Dual Effects of Intravenous Anesthetics on the Function of Norepinephrine Transporters

Koji Hara, M.D., Ph.D.,* Nobuyuki Yanagihara, Ph.D.,† Kouichiro Minami, M.D., Ph.D.,*
Hideyasu Hirano, M.D., Ph.D.,‡ Takeyoshi Sata, M.D., Ph.D.,§ Akio Shigematsu, M.D., Ph.D.,∥
Futoshi Izumi, M.D., Ph.D.#

Background: Norepinephrine transporters (NETs) terminate the neuronal transmission of norepinephrine, which is released from noradrenergic neurons. To investigate the interaction with NET, the authors examined the effects of short- and long-term treatment with anesthetics on the activity and mRNA level of NET.

Methods: To assay [³H]norepinephrine uptake, bovine adrenal medullary cells in culture were incubated with [³H]norepinephrine in the presence of intravenous anesthetics, including propofol, thiamylal, and diazepam. To study the direct interaction between the anesthetics and NET, the effect of propofol on the binding of [³H]desipramine to the plasma membrane was examined. To study the long-term effect of anesthetics, [³H]norepinephrine uptake by cells pretreated with propofol for 6–24 h and [³H]desipramine binding after pretreatment for 12 h were measured. Simultaneously, we examined the effect of anesthetics on the expression of NET mRNA using the reverse transcriptase–polymerase chain reaction.

Results: All of the intravenous anesthetics inhibited [³H]norepinephrine uptake in a concentration-dependent manner. The active concentrations of propofol (1–3 μM) and thiamylal ($\leq$ 30 μM) were similar to those encountered clinically. The kinetic analysis revealed that all the anesthetics noncompetitively inhibited [³H]norepinephrine uptake. Propofol inhibited [³H]desipramine binding with a potency similar to that observed in [³H]norepinephrine uptake. Scatchard analysis showed that propofol competitively inhibited [³H]desipramine binding. On the other hand, long-term treatment of cells with propofol (10 μM) enhanced the NET functional activity and [³H]desipramine binding, and also increased the level of NET mRNA.

Conclusions: These results suggest that intravenous anesthetics have a dual effect on NET; short-term treatment causes inhibition, whereas long-term treatment leads to up-regulation. The interaction of intravenous anesthetics with NET may modulate the neuronal transmission of norepinephrine during anesthesia. (Key words: Noradrenergic; target protein; tricyclic antidepressant.)

THE anesthetic action induced by general anesthetics is a complex phenomenon. Its important component appears to be an alteration of synaptic transmission in the central nervous system.[1,2] A large number of recent studies have led to the proposal that actions on postsynaptic neurotransmitter receptors or presynaptic neurotransmitter release account for the dominant central nervous system–depressant effects of anesthetics.[3,4] On the other hand, several lines of evidence have shown that some anesthetics also interact with presynaptic transporters to alter the uptake of neurotransmitters. For example, anesthetics inhibit the uptake of γ-aminobutyric acid,[5] dopamine,[6] and serotonin[7] by rat brain synaptosomes. It is well known that ketamine suppresses the uptake of norepinephrine by sympathetic neurons,[8–10] which may explain its sympathetic stimulation. This evidence suggests that neurotransmitter transporters are also a target site for anesthetics.

Norepinephrine transporters (NETs) located in the presynaptic membranes of noradrenergic nerve terminals mediate the termination of neurotransmission by the reuptake of norepinephrine released into the extracellular milieu.[11] Human NET was the first NET cloned, and its mRNA was localized in the brainstem and adrenal gland.[12] Bönisch's group cloned bovine adrenal medullary NET and reported that its pharmacologic properties were similar to those of the NET in central and peripheral noradrenergic neurons.[13,14] Bovine NET expression in adrenal medullary cells has been used as a model system to study the effect of drugs on NET in noradrenergic neurons.[15–17]

Recently, we reported that ketamine inhibits [³H]norepinephrine uptake *via* an interaction with NET at a site partly overlapping the binding site of desipramine,[17] a selective inhibitor of NET. In the present study, we further examine the effects of other intravenous anesthetics (propofol, thiamylal, and diazepam) on NET in cultured bovine adrenal medullary cells. Comparing present results with those for ketamine, we searched for a putative common site for intravenous anesthetics on the NET. We also studied the effects of long-term treatment with propofol on the [³H]norepinephrine uptake, [³H]desipramine binding, and mRNA level of NET.

