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α-Adrenergic and Cholinergic Receptor Agonists Modulate Voltage-Gated Ca^{2+} Channels

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We investigated the effect of α-adrenergic and cholinergic receptor agonists on Ca^{2+} current in adult rat trigeminal ganglion neurons using whole-cell patch clamp methods. The application of acetylcholine, carbachol, and oxotremorine (50 μM each) produced a rapid and reversible reduction of the Ca^{2+} current by 17±6%, 19±3%, and 18±4%, respectively. Atropine, a muscarinic antagonist, blocked carbachol-induced Ca^{2+} current inhibition to 3±1%. Norepinephrine (50 μM) reduced Ca^{2+} current by 18±2%, while clonidine (50 μM), an α_2-adrenergic receptor agonist, inhibited Ca^{2+} current by only 4±1%. Yohimbine, an α_2-adrenergic receptor antagonist, did not block the inhibitory effect of norepinephrine on Ca^{2+} current, whereas prazosin, an α_1-adrenergic receptor antagonist, attenuated the inhibitory effect of norepinephrine on Ca^{2+} current to 6±1%. This pharmacology contrasts with α_2-adrenergic receptor modulation of Ca^{2+} channels in rat sympathetic neurons, which is sensitive to clonidine and blocked by yohimbine. Our data suggest that the modulation of voltage dependent Ca^{2+} channel by norepinephrine is mediated via an α_1-adrenergic receptor. Pretreatment with pertussis toxin (250 ng/ml) for 16 h greatly reduced norepinephrine- and carbachol-induced Ca^{2+} current inhibition from 17±3% and 18±3% to 2±1% and 2±1%, respectively. These results demonstrate that norepinephrine, through an α_1-adrenergic receptor, and carbachol, through a muscarinic receptor, inhibit Ca^{2+} currents in adult rat trigeminal ganglion neurons via pertussis toxin sensitive GTP-binding proteins.

Key Words: α-adrenergic receptor and muscarinic receptor, Ca^{2+} currents, Pertussis toxin-sensitive GTP binding proteins, Rat trigeminal ganglion neurons

INTRODUCTION

Ca^{2+} is an important regulator for many neuronal functions. Voltage-gated Ca^{2+} channels play a key role in the control of free cytosolic Ca^{2+}. The modulation of voltage-gated Ca^{2+} channel is probably an important component in the mechanism underlying the regulation of excitability and neurosecretion (Miller, 1987). Recent reports showed that the activity of voltage-gated Ca^{2+} channel can be regulated by a wide variety of neurotransmitters and neuropeptides including acetylcholine, γ-aminobutyric acid, norepinephrine, opioids, and neuropeptide Y (Deisz & Lux, 1985; Forscher et al, 1986; Gross & Macdonald, 1987; Rosenthal et al, 1988; Sah, 1990; Schroeder et al, 1991). For example, in the central nervous system (CNS) such as hippocampal and spinal cord neurons, α-adrenergic receptor agonists as well as muscarinic receptor agonists decrease voltage-gated Ca^{2+} currents (G wiler & Brown, 1987; Toseli & Lux, 1989; Sah, 1990). Similarly, in peripheral nervous system (PNS) such as rat dorsal root ganglionic neuron and sympathetic neurons, muscarinic receptor agonists as well as adrenergic receptor agonists also

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decrease voltage-gated Ca^{2+} currents (Bernheim et al, 1991; Formenti & Sansone, 1991).

The inhibition of voltage-gated Ca^{2+} channels by receptor agonists is probably mediated by GTP-binding proteins (G proteins) in many cases (Gilman, 1987). For example, it has been shown that inhibition of Ca^{2+} current by acetylcholine, norepinephrine, neuropeptide Y, and bradykinin can be largely abolished by pretreating the cells with pertussis toxin (PTX), which inactivates the GTP binding proteins, Go and Gi or by intracellular application of guanine nucleotide analogues such as GDPβS (Lipscombe et al, 1989; Toseli and Lux, 1989; Schofield, 1991). We have previously shown that PTX-sensitive GTP binding proteins are involved in the inhibition of voltage- dependent Ca^{2+} channels by α_2-adrenergic receptor and muscarinic receptor agonists in cultured spinal cord-dorsal root ganglion cells (Nah et al, 1993). In addition, protein kinases such as protein kinase A or protein kinase C are also involved in signal transduction of Ca^{2+} channel regulation, but which is known to depend on cell systems (Hille, 1992).

