Muscarinic receptors couple to modulation of nicotinic ACh receptor desensitization in myenteric neurons

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Brown, Erika N., and James J. Galligan. Muscarinic receptors couple to modulation of nicotinic ACh receptor desensitization in myenteric neurons. Am J Physiol Gastrointest Liver Physiol 285: G37–G44, 2003. First published April 2, 2003; 10.1152/ajpgi.00053.2003.—Signaling mechanisms coupled to activation of different neurotransmitter receptors interact in the enteric nervous system. ACh excites myenteric neurons by activating nicotinic ACh receptors (nAChRs) and muscarinic receptors expressed by the same neurons. These studies tested the hypothesis that muscarinic receptor activation alters the functional properties of nAChRs in guinea pig small intestinal myenteric neurons maintained in primary culture. Whole cell patch-clamp techniques were used to measure inward currents caused by ACh (1 nM) or nicotine (1 nM). Currents caused by ACh and nicotine were blocked by hexamethonium (100 μM) and showed complete cross desensitization. The rate and extent of nAChR desensitization was greater when recordings were obtained with ATP/GTP-containing compared with ATP/GTP-free pipette solutions. These data suggest that ATP/GTP-dependent mechanisms increase nAChR desensitization. The muscarinic receptor antagonist scopolamine (1 μM) decreased desensitization caused by ACh but not by nicotine, which does not activate muscarinic receptors. Phorbol 12,13-dibutyrate (10–100 nM), an activator of protein kinase C (PKC), but not 4-α-phorbol 12-myristate 13-acetate (a PKC inactive phorbol ester), increased nAChR desensitization caused by ACh and nicotine. Forskolin (1 μM), an activator of adenylate cyclase, increased nAChR desensitization, but this effect was mimicked by dideoxyforskolin, an adenylate cyclase inactive forskolin analog. These data indicate that simultaneous activation of nAChRs and muscarinic receptors increases nAChR desensitization. This effect may involve activation of a PKC-dependent pathway. These data also suggest that nAChRs and muscarinic receptors are coupled functionally through an intracellular signaling pathway in myenteric neurons.

enteric nervous system; electrophysiology; intracellular signaling

IT HAS BEEN KNOWN for some time that “cross-talk” between intracellular signaling pathways activated by different classes of cell surface receptors is a mechanism for integration of information coming into individual cells, including neurons (12, 24). Typically, these interactions involve G protein-linked receptors and two or more intracellular signaling pathways composed of multiple enzymatic processes and diffusible signaling molecules (12, 23). Recently, it has been shown that there are interactions between different ligand-gated ion channels that result in inhibition of signals mediated by each receptor type. This interaction does not involve diffusible second messenger molecules but instead may involve direct protein-protein interaction. For example, in myenteric neurons, nicotinic ACh receptors (nAChRs) and P2X receptors exhibit cross inhibition when these receptors are activated simultaneously (9, 26). In the submucosal plexus, a similar direct inhibitory interaction occurs between P2X receptors and 5-HT3 receptors (1).

ACh, acting at nAChRs and at M1 muscarinic receptors, is an important excitatory neurotransmitter in the enteric nervous system (ENS; see Refs. 6, 7, 15, 16). M1 muscarinic receptors are G protein-coupled receptors that link to activation of phospholipase C (PLC) and generation of inositol trisphosphate and diacylglycerol (DAG; see Ref. 19). DAG activates protein kinase C (PKC), and PKC can phosphorylate a variety of intracellular targets, including ion channels (21). M1 muscarinic receptors mediate some slow excitatory postsynaptic potentials (sEPSPs) in the ENS (17, 18). Furthermore, drugs that inhibit the PLC-PKC signaling pathways can inhibit sEPSPs, whereas drugs that activate this system can mimic sEPSPs (2). The sEPSPs are associated with a long-lasting increase in excitability. ACh acting at nAChRs mediates most fast excitatory postsynaptic potentials (fEPSPs) in the ENS (17, 18). There are neurons in the ENS that receive dual excitatory synaptic input mediated by ACh acting at nAChRs and muscarinic receptors (17, 18). The long-lasting increase in excitability subsequent to muscarinic receptor activation can potentiate fEPSPs mediated by nAChRs on the same neuron. This is a mechanism by which two different receptors can interact in the ENS. However, it is also known that nAChRs are targets for phosphorylation, which alters desensitization and other functional properties of the nAChR (4, 5, 8, 20). It is not known if the functional properties of nAChRs in the ENS can be modified after activation of intracellular signaling pathways. In the present study, we tested the hypothesis that simultaneous activation...
of nAChRs and muscarinic receptors is associated with an alteration in desensitization of nAChRs expressed by the same myenteric neurons. Data from these studies would provide evidence that different types of receptor for the same transmitter can interact functionally and therefore modify interneuronal signaling.

