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Characterization of Acetylcholine-induced Currents in Male Rat Pelvic Ganglion Neurons

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The pelvic ganglia provide autonomic innervations to the various urogenital organs, such as the urinary bladder, prostate, and penis. It is well established that both sympathetic and parasympathetic synaptic transmissions in autonomic ganglia are mediated mainly by acetylcholine (ACh). Until now, however, the properties of ACh-induced currents and its receptors in pelvic ganglia have not clearly been elucidated. In the present study, biophysical characteristics and molecular nature of nicotinic acetylcholine receptors (nAChRs) were studied in sympathetic and parasympathetic major pelvic ganglion (MPG) neurons. MPG neurons isolated from male rat were enzymatically dissociated, and ionic currents were recorded by using the whole cell variant patch clamp technique. Total RNA from MPG neuron was prepared, and RT-PCR analysis was performed with specific primers for subunits of nAChRs. ACh dose-dependently elicited fast inward currents in both sympathetic and parasympathetic MPG neurons (EC_{50}; 41.4 μM and 64.0 μM, respectively). ACh-induced currents showed a strong inward rectification with a reversal potential near 0 mV in current-voltage relationship. Pharmacologically, mecamylamine as a selective antagonist for $\alpha 3\beta 4$ nAChR potently inhibited the ACh-induced currents in sympathetic and parasympathetic neurons (IC_{50}; 0.53 μM and 0.22 μM, respectively). Conversely, α-bungarotoxin, α-methyllycaconitine, and dihydro-β-erythroidine, which are known as potent and sensitive blockers for $\alpha 7$ or $\alpha 4\beta 2$ nAChRs, below micromolar concentrations showed negligible effect. RT-PCR analysis revealed that $\alpha 3$ and $\beta 4$ subunits were predominantly expressed in MPG neurons. We suggest that MPG neurons have nAChRs containing $\alpha 3$ and $\beta 4$ subunits, and that their activation induces fast inward currents, possibly mediating the excitatory synaptic transmission in pelvic autonomic ganglia.

Key Words: Autonomic ganglia, Nicotinic acetylcholine receptor, Ionic currents, Reverse transcriptase polymerase chain reaction

INTRODUCTION

The major pelvic ganglia (MPG) receive presynaptic inputs from sympathetic hypogastric nerve and parasympathetic pelvic nerve, and provide autonomic innervation to lower bowel, urinary bladder, prostate, and penis (Keast, 1999). Physiologically, these ganglia play important roles in various autonomic reflexes, including micturition and penile erection (de Groat & Booth, 1993). In pathologic conditions, such as bladder outlet obstruction and erectile dysfunction, structural and functional alterations in MPG neuron have been observed (Mills et al, 1992). Because of their relatively simple anatomy and consequent ease of isolation, manipulation and quantification, MPG have been used as a model system for studying physiological and pathophysiological aspects of neural control of pelvic viscera.

A peculiar feature of MPG that differentiates them from other autonomic ganglia is the colocalization of both sympathetic and parasympathetic postganglionic neurons within the same ganglion capsule (Keast, 1999). These two types of neurons act antagonistically to each other, such as contracting or relaxing the same urogenital muscles (de Groat & Booth, 1993). According to earlier studies, MPG neurons with sympathetic phenotypes (based on tyrosine hydroxylase immunoreactivity) are larger, express T-type Ca^{2+} channels, and are highly modulated by α_2-adrenoceptor (Zhu et al, 1995; Park et al, 2001). Moreover, $GABA_A$ and neuropeptide Y (NPY) receptors are expressed exclusively in sympathetic neurons (Cha et al, 2001; Kong et al, 2001), whereas ATP-sensitive K^+ channels are in parasympathetic neurons (Park et al, 2002). The differences in morphological, immunohistochemical and electrophysiological properties enable us to discriminate the sympathetic

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ABBREVIATIONS: ACh, acetylcholine; MPG, major pelvic ganglion; nAChR, nicotinic acetylcholine receptor; a-BgTx, a-bungarotoxin; MLA, α-methyllycaconitine; DHβE, dihydro-β-erythroidine.

neurons from parasympathetic MPG ones.

