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Differential Effects of Etomidate, Propofol, and Midazolam on Calcium and Potassium Channel Currents in Canine Myocardial Cells

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Background: **Intravenous anesthetics etomidate, propofol, and midazolam produce negative inotropic effects of various degrees. The mechanism underlying these differences is largely unknown.**

Methods: **The effects of intravenous anesthetics on L-type Ca^{2+}, transient outward and inward-rectifier K^+ channel currents (I_{Ca}, IK_{to}, and IK_1) were compared in canine ventricular cells using the whole-cell voltage-clamp technique. I_{Ca} and I_K were elicited by progressively depolarizing cells from −40 to +40 mV, and from −90 to +60 mV, respectively. The peak amplitude and time-dependent inactivation rate of I_{Ca} and I_K were measured before, during, and after the administration of equimolar concentrations (5, 30, or 60 μM) of etomidate, propofol, or midazolam.**

Results: **Exposure to etomidate, propofol, and midazolam produced a concentration-dependent inhibition of I_{Ca}. Midazolam was the most potent intravenous anesthetic; at 60 μM, etomidate, propofol, and midazolam decreased peak I_{Ca} by 16 ± 4% (mean ± SEM), 33 ± 5%, and 47 ± 5%, respectively. Etomidate, propofol, and midazolam given in a 60-μM concentration decreased IK_{to} by 8 ± 3%, 9 ± 2%, and 23 ± 3%, respectively. IK_1 was decreased by 60 μM etomidate and midazolam by 20 ± 6% and 14% ± 5%, respectively. Propofol had no effect on IK_1.**

Conclusions: **At equimolar concentrations, intravenous anesthetics decreased the peak I_{Ca}, I_{Kto}, and I_{K1} with various degrees of potency. Effects of anesthetics on I_{Ca} were significantly greater compared with their effects on K^+ currents. These findings suggest that the negative inotropic actions of etomidate, propofol, and midazolam are related, at least in part, to decreased I_{Ca}. Some effects, such as I_K inhibition, may partially antagonize effects of decreased I_{Ca}. Indeed, the final effect of these intravenous anesthetics on myocardium will be the sum of these and other sarcolemmal and intracellular effects. (Key words: Anesthetics, intravenous: etomidate; midazolam; propofol. Animal: dog. Current: calcium; potassium. Tissue: myocardium; ventricular.)**

INTRAVENOUS anesthetics etomidate, propofol, and midazolam are used to induce and maintain general anesthesia and to provide sedation during local and regional anesthesia. These agents can depress cardiovascular function in humans[1-3] and animals.[4,5]

The mechanisms underlying *in vivo* cardiovascular depression by intravenous anesthetics are not well understood but probably include a reduction in afterload[6,7] and preload[8,9] and direct myocardial depression.[10,11] Although the mechanisms of intravenous anesthetic-induced negative inotropic effects appear to be diverse,[6,11-14] increasing evidence suggests that these agents exert direct negative inotropic actions *in vivo*[1-5] and *in vitro*.[13,15,16]** Because changes in contractile force reflect an interaction between Ca^{2+} influx and K^+ efflux through the sarcolemma, Ca^{2+} release and sequestration by the sarcoplasmic reticulum, activity of membrane Ca^{2+} and K^+ pumps, and the Ca^{2+} sensitivity of the contractile proteins, it is possible that these agents may interfere with any one of these steps, thus decreasing contractility. Although all intravenous anesthetics have negative inotropic effects, researchers frequently contend that etomidate

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**Rusy BF, Thomas-King PY, King GP, Komai H: Effects of propofol on the contractile state of isolated rabbit papillary muscles under various stimulation conditions (Abstract). ANESTHESIOLOGY 1990; 73:A560.

is the least potent.[4,11]†† Preliminary studies implicate sarcolemmal ion channels as a potential site of intravenous anesthetic-induced negative inotropic action.[13]**‡‡

Our goal was to gain greater insight into the mechanisms underlying the negative inotropic effects of intravenous anesthetics by evaluating and comparing the effects of etomidate, propofol, and midazolam on the high-threshold Ca^{2+} current (I_{Ca}), low-threshold transient K^+ current (IK_{to}), and the inward-rectifier K^+ current (IK_1) in single canine ventricular cells using the whole-cell patch-clamp technique.

