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Differential Effects of Thiopental on Neuronal Nicotinic Acetylcholine Receptors and P_{2X} Purinergic Receptors in PC12 Cells

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Background: **PC12 cells, derived from rat pheochromocytoma, express neuronal nicotinic acetylcholine receptors (nAchRs) and P_{2X} purinergic receptors, both of which resemble the receptors in postganglionic sympathetic neurons. The former is the established and the latter is the putative receptor to mediate fast synaptic transmission. The authors investigated effects of thiopental on these two ligand-gated ion channels.**

Methods: **Whole cell currents were recorded in PC12 cells without treatment of nerve growth factor, using conventional whole cell patch clamp technique. Nicotine or adenosine triphosphate (ATP) 30 μM was applied for 4–5 s in the absence or presence of thiopental 3–300 μM.**

Results: **Nicotine induced the rapidly decaying inward current at −60 mV, which exhibited the characteristics of the neuronal nAchR-mediated current. Thiopental inhibited the nicotine-induced inward current and accelerated the current decay in a dose-dependent manner, resulting in the greater effects on the steady current than the peak current. IC50s for the peak and steady current were 56.7 and 7.4 μM, when the anesthetic was coapplied with nicotine. Thiopental's inhibition was not associated with a change in the reversal potential and was voltage-independent at membrane potential of −30 to −70 mV. Most of thiopental's effects seemed to require channel opening. In contrast to the nicotine-induced current, thiopental had little effect on the current elicited by ATP.**

Conclusions: **Thiopental, whose reported EC50 for general anesthesia is 25 μM, inhibited the neuronal nAchR-mediated current but not the P_{2X} receptor-mediated response in PC12 cells at clinically relevant concentrations. Inhibition may result in suppression of synaptic transmission in sympathetic ganglia. (Key words: Anesthetics, intravenous: thiopental. Ion channels. Measurement techniques: patch clamp; PC12 cells. Receptors: nicotinic; purinergic.)**

NEURONAL nicotinic acetylcholine receptors (neuronal nAchRs) widely expressed in central and peripheral neurons are distinct from muscle nAchRs in terms of subunit composition, electrophysiology, and pharmacology.[1,2] For example, although muscle and neuronal receptors are composed of five subunits and muscle-type receptors consist of four different subunit types, neuronal counterparts require only one or two subunit types to form functional receptors.[2] Neuronal nAchRs mediate fast synaptic transmission in autonomic ganglia. Additionally, increasing evidence indicates that pre- and postsynaptic neuronal nAchRs play functional roles in central nervous system.[2,3] Although many studies showed the inhibitory effects of anesthetics on muscle nAchRs,[4,5] there have been few electrophysiologic studies investigating the anesthetic effects on neuronal nAchRs in mammalian cells.

Inhibitory effects by barbiturates on the nicotine-induced catecholamine release have been reported in adrenal medullary cells, suggesting inhibition of neuronal nAchRs.[6,7] The electrophysiologic studies using spectral analysis of macroscopic current and single channel recording revealed inhibitory effects on neuronal nAchRs by pentobarbitone and methohexitone.[8,9] However, direct stimulatory actions on $GABA_A$ receptor channels by the anesthetics could affect the effects on nicotinic channels because chromaffin cells possess functional $GABA_A$ receptor channels.[10,11] In contrast, PC12 cells, rat pheochromocytoma cell line, express ganglion type neuronal nAchRs[12–14] but lack functional $GABA_A$ receptor channels without treatment of nerve growth factors (NGF).[15,16]

In many tissues, extracellular adenosine 5′-triphosphate (ATP) has been shown to act as a transmitter or a

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cotransmitter and to activate purine P_2 receptors, which consist of two classes of receptors: G protein-linked receptors (mainly P_{2Y}) or ligand-gated ion channel (P_{2X}).[17] In the sympathetic and central nervous systems, it has been known that ATP is released from nerve terminals and binds to postsynaptic P_{2X} receptors, resulting in fast excitatory synaptic current,[18,19] and that nAchRs and P_{2X} receptors are often colocalized in the same neuron.[18-20] PC12 cells also express P_{2X} receptors and have been extensively studied on characteristics of these receptors.[21-23] However, effects of barbiturates on P_{2X} receptors have not been clarified.

Nicotinic acetylcholine receptors and P_{2X} receptors in PC12 cells resemble those expressed in rat postganglionic sympathetic neurons.[14,24-26] Acetylcholine is an established neurotransmitter, and ATP is a putative neurotransmitter in sympathetic ganglia. We used undifferentiated PC12 cells to study effects of thiopental on these two ligand-gated ion channels. The main aims of this study are (1) to study the effects of thiopental on neuronal nAchRs without potential contamination from direct actions on $GABA_A$ receptors using whole cell voltage clamp recording and (2) to compare this effect with that on the P_{2X} purinergic receptor-mediated current.

Materials and Methods

Cell Culture

PC12 cell line was provided by Japanese Cancer Research Resources Bank-Cell Bank. Cells were grown in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum and 5% horse serum plus 0.3 mg/ml glutamine, 50 U/ml penicillin, and 100 μg/ml streptomycin. Cells were maintained in 25-cm^2 flasks in a 95% air, 5% CO_2 atmosphere at 37°C. For the experiment, cells were plated on collagen-coated cover slips and used after additional 2-4day culture.

