Effects of sevoflurane on contractile response and electrophysiological properties in single cardiac myocytes of dog

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Effects of sevoflurane on contractile response and electrophysiological properties in single cardiac myocytes of dog

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Sevoflurane is a potent anesthetic that is widely used due to its efficacy in preventing arrhythmias associated with epinephrine. The mechanism of negative inotropic effect of sevoflurane was examined in the present study on canine single ventricular myocytes. The effects of sevoflurane on contractile response, membrane potential, membrane ionic currents and intracellular cyclic AMP were studied. Contractions were measured by a video-edge detector and membrane ionic currents were recorded using whole-cell voltage clamp technique. Sevoflurane, in concentrations ranging from 1.0% to 4.0%, decreased contraction of single ventricular cells. These inhibitory effects on the contractile response were dose-dependant and reversible. In normal Tyrode solution, sevoflurane decreased the plateau of action potentials and shortened the durations. In high-K^+ (26mM) Tyrode solution, the duration of slow action potential was shortened.

Voltage-clamp experiment with single canine ventricular cells

revealed that sevoflurane reduced the peak Ca^{2+} currents (I_{Ca}). These inhibitory effects of sevoflurane on I_{Ca} did not show use-dependent block. On the other hand, sevoflurane depressed Na^{+} currents (I_{Na}) only in high concentration (4.0%) and had no effect on the inward rectifier K^{+} currents (I_{K1}). The inhibition of I_{Ca} was not associated with changes in intracellular concentration of cyclic AMP except at a high dose (4.0%). These results suggest that the negative inotropic effect of sevoflurane is mediated via inhibition of I_{Ca} in canine ventricular cells. (Key words: Anesthetics, volatile, sevoflurane. canine heart; single cell; calcium current; sodium current; cyclic AMP)

Introduction

Sevoflurane (fluoromethyl 2,2,2-trifluoro-1-[trifluoromethyl] ethyl ether, SV) is a rapid-acting, potent, inhalational anesthetic and often used clinically today. Rapid uptake and elimination of sevoflurane are due to a low blood / gas partition coefficient reported to be 0.6[1]. Unlike other inhaled anesthetic agents such as halothane and isoflurane, SV is much safer in preventing arrhythmias due to epinephrine[2] and has a lower incidence of hepatic toxicity[3]. However, there are reports of circulatory depression by SV[4-6]. This effect is also observed with halothane and isoflurane and could be due to inhibition of intracellular Ca^{2+} release or inhibition of Ca^{2+} influx. The mechanism by which SV produces circulatory depression is not known. Previously, we reported that SV-induced inhibition of canine papillary muscle contraction may be due to a reduction of plateau and duration of the slow action potential[7]. However, detailed description of the

effects of SV on membrane currents are required to better understand its pharmacological effect. These studies were carried out to examine the effects of SV on contractile responses and membrane currents in canine single ventricular cells using whole-cell voltage clamp.

Materials and Methods

Cell isolation

Twenty three adult mongrel dogs of either sex weighing 8-12kg were anesthetized with ketamine (25mg/kg, intramuscular injection). Heparin (5000U) and pentobarbital (20mg/kg) were administered intravenously and the chest was opened. Hearts were excised immediately, and Tyrode solution (37°C) was perfused via the left coronary artery for 15 minutes. [Tyrode solution contained (in mM) NaCl:135, KCl:4, Na_2HPO_4:0.33, Dextrose:10, HEPES:10, $MgCl_26H_2O$:1, $CaCl_2$:1.8 (pH 7.4 by NaOH).] Then, calcium free Tyrode solution containing 0.05% collagenase was perfused for 30 minutes. After the treatment with the enzyme, left ventricle was minced into small pieces and agitated in calcium free Tyrode solution containing 0.05% collagenase and 0.006% trypsin[8] until single cells were released. The solution was then filtered and single cells resuspended in Ca^{2+}-free Tyrode solution. A total of 158 single

cells were used in this study.

Application of sevoflurane

SV was applied by bubbling 100% oxygen gas into Tyrode solution through a SV vaporizer (Sevotec 3, Ohmeda, West Yorkshire, England). The concentration of SV was monitored by anesthetic gas analyzer (CAPNOMAC, Datax, Helsinki, Finland). All experiments were performed at 37°C.

Effects of SV on contractile response, membrane potential, membrane ionic currents and intracellular cyclic AMP concentration were studied at SV concentration of 1.0, 2.0 and 4.0%. Comparisons between group data were made with Student's *t* test for unpaired samples and Kruskal-Wallis test was used to determine dose-dependency. All results are presented as mean±SD. Data were evaluated statistically significant at $P<0.05$.

Electrophysiological techniques

Dispersed cells were placed in a perfusion chamber (37°C) on the stage of an inverted microscope (IMT-2, Olympus, Tokyo, Japan) equipped with Nomarski optics. At 600X magnification, a hydraulic micromanipulator (MO-103, Narishige, Tokyo, Japan) was used to position glass microelectrodes with tip resistances of 3-5MΩ on the membranes of single ventricular cells. The pipette solution contained (in mM) potassium gluconate:140, ATP:2, HEPES:5, EGTA:0.15 (pH 7.2 by KOH). In order to maintain the cytoplasmic constituents and avoid 'run-down' of Ca^{2+} currents, the perforated patch technique was used[9]. This technique involves adding the antibiotic, nystatin, to the recording pipette. The transmembrane pores formed by nystatin allow monovalent ions in the pipette to equilibrate with the myoplasm. Large anions and/or molecules cannot pass through the nystatin induced pores thus effectively trapping them in the cytosol. The resulting lack of 'wash-out' of the

cytoplasmic factors appears to prevent the 'run-down' of I_{Ca}.

Contraction of single cells was initiated by extracellular field stimulation (0.5Hz, 15V, 1msec). Unloaded cell shortening was measured on-line using a video edge detector. A miniature CCD camera (FCD-725, Olympus, Tokyo, Japan) was attached to the camera port of the inverting microscope and the output was fed to a video edge detector (Crescent Electronics, Sandy, Utah, U.S.A.). Records of cell shortening were recorded by the digital recording system (RP-880, NF Electronic Instruments, Yokohama, Japan). Action potentials induced by intracellular stimulation were also recorded with the digital recording system.

