—Originals—

Bupivacaine combined with isoflurane synergistically depressed myocardial contractility in rat working heart preparation

Takero Takamura, Atsuhiko Sakamoto and Ryo Ogawa

Department of Anesthesiology, Nippon Medical School

Abstract

It is well known that both local and volatile anesthetics depress myocardial functions. This study was performed to evaluate the combined effects of commonly utilized local anesthetics (bupivacaine, mepivacaine, and lidocaine) and a volatile anesthetic (isoflurane) on heart rate and myocardial contractility in an isolated rat working heart preparation using each local anesthetic at 0 to 3×10^{-4} (mol/L) with or without 1 MAC (minimum alveolar concentration) of isoflurane. The three local anesthetics depressed the heart rate and myocardial contractility dose-dependently. Bupivacaine depressed the heart rate more than the other two local anesthetics. One MAC isoflurane showed significant negative inotropic effects, and accelerated the effects of the local anesthetics. By using isobolographic analysis, we concluded that the combined effect of isoflurane and bupivacaine on myocardial negative inotropism was synergistic, while the effects of isoflurane and mepivacaine or lidocaine were additive. (J Nippon Med Sch 1997 ; 64 : 292—297)

Key words: rat working heart, bupivacaine, isoflurane, myocardial functions, synergistic effect

Introduction

A high plasma concentration of bupivacaine is cardiotoxic. The toxicity of bupivacaine and lidocaine has been examined in animal preparations¹⁻⁴. It has also been suggested that volatile anesthetics, including isoflurane, depress myocardial functions⁵⁻¹¹.

Recently, regional anesthesia has been utilized to anesthetize patients in combination with general anesthesia, and it is possible that local anesthetic intoxication will occur during general anesthesia. However, the effects of the combination of two kinds of anesthetics have not been clarified. We investigated the combined effects of isoflurane and commonly utilized local anesthetics on heart rate and myocardial contractility in a working rat heart preparation.

Materials and Methods

The ethical committee on animal study at Nippon Medical School approved the experimental design.

Thirty male Sprague-Dawley rats each weighing 280-300 g were anesthetized with sodium pentobarbital 50 mg/kg given ip. The heart was excised and immersed in ice-cold Krebs-Henseleit bicarbonate buffer (KHB) solution, consisting in mmol/ml of NaCl 118, KCl 4.7, CaCl₂ 1.25, MgSO₄ 1.25, KH₂PO₄ 1.25, NaHCO₃ 24, and glucose 15, equilibrated with 95% oxygen and 5% carbon dioxide. An isolated heart perfusion apparatus (K-884600, Kontes, New Jersey, USA) was used to perfuse the rat heart¹². The aorta was cannulated and 37°C-KHB solution was perfused according to the Langendorff procedure. The atrium was cannulated via the pulmonary vein within 5 min of retrograde perfusion.

Correspondence to Takero Takamura, Department of Anesthesiology, Nippon Medical School Hospital, 1-1-5 Sendagi, Bunkyo-ku, Tokyo 113, Japan

The Langendorff mode was then converted to a working heart mode by switching the supply of perfusate from the aorta to the left atrial cannulation (filling pressure: 15 cmH₂O). Under this procedure, the left ventricle began spontaneously to eject the perfusate through the aortic cannula against the resistance made by a fine tube.

The hearts were randomly assigned to six groups; three local anesthetic group [bupivacaine (n=5), mepivacaine (n=5), lidocaine (n=5)], and three isoflurane added groups [isoflurane plus bupivacaine (n=5), isoflurane plus mepivacaine (n=5), and isoflurane plus lidocaine (n=5)].

In the local anesthetic groups, local anesthetic was added to the perfusate at concentrations of 10^{-6} , 3×10^{-6} , 10^{-5} , 3×10^{-5} , 10^{-4} , and 3×10^{-4} (mol/L) at intervals of 5 min after a stabilization period of 20 min. In each isoflurane added group, 1 MAC concentration of isoflurane was bubbled into the KHB solution through a calibrated vaporizer. The hearts were perfused for 10 min with a solution containing isoflurane after a stabilization period of 10 min, after which, local anesthetics were added in the manner described above.