Electrophysiology

Membrane currents were measured by conventional whole cell voltage clamp method.[27] Cells on the cover slips were placed in a recording bath with an approximate volume of 1.5 ml and continuously perfused at the rate of 1-2 ml/min with a standard external solution containing (in mM), NaCl, 140; KCl, 5.4; $CaCl_2$, 1.8; $MgCl_2$, 1.0; N-2-hydroxy ethylpiperazine-N′-2-ethanesulphonic acid (HEPES), 10; and glucose, 11.1 (pH was adjusted to 7.4 with NaOH). Heat-polished patch pipettes had tip resistance of 2-7 MΩ when filled with an intracellular solution containing (in mM) CsCl, 150; HEPES, 10; ethylene glycol-bis-(β-aminoethyl ether) tetraacetic acid, 5; Mg-ATP, 2 (pH, 7.3 with CsOH). Cells were voltage clamped at −60 mV with a patch clamp amplifier (CEZ 2300, Nihon Koden, Tokyo, Japan) unless otherwise stated. Whole cell currents were filtered at 0.2 KHz with Bessel filter and digitized at 0.5 kHz in most of the cases, or they were filtered at 0.2 KHz with Gaussian filter and digitized at 1 KHz in a few cases. The currents were stored and analyzed on a computer using Pclamp software (Axon Instruments, Foster City, CA). For display of the current traces, the data were 10 times reduced by averaging 10 points to yield a single point. All experiments were performed at room temperature (22-25°C)

Drug Application

Nicotine or ATP-Na_2 of 30 μM in the external solution was applied to cells using a rapid application technique described as the "Y-tube" method.[28] The tip of the Y-tube was made by a glass micropipette (Microcaps, 2 μl, Drummond) with about 100 μm-opening and was positioned about 500 μm from the recorded cell. This method enabled the complete exchange of the external solution surrounding the cell around 100 ms, as estimated by recording the liquid junction current produced at an open patch pipette. The agonists with or without anesthetics were applied for 4-5 s, and each application was separated by 5 min. Thiopental was coapplied with the agonists using the same method. For preincubation with the anesthetic or the antagonists, the external solutions containing the drugs were 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 the measurement.

Nicotine of 100 or 300 μM was also tested to see if higher concentration of nicotine can overcome the inhibition by thiopental.

Current-voltage Relationship

Instantaneous current-voltage curves were obtained for nicotine 30 μM alone and for nicotine with thiopental 30 μM after preincubation of thiopental. A ramp pulse of +30 to −70 mV (100 mV/200 ms) was applied to a cell every 200 ms,[29] and current traces near the peak current were subtracted from those in the absence of agonist. Five cells that exhibited slow desensitization were chosen for this experiment to avoid a large decline of the current during the ramp pulse.

Drugs

Drugs used in the current study included (−)-nicotine, hexamethonium, suramin (Wako, Osaka, Japan), ATP-Na_2, ATP-Mg (Sigma, St Louis, MO), and thiopental sodium (Tanabe, Osaka, Japan). Thiopental sodium was dissolved in distilled water to make 100 mM stock solution and diluted with the external solution to the designated concentration.

Data Analysis

We measured the peak current and the steady current, which was defined as the average of the preceding 50 points at 4 s during agonist application. Because nicotine-elicited currents declined with each application of agonist, the response in the presence of thiopental was compared with the average of the elicited current before and after thiopental application. To verify this procedure, nicotine was applied successively three times with an interval of 5 min, and the second response was compared with the average of the first and third responses. The same procedure was applied for the ATP-induced current.

The decaying phases of the nicotine-induced current were fitted either to a single or a double exponential function of the following form by simplex method using Axograph software (Axon Instruments, Foster City, CA):

$$I = I_{final} + \sum I_i \times \exp(-t/\tau_i)$$

where I is the total peak current, I_{final} is the residual current during the steady state condition, I_i is the peak current amplitude of the each component, and τ_i is the time constant of the corresponding component. Goodness of fit was compared by chi-square test between a single and a double exponential models. The time constants of the decay were measured for the baseline responses just before the thiopental administration (Baseline) and the responses in the presence of thiopental (Thiop). These measurements were done for the experiments with thiopental preincubation and for the ones wherein nicotine alone was applied successively. The ratios of the time constants for two successive responses (Thiop/Baseline) were calculated to see the time-dependent changes and the effects of thiopental. Desensitization was also evaluated by calculating the percent decay of the current (% current decay) defined by the equation[30]:

$$\%\ \text{current decay} = (I_{peak} - I_{steady})/I_{peak} \times 100$$

where I_{peak} denotes the peak current and I_{steady} denotes the steady current.

Statistical Analysis

Data are expressed as mean ± SEM; unpaired *t* test and analysis of variance (ANOVA) followed by Dunnett's test were used to estimate the significance when appropriate. *P* less than 0.05 was considered to be significant.

Results

The Nicotine-induced Current

Nicotine elicited inward currents at −60 mV. The nicotine-induced current decayed rapidly during agonist application, consistent with other studies, as a result of desensitization.[13,29,31] The peak current varied greatly with recorded cells, from 50 to 800 pA. When nicotine was applied successively, the peak and steady current declined with each application, so that the third response accounted for 72 ± 9% for the peak current and 66 ± 13% for the steady current of the first response (n = 7). The magnitude of decline seemed to depend on the interval between the applications of the agonist, *i.e.,* the short interval enhanced the decline. Therefore, some reversible processes such as desensitization and reversal from desensitization should contribute to this phenomenon at least in part. Because the second response was close to the average of the first and the third response, 100 ± 3% for the peak current and 102 ± 6% for the steady current (n = 7), the effects of thiopental were evaluated by comparison with the average of the control responses before and after administration of thiopental.

The nicotine-induced current was strongly depressed by 100 μM hexamethonium, a competitive antagonist for neuronal nAch receptors. The peak and steady current was 17 ± 3% and 10 ± 2% of the average of the control responses when 100 μM hexamethonium was preperfused and coapplied (n = 4).