Materials and Methods

Materials

Eagle's minimum essential medium (MEM) was obtained from Nissui Pharmaceuticals (Tokyo, Japan). Fetal calf serum, l-norepinephrine, pargyline hydrochloride, and l-ascorbic acid were obtained from Nacalai Tesque (Kyoto, Japan). Collagenase was obtained from Nitta

Zerachin (Osaka, Japan). 2,6-Diisopropylphenol (propofol) was obtained from Tokyo Kasei (Tokyo, Japan). Diazepam hydrochroride was a gift from Takeda Chemical Industries (Osaka, Japan). Thiamylal sodium was obtained from Sankyo Co. (Osaka, Japan). Desipramine hydrochloride was obtained from Sigma (St. Louis, MO), and nisoxetine hydrochloride was obtained from Research Biochemicals International (Natick, MA). l-[7,8-3[^{3}H]Noradrenaline (34.0 Ci/mmol) was obtained from Amersham International (Buckinghamshire, United Kingdom), and [benzene ring, 10,11-[^{3}H]-desmethylimipramine (desipramine) hydrochloride (73.0 Ci/mmol) was obtained from New England Nuclear (Boston, MA). 2,6-Diisopropylphenol was diluted with dimethyl sulfoxide for experiments. Dimethyl sulfoxide at the concentrations used for experiments had no effect on [^{3}H]norepinephrine uptake and [^{3}H]desipramine binding. Thiamylal sodium and diazepam hydrochroride were dissolved with distilled water.

Isolation and Culture of Adrenal Medullary Cells

Adrenal medullary cells were isolated from bovine adrenal medulla as described previously.[18] The cells were plated at 4×10^6 cells per dish (Falcon, 35 mm) in Eagle's MEM containing 10% fetal calf serum, 60 μg/ml aminobenzylpenicillin, 100 μg/ml streptomycin, 0.3 μg/ml amphotericin B, and 3.0 μM cytarabine.[15] The cells were cultured in 5% CO_2–95% air at 37°C and used for experiments between 2 and 4 days of culture.

[^{3}H]Norepinephrine Uptake by the Cells

Cultured cells (4×10^6 per dish) were incubated at 37°C for 15 min in oxygenated Krebs-Ringer phosphate buffer containing 100 μM pargyline, 100 μM ascorbic acid, and 500 nM [^{3}H]norepinephrine in the presence or absence of propofol (0.1–300 μM), thiamylal (3–1,000 μM), or diazepam (3–1,000 μM). Pargyline is a monoamine oxidase inhibitor that prevents the enzymatic decomposition of norepinephrine in the cells. Ascorbic acid is an antioxidant of norepinephrine. Krebs-Ringer phosphate buffer was composed of 154 mM NaCl, 5.6 mM KCl, 1.1 mM $MgSO_4$, 2.2 mM $CaCl_2$, 0.85 mM NaH_2PO_4, 2.15 mM Na_2HPO_4, and 10 mM glucose, adjusted to pH 7.4. For the kinetic analysis of [^{3}H]norepinephrine uptake, the cells were incubated with increasing concentrations (1–30 μM) of [^{3}H]norepinephrine in the presence or absence of 100 μM propofol, thiamylal, or diazepam. After incubation, the cells were rapidly washed four times with 1 ml ice-cold buffer and solubilized in 1 ml Triton X-100 (10%; Nacalai Tesque, Kyoto, Japan). The radioactivity in the solubilized cells was counted by a liquid scintillation counter (LSC-3500E; Aloka, Tokyo, Japan). Nonspecific uptake was determined in the presence of 10 μM desipramine, and specific uptake was obtained by subtracting the nonspecific uptake from

the total uptake. The desipramine-sensitive uptake was $92 \pm 3\%$ (n = 12) of the total uptake.

[^{3}H]Desipramine Binding to Plasma Membranes

Plasma membranes isolated from bovine adrenal medulla were prepared as described previously.[19] The binding of [^{3}H]desipramine was determined by incubation of membranes (10 μg protein) suspended in buffer B (composition: 135 mM NaCl, 10 mM Tris-HCl (pH 7.4), 5 mM KCl, 1 mM $MgSO_4$) for 30 min at 25°C. The incubation medium (final volume, 250 μl) contained [^{3}H]desipramine (2–24 nM), and in some experiments also contained propofol (0.1–300 μM). After incubation, binding was terminated by the addition of 2 ml ice-cold buffer B and rapid filtration of the membrane suspension under vacuum through Whatman GF/C glass fiber filters (Whatman, Maidstone, United Kingdom). The filters were rapidly washed twice with 2 ml ice-cold buffer B and were placed in counting vials containing a scintillation cocktail. The radioactivity was counted in Aloka LSC-3500E. Nonspecific binding was determined in the presence of 10 μM nisoxetine, a selective NET inhibitor, and specific binding was obtained by subtracting the nonspecific binding from the total binding.