Whole-cell voltage clamp technique was employed to measure ionic currents by using the patch clamp amplifier (Yale MK-V, New Haven, U.S.A.)[10]. All data were recorded on PCM recording system (PCM DP16, Shoshin EM Corp., Okazaki, Japan).

Assessment of intracellular cyclic AMP concentration

Isolated canine ventricular cells were suspended in normal Tyrode solution at a density of 1-2mg protein / ml. The protein concentration of the suspension was estimated by the method of Lowery with bovine serum albumin as the standard[11]. SV was vaporized by 100% oxygen gas and bubbled into the cell suspension for 10 minutes. At the end of the period, 450 μl of bubbled cell suspension was sampled. Then 25 μl of 2 N HCl and 25 μl of 100mM EDTA were added to the mixture, and the incubation tube was immersed in boiling water for 10 minutes to extract cyclic AMP, as described by Hazeki and Ui[12]. After a brief centrifugation of the acidified suspension at 3000 rpm for 10 minutes, the supernatant was subject to the radioimmunoassay by means of cyclic AMP assay kits (Yamasa Syoyu, Chiba, Japan). The cyclic AMP level was expressed as picomoles per milligram of protein.

Results

Previously, we demonstrated the negative inotropic effect of SV on multicellular preparation of the isolated canine ventricles[7]. In this study, we have directly measured the shortening of unloaded single cells from canine ventricles. SV dose-dependently decreased contractions induced by electrical stimulation in single canine ventricular cells (Fig.1, Table 1). These depressant effect were maximal within 5-7 minutes and recovered to control level in 10-15 minutes after wash out of SV.

As shown in figure 2 and presented in table 2, SV did not alter the resting membrane potential in normal Tyrode solution. On the other hand, SV decreased the plateau height of action potentials (Fig.2) and shortened the duration of action potentials. 30% repolarization (from plateau height) was reduced by approximately 377 msec at 2.0% SV. These results suggest that SV is likely to alter Ca^{2+} homeostasis. In order to examine the effects of SV on transmembrane

Ca^{2+} flux, the cells were bathed in high-K^+ (26mM) Tyrode solution containing 10^{-6} M isoproterenol. Under these conditions, the membrane potential was depolarized to -40 mV (and therefore inactive sodium currents). SV had no effect on the resting membrane potential, however, the duration of the action potential was markedly reduced (Fig.3, Table 3).

The effects of SV on I_{Ca} were examined using the whole-cell voltage clamp technique. Calcium currents were obtained by applying depolarizing pulses from a holding potential of -30mV or -40mV. Fig.4 shows dose-dependent inhibition of peak I_{Ca} by SV. The current-voltage relationship for peak I_{Ca} is shown in fig.5. The amplitude of peak I_{Ca} was inhibited by SV in a dose-dependent manner. I_{Ca} were measured in the absence of SV with test depolarization once every 5 seconds. Depolarization were then discontinued, drug was applied, and after a 10 minutes quiescent period, depolarizing test pulses were resumed. In this procedure, SV (2.0%) did not show an

use-dependent depression of peak I_{Ca} in single canine ventricular cells (Fig.6).

The effects of SV on I_{Na} were examined by applying a series of test pulses from a holding potential of -70mV to more positive potentials. Figure 7 shows the effect of SV on a series of I_{Na} recordings elicited by test pulses from -80 to -30mV in a single canine ventricular cell. I_{Na} were decreased only in 4.0% SV (1.0%:98.6±3.1, 2.0%:92.6±5.8, 4.0%:85.2±6.3* % of control, n=5, *$P<0.05$).

I_{K1} were measured by stepwise hyperpolarizing pulses from holding potential of -70mV to more negative command potentials. The experiment illustrated in figure 8 showed no change of I_{K1}.

In order to examine whether the effects of SV were due to changes in cyclic AMP, cyclic AMP levels were measured using radioimmunoassay. Table 4 shows that only at 4.0% SV, cyclic AMP levels were decreased.

Discussion

The present experiments provide information concerning the actions of SV on I_{Ca} using both electrophysiological and biochemical techniques. First, we examined effects of SV on contractile responses in single canine ventricular cells. Our results showed that contractions of single cells are decreased by SV (1.0-4.0%). Subsequently, we studied action of SV on membrane potentials in single ventricular cells. Following SV administration, the plateau phase and duration of the action potential were significantly decreased without change in the resting potential. Moreover, SV decreased duration of the slow action potential induced by high-K^+ (26mM) Tyrode solution. Slow action potentials recorded from partially depolarized myocytes results primarily from the entrance of Ca^{2+} into the cell presumably the L-type of Ca^{2+} channels. These results are similar to those of halothane, isoflurane and enflurane in multi-cellular preparation[13)14)]. Recently, Bosnjak et al reported

that both halothane and isoflurane produced a similar degree of inhibition of I_{Ca} elicited by the voltage-clamp studies in single guinea pig ventricular cells[15]. Our study in the first to show that SV produce a concentration-dependent depression of peak I_{Ca} without shifting the current-voltage relationship for Ca^{2+} channel activation. Therefore, the negative inotropic effect of SV on the myocardium are probably related, at least in part, to the depression of I_{Ca} similar to other inhalational agents[16-18]. In addition, we found that sevoflurane did not show use-dependent block (accentuation of drug inhibition by repetitive membrane depolarization) in single cells. These inhibitory blocking mechanism of I_{Ca} are different from thiopental or D600, an organic Ca^{2+} channel blocker. However, these results do not exclude the possibility that the inhibitory effects on myocardial contractility by SV may be due to affects on Na^{+} / Ca^{2+} exchange or intracellular Ca^{2+} stores[6]. To define a possible site at which SV acts, we measured

the cyclic AMP concentration of single cells using radioimmunoassay. The results show that at concentrations of SV that reduce I_{Ca}, cyclic AMP levels were not altered. These results differ from those in response to halothane[19]. I_{K1}, which regulates the resting membrane potential, was not significantly modified by SV (1.0-4.0%) consistent with no change in the resting membrane potential. On the other hand, only high concentration of SV (4.0%) significantly reduced I_{Na} in single cells. Thus, the depressant effect of SV on contractility in cardiac myocardium cannot be attribute to inhibition of Na^+ channels.