Effects of Thiopental on the Nicotine-induced Current

Thiopental alone induced no current responses at 100 or 300 μM in tested cells (n = 6 for both). 300 μM is about 10 times higher than reported EC50 for general anesthesia and the maximum concentration used in the present study.[32] Thiopental, 3–300 μM, when coapplied with nicotine, inhibited the peak and steady current in a dose-dependent manner (fig. 1). The inhibition was much stronger for the steady current than for the peak current. Thiopental, 100 μM, almost completely abol-

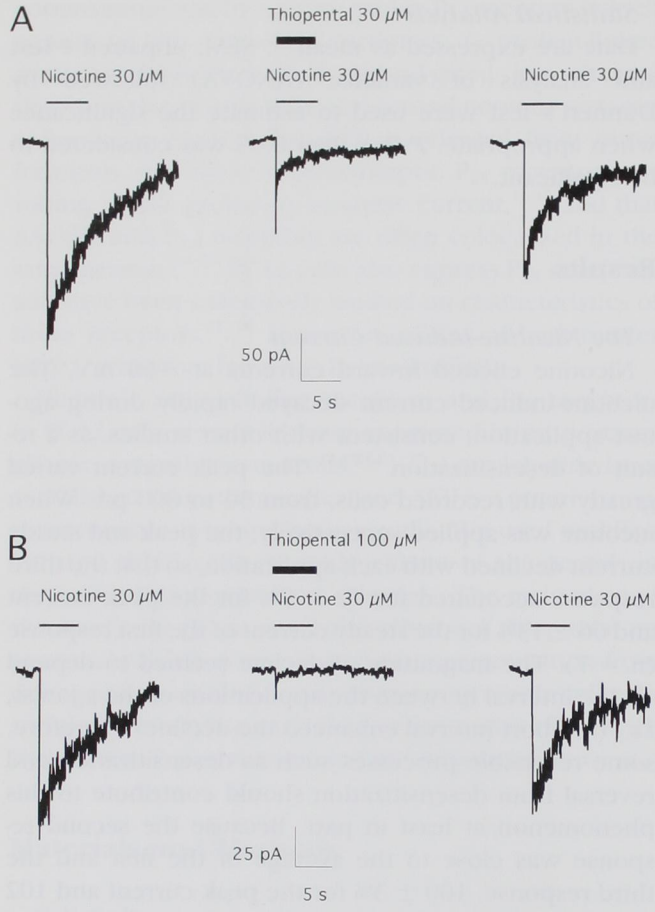

Fig. 1. Inhibition by thiopental of the current evoked by 30 μM nicotine in PC12 cells. Cells were held at −60 mV. Nicotine was applied during the period indicated by *light horizontal bar*. *Thiopental 30 (A)* and 100 μM (*B*) was coapplied with nicotine as indicated by *thick bar*. Thiopental suppressed the nicotine-induced current reversibly, with greater effects on the steady current than on the peak current. Thiopental, 100 μM, almost completely abolished the steady current.

ished the steady current. Regarding reversibility of inhibition, the ratio of the postcontrol responses after thiopental to the precontrol values was not different from that of three successive nicotine applications, indicating that nicotine responses recovered fully from thiopental's inhibition after a 5-min interval (fig. 2). IC50s for the peak and steady current were 56.7 ± 10.1 and 7.4 ± 1.4 μM, respectively (fig. 3A).

When cells were preincubated with thiopental and then nicotine and thiopental were coapplied, inhibition of the peak current was still much smaller than that of the steady current. The relative peak currents in the presence of thiopental with or without preincubation were not different from each other at any dose of thiopental except for 3 μM, wherein the relative peak current without preincubation was significantly smaller than that with preincubation. The magnitudes of blockade of the steady currents were also similar regardless of the presence or absence of preincubation. IC50s for the peak and steady current were 49.2 ± 11.1 and 6.1 ± 0.6 μM, when thiopental preincubation was performed (fig. 3B). There were no statistically significant differences in IC50 or Hill coefficient for the peak and

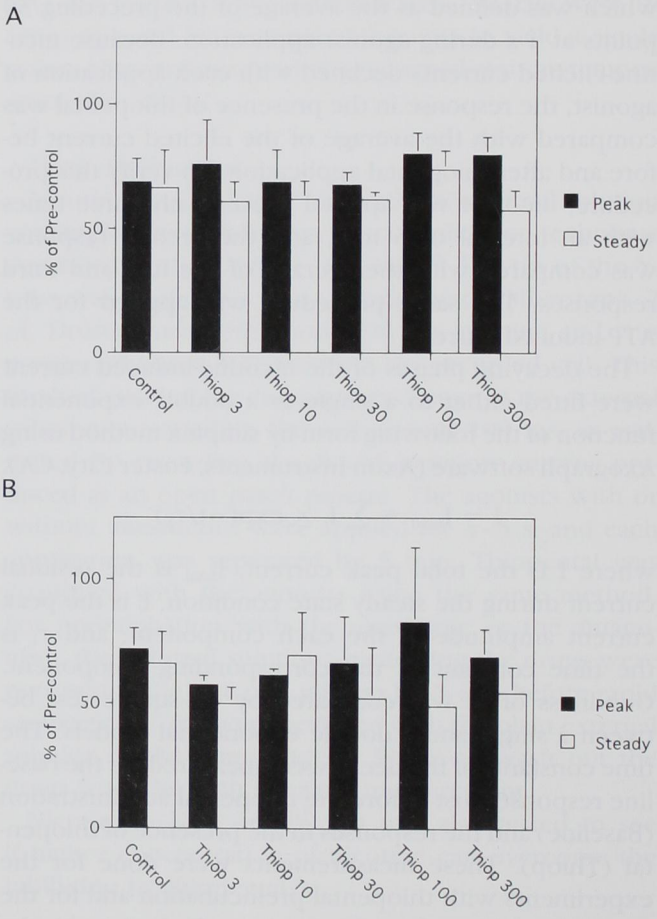

Fig. 2. Comparison of recovery from thiopental's inhibition among different conditions. In the control group, nicotine was applied successively three times with 5-min interval; in the thiopental groups, nicotine with thiopental of designated dose was applied between two nicotine applications. The third nicotine-induced responses were normalized to the first baseline responses (% of precontrol) to see the natural decline of the nicotine responses in the control group and the extent of recovery from inhibition in the thiopental groups. In the absence (*A*) or the presence of (*B*) preincubation of thiopental, % of precontrol were not different from that of the control condition at any dose of thiopental, indicating that recovery from thiopental's inhibition was complete. Each column represents mean with SEM from four to seven experiments.

