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Effect of Propofol, an Intravenous Anesthetic Agent, on K_{ATP} Channels of Pancreatic β-cells in Rats

Eun Jee Park, Dae-Kyu Song[1], Jae Kyu Cheun, Jung In Bae, Won-Kyung Ho[2], and Yung E Earm[2]

Department of [1]Physiology and Anesthesiology, Keimyung University School of Medicine, Taegu 700−712; [2]Department of Physiology, Seoul National University College of Medicine, Seoul 110−799, Korea

ATP-sensitive potassium channels (K_{ATP} channels) play an important role in insulin secretion from pancreatic beta cells. We have investigated the effect of propofol on K_{ATP} channels in cultured single pancreatic beta cells of rats. Channel activity was recorded from membrane patches using the patch-clamp technique. In the inside-out configuration bath-applied propofol inhibited the K_{ATP} channel activities in a dose-dependent manner. The half-maximal inhibition dose (ED50) was 48.6 ± 8.4 μM and the Hill coefficient was 0.73 ± 0.11. Single channel conductance calculated from the slope of the relationship between single channel current and pipette potential ($+20 \sim +100$ mV) was not significantly altered by propofol (control: 60.0 ± 2.7 pS, 0.1 mM propofol: 58.7 ± 3.5 pS). However, mean closed time was surely increased. Above results indicate that propofol blocks the K_{ATP} channels in the pancreatic beta cells in the range of its blood concentrations during anesthesia, suggesting a possible effect on insulin secretion and blood glucose level.

Key Words: Propofol, ATP-sensitive potassium channel, Patch-clamp technique, Single channel study, Pancreatic beta cell

INTRODUCTION

Propofol (2,3-diisopropylphenol, Diprivan) has been widely used as an intravenous general anesthetic because of its rapid induction and recovery, and minimal side effects. Most anesthetics, volatile or intravenous, are known to modulate the various ion channels in different organs. Propofol is able to trigger directly $GABA_A$ receptor activation in neurons and thereby acts as a sedative (Concas et al, 1992; Hara et al, 1993; Uchida et al, 1997; Stapelfeldt & Oleszewski, 1998). In the heart, the L-type Ca channels are inhibited by propofol (Luk et al, 1995; Buljubasic et al, 1996; Yang et al, 1996; Zhou et al, 1997) which is a major side effect and results in bradycardia and a negative inotropic effect. In addition, propofol directly affects Na channels and voltage-dependent K channels in the ventricular myocytes (Heath & Terrar, 1996; Morey et al, 1997; Wu et al, 1997; Zhou et al, 1997; Saint, 1998; Saint & Tang, 1998), neurons (Ratnakumari & Hemmings, 1996; 1997; Friederich & Urban, 1998), lymphocytes (Mozrzymas et al, 1996), and chondrocytes (Mozrzymas et al, 1994). K channels play important roles in resting membrane potential and repolarization of the excitable and endocrine cells. However, there is no report to date about the action of propofol on ATP-sensitive K channels (K_{ATP} channels) which are contributing to modulation of neurotransmitter release at the brain synapse, vascular tone, epithelial electrolyte transport, and glucose-induced insulin secretion (Nichols & Lederer, 1991; Ashcroft, 1998). One report recently showed that propofol impaired the relaxation produced by K_{ATP} channel openers in the rat aorta (Kinoshita et al, 1998). The blood glucose level is an issue in that glucose utilization in the tissues may be

Corresponding to: Yung E Earm, Department of Physiology, Seoul National University College of Medicine, 28 Yonkeun-Dong, Chongno-Ku, Seoul 110-799, Korea. (Tel) 02-740-8224, (Fax) 02-763-9667, (E-mail) earmye@plaza.snu.ac.kr

decreased during general anesthesia (Cavazzuti et al, 1991; Myles et al, 1995). We demonstrates that the K_{ATP} channels in rat pancreatic beta cells are inhibited by propofol in the range of its plasma concentration during anesthesia, suggesting that propofol may be influential in glucose-induced insulin secretion.

