The role of adenosine triphosphate-regulated potassium channels in propofol–induced beneficial effect on contractile function of hypercholesterolemic isolated rabbit hearts

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ABSTRACT

Objective: To investigate the role of adenosine triphosphate-regulated potassium (K_{ATP}) channels in the propofol-induced changes in the contractile function of hypercholesterolemic rabbit hearts.

Methods: This study was carried out in the Department of Pharmacology Laboratory, Faculty of Medicine, Dokuz Eylul University, Izmir, Turkey during the period January to December 2003. Twenty-two isolated rabbit hearts were grouped into 4. Group I (n=6) were infused with 50 µM propofol during a 60 minutes perfusion. Group II (n=6) were also infused with 100 µM propofol over the same period. Group III (n=5) was perfused with solutions containing 10 µM glybenclamide and group IV (n=5) 100 µM diazoxide for 5 minutes before and during a 60 minutes infusion with 100 µM propofol.

Results: The 50 µM propofol infusion decreased left ventricular pressure (LVP) significantly ($p<0.05$) but it did not change dP/dt_{max} and dP/dt_{min}. The 100 µM propofol infusion caused a significant increase in LVP at 20 minutes. Furthermore, a 100 µM propofol infusion resulted in a significant increase in maximal positive left ventricular pressure (dP/dt_{max}) and maximal negative left ventricular pressure (dP/dt_{min}) compared to baseline ($p<0.05$). The increase in dP/dt_{max} and dP/dt_{min} induced by 100 µM propofol was inhibited by glybenclamide ($p<0.05$), a K_{ATP} channel blocker, but was not affected by diazoxide ($p>0.05$), a K_{ATP} channel opener.

Conclusion: The activation of K_{ATP} channels seems to be one of the mechanisms by which propofol induced beneficial effect on contractility of myocardium in hypercholesterolemic rabbit hearts.

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The opening and closing of adenosine triphosphate-regulated potassium (K_{ATP}) channels is involved in several cardiovascular adaptive responses. Opening of these channels in cardiac myocytes produces the reduction of infarct size or improvement of post ischemic contractile function.[1,2] Adenosine triphosphate-regulated potassium channel agonists enhance the functional recovery of post-ischemic reperfused myocardium in vivo, and these effects are blocked by selective K_{ATP} channel antagonists such as glybenclamide (glyburide).[3] Previous experimental study demonstrated that the actions of volatile anesthetics, such as isoflurane, on the functional recovery of stunned myocardium are attenuated by a K_{ATP} channels antagonist, glybenclamide. In this study, it was concluded that these actions were mediated by isoflurane-induced activation of K_{ATP} channels.[4,5]

Propofol, an intravenous anesthetic agent, has a chemical structure similar to antioxidants such as vitamin E.[6] It has been found to protect the myocardium against injury induced by both exogenous hydrogen peroxide[7] and ischemia-reperfusion.[8,9] It is suggested that this cardioprotective effect might be due to a number of factors including preservation of energy levels during ischemia, alteration of intracellular calcium concentration, inhibition of oxygen free radicals and increased coronary flow.[7-11]

According to a previous study, increasing concentrations of propofol induced concentration and time-dependent inhibition in contractile function of myocardium in hearts of rabbits fed with standard diets. However, in the same study, high dose of propofol did not induce any cardiac depressant effect in hypercholesterolemic isolated rabbit hearts.[12] In the current study, using hypercholesterolemic isolated rabbit heart, we determined the role of K_{ATP} channels in propofol–induced beneficial effect on contractile function of myocardium.