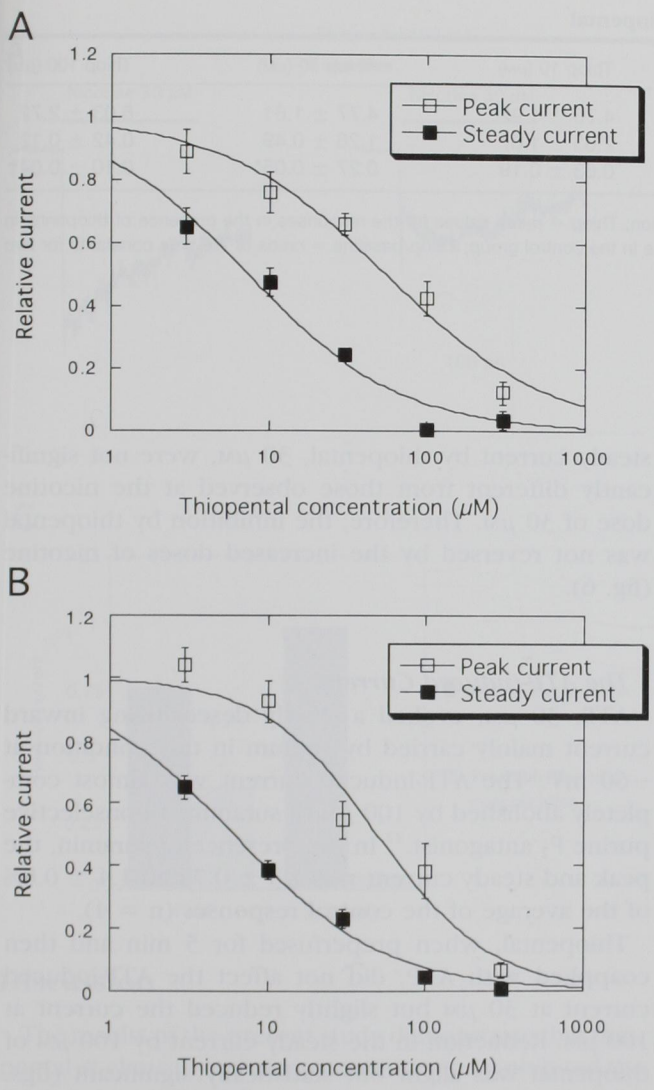

Fig. 3. The concentration–inhibition curve for thiopental on the nicotine-induced current. The currents in the presence of thiopental were normalized to the average of the control currents before and after thiopental and plotted against the concentration of thiopental. A least-squares fit was performed using the equation: $I = 1 - C^n/(C^n + Kd^n)$, where I: relative current, C: concentration of thiopental, n: the Hill coefficient, Kd: the dissociation constant. Without preincubation (*A*), the fitting procedure gave a Kd of 56.7 ± 10.1 μM, a Hill coefficient of 0.83 ± 0.13 for the peak current, and gave a Kd of 7.4 ± 1.4 μM and a Hill coefficient of 0.9 ± 0.16 for the steady current. With preincubation (*B*), it gave Kd of 49.2 ± 11.1 and 6.1 ± 0.6 μM and Hill coefficients of 1.19 ± 0.29 and 0.9 ± 0.08 for the peak and steady current. r^2 was 0.96 in (*A*) and 0.99 in (*B*), respectively. Each point represents mean of four to six experiments, and error bar indicates SEM.

steady current measured with thiopental preincubation when compared with the values obtained without preincubation.

Desensitization of the Nicotine-induced Current

The decay phases of the nicotine-induced current with and without thiopental were well fitted to single exponential functions. Because fitting to double exponential curve did not improve the adequacy of the fit, single exponential models were used. Although the absolute values of the time constants of the decay varied greatly cell to cell for the control responses (from 0.73 to 10.79 s), they did not change significantly with the repeated nicotine applications as judged by the ratio of the time constants of two successive nicotine responses. There was a tendency that the time constants decreased with increasing concentration of thiopental, although these changes did not reach a statistical significance because of the great variability. When changes in the ratio of the time constants were analyzed with respect to thiopental concentrations, thiopental clearly produced a dose-dependent decrease in the ratio of Thiop/Baseline, indicating that the anesthetic accelerated the decay rate in a dose-dependent manner (table 1). These changes were fitted to the empirical Hill equation (ratio = $IC50^n/(C^n + IC50^n)$, where C is thiopental concentration; n is Hill coefficient). Fitting was found to be very good ($r^2 = 0.96$) and gave a IC50 of 13.3 μM ± 3.2 and an n of 1.05 ± 0.27.

The % current decay remained unchanged with the repeated applications of nicotine. Thiopental dose-dependently increased % current decay (table 2). Increases in % current decay from the value for nicotine alone were analyzed by fitting to the following equation:

$$\%\ \text{current decay} - 54.8 = 45.2 \times C^n/(C^n + EC50^n)$$

where 54.8 is the mean value at the control condition, 45.2 is the maximal value, C is thiopental concentration, and n is Hill coefficient. The equation described the thiopental-induced increase in % current decay very well ($r^2 = 0.98$). EC50 and Hill coefficient were estimated to be 9.1 ± 1.9 μM and 0.73 ± 0.14, respectively.

These analyses showed that thiopental dose-dependently accelerated the current decay and that thiopental concentrations producing the half maximal effects were slightly higher but comparable to IC50 for inhibition of the steady current.