Long-term Treatment of Cells with Propofol for [^{3}H]Norepinephrine Uptake

Cells were preincubated with or without propofol (1–30 μM) for 6–24 h. After preincubation, the cells were washed with 2 ml Eagle's MEM and stood for another 3 h in a culture chamber to completely wash out propofol. The cells were then incubated with [^{3}H]norepinephrine (500 nM) for 15 min, and [^{3}H]norepinephrine uptake by the cells was evaluated as previously described.

Long-term Treatment of Cells with Propofol for [^{3}H]Desipramine Binding

After treatment of cells with 10 μM propofol for 12 h and subsequent washing with 1 ml MEM and standing for 1 h, cells were collected and crashed by a homogenizer (Ultra-Turrax T8; IKA Labortechnik, Staufen, Germany) for 60 s in a lysis buffer (10 mM Tris-HCl [pH 7.4], 2 mM $MgCl_2$, 1 mM dithiothreitol, 1 mM phenylmethylsulphonyl fluoride) and centrifuged at 1,000g for 10 min at 4°C. The supernatant was centrifuged at 60,000g for 30 min. The final pellet containing plasma membranes was suspended in the binding buffer (50 mM Tris-HCl [pH 7.4], 300 mM NaCl, 5 mM KCl).[16] The protein of isolated plasma membranes was quantified by the method of Lowry *et al.*[20] The binding of [^{3}H]desipramine was determined by incubation of membranes (100 μg protein) suspended in buffer B for 30 min at 25°C as described previously.
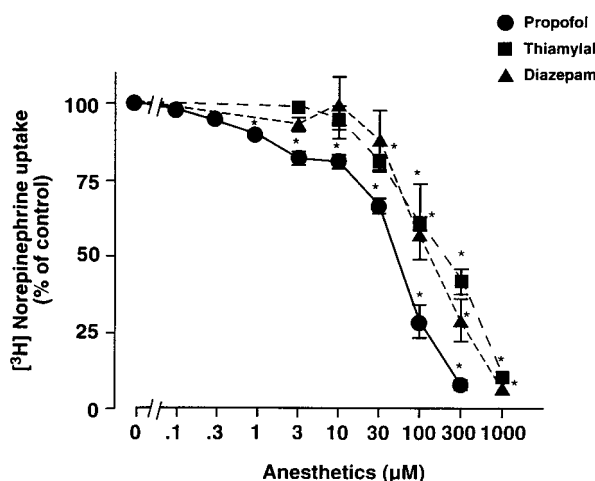
Fig. 1. Effects of intravenous anesthetics on desipramine-sensitive [³H]norepinephrine uptake. Cultured cells (4 × 10⁶ per dish) were incubated at 37°C for 15 min with 500 nm [³H]norepinephrine in the presence or absence of propofol (0.1–300 µM), thiamylal (3–1,000 µM), or diazepam (3–1,000 µM). The results are expressed as a percentage of control values. The results are the mean (±SD) of four separate experiments conducted in duplicate. *P < 0.05 compared with control.

Assay of Norepinephrine Transporter mRNA Expression by Reverse Transcriptase–Polymerase Chain Reaction

Poly(A)⁺RNA was isolated from control or propofol-treated cells by guanidine hydrochloride, ethanol fractionation, chloroform–isobutanol extraction, and oligo(dT) cellulose column separation as previously described.[21] The first-strand cDNA was synthesized from Poly(A)⁺RNA using a first-strand cDNA synthesis kit (Amersham Pharmacia Biotech, Piscaway, NJ). The obtained cDNA was amplified by polymerase chain reaction. Each sample was assayed for NET and β-actin mRNAs using their specific primers. The sense and antisense primers for NET were 5′-CTGACCAGCACCAT-CAACTGT-3′ and 5′-GTGAAGAGTTTCCGGTGTCGC-3′, and those for β-actin were 5′-TGGAGAAGAGCTAT-GAGCTGCCTG-3′ and 5′-GTGCCACCAGACAGCACTGT-GTTG-3′, respectively. Polymerase chain reaction using primers for NET or β-actin and a Takara Ex Taq kit (Takara, Otsu, Japan) was conducted with an automatic thermal controller (PC-800; Astec, Fukuoka, Japan). The thermocycling conditions were as follows: 28 cycles of 94°C for 1 min, 62°C for 30 s, 72°C for 20 s for NET; and 20 cycles of 94°C for 15 s, 66°C for 15 s, 72°C for 30 s for β-actin. The resultant polymerase chain reaction products were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and visualized by GelStar Nucleic Acid Gel Stain (Takara, Otsu, Japan). Fluorescence intensity of the bands was quantified with a Fuluoroimage Analyzer (BAS 3000; Fujifilm, Tokyo, Japan).