Materials and Methods

Preparation of Single Ventricular Cells

These experiments were approved by the Medical College of Wisconsin Animal Care Committee, and all experimental procedures strictly conformed to the standards of American Association for Accreditation of Laboratory Animal Care. Adult mongrel dogs weighing 15 to 25 kg were placed in a Plexiglas box and anesthetized with halothane. After surgical anesthesia was attained, the trachea was intubated and the lungs were ventilated with 1.5% halothane in oxygen. The chest was opened and the heart was excised rapidly and placed in cold Krebs' solution. Thin strips of the ventricular tissue were cut into 5-mm lengths and placed in cold cardioplegia solution composed of 10 mM NaCl, 60 mM KCl, 5 mM $MgCl_2$, 20 mM glucose, 100 mM sucrose, 5 mM pyruvate, 20 mM taurine, 5 mM N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES), and 0.5 mM disodium salt of ethylenediamine tetraacetic acid (Na_2-EDTA). The washed ventricular segments were placed in Ca^{2+}-free Tyrode's solution composed of 140 mM NaCl, 5.4 mM KCl, 5 mM $MgCl_2$, 5.5 mM glucose, 5 mM HEPES, 4 mg/ml collagenase (Type I, 161 units/mg; Worthington, Freehold, NJ), 2 mg/ml bovine albumin (Sigma Chemical Co., St. Louis, MO), 40 μM $CaCl_2$, 5 mM pyruvic acid, and 5.5 mM disodium salt of adenosine triphosphate (Na_2-ATP); the pH was adjusted to 6.2 with NaOH. The solution containing ventricular tissue was incubated at 37°C for 1 to 2 h in a slow shaker. After this incubation, single ventricular cells were washed three times in cold potassium glutamate (K-glutamate) solution composed of 130 mM K-glutamate, 5.7 mM $MgCl_2$, 5 mM HEPES, 5.5 mM glucose, 5 mM Na_2-ATP, and 0.12 mM Na_2-EDTA; the pH was adjusted to 7.4 with KOH. Dispersed cells were stored in K-glutamate solution at 4°C before use.

Voltage-clamp Recording

A drop of dispersed single canine ventricular cells was placed in a perfusion chamber (22°C) on the stage of an inverted microscope (Olympus IMT-2; Leeds Instruments, Minneapolis, MN) equipped with modulation contrast. At 500× magnification, a hydraulic micromanipulator (Narishige, Tokyo, Japan) was used to position heat-polished borosilicate patch pipettes with tip resistance of 4 to 6 MΩ on the membrane of ventricular cells. High-resistance seals (3 to 30 GΩ) were formed, after which the pipette patch was removed by negative pressure to obtain the electrical access to the whole cell as previously described.[17] Whole-cell currents were elicited by 200-ms depolarizing pulses generated by a computerized system (pClamp software; Axon Instruments, Burlingame, CA) every 5 to 10 s. The currents were amplified by a List EPC-7 patch-clamp amplifier (Adams & List Associates, Great Neck, NY), and the amplifier output was low-pass filtered at 500 Hz. All data were digitized (sampling rate = 10,000/s) and stored on a hard disk to permit analysis at a later time. For the I_{Ca}, the leak and capacitative currents were subtracted from each record by linearly summating scaled currents obtained during 10-mV hyperpolarizing pulses.

Recording Solutions

The external solution used to measure I_{Ca} contained: 10 mM $BaCl_2$, 135 mM tetraethylammonium chloride, 1 mM $MgCl_2$, 10 mM glucose, and 10 mM HEPES (pH = 7.4). The pipette solution used to measure I_{Ca} contained: 130 mM CsCl, 5 mM adenosine triphosphate (magnesium salt), 5 mM ethyleneglycol-bis-(β-aminoethyl ether) N,N,N′,N′-tetraacetic acid (EGTA), 10 mM HEPES, 1 mM $MgCl_2$, and 10 mM glucose (pH = 7.2).

