$  ( $P < 0.05$  compared to control).  $I_p$ -voltage plots showed that in the presence of AK-C, T currents were evoked over a similar voltage range compared to controls (Fig. 1,B2), but the  $I_{pmax}$  was evoked at slightly more positive  $V_c$  than in controls.

These results confirmed our previous observations<sup>18,19</sup> and in the next experiments we tested the effect of ATP- $\gamma$ -S on the calcium current components of nodose neurons. Currents evoked in the presence of 2.5 mM ATP- $\gamma$ -S, included in the recording pipette with 2.5 mM ATP, were similar to control currents during the first 5–7 min of recording, but declined rapidly thereafter (Fig. 1, C1). The currents evoked from  $V_h = -80$  mV were affected to a greater extent than those evoked from  $V_h = -40$  mV. In the presence of ATP- $\gamma$ -S,  $I_p$  values of currents evoked from  $V_h = -80$  mV declined to  $36 \pm 5\%$  of  $I_{pmax}$  in 15 min ( $n = 10$  neurons; different than control,  $P < 0.05$ ), but  $I_p$  values of currents evoked from  $V_h = -40$

mV declined from  $64 \pm 3\%$  to  $22 \pm 4\%$  of  $I_{pmax}$  ( $n = 9$ ; no significant difference from control).

Currents evoked in the presence of ATP- $\gamma$ -S also activated more slowly after the onset of the depolarizing voltage command. In control currents, evoked at  $V_c = +10$  mV from  $V_h = -80$  mV,  $I_p$  occurred 10–15 ms after the onset of the voltage command; in the presence of ATP- $\gamma$ -S,  $I_p$  occurred later, averaging  $42 \pm 3$  ms in 10 neurons 15 min after patch rupture.

$I_p$ -voltage plots were also derived from currents evoked in the presence of ATP- $\gamma$ -S (Fig. 1,C2). The T current was evoked at similar potentials as in control neurons. The current evoked at more positive  $V_c$ , however, was smaller than in controls (as shown above) and the maximal  $I_p$  was achieved at more positive  $V_c$ , typically  $+20$  mV. Similar results were obtained in 5 other neurons.

We next tested the effect of ATP- $\gamma$ -S on the T current. Currents were evoked from  $V_h$  of  $-80$  or  $-90$  mV at  $V_c$  of  $-65$  to  $-10$  mV in 5-mV increments. As described above, T currents evoked in control neurons were stable throughout the recording, and were evoked at  $V_c$  at or positive to  $-50$  mV, with maximal currents occurring at about  $-20$  mV (Fig. 2). Typically, there was a small shift of the voltage range of activation as the recording progressed, about  $-5$  mV (not shown)<sup>19</sup>. T currents evoked in the presence of ATP- $\gamma$ -S were similar to

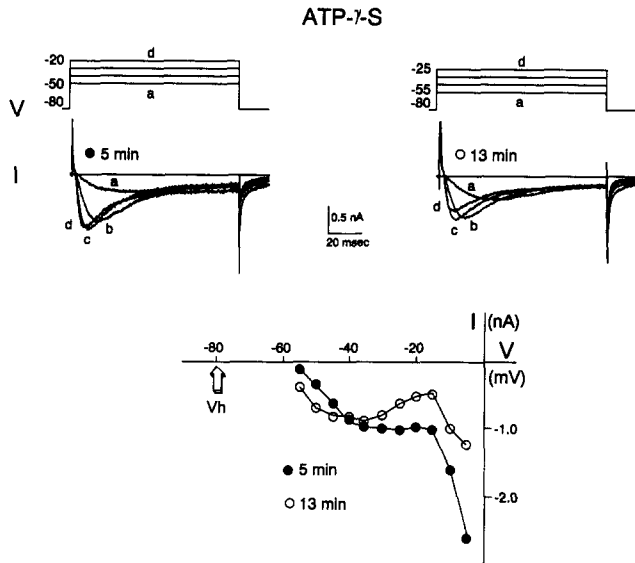


Fig. 2. T currents were reduced in the presence of ATP- $\gamma$ -S. T currents were evoked at 3–5 min intervals from  $V_h$  or  $-80$  or  $-90$  mV at  $V_c$  ranging from  $-65$  mV to  $-10$  mV in the presence of ATP- $\gamma$ -S (top). In this figure, only selected traces (a–d) are shown. At the more depolarized  $V_c$ , an N current component was invariably present. The illustrated currents were recorded before (5 min) and after (13 min) the effect of ATP- $\gamma$ -S became evident ( $\bullet$  and  $\circ$ , respectively).