Data Analysis

All values are expressed as mean ± SD. Statistical analysis was conducted by one-way analysis of variance followed by a Dunnett t test for multiple comparisons in figure 1 and by paired Student t test for comparisons of the values of Michaelis constant (K_m), maximal velocity (V_{max}), dissociation constant (K_d), and maximal binding (B_{max}) in figures 2 and 3 and table 1. In figures 4 and 5, analysis was performed using one-way analysis of variance followed by a Dunnett t test, and in figure 6 by paired Student t test for comparisons of the values of K_d and B_{max}. In figure 7, one-way analysis of variance followed by a Dunnett t test was used. Differences were considered as statistically significant at P less than 0.05.

Results

Effects of Intravenous Anesthetics on [³H]Norepinephrine Uptake

Uptake of [³H]norepinephrine was linearly related to cell density (0.2–4 × 10⁶ cells) and incubation time (10–40 min).[17] Thus, the assay condition (4 × 10⁶ cells, 15 min) selected for the subsequent experiments was well within the linear range of [³H]norepinephrine uptake. All anesthetics, including propofol, thiamylal, and diazepam, inhibited [³H]norepinephrine uptake in a con-

Fig. 2. Saturation curve of [³H]norepinephrine uptake. (A) Cells were incubated with or without 100 µM propofol, thiamylal, or diazepam, and in the presence of various concentrations (1–30 µM) of [³H]norepinephrine at 37°C for 15 min. The results are the mean (±SD) of four separate experiments conducted in duplicate. (B) Eadie-Hofstee analysis of [³H]norepinephrine uptake. The data were obtained from fig. 2A. V/S = velocity/substrate.
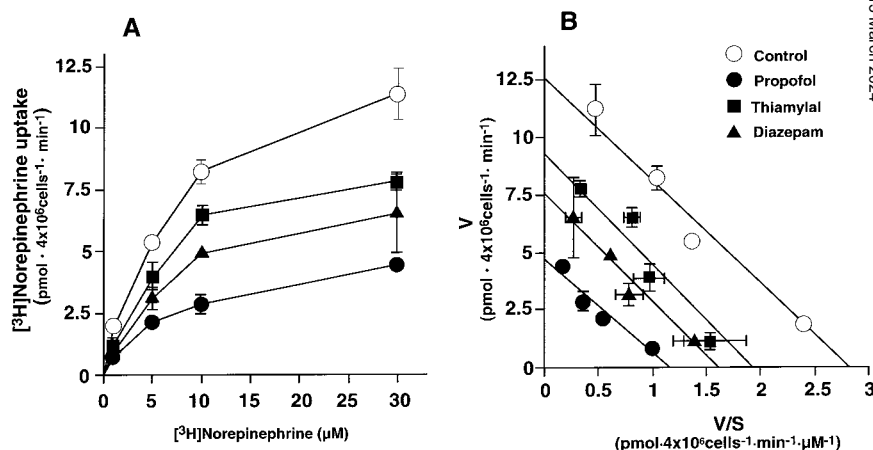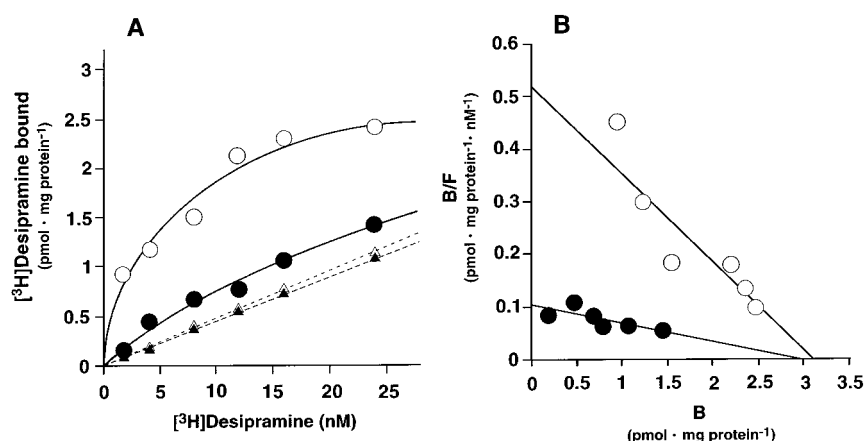
Fig. 3. Saturation curve of nisoxetine-sensitive [³H]desipramine binding to plasma membranes. (A) Plasma membranes (10 μg protein) from bovine adrenal medulla were incubated at 25°C for 30 min with (filled circles) or without (open circles) propofol (60 μM) and in the presence of increasing concentrations of [³H]desipramine (2–24 nM). Nonspecific bindings are expressed as dotted lines in the presence (filled triangles) or absence (open triangles) of propofol. Experiments were conducted in duplicate and repeated five times with essentially equivalent results. (B) Scatchard plot analysis of [³H]desipramine binding. The data were obtained from fig. 3A. B/F = bound/free.