The external solution used to measure I_K contained: 135 mM NaCl, 5.5 mM dextrose, 0.5 mM $MgCl_2$, 4 mM KCl, and 2 mM $CaCl_2$ (pH = 7.4). The pipette solution used to measure I_K contained: 125 mM potassium aspar-

††Williams JP, McArthur DJ, Walker EW, Rietsema K, Teunissen E, Bonnenkamp H, Goderie P, Stanley HT: A comparison of the hemodynamics of Diprivan (propofol), thiopental, and etomidate for induction of anesthesia in patients with coronary artery disease. Semin Anesth 1988; 7:112–5.

‡‡Wegrzynowicz ES, Matsuda J, Volk K, Shibata E, Wachtel R: Propofol decreases voltage activated calcium currents in rabbit heart myocytes (Abstract). ANESTHESIOLOGY 1990; 73:A592.

tate, 20 mM KCl, 10 mM EGTA, 5 mM adenosine triphosphate (magnesium salt), 1 mM $MgCl_2$, and 5 mM HEPES (pH = 7.2).

Drug Effects

Repetitive current-voltage curves were obtained in the control solution to monitor time-dependent changes in I_{Ca} and I_K, followed by exposure to 5, 30, or 60 μM etomidate, midazolam, or propofol. Effects of anesthetics on I_{Ca} and I_K were complete within 6 min and were reversible with washout.

The recommended intravenous doses to induce anesthesia are approximately 0.2 to 0.4 mg/kg for midazolam, 0.3 mg/kg for etomidate, and 2.5 mg/kg for propofol. It was reported that plasma protein-bound fraction of midazolam and propofol is 97%, whereas for etomidate it is 76%. Peak plasma concentrations during induction with these doses vary widely but are reported to be approximately 0.5 to 3 μM for midazolam,[1,18-20] 3 μM for etomidate,[21] and 50 μM for propofol.[22,23]§§ For our study, intravenous anesthetics were obtained commercially in their vehicles (2 mg/ml etomidate, 5 mg/ml midazolam, and 10 mg/ml propofol). Each anesthetic was diluted in a given extracellular solution to make 1 mM stock solution that was divided into aliquots, frozen at −15°C, and thawed for daily use. Measured volumes of the 1-mM concentration of each drug were diluted into a known volume of perfusate to obtain perfusate drug concentrations of 5, 30, and 60 μM. Cells were randomly exposed to one of the anesthetic agents, at one of three concentrations. 4-aminopyridine (4-AP; Sigma Chemical Co.) and barium chloride (Ba^{2+}; Fisher Scientific, Itasca, IL) were dissolved directly in the recording solution. Nifedipine (Sigma Chemical Co.) was dissolved in 70% ethanol to make 1 mM stock solution, yielding a final solvent concentration in the recording solution of 0.07%, which by itself did not affect the current. Cobalt chloride (Co^{2+}; Sigma Chemical Co.) was dissolved directly in the recording solution. Tetrodotoxin (TTX; Sigma Chemical Co.) was dissolved in the recording solution to make 1 mM stock solution.

Vehicle preparations of etomidate (350 mg/mL propylene glycol in H_2O), propofol (Intralipid; 100 mg/ml soybean oil, 22.5 mg/ml glycerol, and 12 mg/ml egg lecithin in water), and midazolam (0.01% disodium edetate and 1% benzyl alcohol in water, adjusted to pH of 3 with 0.1 $NHCl_3$) were also prepared and administered in separate groups of cells (n = 2 to 4) at the concentrations that would correspond to the highest drug concentration given in this study (60 μM) over the same time period (6 to 10 min). None of the drug vehicles had any effect (change <5%) on either I_{Ca}, IK_{to}, or IK_1.

Statistical Analysis

All currents are expressed as means ± SEM and were analyzed by two-way analysis of variance. If the F-test showed significance, Fisher's test for least significant differences was performed, with the level of significance designated at $P \leq 0.05$.