Nicotinic acetylcholine receptors (nAChRs) are extensively located in the neuro-muscular and inter-neuronal junctions (Lukas et al, 1999). Especially in autonomic neurons, the nAChRs act as a major mediator of both sympathetic and parasympathetic synaptic transmissions (Skok, 2002). Structurally, the nAChRs are composed of five subunits to form homo- or hetero-pentamer, and considered as a member of ligand-gated ion channel superfamily along with $GABA_A$, glycine, and $5\text{-}HT_3$ receptors (Rust et al, 1994). Molecular cloning has identified nine α (2~10) and three β (2~4) subunits of nAChR in neuronal cells which are assembled in numerous combinations to form functional receptors (Lukas et al, 1999; De Biasi, 2002). The composition of subunits is the principal determinant of the properties of nAChR, including agonist and antagonist potencies, activation and inactivation kinetics, and Ca^{2+} permeability. Therefore, identification of the subunit composition may provide insights into the role of nAChR in the modulation of neuronal excitability and synaptic transmission. To date, however, the biophysical characteristics and the molecular nature of nAChRs, as a major mediator of autonomic neurotransmission, in the pelvic ganglia had not yet been investigated.

In the present study, thus, we used patch-clamp and RT-PCR techniques to identify which subtypes of nAChRs are expressed and function in the pelvic ganglion neurons. Our data suggest that nAChRs in MPG neurons contain mainly $\alpha3$ and $\beta4$ subunits, and that their activation induces fast inward currents which may be involved in the excitatory synaptic transmission of sympathetic and parasympathetic pelvic autonomic ganglia.

METHODS

Preparation of MPG neurons

MPG neurons were enzymatically dissociated, as described previously (Zhu et al, 1995; Park et al, 2001). Isolated MPG neurons were plated onto culture dishes coated with poly-L-lysine and incubated with minimal essential medium, containing 10% fetal calf serum and 1% penicillin-streptomycin (all from Life Technologies, Grand island, NY, USA), in a humidified 95% air-5% CO_2 incubator at 37°C. In most cases, neurons were used within 24 hours after plating.

Electrophysiology

The ionic currents of MPG neurons were recorded by using the dialyzed or perforated whole-cell patch clamp technique. Patch electrodes were fabricated from a borosilicate glass capillary (BF150-117-15, Sutter Instrument Co., San Rafael, CA, USA), using a P-97 Flaming Brown micropipette puller (Sutter Instrument Co.). The patch electrodes were fire-polished on a microforge (Narishige, Tokyo, Japan), and had resistances of 1.5~2.5 MΩ, when filled with the internal solution described below. An Ag/AgCl wire was used to ground the bath. The cell membrane capacitance and series resistance were electronically compensated (>80%) by using the patch clamp amplifier (EPC-9, Instrutech Corp., NY, USA). Voltage protocol generation and data acquisition were performed, using the Pulse/Pulsefit (v8.50) software (Heka Elektronik, Lambrecht, Germany) on an IBM computer. Current traces were filtered at 2~5 kHz, using the 4-pole bessel filter, in the clamp amplifier and stored on the computer hard drive for later analysis.

RT-PCR analysis

Total RNA from dissociated MPG neurons was prepared, using a modified guanidinium thiocyanate-phenol-chloroform extraction method (Chomczynski & Sacchi, 1987). Synthesis of the first strand of cDNA was performed in an RT-PCR buffer, containing 2 μg of total RNA, 25 nmoles dNTP, 0.5 μg of random hexamer, 20 U of RNase inhibitor and 200 U of murine leukemia virus reverse transcriptase (all from Promega, WI, USA) in a final volume of 25 μl at 37°C for 60 min. Specific sense and antisense primer pairs, based on rat nACh receptor sequences deposited in the