MATERIALS AND METHODS

Primary culture of myenteric neurons. The procedures used in these studies are similar to previously published methods using similar preparations (9, 26, 27). Newborn (1 to 2 days old) guinea pigs were anesthetized by halothane inhalation, stunned, and exsanguinated by severing major neck blood vessels. The entire small intestine was placed in 4°C Krebs bicarbonate buffer of the following composition (in mM): 117 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2 MgCl₂, 1.2 NaH₂PO₄, 25 NaHCO₃, and 11 glucose. The longitudinal muscle myenteric plexus was stripped free using a moist cotton swab and then was cut into 5-mm pieces. Dissected tissues were divided into two equal aliquots, and each aliquot was transferred to 1 ml of sterile-filtered Krebs solution containing 1,600 units of trypsin (Sigma Chemical, St. Louis, MO) for 30 min at 37°C. After trypsin incubation, tissues were triturated 30 times through a fire-polished Pasteur pipette and centrifuged at 900 g for 10 min using a bench-top centrifuge. The supernatant was discarded, and the pellet was resuspended in 1 ml Krebs solution containing 4,000 units crab hepatopancreas collagenase (Calbiochem-Novabiochem, La Jolla, CA). The suspension was triturated using a fire-polished Pasteur pipette and then centrifuged for 10 min. The pellet was resuspended in Eagle's MEM containing 10% FBS, 10 μM gentamicin, 100 U/ml penicillin, and 50 mg/ml streptomycin (all from Sigma). Aliquots (200 μl) were plated on sterile, poly-lysine (mol wt 30,000–70,000; Sigma)-coated glass cover slips placed in 35-mm plastic culture dishes containing 3 ml MEM. After 2 days of incubation, cytotoxic arabanosite (10 μM) was added to the MEM to limit smooth muscle and fibroblast proliferation. Cultures were maintained at 37°C in a tissue culture incubator containing a 5% CO₂ atmosphere. Cultures were maintained up to 2 wk after plating with medium replacement every 2 days.

Whole cell patch-clamp recordings. The glass cover slips containing neurons was removed from the culture dishes and placed in a Plexiglas recording chamber (3 ml volume) with a glass bottom. The recording chamber was placed on the stage of an inverted microscope, and neurons were viewed using Hoffman modulation contrast optics. The extracellular solution was the standard Krebs solution described above. The Krebs solution was superfused through the recording chamber at a flow rate of 4 ml/min. Fire-polished patch-clamp pipettes were fabricated from borosilicate glass capillary tubes (World Precision Instruments, Sarasota, FL). The fire-polished pipettes had a tip resistance of 3–7 MΩ. Electrode liquid junction potentials and series resistance were compensated. The standard intracellular (pipette) solution for patch-clamp recording was as follows (in mM): 160 CsCl, 2.9 MgCl₂, 10 EGTA, 10 HEPES, 0.5 ATP, and 0.25 GTP; pH was adjusted to 7.4 using CsOH. The CaCl₂-to-EGTA ratio yielded a resting level of free Ca²⁺ concentration of <100 nM. Experiments were performed using an Axopatch 200B patch-clamp amplifier, a Digidata 1200 analog-to-digital converter, and pCLAMP 6.01 programs for acquisition, storage, and analysis of data (Axon Instruments, Burlingame, CA). Data were filtered at 2 kHz using a four-pole Bessel filter (Warner Instruments, New Haven, CT) and digitized at a rate of 5 kHz and stored on the computer hard drive. Unless otherwise indicated, the holding potential was −60 mV.

Drug application. Drugs were applied via flow tubes gated by computer-controlled solenoid valves. Four of these flow-through tubes were glued together at the tip, and each was connected via polyethylene tubing to a reservoir syringe (10 ml) containing a known concentration of drug. The reservoir platform was positioned above the recording chamber so that drugs were gravity fed through the flow tubes. Platform height was adjusted to provide a flow rate of 0.1 ml/min. The flow tube array was mounted on a micromanipulator, and drugs were applied by positioning the flow tubes over the neuron. This method permitted rapid adjustments between control and drug-containing solution (equilibrium <100 ms).

