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Thiopental inhibits function of different inward rectifying potassium channel isoforms by a similar mechanism

Angélica López-Izquierdo, Daniela Ponce-Balbuena, Tania Ferrer, Aldo A. Rodríguez-Menchaca, José A. Sánchez-Chapula *

Unidad de Investigación "Carlos Méndez" del Centro Universitario de Investigaciones Biomédicas de la Universidad de Colima, 28045, Colima, Col. México

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ABSTRACT

Thiopental is a well-known intravenous barbiturate anesthetic with important cardiac side effects. The actions of thiopental on the transmembrane ionic currents that determine the resting potential and action potential duration in cardiomyocytes have been studied widely. We aimed at elucidating the characteristics and mechanism of inhibition by thiopental on members of the subfamily of inward rectifying Kir2.*x* (Kir2.1, 2.2 and 2.3), Kir1.1 and Kir6.2/SUR2A channels. These inward rectifier potassium channels were transfected in HEK-293 cells and macroscopic currents were recorded in the whole-cell and inside-out configurations of the patch-clamp technique. Thiopental inhibited Kir2.1, Kir2.2, Kir2.3, Kir1.1 and Kir6.2/SUR2A currents with similar potency; in whole-cell experiments 30 μM thiopental decreased Kir2.1, Kir2.2, Kir2.3 and Kir1.1 currents to 55 ± 6, 39 ± 8, 42 ± 5 and 49 ± 5% at −120 mV, respectively. Point mutations on Kir2.3 (I213L) or Kir2.1 (L222I) did not modify the potency of block. Thiopental inhibited all Kir channels in a concentration-dependent and voltage-independent manner. Also, the time course of thiopental inhibition was slow ($T_{1/2}$ ~ 4 min) and independent of external or internal drug application. However, in the presence of PIP_2, inhibition by thiopental on Kir2.1 was significantly decreased. Thiopental at clinically relevant concentrations significantly inhibited all Kir channels evaluated in this work. The reduction of thiopental effects during PIP_2 treatment suggests that thiopental inhibition on Kir2.1 channels is related to channel–PIP_2 interaction.

1. Introduction

Thiopental is a short-acting barbiturate that is used intravenously for anesthesia induction. A number of studies have shown that the administration of thiopental at doses within the clinical anesthetic range reduces myocardial contractility and depresses cardiovascular function (Park and Lynch, 1992; Rusy and Komai, 1987). The EC_{50} thiopental concentration for general anesthesia, defined as the lack of response to a painful stimulus, taking into account the total plasma concentration, together with a value for protein binding of 85%, gives a free aqueous concentration of 25 μM (Franks and Lieb, 1994). Like many other anesthetic agents, thiopental may produce dysrhythmias, cardiac arrests, and death in the perioperative period (Atlee, 1997). Thiopental at clinically relevant concentrations markedly prolongs the corrected QT interval during anesthetic induction (McConachie et al., 1989). In single ventricular myocytes thiopental prolongs cardiac repolarization by suppressing several cardiac transmembrane currents, including the L-type Ca^{2+} current, the inward rectifier K^+ current (I_{K1}) and the slow delayed rectifier K^+ current (I_{Ks}) with no effect on the rapidly activating component of I_K (Carnes et al., 1997; Heath and Terrar, 1996; Sakai et al., 1996). Likewise, thiopental induces action potential duration prolongation in isolated ventricular cardiomyocytes of guinea pig and rabbit. These changes may be attributed, at least in part, to inhibitory actions on I_{K1} and I_{Ks} (Heath and Terrar, 1996; Martynyuk et al., 1999).

Similar to various drugs, little is known about the molecular mechanism(s) by which thiopental inhibits these currents. Recently, we demonstrated that the basic antimalarial drug chloroquine inhibited I_{K1} in a voltage-dependent manner suggesting that chloroquine blocks Kir2.1 channels by plugging the cytoplasmic conduction pathway (Rodriguez-Menchaca et al., 2008). On the other hand, the effect of the acidic drug, thiopental on I_{K1} has been shown to be voltage-independent (Pancrazio et al., 1993), which suggests a different mechanism of action from chloroquine. The potency of barbiturate-induced depression of I_{Kl} has been found to be related to the partition coefficient, suggesting that lipophilicity is an important factor in this effect (Pancrazio et al., 1993). Evidence has emerged indicating that anesthetics can exert direct effects on membrane proteins presumably at hydrophobic intramembranous sites (Trudell and Bertaccini, 2002).

