Frequency-dependent Effects of Propofol on Atrioventricular Nodal Conduction in Guinea Pig Isolated Heart

Mechanisms and Potential Antidysrhythmic Properties

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Background: The use of propofol has been associated with episodes of bradycardias. The mechanism(s) underlying these phenomena are not well defined. Therefore we investigated (1) the chronotropic and dromotropic effects of propofol, (2) the frequency-dependent effects of propofol on the atrioventricular (AV) node, and (3) the physiologic mechanism(s) underlying propofol’s effects on AV nodal conduction.

Methods: Guinea pig isolated, perfused hearts were instrumented for measurement of atrial rate and AV nodal conduction time in spontaneously beating hearts, or stimulus-to-His bundle (S-H) intervals in atrially paced hearts. In addition, the Wenckebach cycle length, effective refractory period and S-H interval prolongation to an abrupt increase in pacing rate were measured to further define propofol’s dromotropic effects and frequency-dependent behavior.

Results: Propofol, in a concentration-dependent manner, (1) slowed atrial rate and AV nodal conduction time in spontaneously beating hearts, (2) prolonged the S-H interval in atrially paced hearts, and (3) prolonged Wenckebach cycle length and effective refractory period. The negative dromotropic effect of propofol was greater during atrial pacing than in spontaneously beating hearts. Furthermore, this effect was enhanced at faster pacing rates, indicating frequency-dependent behavior. Atropine significantly antagonized propofol-induced S-H interval prolongation. The results of competition binding studies also supported a M1-muscarinic receptor-mediated mechanism.

Conclusions: We conclude that in the isolated guinea pig heart, propofol slows atrial rate and depresses AV nodal conduction in a concentration-dependent manner. The negative dromotropic effect of propofol shows frequency dependence and is predominantly mediated by M1-muscarinic receptors. Given the marked rate dependence of AV nodal actions, this anesthetic agent may impart antidysrhythmic protection to those patients susceptible to supraventricular tachycardias. (Key words: Anesthetics, intravenous, propofol. Heart, arrioventricular node: dromotropic effect; frequency dependence. Receptors: muscarinic.)

The cardiovascular effects of propofol (2,6-diisopropylphenol), an intravenous anesthetic agent commonly used for general anesthesia or conscious sedation, have been actively investigated. Although propofol is known to reliably cause dose-dependent reductions in blood pressure, its effects on heart rate and rhythm are not uniform. For example, the administration of propofol in humans has been associated with episodes of various bradycardias. These include sinus bradycardia, atrioventricular (AV) block, and asystole. In addition, propofol has been associated with the conversion of supraventricular tachycardia to normal sinus rhythm. Although different mechanisms have been proposed, the definitive mechanism(s) whereby propofol may mediate these events remain unclear. Prior investigations have demonstrated that propofol has both indirect...
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and direct cardiovascular effects. Its indirect effects include modulation of autonomic nervous system tone and alteration of the baroreceptor reflex sensitivity. Because propofol has been shown to lack central vagolytic activity and may actually exert a central vagotonic or sympatholytic effect, its autonomic effects may predispose certain patients to the development of bradycardias. Its bradydysrhythmic effects of propofol may be potentiated in the presence of different drugs (e.g., succinylcholine, fentanyl, vecuronium, or neostigmine) that exhibit vagotonic or lack vagolytic activities as well as in those individuals possessing an increased vagal tone.1-7 The administration of anticholinergic drugs (e.g., atropine) has been shown to mitigate or even reverse these effects.1-7 Modulation of baroreceptor reflex sensitivity is another potential indirect effect of propofol on the cardiovascular system. Although some studies have suggested that propofol alters baroreceptor reflex sensitivity, this finding remains controversial. Whereas Sellgren et al.9 and Cullen et al.10 have shown that baroreceptor reflex sensitivity was maintained during propofol anesthesia, numerous other studies have documented a propofol-induced attenuation of baroreceptor reflex sensitivity.11-15 In addition to the indirect actions of propofol, the drug also exhibits direct cardiac effects. Although several studies have shown that propofol directly depresses atrial conduction and sinus nodal activity,16-18 we know of only one that has examined the effect of propofol on AV nodal conduction.17 Stowe et al. found that propofol slowed AV nodal conduction during constant atrial pacing in a concentration-dependent manner.17 However, none of these prior studies investigated the cellular basis of propofol’s effects on sinoatrial and AV nodal function.

During episodes of supraventricular tachycardias, a critical function of the AV node is to filter supraventricular atrial impulses down to a rate that still allows adequate diastolic filling time. An important physiologic feature of AV nodal transmission is its frequency dependence.10 As the atrial pacing rate is increased, conduction of the electrical impulse through the AV node becomes progressively slower until conduction fails. In this manner, failure of impulse conduction through the AV node protects the ventricles from abnormally high rates of atrial electrical activity. The ideal drug to treat supraventricular tachycardias should have minimal effect at normal heart rates but should markedly slow AV nodal conduction time during tachycardias. This is the basis for the high efficacy of antidysrhythmic drugs such as adenosine and diltiazem in terminating supraventricular tachycardias (albeit by different mechanisms) but in having minimal effects during normal sinus rhythm.22-24 We hypothesized that propofol may exhibit unique pharmacologic properties that depress AV nodal conduction in a frequency-dependent manner. Therefore, in a guinea pig isolated heart model, we (1) further characterized the direct chronotropic and dromotropic effects of propofol, (2) determined the frequency-dependent effects of propofol on the AV node, and (3) investigated the physiologic mechanism(s) underlying its effect on AV nodal conduction. To facilitate interpretation of the functional responses of hearts to propofol, parallel binding studies were carried out to also evaluate the pharmacologic properties (e.g., potency and affinity) of the drug.