In summary, our study indicates that the decrease of myocardial contractile response in single canine cardiac cells is related, at least in part, to inhibition of I_{Ca} at the sarcolemma and not through changes in intracellular cyclic AMP levels.

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	contraction (% of control)
1.0 % SV	67.7±8.8
2.0 % SV	43.0±8.5
4.0 % SV	20.1±4.9

Table 1

Effects of sevoflurane (SV) on contractile response induced by electrical stimulation.

Contractions were decreased dose-dependently ($P<0.01$, Kruskal-Wallis test).

Data are presented as mean ± SD, n=8.

	control	1.0% SV	2.0% SV	4.0% SV
RMP (mV)	-76.0±1.8	-75.4±1.7	-75.0±1.9	-74.7±1.9
Plateau height (mV)*	29.9±1.5	24.6±1.7	20.5±1.1	19.1±0.9
Duration 30% (ms)*	689±44.6	487±55.9	377±47.0	215±22.7
Duration 90% (ms)*	861±30.5	775±34.1	648±34.7	427±40.4

Table 2

Effects of sevoflurane (SV) on membrane potentials in normal Tyrode solution.

RMP: resting membrane potential

*Data are presented as mean ± SD, n=12 (control: n=36), *P<0.01 (Kruskal-Wallis test).*

	control	1.0% SV	2.0% SV	4.0% SV
RMP (mV)	-40.4±2.3	-40.6±2.9	-40.4±2.7	-39.9±2.6
Duration 30% (ms)*	668±28.9	534±27.6	340±26.1	212±21.1
Duration 90% (ms)*	776±26.1	682±29.1	508±16.3	352±23.1

Table 3

Effects of sevoflurane (SV) on membrane potentials in high-K^+ Tyrode solution.

*Data are presented as mean ± SD, n=8 (control: n=24), *P<0.01 (Kruskal-Wallis test).*

	cyclic AMP
control	1.07±0.16
1.0 % SV	1.06±0.17
2.0 % SV	1.05±0.16
4.0 % SV	0.85±0.09*

Table 4

Effects of sevoflurane (SV) on intracellular cyclic AMP (pmol•mg^{-1} protein) concentration.

*Data are presented as mean ± SD, n=5, *P<0.05.*

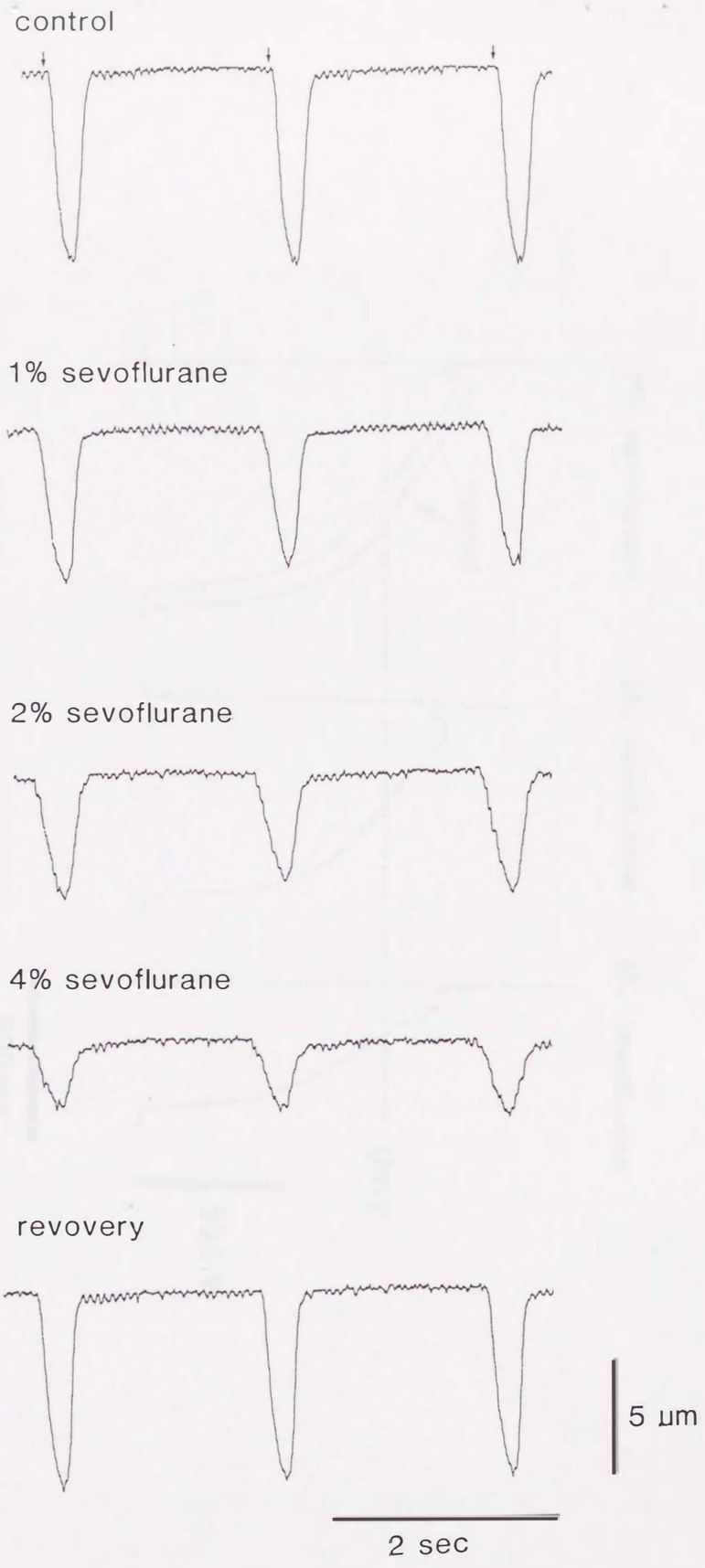


Fig.1

Cell shortening traces recorded by extracellular field stimulation (0.5Hz, 1msec, 15V) through Ag-AgCl electrodes in the absence (control), in the presence of sevoflurane (1%, 2%, 4%) and 10 minutes after washed off sevoflurane (recovery) in single canine ventricular cell.

