Mechanisms of modulation of neuronal nicotinic receptors by substance P and OAG

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Andoh, Tomio, Hideki Itoh, Itaru Watanabe, Toshio Sasaki, and Tomoko Higashi. Mechanisms of modulation of neuronal nicotinic receptors by substance P and OAG. Am J Physiol Cell Physiol 281: C1871–C1880, 2001.—Substance P is known to modulate neuronal nicotinic acetylcholine receptors (nAChRs) in the sympathetic nervous system. There are two conflicting proposals for the mechanism of this effect, an indirect action mediated by protein kinase C (PKC) and a direct interaction with receptor subunits. We studied the mechanisms of this effect in PC-12 cells. Substance P enhanced the decay of the nicotine-induced whole cell current. This effect was fast in its onset and was not antagonized by guanosine 5′-O-(2-thiodiphosphate), a G protein blocker, or staurosporine, a nonselective PKC blocker. Staurosporine failed to reverse the inhibition by 1-oleoyl-2-acetyl-sn-glycerol (OAG), a synthetic diacylglycerol analog known to activate PKC. The inhibitory effects of the peptide and OAG were preserved in excised patches, but substance P applied to the extra patch membrane was ineffective in the cell-attached patch configuration. We conclude that substance P modulates neuronal nAChRs most likely by direct interactions with the receptors but independently from activation of PKC or G proteins and that PKC does not participate in modulation by OAG.

STANDARDIZED Nomenclature: ion channel; neuropeptide; diacylglycerol; PC-12 cells; 1-oleoyl-2-acetyl-sn-glycerol

Substance P has been known to modulate neuronal nicotinic acetylcholine receptors (neuronal nAChRs) in a number of preparations such as sympathetic (33) and parasympathetic (19) neurons, adrenal chromaffin cells (6), and PC-12 cells derived from rat pheochromocytoma cells (27, 28, 32). Substance P enhances the agonist-induced desensitization of nAChRs in these cells without the involvement of neurokinin receptors (28). This modulation is considered to be physiologically relevant, since the substance P-containing fibers have been identified in sympathetic ganglia and adrenal medulla (33, 35). Simmons et al. (29) have shown that substance P acts via an intracellular second messenger system, since nAChRs, monitored by cell-attached patches, are modulated by the peptide bath applied to the extra patch membrane in chick sympathetic neurons. They suggested that protein kinase C (PKC) is a likely candidate to mediate the effect of substance P because modulation of nAChR desensitization was blocked by staurosporine, and activators of PKC mimicked the substance P-induced effects. This proposal is further supported by the findings that substance P stimulates phosphatidylinositol metabolism and leads to an activation of PKC in other systems (25).

However, other studies suggest that substance P modulates nAChRs by a direct interaction with the receptor subunits (4, 30, 31). Stafford et al. (30) demonstrated that two domains of the β2-subunit control the affinity of substance P to neuronal nAChRs, suggesting a direct interaction. In addition, a line of evidence indicates that substance P directly binds to Torpedo nicotinic receptors (4).

Besides substance P, neuronal nAChRs are modulated by other neuropeptides such as calcitonin gene-related peptide (CGRP) and dynorphins (10, 24). CGRP has been found to inhibit the current mediated by neuronal nAChRs by two distinct pathways in rat adrenal chromaffin cells: fast block, most likely by direct effects, and slow inhibition through G protein-coupled receptor activation (10). We recently reported that dynorphin A also inhibits neuronal nAChRs by a direct interaction leading to a reduction of channel open time in PC-12 cells (15, 24). These observations prompted us to clarify whether substance P directly inhibits neuronal nAChRs or whether second messengers mediate this effect. We found that modulation of nicotinic receptors by substance P does not require diffusible cytoplasmic factors in PC-12 cells. We also found that 1-oleoyl-2-acetyl-sn-glycerol (OAG), a synthetic diacylglycerol analog known to activate PKC (5, 23), inhibits neuronal nAChR-mediated current without the involvement of PKC activation.

MATERIALS AND METHODS

Cell preparation. PC-12 cells were cultured as previously described (1). For the experiments, cells were plated on collagen- and poly-L-lysine-coated coverslips, and they were used after treatment with 100 ng/ml of 2.5S nerve growth factor (NGF; Takara Shuzo, Shiga, Japan) for 4–6 days.