Materials and Methods

Chemicals

Propofol (2,6 diisopropylphenol) was obtained commercially as Diprivan (Zeneca Pharmaceuticals, Wilmington, DE), a sterile, nonpyrogenic emulsion containing 10 mg/ml propofol (molecular weight [MW] = 178.3). 8-cyclopentyl-1,3-dipropylxanthine (CPX) (MW = 504.4) was purchased from Research Biochemicals (Natick, MA). Atropine (MW = 676.8), indomethacin (MW = 357.8) and S(+)-(imino)nortokaamino)methyl)-ornithine (l-NOARG) (MW = 219.2) were purchased from Sigma Chemical (St. Louis, MO). Stock solutions of the drugs were dissolved in perfusion medium and infused to achieve the desired perfusate concentration. [3H]R(-)-Quinuclidinyl benzilate ([3H]QNB) and [3H]8-cyclopentyl-1,3-dipropylxanthine ([3H]CPX) were purchased from New England Nuclear (Doraville, GA).

Isolated Perfused Hearts

Before the study was begun, all protocols were reviewed and approved by the Animal Use Committee of the University of Florida Health Sciences Center. Guinea pigs of either sex weighing 250-300 g were anesthetized with halothane and killed by cervical dislocation. Hearts were quickly removed and rinsed in

ice-cold Krebs-Henseleit solution. The aorta was cannulated for perfusion of the coronary arteries at a constant flow rate of 8 ml/min with Krebs-Henseleit solution perfused with 95% oxygen and 5% carbon dioxide. The oxygen tension, temperature, and pH of the Krebs-Henseleit solution were maintained at 500–600 mmHg, 35.5 ± 0.5°C, and 7.3–7.4, respectively.

To facilitate pacing of the heart and measurement of the His bundle electrogram, the sinoatrial nodal region (including vena cava) and part of the right atrium were excised.25-27 Hearts were electrically paced at a cycle length (interval between two successive pacing stimuli) of 300 ms (unless otherwise indicated) by means of a bipolar electrode placed on the atrium.

An interval generator (model A510, World Precision Instruments, Sarasota, FL) delivered the stimuli through a stimulus isolation unit (model A360, World Precision Instruments) as square wave pulses 3 ms in duration and at least twice the threshold intensity. AV nodal conduction time was measured from His bundle electrograms during constant atrial pacing. The stimulus-to-His bundle (S-H) interval was used as index of AV nodal conduction time and was measured visually from an oscilloscope, or with a Zeos 486 DX2-66 computer as previously described.27

To determine chronotropic and dromotropic effects of propofol in spontaneously beating guinea pig isolated hearts, an unipolar extracorollular electrode was recorded from the surface of the right atrium with a polytetrafluoroethylene-coated stainless steel electrode. Atrial rate and AV nodal conduction time were determined from measurements of atrial cycle length and AV intervals, respectively. The A-V interval was defined as the time from the onset of atrial activation in the electrogram to the onset of ventricular activation.

After completion of dissection and instrumentation, the hearts were allowed to equilibrate for 30 min before the experiments were begun. Experimental interventions were always preceded and followed by measurements of atrial rates (spontaneously beating preparations) or of S-H intervals (atrially paced preparations). Whenever the pre- and postintervention values differed by more than 15%, the intervening data was discarded. In the event an intervention caused second-degree AV block, the longest S-H interval allowing 1:1 AV conduction immediately before the onset of AV block was considered the maximum dromotropic effect and that value was used for data analysis.

Cardiac Membrane Preparation
Dissected ventricular tissues were minced and homogenized (Polytron, Brinkman Instruments, Westburg, NY) for 10–15 s in 10 volumes of ice-cold buffer (50 mm tris(hydroxymethyl)aminomethane, Tris-HCl, pH 7.4). The homogenate was spun in a centrifuge at 48,000 g for 15 min at 4°C to pellet membranes. The pellet was resuspended in 50 ml of buffer and spun again, then the pellets were washed twice more by resuspension and centrifugation. The final pellet was resuspended in 50 ml of the buffer used for the assays. Membrane suspensions were stored at 80°C. Protein content was determined by the Bradford protein dye-binding method (Bio-Rad, Cambridge, MA) using bovine serum albumin as standard.

Radioligand Binding Assay
Competition assays to determine the affinities of propofol for the M1 muscarinic cholinergic receptor and for the A1 adenosine receptor were performed with 100 µl aliquots of the membrane suspension (0.2–0.7 µg) incubated at 36°C in 50 mm Tris-HCl buffer (pH 7.4), containing adenosine deaminase (5.0 U/ml) and one of the following radioligands: 0.1 µM [3H]-cyclopentyladenosine (CPX) for the A1 adenosine receptor and 0.1 µM [3H]QNB for the M1 muscarinic cholinergic receptor.28 Binding parameters from the competition assays were determined using the computer program LIGAND (Biosoft, Cambridge, UK). The value of the equilibrium dissociation constant (Kd) for displacement of each radioligand by propofol was calculated using the Cheng-Prusoff transformation.29

Data Analysis
All measurements are reported as the mean ± SEM. To determine the concentration of propofol causing half-maximal negative dromotropic effect (EC50) or concentration of propofol causing half-maximal atrial rate slowing for effects of propofol on AV nodal conduction time, S-H interval, and heart rate, propofol concentration–response relations were fitted using a nonlinear (Marquardt-Levenberg) regression algorithm to a parabolic (equation 1) and a dose-response logistic equation (equation 2), respectively (Table Curve program, Jandel Scientific, San Rafael, CA).