centration-dependent manner (fig. 1). Control values (absent of anesthetics) were 1.12 ± 0.04 pmol · 4×10^6 cells^{-1} · min^{-1}. From the results of figure 1, each the half-maximal inhibition concentration (IC_{50}) for [³H]norepinephrine uptake by intravenous anesthetics was calculated. Because the inhibition curve by propofol was biphasic, the data of propofol were examined using a modified Scatchard analysis[22] (data not shown), and it was resolved in two components (table 1). Incubation of the cells with increasing concentrations of [³H]norepinephrine (1–30 μM) showed that [³H]norepinephrine uptake was a saturable process (fig. 2A). From Eadie-Hofstee analysis (fig. 2B), all anesthetics produced a significant reduction in the V_{max} without altering the K_m values (table 1). The kinetic analysis of additional experiments in clinical relevant concentration (5 μM of propofol) showed that low concentration of propofol significantly ($P < 0.05$) changed V_{max} (control, 10.5 ± 0.4 pmol · 4×10^6 cells^{-1} · min^{-1}; propofol, 8.7 ± 0.3 pmol · 4×10^6 cells^{-1} · min^{-1}) but did not change K_m (control, 4.9 ± 0.4 μM; propofol, 5.1 ± 0.3 μM; data not shown), suggesting noncompetitive inhibition similar to that observed with a high concentration of propofol.

Effect of Propofol on [³H]Desipramine Binding to Plasma Membranes Isolated from Bovine Adrenal Medulla

A specific binding of [³H]desipramine was found to be saturable (fig. 3A). Scatchard analysis in control [³H]desipramine binding showed a single population of binding site with an apparent K_d of 6.05 ± 0.23 nM and B_{max} of 3.11 ± 0.16 pmol/mg protein (fig. 3B). Propofol (60 μM) inhibited [³H]desipramine binding by increasing the K_d value to 29.0 ± 4.0 μM ($P < 0.05$) without any change in the B_{max} value (2.84 ± 0.26 pmol/mg protein; fig. 3B). We also performed the experiment with a clinically relevant concentration. Low concentration (5 μM) of propofol changed K_d without altering B_{max} (data not shown), suggesting competitive inhibition as observed with a high concentration of propofol. As shown in figure 4, propofol inhibited the binding of [³H]desipramine in a concentration-dependent manner (1–300 μM). The inhibition curve appeared to be biphasic. When the
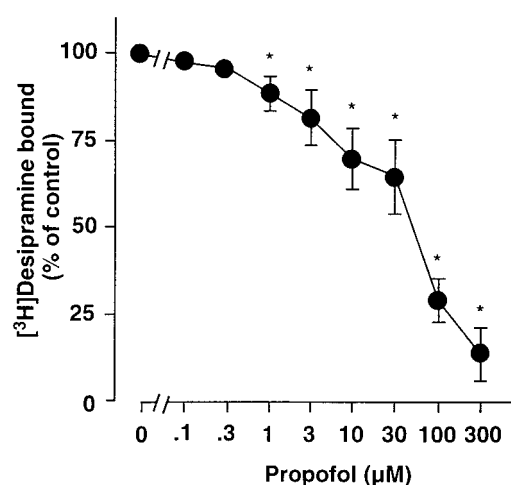
Table 1. Effects of Various Intravenous Anesthetics on the Kinetic Parameters for [³H]Norepinephrine Uptake

Treatment	IC_{50} (μM)	K_m (μM)	V_{max} (pmol · 4×10^6 cells^{-1} · min^{-1})
Control	—	4.4 ± 0.3	12.5 ± 0.9
Propofol	0.9 ± 0.1, 49 ± 3	4.0 ± 0.4	4.8 ± 0.5*
Thiamylal	182 ± 16	4.9 ± 0.3	9.5 ± 0.6*
Diazepam	140 ± 13	4.7 ± 0.5	7.6 ± 0.6*

IC_{50} values were calculated from the data in figure 1. The maximal velocity (V_{max}) and the apparent Michaelis constant (K_m) were calculated by Eadie-Hofstee analysis of the saturation curves in the absence of a drug (control) or in the presence of 100 μM propofol, thiamylal, or diazepam. The results are mean (± SD) of four separate experiments performed in duplicate.