Methods. *Preparation and measurements.* This study was carried out in the Department of Pharmacology Laboratory, Faculty of Medicine, Dokuz Eylul University, Izmir, Turkey during the period January to December 2003. All experimental procedures and protocols used in this investigation were approved by the Animal Use Committee of the Dokuz Eylul University School of Medicine. New Zealand white male rabbits (n=22) weighing between 1600 and 2500 g (mean 1994 ± 88.1 g) were used. Rabbits were fed with a diet containing cholesterol (1% w/w) for one month. All animals received standard amounts (150 g/day) of rabbit chow pellets. Prior to the study, ear vein blood samples were taken (1 mL) and cholesterol levels were measured by an autoanalyzer (Hitachi 912 analyzer, Germany) with a commercial kit (Cholesterol reagent, RAICHEM, USA). Serum cholesterol was measured before feeding the animals with high cholesterol diets. Each rabbit was given a dose of 1000 IU/kg heparin and anesthetized with 60 mg/kg of intravenous thiopental. After thoracotomy, the heart and aortic arch were rapidly excised and placed in a cold Krebs-Henseleit bicarbonate buffer solution (+4°C). The hearts were perfused via retrograde cannulation of the aorta with filter (25 µm pore size) inline at a constant- flow perfusion of 30 mL/min and bath temperature of 37°C. Each perfusate solution was bubbled with a gas mixture of 95% O_2 and 5% CO_2.[12] The perfusate had the following composition (in mM): NaCl 118, KCl 4.7, $NaHCO_3$ 25, $CaCl_2$ 2, $MgSO_4$ 1.2, KH_2PO_4 1.2, and glucose 11.

For measurement of left ventricular pressure (LVP), a saline-filled balloon connected to a pressure transducer was inserted into the left ventricle cavity via the left atrium. The balloon volume was adjusted to maintain a diastolic LVP of 0 mm Hg during the initial period and was not altered during the experiments. Spontaneous heart rate (HR), LVP, maximal positive left ventricular pressure (dP/dt_{max}) and maximal negative left ventricular pressure (dP/dt_{min}) were recorded continuously on a data acquisition system (BIOPAC, MP30B-CE, 206B1564; USA).

The following pharmaceuticals were used: propofol (Diprivan®; Zeneca, UK), glybenclamide diazoxide and dimethyl sulfoxide (Sigma Chemical Company, St. Louis, USA), thiopental sodium (I.E. Ulugay Pharmacia, Turkey) and heparin sodium (Liquemine®; Roche, Sweden). Propofol was diluted with Krebs-Henseleit bicarbonate buffer solution to obtain propofol concentrations of 50 and 100 µM and was directly infused at a constant flow rate of 0.5 mL/min with an infusion pump (Harvard Apparatus, UK). Glybenclamide and diazoxide were dissolved in dimethyl sulfoxide (DMSO). The final concentration of DMSO was 0.1% in the experimental solutions.[13]

Experimental protocol. **Figure 1** summarizes the protocol for each of the 4 experimental groups. All hearts were allowed to stabilize for 15 minutes before the starts of the experiments. While hearts were infused with 50 µM propofol in group I (n=6), in group II (n=6) hearts were infused with 100 µM propofol for 60 minutes. In group III (n=5) and group IV (n=5), 10 µM

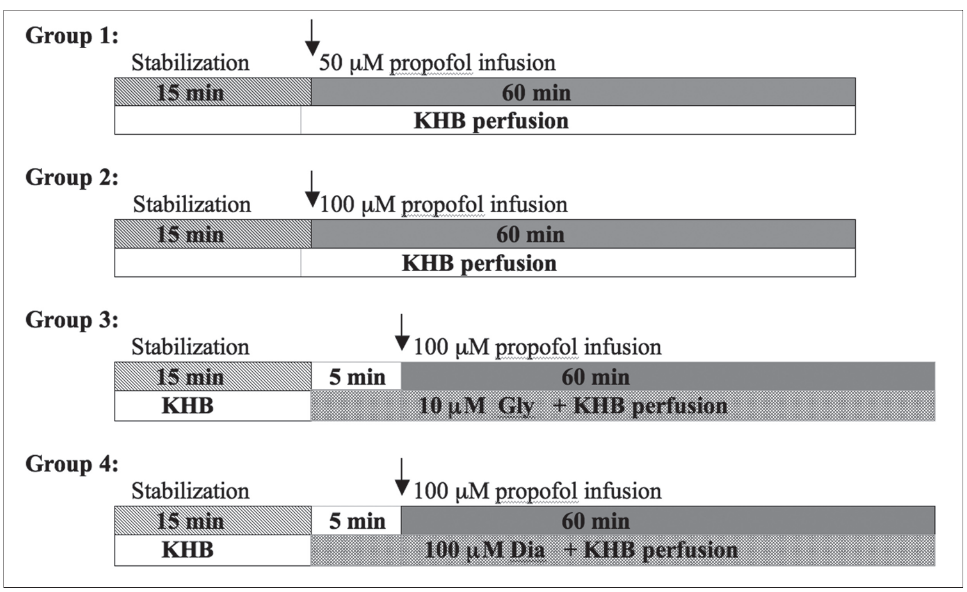

Figure 1 - Schematic presentation of experimental protocol. KHB = Krebs-Henseleit bicarbonate buffer, Gly = Glybenclamide, Dia = Diazoxide.