Drugs. All drugs were obtained from Sigma Chemical. ACh and nicotine were prepared as 1 M stock solutions in deionized water. Phorbol 12,13-dibutyrate (PDBu), 4-α-PMA, forskolin, and dideoxyforskolin (DDF) were prepared as stock solutions in 50% (vol/vol) ethanol-deionized water and were then diluted to working concentrations in Krebs buffer. The final ethanol concentration in the buffer did not exceed 0.05%.

Statistics. All data were expressed as means ± SE, and n values refer to the number of neurons from which data were obtained. Differences between treatment groups were established using Student’s t-test for paired and unpaired data, and one-way ANOVA and the Student-Newman-Keuls test for multiple comparisons. A P value <0.05 was taken as the level of statistical significance.

RESULTS

ACh and nicotine activate nAChRs in myenteric neurons. ACh (1 mM) caused rapidly developing and desensitizing inward currents in >90% of neurons tested (Fig. 1A). ACh caused inward currents that did not decline in amplitude over 20 min, the time course of most experiments done here. In eight neurons, the initial ACh response was 1.3 ± 0.3 nA, whereas at 20 min the response amplitude was 1.5 ± 0.3 nA (P > 0.05). These data are similar to those published previously (27). ACh-induced currents were the result of activation of nAChRs because they were mimicked by nicotin (1 mM; Fig. 1A). In addition, ACh- and nicotine-induced currents were blocked completely and reversibly by the nAChR antagonist hexamethonium (Fig. 1B). ACh and nicotine also activated the same receptors as responses caused by nicotine, and ACh exhibited cross desensitization. In these experiments, ACh (1 mM) was applied for 2 s, and, after a 5-min recovery, nicotine was applied for 2 s. After an additional 5 min of recovery, ACh was applied again for 7 s, and the inward current decayed to a steady-state level during this time (see below). Nicotine was applied again at this point, and the nicotine-induced current was reduced significantly (Fig. 1C). After a 5-min recovery period, the ACh response returned to near its initial amplitude (Fig. 1C).

Modulation of nAChR desensitization during muscarinic receptor activation. ACh-induced currents decayed in the continued presence of agonist, and the ACh-induced current reached a steady-state level within 7 s (Fig. 2, A and B). The time course of desensitization was quantified as the time to half decay (T½)
The inward current was then measured at 1-s intervals until the current reached a steady-state level ($I_{\text{steady \ state}}$; Fig. 2A). The amount of nAChR desensitization was quantified by calculating the ratio of $I_{\text{steady \ state}}$ versus $I_{\text{peak}}$ in the same neuron (Fig. 2A). ACh-induced currents declined by one-half in <3 s and reached a steady-state level of ~77% of the initial peak current (Fig. 2B; Table 1).

Desensitization of nAChRs can be modulated by a variety of intracellular signaling pathways and receptor phosphorylation (20). Therefore, we tested the effects of removing ATP and GTP from the pipette solution on the rate and extent of nAChR desensitization in myenteric neurons. The peak amplitude of the ACh-induced current recorded using an ATP/GTP-free pipette solution was not different from the peak amplitude obtained using ATP/GTP-containing pipette solutions (Table 1). Furthermore, the ACh-induced current recorded without ATP/GTP in the recording pipette was stable in amplitude over a 20-min recording period.

Table 1. Properties of nAChR desensitization under different recording conditions

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$n$</th>
<th>Peak Current, nA</th>
<th>$T_{1/2}$, s</th>
<th>$I_{\text{steady \ state}}/I_{\text{peak}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACh (1 mM)</td>
<td>10</td>
<td>$1.4 \pm 0.2$</td>
<td>$2.5 \pm 0.3$</td>
<td>$0.29 \pm 0.03$</td>
</tr>
<tr>
<td>Control Without ATP/GTP</td>
<td>10</td>
<td>$1.8 \pm 0.4$</td>
<td>$3.9 \pm 0.4^*$</td>
<td>$0.44 \pm 0.04^*$</td>
</tr>
<tr>
<td>Scopolamine (1 μM)</td>
<td>10</td>
<td>$1.2 \pm 0.2$</td>
<td>$5.0 \pm 0.4^*$</td>
<td>$0.55 \pm 0.04^*$</td>
</tr>
<tr>
<td>Nicotine (1 mM)</td>
<td>7</td>
<td>$1.3 \pm 0.2$</td>
<td>$3.8 \pm 0.4$</td>
<td>$0.4 \pm 0.04$</td>
</tr>
<tr>
<td>Scopolamine (1 μM)</td>
<td>6</td>
<td>$1.3 \pm 0.3$</td>
<td>$4.1 \pm 0.8$</td>
<td>$0.39 \pm 0.03$</td>
</tr>
</tbody>
</table>