control T currents within the first 5 min of the recording (Fig. 2, filled circles), but thereafter T currents were smaller, particularly those currents evoked at more depolarized potentials (Fig. 2, open circles). This effect, although present in all neurons tested, could not be readily quantified due to the difficulty in determining

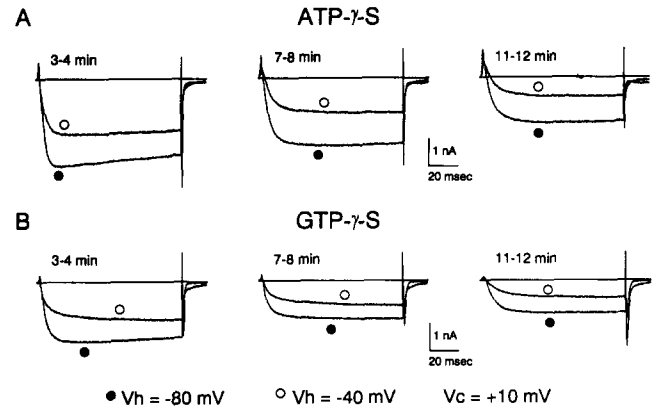


Fig. 3. ATP- $\gamma$ -S and GTP- $\gamma$ -S had similar effects on calcium currents. Currents were evoked from  $V_h = -80$  mV ( $\bullet$ ) and  $-40$  mV ( $\circ$ ) at  $V_c = +10$  mV in the presence of ATP- $\gamma$ -S (A) or GTP- $\gamma$ -S (B). The position of the circle indicates  $I_p$ .

unequivocally if a stable maximal T current amplitude had been achieved. In one experiment, for example, T current was decreased 16–60% in 4 neurons, with a mean reduction of  $34 \pm 9\%$ , measured 10–15 min after patch rupture.

These effects of ATP- $\gamma$ -S on calcium currents were strikingly similar to those of certain neurotransmitters or GTP- $\gamma$ -S<sup>2,7,19,35</sup>. In our next experiments, therefore, we compared the effects of ATP- $\gamma$ -S and GTP- $\gamma$ -S on currents containing the N and L components (Fig. 3A, B). Currents evoked in the presence of either compound declined faster than control currents, and the time  $I_p$  was increased to a similar extent. For example, in 2 different experiments,  $I_p$  values of currents evoked from  $V_h = -80$