A needle type cannula (23 G) was placed in the left ventricular cavity transmurally and was connected to a transducer (Blood Pressure Amplifier AP-641 G, Nihon Kohden, Tokyo, Japan) and a pressure processor (EQ-601 G, Nihon Kohden, Tokyo, Japan). The pressure waves were differentiated by computer. Heart rate (HR: bpm), left ventricular pressure (LVP: mmHg) and peaks in differentiation of LVP by time (dp/dt max: mmHg/sec) were recorded at the control period, 10 min after exposure to isoflurane, and every 5 min after the administration of local anesthetics.

To determine whether the depression of myocardial function by the combination of isoflurane and local anesthetics is additive or synergistic, isobolographic analysis was performed according to the method of Tallarida et al¹³. The theoretical concentration of local anesthetics and of bubbled isoflurane which reduced dp/dt max to 50% of the control (ED₅₀), as well as theoretical 95% confidence intervals (CIS), were computed¹³. The effects of isoflurane at concentrations of 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, and 4.5 MAC on the same isolated rat working heart preparation were also measured to obtain the

theoretical ED₅₀ value of isoflurane. The theoretical ED₅₀s were then plotted on X and Y axes, and the diagonal were drawn between two theoretical ED₅₀ lines regarded as the theoretical additive line. When the points experimentally obtained fell below the theoretical additive line, the points indicated the synergistic action on the myocardium.

The values were represented by mathematical means and central tendency by standard deviations. The comparisons of means among the three groups were performed using one-way ANOVA. Fisher's PLSD test was applied to assess differences between two groups if ANOVA denied the uniformity of means. A p-value under 5% was considered significant.

Results

There were no significant differences in the values obtained in the control period among the six groups.

Fig. 1 shows the concentrations of local anesthetics which stopped the spontaneous contraction of heart preparations. In the preparations which were not exposed to volatile anesthetic agents, the concentrations decreased from lidocaine, mepivacaine to bupivacaine. There was a 30-fold difference between lidocaine and bupivacaine. Similar results were obtained in the preparations exposed to local anesthetics combined with isoflurane. However, the trend for the concentration of local anesthetics to cause cardiac standstill was lower in the groups with isoflurane than in the groups without isoflurane (Fig. 1).

Fig. 1 Concentrations of local anesthetics which stopped spontaneous contraction of heart preparations (all samples). B: bupivacaine, M: mepivacaine, L: lidocaine, I: isoflurane, Concentration: concentration of local anesthetics.

Three local anesthetics decreased heart rate dose-dependently without isoflurane (**Fig. 2** above). The heart rate in the bupivacaine group was reduced significantly compared to the lidocaine group at 10^{-5} and 3×10^{-5} mol/L. Also the heart rate in the mepivacaine group was reduced significantly compared to the lidocaine group at 3×10^{-4} mol/L. No change in heart rate was observed when isoflurane was added to the perfusate, and similar changes in heart rate were observed among the three groups with local anesthetics and isoflurane (**Fig. 2** below).

Fig. 2 Effect of local anesthetics on heart rate with isoflurane (below) and without isoflurane (above) (mean $\pm$ SE). B: bupivacaine, M: mepivacaine, L: lidocaine, I: isoflurane, C: control time, (I) The time when isoflurane exposure was started, Concentration: concentration of local anesthetics, HR: heart rate. # $P < 0.05$ vs lidocaine; ## $P < 0.05$ vs mepivacaine, * $P < 0.05$ vs isoflurane plus lidocaine; ** $P < 0.05$ vs isoflurane plus mepivacaine.

There was no significant difference in heart rate between the local anesthetics with or without isoflurane. No premature contraction was observed in any of the six groups until just before cardiac standstill.

The values of dp/dt max decreased in parallel to the concentration of isoflurane bubbled into the perfusate, and the theoretical ED₅₀ was 2.73 MAC (95% CIS: 2.70-2.79) (**Table 1**). At 1 MAC isoflurane depressed dp/dt max from 4066 ± 274 (mmHg/sec) to 3566 ± 349 (mmHg/sec) (the average of the three local anesthetic groups) significantly (**Fig. 3** below, **Fig. 4**). Local anesthetics reduced cardiac contractility dose-dependently; however, there was no significant difference between the three local anesthetics (**Fig. 3** above), even though the theoretical ED₅₀ and 95% CIS were different among the three local anesthetics (**Table 1**).