Data are means ± SE of measurements obtained from $n$ neurons. Data were analyzed using ANOVA and Student-Newman-Keuls multiple-comparisons test. nAChR, nicotinic ACh receptor; $T_{1/2}$, time to half decay; $I_{\text{steady \ state}}$, current at steady-state level; $I_{\text{peak}}$, peak current. *Significantly different from control ACh groups ($P < 0.05$). †Significantly different from the group without ATP/GTP.
When an ATP/GTP-free pipette solution was used, the initial ACh-induced current was $1.5 \pm 0.5 \text{nA}$, whereas 20 min after establishing the whole cell recording the ACh response amplitude was $1.4 \pm 0.5 \text{nA}$ ($n = 6$, $P > 0.05$). When recordings were obtained using an ATP/GTP-free pipette solution, the $T_{1/2}$ for decay of the ACh-induced current increased to 4 s, whereas the steady-state current was $\sim 60\%$ of the initial peak current (Fig. 2B and Table 1).

Myenteric neurons express muscarinic cholinergic receptors that can couple to activation of one or more intracellular signaling pathways, leading to excitation of myenteric neurons. It is possible that simultaneous activation of muscarinic receptors by ACh could modulate nAChR function. Therefore, we tested the effects of scopolamine (1 \text{ \mu M}), a muscarinic receptor antagonist, on the rate and extent of nAChR desensitization recorded using an ATP/GTP-containing pipette solution. The peak amplitude of the ACh-induced current recorded in the presence of scopolamine was not different from that recorded under control conditions (Table 1). Furthermore, it was found that scopolamine decreased both the rate and extent of nAChR desensitization (Fig. 2B and Table 1). Scopolamine also increased the rate of recovery from desensitization of nAChRs caused by ACh. This conclusion is based on studies in which ACh (1 mM) was applied for 7 s to cause nAChR desensitization, and ACh was reapplied at several time points after the initial response to determine the time course of recovery from desensitization (Fig. 3A). Under control recording conditions, the ACh response had a time to one-half recovery of $0.1 \pm 0.04 \text{min}$ ($n = 7$), whereas in the presence of scopolamine (1 \text{ \mu M}) the time to one-half recovery was reduced to $0.03 \pm 0.01 \text{min}$ ($n = 6$, $P < 0.05$; Fig. 3B).

Scopolamine does not alter nAChR desensitization caused by nicotine. It is possible that scopolamine could alter the rate and extent of nAChR desensitization through mechanisms other than blockade of muscarinic receptors. To rule out this possibility, we tested the effects of scopolamine on currents caused by nicotine, which will not activate muscarinic receptors. It was found that both the time course and extent of nAChR desensitization caused by nicotine were unaffected by scopolamine (Fig. 4A and Table 1). Scopolamine also did not alter the time course of recovery from desensitization caused by nicotine (Fig. 4B). The time to one-half recovery from nicotine desensitization under control conditions was $0.67 \pm 0.1 \text{min}$, whereas in the presence of scopolamine this value was $0.51 \pm 0.1 \text{min}$ ($P > 0.05$).

Bethanechol increases nAChR desensitization. The data presented above indicate that muscarinic receptor activation can increase nAChR desensitization. This issue was investigated further by using nicotine to selectively activate and desensitize nAChRs in the absence and presence of the muscarinic receptor antagonist bethanechol (BeCh; 100 \text{ \mu M}). It was found that under control conditions, the amount of desensitization in the same neuron was stable on successive nicotine applications (Fig. 5, left). However, when neurons were treated with BeCh, the amount of nAChR desensitiza-
tion caused by nicotine increased significantly (Fig. 5, right). BeCh did not change the amplitude of the peak nicotine-induced current. The control peak nicotine current was 1.7 ± 0.5 nA, whereas in the presence of BeCh this value was 1.8 ± 0.6 nA (P < 0.05, n = 5).