Effects of Thiopental Preincubation Only

When cells were preincubated with thiopental, 100 μM, and then only nicotine was applied, the peak current was slightly reduced, but inhibition of the steady

Table 1. Time Constants of the Current Decay with or without Thiopental

	Control	Thiop 3 (μM)	Thiop 10 (μM)	Thiop 30 (μM)	Thiop 100 (μM)
Baseline (s)	3.79 ± 1.58	3.69 ± 0.65	4.76 ± 1.92	4.77 ± 1.61	6.03 ± 2.72
Thiop (s)	3.29 ± 1.44	2.17 ± 0.20	2.63 ± 1.07	1.26 ± 0.49	0.42 ± 0.12
Thiop/baseline	1.14 ± 0.30	0.78 ± 0.25	0.63 ± 0.19	0.27 ± 0.05*	0.10 ± 0.04†

Baseline = mean values for the baseline responses before thiopental administration; Thiop = mean values for the responses in the presence of thiopental in the thiopental groups and for the responses to the second applications of nicotine in the control group; Thiop/baseline = ratios of the time constants for two successive responses.

Values are mean ± SE. There were 4 to 7 experiments for each condition.

* $P < 0.05$ *versus* Thiop/Baseline of the control group.

† $P < 0.01$ *versus* Thiop/Baseline of the control group.

current was minimal (figs. 4A and 4B). Blockade by preapplied thiopental was significantly smaller for the peak and steady current than that by preincubation plus coapplication of thiopental, suggesting that channel opening by nicotine enhanced thiopental's effect (fig. 4C).

The Current-Voltage Relationship

The nicotine-induced current exhibited strong inward rectification and reversed around 0 mV, as previously described.[13,31,33] Thiopental depressed the inward current without any obvious changes in the reversal potential (fig. 5). The magnitudes of depression of the inward current were 68 ± 4, 77 ± 2, and 71 ± 4% at −30, −50, and −70 mV from the analysis of five pairs of the current-voltage curves. These values were statistically insignificant among three membrane potentials, suggesting that the thiopental's inhibition is voltage-independent from −30 to −70 mV. However, thiopental's effects seemed small at the more depolarizing potential.

Higher Concentration of Nicotine

When the dose of nicotine was increased to 100 or 300 μM, the magnitudes of depression of the peak or steady current by thiopental, 30 μM, were not significantly different from those observed at the nicotine dose of 30 μM. Therefore, the inhibition by thiopental was not reversed by the increased doses of nicotine (fig. 6).

The ATP-induced Current

ATP, 30 μM, evoked a slowly desensitizing inward current mainly carried by sodium in this condition at −60 mV. The ATP-induced current was almost completely abolished by 100 μM of suramin, a nonselective purine P_2 antagonist.[22] In the presence of suramin, the peak and steady current was 1.3 ± 0.7 and 1.4 ± 0.8% of the average of the control responses (n = 4).

Thiopental, when preperfused for 5 min and then coapplied with ATP, did not affect the ATP-induced current at 30 μM but slightly reduced the current at 100 μM. Reduction in the steady current by 100 μM of thiopental was slight but statistically significant (figs. 7A and 7B). Thiopental induced no remarkable changes in the current-voltage relationship of the ATP-induced peak current at membrane potential from −70 to 30 mV (fig. 8).

Table 2. Percent Current Decay of the Nicotine-induced Current with or without Thiopental

	Control	Thiop 3 (μM)	Thiop 10 (μM)	Thiop 30 (μM)	Thiop 100 (μM)
Baseline (%)	53.34 ± 5.7	41.61 ± 3.92	54.96 ± 11.31	57.26 ± 9.78	60.07 ± 4.72
Thiop (%)	54.8 ± 6.54	67.34 ± 3.13*	81.32 ± 4.06*†	83.05 ± 4.72†‡	94.7 ± 2.06†‡

Baseline = mean values for the baseline responses before thiopental administration; Thiop = mean values for the responses in the presence of thiopental in the thiopental groups and for the responses to the second applications of nicotine in the control group.

Values are mean ± SE. There were 4 to 7 experiments for each condition.

* $P < 0.05$ *versus* baseline in the same group.

† $P < 0.05$ *versus* corresponding value of the control group.

‡ $P < 0.01$ *versus* baseline in the same group.

