Inotropic Effects of Propofol, Thiopental, Midazolam, Etomidate, and Ketamine on Isolated Human Atrial Muscle

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Background: Cardiovascular instability after intravenous induction of anesthesia may be explained partly by direct negative inotropic effects. The direct inotropic influence of etomidate, ketamine, midazolam, propofol, and thiopental on the contractility of isolated human atrial tissue was determined. Effective concentrations were compared with those reported clinically.

Methods: Atrial tissue was obtained from 16 patients undergoing coronary bypass surgery. Each fragment was divided into three strips, and one anesthetic was tested per strip in increasing concentrations (10^{-4} to 10^{-3} M). Strips were stimulated at 0.5 Hz, and maximum isometric force was measured. Induction agents were studied in two groups, group 1 (n = 7) containing thiopental, midazolam, and propofol, and group 2 (n = 9) consisting of etomidate, ketamine, and propofol.

Results: The tested anesthetics caused a concentration-dependent depression of contractility resulting in complete cessation of contractions at the highest concentrations. The IC_{50} (mean ± SEM; μM) for inhibition of the contractility were: thiopental 43 ± 7.6, propofol 235 ± 48 (group 1), and 246 ± 42 (group 2), midazolam 145 ± 54, etomidate 133 ± 15, and ketamine 303 ± 54.

Conclusions: This is the first study demonstrating a concentration-dependent negative inotropic effect of intravenous anesthetics in isolated human atrial muscle. No inhibition of myocardial contractility was found in the clinical concentration ranges of propofol, midazolam, and etomidate. In contrast, thiopental showed strong and ketamine showed slight negative inotropic properties. Thus, negative inotropic effects may explain in part the cardiovascular depression on induction of anesthesia with thiopental but not with propofol, midazolam, and etomidate. Improvement of hemodynamics after induction of anesthesia with ketamine cannot be explained by intrinsic cardiac stimulation. (Key words: Anesthetics, intravenous: etomidate; ketamine; midazolam; propofol; thiopental. Heart, atria: human; Isometric contraction; inotropic effect.)

INTRAVENTRICULAR induction of general anesthesia often is associated with hypotension. Several mechanisms have been thought responsible for the decreased blood pressure, including a direct effect on the contractility of the myocardium. Indirect factors, such as concomitant changes in preload and afterload of the heart, sympathetic activity, baroreflex activity, and central nervous system activity may have the direct effects of anesthetics on contractility difficult to measure in vivo, although relative heart rate and load-independent indexes of contractility can be derived from a series of pressure-volume diagrams of the left ventricle.

Measurement of the intrinsic myocardial contractility is more accurately performed in an in vitro model. In different animal species, the in vitro effects of propofol, thiopental, etomidate, and ketamine on the contractility of isolated cardiac tissue have been determined. The results of these studies show a variable degree of negative inotropic action in papillary or left atrial muscle. In contrast, etomidate did not induce a significant inotropic effect in papillary muscle of hamster, whereas positive inotropy was demonstrated in rats and ferrets after ketamine administration. Midazolam has not yet been studied in isolated myocardium. To the best of our knowledge, however, there are no in vitro studies concerning the inotropic influence of intravenous anesthetics on hu-
man myocardium. The purpose of this study was to evaluate the direct inotropic effects of thiopental, propofol, midazolam, etomidate, and ketamine on isometric contractions of isolated human atrial tissue.

Materials and Methods

This study was approved by the institutional Ethical Committee, and informed consent was obtained from patients scheduled for routine coronary artery bypass surgery (CABG). Ventricular function was assessed using heart catheterization and found to be normal in all subjects (ejection fraction over 0.50 and absence of regional wall motion abnormalities). We studied atrial tissue from 16 male patients. In 15 patients, preoperative medication consisted of β₁-adrenoceptor antagonists, nitrates, and calcium channel blocking agents. One patient had only a β blocker. All cardiac medication was continued and administered the day of surgery. All patients received similar anesthesia and surgery for coronary artery bypass graft. Anesthesia was induced using sufentanil and midazolam, with pancuronium administered to facilitate tracheal intubation, and maintained using a continuous infusion of midazolam and sufentanil. During atrial cannulation, a small sample of the atrial tissue was removed, stored in sterile buffer solution and immediately transported to the laboratory. None of the patients received inotropic support before the atrial tissue was removed.