Results

Calcium Current

Calcium current (I_{Ca}) was generated by depolarizing pulses (10 mV; 200 ms) from a holding potential of −40 mV to a command potential as high as +40 mV. All experiments were performed with Ba^{2+} (10 mM) as the charge carrier in place of external Ca^{2+} (2 mM); this substitution increased the peak of I_{Ca} and decreased the rate of inactivation. In 64 cells (each group containing 5 to 9 cells), the threshold activation occurred at approximately −30 mV and the maximal activation was reached between −10 and 0 mV. This I_{Ca} resembled high-threshold, long-lasting (L-type) Ca^{2+} channel current previously described by researchers in our laboratory[24] and by others.[25-27] Figure 1 (upper panel) illustrates the effects of 60 μM etomidate (A), propofol (B), and midazolam (C) on the peak I_{Ca} elicited by the test pulse from a holding potential of −40 mV to 0 mV in three canine ventricular cells. Peak I_{Ca} (fig. 1A-C, lower panel) was plotted as a function of membrane potential to analyze the effects of etomidate (A), propofol (B), and midazolam (C) on the current-voltage relations for I_{Ca} activation. Figure 1D summarizes decreases of the peak I_{Ca} amplitude by all three anesthetics. Etomidate at concentrations of 5 μM, 30 μM, and 60 μM decreased the peak I_{Ca} amplitude by 3 ± 3% (n = 7, NS), 10 ± 6% (n = 7, NS) and 16 ± 4% (n = 6; $P \leq 0.05$), respectively. Propofol at concentrations of 5 μM, 30 μM, and 60 μM also decreased the peak I_{Ca} amplitude by 10 ± 3% (n = 6, NS), 21 ± 4% (n = 9; $P \leq 0.05$), and 33 ± 6% (n = 9; $P \leq 0.05$), respectively. In a dose-dependent manner, 5 μM, 30 μM, and 60 μM midazolam decreased the

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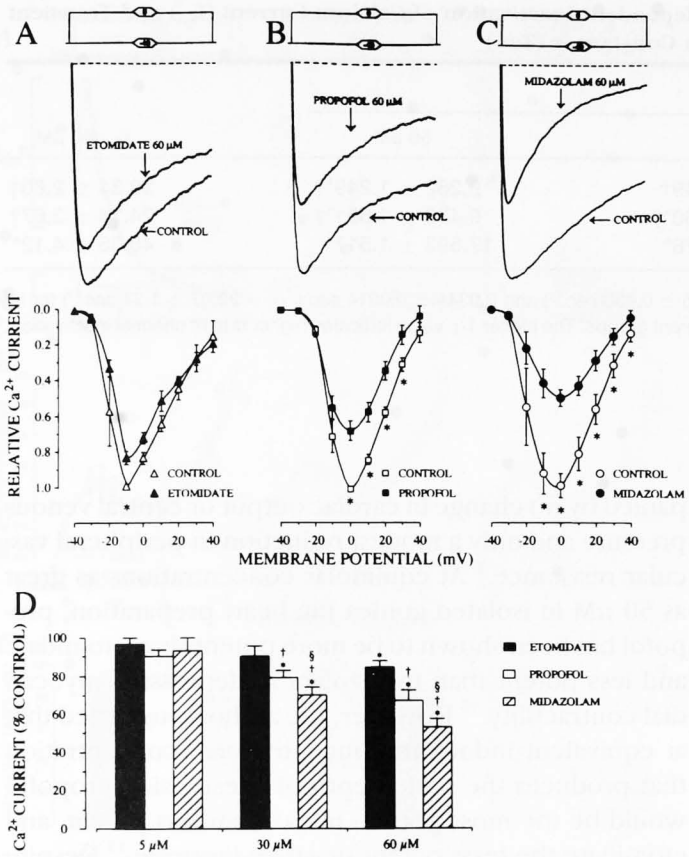

Fig. 1. Effects of 60 μM etomidate (*A*), propofol (*B*), and midazolam (*C*) on inward Ca^{2+} channel current (I_{Ca}) elicited by depolarizing pulse from a holding potential (HP) of −40 to 0 mV in three ventricular cells (*upper*), and corresponding current-voltage relationships (*lower*). The control current-voltage relationship for activation of I_{Ca} (expressed as a fraction of control maximal I_{Ca}) was reduced by all three anesthetics. *$P \leq 0.05$ *versus* anesthetic. Concentration-dependent decrease of the peak I_{Ca} amplitude (expressed as a percentage of control) by 5, 30, and 60 μM etomidate, propofol, and midazolam in a total of 64 canine ventricular cells is shown in panel *D*. *$P \leq 0.05$ *versus* control; †$P \leq 0.05$ *versus* etomidate; §$P \leq 0.05$ midazolam *versus* propofol.