Trigeminal ganglion neurons as well as dorsal root ganglion neurons convey somatosensory informations from peripheral tissues to the central nervous system. However, a large amount of information, as mentioned above, on neurotransmitter-mediated voltage-gated Ca^{2+} channels has been obtained from studies of dorsal root ganglion neurons. It is surprising that pharmacological properties of trigeminal ganglion neurons are not well examined even though there are important clinical aspects such as trigeminal neuralgia (Kerr, 1979). Here we show, using adult rat trigeminal ganglion cells in culture, that α-adrenergic receptor and cholinergic receptor agonists inhibit voltage-gated Ca^{2+} currents mediated by PTX-sensitive GTP binding proteins

METHODS

Materials

Acetylchoine, carbachol, oxotremorine, atropine, norepinephrine, yohimbine, and prazosin were purchased from Sigma Chemical Co. (St. Louis, MO). Pertussis toxin was purchased from List Biological Laboratories (Campbell, CA). Media and serum were obtained from GIBCO (Grand Island, NY). All other drugs and chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

Cell cultures

Trigeminal ganglion neurons were prepared with slight modification (Goldenberg & De Boni, 1983) as follows: after decapitation of an adult Sprague-Dawley rat (150~250 g), trigeminal nerves containing trigeminal ganglia were dissected and placed in Ca^{2+}/Mg^{2+}-free Hanks solution (Sigma Chemical Co. St. Louis, MO) containing 0.1% collagenase (Cls II Cooper Biomedical, Malvern, PA). The tissue was minced with iridectomy scissors and incubated at 37°C for 90 min. After digestion, the tissue was dissociated through a fire-polished Pasteur pipette. To remove non-neuronal cells and myelin debris, the dissociated tissue suspension was added onto a two-layer, freshly prepared Percoll gradient (densities 1.048 and 1.077 g/ml, respectively), then subjected to density gradient centrifugation (10 min, 800 g). The interphase (density 1.048 g/ml) fraction was removed and washed with Ca^{2+}/Mg^{2+}-free Hanks solution twice. After final centrifugation (10 min, 800 g), the cell pellet was suspended in cell culture medium, seeded onto poly-D-lysine and laminin coated-dishes, and cultured at 37°C (95% air and 5% CO_2). Culture medium was as follows: Ham's F12 (GIBCO) with 10% fetal calf serum (Hyclone Laboratories, Logan, UT), 100 units/ml penicillin, 100 μg/ml streptomycin, 5 mg/ml glucose, and 100 ng/ml nerve growth factor (Sigma). Cells were studied within the first 2 days in culture.

Recordings and experimental solutions

All recordings were done in a standard whole-cell patch clamp (Hamil et al, 1981) at room temperature. Test pulses of 200 ms duration were applied every 15 s. Test pulses of 1 s duration were applied at every 30 or 40 s to avoid accumulating inactivation. Records were filtered at 2 kHz with an 8-pole Bessel filter. Leak, capacity, and other contaminating currents were eliminated by subtracting recordings in 1 mM Cd^{2+}, a selective Ca^{2+} channel blocker. Solutions were applied locally to the cell under study using an array of 6 Drmmond 1 μl disposable micropipettes ("microcaps"; VWR) glued together side by side, supported on a plastic coverslip, and mounted on a rod attached

to a manipulator. The back ends of the glass pipettes were connected with polyethylene tubing and stopcocks to solution reservoirs. The delivery end was positioned within 150 μm from cell. Solution, either control, receptor agonists, other receptor antagonists, or kinase activators flowed over the cell throughout the recording. Except where indicated, the extracellular solution contained 5 mM $CaCl_2$, 150 mM NaCl, 5 mM KCl, 2 mM $MgCl_2$, 10 mM HEPES (titrated to pH 7.4 with TEA-OH), and 0.1% glucose. Compared to Na^+-free solutions, this Na^+-containing solution diminishes the rate of Ca^{2+} channel rundown during prolonged recordings. The recording electrode contained 100 mM CsCl, 1 mM Na_2ATP (equine; Sigma), 0.3 mM Na_3GTP (Aldrich Chemical Company), 10 mM EGTA, 2.5 mM $MgCl_2$, 1.5 mM $CaCl_2$, 80 mM leupeptine, 8 mM creatine phosphate (Na salt) and 40 mM HEPES (pH 7.0). Unless otherwise indicated, Ca^{2+} current amplitude was measured by averaging the data between 180 ms and 200 ms after the onset of the pulse. This procedure avoids contamination due to voltage-gated Na^+ current.

Data were presented as means S.E.M. Statistical significance was measured by a paired Student's t-test.

