Bupivacaine-induced slow-inward current inhibition: a voltage clamp study on frog atrial fibres

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The effects of various concentrations of bupivacaine on the characteristics of the slow-inward current (isi) were studied over a ten-minute period on isolated frog atria. At a concentration of 10^{-7} M, bupivacaine did not modify isi. At 10^{-6} M, the maximal amplitude of the slow-inward current (i max) was depressed by 11 per cent. At 10^{-5} M, i max was depressed by 24.5 per cent, the time-to-peak current value (tpeak) was increased by 13.4 per cent and the inactivation time constant (τ in) by 29.8 per cent. At 10^{-4} M, i max was depressed by 32.9 per cent, tpeak increased by 30.4 per cent and τ in by 58.7 per cent. In conclusion, bupivacaine produced only moderate inhibition of the slow-inward current. The findings might explain the decline in sinus impulse formation with sinus bradycardia, and the slowing of atrio-ventricular node conduction produced by bupivacaine. However, the decrease in contractility previously reported does not seem to be due only to inhibition of the slow-inward current.

Les effets de la bupivacaïne sur les caractéristiques du courant entrant lent (isi) ont été étudiés sur une période de 10 min à différentes concentrations sur l'oreillette isolée de grenouille. A 10^{-7} M aucune modification de isi n'est constatée. A 10^{-6} M, l'amplitude maximale de isi (i max) est diminuée de 11 pour cent. A 10^{-5} i max diminue de 24.5 pour cent, le temps au pic (τ pic) augmente de 13.4 pour cent et la constante de temps d'inactivation (τ in) de 29.8 pour cent. A 10^{-4} M, i max diminue de 32.9 pour cent, t pic augmente de 30.4 pour cent et τ in de 58.7 pour cent. En conclusion, la bupivacaïne inhibe le courant entrant lent d'une manière peu importante. Ceci peut expliquer les anomalies constatées au niveau de la baisse de l'influx sinusal et du ralentissement de la conduction dans le nœud auriculo-ventriculaire. Cependant, la baisse importante de la contractilité constatée dans la littérature ne semble pas être due à l'inhibition de ce courant.

Key words
ANAESTHETICS, LOCAL: bupivacaine;
HEART: voltage clamp, Ca^{++} current.

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Cardiovascular collapse and cardiac arrhythmias have been described in clinical reports and animal experiments following high doses of bupivacaine.[1] Ventricular arrhythmias, sinus bradycardia and sinus arrest, atrioventricular (AV) blocks, and junctional tachycardia have been described.[2–4] Moreover, the progressive increase in plasma levels of bupivacaine in anaesthetized dogs is responsible for the electrophysiological alterations of the intra-cardiac conductive pathways.[5,6] At low concentrations ($<3\ \mu g \cdot ml^{-1}$), bupivacaine inhibits atrial and infranodal conduction velocities.[2,6,7] This effect is due to a rapid, dose-dependent depression of the fast action potential Vmax by inhibition of the fast-inward sodium current.[7–9] At high plasma levels ($>3\ \mu g \cdot ml^{-1}$), bupivacaine alters sinus function and AV node conduction.[2,3,6] These effects might be due to inhibition of the slow-inward current (isi) by bupivacaine. Indeed, slow action potential Vmax of the sinus and AV nodes depends on activation of isi.[10,11] Moreover, this calcium inhibitory effect might be responsible for the major effect on contractility that bupivacaine induces at toxic doses.[2,12–16] Three reports have confirmed that bupivacaine inhibits isi.[12,17,18] However, the importance of this inhibition remains a subject of discussion. The object of the present study was to investigate the effects of different concentrations of bupivacaine on isi of frog atrial fibres by the double sucrose gap technique.

Methods

The effects of bupivacaine on the electrical activity of the

cardiac cell were studied using the double sucrose gap technique.[19] According to the method, using vaseline seal partitions, described by Rougier et al.,[20] fibres 150 microns in diameter and 2 to 3 mm long, were isolated from the frog atrium and mounted in a double sucrose gap compartment bath.[21] Briefly, in the sucrose gap chamber, a short segment (100–200 μm) at the middle of the preparation (artificial node of Ranvier) is isolated from the ends of the fibres by two streams of isosmotic sucrose solution which filled the extracellular space. The central pool (test pool) can be perfused with physiological fluid or test solution. In the test pool, the inside potential of the membrane is clamped and the stimulating voltage applied outside generates a current flow across the membrane.