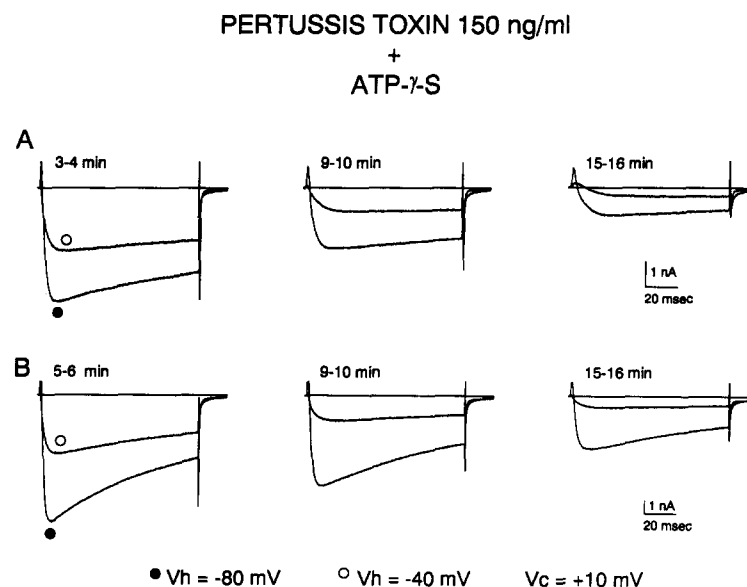


Fig. 4. Pretreatment with PTX reduced or blocked the effect of ATP- $\gamma$ -S. Recordings from 2 neurons are shown, in which currents were evoked from  $V_h = -80$  mV ( $\bullet$ ) and  $-40$  mV ( $\circ$ ) at  $V_c = +10$  mV. Both neurons were pretreated with 150 ng/ml PTX for 16 h, and recordings were made in the presence of ATP- $\gamma$ -S with either 50 ng/ml (A) or 150 ng/ml (B) PTX included in the recording pipette.

mV in the presence of GTP- $\gamma$ -S (Fig. 3B) had declined to  $42 \pm 7\%$  ( $n = 6$  neurons) and  $38 \pm 8\%$  ( $n = 4$  neurons) of  $I_{pmax}$  12 and 15 min after patch rupture, respectively. The time to  $I_p$  was increased to  $56 \pm 10$  ms 15 min after patch rupture, a value similar to that found in currents recorded in the presence of ATP- $\gamma$ -S (Fig. 3A).

One difference between recordings with ATP- $\gamma$ -S and GTP- $\gamma$ -S was the greater time required for the effects of ATP- $\gamma$ -S to become apparent. In all neurons tested, the effect of GTP- $\gamma$ -S was evident within the first 3 min of the recording (Fig. 3B), whereas the effect of ATP- $\gamma$ -S was not evident until about 7 min (Fig. 3A).

The delayed response to ATP- $\gamma$ -S suggested that ATP- $\gamma$ -S may have reduced calcium currents indirectly. Since the effect of ATP- $\gamma$ -S was similar to that of GTP- $\gamma$ -S, we postulated that ATP- $\gamma$ -S may have been acting similarly, i.e., via a G protein<sup>7,19</sup>. If this were the case, PTX, which blocks the effect of GTP- $\gamma$ -S<sup>30</sup> on calcium currents<sup>7,19</sup>, should reduce or abolish the effect of ATP- $\gamma$ -S. The rate of calcium current run-down was increased in neurons pretreated with PTX. For example, in 3 neurons from which calcium currents were recorded for greater than 15 min in the absence of ATP- $\gamma$ -S,  $I_p$  declined to  $72 \pm 6\%$  of  $I_{pmax}$  during a 10-min recording, compared to  $73 \pm 5\%$  during a 14-min recording in control neurons (see Fig. 1)<sup>19</sup>. Current run-down was not changed in the presence of ATP- $\gamma$ -S, but PTX pretreatment reduced or blocked the effect of ATP- $\gamma$ -S in 6 neurons (Fig. 4). In all of these cases, the increase in time to  $I_p$  was less and occurred later in the recording than in the absence of PTX (compare Fig. 1 and 4).

## DISCUSSION

The results of this study show that ATP- $\gamma$ -S had profound effects on neuronal calcium currents, but it is improbable that these effects were due to ATP- $\gamma$ -S acting as a phosphate donor for endogenous AK. This becomes apparent when the effects of ATP- $\gamma$ -S and AK-C are compared. AK-C had no effect on T current, but prevented the loss of the labile (N and L) calcium current components usually observed during recordings using the whole cell voltage-clamp technique. This finding suggests that current run-down may be a direct result of intracellular 'dialysis'<sup>27</sup> and that AK-mediated phosphorylation of calcium channels or associated proteins may prevent channel inactivation or run-down<sup>1,9,18,26</sup>. The greater effect of AK-C on currents evoked from  $V_h = -80$  mV suggests that the N current component may be affected by AK-C more than the L current component<sup>18</sup>.