* $P < 0.05$ compared with control.

Fig. 4. Effect of various concentrations of propofol on [³H]desipramine binding. Plasma membranes were incubated with [³H]desipramine (10 nM) at 25°C for 30 min in the presence or absence of propofol (0.1–300 μM). Control values (0 μM propofol) for specific binding were 2.26 ± 0.11 pmol/mg protein. The results are the means (±SD) of four separate experiments conducted in duplicate. *$P < 0.05$ compared with control.

Fig. 5. The effect of long-term treatment with propofol on [³H]norepinephrine uptake. (A) Time course of preincubation with propofol (1 μM) for [³H]norepinephrine uptake. After preincubation with or without propofol for the period indicated, the cells were cultured for another 3 h in propofol-free medium and then incubated at 37°C for 15 min in the presence of [³H]norepinephrine (500 nM). The values of the control group at 6, 12, and 24 h were 1.3 ± 0.2, 1.2 ± 0.2, and 1.2 ± 0.1 pmol · 4 × 10⁶ cells⁻¹ · min⁻¹, respectively. (B) Effect of preincubation with various concentrations of propofol on [³H]norepinephrine uptake. The results are the mean (±SD) of four separate experiments conducted in duplicate. *P < 0.05 compared with control.
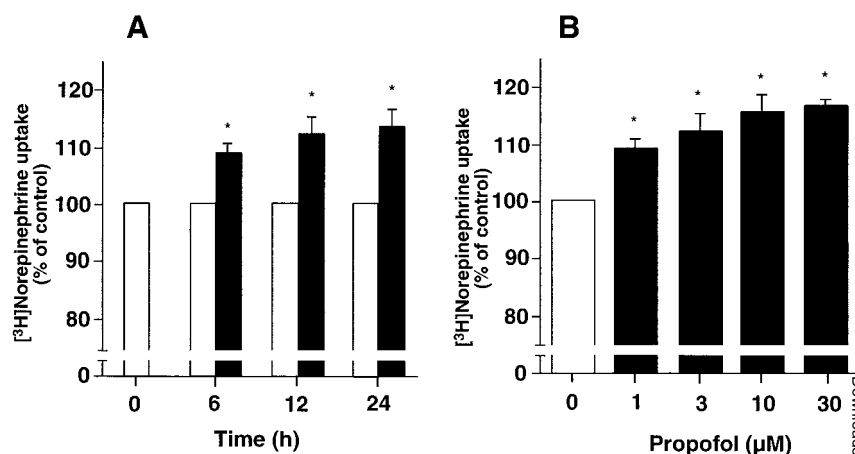
data were examined by a modified Scatchard analysis[22] (data not shown), it showed two components with IC_{50} values of 2.8 ± 0.3 μM and 50 ± 6 μM. Furthermore, inhibitory constant (K_i) values of 0.9 ± 0.1 μM and 16 ± 2 μM for inhibition of [³H]desipramine binding by propofol were calculated,[23] respectively.

Effect of Long-term Treatment with Propofol on [³H]Norepinephrine Uptake, [³H]Desipramine Binding, and Norepinephrine Transporter mRNA Expression
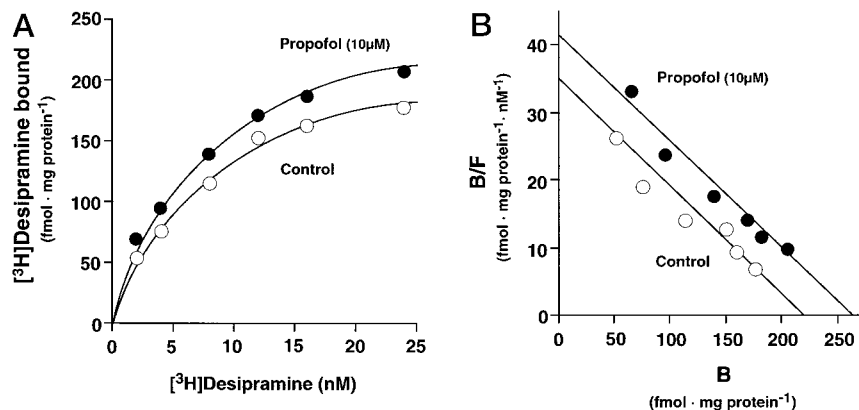
Long-term treatment of cells with propofol caused time- (6–24 h) and concentration (1–10 μM)-dependent increases in [³H]norepinephrine uptake (figs. 5A and 5B). In the assay of [³H]desipramine binding, specific binding of [³H]desipramine to the plasma membranes prepared from control and propofol-treated cells was saturable with increasing concentrations of [³H]desipramine (2–24 nM; fig.6A). Scatchard analysis showed that 10 μM propofol produced a significant (P < 0.05) increase in the B_{max} (control, 230 ± 18 fmol/mg protein; propofol, 270 ± 13 fmol/mg protein) without any change in the K_d (control, 6.6 ± 0.3 nM; propofol, 6.3 ± 0.4 nM; fig. 6B). In the assay of mRNA expression, polymerase chain reaction with NET primers and β-actin primers yielded single bands corresponding to approxi-