RESULTS

Time course of norepinephrine- and carbachol-induced inhibition in Ca^{2+} currents

Fig. 1, 2 show the effect of α-adrenergic and muscarinic receptor agonists on voltage-gated Ca^{2+} current in adult rat trigeminal ganglion neuron using whole-cell recording technique. Under the control conditions, depolarizing voltage steps to +10 mV from a holding potential of −70 mV evoked an inward current during 200 ms in most tested cells. Application of norepinephrine or carbachol reduced the current amplitude (18.2% and 19.3%, respectively at 10 mV; $P<0.001$, n=17). The prolonged inhibition of Ca^{2+} currents was shown during the continued presence of norepinephrine (data not shown). Inhibition by norepinephrine was quickly recovered by washing the drug. This inhibition did not desensitize during prolonged and repeated application of norepinphrine. Clonidine, an α_2-adrenergic receptor agonist, had no effect (Fig. 1). As shown in figure 6, acetylcholine and oxotremorine (each 50 μM) also inhibited voltage-gated Ca^{2+} currents with similar manner of carbachol. Inhibition by muscarinic receptor agonists also was quickly recovered by removing the drugs and did not desensitize during prolonged and repeated application of these agonists (data not shown).

Current-voltage (I-V) relationships of α-adrenergic and muscarinic receptor agonists induced inhibition of Ca^{2+} currents

The current-voltage (I-V) relationships showed that the inward Ca^{2+} current first appeared at potentials more positive than −30 mV and became maximal in its amplitude at 0 mV. Application of norepinephrine

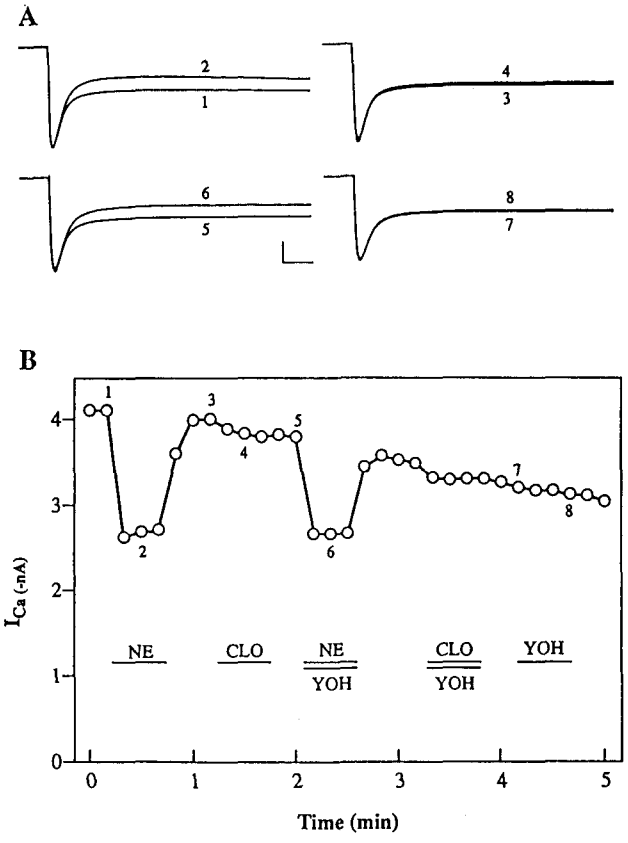

Fig. 1. Effects of α-adrenergic receptor agonists and antagonists on Ca^{2+} currents. A. Ca^{2+} currents (traces) elicited by steps to +10 mV from at times indicated in the graph (B) of current versus time. B. The solid bars indicate the applications of α-adrenergic receptor agonist, norepinephrine (NE) or clonidine (CLO) (50 μM) alone or together with antagonist, yohimbine (YOH) (10 μM). The scale bars: 2 nA, 4 ms

or carbachol reduced Ca^{2+} current amplitude over the potential range −20 to +30 mV (Fig. 3A and 3B, open triangle or closed circle, respectively). The greatest decrease in Ca^{2+} current amplitude after treatment of each agonist apppeared at 0 mV which is a peak of the I-V curve. The addition of 1 mM Cd^{+} during the perfusion of cell blocked both high- and low-threshold inward Ca^{2+} currents in trigeminal gainglion neurons. These results indicate that these inward Ca^{2+} current were produced by activation of voltage-gated Ca^{2+} channels. As shown in histograms of Fig. 4, α-adrenergic and muscarinic agonists caused inhibition of the Ca^{2+} currents in the variable ranges among 30 and 77 cells, respectively. Ca^{2+} currents were not affected (less than 5% inhibition) by norepinephrine in 3% of the tested cells and also by muscarinic agonists in 15%.