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Current recording. Nicotine-evoked currents were recorded at room temperature in either whole cell, outside-out, or cell-attached configurations by using AXOPATCH 200A (11). Heat-polished patch pipettes had a tip resistance of 2–7 MΩ when filled with an intracellular solution. The coverslips were placed in a recording bath with an approximate volume of 1.5 ml and continuously perfused at the rate of 2 ml/min with an external solution. Current responses were evoked by application of nicotine using a rapid application technique described as the “Y-tube” method (21). The tip of the Y-tube was positioned about 300 μm from the recorded cell. This method enabled the complete exchange of the external solution surrounding the cell within 50–150 ms, as estimated by recording the liquid junction current produced at an open patch pipette. The series resistance ranged from 8 to 23 MΩ and was not compensated.

Recording solutions and materials. The extracellular perfusion solution consisted of (in mM) 140 NaCl, 5.4 KCl, 1.8 CaCl₂, 1.0 MgCl₂, 10 HEPEs, and 11.1 glucose (pH was adjusted to 7.4 with NaOH). For whole cell current recordings, the intracellular pipette solution contained (in mM) 150 CsCl, 10 HEPEs, 5 EGTA, 2 ATP-Mg, and 0.3 GTP-Na₂ (pH 7.3 with CsOH). GTP-Na₂ was replaced with 2 mM guanosine 5′-O-(2-thiodiphosphate) (GDPβS) for blockade of GTP-binding proteins (G protein) in some experiments. Stauroporine was added to the standard pipette solution at a final concentration of 0.1 mM for blockade of PKC in other cases. The measurements started >15 min after the whole cell mode was established in these experiments. For outside-out patch recordings, ATP-Mg, and GTP-Na₂ were omitted in the intracellular solution. For cell-attached patch recordings, the perfusion solution consisted of (in mM) 140 NaCl, 2.7 KCl, 10 CaCl₂, 1.0 MgCl₂, and 10 HEPEs (pH was adjusted to 7.4 with NaOH). The pipette solution was the same as the perfusion solution except that the concentration of CaCl₂ was lowered to 1.0 mM, and 5 μM nicotine was included. (–)-Nicotine was purchased from Wako (Osaka, Japan); ATP-Mg, GTP-Na₂, GDPβS, and OAG were from Sigma (St. Louis, MO); and substance P was from Research Biochemicals. OAG was freshly suspended with the external solution by sonication to make 85 μM solution immediately before use.

Whole cell recording. In whole cell current recording, NGF-treated PC-12 cells were voltage clamped at −60 mV, and 30 μM nicotine with or without 10 μM substance P was applied for 5 s. The recordings were made in isolated cells without connection to the surrounding cells, and each application was separated by 5 min. The currents were low-pass filtered at 1 kHz and digitized at 5 kHz. For preincubation with substance P, the external solution containing the peptide was perfused at the rate of 5 ml/min for 5 min before rapid application. Cells were perfused with the plain external solution at the same rate for 5 min to wash out the drugs from the bath after measurement. We measured the peak and the nondesensitized current, which was defined as the average of the last 50 points during 5-s agonist application. Because nicotine-induced currents slightly declined with each application of nicotine, the response in the presence of substance P was normalized to the average of the elicited currents before and after the peptide application. The relative current in the presence of substance P was compared with control, which is the second response normalized to the average of the first and third responses in three successive nicotine applications with an interval of 5 min. Desensitization was evaluated by calculating the percentage decay of the current (%current decay) during agonist application, defined by the following equation (33):%current decay = (peak current − nondesensitized current)/peak current × 100

We also studied the effects of OAG, an activator of PKC, on the nicotine-induced current in the same way as substance P. Because the effect of OAG was not fully reversible, the current in the presence of OAG was normalized to the response immediately before OAG. The second response, normalized to the first response among three successive responses induced by nicotine, served as control in these cases.

Outside-out patch recordings. Single channel recordings were obtained from outside-out patches excised from NGF-treated cells. Nicotine (3 μM) in the absence and presence of 10 μM substance P or 10 μM OAG was applied for 30 s at the membrane potential of −60 mV, and each application was separated by 2 min. Substance P or OAG was applied without preincubation. The signals were filtered at 1 kHz (Bessel) and digitized at 5 kHz. The pCLAMP software package (Axon Instruments) was used to obtain all points amplitude histograms and the mean currents. Opening and closing of the channels were detected using 50% threshold criterion. Events <1 ms were ignored. The mean currents were obtained by integrating the current records during agonist application (30 s) and dividing the integral by the time of agonist application (30 s).