Ringer-type physiological solution was used with the following composition: 110 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, 5 mM HEPES (biological buffer). The pH was adjusted to 7.4 with NaOH. The solution was maintained at room temperature (18–20°C). The slow kinetic current was studied after inhibition of the i fast current by the addition of 3×10^{-7} M tetrodotoxin to the medium. Bupivacaine was added to the physiological fluid prior to the experiment in order to obtain test solutions at concentrations of 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M.

The membrane was clamped at its resting potential and depolarized (50–60 mV) until the maximal inward current was obtained using low-resistance calomel half-cells of good stability. A period of stabilization was allowed for 20 min. During this period, the preparation was paced at the same frequency (0.1 Hz), as that used during the experiment. All unstable fibres were rejected. Infusion of bupivacaine was then started at 0.375 ml · min^{-1}; the same rate was used for the reference medium. After the initial control, measurements were recorded at ten minute intervals on a Tektronix 2230 oscilloscope and then transferred to a computer where they were analyzed using a specific program. The computer program determined the maximal amplitude of the slow kinetic current (i max, the difference between the baseline and the inward current peak), the time-to-peak value (t peak, the time between the beginning of stimulation and the peak current) and the inactivation time constant (τin).

Statistical analyses were performed using ANOVA and Student's paired t test. $P < 0.05$ was considered the minimal level of significance.

Results

The results are shown in the Table. Bupivacaine decreased the isi amplitude (i max) in a concentration-dependent manner. It also reduced the kinetics of activation and inactivation because t peak and τin respectively increased. Bupivacaine 10^{-7} M did not modify the amplitude, the time-to-peak value or the inactivation time

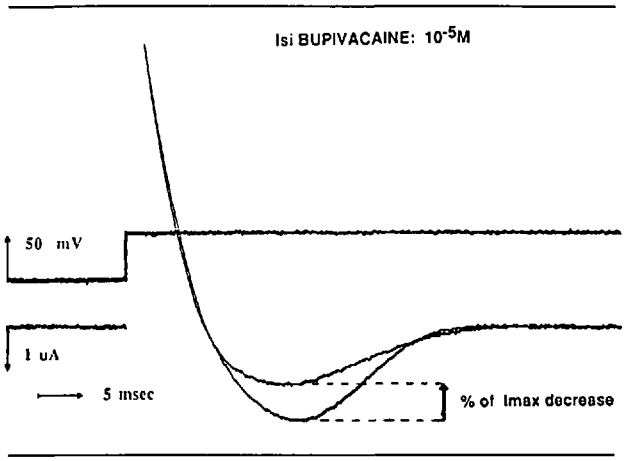

FIGURE Effects of 10^{-5}M bupivacaine on the slow-inward current isi before and 10 min after the beginning of infusion. Upper trace: voltage deflection from the resting potential. Lower traces: slow inward current shapes before (greater one) and after (the smaller one) bupivacaine effect.

constant of the current. At 10^{-6} M bupivacaine, only the maximal current amplitude was significantly reduced (i max = − 11 per cent, $P < 0.05$); t peak was not modified. In contrast, although there was an 18.8 per cent increase in τin, this was not statistically significant. At 10^{-5} and 10^{-4} M (Figure), bupivacaine modified all the variables studied: at 10^{-5} i max was decreased by 24.5 per cent ($P < 0.01$); the time-to-peak value and τin were increased by 13.4 per cent ($P < 0.01$) and 29.8 per cent ($P < 0.05$), respectively. At 10^{-4}, i max was decreased by 32.9 per cent ($P < 0.001$), t peak and τin were increased by 30.4 per cent ($P < 0.05$) and 58.7 per cent ($P < 0.01$) respectively. Bupivacaine-induced isi inhibition seemed to be prolonged because twenty minutes after the washout, recuperation was not observed in any of the fibres.

TABLE Slow-inward current after bupivacaine exposure

Bupivacaine *concentration*	Time *(min)*	I max *(μA)*	t peak *(msec)*	*p in (msec)*
10^{-7} · M	0	0.95 ± 0.06	8.3 ± 1.2	18.0 ± 2.4
(*n* = 7)	10	0.92 ± 0.06	8.6 ± 1.2	17.8 ± 2.4
10^{-6}	0	0.87 ± 0.08	5.9 ± 0.7	16.6 ± 1.9
(*n* = 6)	10	0.77 ± 0.07*	6.1 ± 0.7	19.8 ± 2.6
10^{-5}	0	0.94 ± 0.08	9.6 ± 0.8	25.5 ± 1.8
(*n* = 6)	10	0.72 ± 0.09†	10.8 ± 0.7†	33.4 ± 3.5*
10^{-4}	0	0.99 ± 0.07	7.6 ± 0.6	20.5 ± 1.0
(*n* = 10)	10	0.66 ± 0.06‡	9.9 ± 1.0*	32.4 ± 2.9†

Results are expressed as mean ± SEM. Asterisks represent significance of the difference between the values at 10 min and 0 min; *, $P < 0.05$; †$P < 0.01$; ‡$P < 0.001$.
I max = maximum amplitude of slow-inward current, *t* peak = time to peak-value, in = coefficient inactivation time constant.