glybenclamide and 100 µM diazoxide were, respectively, added to the perfusate solutions 5 minutes before starting the 60 minutes 100 µM propofol infusion to the hearts.[13] Glybenclamide or diazoxide perfusion continued during the propofol infusion. The LVP, HR, dP/dt_{max} and dP/dt_{min} were recorded at baseline just before starting and every 10 minutes during the 60 minutes propofol infusion.

Statistical analyses. Results were expressed as mean ± standard error of the mean (SEM). Statistical analyses of data within groups and between groups (drug effects) were evaluated by Wilcoxon matched pairs test and Mann Whitney U test, respectively. Differences among groups were performed using Kruskal-Wallis test followed by Dunn's Multiple Comparison tests for more than 2 groups. (GraphPad Instat™, 1990-1994, GraphPad Software V2.05a 9342, USA). For all studies, p values of <0.05 were considered to be statistically significant.

Results. After one month of the high cholesterol diet, total serum cholesterol levels were increased significantly from 78.8 ± 6.7 to 724.1 ± 69.0 mg/dL ($p<0.05$). There were no significant differences in baseline LVP, HR, dP/dt_{max} and dP/dt_{min} among the experimental groups.

Effects of drugs on LVP in hypercholesterolemic isolated rabbit heart. In group I, 50 µM propofol infusion resulted in a significant decrease in LVP at 10, 20, 30, 40, 50, and 60 minutes compared to baseline values ($p<0.05$, **Table 1, Figure 2**). In group II, the 100 µM propofol infusion did not produce any changes in LVP significantly compared to baseline values at 10, 30, 40, 50 and 60 minutes, but resulted in a significant increase in LVP compared to baseline at 20 minutes ($p<0.05$, **Table 1, Figure 2**). The 50 µM propofol infusion produced a significant decrease in LVP at all time points when compared to the 100 µM propofol infusion ($p<0.05$, **Table 1, Figure 2**). Neither the glybenclamide nor the diazoxide perfusion caused a significant change on the effect of 100 µM propofol infusion in LVP ($p>0.05$).

Effects of drugs on HR in hypercholesterolemic isolated rabbit heart. The 50 µM or 100 µM propofol infusion did not result in any significant change of the HRs when compared to baseline values ($p>0.05$, **Table 1, Figure 3a**). In the presence of glybenclamide or diazoxide perfusion, 100 µM propofol infusion did not cause any significant difference in HRs ($p>0.05$, **Table 1, Figure 3a**).

Effects of drugs on dP/dt_{max} and dP/dt_{min} in hypercholesterolemic isolated rabbit heart. In group I, the 50 µM propofol infusion did not change the dP/dt_{max} and dP/dt_{min} significantly compared to baseline

Table 1 - Hemodynamic effects for each group.