Equation 1: \( y = a + bx^2 \), where \( y = AV\) nodal conduction time or S-H interval; \( x = \) the concentration of propofol (micromolar); and \( a \) and \( b \) are curve-fitting parameters.

Equation 2: \( y = d + \frac{(a - d)}{1 + \frac{x}{c}} \), where \( y = \) atrial rate (beats per minute) of propofol (micromolar); \( a = \) maximum use of atrial rate (beats per minute) of control; \( b = \) Hill coefficient; \( c = \) concentration (micromolar) causing half-maximal depression of atrial rate; \( d = \) extrapolated minimum value (beats per minute). The two-tailed Student’s t test was used to analyze paired data. The one-way analysis of variance followed by the Bonferroni post-hoc test was used to analyze unpaired data.

Protocols
Chronotropic Effect of Propofol: To determine the chronotropic effects of propofol, the experiments were carried out in each of five separate preparations from the same hearts. The effects of propofol on the atrial conduction time were recorded via the application of a single pulse to the right atrium, the heart rate (i.e., prolongation of the S-H interval during pacing of the heart at an identical cycle length) was measured, and the rate of increase of perfusate concentration of propofol was calculated.

Dromotropic Effect of Propofol: In the experiments (n = 4), a condition for the negative dromotropic effects (i.e., prolongation of the S-H interval during pacing of the heart at an identical cycle length) of propofol was achieved by perfusing the preparation with 25 or 50 µm propofol. The effect of each concentration of propofol was calculated using the Cheng-Prusoff transformation.29

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Equation 2: \( y = d + (a - d)/(1 + (x/c)^b) \), where \( y \) = atrial rate (beats per minute); \( x \) = concentration of propofol (micromolar); \( a \) = extrapolated maximum value of atrial rate (beats per minute); \( b \) = apparent Hill coefficient; \( c \) = concentration of propofol (micromolar) causing half-maximal decrease in atrial rate; and \( d \) = extrapolated minimum value of atrial rate (beats per minute).

The two-tailed Student’s \( t \) distribution was used to analyze paired data. The one-way repeated measures analysis of variance followed by Student-Newman-Keuls testing was used to analyze multiple comparisons among paired control and interventions. Differences between or among group means were considered significant at the level of \( P < 0.05 \).

Protocols

Chronicotropic Effect of Propofol. Experiments to determine the chronicotropic effect of propofol were carried out in each of five spontaneously beating hearts. In the same hearts, the effect of propofol on AV nodal conduction time was also determined. After control atrial and ventricular electrograms were recorded, successively larger doses of propofol were infused to achieve perfusate concentrations ranging from 1 to 100 \( \mu M \). The effects of propofol on atrial rate and AV nodal conduction time were recorded simultaneously. For each heart, the relation between slowing of atrial rate and prolongation of AV nodal conduction time at each concentration of propofol was determined.

Dromotropic Effect of Propofol. In this series of experiments (n = 4), a concentration–response relation for the negative dromotropic effect of propofol (i.e., prolongation of the S-H interval) was obtained during pacing of hearts at an atrial cycle length of 300 ms. After a control His bundle electrogram was recorded, propofol was administered at successively larger concentrations starting at 1 \( \mu M \) and ending at 100 \( \mu M \). The effect of a given concentration of propofol was measured when the response had reached a steady state (at 20–30 min after onset of infusion).

In addition, atrial cycle length–response relations for the negative dromotropic effects of propofol were obtained in a series of six hearts. Effects were noted in the absence of propofol and then in the presence of 10 and 25 \( \mu M \) propofol as the atrial cycle length was incrementally decreased from 400 to 200 ms.

To investigate further propofol’s negative dromotropic effect and its frequency-dependent behavior, determinations were made of the Wenckebach cycle length (WCL), the effective refractory period (ERP) of the AV node, and the prolongation of the S-H interval in response to an abrupt and transient increase in atrial pacing rate. The following three programmed stimulation protocols were used.

Wenckebach Cycle Length. The WCL was determined by decreasing the atrial pacing cycle length in 3-ms steps every 10 stimuli until second-degree AV block occurred. The longest basic cycle length (S1,S1 interval) for a stimulus that failed to conduct through the AV node and produce a His bundle response was defined as the WCL. After the control cycle length was determined, the effects of 10 \( \mu M \) and 25 \( \mu M \) propofol were determined.

Premature Stimulus Protocol. The right atrium was stimulated at a fixed S1,S2 interval of 300 ms. After a train of 15 stimuli (S1), a single premature (test) stimulus (S2) was introduced and the S-H interval was determined. The coupling interval (S1,S2) between the last S1 and the S2 was progressively shortened in 3-ms steps after every train of stimuli. The longest S1,S2 interval for a stimulus that failed to conduct through the AV node and produce a His bundle response was defined as the AV nodal ERP. After the control refractory period was determined, the effects of 10 and 25 \( \mu M \) propofol were determined.

Single-step Protocol (Tachycardia Experiments). This stimulation protocol consisted of an abrupt, transient (1-min) decrease in atrial pacing cycle length. After 30 s of pacing at a fixed atrial cycle length, pacing at an atrial cycle length of 7% greater than the WCL was begun and maintained for 1 min, followed by a return to the original pacing cycle length. The 1-min duration of pacing was shown in a previous study to be sufficient in most cases for the AV nodal conduction time to achieve steady state, regardless of the baseline atrial pacing cycle length. In each heart in this series (n = 9), a maximum of three pacing protocols were performed: pacing in the presence (control) and in the presence of 10 and 25 \( \mu M \) propofol.