mately 0.3-kb and 0.2-kb fragments, respectively (fig. 7). The band for NET mRNA was sequenced and found to be identical to the reported bovine NET[13] (data not shown). Furthermore, propofol (30 μM) increased the NET mRNA level by 2.2- and 2.0-fold at 12 and 24 h, respectively (fig. 7A). The increase in NET mRNA level produced by propofol was concentration-dependent (10–30 μM; fig. 7B). Each result of NET mRNA was normalized with β-actin mRNA.

Discussion

We demonstrated that all the intravenous anesthetics used in this study inhibited [³H]norepinephrine uptake by cultured adrenal medullary cells. The rank order of the potency to suppress [³H]norepinephrine uptake was propofol, diazepam, and thiamylal. The peak plasma concentration of propofol was reported to be approximately 50 μM after bolus administration.[24] However, the steady state free plasma concentration of propofol may not exceed 2 μM because 98% binds to plasma proteins. In addition, taking protein binding into account, the clinically relevant concentrations of diazepam and thiopental are approximately 1 μM[25] and 25 μM,[3] respectively, and the plasma concentration of the latter is similar to that of thiamylal.[26] At these clinically relevant

Fig. 6. The effect of long-term treatment with propofol on nisoxetine-sensitive [³H]desipramine binding. (A) Plasma membranes (100 μg protein) isolated from cells pretreated with (filled circles) or without (open circles) propofol (10 μM) for 12 h were incubated at 25°C for 30 min in the presence of increasing concentrations of [³H]desipramine (2–24 nM). Experiments were conducted in duplicate and repeated four times with essentially equivalent results. (B) Scatchard plot analysis of [³H]desipramine binding. The data were obtained from fig. 6A.
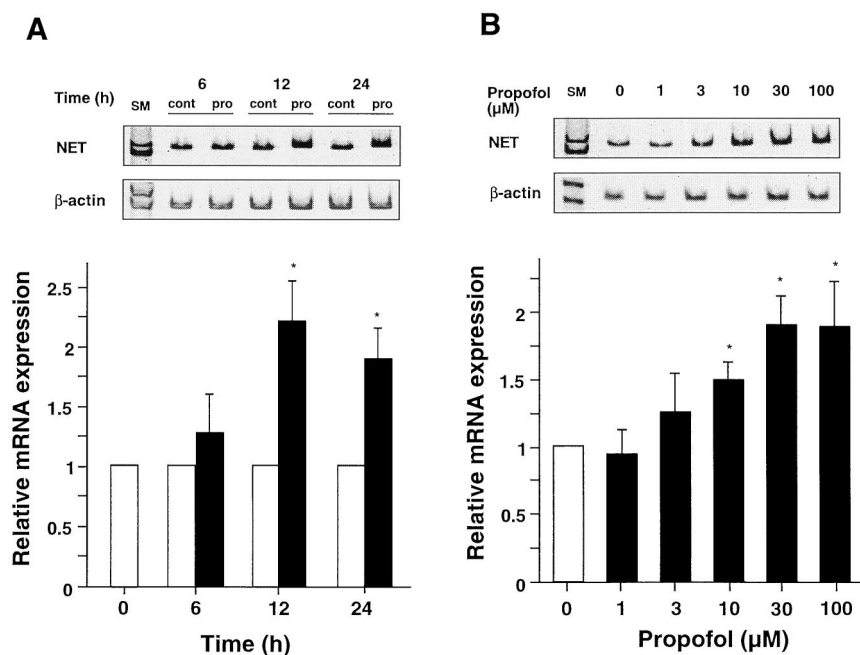
A