Propofol (µM)	N	Baseline	10 min	20 min	30 min	40 min	50 min	60 min
LVP (mmHg)								
50	6	101.7 ± 7.8	75.8 ± 4.0*,+	72.6 ± 4.6*,+	71.5 ± 4.5*,+	67.1 ± 7.4*,+	67.3 ± 7.7*,+	66 ± 6.7*,+
100	6	126.9 ± 13.4	141.7 ± 18.7	137.7 ± 14.9*	126 ± 13.8	124.9 ± 16.3	125.1 ± 15.3	123.4 ± 15.2
Gly + 100	5	93.2 ± 6.1	94.9 ± 4.8	94.5 ± 5.6	95.2 ± 5.7	94.7 ± 7	98.9 ± 12.6	100.8 ± 16.2
Dia + 100	5	117.6 ± 12.3	119.1 ±10.6	124.9 ± 9.9	125.2 ± 9.9	125.5 ± 10.2	127.2 ± 10.8	127.3 ± 10.7
HR (beat/min)								
50	6	127 ± 12.6	136 ± 9.3	137 ± 8.8	139 ± 7.7	139 ± 7.9	137 ± 9.1	134 ± 8.6
100	6	158 ± 9.1	160 ± 11.1	158 ± 10.2	161.7 ± 9.6	1630 ± 9.1	163 ± 9.1	160 ± 9.8
Gly + 100	5	145.2 ± 4.3	136.8 ± 6.9	136.8 ± 6.9	136.8 ± 6.9	139.2 ± 7.9	133.2 ± 12.5	136.8 ± 6.9
Dia + 100	5	132 ± 12	132 ± 12	126 ± 11.2	126 ± 11.2	132 ± 7.3	138 ± 12	132 ± 15.3
dP/dt_{max} (mmHg/s)								
50	6	1373 ± 94.9	1335 ± 128.8	1379 ± 114.9	1405 ± 125.3	1380 ± 154.5	1390 ± 150.5	1421 ± 126
100	6	1414 ± 156.9	1819 ± 236.3*	1861 ± 123.2*,[I]	1988±150.4*,[I]	1996±178.1*,[I]	2035 ± 185.5*,[I]	1995 ± 191*,[I]
Gly + 100	5	927.8 ± 89.5	1018 ± 102.1++	1002 ± 100.7++	1034 ± 117.1++	1007 ± 125.9++	727.5 ± 162.7++	672.9 ± 168.5++
Dia + 100	5	1383 ± 129.5	1817 ± 245	1931 ± 245.6***	1952 ± 238***	1964 ± 253.4***	2002 ± 271.1***	1997 ± 281.5***
dP/dt_{min} (mmHg/s)								
50	6	1123 ± 64.9	1206 ± 69.8	1191 ± 99.6	1230 ± 80.9	1156 ± 124.4	1189 ± 106.4	1230 ± 105.3
100	6	1466 ± 171.1	1787±186.9*,[I]	1889 ± 116.8*,**	1919 ± 108.8*,**	1869±149.2*,[I]	1907 ± 149.5*,[I]	1884 ± 140.2*,**
Gly + 100	5	906.8 ± 40.2	855.2 ± 28.3[II]	829.5 ± 66.1++	830.3 ± 70.9++	880.5 ± 90.3++	710.9 ± 86.7++	676.6 ± 57.8++
Dia + 100	5	1437 ± 160.2	1653 ± 252.7	1945 ± 279.6***	1922 ± 272.8***	1947 ± 277.1***	1934 ± 268.9***	1939 ± 281.6***

Data are mean ± SEM. LVP - left ventricular pressure, HR - heart rate,
dP/dt_{max} - maximal positive left ventricular pressure derivative, dP/dt_{min} - maximal negative left ventricular pressure derivative.
*Significantly ($p<0.05$) different from baseline +, ($p<0.05$) different from 100 µM propofol. [I],
**($p<0.05$, $p<0.01$, respectively) different from 50 µM propofol ++, [II] , ($p<0.05$, $p<0.01$, respectively) different from 100 µM propofol.
***($p<0.05$) different from glybenclamide.

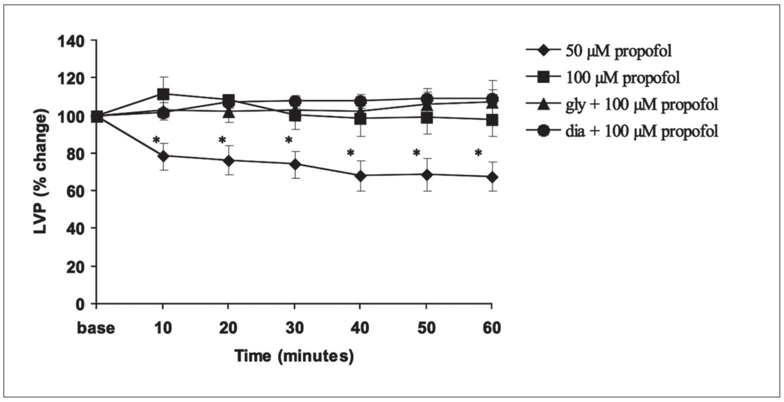

Figure 2 - Effect of the drugs on left ventricular pressure in hypercholesterolemic isolated rabbit heart. LVP= Left ventricular pressure, Gly= Glybenclamide, Dia=Diazoxide. *$p<0.05$ different from 100 µM propofol..