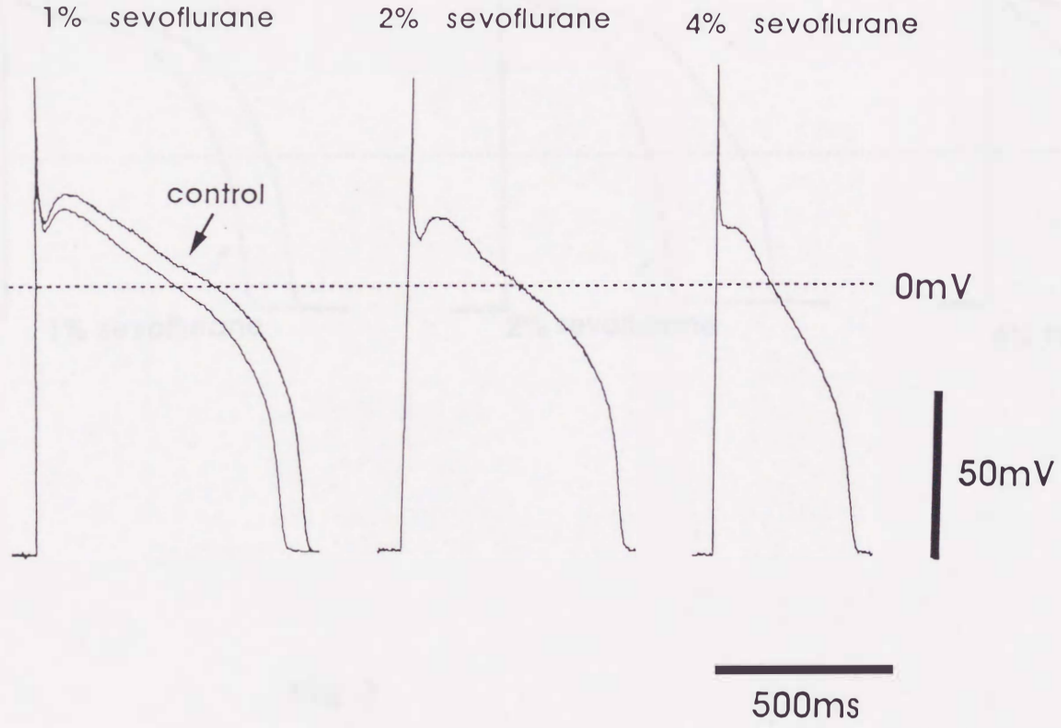


Fig.2

Effects of sevoflurane (1%, 2%, 4%) on normal action potentials induced by electrical stimulation (0.2Hz, 500μsec, 15V) in single left ventricular cells in normal Tyrode solution. Sevoflurane was applied to the bath.

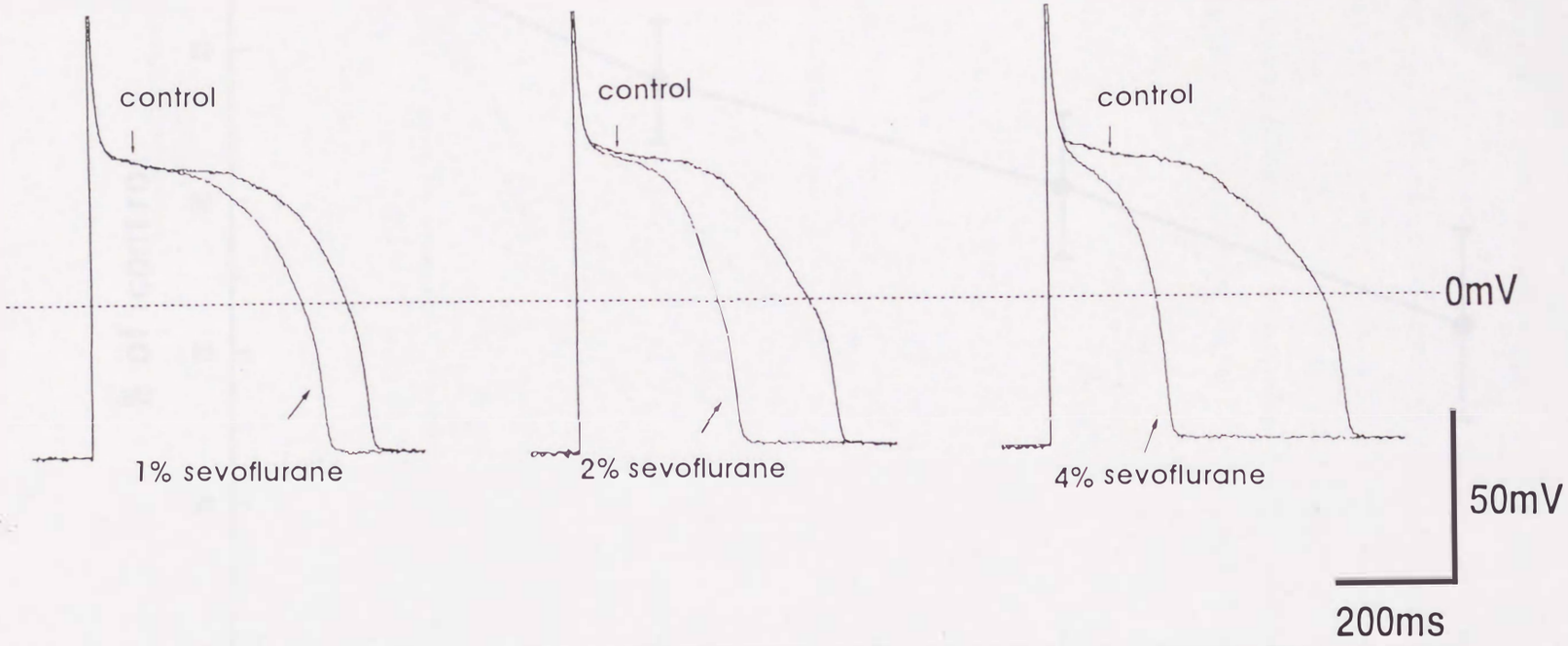


Fig.3

Effects of sevoflurane (1%, 2%, 4%) on slow action potentials induced by electrical stimulation (0.2Hz, 3msec, 15V) in high-K^+ (26mM K^+) Tyrode solution and in the presence of 10^{-6}M isoproterenol.