peak I_{Ca} amplitude by 7 ± 7% (n = 7, NS), 30 ± 4% (n = 5; $P \leq 0.05$), and 47 ± 5% (n = 8; $P \leq 0.05$), respectively.

At equimolar concentrations, midazolam was most potent among the intravenous anesthetics in inhibiting I_{Ca}. The effects of these agents on I_{Ca} were voltage independent (*i.e.*, there was no difference in percentage decrease among different voltages).

Further analysis of the current traces produced by the depolarization step from a holding potential of −40 mV to 0 mV revealed that all three anesthetics not only decreased the peak I_{Ca} but also hastened the inactivation of I_{Ca} and thus further contributed to a decrease of total Ca^{2+} influx. The inactivation phase of I_{Ca} trace was best fitted with a single exponential curve with an average control inactivation constant (τ) of 0.185 ± 0.013 s. Although all three anesthetics decreased the inactivation constant at 60 μM, etomidate given at 5 and 30 μM did not significantly alter the inactivation constant (table 1). Although propofol significantly decreased τ at two higher concentrations, it produced a significantly smaller ($P \leq 0.05$) decrease compared with the same concentration of midazolam. The effects of intravenous anesthetics on I_{Ca} were readily reversible with washout.

Potassium Current

During the 200-ms depolarizing pulses from −90 mV to consecutively more positive voltages as high as +60 mV, cells showed transient outward K^+ current (IK_{to}) and an inward-rectifier K^+ current (IK_1). To measure IK_{to} and IK_1, all experiments were done in the presence of 20 μM tetrodotoxin (TTX) and 2 mM cobalt chloride (Co^{2+}) in the external solution to block Na^+ and Ca^{2+} currents, respectively. Addition of nifedipine (1 μM) in the external solution produced no significant changes in I_K amplitude, indicating that this current is not influenced by the Ca^{2+} influx *via* the L-type Ca^{2+} channel. Voltage-dependent IK_1 was activated approximately at voltages negative to −20 mV and was completely abolished by 1 mM Ba^{2+} in the external solution, as described previously.[28-30] Voltage-dependent IK_{to} was identified as IK_{to_1} subtype because it was blocked by 2 mM 4-aminopyridine (4-AP) in the external solution.[31-35]

Figure 2A shows actual recordings of the peak IK_1 and IK_{to} elicited by depolarizing pulses from a holding potential of −90 to −50 mV, and from −90 to +60 mV, respectively, in control solution and during exposure to 60 μM etomidate, propofol, and midazolam in three ventricular cells. Peak IK_{to} and IK_1 were plotted as a function of membrane potential to analyze the mean anesthetic effect on the current amplitude (fig. 2B). At equimolar concentrations, etomidate, propofol, and midazolam caused reversible decreases in the peak IK_{to} amplitude (at +60 mV) by 8 ± 3% (n = 8; $P \leq 0.05$), 9 ± 2% (n = 6; $P \leq 0.05$), and 23 ± 3% (n = 15; $P \leq 0.05$), respectively. At the same concentration, etomidate and midazolam produced reversible decreases in the peak IK_1 amplitude (at −50 mV) by 20 ± 6% (n = 8; $P \leq 0.05$), and 14 ± 5% (n = 15; $P \leq 0.05$). Propofol had no effect on I_{K1} (n = 6). The effects of intravenous anesthetics on IK_{to} and IK_1 were not voltage dependent.