Experimental Design

In the laboratory, the atrial tissue was split into three strips, measuring 7.8 ± 0.9 mm long and having a cross-sectional area of 2.8 ± 0.3 mm² without differences between groups (analysis of variance), and mounted in a temperature-controlled (30°C) chamber for isometric contraction as described earlier. Strips were superfused with an oxygenated buffer consisting of (in mm): NaCl 125, CaCl₂ 1.2, KCl 6, NaH₂PO₄ 1.2, MgCl₂ 2.5, hydroxyethylpiperazineethane sulfonic acid (HEPES) 10, and glucose 11 (pH 7.40 ± 0.05). They were stimulated at optimal preload at 0.5 Hz with rectangular pulses of 5 ms duration, and an intensity of 10% above threshold. The peak value of the developed force was measured using a force transducer (HBM, Hamburg, Germany). After 15 min of transport bathing and 45 min of superfusion with stabilization, the muscle strips showed a constant isometric contraction on stimulation, which remained stable for many hours.

The combined effects of the transport, the bathing procedure, the superfusion, and the stabilization period before the start of the actual experiment most likely allowed for the washout of any residual effects of anesthetics and patient medication. All experiments were carried out with the initial muscle lengths set at that at which force development was maximal. The mean force generated at optimal length was 14.6 ± 1.9 mN mm⁻² without differences between groups (analysis of variance). Thiopental, propofol solved in Intralipid (10%; Zeneca, Ridderkerk, The Netherlands), midazolam, etomidate solved in propylene glycol or ketamine were incrementally added to obtain cumulative concentrations of 10⁻⁶ to 10⁻² m. Before the maximal contraction was recorded, muscle strips were exposed to each concentration until a steady state was reached for at least 5 min. To facilitate comparison of the anesthetics, one drug was tested on each available strip. Because most atrial strips were too small to be split into five pieces, three atrial strips per patient were prepared. To obtain a standard, the effect of one of the anesthetics (propofol) was tested in muscle strips from all patients. Thus group 1 consisted of propofol, thiopental, and midazolam, group 2 of propofol, etomidate, and ketamine. To test tissue stability, three muscle strips were stimulated in the buffer solution without addition of any drug. After equilibration, the developed force of these strips was stable within ±3% during 2.5 h, which was the average time for each experiment.

Statistical Analysis

The baseline isometric force without anesthetic was normalized to 100%. All values are expressed as mean ± SEM. Mean concentration-response curves were fitted using a logistic regression model (Sigmaplot 4.1, Jandel) and compared with analysis of variance. Concentration-response curves of individual patients were fitted by a similar procedure and IC₅₀,₈ were compared using the Student’s t-test. IC₅₀ was defined as the concentration at which 50% of the maximal effect is obtained. e.g., 50% of the maximal suppression of contractility from the baseline value. P < 0.05 was considered to be significant.

Results

A typical example of the changes in isometric force in response to a step by step increase in concentrations of propofol is shown (fig. 1). Propofol, thiopental,
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midazolam, ketamine, and etomidate demonstrated a concentration-dependent inhibition of atrial muscle contraction, eventually leading to the complete cessation of contractions in all strips tested. Concentrations resulting in complete inhibition of contraction ranged between 3.10^{-9} and 3.10^{-3} M (figs. 2A–2F). Concentration-response curves representing inhibition of the contractile force were plotted for each set of experiments and compared to the reported clinical concentration range (figs. 2A–2E). Thiopental, and to a lesser extent, ketamine, was found to inhibit contractility in the clinical range. The concentration-response curves for propofol obtained in both groups I and II did not differ significantly (table 1). The concentration-response curve of all propofol experiments is depicted (fig. 2A).

To compare the characteristics of the inhibition of atrial contraction by the various anesthetics, the IC_{50} of the concentration-response relationship was determined in each strip by fitting the measured data with a logistic function (table 1). The IC_{50} of etomidate and midazolam did not differ. Propofol and ketamine possessed a significantly greater IC_{50}, whereas that for thiopental was significantly less, both compared to etomidate and to midazolam (table 1). Thus, the ranking order of inhibitory effects of the anesthetic agents as judged by their IC_{50} values was: ketamine and propofol < etomidate < midazolam < thiopental (table 1).