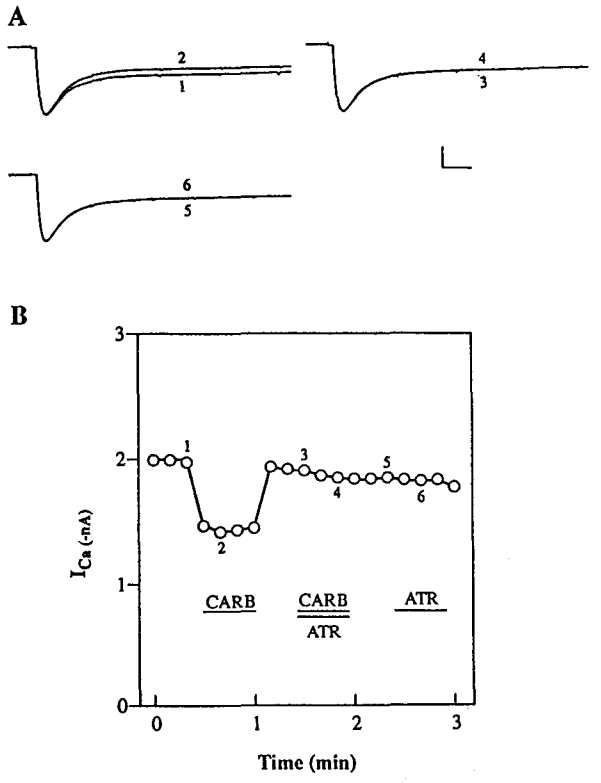

Fig. 2. Effects of muscarinic receptor agonist and antagonist on Ca^{2+} currents. A. Ca^{2+} currents (traces) elicited by steps to +10 mV from at times indicated in the graph (B) of current versus time. B. The solid bars indicate the applications of muscarinic agonist, carbachol (CARB) (50 μM) alone or together with antagonist, atropine (ATR) (1 μM). The scale bars: 2 nA, 4 ms

Effect of α-adrenergic or muscarinic receptor antagonists

In the histograms illustrated in Fig. 6A, yohim-

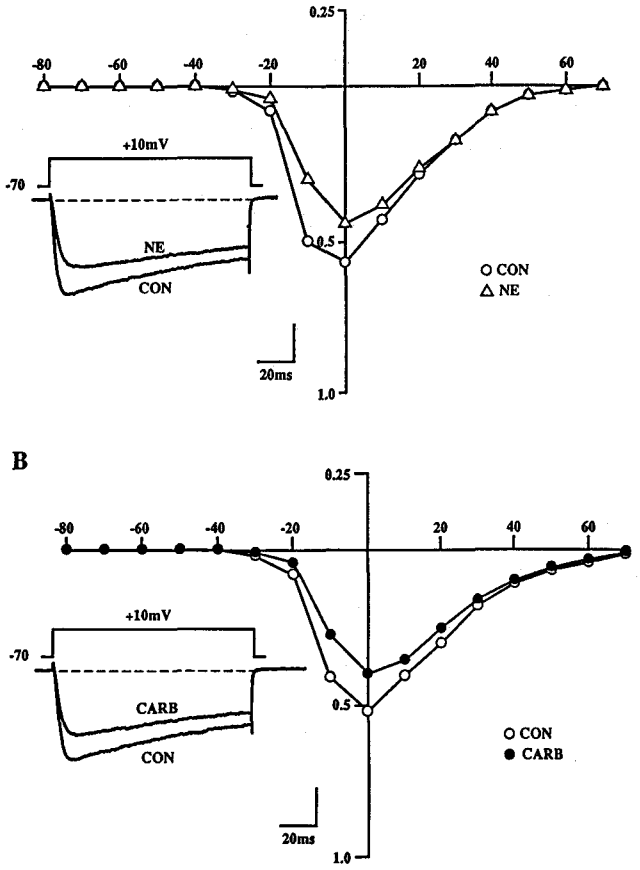

Fig. 3. Effect of norepinephrine and carbachol (50 μM) on Ca^{2+} currents of trigeminal ganglion neurons. (A) Leak-subtracted current-voltage (I-V) curves of Ca^{2+} currens measured 20 ms after the onset of the depolarizing pulses in control (o) and in the presence of norepinephrine (50 μM). (△) (B) Leak-subtracted current-voltage (I-V) curves of Ca^{2+} currens measured 20 ms after the onset of the depolarizing pulses in control (o) and in the presence of carbachol (50 μM) (●). Insets; Ca^{2+} current records in the absence (control, CON) and presence of norepinephrine (NE) or carbachol (CARB) elicited from a holding potential of -70 mV by depolarizing steps to +10 mV. Currents traces and I-V curves are from the same cell. Extracellular solution for figure 1A contained 5 mM CaCl2, 135 mM tetraethylammonium-Cl, and 10 mM HEPES (titrated to pH 7.4 with TEA-OH). Leak, capacity, and other contaminating currents were eliminated by subtracting recordings in 1 mM Cd^{2+}, a selective Ca^{2+} channel blocker.

bine, an α_2-adrenergic receptor antagonist, did not block significantly the inhibitory effect of norepinphrine on Ca^{2+} current. Even high concentration of yohimbine (50 μM) did not block the inhibitory effect of norepinephrine on Ca^{2+} currents. Prazosin (10 μM), an α_1-adrenergic antagonist, significantly blocked the inhibitory action of norepinephrine. As shown in Fig. 2 and 6B, atropine (1 μM) significantly blocked the inhibitory action of carbachol on Ca^{2+} currents, indicating the involvement of muscarinic receptor in this action.