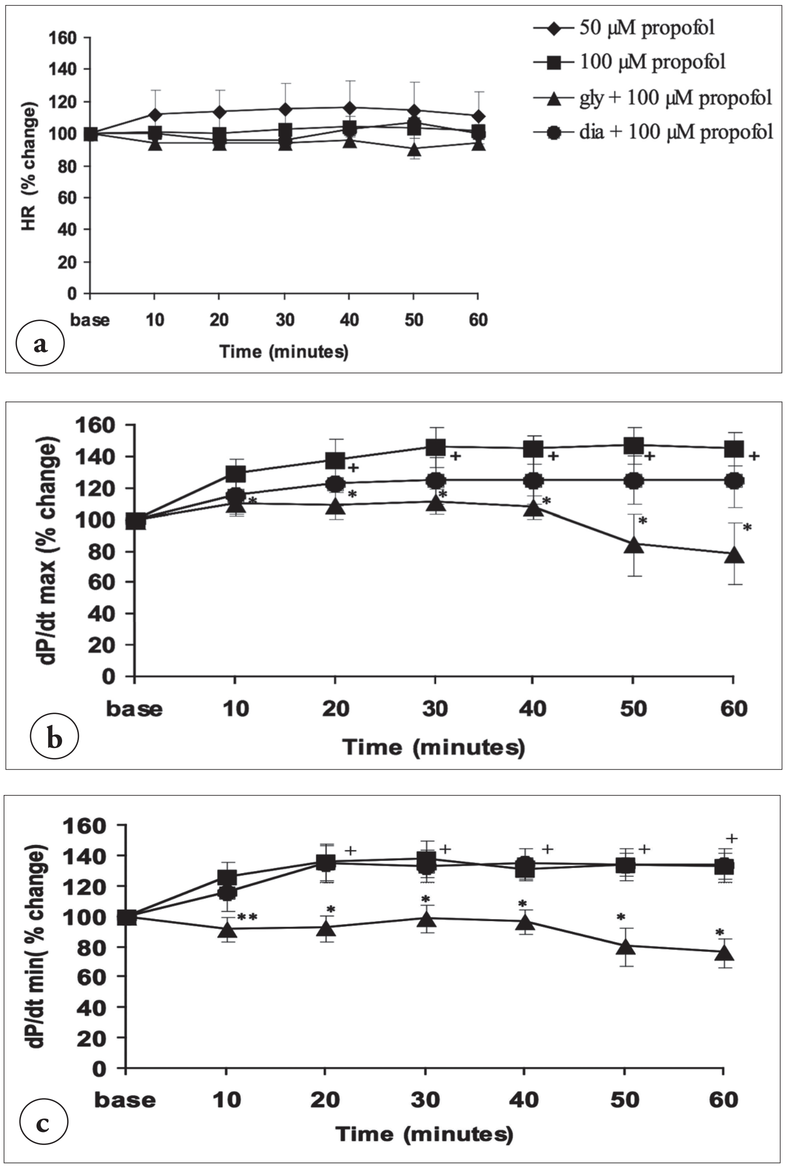

Figure 3 - Effect of the drugs on heart rate **a**), dP/dt_{max} **b)** and dP/dt_{min} **c)** in hypercholesterolemic isolated rabbit hearts. HR - Heart rate, dP/dt_{max} - maximal positive left ventricular pressure, dP/dt_{min} - maximal negative left ventricular pressure, Gly - Glybenclamide, Dia - Diazoxide. Because 50 µM propofol infusion did not cause any statistically significant change, it is not shown in Figures 3b and 3c. *$p<0.05$, **$p<0.01$ different from 100 µM propofol + $p<0.05$ different of diazoxide from glybenclamide.

values (p>0.05, **Table 1**). In group II, 100 µM propofol infusion resulted in a significant increase in dP/dt_{max} and dP/dt_{min} at all time points compared to baseline values, and group I (p<0.05, **Table 1, Figure 3b** and **Figure 3c**). The increases in dP/dt_{max} and dP/dt_{min} observed with the 100 µM propofol infusion in group II were abolished by 10 µM glybenclamide perfusion (in group III) (**Table 1, Figure 3b** and **Figure 3c**). Diazoxide produced no such effects (**Table 1, Figure 3b** and **Figure 3c**).