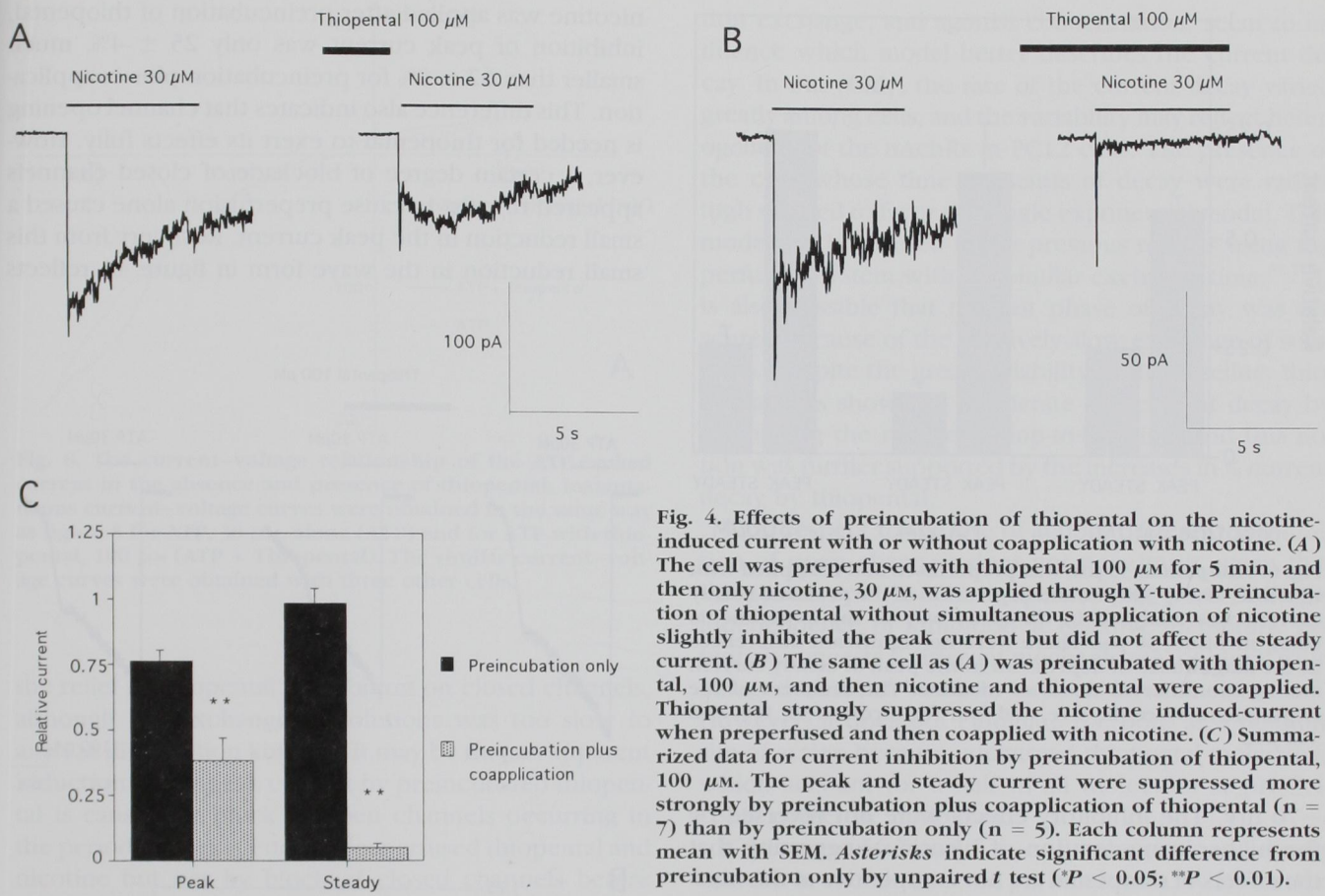

Fig. 4. Effects of preincubation of thiopental on the nicotine-induced current with or without coapplication with nicotine. (*A*) The cell was preperfused with thiopental 100 μM for 5 min, and then only nicotine, 30 μM, was applied through Y-tube. Preincubation of thiopental without simultaneous application of nicotine slightly inhibited the peak current but did not affect the steady current. (*B*) The same cell as (*A*) was preincubated with thiopental, 100 μM, and then nicotine and thiopental were coapplied. Thiopental strongly suppressed the nicotine induced-current when preperfused and then coapplied with nicotine. (*C*) Summarized data for current inhibition by preincubation of thiopental, 100 μM. The peak and steady current were suppressed more strongly by preincubation plus coapplication of thiopental (n = 7) than by preincubation only (n = 5). Each column represents mean with SEM. *Asterisks* indicate significant difference from preincubation only by unpaired *t* test (*$P < 0.05$; **$P < 0.01$).

Discussion

The results of the present study demonstrate that thiopental at clinically relevant concentrations inhibited the neuronal nAchR-mediated current but not the P_{2X} purinergic receptor-mediated response in PC12 cells.

Thiopental at high concentrations did not induce any current consistent with the absence of functional $GABA_A$ receptors in PC12 cells as previously described,[16] whereas high concentration of barbiturates elicited an inward current in adrenal chromaffin cell under the similar conditions.[8,10] Therefore, a potential influence from direct effects of thiopental on $GABA_A$ receptor can be excluded in this study.

Inhibition by Thiopental on Neuronal nAchRs

Thiopental reversibly depressed the nicotine-induced current and augmented desensitization in a dose-dependent manner. The reversal potential of the nicotine-induced current was not changed, and inhibition was voltage-independent at membrane potential of −30 to

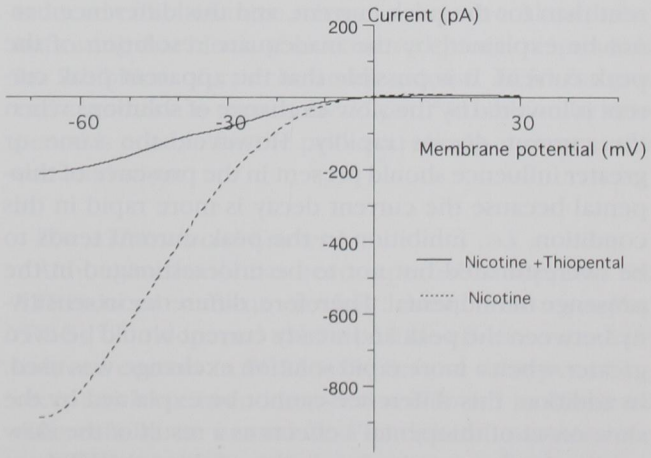

Fig. 5. The current–voltage relationship of the nicotine-induced current in the absence and presence of thiopental. Instantaneous current–voltage curves were obtained for nicotine, 30 μM, alone (Nicotine) and for nicotine with thiopental, 30 μM, after preincubation of thiopental (Nicotine + Thiopental) as described in method. The similar current–voltage curves were obtained with four other cells.