I_{K1} is the major determinant of the resting membrane potential in the heart and participates in the terminal phase of action potential repolarization (Lopatin and Nichols, 2001). I_{K1} is conducted by homo- and/or hetero-tetrameric channels formed by coassembly of the Kir2.*x* subfamily of proteins (Kir2.1–Kir2.3). Kir2.1 mRNA was much more abundant than other Kir species in both atria and ventricles (Wang

* Corresponding author. Av. 25 de Julio 965 Col. Villas San Sebastián CP 28045, Colima, Col. México. Tel./fax: +52 3123161129.
E-mail address: sancheza@ucol.mx (J.A. Sánchez-Chapula).

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et al., 1998). Recent studies indicate that the activity of Kir channels strongly depends on phosphatidylinositol 4,5-bisphosphate (PIP_2). Kir2.1 and Kir1.1 interact strongly with PIP_2 (Du et al., 2004), whereas Kir2.3 and Kir6.2, which forms the pore domain of the K_{ATP} channel have weak interactions with PIP_2.

Here we investigated the mechanism of the inhibitory effect of thiopental on Kir2.1, Kir2.1 (L222I), Kir2.2, Kir2.3, Kir2.3 (I213L), Kir1.1 and Kir6.2/SUR2A channels. We hypothesized that thiopental can affect Kir2.1 channel by interfering with channel–PIP_2 interaction.

2. Materials and methods

2.1. Molecular biology and cell transfection

Kir2.1 cDNA (kind gift of C. Vandenberg) and Kir1.1, Kir2.2, Kir2.3, Kir2.1 (L222I), and Kir2.3 (I213L) (kind gift of D. Logothetis) were subcloned into pcDNA3.1(+) plasmid (Invitrogen, La Jolla, CA). Kir6.2 and the sulphonylurea receptor 2A (SUR2A) cDNAs were subcloned into pCMV6b plasmid. Mutations were made using the QuikChange Site-Directed Mutagenesis kit (Stratagene, La Jolla, CA). All mutations were confirmed by direct DNA sequencing. All Kir constructs were expressed in HEK-293 cells as described (Kubo and Murata, 2001). Cells were transfected with Lipofectamine 2000 reagent (Invitrogen La Jolla, CA) according to the manufacturer's instructions.

2.2. Current recordings in HEK-293 cells

Macroscopic currents were recorded in the whole-cell and inside-out configurations of the patch-clamp technique (Hamill et al., 1981) using an Axopatch-200B amplifier (Molecular Devices Corp). Data acquisition and command potentials were controlled by pClamp 9.0 software (Molecular Devices Corp.). Patch pipettes with a resistance of 1–2 MΩ were made from borosilicate capillary glass (WPI, Sarasota, FL). All experiments were performed at 22 °C.

For whole-cell recordings, the internal solution contained (in mM): 110 KCl, 10 HEPES, 5 K_4BAPTA, 5 K_2ATP and 1 $MgCl_2$; pH 7.2. The standard bath solution contained (in mM): 130 NaCl, 4 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 10 HEPES and 10 glucose; pH 7.4. Action potential voltage-clamp recordings were performed in HEK-293 cells using the action potential recorded from an isolated feline endocardial ventricular myocyte as the command signal. Kir2.1, Kir2.2, Kir2.3, Kir2.1 (L222I), Kir2.3 (I213L) and Kir1.1 currents are represented as the current sensitive to block by 2 mM $BaCl_2$.

Inside-out patches were recorded using a fluoride, vanadate and pyrophosphate (FVPP)–potassium solution on both sides of the patch containing (in mM): 123 KCl, 5 K_2EDTA, 7.2 K_2HPO_4 and 8 KH_2PO_4, 0.1 Na_3VO_4, 5 KF, 10 $K_4P_2O_7$; pH 7.2, to prevent current run down (Huang et al., 1998). This solution was Mg^{2+}- and spermine-free. The pH 5.0 condition was sufficient to abolish any detectable current through Kir2.*x* channels, and off-line subtraction of pH 5 currents was used to subtract endogenous currents.