Cell-attached patch recordings. Single channel currents were also recorded in the cell-attached configuration using NGF-treated cells. Nicotine (5 μM) was included in the pipette solution, and the command potential was kept at 0 mV. After the control response was recorded, 10 μM substance P was applied to the extra patch membrane for 2.5 min. Channel activity was recorded until 3 min after the end of the application. Single channel currents were analyzed in the same way as outside-out patch recordings, and the mean currents were measured for the following three periods of 30 s: the control period immediately before the application of substance P, the last 30 s during the application (2 min after the beginning of the application), and 5 min after the beginning of the application. We also recorded nicotine-induced single channel currents and measured the mean currents, applying plain external solution instead of substance P, to see time-dependent changes.

Statistical analysis. Data are expressed as means ± SE. Statistical analysis was made by unpaired t-test or analysis of variance, followed by the Bonferroni multiple-comparison test, to estimate the significance when appropriate. P < 0.05 was considered to be significant.

RESULTS

Inhibition of whole cell current by substance P. Nicotine elicited inward currents that decayed rapidly during nicotine application due to desensitization at the membrane potential of −60 mV. Substance P alone produced no current responses in tested cells at 10 μM. Substance P (10 μM) depressed the whole cell current elicited by 30 μM nicotine reversibly and augmented the current decay when it was coapplied with nicotine after preincubation (Fig. 1A). The magnitude of inhibition was much greater for the nondesensitized current than that for the peak current (Fig. 2A and B). When substance P was coapplied with nicotine without preincubation, it similarly inhibited the nicotine-induced current (Fig. 1B). However, the magnitude of inhibition of the peak current was significantly smaller than
that caused by preincubation plus coapplication, whereas the inhibitory effects on the nondesensitized current were not influenced by the presence or absence of preincubation (Fig. 2, A and B). When nicotine alone was applied after a 5-min preincubation of substance P, inhibition of the nondesensitized current was much weaker than that caused by preincubation plus coapplication, while the magnitude of the peak current inhibition did not change (Figs. 1C and 2, A and B). These results indicate that the onset of inhibition by substance P is fast because preincubation is not required for the suppression of the nondesensitized current, and they also suggest that channel opening is required for substance P to exert its full effects.

We then studied the possibility of the involvement of G proteins and PKC in the inhibitory effects of substance P. Inclusion of GDPβS or staurosporine to the intracellular solution did not significantly affect the magnitudes of inhibition caused by 10 μM substance P (Fig. 2, A and B). Substance P accelerated the current decay during nicotine application, resulting in an increase in %current decay. This effect was not antagonized by GDPβS or staurosporine (Table 1). Staurosporine failed to reverse the substance P-induced effect, even at 1.0 μM (n = 3, data not shown). These results indicate that G proteins or PKC is not involved in the modulation of neuronal nAChRs by substance P.

Fig. 1. Inhibition of nicotine-induced whole cell current by substance P. Records shown are evoked currents in single PC-12 cells to 5-s pulses of 30 μM nicotine before, during, and after application of substance P. A: 10 μM substance P was coapplied with nicotine after a 5-min preincubation. B: substance P and nicotine were coapplied without pretreatment of substance P. Substance P depressed the nicotine-induced current and accelerated current decay similarly in these conditions, resulting in greater inhibition of the nondesensitized current than the peak current. C: nicotine alone was applied after a 5-min preincubation of substance P. Reduction of nondesensitized current by preincubation alone was smaller than that by coapplication. All these effects of substance P were reversible. Holding potential was −60 mV. Nicotine was applied as indicated by thick horizontal bars with an interval of 5 min. Substance P was applied as indicated by dotted lines.
Effects of substance P on single channel currents. To further investigate the mechanism of the inhibition, we studied the effects of substance P on the single channel current in outside-out patches and the cell-attached mode. In the outside-out configuration, the patches were exposed to 3 μM nicotine, a concentration that results in little desensitization, and 10 μM substance P was applied without preincubation. First, nicotine was applied for 30 s, and then nicotine with or without substance P was applied again after a 2-min interval. As illustrated in Fig. 3A, substance P consistently inhibited the nicotine-induced single channel activity. The amplitude histogram showed that substance P did not decrease the amplitude of the unitary current (Fig. 3B). Although the mean current of the second response normalized to that of the first response was close to 1.0 when nicotine alone was applied twice, the relative mean current was significantly reduced when substance P was coapplied with nicotine (Fig. 3C). Open probability was also reduced by the peptide, and the onset of the action seemed within a few seconds (Fig. 3D). The relative mean current in the presence of substance P was comparable to the relative nondesensitized current without preincubation but in the presence of substance P, suggesting that the magnitude of inhibition observed in excised patches is similar to that in whole cell recordings. These results indicate that the inhibitory effects of substance P do not require diffus-
ible intracellular components. The channel activity did not last long enough to obtain a sufficient number of events for further kinetic analysis.