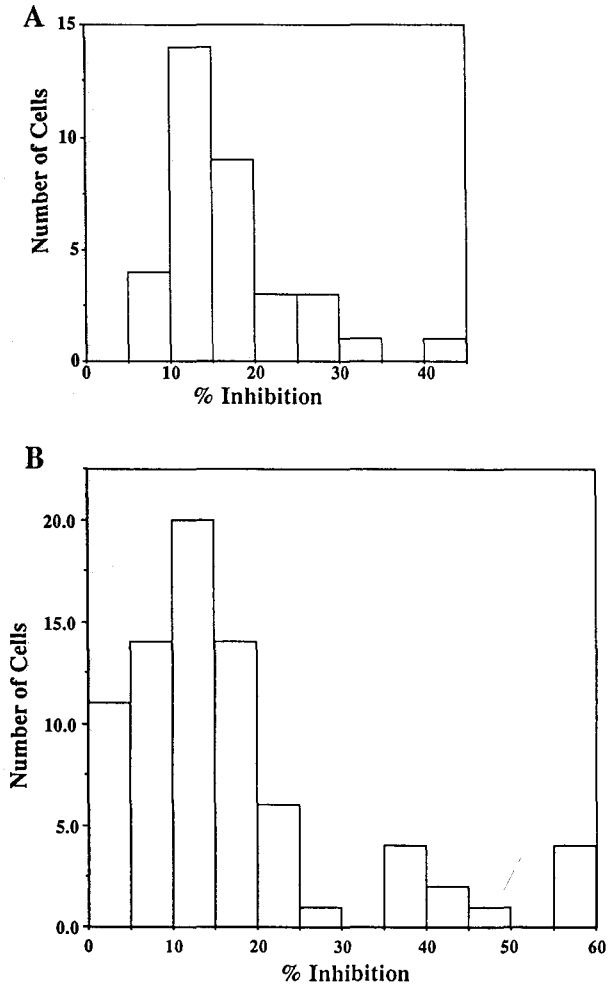

Fig. 4. Inhibition of Ca^{2+} channels by α-adrenergic receptor or muscarinic receptor agonists. Histograms showing distribution of Ca^{2+} currents inhibition on 30 cells in the presence of 50 μM norepinephrine (A) and on 77 cells in the presence of 50 μM muscarinic agonists including acetylcholine, carbachol, and oxotremorine (B). Percent inhibition is calculated as 100[(C-A)/C], where A is the peak current in the presence of receptor agonist, and C is the peak current just before agonist application. Ca^{2+} currents records were measured from a holding potential of −70 mV by depolarizing steps to +10 mV.

Effects of pertussis toxin pretreatment and protein kinase activators

To study whether the modulation of Ca^{2+} current by norepinephrine and carbachol is mediated via PTX sensitive GTP-binding proteins, cells were treated with PTX (250 ng/ml, 16 h). Treatment of PTX did not change mean Ca^{2+} current from both control and PTX-treated cells (data not shown). However, as shown in Fig. 7, the inhibitory effect of carbachol and

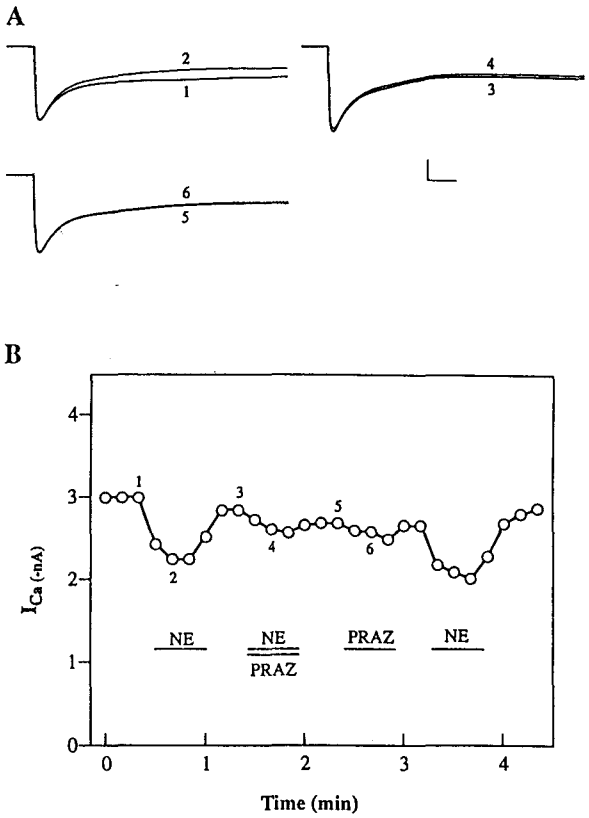

Fig. 5. Effect of α_1-adrenergic receptor antagonist on norepinphrine induced Ca^{2+} current inhibition. A. Ca^{2+} currents (traces) elicited by steps to +10 mV from at times indicated in the graph (B) of current versus time. B. The solid bars indicate the applications of α-adrenergic receptor agonist, norepinephrine (50 μM) alone or together with antagonist, prazosin (10 μM). The scale bars: 2 nA, 4 ms