METHODS

Preparation of single beta cells

Islets of Langerhans were isolated by a collagenase digestion technique from the pancreas. Under ether anesthesia, neutral red solution (3 ml, 1 in 1000 w/v in 0.9 % saline) was injected through the abdominal aorta after cutting the inferior vena cava since this stain is selective for islet tissue (Gray et al, 1983). Collagenase (1.2 mg/ml Hank's balanced salts solution) solution was perfused into the pancreatic ducts retrogradely through the common bile duct. The dissected pancreas was incubated in 37°C water bath for 33 min. The dispersion of islet cells was accomplished by the modified method of Gray et al (Gray et al, 1983). Dispersed single cells were then moved into a RPMI 1640 medium (glucose concentration 11.1 mM) supplemented with fetal calf serum (10 %), penicillin (100 unit/ml), and streptomycin (0.1 mg/ml). They were cultured on small cover glasses (10 mm × 3 mm) at 37°C in a humidified incubator supplied with 5% CO_2 and balanced air. Individual cover glasses were transferred into the bath chamber on an inverted microscope (Carl Zeiss Co, Jena, Germany) for patch-clamp experiments.

Electrophysiological recording

This study used the inside-out mode of the patch clamp technique (Hamill et al, 1981). Pipettes were pulled from borosilicate glass and coated with Sylgard resin (Dow Corning, Midland, MI, USA) near the tip, fire-polished and had resistance between 5 and 10 MΩ. Single channel currents were recorded using an Axopatch 200 A patch-clamp amplifier (Axon Instruments Inc, Foster, CA, USA). During experiments the current and voltage signals were stored on videotape via a VR-10B pulse code modulator (Instrutech Co, Great Neck, NY, USA) and later analyzed by pClamp 6.04 software (Axon Instruments Inc). The data was filterd at 1 KHz and sampled at 5 KHz.

Analysis of single-channel data

The channel activity was expressed as the mean patch current ($I=N\times P_o$), where N and P_o represent the number of channels in the patch membrane and the open probability of the channels, respectively. The relative channel activity in the presence of propofol was expressed as I/I_c, where I_c is the mean patch current recorded in the control solution.

The open and closed times were determined by the 50% threshold method (Colquhoun & Sigworth, 1983). The open time distributions could be well fitted by single exponential curves using the simplex least squares method, but the closed time distributions were fitted with double exponential functions:

$$F(t)= (\alpha_1/\tau_1)\exp(-t/\tau_1)+(\alpha_2/\tau_2)\exp(-t/\tau_2)$$

where α is the area of each component, t is dwell time, τ is time constant.

The dose-response curve for the ED_{50} and the Hill coefficient were obtained by fitting the data with the following function:

$$y=1/(1+(\chi/\chi_0)^p)$$

where χ_0 represents the concentration of half-maximal inhibition (ED_{50}), p is the Hill coefficient.

Experimental solutions

The external (bath) solution in the inside-out experiment was (mM) 135 KCl, 10 NaCl, 0.1 $CaCl_2$, 2 $MgSO_4$, 1 EGTA, 0.1 μM ATP, 5 HEPES (pH 7.2 with KOH). The pipette solution consisted of (in mM) 140 KCl, 5 $CaCl_2$, 5 HEPES, 5 $MgSO_4$ (pH 7.3 with KOH). Electrophysiological experiments were performed at room temperature (22~25°C). All chemicals except propofol (Zeneca Co, Macclesfield, UK) and intralipid (Intralipos®, Green Cross Pharmaceutical Co, Seoul, Korea) were from Sigma Chemical Co. (St Louis, MO, USA).

RESULTS

Concentration dependence of block by propofol

Fig. 1A shows the typical trace illustrating the blockade of K_{ATP} channels in a single beta cell by the

bath application of propofol in the inside-out mode. When the cell was voltage-clamped at pipette potential (Vp)+70 mV, inward single K channel currents were shown as upward deflections. The K channels to yield these currents should be the K_{ATP} channels because they were totally blocked by 2 mM ATP. Propofol was able to suppress the K_{ATP} channels as ATP did. As the concentration of propofol was increased the currents were further inhibited dose-dependently. Intralipid as vehicle alone had no effect (data not shown). The degree of blockade in 2 additional cells to the change of propofol concentrations is shown in the form of a dose-response curve (Fig. 1B). The best fit of the Logistic equation gave a value for the ED_{50} of 48.6±8.4 μM and the Hill coefficient of 0.73±0.11. The effective range of propofol on the K_{ATP} channel activity was within the blood concentration to be employed during general anesthesia.