Table 1. Effects of Midazolam, Etomidate, and Propofol on Time-dependent Inactivation of Calcium Current (I_{Ca}) and Transient Outward Potassium Current (I_{Kto}) as Characterized by Inactivation Constant, τ (Tau)

	I_{Ca}			
Drug	5 μM	30 μM	60 μM	I_{Kto} (60 μM)
Etomidate	5.341 ± 0.885†	6.421 ± 0.449†	9.262 ± 1.249*†	30.34 ± 2.60†
Propofol	6.51 ± 0.820	8.338 ± 1.260*†	9.432 ± 1.591*†	24.14 ± 3.67†
Midazolam	7.244 ± 1.345*	10.638 ± 1.828*	17.593 ± 1.517*	45.35 ± 4.12*

Data are shown as $1/\tau$. The control τ values were 0.185 ± 0.013 sec ($1/\tau$ = 5.405 ± 0.430 sec^{-1}) and 0.0344 ± 0.0014 sec ($1/\tau$ = 29.07 ± 1.37 sec^{-1}) for I_{Ca} and I_{Kto}, respectively. The control values were not significantly different among different groups. The higher $1/\tau$ value indicates higher rate of channel inactivation.

* $P \leq 0.05$ *versus* initial control.

† $P \leq 0.05$ *versus* midazolam.

The decrease of IK_{to} and IK_1 amplitude by intravenous anesthetics was completely reversed with washout.

At equimolar concentrations, all three anesthetics significantly decreased IK_{to}; however, midazolam was more potent than etomidate and propofol (fig. 2C). At the same concentration, etomidate and midazolam produced similar and statistically significant decreases in IK_1, whereas propofol had no effect (fig. 2D).

Similar to I_{Ca}, IK_{to} current that was obtained by a depolarization step from a holding potential of −90 mV to +60 mV was also fitted with a single exponential curve to study its inactivation kinetics. The control inactivation constant (τ) was 0.034 ± 0.0014 s. Neither etomidate nor propofol changed the inactivation constant but midazolam decreased the inactivation constant by nearly 40% (table 1).

Discussion

Intravenous anesthetics cause various degrees of cardiovascular depression *in vivo*[1-5] and *in vitro*.[13,15,16**] The differences in cardiovascular depression could result from their differential effects on systemic vascular resistance,[6,7] venous capacitance,[8,9] the autonomic nervous system, and the heart.[10,11]

Etomidate produces only minimal effects on cardiovascular dynamics[2,11] and therefore is widely recommended for patients with compromised cardiac function and hypotension. On the other hand, propofol produces cardiovascular depression to a larger extent than does etomidate,[4,11**] and thus propofol should be used cautiously in patients with hypovolemia. It was reported that induction of anesthesia with midazolam, even in patients with limited coronary flow, was accompanied by no change in cardiac output or central venous pressure and only a modest reduction in peripheral vascular resistance.[7] At equimolar concentrations as great as 50 μM in isolated guinea pig heart preparation, propofol has been shown to be more potent than etomidate and less potent than midazolam in depressing myocardial contractility.[15] However, the authors suggested that at equivalent induction concentrations (concentration that produces the same depth of anesthesia), propofol would be the most potent, midazolam less potent, and etomidate the least potent negative inotrope.[15] Despite the extensive literature documenting differences in negative inotropic effects among intravenous anesthetics, very little is known about the mechanisms underlying these differences.

Our purpose in this study was to identify and compare the actions of etomidate, propofol, and midazolam on macroscopic Ca^{2+} and K^+ currents in isolated canine ventricular myocytes to identify possible mechanisms for the observed differences in negative inotropic effects of these anesthetics.

Inward Ca^{2+} current (I_{Ca}), transient outward K^+ current (IK_{to}), and inward-rectifier K^+ current (I_{K1}) contribute to the electrical activity in human,[36] rabbit[37] and canine ventricular myocytes[38] and are the major determinants of the action potential duration in these cells.[34,39] The efflux of K^+ through K^+ channels is functioning as an important modulator of the action potential in canine ventricular myocytes,[35,40,41] offsetting Ca^{2+} current and thus preventing early slow-response action potentials[42] while maintaining a high resting membrane potential in latent pacemaker cells.[43]

Our results show that etomidate, propofol, and midazolam reduce the amplitude of I_{Ca}, IK_{to}, and IK_1 in these