Specificity of the Negative Dromotropic Effect of Propofol. In this series of experiments, hearts were perfused with a concentration of propofol that caused a stable S-H interval prolongation of approximately 15 ms. After propofol had prolonged the S-H interval to the new steady-state value, antagonism of the negative dromotropic effects of propofol was attempted with either the muscarinic receptor antagonist atropine at a concentration of 1 \( \mu M \) (n = 7), an A1-adenosine receptor antagonist CPX at a concentration of 5 \( \mu M \) (n = 6), or...
nitric oxide synthetase inhibitor L-NOARG at a concentration of 100 μM (n = 4) or a cyclooxygenase inhibitor indomethacin at a concentration of 2.8 μM (n = 4).

**Propofol and Acetylcholine Release.** To evaluate whether propofol's negative dromotropic effect was caused by a direct action of propofol on the M₂-muscarinic receptor in the AV node or by an indirect action, such as γ-aminobutyric acid–induced acetylcholine release, the following experiment was also performed. In guinea pig isolated hearts (n = 4), a steady state S-H interval prolongation of 15–20 ms was achieved with propofol. Acetylcholinesterase at a concentration of 5 units/ml, which completely reversed an equivalent S-H interval prolongation caused by acetylcholine, was then started. S-H intervals during acetylcholinesterase infusion were then compared with those before beginning the infusion.

**Results**

In the range of propofol concentrations studied, stimulus-to-atrial and His bundle-to-ventricular intervals did not lengthen significantly from control values, indicating that atrial and His bundle–Purkinje conduction were not altered by propofol. Therefore, changes in the A-V interval are an accurate reflection of the effect of propofol on AV nodal conduction time in spontaneously beating hearts. Likewise, S-H intervals will accurately reflect AV nodal conduction time in atrially paced hearts.

**Chronotropic Effect of Propofol**

The rate of spontaneous beating of isolated hearts (n = 5) was 185 ± 8 beats/min. Propofol caused a concentration-dependent slowing of spontaneous atrial rate (fig. 1A). The EC₅₀ value for propofol-induced slowing of spontaneous atrial rate was 9.1 ± 0.5 μM. The Hill coefficient (b) was 1.6 ± 0.2.

**Dromotropic Effect of Propofol**

Propofol caused a concentration-dependent prolongation of AV nodal conduction time of spontaneously beating hearts (n = 5) (fig. 1B). The maximal effect of propofol (100 μM) was a prolongation of AV nodal conduction time to 109 ± 16 ms from a baseline value of 65 ± 2 ms; propofol (100 μM) caused AV block in 2 out of 5 hearts. The threshold and EC₅₀ values for prolongation of AV nodal conduction time by propofol were 25 and 51.8 ± 2.7 μM, respectively (fig. 1B).

The negative dromotropic action of propofol was also investigated in hearts paced at an atrial cycle length of 300 ms (n = 4). In atrially paced hearts, as in spontaneously beating hearts, propofol prolonged the S-H interval in a concentration-dependent manner (fig. 2).

Evidence that the negative chronotropic effect of propofol was predominantly mediated by acetylcholine receptors and not by a calcium or prostanoid receptor antagonist 1: The S-H interval of hearts paced at a cycle length of 300 ms was prolonged by propofol (25–50 μM). A selective receptor antagonist of both cardiac and vascular M₂-muscarinic receptors, atropine, prevented this prolongation (fig. 2A). A selective receptor antagonist of cardiac and vascular acetylcholine receptors, hexamethonium, had no effect on the negative chronotropic effect of propofol. Furthermore, an atropine-resistant effect failed to attenuate the negative chronotropic effect of propofol caused by chronic administration of NG-nitro-L-arginine methyl ester (L-NAME) (fig. 2A). In four control (absent atropine) and six propofol-treated (absent atropine) guinea pig isolated hearts, atropine-resistant AV nodal conduction times were significantly prolonged from baseline S-H intervals (data not shown).

**Frequency-dependent Effect of Propofol on Atrioventricular Nodal Conduction Time**

The prolongation of the S-H interval by propofol was greater at faster heart rates (i.e., behavior was frequency dependent), as was delayed atrial conduction; however, the frequency-dependent prolongation of the S-H interval by propofol was less than that by atropine. In all experiments, the effects of propofol were frequency dependent (fig. 3). The concentration–response curves for the negative chronotropic and dromotropic effects of propofol at 100 μM were frequency dependent. Atropine-resistant AV nodal conduction times in hearts paced at 100 Hz were significantly greater than those in hearts paced at 60 Hz (fig. 3B). The frequency-dependent prolongation of AV nodal conduction time by propofol to a sudden increase in heart rate (tachycardia experiments) was seen in one of 10 hearts.

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Evidence that the negative dromotropic effect of propofol was predominantly mediated by \(M_2\)-muscarinic receptors and not by \(\alpha_1\)-adrenergic receptors, nitric oxide or prostaglandins is presented in Table 1. The S-H interval of hearts paced at an atrial cycle length of 300 ms was prolonged approximately 15 ms by propofol (25–50 \(\mu\)M). The \(M_2\)-muscarinic cholinergic receptor antagonist atropine (1 \(\mu\)M) significantly but not completely antagonized propofol-induced S-H interval prolongation. In contrast, a selective \(\alpha_1\)-adrenergic receptor antagonist CPX (5 \(\mu\)M), a nitric oxide synthetase inhibitor t-NOARG (100 \(\mu\)M) and a cyclooxygenase inhibitor indomethacin (2.8 \(\mu\)M) had no effect on the negative dromotropic effect of propofol. Furthermore, because acetylcholinesterase failed to attenuate the 15–20 ms S-H interval prolongation caused by propofol, the negative dromotropic effect of propofol can be attributed to direct activation of \(M_2\)-muscarinic receptors and not to indirect release of endogenous acetylcholine (data not shown). In four control (absence of propofol) guinea pig isolated hearts, atropine (5 \(\mu\)M) did not change baseline S-H intervals (data not shown).