Discussion

This study clearly documents a concentration-dependent negative inotropic effect of propofol, thiopental, midazolam, etomidate, and ketamine in isolated human atrial tissue. The negative inotropic potency of the intravenous anesthetics as found in this study was plotted together with those obtained in isolated myocardium of various animal species (fig. 3).

Propofol

This study demonstrates a clear negative inotropic action of propofol on isolated human atrial tissue. The inhibitory potency of propofol on human atrial tissue contractility is in agreement with that obtained in guinea pig and ferret papillary muscle and close to that of guinea pig right ventricular tissue and guinea pig left atrial muscle (fig. 3). In contrast, propofol was devoid of substantial negative inotropic action in rat papillary muscle, hamster papillary muscle, and in situ canine heart preparations. Evidence suggests that only the free fraction of a drug is active. As protein binding of propofol exceeds 95%, free fractions of propofol are less

Fig. 1. Typical experiment showing the force traces of an isometric twitch of human atrial tissue during exposure to increasing concentrations of propofol (15–1,500 \mu M).

Fig. 2. Comparative effects of increasing concentrations of anesthetic on isometric contractions of human atrial tissue induced by field stimulation. Data are mean ± SEM. Curves were plotted using logistic regression. Hatched squares indicate the clinical concentration range during anesthesia. Coarse hatching (left) represents the total concentration and fine hatching (right) shows the free fraction. (A) Propofol (n = 16). (B) Thiopental (n = 7). (C) Midazolam (n = 7). (D) Ketamine (n = 9). (E) Etomidate (n = 9). (F) Combined plot showing the concentration-response curve of the five anesthetics. For statistical analysis, see table 1.
Table 1. Inhibitory Effects

<table>
<thead>
<tr>
<th>Anesthetic</th>
<th>n</th>
<th>IC₅₀ (mean ± SEM) (µM)</th>
<th>Slope</th>
<th>Maximal Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiopental</td>
<td>7</td>
<td>43 ± 7.6°</td>
<td>3.1 ± 0.7°</td>
<td>100</td>
</tr>
<tr>
<td>Etomidate</td>
<td>9</td>
<td>133 ± 12.7°</td>
<td>3.3 ± 0.6°</td>
<td>100</td>
</tr>
<tr>
<td>Midazolam</td>
<td>7</td>
<td>145 ± 53.9°</td>
<td>1.4 ± 0.3</td>
<td>90</td>
</tr>
<tr>
<td>Propofol I</td>
<td>7</td>
<td>235 ± 47.8°</td>
<td>2.1 ± 0.6</td>
<td>100</td>
</tr>
<tr>
<td>Propofol II</td>
<td>9</td>
<td>245 ± 42.1°</td>
<td>1.9 ± 0.4</td>
<td>100</td>
</tr>
<tr>
<td>Ketamine</td>
<td>9</td>
<td>303 ± 54.3°</td>
<td>2.07 ± 0.3</td>
<td>100</td>
</tr>
</tbody>
</table>

IC₅₀ of groups I (N = 7) and II (N = 9). The data per experiment were fitted by logistic regression, and each IC₅₀ value was calculated. The mean (± SEM) IC₅₀ was calculated from the individual experiments. The differences between propofol in groups I and II are not significant. (Student’s t-test).

The IC₅₀ values of thiopental, ketamine, and propofol were significantly different from those of etomidate and midazolam (*P < 0.05). The slopes of thiopental and etomidate were significantly different (*P < 0.05).

than 1 µg·ml⁻¹ and far from the range of in vitro cardiac depression observed in this study. Thus, intrinsic depression of cardiac contractility seems to a lesser extent involved in the cardiovascular depression of propofol as observed in vivo. ⁵⁻⁷⁻⁹