Isoflurane accelerated the reduction of myocardial contractility. There was a significant difference between the groups with and without isoflurane at any concentration of the three local anesthetics (**Fig. 4**). Bupivacaine at a concentration of 3×10^{-5} mol/L with isoflurane showed a significantly lower dp/dt max than mepivacaine or lidocaine with isoflurane (**Fig. 3**). The theoretical ED₅₀ of bupivacaine with isoflurane was markedly lower than bupivacaine alone; however, there was no marked difference between the mepivacaine and lidocaine groups with and without isoflurane (**Table 1**).

Isobolographic analysis revealed that bupivacaine had a synergistic negative inotropic effect in combination with isoflurane, and lidocaine and mepivacaine had additive negative inotropic effects on the myocardium (**Fig. 5**).

Table 1 The ED₅₀ values and 95% confidence intervals of negative inotropic effects of three local anesthetics, isoflurane, and the local anesthetics in combination with isoflurane

	n	ED ₅₀	95% CIS
B	5	1.15×10^{-4}	$8.13 \times 10^{-5} - 1.90 \times 10^{-4}$ (mol/L)
M	5	3.39×10^{-4}	$2.30 \times 10^{-5} - 8.10 \times 10^{-4}$ (mol/L)
L	5	2.00×10^{-3}	$1.00 \times 10^{-3} - 7.20 \times 10^{-3}$ (mol/L)
B + I	5	1.95×10^{-5}	$1.41 \times 10^{-5} - 6.03 \times 10^{-5}$ (mol/L)
M + I	5	3.98×10^{-4}	$2.75 \times 10^{-4} - 6.60 \times 10^{-4}$ (mol/L)
L + I	5	2.95×10^{-3}	$1.31 \times 10^{-3} - 9.80 \times 10^{-3}$ (mol/L)
I	15	2.73	2.70-2.79 (MAC)

B: bupivacaine, M: mepivacaine, L: lidocaine, I: isoflurane.

Fig. 3 Effect of local anesthetics on dp/dt max with isoflurane (below) and without isoflurane (above) (mean $\pm$ SE). B: bupivacaine, M: mepivacaine, L: lidocaine, I: isoflurane, C: control time, (I) The time when isoflurane exposure was started, Concentration: concentration of local anesthetics, HR: heart rate. * $P < 0.05$ vs isoflurane plus lidocaine, ** $P < 0.05$ vs isoflurane plus mepivacaine.

Discussion

The contractile control system in the heart is based on the cell's ability to control Ca^{2+} movements. There are three major cellular sites involved in contraction: sarcolemma, sarcoplasmic reticulum and myofilaments. In the sarcolemma of the cardiac muscles, two types of Ca^{2+} channels have been identified: transient (T) and long-lasting (L) channels.

Isoflurane has been reported to inhibit both L-type Ca^{2+} channels and $\text{Na}^+/\text{Ca}^{2+}$ exchange^{5,11}, and decrease the Ca^{2+} store in the sarcoplasmic reticulum, decreasing the release of Ca^{2+} in the rat heart¹⁴ and the rabbit heart⁹. Also, local anesthetics, as the Na^+ channel blocker, decrease the inward Ca^{2+} current mediated by the mechanism of $\text{Na}^+/\text{Ca}^{2+}$ exchange^{15,16}, and those such as bupivacaine and lidocaine also decrease Ca^{2+} release from the sarcoplasmic reticulum directly^{3,17}, so that the myocardial contraction is depressed. Actually, in the present study, 1 MAC isoflurane reduced myocardial contractility by 12.3% [from 4066 ± 274 (mmHg/sec) to 3566 ± 349 (mmHg/sec)], and three local anesthetics dose-dependently depressed myocardial

Fig. 4 Comparison among the local anesthetic groups with and without isoflurane: bupivacaine (above), mepivacaine (middle), lidocaine (below) (mean $\pm$ SE). B: bupivacaine, M: mepivacaine, L: lidocaine, I: isoflurane, C: control time, (I) The time when isoflurane exposure was started, Concentration: concentration of local anesthetics, HR: heart rate. * $P < 0.05$ vs the groups without isoflurane.

contractility, expressed by dp/dt max in the working heart preparations.