Phorbol ester-induced modulation of nAChR desensitization. Myenteric neurons express M₁-type muscarinic receptors that couple via G₄ to the phosphatidylinositol (PI)-dependent signaling pathway and activation of PKC (19). Therefore, PDBu was used to activate PKC to investigate a role for this enzyme in modulating nAChR desensitization. ACh (1 mM; in the presence of 1 μM scopolamine) or nicotine (1 mM) was applied after pretreating neurons with vehicle (0.05% ethanol) or PDBu (10–300 nM). It was found that PDBu increased the maximum nAChR desensitization occurring during a 7-s application of ACh (Fig. 6A) or nicotine (Fig. 6B). However, PDBu did not change the amplitude of the peak ACh- or nicotine-induced current (Table 2). To test that PDBu was not acting via a PKC-independent mechanism to alter nAChR desensitization, a PKC-inactive phorbol ester analog, 4-α-PMA, was tested. 4-α-PMA (10–300 nM) did not alter the amount of nAChR desensitization caused by either ACh (Fig. 6A) or nicotine (Fig. 6B). Furthermore, 4-α-PMA did not change the peak ACh- or nicotine-induced currents (Table 2).

### DISCUSSION

ACh is the principal excitatory neurotransmitter in the ENS, and ACh acts at nAChRs and M₁ muscarinic receptors expressed by the same neurons to cause synaptic excitation (6, 7, 17, 18). The different time course and signaling pathways coupled to nAChRs and M₁ muscarinic receptors provide a basis for integration of synaptic input and output of the neuron receiving the mixed cholinergic input. In addition, the data from the present study indicate that activation of musca-
nAChR-mediated inward currents in myenteric neurons. Previous studies have shown that ACh acts at nAChRs to cause an inward current in myenteric neurons maintained in primary culture (24). These results were confirmed in the present study where it was shown that ACh caused a rapidly developing and desensitizing inward current that was blocked completely by the nAChR antagonist hexamethonium. The ACh-induced current was mimicked by the selective nAChR agonist, nicotine, and the nicotine-induced current was also blocked by hexamethonium. In addition, the ACh- and nicotine-induced currents cross-desensitized, indicating that these responses were mediated at the same population of nAChRs. The amplitude of the ACh-induced current is stable over the period of at least 20 min (see also Ref. 27), and omitting ATP and GTP from the recording pipette solution did not alter the peak ACh current over this time period. However, it was found that the time course and extent of nAChR desensitization were altered by omitting ATP and GTP at least 20 min (see also Ref. 27), and omitting ATP and GTP from the recording pipette solution did not alter the ACh-induced current is stable over the period of at least 20 min (see also Ref. 27), and omitting ATP and GTP from the recording pipette solution did not alter the peak ACh current over this time period. However, it was found that the time course and extent of nAChR desensitization were altered by omitting ATP and GTP from the pipette solution. This result suggests that ATP- and or GTP-dependent processes modify nAChR desensitization. Because ATP and GTP are substrates for kinase-dependent reactions, phosphorylation of the nAChR is a mechanism by which desensitization can be modulated (8, 16).

Muscarinic receptor activation increases nAChR desensitization. Myenteric neurons express both nAChRs and M₁ muscarinic receptors (6, 7, 17, 18). M₁ muscarinic receptors are G protein-coupled receptors that are linked to activation of PLC and PI hydrolysis (19). PI hydrolysis can lead to release of DAG and PKC activation (21). The data from the present study indicate that simultaneous activation of nAChRs and muscarinic receptors increases the amount of nAChR desensitization occurring during a 7-s application of ACh. This conclusion is based on the result showing that the addition of scopolamine, a muscarinic receptor antagonist, to the extracellular solution decreased the rate and extent of nAChR desensitization caused by ACh. Therefore, simultaneous activation of muscarinic receptors modulates the function of nAChRs expressed by the same neuron. M₁ muscarinic receptors mediate eEPSPs in the myenteric plexus (17, 18). The M₁-
mediated sEPSP is the result of an inhibition of potassium channels that are open near the resting membrane potential (18). The pipette solution used in the present study contained cesium ions to block all potassium channels. Therefore, changes in resting conductance of the neurons would not contribute to the muscarinic receptor-mediated change in nAChR desensitization.

Scopolamine could have effects on myenteric neurons that are independent of muscarinic receptor antagonism. For example, scopolamine could interact directly with the nAChR ion channel to alter its functional properties (14). To test the possibility that scopolamine altered nAChR desensitization by a muscarinic receptor-independent mechanism, nicotine was used as an nAChR agonist. Because nicotine does not activate muscarinic receptors, scopolamine should not alter nAChR desensitization if it acts only at muscarinic receptors. It was found that the rate and extent of nicotine-induced nAChR desensitization was unaffected by scopolamine; therefore, scopolamine did not interact with the nAChR via a muscarinic receptor-independent mechanism.