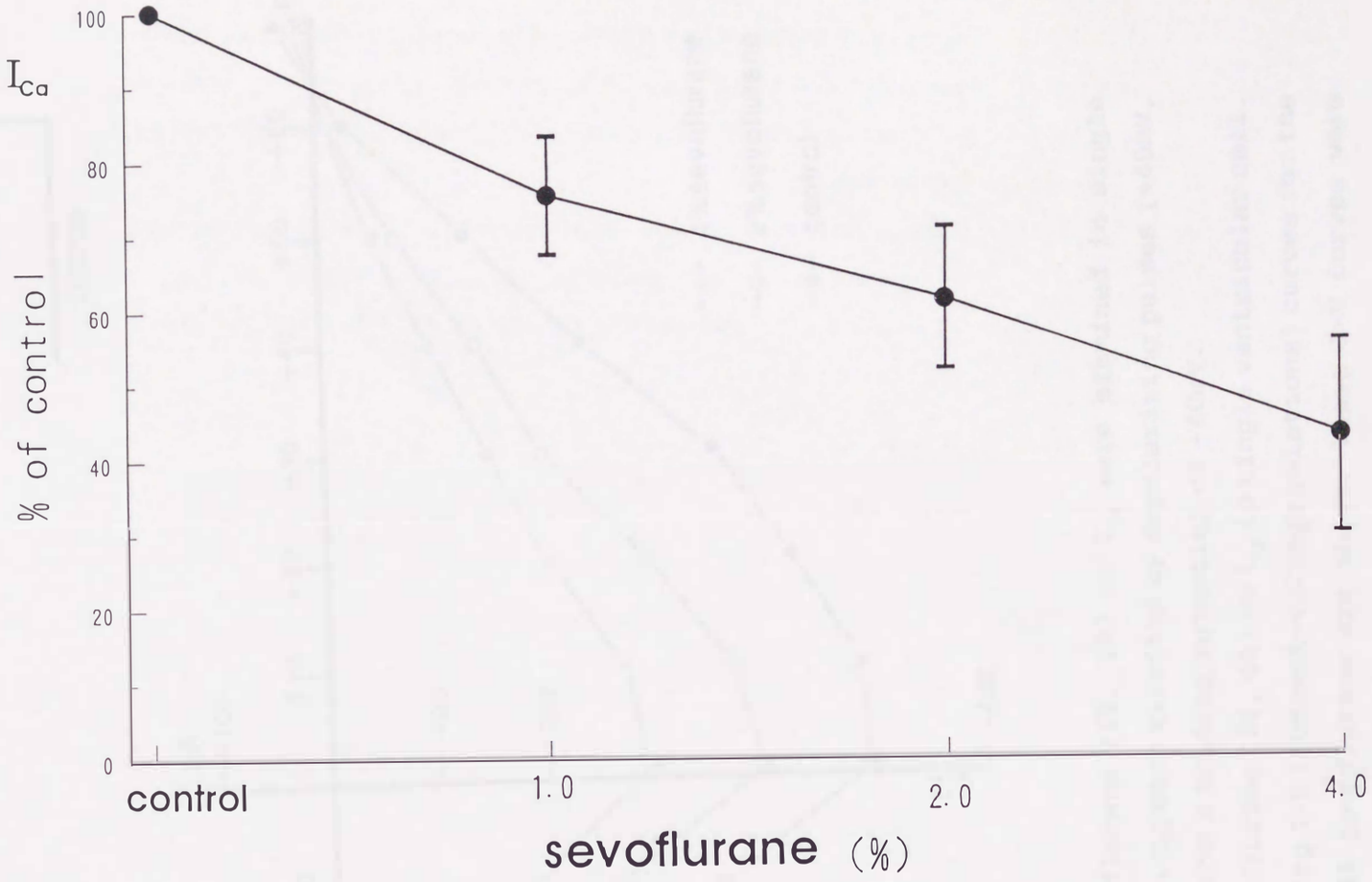


Fig.4

Dose-response curve relating to the inhibitory effect of sevoflurane on I_{Ca} in single ventricular cells. I_{Ca} were decreased dose-dependently from 1% sevoflurane ($P<0.01$, Kruskal-Wallis test). Data are presented as mean+SD, n=12.