Table 1. PCR primer sequences

Primer		Sequence (5' to 3')	Position	Size (bp)	GeneBank accession #
ACh $\alpha2$	Sense	TGC CCA GGT GGC TGA TGA TGA ACC	1356~1379	301	NM_133420
	Antisense	GCT TTC TGT ATT TGA GGT GAC AGC	1656~1633		
ACh $\alpha3$	Sense	AAC CTG CTC CCC AGG GTC ATG TTT	1174~1197	301	NM_052805
	Antisense	CAC TTT GGA TGG CTT CTT TGA TTT	1474~1451		
ACh $\alpha4$	Sense	GTC AAA GAC AAC TGC CGG AGA CTT	1105~1128	301	NM_024354
	Antisense	TGA TGA GCA TTG GAG CCC CAC TGC	1405~1382		
ACh $\alpha5$	Sense	GTG GAT TTA GTG AGC AGT CAT GCA	1478~1501	299	NM_017078
	Antisense	TTT GGG GGG AGT TTT AAA TAG TCT	1776~1753		
ACh $\alpha7$	Sense	AAC TGG TGT GCA TGG TTT CTG CGC	1031~1054	300	NM_012832
	Antisense	AGA TCT TGG CCA GGT CGG GGT CCC	1330~1308		
ACh $\beta2$	Sense	ACG GTG TTC CTG CTG CTC ATC	1014~1034	507	NM_019297
	Antisense	CAC ACT CTG GTC ATC ATC CTC	1523~1503		
ACh $\beta3$	Sense	GAA GAT GTG GAT ACA TCG TTT CCA	1545~1568	299	NM_133597
	Antisense	GAG CAG AGG GAG TAG TTC AGG AAC	1843~1820		
ACh $\beta4$	Sense	ATG AAG CGT CCC GGT CTT GAA GTC	1096~1119	301	NM_052806
	Antisense	GGT CAT CGC TCT CCA GAT GCT GGG	1396~1373		

GenBank, were used (Liu et al, 1998; Table 1). Single stranded cDNA products were denatured at 94°C for 5min, and then subjected to PCR amplification (35 cycles). Each PCR cycle consisted of denaturing at 94°C for 30 sec, annealing at 62°C for 30 sec, and extension at 72°C for 1 min in a PCR amplifier (Minicycler™, MJ Research Inc., MA, USA). PCR buffer (50 μl) contained the transcribed cDNA, 10 pmoles of primers, 10 nmoles of dNTP, and 1.25 U of Taq polymerase (Perkin-Elmer, CT, USA). The resultant PCR products were separated and visualized on a 1.1% agarose gel containing ethidium bromide.

Solution and Drugs

The internal solution to fill the patch electrode contained (in mM): 30 KCl, 100 K-gluconate, 10 HEPES, 10 glucose, 10 EGTA, 10 tris-phosphocreatine, 1.2 $MgCl_2$, 5 MgATP, and 0.3 Na_2-GTP (pH 7.2). The external solution contained (in mM): 135 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 5 HEPES, and 10 glucose (pH 7.4). Drugs were applied to single neurons via a perfusion valve control system (VC6M; Warner Instrument Inc., Hamden, CT, USA), and the outlet of the perfusion system was located within 100 μm of the cell. The bath superfusion rate was approximately 1~2 ml/min. All experiments were performed at room temperature (20~24°C). Drugs used in experiments were obtained as follows: α-methyllycaconitine from Tocris Cookson Inc. (Bristol, UK) and acetylcholine, mecamylamine, dihydro-β-erythroidine from Sigma Chemical Co. (St. Louis, MO, USA). For stock solutions (10 mM~1 M), all drugs were dissolved in distilled water.

Data Analysis

The concentration-response curves, EC_{50}, and IC_{50} values were obtained by using the Prism (v3.0) software (GraphPad software Inc., San Diego, CA, USA). Data were presented as means±SEM. Statistical significance was determined, using Student's *t*-test, and $p<0.05$ was considered significant.

RESULTS

Characteristics of ACh-induced currents in sympathetic and parasympathetic MPG neurons.

In the present study, we were able to distinguish the sympathetic neurons from parasympathetic MPG ones by using the criteria previously established (Park et al, 2001). Compared with the parasympathetic neurons (19.6±1.3 pF; n=34), the sympathetic neurons (55.0±3.8 pF; n=34) have relatively larger cell size, as measured in capacitance ($p<0.001$). In addition, the sympathetic neurons express T-type Ca^{2+} channels that generate anodal break rebound spike (Lee et al, 2002) and ionotropic $GABA_A$ receptors whose activation evokes depolarization (Kong et al, 2001). We also found that the sympathetic neurons showed tonic firing (20 out of 22 neurons) in response to a long depolarizing current injection, unlike parasympathetic neurons which showed 'phasic' firing (14 out of 19 neurons, data not shown).