norepinephrine on Ca^{2+} current was greatly attenuated by PTX pretreatment ($P<0.001$). The inhibition of Ca^{2+} current induced by carbachol in control and PTX treated cells were $18\pm3\%$ and $2\pm1\%$, respectively. The inhibition of Ca^{2+} current induced by norepinephrine in control and PTX treated cells were $17\pm3\%$ and $2\pm1\%$, respectively. These results suggest that the inhibitory effects of α-adrenergic receptor and muscarinic receptor agonists on Ca^{2+} current are coupled to voltage-gated Ca^{2+} channels via PTX-sensitive GTP binding proteins (Fig. 7). In addition to the involvement of PTX- sensitive GTP binding proteins in the inhibition of Ca^{2+} channels by norepinephrine or carbachol, we tested whether protein kinase A or protein kinase C was involved in the modulation of Ca^{2+} channels induced by α-adrenergic or cholinergic receptor agonists. However, treatment of forskolin or phorbol esters such as TPA did not affect the inhibitory effects on Ca^{2+} channels induced by norepinephrine or carbachol (data not shown).

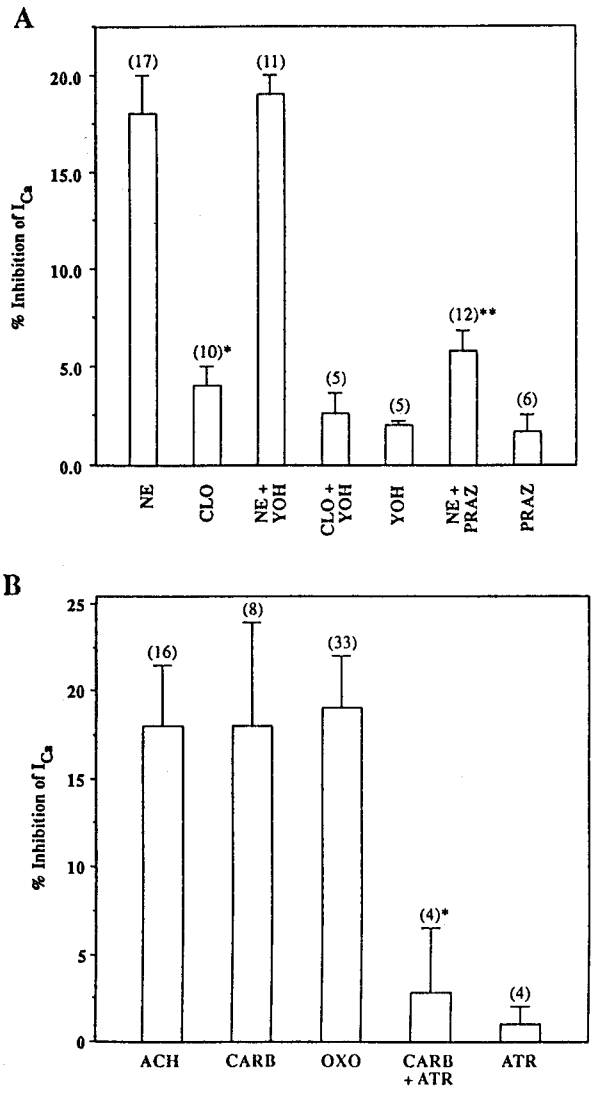

Fig. 6. Effect of α-adrenergic receptor or muscarinic receptor antagonists on norepinephrine- or carbachol-induced inhibition of Ca^{2+} currents. (A) The histograms indicate inhibition of Ca^{2+} currents induced by norepinephrine (NE) alone, or together with yohimbine (YOH), or together with prazosin (PRAZ). (B) The histograms indicate inhibition of Ca^{2+} currents induced by carbachol (CARB) alone, or together with atropine (ATR). Values are means S.E.M. The number of cells tested are shown in parentheses. *Significantly different from atropine (ATR)-untreated cells or norepinephrine-treated cells ($P<0.01$). **Significantly different from prazosin-untreated cells ($P<0.01$).