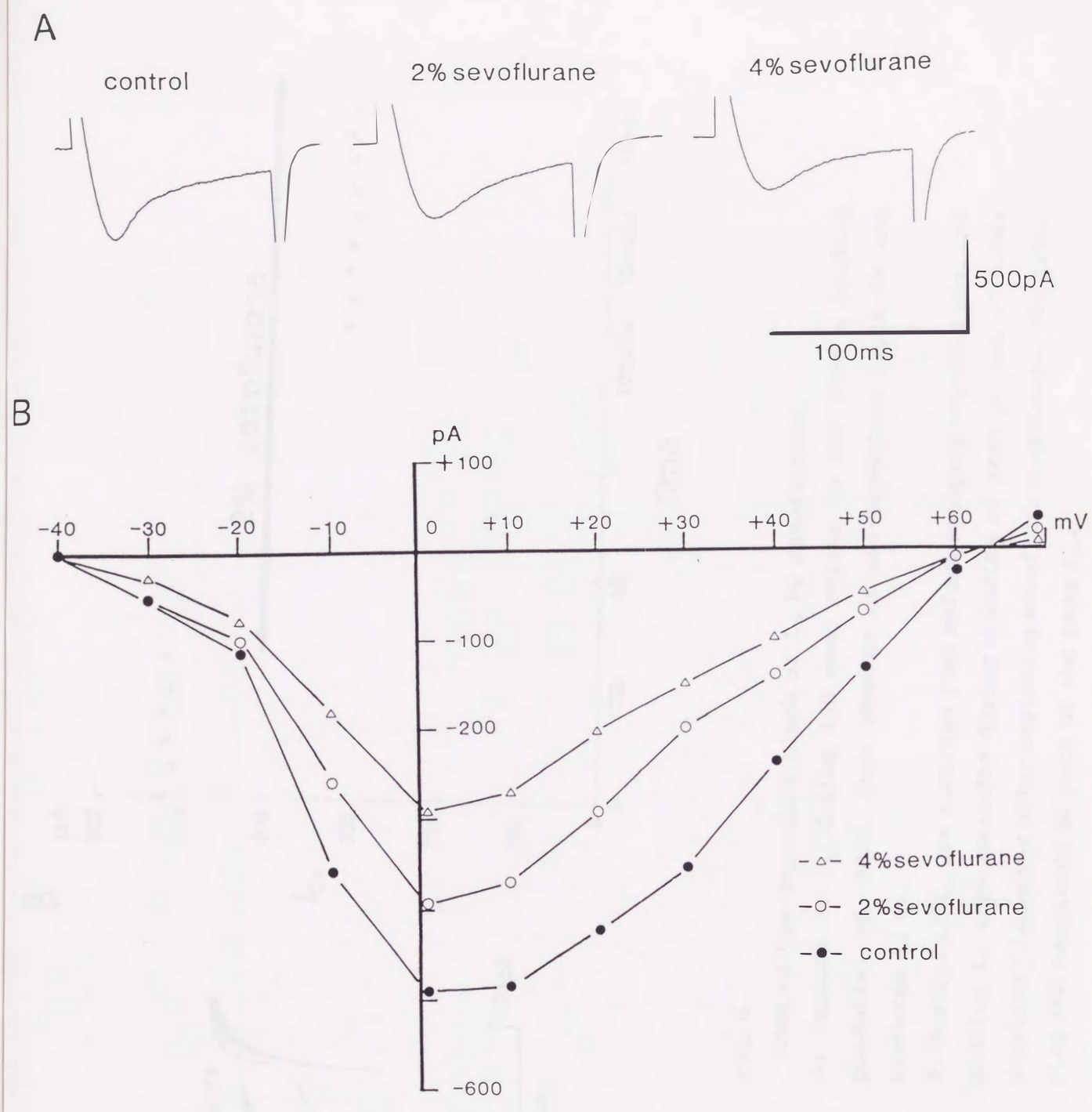


Fig.5

Effects of sevoflurane (2%, 4%) on I_{Ca} were examined in single ventricular cells. I_{Ca} were elicited by depolarizing pulses (+40mV, 100msec, 0.2Hz) from a holding potential of -40mV.

A: Effects of sevoflurane (2%, 4%) on I_{Ca} in single ventricular cell.

B: The corresponding I-V (current-voltage relations) curves for the control and 2%, 4% sevoflurane are shown. These I-V curves were plotted as peak inward current or minimum outward currents at each command potential.