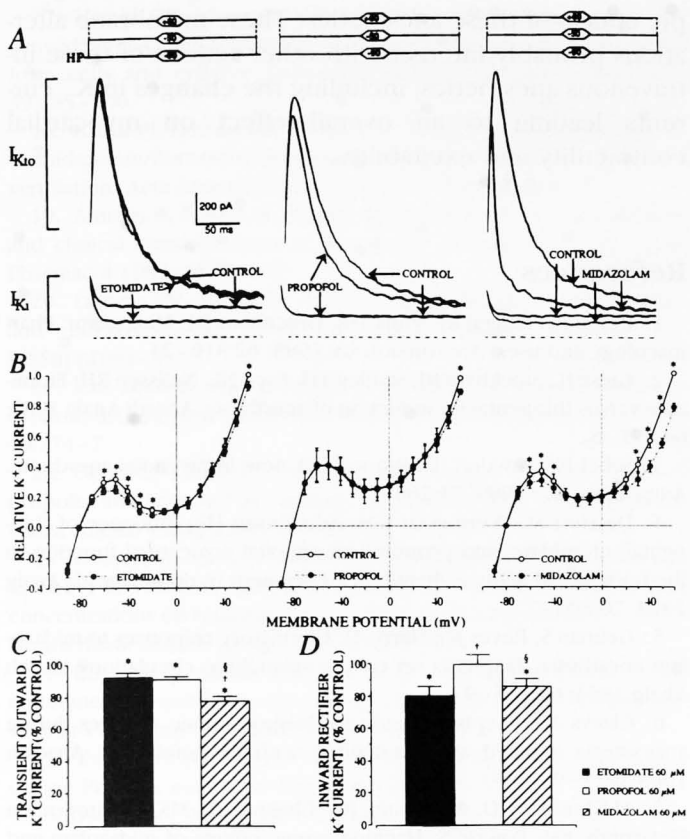

Fig. 2. Effects of 60 μM etomidate, propofol, and midazolam on the peak transient K^+ channel current amplitude (IK_{to}) elicited by depolarizing pulse from a holding potential (HP) of −90 to +60 mV, and the peak inward-rectifier K^+ channel current amplitude (IK_1) elicited by a depolarizing pulse from a holding potential (HP) of −90 to −50 mV in three ventricular cells (*A*). The control current-voltage relation for the activation of IK_{to} (expressed as a fraction of control maximal K^+ current) at voltages positive to −20 mV, and IK_1 at voltages negative to −20 mV, under control conditions and in the presence of 60 μM etomidate (*B*, left), propofol (*B*, center), and midazolam (*B*, right). *$P \leq 0.05$ *versus* anesthetic. The decrease (expressed as a percentage of control) of the peak IK_{to} amplitude (*C*), and maximum IK_1 amplitude (*D*) by 60 μM etomidate, propofol, and midazolam in a total of 29 canine ventricular cells. *$P \leq 0.05$ *versus* control; †$P \leq 0.05$ *versus* etomidate; §$P \leq 0.05$ midazolam *versus* propofol.

cells. Furthermore, these three channel types were not equally sensitive to block by these anesthetics, and I_{Ca} was blocked more effectively than IK_{to} or IK_1. Midazolam was the most potent in decreasing peak I_{Ca}, whereas etomidate was the least potent. A recent study using the whole-cell and single-channel recordings in guinea pig ventricular myocytes found that 4.4 and 27.4 μM etomidate decreased the whole-cell L-type I_{Ca}.[27] Similar concentrations of etomidate did not produce attenuation of I_{Ca} in this study, suggesting the possibility of differences between species (guinea pig *vs.* dog). Another possibility is that the large decrease in I_{Ca} observed by Takahashi and Terrar[44] is partially due to a significant I_{Ca} rundown in guinea pig myocytes as reported by the same authors. The relative potency of intravenous anesthetics in depressing I_{Ca} correlates well with *in vitro* and *in vivo* depression of contractility, supporting the hypothesis that the block of I_{Ca} is one of the most important factors in determining the negative inotropic effect of intravenous anesthetics. Effects of all three anesthetics on I_{Ca} were dose dependent and readily reversible.