2.3. Drug

Thiopental (Laboratorios Pisa, S.A. de C.V. México) was dissolved directly in the external solution at the desired concentration. HEK-293 cells were exposed to drug solutions until steady-state effects were achieved. The effects of thiopental were measured 8–10 min after bath application in whole-cell experiments and inside-out patches. To determine the concentration–effect relationships, single cells were exposed to cumulative concentrations of the drug.

2.4. Data analysis and statistics

Patch-clamp data were processed by using Clampfit 9.0 (Molecular Devices, Union City, CA, USA) and then analyzed in Origin 6.0 (Origin Lab Northampton, MA). Data are presented as mean ± S.E.M. (n = number of cells). For whole-cell experiments, currents were normalized to the current recorded at −120 mV under control conditions; for inside-out experiments, currents were normalized to the current recorded at −80 mV under control conditions. The fractional block of current (f) was plotted as a function of drug concentration ([D]) and the data fit with a Hill equation:

$$f = 1 / \left\{1 + (IC_{50} / [D]^{nh}\right\}$$

to determine the half-maximal inhibitory concentration (IC_{50}) and the Hill coefficient, nh.

Statistical analyses were performed using the Statistica software package, version 10.0 (Tulsa, OK). Concentration–response data were tested for significance by Student's t-test applying Bonferroni's correction for multiple comparisons. Student's t-test was applied to compare individual data sets. A two-tailed probability value (P) of less than 0.05 was considered statistically significant.

3. Results

We investigated the mechanism of the inhibitory effects of thiopental on Kir2.1, Kir2.1 (L222I), Kir2.2, Kir2.3, Kir2.3 (I213L), Kir1.1 and Kir6.2/SUR2A channels. In the following section we use Kir2.1 as the prototype to illustrate the effects of the drug. In subsequent sections we display representative data for all the other Kir channels studied.

3.1. Thiopental inhibits current through Kir2.1 channels

The effects of thiopental on Kir2.1 channels were investigated in the whole-cell configuration in transfected HEK-293 cells. Kir2.1 currents were obtained under control conditions and in the presence of varying thiopental concentrations. Hyper- and depolarizing pulses, 4 s in duration and applied between −120 and −20 mV, elicited large inward currents but small outward currents, which is typical of the strong inward rectifier Kir2.1 channel (Fig. 1A). Fig. 1B shows normalized current–voltage relationships in control and after exposure to varying concentrations of thiopental for 10 min. Thiopental significantly decreased current amplitude in a concentration-dependent manner. At −120 mV, cumulative addition of thiopental decreased Kir2.1 current 44 ± 11, 65 ± 4 and 82 ± 5% at concentrations 10, 30, and 100 μM, respectively (n = 5; P<0.05, for all concentrations).

3.2. Thiopental inhibits Kir2.1 currents elicited by action potential clamp

The effect of thiopental (10–300 μM) was also studied on Kir2.1 currents elicited by action potential (AP) clamp, we used an AP from endocardial single cat ventricular myocytes as the command signal for voltage-clamp measurements, AP command signals were applied at a frequency of 1 Hz. As expected, Kir2.1 current was small or absent during the plateau phase of the AP, but then rapidly increased during repolarization and declined in early diastole. Thiopental inhibited the peak outward current in a concentration-dependent manner (see Fig. 2A). Fig. 2B shows the concentration–response curve for the peak outward current amplitude after 10 min of perfusion with thiopental. The IC_{50} of thiopental on Kir2.1 current elicited by AP voltage-clamp experiment was 32 ± 3 μM (n = 5).

3.3. Time course and potency of thiopental on Kir2.1 currents were similar for external and internal drug application

To determine whether thiopental inhibits Kir2.1 currents acting from the cytoplasmic or extracellular surface, we tested the effect of 30 μM thiopental on Kir2.1 current in excised inside-out patches.