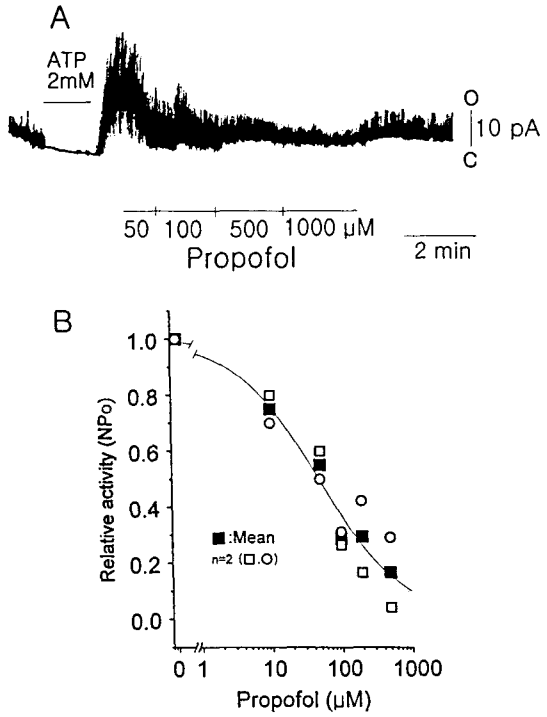

Fig. 1. Effect of propofol on single K_{ATP} channel activities in pancreatic β-cells of rats. (A) Patch-clamp recording was performed using the inside-out mode. Propofol was applied to the internal surface of the patch. Pipette potential (Vp)=+70 mV. ATP (2 mM), applied intracellulary, completely inhibited the channel activity. After recovery, subsequent application of propofol decreased the channel activities in a dose-dependent fashion. (B) Dose-response relationship of propofol on single K_{ATP} channel activities. Relative activity was calculated by normalizing NP_o obtained in varying concentrations of propofol to NP_o measured in standard solution. The continuous line is a fit using the Logistic equation. The half-maximal inhibition dose (ED_{50}) is 48.6 μM, and the Hill coefficient (p) is 0.73.

Voltage dependence of propofol effect

To test whether the blocking effect of propofol is affected by the holding potential the pipette potential was changed between −80 and +100 mV under the constant (0.1 mM) propofol concentration (Fig. 2). Neither the channel activity (B), nor the channel amplitude (C) were influenced by the voltage, confirming that the inhibitory effect of propofol on the K_{ATP} channels was voltage independent. Single channel conductances (C) were 60.0±2.7 pS in the control (n=3), 58.7±3.5 pS in 0.1 mM propofol (n=3) in the

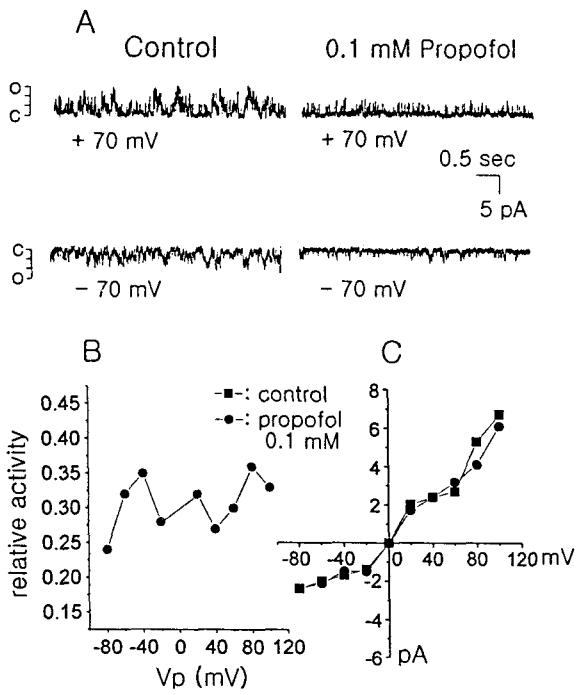

Fig. 2. Membrane potential and effect of propofol on single K_{ATP} channel activities. (A) The inside-out patch currents voltage-clamped at +70 mV (upper trace) and −70 mV (lower trace) are shown. (B) Changes in relative activities of K_{ATP} channels in inside-out patches at Vp between −80 and +120 mV. (C) The plot of the unitary current-voltage relationship in the control and 0.1 mM of propofol.