Thiopental

The inhibitory potency of thiopental found in human atrial strips generally corresponds to that found in excised animal myocardial tissue (fig. 3). ⁵⁻⁷⁻⁹⁻¹¹ A lower potency has been observed in right ventricular tissue of the ferret ¹² and in isolated whole heart preparations in guinea pig. ²⁸ The plasma concentration of thiopental at clinical induction of anesthesia reaches a peak of approximately 100 µg·ml⁻¹ and returns to 10 µg/ml after 5 min. ²⁹ These data on plasma concentration are supported by a more recent study of clinical anesthetic depth at different thiopental concentrations. ³⁰ Thus, these studies suggest that the concentration of thiopental at induction varies between 10 and 100 µg·ml⁻¹. Because about 75% of serum thiopental is bound to protein, the actual concentration of free drug at induction may range between approximately 2.5 and 25 µg·ml⁻¹. In this concentration range, a significant inhibitory contractility was found in human atrial strips. Therefore, our results suggest that direct negative inotropic action of thiopental on human heart may be involved in the cardiovascular depression observed after induction with this agent. ³⁻¹¹

Midazolam

The negative inotropic effect of midazolam was between that of thiopental and propofol. No comparison can be made with data from isolated preparations of animal myocardium, because midazolam studies are not available. Midazolam, however, was one third as depressing on human heart as was observed in isolated whole rat heart. ³² The concentration range of midazolam after anesthetic induction varies between 0.5 and 1.5 µg·ml⁻¹. ³³ Protein binding of midazolam is more than 90%. Therefore, the free fraction probably does not exceed 0.1 – 0.2 µg·ml⁻¹ suggesting that during clinical use, midazolam does not exhibit a negative inotropic action. This is supported by in vitro human and animal studies, in which hemodynamic effects of midazolam are attributed to changes in preload and afterload. ³⁻¹⁻³⁴

Etomidate

Etomidate showed a negative inotropic effect in human atrial strips with a potency similar to that found

Fig. 3. Plots of the potency of negative inotropic effects of anesthetics on human atrial tissue with data from animal experiments. IC₅₀ values from this study on the x-axis are plotted against comparable data from animal studies on the y-axis. Most data are from in vitro studies of isolated heart preparations and some from other forms of isolated experiments. Data are from various studies using different experimental conditions. The nature of the data is provided in symbols: circle = contractility studies of isolated ventricular tissue; square = contractility studies of isolated atrial muscle; diamond = other studies of contractility in models of isolated heart preparations. Species are depicted in the symbols and abbreviated as follows: R = rabbit; G = guinea pig; F = ferret; D = dog. Data are from the following studies: propofol, references 5, 6, 8, and 11; thiopental, references 5, 9–11, 12, and 28; midazolam, reference 32; etomidate, references 13, 14, and 35; and ketamine, references 15–17.

Ketamine

Ketamine was characterized with respect to its negative inotropic effect on human atrial strips. Both negative and biphasic contractility in vitro. Resistance to the negative inotropic effect on rabbit ³³ and guinea pig ³⁰ and ferret papillary muscle ³⁰ is low. In contrast, hamster ³³⁻¹ and rat ventricle, negative inotropic action observed on ferret is attributed to the effect of noradrenaline release on concentration of ketamine after induction. ³²⁻¹⁻³⁻¹ and thus the free fraction may reach a threshold for greater plasma concentration. This results in a greater negative inotropic effect. However, because key cardiovascular system is known to be highly sensitive to the clinical conditions the action of ketamine may promote cardiac depression. ³⁻¹⁻³⁻¹

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for midazolam. In isolated papillary muscle, perfused by a conscious donor dog, the measured potency of etomidate was similar to that found in this study. In rabbits and ferrets, etomidate showed substantial negative effects on contractility of papillary muscle, but in a lower concentration range (fig. 3). In contrast, in rat and frog, etomidate produced only minimal effects on contractility. After induction of anesthesia with etomidate (0.5 mg·kg⁻¹), its plasma concentration 4 min after the bolus injection was 0.3 μg·ml⁻¹. The concentration 1 min after intravenous administration is probably greater. As protein binding of etomidate is 75%, the concentration range of the free fraction of etomidate will not exceed 1 μg·ml⁻¹. Thus, etomidate is assumed to be devoid of a negative inotropic action on human cardiac muscle during clinical use. This is supported by the observation that etomidate produces the least variation in hemodynamics of the intravenous anesthetics most often used. Ketamine

Ketamine was characterized by the lowest potency with respect to its negative inotropic action in isolated human atrial strips. Previous studies have described both negative and biphasic effects of ketamine on contractility in vitro. Results of the current study are comparable to the negative inotropic effects of ketamine on rabbit and guinea pig ventricular muscle, and ferret papillary muscle after administration of bupranolol. In contrast, inotropic effects of ketamine on hamster and rat ventricle are positive. Positive inotropic action observed in other species such as the ferret is attributed to the ketamine-induced blockade of noradrenaline reuptake.