**Frequency-dependent Effect of Propofol on Atrioventricular Nodal Conduction**

The prolongation of the S-H interval caused by propofol was greater at faster than at slower atrial pacing rates (i.e., behavior was frequency dependent). In a series of six hearts, the S-H interval varied inversely with the atrial cycle length over a range of 400–200 ms; propofol augmented this effect in a concentration-dependent manner (Fig. 3).

To further characterize the negative dromotropic properties of propofol, its effects on WCL (\(n = 10\)) and AV nodal ERP (\(n = 9\)) were determined (Table 2). The WCL, the atrial pacing cycle length at which second-degree AV block occurs, was significantly and concentration-dependently prolonged by propofol. In the absence of propofol, the WCL was 173 ± 3 ms, and it was increased to 192 ± 5 and 222 ± 6 ms in the presence of 10 and 25 \(\mu\)M propofol, respectively. Likewise, the AV nodal ERP was prolonged by propofol. The control AV nodal ERP was 127 ± 3 ms, and it was increased to 149 ± 5 and 164 ± 5 ms in the presence of 10 and 25 \(\mu\)M propofol, respectively.

The frequency-dependent negative dromotropic effect of propofol to a sudden increase in atrial pacing rate (tachycardia experiments) was investigated in a separate series of ten hearts. In these experiments, prolongation of the S-H interval in response to an abrupt and transient increase in atrial rate was measured in the absence and presence of 10 and 25 \(\mu\)M propofol (Figs. 4 and 5). In the example shown in figure 4, the atrial pacing cycle length was decreased in a single step from 300 to 7% greater than the WCL (165 ms) and held there for 60 s. At an atrial cycle length of 300 ms, 10 \(\mu\)M propofol caused a 2 ms prolongation of the S-H interval (from 39 to 41 ms), whereas the same concentration of propofol at an atrial cycle length of 165 ms caused a 16 ms prolongation of the S-H interval, from 60 to 76 ms. Hence, the frequency-dependent ratio between S-H interval prolongation at fast and slow pacing rates (short [165 ms] and long [300 ms] atrial pacing cycle lengths) in the presence of 10 \(\mu\)M propofol was 16/2, or 8.0. A ratio greater than 1 indicates a greater effect at a faster than at a slower pacing rate. Figure 5 summarizes the results showing the frequency-dependent prolongation of the S-H interval by propofol. The magnitude and time course of the S-H interval prolongation were greater at an atrial pacing cycle length of 7% greater than the WCL than at 300 ms and at the higher concentration (25 vs. 10 \(\mu\)M) of propofol (Fig. 4). Likewise, as illustrated in figure 5, the ratios between S-H interval prolongation at fast and slow pacing.

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Table 1. Antagonism of the Negative Dromotropic Effect of Propofol

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>Control</th>
<th>Propofol</th>
<th>Propofol + Antagonist</th>
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<tbody>
<tr>
<td>Atropine (1 μM)</td>
<td>45.6 ± 1.3 (7)</td>
<td>63.2 ± 2.8 (7)*</td>
<td>50.1 ± 1.9 (7)†</td>
</tr>
<tr>
<td>CPX (5 μM)</td>
<td>43.8 ± 1.1 (6)</td>
<td>56.4 ± 1.6 (6)*</td>
<td>56.2 ± 1.7 (6)*</td>
</tr>
<tr>
<td>L-NQARG (100 μM)</td>
<td>45.0 ± 1.4 (4)</td>
<td>60.3 ± 2.1 (4)*</td>
<td>60.9 ± 2.1 (4)*</td>
</tr>
<tr>
<td>Indomethacin (2.8 μM)</td>
<td>42.3 ± 0.3 (4)</td>
<td>54.9 ± 1.5 (4)*</td>
<td>55.0 ± 1.4 (4)*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; values in parentheses are no. of experiments.
* P < 0.05. Propofol and Propofol + Antagonist versus Control.
† P < 0.05. Propofol + Antagonist versus Propofol.

Table 2. Frequency-dependent Effects of Propofol on AV Nodal Conduction

<table>
<thead>
<tr>
<th>Control</th>
<th>Propofol (10 μM)</th>
<th>Propofol (25 μM)</th>
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</thead>
<tbody>
<tr>
<td>WCL (ms)</td>
<td>173 ± 3 (7)</td>
<td>192 ± 5 (7)*</td>
</tr>
<tr>
<td>ERP (ms)</td>
<td>127 ± 3 (6)</td>
<td>149 ± 5 (6)*</td>
</tr>
</tbody>
</table>

Data show the effect of propofol (10 and 25 μM) on Wenckebach cycle length (WCL) and effective refractory period (ERP). Data shown are mean ± SEM; values in parentheses are no. of experiments.
* P < 0.05. Propofol (10 μM) and Propofol (25 μM) versus Control.
† P < 0.05. Propofol (25 μM) versus Propofol (10 μM).

Discussion

A major finding of this study was that propofol displayed marked AV nodal conduction block in guinea pig hearts. This effect was dose-dependent and similar to that observed using nitroglycerin. A novel finding was that propofol may be beneficial in those patients with atrial tachycardia.
PROPOFOL AND ATRIOVENTRICULAR NODE

![Diagram](image)

Fig. 4. Effect of propofol on stimulus-to-His bundle (S-H) interval during rapid atrial pacing of a guinea pig isolated perfused heart. Atrial cycle length was abruptly shortened from 300 ms to 7% greater than the Wenckebach cycle length (WCL) for a 60-s period between 30 and 90 s. An example of progressive prolongation of the S-H interval (S-H-I) in the absence of propofol and in the presence of 10 and 25 μM propofol as a function of time after an abrupt increase in rate of pacing is shown. In comparison with control, in the presence of 10 μM propofol, S-H-I was greater and did not reach a steady state. In the presence of 25 μM propofol, second-degree atrioventricular block ensued immediately after initiation of fast atrial pacing.