As illustrated in Fig. 1A and B, ACh (10 μM) produced a strong inward rectification in current-voltage (I~V) relationship. The reversal potential of ACh-induced currents was near 0 mV, which is consistent with previous reports (Mathie et al, 1990; Zhou et al, 2002). The peak amplitudes of currents activated by ACh showed a dose-dependency ranging up to 1mM, and the potency (EC_{50}) for current activation in sympathetic MPG neurons (41.4 μM) was slightly higher than in parasympathetic neurons (64.0 μM). The current density induced by ACh (1 mM) was similar between these two groups (sympathetic vs. parasympathetic neuron; 0.31±0.19 vs. 0.40±0.22 nA/pF, n=8). At higher than 1mM ACh concentration, the peak amplitude of currents was saturated. Instead, the decay

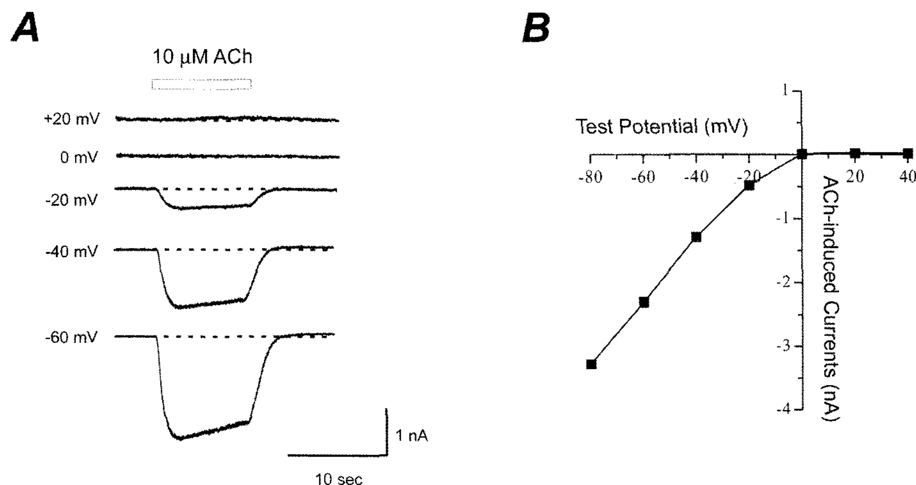

Fig. 1. Current-voltage relationship induced by acetylcholine in male rat MPG neurons. A: representative traces of whole-cell currents induced by acetylcholine (ACh; 10 μM) at different holding potentials (+20, 0, −20, −40, and −60 mV, respectively) in sympathetic MPG neuron (42.86 pF and showing tonic firing). B: current-voltage relationship of ACh-induced currents. The amplitudes of peak currents induced by ACh are plotted as a function of holding potential.

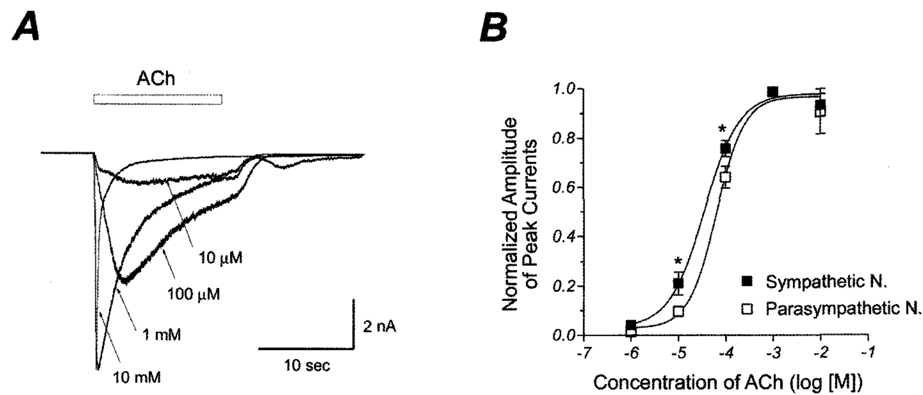

Fig. 2. Concentration-response relationship induced by acetylcholine in MPG neurons. A: representative traces of inward currents evoked by different concentrations of ACh in sympathetic MPG neuron (32.84 pF; showing tonic firing and large GABA- induced currents) at holding potential of −80 mV. B: concentration-response relationship of the peak amplitude induced by ACh in sympathetic (■; n=8) and parasympathetic (□; n=7) MPG neurons. Data are presented as means±SEM and * denotes $p<0.05$.