In contrast, ATP- $\gamma$ -S reduced calcium currents to an extent greater than that produced during run-down in control recordings. This effect was not merely due to the

reduced concentration of ATP in the recording pipette, as the rate of current run-down was similar whether 2.5 or 5 mM ATP was included in the recording pipette solution (RAG and RLM, unpublished observations). Others have reported that ATP- $\gamma$ -S and AK-C have similar effects on L-type currents<sup>1,9</sup>, but in the present experiments the effect of ATP- $\gamma$ -S was opposite to that of AK-C. Like AK-C, ATP- $\gamma$ -S had a greater effect on currents containing the N current component, with little effect on currents evoked from  $V_h = -40$  mV, containing the L current component primarily. Thus, in nodose ganglion neurons, which contain multiple calcium current components, the major effect of ATP- $\gamma$ -S was likely on the N current component.

ATP- $\gamma$ -S also reduced T currents, although this was not apparent throughout the voltage range of activation of this current component. One possible explanation is that the voltage range of activation of the N current overlapped sufficiently with that of the T current so that at  $V_c$  of  $-30$  to  $-10$  mV there was a small N current component not readily distinguished from T currents, e.g., by a slower rate of current inactivation. In this way, an apparent effect of ATP- $\gamma$ -S on the T current at the more depolarized  $V_c$  of its activation range would, in fact, be the result of an effect on the N current component. Another possibility is that ATP- $\gamma$ -S prevents T channel opening, an effect only seen at more depolarized  $V_c$  when a larger proportion of T channels open. Further studies, using single channel recording, will be required to resolve this issue.

The effect of ATP- $\gamma$ -S, unlike that of GTP- $\gamma$ -S, was delayed several minutes, but otherwise the effects of the two compounds were similar. PTX reduced or blocked the effects of both compounds on calcium currents, suggesting that their effects were produced as the result of activation of  $G_i$ - or  $G_o$ -type G proteins. This mechanism might account for the reduction of calcium current by both ATP- $\gamma$ -S and GTP- $\gamma$ -S, but it does not readily explain the delay in the onset of the ATP- $\gamma$ -S effect. One possibility is that ATP- $\gamma$ -S reduced calcium currents by acting as a phosphate donor for kinase other than AK. Activators of protein kinase C, for example, reduce calcium currents<sup>16,33</sup>, an effect which is also PTX-sensitive<sup>16</sup>. Another possibility is that GTP- $\gamma$ -S, as a low-level contaminant in ATP- $\gamma$ -S, produced its effects on calcium currents relatively slowly. We consider this unlikely, however, as the ATP- $\gamma$ -S used in these experiments was manufactured from ATP and was therefore free of contaminating guanosine bases (by HPLC, performed by the manufacturer). Finally, it is possible that ATP- $\gamma$ -S was converted to GTP- $\gamma$ -S intracellularly. A recent report has shown that conversion of ATP- $\gamma$ -S to GTP- $\gamma$ -S occurs in heart cells<sup>32</sup>. In that preparation,

ATP- $\gamma$ -S activated potassium currents with a time delay similar to that observed in the present experiments. Further studies will be required, but the present results are consistent with the idea that the ATP- $\gamma$ -S-induced reduction of neuronal calcium currents was due to its intracellular conversion to GTP- $\gamma$ -S.

In summary, we have shown that ATP- $\gamma$ -S reduced and AK-C enhanced neuronal calcium currents. Thus, ATP- $\gamma$ -S cannot be acting solely as a phosphate donor for AK-mediated actions on calcium channels, a finding which raises a caveat regarding the specificity of this compound in whole cell experiments. Our results confirm that the cyclic AMP-AK system regulates calcium channel activity, and suggest, in addition, that neuronal N channels may be more sensitive to AK-mediated modulation than L channels. This is of particular interest in

light of the relatively selective effect of certain neurotransmitters on the N current<sup>2,15,17,28,35</sup>. In contrast, the effect of ATP- $\gamma$ -S, like those of neurotransmitters, was dependent on G proteins, perhaps because of its intracellular conversion to GTP- $\gamma$ -S. Taken together, these results suggest that neuronal calcium channels are regulated by multiple independent G protein-dependent pathways.

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