**Discussion**

A major finding of this study is that propofol exhibits potentially useful antidysrhythmic properties. In experiments carried out in guinea pig isolated hearts, propofol displayed marked frequency-dependent depression of AV nodal conduction. These rate-dependent properties were concentration-dependent, and appear to be predominantly mediated by cardiac M<sub>2</sub> muscarinic cholinergic receptors. Adenosine, nitric oxide and prostaglandins did not modify the depressant effect of propofol on AV nodal conduction. Although acting by different cellular mechanisms, the magnitude of propofol's frequency dependence was quantitatively similar to that observed using the antidysrhythmic agents diltiazem and adenosine. The results of our study suggest that propofol may be a rational anesthetic selection in those patients predisposed to supraventricular tachycardias.

**Negative Chronotrophic and Dromotropic Effects of Propofol**

The marked concentration-dependent negative chronotropic and dromotropic effects of propofol may confer important antidysrhythmic properties to this anesthetic agent. These findings confirm and expand upon those of a prior study. Although no direct comparisons were made among anesthetic induction agents in the current study, Stowe et al. showed that the negative chronotropic and dromotropic effects of propofol are greater than those of thiopental, ketamine, midazolam and etomidate. Similarly, in spontaneously beating guinea pig hearts, propofol slowed atrial rate and prolonged AV nodal conduction time in a concentration-dependent manner (fig. 1). In these hearts, the average EC<sub>50</sub> value to slow heart rate (negative chronotropic effect) was approximately sixfold less than that to slow AV nodal conduction (negative dromotropic effect). It is well known that atrial rate modulates AV nodal conduction time. AV nodal conduction time increases progressively as atrial rate is increased. Because propofol slows atrial rate, is not surprising that in unpaced hearts (i.e., spontaneously beating hearts), propofol caused significantly less prolongation of S-H interval than in paced hearts (fig. 2) and second-degree AV block occurred much less frequently than in paced hearts at equimolar concentrations of propofol. Therefore, modulation of AV nodal conduction by atrial rate and the finding that the potency of propofol to slow heart rate (EC<sub>50</sub> = 9.1 ± 0.05 μM) is sixfold less than that to prolong AV nodal conduction (EC<sub>50</sub> = 51.8 ± 27 μM) can fully explain the greater sensitivity of the AV node in atrially paced hearts to the negative dromotropic effect of propofol. It is therefore anticipated that during normal sinus rhythm, the predominant direct effect of propofol would be to decrease heart rate (sinus bradycardia).

**Mechanism of Propofol’s Negative Dromotropic Effect**

The depression of AV nodal conduction time caused by propofol is predominantly mediated by the M<sub>2</sub> muscarinic cholinergic receptor. This conclusion is based on two experimental findings: (1) the negative dromotropic effect of propofol was significantly attenuated by the muscarinic receptor antagonist atropine (table 1), and (2) propofol displaced binding of the radiolabeled muscarinic receptor antagonist [<sup>3</sup>H]QNB to ventricular membranes in a concentration-dependent manner (figs. 6A and 6B). The EC<sub>50</sub> value of 51.8 μM for the negative dromotropic effect of propofol in guinea pig hearts is similar to the K<sub>i</sub> value of propofol calculated from displacement of [<sup>3</sup>H]QNB binding to guinea pig ventricular muscarinic receptors (65.5 ±

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concentrations of propofol, the AV nodal effects of propofol are not mediated by \( A_1 \)-adenosine receptors. Studies have implicated other cellular messengers (e.g., nitric oxide and prostaglandins) as important mediators of the vasodilatory effects of propofol. \(^{34,35}\) However, we found no evidence that prostaglandins or nitric oxide modulate the effects of propofol on AV nodal conduction. That is, neither indomethacin, an inhibitor of prostaglandin synthesis, nor L-NOARG, an inhibitor of nitric oxide production, attenuated the negative dromotrophic effect of propofol. Furthermore,

Fig. 5. Frequency-dependent prolongation of stimulus-to-His bundle interval by propofol. The ratio of S-H prolongation at atrial pacing cycle lengths (ACL) of 300 ms (baseline) and 7% greater than the WCL (maximum) are shown in the absence of propofol and with 10 \( \mu \)M propofol. Bars = mean and SEM of single determinations from nine hearts. * \( P < 0.05 \), maximum versus baseline; † \( P < 0.05 \), maximum (control) versus maximum (propofol 10 \( \mu \)M).

8.0 \( \mu \)M). Additionally, as expected, the potent muscarinic receptor antagonist atropine readily displaced \(^{[3]H}QNB\) from \( M_2 \)-muscarinic cholinergic receptors with a \( K_d \) value of 1.7 nM (fig. 6B).