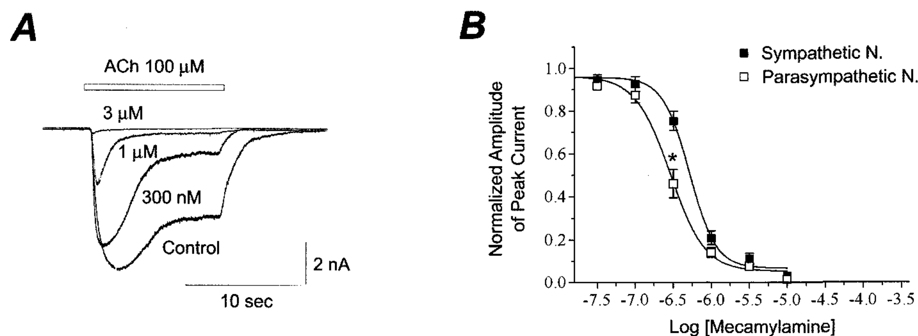

Fig. 3. Effects of mecamylamine on ACh-induced currents in MPG neurons. A: Representative traces of ACh-induced currents in the presence of various concentrations of mecamylamine (0.3~3 μM) in sympathetic MPG neurons. B: Concentration-response curves for inhibition of ACh-induced currents by mecamylamine in sympathetic (■) and parasympathetic (□) MPG neurons. The amplitudes of peak current were plotted against the concentration of mecamylamine after normalizing to each value measured in the absence of mecamylamine. Data are presented as means±SEM (n=6~8) and * denotes $p<0.05$.

rate became strikingly faster with consequent reduction in the net charge influx during acetylcholine application (Fig. 2).

Effects of nAChRs blockers on ACh-induced currents

Several selective blockers are currently available for discriminating the compositions of nAChRs. For examples, α-bungarotoxin (α-BgTx) and α-methyllycaconitine (MLA) are known to potently block the α7 subunit-containing nAChRs (Lukas et al, 1999; Narahashi et al, 1999). Receptors, composed of $\alpha4\beta2$ or $\alpha3\beta2$, are more sensitive to dihydro-β-erythroidine (DHβE), while those containing $\alpha3\beta4$ have high affinities to low doses of mecamylamine (Albuquerque et al, 1997). As shown in Fig. 3, ACh-induced currents were inhibited by mecamylamine dose-dependently in rat MPG neurons. Unlike DHβE, mecamylamine remarkably accelerated inactivating rates of ACh-induced currents, and the IC_{50} value of mecamylamine was lower in parasympathetic neurons than in sympathetic neurons (sympathetic: 250 nM, parasympathetic: 80 nM). DHβE also reduced ACh-induced currents dose-dependently. However, the IC_{50} value was much higher than those reported for selective blockade of $\alpha4\beta2$ or $\alpha3\beta2$ receptors (Chavez-Noriega et al, 1997). DHβE affected the ACh-induced currents equally in both sympathetic and parasympathetic neurons (Fig. 4). The selective antagonists for α7 nAChR, α-BgTx and MLA exerted negligible effects at below micromolar concentrations, suggesting that MPG neurons do not express functional α7 nAChR (Table 2).

RT-PCR analysis for subunits of nAChRs

To identify subunits of nAChRs expressed in MPG neurons, we performed RT-PCR with specific primers for α and β subunits (Table 1). As shown in Fig. 5, the

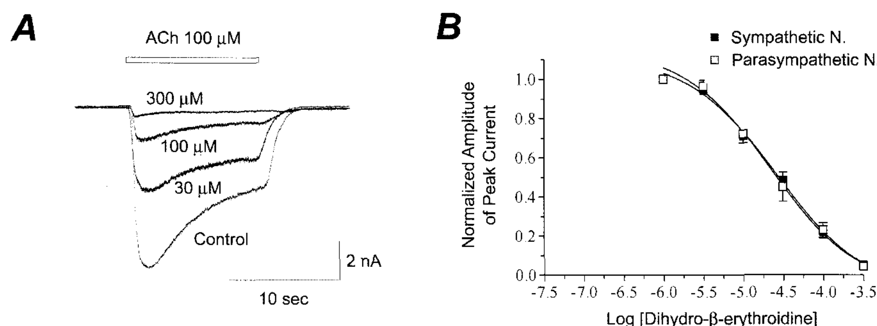

Fig. 4. Effects of dihydro-β-erythroidine on ACh-induced currents in MPG neurons. A: Representative traces of ACh-induced currents in the presence of various concentrations of dihydro-β-erythroidine (30~300 μM) in sympathetic MPG neurons. B: Concentration- response curves for inhibition of ACh-induced currents by dihydro-β-erythroidine in sympathetic (■) and parasympathetic (□) MPG neurons. The amplitudes of peak current were plotted against concentration of dihydro-β-erythroidine after normalizing to each value measured in the absence of dihydro-β-erythroidine. Data are presented as means±SEM (n=5~6) and * denotes $p<0.05$.