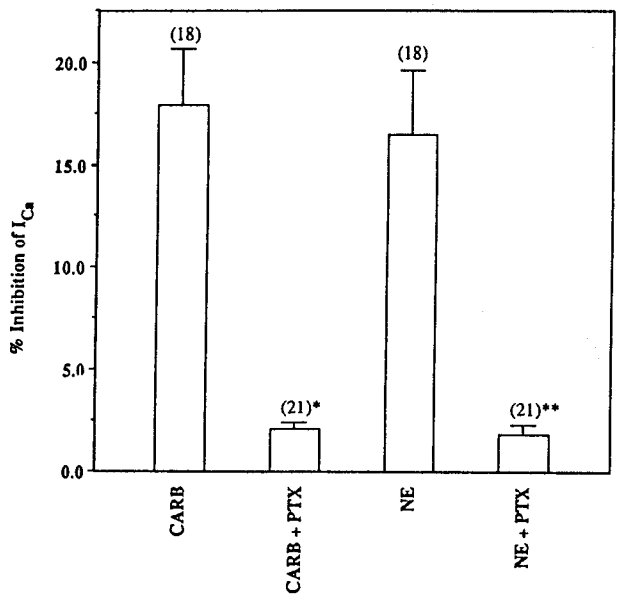

Fig. 7. Effect of pertussis toxin pretreatment on the inhibition of Ca^{2+} currents by norepinephrine and by carbachol. The histograms indicate inhibition of Ca^{2+} currents induced by norepinephrine or carbachol (each 50 μM) application in neurons recorded from control and pertussis toxin-treated cultures (250 ng/ml, 16 h). Values are means S.E.M. The number of cells tested are shown in parentheses. *Significantly different from the pertussis toxin-untreated cells ($P<0.001$). **Significantly different from the pertussis toxin-untreated cells ($P<0.004$).

DISCUSSION

We demonstrated that cultured trigeminal ganglion neurons contain voltage-gated Ca^{2+} channels. The activity of these channels is inhibited by α-adrenergic and cholinergic receptor agonists. These respective receptor agonists produce a rapid and reversible reduction of voltage-gated Ca^{2+} currents in adult rat trigeminal ganglion neurons (Figs 1, 2). The variability of the magnitude of inhibition of Ca^{2+} currents responding to α-adrenergic and muscarinic receptor agonists imply that trigeminal ganglion neurons as well as dorsal root ganglion neurons also contain a heterologous population of cells (Fig. 4).

Relatively little is investigated about electrophysiological and pharmacological properties of trigeminal ganglion neurons, since it is assumed that the signal transduction pathway of trigeminal ganglion neurons by various neurotransmitters would be similar with those in other sensory neurons such as dorsal root ganglion neurons. Recent report showed that rat trigeminal ganglion neurons cultured contain nicotinic acetylcholine receptors (nAChRs) and dimethylphenylpiperazinium, a nAChRs agonist, induced a inward currents but not by carbachol, a muscarinic acetylcholine receptor (mAChR) agonist (Liu et al, 1993). However, in our experiment, acetylcholine, which interacts both nAChRs and mAChRs, also shows similar extent of inhibition of voltage-gated Ca^{2+} currents in addition to carbachol and oxotremorine (see Figs. 2, 6). These apparent discrepancy may be attributable to the differences in cell size studied, since most of cell size used in this experiment was less than 30 μm in diameter. In another experiment, DAMGO, μ-opiate receptor agonist, also inhibited voltage-gated Ca^{2+} channels activity in the range of 25~30 μm of diameter, which is probably nociceptive neurons (Ninkovic & Hunt, 1983) (data not shown). On the other hand, our results are well consistent with the reported finding in other sensory neurons such as rat dorsal root ganglion, where acetylcholine inhibits voltage-gated Ca^{2+} currents (Formenti & Sansone, 1991).