Discussion

The results indicated that bupivacaine inhibited the slow-inward current in a concentration-dependent manner. This moderate calcium inhibitory effect might explain the sinus bradycardia and the slowing of AV node conduction observed in anaesthetized dogs following bupivacaine infusion. These alterations are markedly less profound than those that affect the atria and the ventricles, structures which are dependent on the fast-inward sodium current to produce their action potential, Vmax.[6,26,27] The effects of bupivacaine on the slow-inward current have been described in three reports. Lynch [12] studied the changes in Vmax of the slow action potentials in guinea pig papillary muscles which had been partially depolarized by a solution enriched in potassium and containing 0.1 μM isoproterenol. At a concentration of 4×10^{-6} M, bupivacaine had no effect on Vmax. At 10^{-5}M, it caused a depression of the Vmax which appeared to be frequency-independent between 0.25 and 1 Hz. Coyle and Sperelakis[17] used the same methodology as Lynch except that the former delivered only a 0.5 Hz frequency and used a higher concentration of isoproterenol (1 μM). These authors did not observe a change in the Vmax of the slow action potentials at 10^{-6} M. In contrast, Vmax was reduced by 50 per cent at 9.1×10^{-6} M bupivacaine and completely abolished at 10^{-4} M. The results obtained by these two groups of investigators were only comparable at 10^{-5}M. The difference between these two studies might have been because Coyle and Sperelakis used a tenfold higher concentration of isoproterenol which could have induced a greater basal slow-inward current. Indeed, the absolute values of Vmax are higher in the latter study than in Lynch's report. Sanchez-Chapula[18] studied isi current directly without using isoproterenol. He observed only a slight decrease (six per cent) by 10^{-5} M bupivacaine at a stimulation rate of 0.05 Hz; at 2×10^{-4} M, however, bupivacaine caused about 90 per cent inhibition. This depression of isi appears to be less important than reported by Coyle and Sperelakis and by Lynch at least at the concentration of 10^{-5} M.

Our results were also obtained directly on the isi current. Bupivacaine activity was observed to begin at 10^{-6} M. However, bupivacaine only caused 32.9 per cent inhibition at 10^{-4} M. The difference between our results and those of others can probably be explained by the difference in species used. Frogs are perhaps less sensitive than mammals to the effects of bupivacaine on the isi current.

Nevertheless, bupivacaine depressed not only the amplitude of the slow-inward current in the frog but also its kinetics. The time necessary to reach the peak of the current (time-to-peak) increased and inactivation occurred more slowly. Consequently, the time for calcium entry into the cells increased. This counterbalanced, at least in part, the decrease in the slow-inward current amplitude. As a result, the important negative inotropic effect induced by bupivacaine is probably due to other mechanisms. In addition, isi is not exclusively due to Ca^{++} but also to Na^{+}. In a series of experiments where Sanchez-Chapula[18] used a sodium-free solution, he observed that bupivacaine at a concentration of 5×10^{-5} M caused a 40 per cent depression in the slow-inward calcium current iCa, whereas the slow inward current isi was decreased by only 25 per cent in normal physiological solution. These findings explain in part the decrease in contractility reported by Lynch even though there was only a slight effect on the slow action potential Vmax. Furthermore, when we used the same voltage clamp technique, we observed that 10^{-5} M verapamil inhibited isi in frog atria by 59 per cent, whereas bupivacaine only caused 24.5 per cent inhibition. Therefore other factors, in particular an inhibition of the energy metabolism, might be implicated in the decline in contractility induced by bupivacaine as suggested in a previous study.[16]

Doubts have been raised concerning the validity of voltage clamping by the double sucrose gap technique.[22] However, it appears that the limitations of the voltage clamp experiments are largely dependent on the type of double sucrose gap device used; partitions with vaseline seals lead to less important errors.[23] Others have shown that double sucrose gap devices allow reasonable accuracy.[24,25]

Taken together, the results show that bupivacaine partially inhibited the slow-inward current. This inhibition appeared to be less important than that of the fast-inward current. The results do not explain the marked decline in contractility observed in all studies reported in the literature and suggest that other mechanisms are involved such as an effect on the energy metabolism of the cardiac myocyte.[28,29] Nevertheless, our findings might explain the sinus bradycardia and slowing of AV node conduction induced by bupivacaine.

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