Another important endogenous cardioinhibitory autacoid that regulates myocardial function is the nucleoside adenosine. Although \( A_1 \)-adenosine receptors and \( M_2 \)-muscarinic receptors are directly coupled (no second messenger involved) to a common pertussis toxin–sensitive G-protein in the heart, \(^{32,35}\) we found no evidence that the \( A_1 \)-adenosine receptor mediates the AV nodal effects of propofol. The failure of CPX to attenuate the S-H interval prolongation caused by propofol strongly suggests that stimulation of \( A_1 \)-adenosine receptors is not responsible for propofol’s negative dromotrophic actions (table 1). Propofol did displace binding of \(^{[3]H}CPX\) to ventricular membranes (fig. 6A), but it did so only at very high concentrations of the anesthetic (\( K_d = 219.0 \pm 21.0 \) nM). This \( K_d \) value, which is almost fourfold higher than the \( K_d \) value of propofol binding to the \( M_2 \)-muscarinic receptor (\( K_d = 65.5 \pm 8.0 \) nM), is much greater than the concentrations used in our functional experiments and does not correspond to clinically relevant concentrations. Thus, at lower

Fig. 6. Displacement of \(^{[3]H}[(\pm)\text{quinuclidinyl benzilate ([(PMQNB)} and \(^{[3]H}8\text{-cyclopentyl-1,3-dipropylxanthine ([H]CPX)} binding to guinea pig ventricular membranes by propofol. (A) Radiolabeled ligand \(^{[3]H}QNB \) 0.1 nM or \(^{[3]H}CPX \) 2.0 nM, crude ventricular membranes and competing unlabeled drug (propofol) were incubated together for 2 h at room temperature. The dissociation constants (\( K_d \)) for propofol displacement of \(^{[3]H]CPX\) and \(^{[3]H}QNB\) binding to guinea pig ventricular membranes were 139 and 65.8 nM, respectively. (B) \(^{[3]H}QNB\) (0.1 nM), crude ventricular membranes, and competing unlabeled drugs (propofol, atropine, and 8-cyclopentyl-1,3-dipropylxanthine [CPX]) were incubated together as described above. The \( K_d \) values for propofol and atropine displacement of \(^{[3]H}QNB\) binding were 52.6 nM and 1.7 nM, respectively. CPX did not displace \(^{[3]H}QNB\) binding. Each symbol = the mean of triplicate determinations.

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A

B

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DRUG CONCENTRATION (M)

PROPOFOL CONCENTRATION (M)

\(^{[3]H}QNB SPECIFIC BINDING (% OF MAXIMUM)

\(^{[3]H}CPX SPECIFIC BINDING (% OF MAXIMUM)

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PROPOFOL

ATROPIINE

CPX

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10\(^{-10}\)

10\(^{-9}\)

10\(^{-8}\)

10\(^{-7}\)

10\(^{-6}\)

10\(^{-5}\)

10\(^{-4}\)

10\(^{-3}\)

10\(^{-2}\)

10\(^{-1}\)

10\(^{0}\)

10\(^{1}\)

10\(^{2}\)
PROPOFOL AND ATRIOVENTRICAL NODE

Propofol appears to act directly at the M2-muscarinic receptor, not indirectly by propofol- or γ-aminobutyric acid-induced endogenous acetylcholine release.

Frequency-dependent Effects of Propofol on Atrioventricular Nodal Conduction

In addition to the negative chronotropic and dromotropic effects of propofol, its frequency-dependent behavior may also impart important antiarrhythmic properties to this drug. Consistent with the depressant effect of propofol on AV nodal conduction time, this drug also prolonged the WCL and AV nodal ERP in a concentration-dependent manner (table 2). WCL and ERP are two distinct but complementary indices of the frequency-dependent effect of drugs on AV nodal conduction. ERP is a measure of the ability of the AV node to block a single premature, supraventricular stimulus. WCL, however, is a measure of the filtering capability of the AV node to sustained supraventricular stimuli, thus taking into consideration the effect of AV nodal accommodation.22 For this reason, WCL is always longer than ERP. Therefore, the increase in ERP and WCL caused by propofol should enhance the filtering capacity of the AV node and thus reduce transmission of premature and sustained rapid atrial impulses, respectively, to the ventricles. The negative dromotropic effect of propofol was greater as the atrial pacing rate was increased (fig. 3). This suggests that the effectiveness of propofol to cause second-degree AV nodal block will increase as a function of the atrial rate and is consistent with the finding that the ratio between S-H interval prolongation at fast and slow pacing rates is increased as the atrial pacing cycle length is shortened (fig. 5). The observations that the magnitude of S-H interval prolongation caused by propofol was greater at faster than at a slower rate of pacing are similar to those reported for calcium channel antagonists22 and adenosine.26,27 In recent studies in our laboratory, we found a frequency-dependent ratio of 12.0, 10.6 and 9.1 for propofol, diltiazem and adenosine, respectively, in guinea pig isolated hearts (data not shown). A drug with a larger frequency-dependent ratio would be expected to be safer and more effective in treating supraventricular tachycardias. The frequency-dependent effects of calcium channel antagonists20-24,36 and adenosine26,27 on AV nodal conduction have been extensively investigated and are thought to explain the prompt and effective control of ventricular rate by these drugs during tachycardias. Anesthetic induction agent such as propofol that has similar frequency-dependent behavior may be advantageous in a subset of patients requiring general anesthesia. In particular, this effect of propofol may provide additional rationale for using it in patients with a history of supraventricular tachycardias or in those patients susceptible to developing paroxysmal supraventricular tachycardias in which the AV node is part of a reentrant circuit.