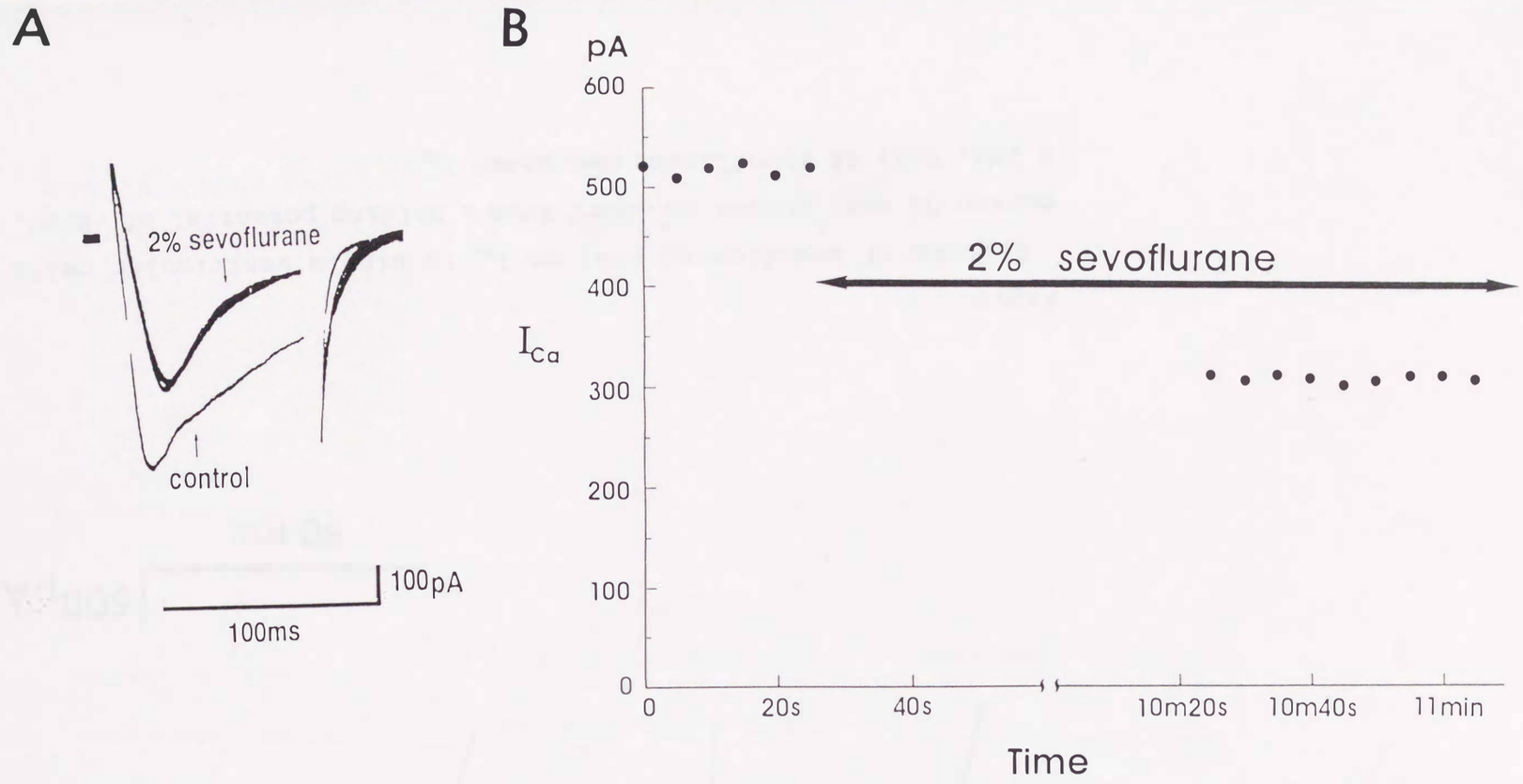


Fig.6

Lack of use-dependent block of I_{Ca} by sevoflurane.

A: Records of I_{Ca} during 100 msec pulses to 0mV from a holding potential of -30mV. Note absence of use-dependent block in the presence of SV.

B: Control of I_{Ca} was recorded just before applying sevoflurane, and smaller I_{Ca} were recorded during a series of test pulses at 0.2Hz starting 10 minutes after beginning sevoflurane exposure. The graph plot the magnitude of each of the peak I_{Ca}.

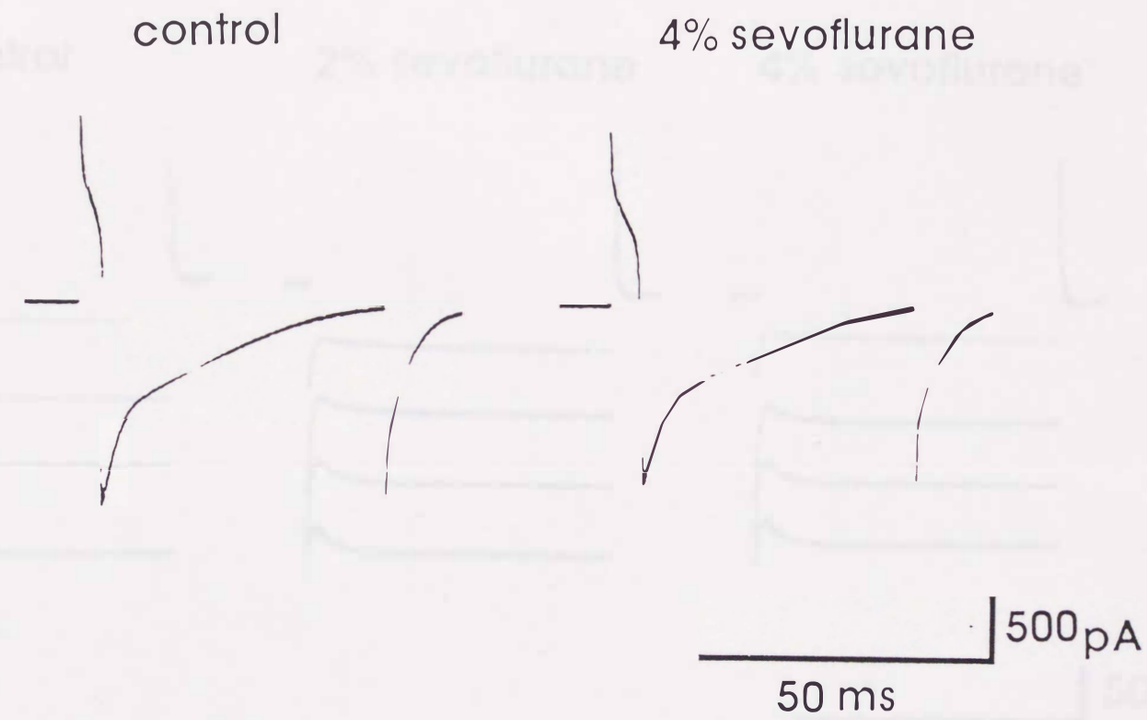


Fig.7

Effects of sevoflurane (4%) on I_{Na} in single ventricular cells during 50 msec pulses to -30mV from a holding potential of -80mV, 0.2Hz. Only 4% sevoflurane decreased I_{Na}.