Table 2. Comparison of the potency of agonist and antagonists for nicotinic acetylcholine receptors (nAChR) in MPG neurons

	Sympathetic neuron	Parasympathetic neuron
Agonist	EC_{50} (μM)	EC_{50} (μM)
Acetylcholine	41.4	64.0
Antagonist	IC_{50} (μM)[a]	IC_{50} (μM)
Mecamylamine	0.53	0.22
dihydro-β-erythroidine	26.2	35.0
α-bungarotoxin	3.14	ND[b]
α-methyllycaconitine	37.8	18.6

[a]Inhibitory effects of antagonists to the amplitude of peak current induced by acetylcholine (100 μM). [b]Not determined.

Fig. 5. RT-PCR analysis of mRNAs encoding the subunits of nAChRs expressed in MPG neurons. Total RNA isolated from MPG neurons was reverse transcribed and amplified by PCR with specific primers to the subunits of nAChRs. The resultant PCR products were visualized on agarose gel, containing ethidium bromide. As an internal control, GAPDH was also amplified. Estimated product size for each subunit is shown in parenthesis. M, DNA size marker.

transcripts for α3 and β4 subunits were found to be abundant in MPG neurons. Other subunits, such as α2, α5, α7, and β2, were also expressed, but in a less extent.

DISCUSSION

The major findings in this study are as follows; 1) ACh induced strong inwardly rectifying currents in both sympathetic and parasympathetic MPG neurons, 2) mecamylamine, as a selective $\alpha3\beta4$ nAChR blocker, potently blocked ACh-induced currents, and 3) among various subunits of nAChR, α3 and β4 were most abundantly expressed in MPG neurons.

In neuro-muscular and inter-neuronal synapses, nAChRs located at post-synaptic membrane act as a major mediator to convey the excitatory presynaptic input. When ACh binds to ligand-binding domain of ionotropic nAChR, central aqueous pore is opened, and Na^+, Ca^{2+} and K^+ permeate through the pore. This inward cationic movement generates membrane depolarization with a consequent increase in excitability. In the present study, the activation of nAChR in MPG neurons induced strong inwardly rectifying currents in current-voltage relationship, which is a characteristic of neuronal nAChRs. In addition, ACh significantly increased intracellular Ca^{2+} concentration in MPG neurons, irrespective to depolarization-induced Ca^{2+} entry or muscarinic activation (data not shown). Conversely, the muscle type nAChRs (containing $\alpha1\beta1\varepsilon\gamma\delta$) show linear current-voltage relationship and low Ca^{2+} permeability (Francis & Papke, 1996; Lukas et al, 1999). As described earlier, the ionic conductance, desensitization rate, and Ca^{2+} permeability vary, depending on the subunit composition of neuronal nAChRs. Therefore, identification of the subunit composition provides useful information about the consequences of the nAChR activation in synaptic modulation. Therefore, we carried out pharmacological and molecular biological experiments in the present study.

To assess the functional contribution of subunits to the native ACh-induced currents in MPG neurons, we tested the effects of selective nAChR blockers. Among various antagonists for nAChRs, mecamylamine, a selective $\alpha3\beta4$ antagonist, potently blocked the ACh-induced currents in MPG neurons. Recently, Zhou et al (2002) reported that mecamylamine-sensitive nAChRs, which are composed of α3, α5, β2 and β4 subunits, are predominantly expressed in guinea pig small intestinal myenteric neurons. In addition to the potent blockade by mecamylamine, the strong inward rectification, the slow inactivation kinetics, and the EC_{50} values of the ACh-induced currents in MPG

neurons are very similar to the results of other experiments, using recombinant $\alpha 3\beta 4$ subunits (Harvey et al, 1996; Nelson et al, 2001; Papke et al, 2001). DHβE, known as a $\alpha 4\beta 2$ antagonist, also inhibited the ACh- induced currents dose-dependently. However, an IC_{50} value of DHβE in MPG neuron was around 25 μM, which is comparable with that (IC_{50}: 14 μM) for $\alpha 3\beta 4$ subunits, but not with that (110 nM) for $\alpha 4\beta 2$ subunits expressed in *Xenopus* oocytes (Chavez-Noriega et al, 1997). Overall, these results supported that $\alpha 3\beta 4$ complex in rat MPG neurons is the main combination of nAChR.