On the contrary, the peptide did not significantly inhibit nicotine-induced currents when applied to the extra patch membrane in cell-attached recordings (Fig. 4A). The mean currents at 2 min and 5 min after the application of substance P or the plain external solution were normalized to those of the control period. The relative mean currents slightly decreased after the
application of substance P; however, the changes did not reach statistical significance (Fig. 4B). There was no significant difference in the relative mean currents between the cells that received substance P and those exposed to the plain external solution. These findings also indicate that the roles of diffusible intracellular messengers are not important in the effects of substance P.

Inhibition of neuronal nAChRs by OAG. OAG (10 μM) also reduced the nicotine-induced whole cell current and accelerated the current decay when it was coapplied with nicotine after preincubation (Fig. 5). The peak and nondesensitized currents were depressed to 64.4 ± 3.7 and 25.4 ± 2.3% of the current immediately before OAG. The inhibition was not reversible after a 5- or 10-min washout. After a 5-min washout, the peak and nondesensitized currents remained 53.5 ± 6.0 and 32.6 ± 5.0% of the response immediately before OAG, while the peak and nondesensitized components of the third response accounted for 91.4 ± 3.1 and 77.6 ± 2.2% of the first response during three repeated nicotine applications. Recovery was significantly depressed after OAG. Inhibitory effects of 10 μM OAG were significantly decreased when preincubation was omitted. There was no significant difference in the magnitude of inhibition by 85 and 10 μM OAG (Fig. 6, A and B). Preincubation of 85 μM OAG alone was as effective as preincubation plus coapplication (data not shown). These findings suggest that OAG similarly inhibits the nicotine-induced current and that the action is slow in its onset and is long lasting, but it does not require channel activation.

Staurosporine included in the pipette solution did not significantly alter inhibitory effects of 10 or 85 μM OAG (Fig. 6, A and B). Enhancement of current decay during nicotine application by 10 or 85 μM OAG was also not affected by staurosporine (Table 2). These results indicate that inhibition of neuronal nAChRs by OAG does not involve PKC activation.

Effects of 10 μM OAG on single channel currents were studied in outside-out patches in the same protocol as that used for substance P. OAG strongly depressed the nicotine-induced single channel activity upon coapplication without preincubation (Fig. 7A). The inhibition seemed to develop gradually during the first several seconds of coapplication, whereas the channel activity was maintained when nicotine alone was applied. OAG coapplied with nicotine reduced open probability and the mean current without changing the amplitude of the unitary current (Fig. 7, B–D). The time course of changes in open probability showed a slow onset of the action of OAG. When data from all studied patches were combined, open probability was 0.189 for the first 10 s and 0.124 for the later 20 s during application of nicotine alone. In the presence of
OAG, it was reduced to 0.059 for the first 10 s and 0.04 for the later 20 s. There was a significant difference in open probability between the early and later phases of the response in the presence of OAG, while this was not the case for control. Therefore, blockade by OAG was significantly enhanced in the later 20 s compared with the first 10 s. The magnitude of reduction of the mean current in excised patches was comparable with that of inhibition of nondesensitized current in whole cell recordings. Preservation of the inhibitory effects of OAG in outside-out patches also indicates that diffusible cytoplasmic factors are not responsible for the inhibition.

DISCUSSION

The results of the present study demonstrate that substance P and OAG both inhibit the neuronal nAChR-mediated current and augment the current decay during agonist application. They also demonstrate that these effects did not require activation of diffusible cytoplasmic factors, including PKC, in PC-12 cells. Lack of the involvement of PKC is inconsistent with earlier studies for both substance P and OAG (9, 29). Staurosporine included in the pipette solution failed to reverse the effects of both compounds in our study. It might be possible that the entire intracellular fluid was not effectively dialyzed with the pipette solution containing staurosporine because of the size and shape of differentiated PC-12 cells. However, this is unlikely because sufficient time elapsed before the measurements were started. Moreover, the finding that the inhibitory effects of substance P and OAG were well preserved in excised patches strongly opposes the involvement of diffusible cytoplasmic factors. As for substance P, the mechanism independent from a second messenger system was also supported by the rapid onset of the effect and the finding that the peptide was ineffective when applied to the extra patch cell membrane in cell-attached recordings.