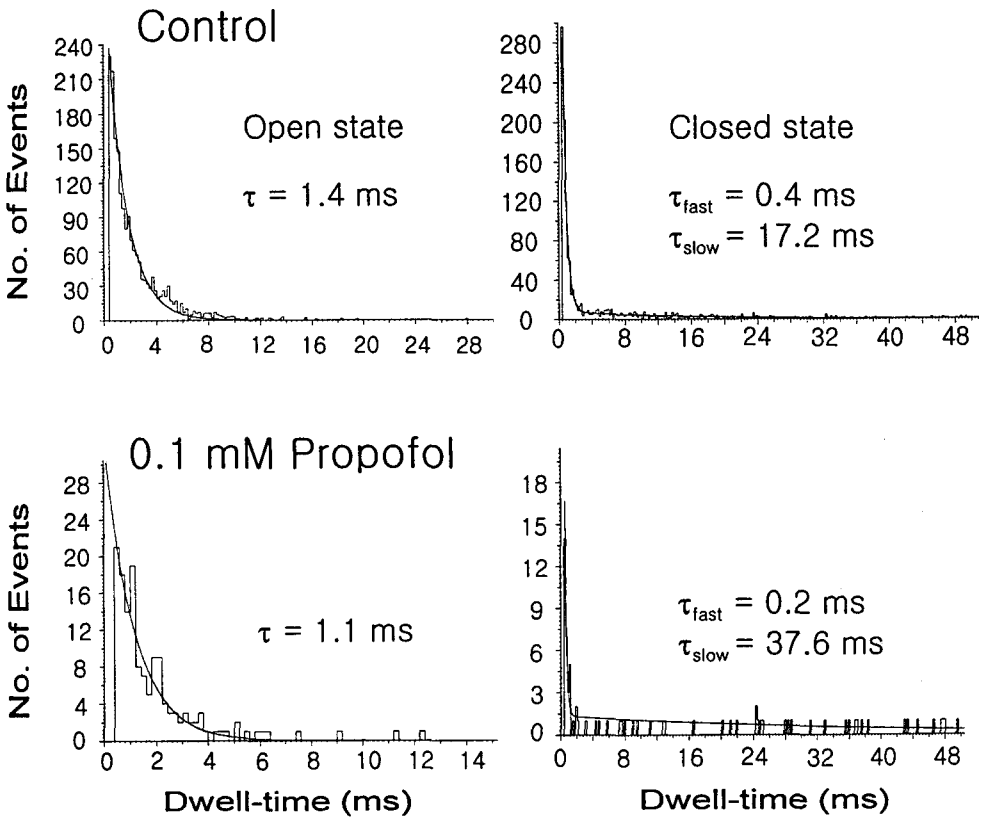

Fig. 3. Histograms of open-time and closed-time distributions of K_{ATP} channels in standard and 0.1 mM propofol solutions. The fitted lines for open- and closed-times were drawn by single- and double-exponential functions, respectively. Pipette potential (Vp) was +70 mV in the inside-out mode. Bin width was 0.2 ms. Data was filtered at 1 KHz.

range of positive pipette potentials (a polarized membrane state).

Effect of propofol on open- and closed-time distributions

Fig. 3 shows the open and closed time distributions of a single K_{ATP} channel in a β cell, and the mean values with two additional cells are summarized in Table 1. The mean open and closed times signify the values to be averaged overall in the individual dwell states. So, the mean closed times include the silent trace between burst events. Propofol was able to increase the mean closed time. However, the fast time constant of the closed time distribution (τ_{fast}, intraburst component of closing) was not increased, whereas τ_{slow} (interburst component of closing) was increased, suggesting that propofol may stabilize the state of long closure. The distributions of the channel current amplitudes in propofol are shown in Fig. 4. The application of 0.1 mM propofol at the resting membrane potential level (Vp=+70 mV) did not show any significant effect on the current amplitudes (control: 4.25±0.81 pA, n=12; propofol:

Table 1. Effects of propofol (0.1 mM) on kinetics of single K_{ATP} channels

	Control (ms)	Propofol (ms)
Mean open time	2.28±2.41	2.18±2.30
Mean closed time	14.11±7.35	127.92±223.19
Open state		
τ	1.57±0.07	1.33±0.12
Closed state		
τ_{fast}	0.54±0.11	0.333±0.13
τ_{slow}	13.48±3.71	80.62±42.99

The mean time constant (τ) of open-time distribution was obtained by single exponentials. Double exponential curves were fitted for the τ_{fast} and τ_{slow} of closed-time distribution. Data represent mean±SE. n=3.