The Na^+ channel block effects of local anesthetics suppress the maximum velocity of the depolarization of Purkinje fibers and ventricular conduction^{18,19}. This produces a disturbance in the myocardial depolarization-repolarization cycle, and reduces heart rate and produces reentry-type arrhythmia^{1,20}. In this study, the three local anesthetics actually depressed the heart rate dose-dependently.

Many studies have shown the relative effects on cardiac functions of bupivacaine, mepivacaine and lidocaine. Komai et al¹ found that the effect of bupivacaine was 14-fold higher than that of lidocaine in slowing the heart rate to 50% of the control, using a rat working heart preparation. Ralph et al² reported that the reductions in heart rate, force of ventricular contraction, and cardiac

Fig. 5 Isobologram of ED_{50} values and 95% confidence intervals of negative inotropic effects of local anesthetic (square on the horizontal axis), isoflurane (square on the vertical axis), or the local anesthetics in combination with isoflurane (circle in the dose field). The bold lines on the axes represent the confidence interval for the single anesthetics. The diagonal line connecting the local anesthetics and isoflurane ED_{50} values (closed square on the axes) is the theoretical additive line, and the point on this line (open square) is the theoretical additive point. When points experimentally obtained fell below the theoretical additive line, the points indicated synergistic action on the myocardium.

output resulting from exposure to 3 $\mu\text{g/mL}$ of bupivacaine exceeded those resulting from exposure to 30 $\mu\text{g/mL}$ of lidocaine in the Langendorff model of guinea pigs. Kashimoto et al⁴ described that 25 $\mu\text{g/mL}$ of bupivacaine in blood concentration caused the same reduction in heart rate and cardiac output as 100 $\mu\text{g/mL}$ of lidocaine in an

isolated rat lung-heart preparation.

The affinity of bupivacaine for the Na^+ channel is stronger than that of lidocaine, and the duration of bupivacaine to the Na^+ channel is longer than that of lidocaines^{3,18}. In this study, also, bupivacaine caused cardiac arrest at lower concentrations and suppressed heart rate more than the other two drugs.

In the case of mepivacaine, it may be that the depressant force of mepivacaine is between that of bupivacaine and lidocaine, judging from the present results.

On the other hand, there was no difference in myocardial contraction among the three local anesthetics. Lynch³ reported that in isolated guinea pig right ventricular papillary muscle preparations the effects of local anesthetics, such as bupivacaine, etidocaine, and lidocaine on the depression of myocardial contractility were frequency-dependent in a reverse manner, that is, the depression of contractility was less significant at higher pacing (2-3 Hz) than at lower pacing (<1 Hz). In our study, the heart rate was relatively high because of spontaneous beats without pacing; therefore, our results may support Lynch's conclusion. On the other hand; Clakson et al²¹ reported that in the same preparations used by Lynch, bupivacaine and lidocaine depressed myocardial contractility in a frequency-dependent manner. Anyhow, these controversial results might be due to several factors, such as the methods of these studies, the type and the situation of the preparation, and the species of animals. Actually, we did not investigate whether myocardial contractility depends on frequency or not.

In the present study the combination of the volatile anesthetic isoflurane and local anesthetics produced a negative inotropic action on the isolated perfused myocardium. The effect was synergistic with bupivacaine and additive with lidocaine or mepivacaine. It is suggested that bupivacaine at high concentrations, as well as isoflurane, has a direct effect on the L-type Ca^{2+} channel²²⁻²⁴; therefore a combination of isoflurane and bupivacaine produces a stronger suppression of myocardial contractility. Our results showed that cardiac standstill due to local anesthetics was enhanced by isoflurane in the following order: lidocaine, mepivacaine, bupivacaine. The difference in potency

suggests that the sites involved in myocardial contraction vary among the three local anesthetics. To make the mechanisms clear, it is necessary that the process of the interactions between isoflurane and each local anesthetic is elucidated in detail, especially the intracellular Ca^{2+} mobilization mechanisms.

In the present study, we used a rat working heart preparation, which is a denervated heart preparation; consequently the results derived from this preparation are not affected by the autonomic nervous system, and are not necessarily directly applicable to the clinical situation. However, combinations of these commonly utilized local and inhalational anesthetics might produce a profound depressant effect on myocardial contractility.

In conclusion, the combination of the volatile anesthetic isoflurane and local anesthetics showed a negative inotropic action in a rat working heart preparation. The effect was synergistic with bupivacaine and additive with lidocaine or mepivacaine.

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