Our findings indicate that inhibition by substance P does not involve cytoplasmic second messengers or G proteins in PC-12 cells. How, then, does this peptide modulate neuronal nAChRs? The rapid onset of the effect favors a direct action on neuronal nAChRs. This notion is consistent with the findings of the study reported by Stafford et al. (30). They showed that the affinity of substance P is strongly influenced by two domains of the β4-subunit of rat nAChRs, an extracellular NH2-terminal domain and another domain containing the M1, M2, and M3 regions using chimeric β-subunit coexpressed with the α3-subunit in Xenopus oocytes. Binding of substance P to the channel is also indicated by affinity labeling of 125I-p-benzoylphenylalanine substance P to the M2 region of the δ-subunit from Torpedo (4). On the contrary, cytoplasmic second messengers, including PKC, are suggested to be involved in modulation by substance P in embryonic chick sympathetic neurons (29). Unlike our results, channel opening is not important for inhibitory effects of substance P on chick neuronal nAChRs because a significant inhibition was observed by pretreatment of the peptide, followed by application of nicotine alone after washout of substance P in the same preparation.

Table 2. Changes in % current decay caused by 1-oleoyl-2-acetyl-sn-glycerol

<table>
<thead>
<tr>
<th>Nicotine, %</th>
<th>Nicotine + OAG, %</th>
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<tr>
<td>OAG (10 μM; n = 6)</td>
<td>33.6 ± 4.1</td>
</tr>
<tr>
<td>OAG (10 μM + staurosporine; n = 8)</td>
<td>39.1 ± 5.1</td>
</tr>
<tr>
<td>OAG (85 μM; n = 8)</td>
<td>36.8 ± 4.0</td>
</tr>
<tr>
<td>OAG (85 μM + staurosporine, n = 4)</td>
<td>28.5 ± 6.7</td>
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Values are means ± SE. Nicotine, % current decay for the response induced by nicotine alone immediately before application of 1-oleoyl-2-acetyl-sn-glycerol (OAG). Nicotine + OAG, % current decay for the response induced by coapplication of nicotine and OAG. *Significantly different from nicotine (P < 0.01). There was no significant difference in the corresponding values among 4 conditions.
Moreover, our results of cell-attached recordings were inconsistent with those obtained in chick sympathetic neurons (29). What factors explain these discrepancies? The concentration of substance P studied in our experiment was 10 μM, which is comparable with that mainly studied in chick neurons (20 μM). Therefore, differences in the concentrations of the peptide would not account for the discrepancies. Differences in spe-

Fig. 7. OAG depressed nicotine-induced single channel activity in outside-out patches. A: representative examples of single channel activity with or without OAG. Top 2 traces are a continuous recording of the first response to 3 μM nicotine alone, showing a period of 20 s, starting from 1 s after beginning the application. Bottom 2 traces depict recording from same patch exposed to nicotine + 10 μM OAG after a 2-min interval. Onset of inhibition by OAG seemed slower than that by substance P. B: amplitude histogram of single channel current in absence or presence of OAG. OAG (10 μM) reduced number of events without changing amplitude of unitary current. Similar changes in amplitude histogram were observed in 4 other patches. C: summary of effects of OAG on mean current. Nicotine alone was applied twice with an interval of 2 min in nicotine group, while the patches were first exposed to nicotine and then to nicotine + OAG after a 2-min interval in OAG group. Mean current of the second response was normalized to that of the first response. Relative mean currents were 115.2 ± 16% for nicotine group and 15.4 ± 3.6% for OAG group (n = 6 and 5 for nicotine and OAG). **P < 0.01, significantly different from nicotine. D: time course of changes in open probability with or without OAG. Data were obtained from another patch different from the one shown in A. Depression of open probability gradually advanced during the first 10 s. Open probability of the channel was reduced from 0.258 ± 0.068 for nicotine alone (control) to 0.045 ± 0.018 for nicotine with OAG (OAG) when data from all studied patches were averaged.
cies and types of cells may explain the discrepancies in a part. Although the exact subunit combinations of nAChRs are not identified in PC-12 cells or chick sympathetic neurons, αβ-containing receptors are likely to be expressed dominantly in both types of cells. However, amino acid sequence identity is not as high for the β4-subunit as for the α3-subunit (74% for β4- vs. 83% for α3-subunit), and there are large differences in pharmacology between rat and chick clones (7, 20, 26). It may be possible that substance P has lower affinity to nAChRs in chick sympathetic neurons than to those in PC-12 cells and that indirect actions are dominant in chick preparations. There may be differences in the extent of modulation by protein kinases among species and types of neurons.