B

Fig. 7. The effect of long-term treatment with propofol on norepinephrine transporter (NET) messenger RNA (mRNA) expression. Poly(A)[+]RNA was isolated from control or propofol-treated cells at the indicated time. Reverse transcriptase–polymerase chain reaction was performed with a thermocycler using a first-strand cDNA synthesis kit and a Takara Ex Taq kit. (*A*) The time course of NET mRNA expression with (closed column) or without (open column) propofol (30 μM) treatment. The upper figures show single bands for the polymerase chain reaction products for NET and β-actin mRNA after separation by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. SM = size markers (310 and 281 base pairs for NET and 234 and 194 base pairs for β-actin); cont = control; pro = propofol. The lower figures show the relative level of NET mRNA expression determined by quantifying the fluorescence intensity of the bands. (*B*) Effect of treatment with various concentrations of propofol for 12 h on NET mRNA expression. The results are the mean (±SD) of four separate experiments conducted in triplicate. *$P < 0.05$ compared with control.

concentrations, propofol and probably thiamylal seem to inhibit NET function to a small but significant degree. As much as 80% of norepinephrine released from presynaptic terminals is believed to be physiologically re-uptaken by the neurons,[27] terminating neurotransmission. Therefore, even a slight inhibition of NET activity by clinical concentrations of anesthetics may enhance neurotransmission.

To address the site of action of propofol on NET, we examined the effects of propofol on the kinetic parameters of [³H]norepinephrine uptake and on [³H]desipramine binding. All of the intravenous anesthetics significantly lowered the V_{max} value of [³H]norepinephrine uptake without changing the K_m value, indicating noncompetitive inhibition. Propofol inhibited the specific binding of [³H]desipramine with a potency similar to that of [³H]norepinephrine uptake. Scatchard analysis revealed that propofol significantly increased the K_d value without affecting the value of B_{max}, indicating competitive inhibition. The present results were also confirmed by a clinical concentration of propofol (see Results). Propofol seems to have biphasic effects on [³H]norepinephrine uptake and [³H]desipramine binding (figs. 1 and 4), suggesting that propofol affects the NET at two sites of action, such as high- and a low-affinity sites. Further study is required to confirm this possibility.

Over the past 10 yr, there has been controversy over whether the site of substrate recognition is identical to that for tricyclic antidepressant binding on monoamine transporters.[28,29] From recent molecular cloning and chimeric dopamine–norepinephrine transporter studies, the current prevailing hypothesis is that there are distinct regions within NET molecules that determine substrate recognition and translocation and antagonist affinity, but these regions may overlap each other.[9,30,31] In the present study, the noncompetitive kinetics of [³H]norepinephrine uptake suggest that propofol interacts with NET at a different site from the norepinephrine recognition site. The competitive inhibition of [³H]desipramine binding by propofol suggests that propofol acts directly on the desipramine binding site. Alternatively, propofol and desipramine may act at different sites on the NET that are allosterically coupled. Thus, a simple interpretation of these results is that propofol interacts with NET, which, in turn, may allosterically lead to a conformational change in the transporter that inhibits transporter function. We previously proposed that ketamine inhibits the transport of norepinephrine by interacting with a site that partly overlaps the desipramine binding site on NET.[17] Propofol inhibited [³H]norepinephrine uptake in a manner very similar to that of ketamine. Taken together, our present results provide further evidence to support the hypothesis that in NET molecules there is a common region or areas in close proximity that are susceptible to some intravenous anesthetics. Further studies using various NET mutants produced by molecular techniques are required to determine the precise site of intravenous anesthetic action on NET.

Our findings explain some of the pharmacologic effects of intravenous anesthetics. For instance, intravenous anesthetics may enhance the action of exogenous or endogenous catecholamines. Indeed, propofol and thiamylal are reported to enhance epinephrine-induced arrhythmias in dogs.[32–34] Furthermore, evidence has emerged that the descending inhibitory system consists of noradrenergic neurons.[35] Tricyclic antidepressants,

including desipramine, that selectively antagonize NET are used to treat the chronic pain that accompanies postherpetic neuralgia, diabetic neuropathy, cancer, and complex regional pain syndrome.[36–38] Their antinociceptive effect is considered to arise partly from enhancing noradrenergic neurotransmission by inhibiting NET in the descending inhibitory system in the brain and spinal cord.[39] Our results raise the possibility that the inhibitory effects of intravenous anesthetics on NET activity during anesthesia have an antinociceptive action.

Propofol is sometimes administered for prolonged periods during surgery. We found that treatment of cells with clinically relevant concentrations of propofol increased the functional activity of NET and [³H]desipramine binding sites. Presently, clinical significance of the phenomenon is not clear. It may be regarded as a compensatory action of propofol that normalizes the norepinephrine level at noradrenergic synapses during anesthesia.

In conclusion, intravenous anesthetics have a dual effect on NET function: short-term treatment produces inhibition, whereas long-term treatment causes up-regulation. Our findings indicate that NET is one of the target proteins of intravenous anesthetics and help to unveil the pharmacologic basis of this interaction for the better understanding of the various actions of intravenous anesthetics.

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