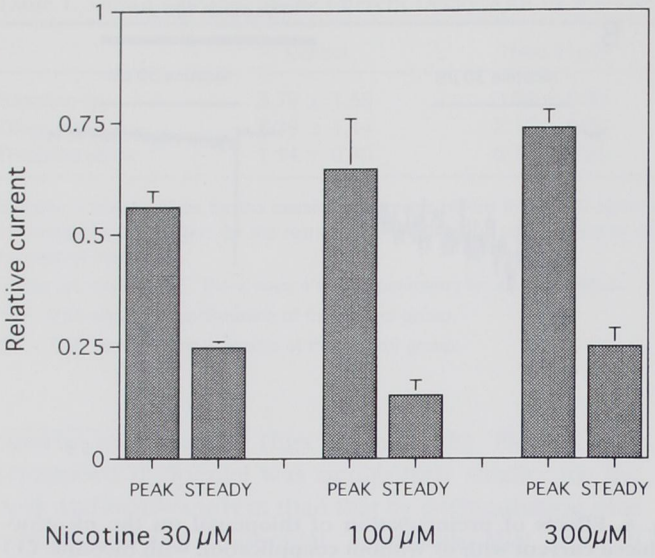

Fig. 6. Thiopental, 30 μM, was preperfused and coapplied with nicotine, 100 or 300 μM, and depression of the peak and steady current was analyzed in the same way as the experiments using nicotine, 30 μM. There were no significant differences in the magnitudes of depression in the peak or steady current among the different doses of nicotine. The numbers of the experiments were six, five, and four for nicotine 30, 100, and 300 μM.

−70 mV. The inhibitory effects were not reversed by the higher concentration of nicotine, suggesting that the effects of thiopental are not competitive at the nicotine-binding sites.

The inhibitory effects were greater for the steady current than for the peak current, and this difference cannot be explained by the inadequate resolution of the peak current. It is possible that the apparent peak current is lowered by the slow exchange of solutions when the current decays rapidly. However, the same or greater influence should present in the presence of thiopental because the current decay is more rapid in this condition, *i.e.*, inhibition in the peak current tends to be overestimated but not to be underestimated in the presence of thiopental. Therefore, difference in sensitivity between the peak and steady current would be even greater when a more rapid solution exchange was used. In addition, this difference cannot be explained by the slow onset of thiopental's effects as a result of the slow application because preincubation of thiopental did not potentiate the magnitude of inhibition of the peak current significantly.

Lack of significant effects of preincubation is attributable to lack of inhibition of closed channels and indicates preferential inhibition of open channels.[34] When nicotine was applied after preincubation of thiopental, inhibition of peak current was only 25 ± 4%, much smaller than 62 ± 9% for preincubation plus coapplication. This difference also indicates that channel opening is needed for thiopental to exert its effects fully. However, a certain degree of blockade of closed channels appeared to exist because preperfusion alone caused a small reduction in the peak current. Recovery from this small reduction in the wave form in figure 4A reflects

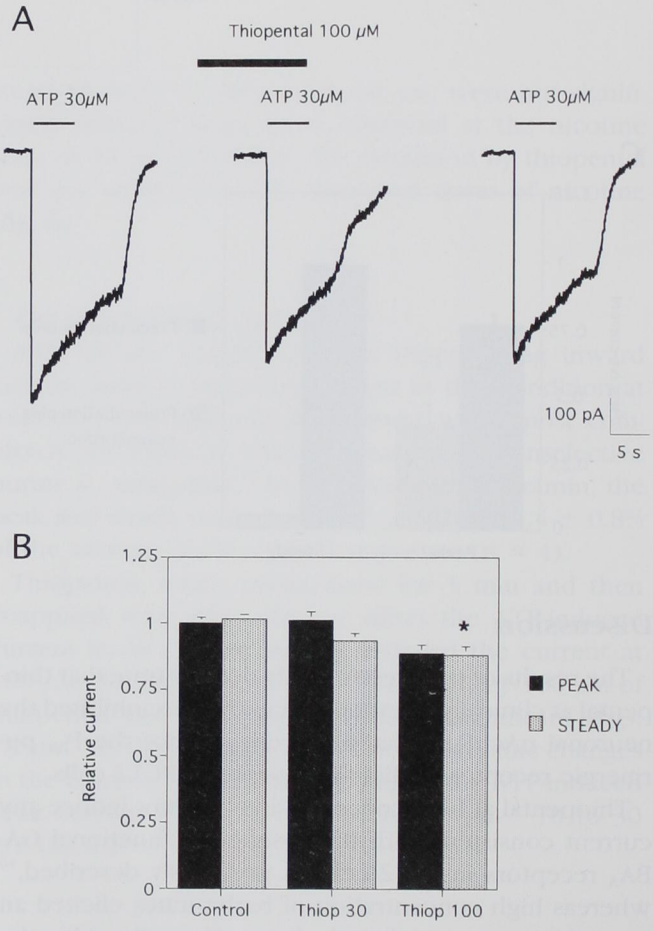

Fig. 7. Effects of thiopental on the ATP-evoked current. (*A*) The current activated by rapid perfusion of ATP, 30 μM, was measured in cell held at −60 mV before and after thiopental (*right* and *left panel*). Thiopental, 100 μM, was applied with ATP after 5 min preperfusion (*middle panel*). (*B*) Summarized data for effects of thiopental on the ATP-evoked current. The current was measured as in (*A*) and normalized to the average of the control current before and after thiopental applications. In the control condition, relative current is the second response normalized to the average of the first and third responses during three successive ATP applications. Each column represents mean and SEM of four to five experiments. An *asterisk* indicates significant difference from the control by Dunnett's test for multiple comparison (*$P < 0.05$).