Using RT-PCR analysis, we confirmed that major subunits expressed in MPG neurons were $\alpha 3$ and $\beta 4$ subunits. In a less extent, other subunits ($\alpha 2$, $\alpha 4$, $\alpha 5$, $\alpha 7$, $\beta 2$ and $\beta 3$) were also expressed in MPG neurons, which is similar to the studies in adrenal chromaffin cells and intestinal myenteric neurons (Nelson et al, 2001; Tachikawa et al, 2001; Zhou et al, 2002). Recently, the $\alpha 7$ subunits were reported to be the most abundant subtype of nAChR in both the central and peripheral nervous system (Cuevas et al, 2000). Even though transcripts for the $\alpha 7$ subunit were also present in the present study, $\alpha 7$ appeared to be not a major part of functional nAChRs in MPG neurons, because of the following two reasons. Firstly, the most obvious evidence is that α-BgTx and MLA at nanomolar ranges scarcely blocked the ACh-induced current. These antagonists are known to show specific and selective blocking activity to the $\alpha 7$ subunit-containing nAChRs with IC_{50} of 100 pM ~ 10 nM (Roth et al, 2000; Virginio et al, 2002). Secondly, the inactivating process of ACh-induced currents in MPG neurons was much slower than that of typical $\alpha 7$ subunit-containing nAChRs which is completed within hundreds of millisecond (Zhang et al, 1994; Chavez-Noriega et al, 1997). Bryant et al (2002) has reported that $\alpha 3\beta 4$ nAChR is inhibited by high concentration of MLA (IC_{50}: 3.8 μM), explaining the nonspecific blocking activity of MLA on the nAChR in MPG neuron (Table 2).

The expression level of each nAChR subunit in autonomic ganglia shows marked diversity, depending on the species and tissues. In chick ciliary ganglion, the ratio of mRNA encoding $\alpha 3$, $\alpha 5$, $\alpha 7$, $\beta 2$ and $\beta 4$ subunits is 3 : 1 : 7 : 1 : 1 (Corriveau & Berg, 1993). Interestingly, the differences in distribution of nAChRs between sympathetic (SCG, solar plexus, and PC-12 cells) and parasympathetic (intracardiac plexus and submucous plexus: see Skok, 2002) ganglion neurons have been reported. The number of cells stained with the antibodies against $\alpha 3$, $\alpha 4$, $\alpha 5$, $\alpha 7$ subunit is 100%, 57.5%, 100% and 100% in rat superior cervical ganglion (SCG), and 31.8%, 46.6%, 14.3% and 21.7% in rat intracardiac ganglion (Skok et al, 1999). In pelvic ganglions, however, there were few meaningful qualitative, but only quantitative, differences in pharmacological properties of nAChRs between the sympathetic and the parasympathetic neurons. Even though EC_{50} for ACh was lower in sympathetic MPG neurons, we could not detect a significant difference in current density (pA/pF) between the two groups.

Xu et al (1999) reported that nAChR $\alpha 3$ or $\beta 2/4$ subunit knock-out mouse shows high incidence of urogenital dysfunctions such as urinary incontinence. Especially, both $\alpha 3$ and $\beta 2/4$ null mutant animals develop severe bladder distension within 2 days after birth (De Biasi, 2002). Clinically, the expression of nAChR $\alpha 3$ subunit has been known to be reduced or absent in patients of megacystis microcolon intestinal hypoperistalsis syndrome (MMHIS), which was identified by in situ hybridization and immunohistochemistry studies. Interestingly, those patients showed severe bladder dilatation in addition to intestinal obstruction. The results of our study in MPG neurons could suggest the molecular mechanisms of these above symptoms (Anneren et al, 1991; Richardson et al, 2001). In autonomic neuropathies producing urogenital problems, high level of autoantibodies to nAChRs was detected in blood (Vernino et al, 2000). Identification of the nAChR subtype to which autoantibodies bind has clinical importance. Therefore, our study to characterize the molecular and functional nature of nAChRs in the pelvic ganglia may provide important basis for understanding the pathogenesis of urogenital diseases and finding their therapeutic targets.

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