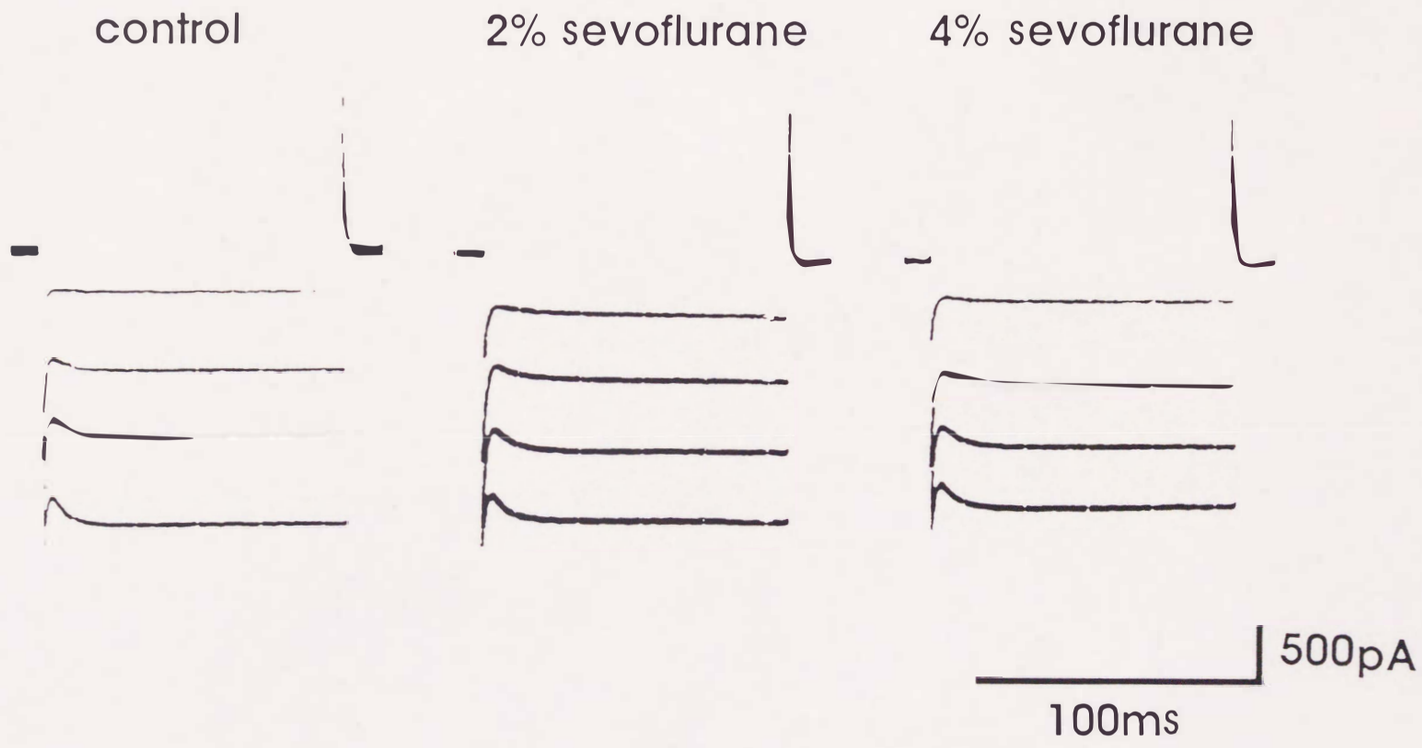
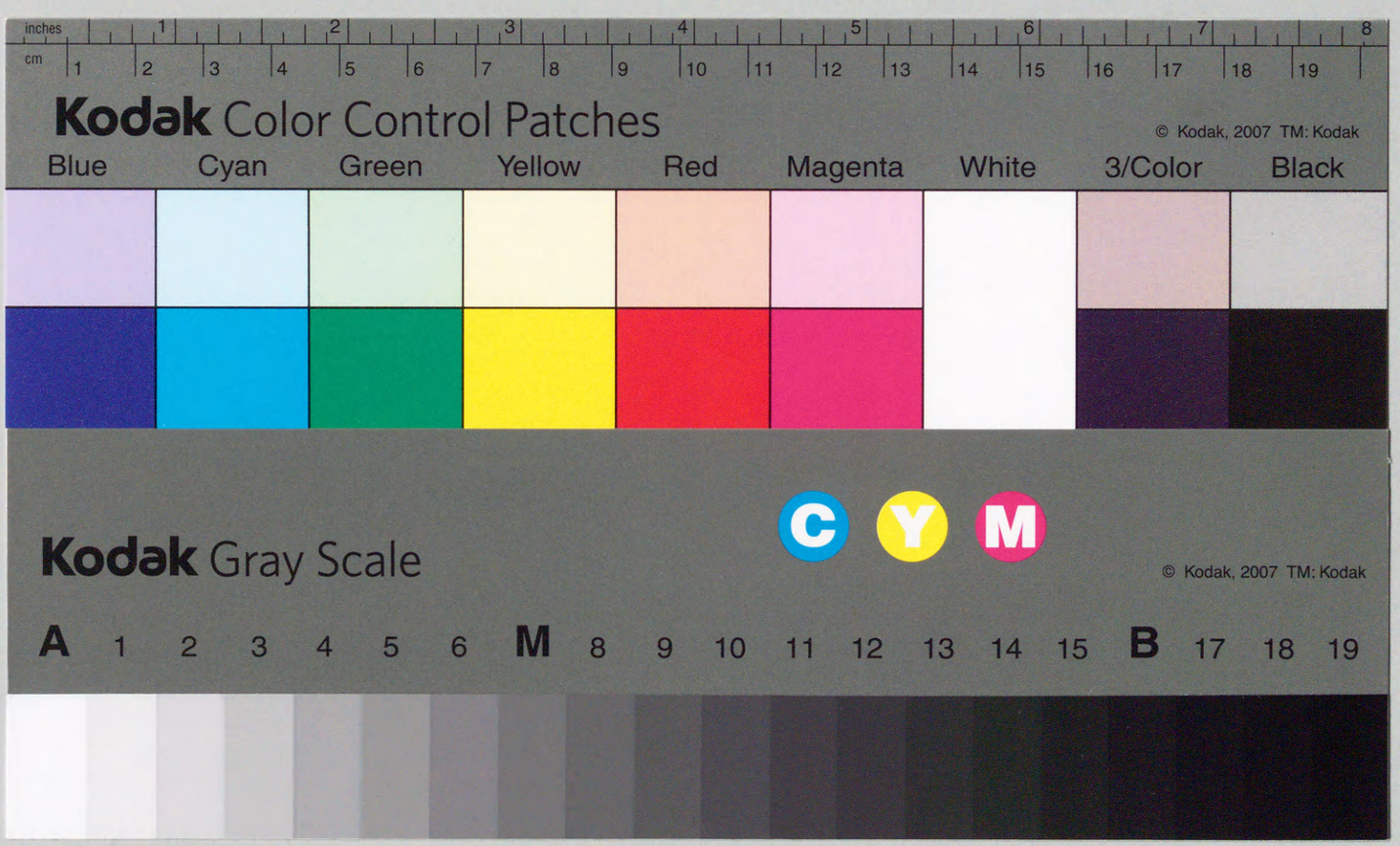


Fig.8

Effects of sevoflurane (2%, 4%) on I_{K1} in single ventricular cell during 100 msec pulses to -80, -90, -100, -110mV from a holding potential of -70mV, 0.2Hz.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