Norepinephrine, an α-adrenergic receptor agonist, also decreases voltage-gated Ca^{2+} currents in chick dorsal root ganglion neurons as well as frog and rat sympathetic neurons (Canfield & Dunlap, 1984; Dunlap & Fischbach, 1978). Interestingly, the pharmacological studies showed that α_2-adrenergic receptor agonist, clonidine, does not inhibit voltage-gated Ca^{2+} current, while phentolamine and yohimbine blocked norepinephrine-induced inhibition of voltage-gated Ca^{2+} currents in chick dorsal root ganglion neurons and frog sympathetic ganglion cells (Canfield & Dunlap, 1984; Lipscombe et al, 1989). In contrast, in rat sympathetic neurons, both clonidine and norepinephrine decrease voltage-gated Ca^{2+} currents and the inhibitory effects of both agonists were blocked by yohimbine (Schofield, 1991). These pharmacological profiles imply that in chick sensory neurons and frog sympathetic neurons norepinephrine inhibits voltage- gated Ca^{2+} currents through distinct subtype of α-adrenergic receptor. Here, we obtained similar results using adult rat trigeminal ganglion neurons. In addition, we found that clonidine (in the range of 50 ~100 μM), an α_2-adrenergic receptor agonist, did not reduce voltage-gated Ca^{2+} channel activity. Yohimbine, an α_2-adrenergic receptor antagonist, also did not block the inhibitory effect of norepinephrine on voltage-gated Ca^{2+} channel activity. However, prazosin, an α_1-adrenergic receptor antagonist, markedly attenuated the action of norepinephrine. How can we explain our results from the classical α_2-adrenergic receptor, which shows higher affinity for yohimbine than prazosin? Several lines of evidences indicate that α_2-adrenergic receptors can be divided into at least three distinct subtypes (see for review, Ruffolo et al, 1993). α_{2A}-adrenergic receptor were found in the kidney, spinal cord and cerebral cortex and showed high affinity for oxymetazoline. α_{2B}-adrenergic receptors were found in NG 108-15 cells and neonatal rat lung and showed high affinity for prazosin and low affinity for oxymetazoline. α_{2C}-adrenergic receptors were found in the opossum OK cell line and spinal cord. Besides ligand binding assays described above, functional studies also support subdivision of α_2-adrenergic receptor. For example, 1 μM prazosin antagonizes norepinephrine-induced inhibition of K^+-induced release of [^{3}H]norepinephrine from rat cortical slices (Nasseri & Minneman, 1987). Moderate concentrations of prazosin also potentiate stimulation-induced release of endogenous norepinephrine from rat submandibular gland (Turner et al, 1984). In present study, the inhibitory effect of norepinephrine was markedly attenuated by prazosin. These results suggest that the inhibitory effect of norepinephrine on voltage-dependent Ca^{2+} currents is coupled to the α_1-adrenergic receptor in adult rat trigeminal ganglion

neurons, since norepinephrine inhibits presynaptically excitatory synaptic transmission mediated through α_1-adrenergic receptor in rat hippocampus (Scanziani et al, 1993). Further experiments may be required to elucidate that a1-adrenergic receptor or subtype of α_2-adrenergic receptor exists in adult rat trigeminal ganglion neurons.

It is generally accepted that PTX has been used to charaterize receptor signal transduction pathway, since PTX catalyzes the NAD-dependent Adp ribosylation of the a subunit of PTX-sensitive GTP binding proteins thereby inactivating GTP binding protein a subunit and attenuating the effects of receptors agonists (Gilman, 1987). For example, using spinal cord and dorsal root ganglion cocultures we reported that α_2-adrenergic, muscarinic and κ opiate agonists inhibited the voltage-gated Ca^{2+} uptake as well as adenylate cyclase activity via PTX sensitive GTP-binding proteins (Attali et al, 1989; Vogel et al, 1989; Nah et al, 1993; Scanziani et al, 1993). Moreover, chronic treatment of adrenergic receptor, muscarinic and κ opiate agonists induced quantitative and qualitative changes in GTP-bindng proteins (Attali & Vogel, 1989; Nah et al, 1993). We found that μ opioid agonists inhibited the voltage-dependent Ca^{2+} currents via PTX-sensitive GTP-binding proteins in adult rat dorsal root ganglion neurons (Schroeder et al, 1991; Wilding et al, 1993). Therefore, the effect of PTX on Ca^{2+} current inhibition induced by α-adrenergic receptor agonist or cholinergic receptor agonist in adult rat trigeminal ganglion neurons were investigated. Interestingly, in our present study, the inhibitory effect of norepinephrine or carbachol was markedly attenuated by PTX pretreatment, suggesting that the inhibitory effect of norepinephrine and carbachol on Ca^{2+} currents is negatively coupled to the voltage-gated Ca^{2+} channels through PTX sensitive GTP-binding proteins.

In the neurons, the signaling between receptor and Ca^{2+} channels regulation at least is divided into two categories. One is that local or direct regulation of Ca^{2+} channels through PTX-sensitive GTP binding proteins (Wilding et al, 1995). The other one is a remote signaling path having the diffusible messenger(s) (Beech et al, 1992). To test whether norepinephrine or carbachol utilizes diffusible second messenger for Ca^{2+} channel regulation, we treated cells with protein kinase activator. Interestingly, in our study protein kinase activators such as forskolin or phorbol ester did not affect the inhibition of Ca^{2+} channels induced by norepinephrine or carbachol (data not shown). These results suggest that both α-adrenergic and cholinergic receptors are negatively coupled to Ca^{2+} channels through PTX-sensitive GTP binding proteins without involvement of diffusible second messegers.

In summary, we demonstrated that α-adrenergic and cholinergic receptor agonists inhibit voltage-gated Ca^{2+} channels through PTX-sensitive GTP binding proteins and this regulation does not require diffusible second messenger in adult rat trigeminal ganglion neurons in culture.

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