In addition to decreasing the peak current, intravenous anesthetics could decrease a total I_{Ca} by increasing the rate of inactivation of open Ca^{2+} channels. Indeed, analysis (single exponential fit) of the inactivation portion of the I_{Ca} revealed that midazolam dramatically increased the rate of I_{Ca} inactivation. Propofol was less potent, whereas only 60 μM etomidate significantly increased the rate of I_{Ca} inactivation. Absence of the effect of 5 μM etomidate on I_{Ca} inactivation rate that we found correlates with the findings of Takahashi and Terrar,[27] who reported no effect of a similar dose of etomidate on the single Ca^{2+} channel kinetics. At 27.4 μM, etomidate reduced the mean open time and increased the mean closed time, favoring the closed state without any effect on conductance.[27] In our study, 30 μM etomidate did not alter the I_{Ca} inactivation rate, which could be consistent with the relatively greater effect seen in their study. The same group reported that a high concentration of propofol (100 μM) also decreased the mean open time and increased the mean closed time without changing I_{Ca} channel conductance.[44] Decreased peak current and increased rate of inactivation by propofol seen in this study are consistent with their observations at the single-channel level. The increased rate of I_{Ca} inactivation suggests that midazolam, and to a lesser degree propofol and etomidate, bind to the open Ca^{2+} channel and through an interaction with the gating mechanism increase the rate of transition of the channel from an open to inactivated state. Further studies investigating frequency and voltage dependence, rate of recovery from inactivation, and the single-channel recordings are needed to determine the exact nature of this interaction.

Midazolam was the most potent and propofol the least potent in depressing IK_{to} in ventricular cells. The decrease in amplitude of IK_{to} by these anesthetics suggests

that these agents may influence the phase 1 repolarization and early plateau phase of the action potential.[31] These alterations of plateau voltage may result in changes in other voltage-dependent currents during the plateau phase, including augmented I_{Ca}, and therefore cytosolic Ca^{2+} concentration, contractility, and duration of the action potential in the ventricular myocytes.[31,38] However, the magnitude of I_K decrease may not be able to overcome a large decrease in I_{Ca}, as suggested by shortened action potential duration in the presence of propofol.[45] It is of great interest that midazolam increased the rate of IK_{to} inactivation, whereas propofol and etomidate did not change channel inactivation. The mechanism(s) underlying similar effects of midazolam on I_{Ca} and IK_{to} inactivation are not clear.

Etomidate exerted the greatest depressant effect on IK_1, whereas propofol had no effect. Baum[46] has reported the lack of propofol's effect on IK_1 in guinea pig ventricular myocytes. Blockade of IK_1 can affect the duration of the action potential and the rate of repolarization because this current is activated during the repolarization of the cardiac action potential.[47] This current plays a critical role in determining the amplitude and shape of the subthreshold response,[29,48] thus influencing the excitability of ventricular myocytes. Therefore, a depressant effect of intravenous anesthetics on the outward I_K would alter resting membrane potential and affect action potential duration and cellular excitability.

Despite the inhibition of both I_{Ca} and I_K, the greater decrease of I_{Ca} may be responsible for the negative inotropic effect observed with these agents. Although midazolam produced the largest decrease of I_{Ca}, the final effect on myocardial contractility would be dramatically attenuated due to a decrease of I_K and its significantly smaller induction dose. Because the induction concentration of midazolam is less than 5 μM, midazolam would probably be free of effects on I_{Ca} when anesthesia is induced.

Etomidate, propofol, and midazolam produced a dose-dependent decrease of Ca^{2+} influx and a type-dependent decrease of K^+ efflux. These anesthetics, particularly midazolam, also increased the rate of I_{Ca} and I_K (only midazolam) inactivation, thus further decreasing the total current. Our data suggest that cardiac depression caused by intravenous anesthetics is due, at least in part, to a decreased amplitude of I_{Ca}, as directly measured by the whole-cell voltage-clamp method. The relative magnitude of a decrease in I_{Ca} correlates well with clinically and experimentally observed negative inotropic effects of these anesthetics. These membrane alterations probably interact with other actions of these intravenous anesthetics, including the changes in K^+ currents leading to an overall effect on myocardial contractility and excitability.

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