Despite using a concentration of atropine (1 μM) that would block more than 99% of the M2-muscarinic cholinergic receptors in the AV node (estimated using Gaddum’s equation12), approximately 25% of the S-H interval prolongation caused by propofol remained: the S-H interval prolongation decreased from 17.6 to 4.5 ms (table 1). It is unlikely that the atropine-insensitive component of propofol-induced AV nodal conduction slowing is caused by “ rundown” of the isolated heart preparation for two reasons. First, the experiments were carried out promptly (within 45 min from the start of retrograde perfusion). Second, the AV nodal effects of propofol that were not antagonized by atropine were reversible upon washout (data not shown). Therefore, it is tempting to postulate that another mechanism is also contributing to the negative dromotropic effect of propofol. As discussed previously, adenosine, acetylcholine, nitric oxide and prostaglandins do not appear to mediate the effects of propofol on AV nodal conduction (table 1). Another major mechanism that could mediate a component of propofol’s negative dromotropic effect is antagonism of the slow-inward L-type calcium channels. Indeed, prior studies have suggested that propofol attenuates the inward calcium flux by modulating the activity of voltage-gated channels in rat aortic and dog coronary vascular rings,43,44 isolated guinea pig papillary muscle15 and ventricular myocytes.46 However, because we did not directly demonstrate that propofol altered calcium currents in the AV node and because there is no a priori reason to assume this finding would be necessarily duplicated in the AV node, we are reluctant to attribute calcium channel antagonism as a component of propofol’s depressant effects on AV nodal conduction. Such a conclusion would be best drawn after performing single-cell electrophysiologic studies designed to measure slow-inward L-type calcium current in N cells of the AV node using a newly developed technique.45

[References]


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Modulation of Propofol's Atrioventricular Nodal Frequency-dependent Effects by Autonomic Innervation

Compared with the various antidyssrhythmic agents used to treat supraventricular tachycardias, propofol appears to have unique pharmacologic properties. Both the direct and indirect cardiac effects of propofol would be expected to promote its frequency-dependent actions on the AV node. Other drugs, such as diltiazem and adenosine, that exhibit potent frequency-dependent effects on AV nodal conduction can cause reflex autonomic changes that attenuate the rate-dependent effects of the drugs. For example, increased sympathetic tone and/or decreased vagal tone would be expected to diminish the rate-dependent effects of calcium channel blockers. Indeed, Nayebpour et al. found that although autonomic influences reduced diltiazem's frequency-dependent effects on AV nodal conduction, these influences were not sufficient to prevent frequency-dependent drug action. On the other hand, unlike the reflex changes observed with diltiazem and adenosine, propofol's indirect cardiovascular effects have been found to cause autonomic changes that would actually enhance the in situ rate-dependent effects of propofol on AV nodal conduction. Therefore, we hypothesize that propofol, by depressing sympathetic outflow, enhancing vagal tone, and altering the baroreceptor sensitivity, should actually exert greater rate-dependent effects in vivo than those observed in the denervated heart model. The definitive answer to this interesting question awaits further study at the whole animal level.

Distribution of Propofol in Blood

Surprisingly, to our knowledge, no prior studies have directly measured the free (physiologically active) concentration of propofol in blood, either in vitro or in vivo. However, several investigations have reported partition coefficients of propofol (octanol/water and octanol/blood) that can be used to indirectly estimate the free concentration of propofol.9,50 Because of the unavailability of directly measured free concentrations of propofol and because of its high degree of protein binding (96–99%),51,52 it is unwarranted to extrapolate in vitro concentrations to those reported during anesthesia. Regardless of the concentration(s) of propofol that induce general anesthesia, this anesthetic drug was found to possess unique pharmacologic properties. The efficacy of propofol to cause frequency-dependent slowing of AV nodal conduction at concentrations that produce minimal effects on baseline conduction times was comparable to that observed with the potent antidyssrhythmic agents, diltiazem and adenosine.

To confirm our results with those of prior in vitro studies with propofol, we used concentrations of propofol in the lower range (10 and 25 μM) of those previously studied. For example, to investigate the in vitro inotropic or electrophysiological effects of propofol, Stowe et al.,17 Park and Lynch,27 and Azuma et al.35 used aqueous propofol concentrations of 0.5–1,000, 30–300, and 10–600 μM, respectively, in their studies.

In conclusion, our results indicate that propofol, by a M2-muscarinic cholinergic receptor-mediated mechanism, depresses AV nodal conduction in a concentration- and frequency-dependent manner in guinea pig isolated heart. Unlike the electrophysiologic profile of two major antidyssrhythmic agents, diltiazem and adenosine, which induce reflex autonomic actions that may attenuate their rate-dependent effects, propofol appears to generate direct and indirect effects on cardiovascular control pathways that may preserve its frequency-dependent effects on AV nodal conduction. Although we are not advocating the routine use of propofol to treat supraventricular tachycardias, our findings do suggest that propofol may have important antidyssrhythmic properties. Future studies that correlate structure-activity relations to the frequency-dependent behavior of similar compounds may lead to the design of new anesthetic agents with greater antidyssrhythmic properties or, alternatively, new antidyssrhythmic drugs that display even greater frequency dependence but lack anesthetic properties.

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References


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Background: Evidence suggests that the nitric oxide pathway in the central nervous system of the rat brain may play a role in regulating blood flow and vasomotion. The purpose of the present study was to determine the effects of halothane and isoflurane on cerebral blood flow and cerebrovascular reactivity to isometric pressure changes.

Methods: Cerebral blood flow (CBF) was measured in the anterior cerebral artery using a laser-Doppler flowmeter. CBF and cerebral vascular reactivity to isometric pressure changes were measured in awake, anesthetized, and paralyzed rats. The effects of halothane (0.5%, 1.0%, and 2.0%) and isoflurane (0.6%, 1.0%, and 2.0%) on CBF and cerebral vascular reactivity were assessed using a paired t-test.

Results: Halothane increased CBF at all concentrations tested, while isoflurane had no significant effect on CBF. Halothane-induced increases in CBF were accompanied by a decrease in cerebral vascular reactivity to isometric pressure changes.

Conclusions: These findings suggest that halothane and isoflurane have different effects on cerebral blood flow and cerebrovascular reactivity.

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