PKC activators, including OAG, have been shown to enhance the current decay of neuronal nAChR-mediated current in chick sympathetic neurons (9); however, evidence supporting the idea that PKC activation mediates the effect is circumstantial: a slow onset of the effect and similarity in effective concentrations for modulation of nicotinic receptors and PKC activation. As for phorbol esters, lack of changes in the current decay by an inactive analog also supported the idea. Again, this idea was not supported by the results of our study. Lack of reversal of the effects of OAG by staurosporine and preservation of the effects in cell-free excised patches strongly oppose the involvement of cytosol PKC. Although we did not obtain a dose-response relationship for the effects of OAG, nicotinic receptors in PC-12 cells seemed more sensitive to OAG than those in chick sympathetic neurons because 10 μM OAG was as effective as 85 μM OAG in our study, while 20 μM OAG exerted only a small effect in chick neurons (9). Inhibition of the non-desensitized current was not reversible after a 5-min washout in PC-12 cells, whereas it was partially reversible in chick neurons (9). The onset of the effect of OAG seemed to be slower than that of substance P in the present study, since inhibition of the non-desensitized current was significantly enhanced by preincubation in whole cell recordings, and a reduction in open probability gradually increased during the first 10 s in excised patch recordings, while those were not the cases for substance P. The slow onset and recovery of the effect of OAG suggest indirect pathways other than diffusible cytoplasmic factors, such as modulation through G proteins or changes in membrane lipid environment. We cannot exclude the possibility of slow channel block, as suggested in the earlier study (9). Diacylglycerol has been shown to perturb phospholipid bilayers and to modulate function of certain membrane proteins independent of PKC activation (2, 8, 17, 36). The effect of OAG on nicotinic receptors may represent another example of effects of diacylglycerol on membrane proteins not mediated by PKC activation.

It has been shown that some types of protein kinases and phosphatases can be closely associated with membrane ion channels and can remain bound when the patch is excised (3, 34). It might be possible that substance P and OAG activate PKC tethered to the excised patch, resulting in modulation of neuronal nAChRs. However, there has been no report suggesting this type of association between nAChRs and PKC, only for other types of ion channels such as Ca2+-activated potassium channels and Ca2+-sensitive nonspecific cation channels (3, 34). If activation of PKC plays an important role in the inhibitory effects of substance P, the peptide applied to the extra patch membrane should have exhibited its effect through activating cytosol PKC. Therefore, the involvement of PKC in excised patch recordings is very unlikely, at least for substance P.

Phosphorylation of nAChRs has been considered to regulate the rate of desensitization of nicotinic receptors (14, 18). As for phosphorylation of muscle receptors by protein kinase A and protein tyrosine kinase, this hypothesis was directly supported by the experiments using cell-free reconstituted channels (12, 13). However, consequences of phosphorylation by PKC have not been directly examined, but merely suggested, by indirect evidence that PKC activators enhance desensitization of muscle type and neuronal type nAChRs (9). A recent study has shown that 4β-phorbol 12,13-dibutyrate, a kind of phorbol ester known to activate PKC, directly modulates Torpedo nAChRs independently of PKC activation (22). Moreover, it has recently been revealed that activation or depression of PKC does not influence the onset of desensitization of neuronal nAChRs but regulates the rate of recovery from desensitization in rat chromaffin cells (16). Therefore, it is not surprising that modulation by OAG or substance P does not involve PKC activation.

When one takes into account the earlier studies, it appears that three neuropeptides, substance P, CGRP, and dynorphin A, modulate neuronal nAChRs, most likely by direct interactions with receptor subunits in rat chromaffin cells and a related cell line (10, 15). It may be interesting to explore whether a common region of receptor subunits is responsible for the effects of these peptides and whether there is any synergistic or antagonistic interactions among these peptides.

In summary, we have studied mechanisms of modulation of neuronal nAChRs by substance P and OAG, which have been considered to exert their effects through activation of PKC. We found that both compounds inhibit the nicotine-induced current independent from diffusible cytoplasmic factors in PC-12 cells.

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