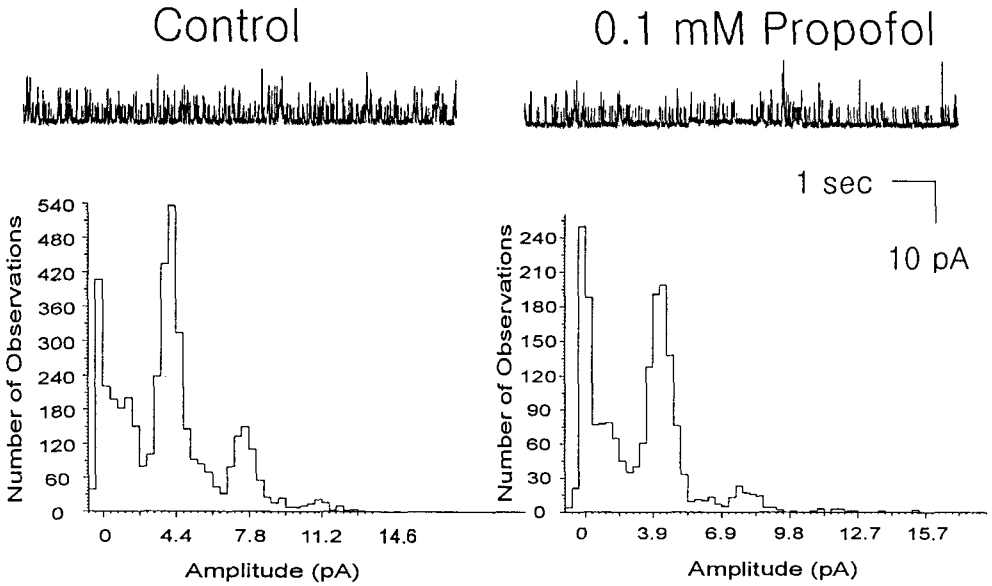

Fig. 4. Effect of propofol on distribution of single K_{ATP} channel amplitudes. *Upper panels,* Representative traces showing the effect of 0.1 mM propofol. *Lower panels,* All point amplitude histograms taken from the data shown in upper panels. No significant difference was found.

4.11±0.73 pA, n=7).

DISCUSSION

Our data clearly demonstrates for the first time that propofol blocks the K_{ATP} channels in a dose-dependent and voltage-independent manner in the pancreatic beta cells of rats. The effect on single channel kinetics shows the increase of mean closed time without affecting mean open time and single channel conductance.

The value of ED_{50} (48.6 μM) obtained in this experiment is within the blood concentration of propofol introduced for maintenance of general anesthesia (10~55 μM) (Shaffer et al, 1988). The delayed rectifier K current (I_K) (Baum, 1993; Heath & Terrar, 1996; Morey et al, 1997) and transient outward current (I_{to}) (Buljubasic et al, 1996; Wu et al, 1997) in the ventricular myocytes are other targets of propofol. The inhibitory effect of propofol on voltage-dependent K channels is also found in lymphocytes (Mozrzymas et al, 1996), PC12 cells (Magnelli et al, 1992) and articular chondrocytes (Mozrzymas et al, 1994). The effective doses of propofol on the K channels in the different organs were common and within its therapeutic plasma concentration (roughly ED_{50} of 10~100 μM except chondrocytes $ED_{50}<6$ μM). This is in harmony with that of the K_{ATP} channels in this study.

The K_{ATP} channel inhibition and resultant Ca influx through voltage-dependent Ca channels can lead to insulin secretion. Because of its higher affinity to plasma protein the actual concentration of propofol reaching the channels on the beta cell membrane is likely to be lower than that of blood (5 μM if 90% is bound). However, it must be pointed out that the greater the degree of propofol purification, the less ED_{50} is likely to appear as the K channels in chondrocytes (Mozrzymas et al, 1994). It seems that the method of purification can be diverse as to the extent of its blocking effect on the K_{ATP} channels. In addition, the possibility that propofol might affect the K_{ATP} channel activity in the pancreatic beta cells could be increased during anesthesia, requesting further propofol dosage (Shaffer et al, 1988; Vandesteene et al, 1988).

Propofol also inhibits I_{Ca} in the heart. The decrease of Ca inflow through L-type Ca channels induces the diminution of myocardial contractility. Although the K_{ATP} channels in the beta cells are the principal mediators in the glucose-induced insulin secretion, the Ca channels in the beta cells responsible for insulin exocytosis might be a target of propofol. If this is

true, actual insulin secretion from the beta cells may be unchanged or even decreased during propofol anesthesia in spite of K_{ATP} channel inhibition of propofol. The degree of glucose utilization is also decreased during anesthesia (Cavazzuti et al, 1991), causing increased blood glucose level. Our results show that the possibility of increased blood glucose level (Myles et al, 1995) in propofol anesthesia is, at least, not on K_{ATP} channels but on other mechanism(s). In addition, for better understanding and safer usage of propofol, this study suggests that its effects on the K_{ATP} channels of other organs, performing various functions with different subunits and ATP sensitivity of K_{ATP} channels (Ashcroft & Gribble, 1998), should be further determined.

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