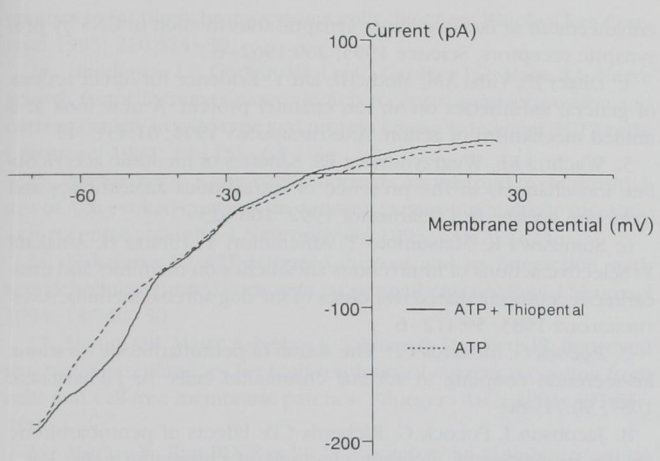

Fig. 8. The current–voltage relationship of the ATP-evoked current in the absence and presence of thiopental. Instantaneous current–voltage curves were obtained in the same way as figure 5 for ATP, 30 μM, alone (ATP) and for ATP with thiopental, 100 μM (ATP + Thiopental). The similar current–voltage curves were obtained with three other cells.

the relief of thiopental's inhibition on closed channels, although the exchange of solutions was too slow to analyze dissociation kinetics. It may be that an apparent reduction in the peak current by preincubated thiopental is caused by block of open channels occurring in the period of coexistence of preperfused thiopental and nicotine but not by block of closed channels before agonist application. A relatively slow exchange time of the drug application system allowed a sufficient time for two of these agents to coexist. Additionally, poor time resolution of the recording condition prevents the definitive conclusions. Therefore, it is not clear how much thiopental acts on closed channels, although most inhibition is exerted on open channels.

Preferential inhibition on open channels is consistent with the results of single channel recording of neuronal nAchRs using bovine chromaffin cells, which demonstrated that pentobarbitone and methohexitone principally shorten the mean open time and induce "flickering".[8,9] The decrease in the exponential time constants of the current decay by thiopental also agree with the decrease in mean channel open time.[35] Barbiturates' inhibition of open channels has been also reported for the non-NMDA receptor-mediated current in rat cortical neuron.[36]

The decay phases of neuronal nAchRs have been analyzed by fitting to one or two exponential functions in several papers.[30,31,37,38] Several factors, such as the subunit composition of the receptors, the speed of solution exchange, and agonist concentration, seem to influence which model better describes the current decay. In our study, the rate of the current decay varied greatly among cells, and the variability may reflect heterogeneity of the nAchRs in PC12 cells. The presence of the cells whose time constants of decay were rather high seemed to favor the single exponential model. This model has been used in the previous reports using the perfusion system with the similar exchange time.[31,37] It is also possible that the fast phase of decay was obscured because of the relatively slow exchange of solutions. Despite the great variability in the baseline, thiopental was shown to accelerate the current decay by calculating the ratio of Thiop-to-Baseline, and this notion was further supported by the increases in % current decay by thiopental.

With respect to the site of action, preferential inhibition of open channels does not necessarily mean that thiopental acts on the ion-permeating pore. Voltage-independence of inhibition at negative membrane potentials suggests a different mechanism(s) of block from charged channel blockers such as local anesthetics.[39] However, it does not eliminate the pore as a possible site of action because uncharged thiopental molecules, which account for a half of all molecules at pH 7.4, could cause the inhibition.

IC50s were 56.7 and 7.4 μM for the peak and steady current in the absence of preincubation. IC50 for the steady current in this study is close to the reported IC50 (7.8 μM) for thiopental's inhibition of the nicotine-induced catecholamine release in dog adrenal medulla,[6] indicating that block of nicotine receptor is responsible for inhibition of catecholamine release. EC50 for general anesthesia is reportedly 25 μM.[32] Inhibition by thiopental is notable for the peak and steady current at this concentration.

The ATP-induced Current

Although ethanol has been reported to suppress the ATP-induced current in frog dorsal root ganglion (DRG),[40,41] thiopental exhibited little effect on the ATP-induced current in PC12 cells. Several subtypes of P_{2X} receptors have been identified and found to distribute differentially in central and peripheral nervous systems.[42,43] It is likely that the dominant subunits in frog DRG and PC12 cells are different because they exhibit different sensitivities to agonists.[21,22,42] Therefore, difference in the subunits and difference in the agents can explain this discrepancy. P_{2X} receptors in rat sympathetic neuron from superior cervical ganglia mostly re-

semble those in PC12 cells.[29] Therefore, it is suggested that thiopental does not much affect P_{2X} receptors in rat sympathetic neurons.

Although the P_{2X} receptors function as a ligand-gated ion channel, there is no primary sequence homology between the P_{2X} receptor and other ligand-gated ion channels such as nicotinic, glutamate, $GABA_A$, and glycine receptor, and the structure of the P_{2X} receptor is considered to be very different from that of other ligand-gated ion channels.[42] The finding that P_{2X} receptors are much less sensitive to thiopental than neuronal nAchRs indicates that P_{2X} receptors lack the structure wherein thiopental can act on with high affinity.

Clinical Implications

We found that neuronal nAchRs in PC12 cells are very sensitive to thiopental, whereas this is not the case for P_{2X} purinergic receptors. The former finding is consistent with the previous studies, demonstrating high sensitivity of molluscan neuronal nAchRs receptors to various anesthetics.[44] In molluscan, neuronal nAchRs exhibit different characteristics from those of mammalian receptors, *i.e.*, some of molluscan receptors show extraordinarily high sensitivity to nicotinic agonists and are permeable to Cl^-.[44] Neuronal nAchRs receptors in PC12 cells resemble the receptors of sympathetic ganglia neuron in terms of ion selectivity, current-voltage relationship, pharmacology, putative subunit composition.[12-14,24,25] Therefore, it is conceivable that thiopental suppresses synaptic transmission in sympathetic ganglia by inhibition of neuronal nAchRs. However, further studies are needed to define anesthetic effects on central nAchRs and the significance of such effects for general anesthesia because central nAchRs are heterogeneous and different from ganglionic counterparts in terms of subunit composition and pharmacology.[2]

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