The data discussed above suggest that simultaneous activation of muscarinic receptors alters nAChR desensitization. To test this hypothesis more directly, nicotine and the muscarinic receptor agonist BeCh were applied simultaneously to individual neurons. When BeCh was coapplied with nicotine, the amount of nAChR desensitization increased compared with the level occurring during the control recording. Taken together, these data indicate that activation of muscarinic receptors increases nAChR desensitization during simultaneous activation of the two cholinergic receptors.

**PKC activation modulates nAChR desensitization.** Because M1 muscarinic receptors couple to generation of DAG (19) and DAG activates PKC (21), we tested the effects of the PKC activator PDBu on nAChR desensitization. PDBu caused a concentration-dependent increase in the amount of nAChR desensitization caused by ACh (in the presence of scopolamine to block muscarinic receptors) and by nicotine. However, PDBu can alter nAChR desensitization by an action that is independent of PKC activation (15). To test this possibility, we used 4-α-PMA, a phorbol ester that does not activate PKC, in our desensitization protocol. 4-α-PMA, in a concentration range identical to that used for PDBu, did not alter nAChR desensitization caused by either ACh or nicotine. These data suggest that PDBu-induced changes in nAChR desensitization were mediated by PKC activation. Although PDBu increased nAChR desensitization, the phorbol ester did not alter the peak currents caused by either ACh or nicotine. This result indicates that PKC activation does not alter the affinity of the nAChR for agonist. In rat cardiac parasympathetic neurons, vasoactive intestinal peptide and pituitary adenylate cyclase-activating peptide (PACAP) both cause an increase in peak nAChR-mediated currents, and this increase in response has been attributed to an increase in affinity of the nAChR for ACh (11). Vasoactive intestinal peptide and PACAP receptors modify nAChR function through a mechanism that is independent of a diffusible second messenger but may use a membrane-delimited but G protein-dependent pathway (11). Also, in rat cardiac parasympathetic neurons, receptors for substance P couple to an inhibition of a subset of nAChRs. This mechanism also does not involve a diffusible, cytosolic second messenger, and it may involve a change in desensitization or the conducting properties of the nAChR ion channel (3). Both of these mechanisms are different from the pathway activated by muscarinic receptors in myenteric neurons where the increase in PKC activity may alter the transition of the nAChR from the open and activated state to one or more desensitized states (20). Because peak currents are not altered by muscarinic receptor activation or treatments that mimic downstream signaling, the change in function of the nAChR is likely to be independent of changes in ACh binding.

Regulation of nAChR desensitization by PKC is not unique to myenteric neurons. Earlier studies done on desensitization of nAChRs at the neuromuscular junction and in sympathetic ganglia demonstrated that PKC activation increases nAChR desensitization (4, 5, 10, 16). Therefore, regulation of desensitization by PKC is a general mechanism by which the function of nAChRs can be modulated. Protein kinase A (PKA) has also been shown to phosphorylate nAChRs and alter desensitization of these receptors (16, 20). On the basis of these previous data, we tested the effects of forskolin, an activator of adenylyl cyclase that can lead to stimulation of PKA. Forskolin increased nAChR desensitization to a similar degree as PDBu; however, forskolin can act as an nAChR ion channel blocker (25). Therefore, we tested the adenylyl cyclase-inactive forskolin analog DDF for its effects on nAChR desensitization. It was found that forskolin and DDF caused similar increases in nAChR desensitization, leading to the conclusion that the effects of forskolin on desensitization are likely to be independent of adenylyl cyclase and PKA activation.

**Functional significance.** There are many examples of cotransmitters released simultaneously by autonomic nerves producing responses in synaptic targets that differ from responses produced by the individual transmitters acting alone (13). These effects can be mediated presynaptically, resulting in either a facilitation or inhibition of transmitter release, or transmitter interactions can be postsynaptic where one transmitter may sensitize the target cell to the actions of the second transmitter (13). In addition, it has been shown previously that nicotinic and P2X receptors and P2X and 5-HT3 receptors exhibit cross inhibition when these receptors are activated simultaneously (1, 9, 27). The data from the present paper indicate that there is a potential for postsynaptic modulation of responses to a single transmitter (ACh) mediated by different classes (nicotinic and muscarinic) of receptor for that transmitter. Muscarinic receptor-mediated acceleration of the desensitization rate of nAChRs could function to limit postsynaptic excitation during periods of high...
cholinergic nerve activity. The delay in recovery from desensitization of the nAChR would serve a similar function.

REFERENCES