In clinical studies, the concentration of ketamine reached 60 μM L⁻¹ at 5 min after induction. Protein binding of ketamine is only 20% and thus the free fraction of ketamine after induction may reach 40–50 μM L⁻¹ at 5 min. However, greater plasma concentrations can be expected at 1–3 min after induction and may reach 100–150 μM. Our results therefore suggest that ketamine may have a modest direct negative inotropic effect after induction. However, because ketamine does not depress the cardiovascular system in vivo, it is likely that this interaction is counteracted by centrally mediated stimulating responses. Nevertheless, in some clinical conditions the direct negative inotropic action of ketamine may produce pronounced cardiovascular depression.

The purpose of this study was to present a quantitative description of the intrinsic effects of clinically used intravenous anesthetics on myocardial contractility in humans. The drugs were tested in the same formulation used clinically. Therefore, possible effects of solvents are also present. The effects of the solvent of etomidate and propofol on contractility of myocardial tissue have been studied previously. Propylene glycol, the solvent of etomidate, did not affect the contraction of papillary muscle of rabbit and ferret. The solvent of propofol, Intralipid (10%) emulsion, did not cause changes in inotropy in guinea pig and rat papillary muscle. In contrast, Cook reported a modest increase of contractility in ferret papillary muscle (10%), possibly due to intralipid serving as metabolic substrate. Therefore, it seems unlikely that the presence of solvents in our experiments influenced the results.

The results of this study must be interpreted in the context of the nonphysiologic conditions of the experiments. Results were obtained at 30°C at a stimulus frequency of 0.5 Hz and may differ from results obtained at a temperature of 37°C and frequencies of 1–2 Hz. HEPES buffer has been reported to affect intracellular bicarbonate concentrations, resulting in a relatively depolarized resting potential and prolonged action potential in guinea pig papillary muscle preparations. However, this effect of HEPES was a minor factor in the current study, because control experiments remained stable for a longer period of time (more than 2 h).

In contrast to earlier studies using different animal models, our patients were receiving β₁-adrenoceptor antagonists and calcium channel blockers. The acute effects of these drugs on contractility are not expected to influence the outcome of this study in view of the extensive washing and equilibrium period before performing the experiments. However, it cannot be ruled out that cellular adaptation after the long-term use of antianginal medication (β₂ blockers, calcium channel blockers and nitrates) affected the inotropic response and thus influenced the results of our measurements. Additional studies are needed to elucidate the influence of long-term medication on the interaction between intravenous anesthetics and contractility.

Furthermore, it is not known whether the results from experiments on human atrial tissue experiments are comparable to those on ventricular tissue, because data on human ventricular tissue are not available. In animal studies, no systematic difference has been observed. Comparison of the data of Azari et al.
tissue with ventricular studies of this species does not show a clear relationship. Comparison of the inotropic action of thiopental suggests a slightly greater sensitivity of guinea pig ventricular tissue. Conversely, propofol showed a similar potency compared to a study of Park et al. and a lower sensitivity of the guinea pig right ventricle in another study, although this may be related to differences in the experimental setup.

In summary, in human atrial tissue, thiopental was the most potent inhibitor of contractile force as compared to midazolam and etomidate, whereas propofol and ketamine were the least inhibitory intravenous anesthetics. Negative inotropic effects in the clinical concentration range were demonstrated for thiopental, and to some extent for ketamine. Thus, cardiovascular depression on induction of general anesthesia with thiopental is based on a direct negative inotropic action on human myocardium. The depression of hemodynamics after induction with propofol, midazolam, or etomidate cannot be explained by a direct negative inotropic action. Finally, it is unlikely that the improved hemodynamics observed clinically with ketamine are caused by an intrinsic action on the myocardium.

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