Discussion. In the present study, a 50 µM propofol infusion caused a significant inhibition of LVP while a 100 µM propofol infusion did not produce any cardiac depressant effect on hypercholesterolemic isolated rabbit hearts. Furthermore, a 100 µM propofol infusion caused a significant increase in dP/dt_{max} and dP/dt_{min} values. These findings suggest that high concentrations of propofol increases myocardial contractility in hypercholesterolemic isolated rabbit hearts. Experimental studies have shown that propofol protects against myocardial injury caused by both exogenous hydrogen peroxide administration[7] and ischemia-reperfusion.[8,9] Our previous study reported that a 100 µM propofol did not cause any significant inhibition on contractile function of myocardium in hypercholesterolemic isolated rabbit hearts.[12] However, Coetzee[14] reported that propofol failed to protect the pig heart from ischemic reperfusion injury, as induced by left anterior descending coronary artery occlusion, but Ko et al[8] reported that 100 µM propofol attenuated ischemia-reperfusion injury in the isolated rat hearts. The observed cardioprotective effect of propofol may be due to preservation of energy levels during ischemia, alteration of intracellular calcium concentration, inhibition of oxygen free radicals or increased coronary flow.[7-11] Cardioprotection related to K_{ATP} channels was initially thought to be via the surface membrane channel (sK_{ATP}) of myocytes. However, some studies have shown that K_{ATP} channels in the mitochondrial inner membrane (mK_{ATP}) are responsible for the protection.[1,2,15,16] In our study, glybenclamide, an inhibitor of both sarcolemmal and mitochondrial K_{ATP} channels,[17] reversed the beneficial effect of 100 µM propofol infusion on dP/dt_{max} and dP/dt_{min}. In addition, diazoxide, a selective mK_{ATP} channel opener, did not alter these effect of 100 µM propofol infusion on dP/dt_{max} and dP/dt_{min}. These findings suggest that K_{ATP} channel activation likely plays a role in the 100 µM propofol-induced beneficial effect on contractile function of myocardium. Contrary to our findings, Mathur et al[18] demonstrated that pretreatment with the K_{ATP} channel blocker, glybenclamide, significantly attenuated the cardioprotection associated with sevoflurane but not with propofol. They suggested that propofol did not provide cardioprotection via the K_{ATP} channels. In other 2 subsequent studies, volatile anesthetics such as isoflurane exerted myocardial protective effects, which were abolished by pretreatment with the selective K_{ATP} channel antagonist glybenclamide. The authors suggested that K_{ATP} channel activation by isoflurane might mediate these cardioprotective effects.[4,5] Activation of K_{ATP} channels in vascular smooth muscle causes vasodilation. Isoflurane in vivo[19] and halothane in vitro[20] have been shown to produce coronary vasodilation through activation of K_{ATP} channels, and this effect was inhibited by glybenclamide. Myocardial protection induced by these agents during ischemia and reperfusion injury may involve K_{ATP} channel activation. Mouren et al[21] showed that while therapeutic concentrations of propofol did not change coronary vascular tone, supratherapeutic concentrations of propofol (100, 300 and 1000 µM) induced a significant increase in coronary blood flow. Ko et al[8] reported that the heart treated with 100 µM propofol, exhibits a significantly increased coronary flow after reperfusion. Propofol also has a direct vasodilator effect on distal coronary arteries in rats,[22] which is primarily endothelium-dependent and mediated by multiple substances, including nitric oxide and a vasodilator prostanoid. In addition, the same authors showed that this vasodilator effect of propofol was not mediated by the opening of the K_{ATP} channels.[22] In our study, propofol may produce coronary vasodilatation through activation of K_{ATP} channels at 100 µM concentration. Increase in coronary flow may improve the perfusion of hypercholesterolemic myocardium and enhance the contractile function of myocardium. However, coronary artery vasodilation and coronary blood flow were not evaluated in this study and a further study in this area is recommended.

In conclusion, the results of this study demonstrate that 100 µM propofol enhances the contractile function of myocardium on hypercholesterolemic isolated rabbit heart, and these effects are abolished by glybenclamide, a K_{ATP} channel antagonist, but not altered with diazoxide, a K_{ATP} channel opener. These findings suggest that this beneficial action of propofol on hypercholesterolemic isolated rabbit heart, at least in part, is via activation of K_{ATP}-channels.

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