

## Desflurane Induces Only Minor $\text{Ca}^{2+}$ Release from the Sarcoplasmic Reticulum of Mammalian Skeletal Muscle

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**Background:** Desflurane is a weaker trigger of malignant hyperthermia than is halothane. There are very few data of the pathophysiologic background of this observation. Therefore, the authors' aim was to investigate the direct effect of desflurane on calcium release in skinned skeletal muscle fibers.

**Methods:** For the measurements, single saponin-skinned muscle fiber preparations of BALB/c mice were used. For  $\text{Ca}^{2+}$  release experiments, liquid desflurane at 0.6 and 3.5 mM was applied to weakly calcium-buffered solutions with no added  $\text{Ca}^{2+}$ . Desflurane was diluted in strongly  $\text{Ca}^{2+}$ -buffered solutions, with  $[\text{Ca}^{2+}]$  between 3.0 and 24.9  $\mu\text{M}$  for  $[\text{Ca}^{2+}]$ -force relations. Force transients were transformed into  $\text{Ca}^{2+}$  transients based on the individual  $[\text{Ca}^{2+}]$ -force relations. As controls, 30 mM caffeine and equimolar sevoflurane were investigated in the same muscle fibers.

**Results:** At 3.5 mM, desflurane induced peak force transients of  $8 \pm 4\%$  (mean  $\pm$  SD) of maximal  $\text{Ca}^{2+}$ -activated force ( $T_{\text{max}}$ ). These peak values were significantly smaller than those in the presence of 3.5 mM sevoflurane ( $24 \pm 10\%$  of  $T_{\text{max}}$ ,  $P < 0.05$ ), and 4 or 5 times smaller than previously reported  $\text{Ca}^{2+}$ -release-induced force transients by equimolar halothane. Calculated peak  $\text{Ca}^{2+}$  transients derived from force transients and induced by 3.5 and 0.6 mM desflurane were significantly smaller than

those induced by 30 mM caffeine. The  $[\text{Ca}^{2+}]$ -force relation was shifted by desflurane, resulting in a  $\text{Ca}^{2+}$ -sensitizing effect. The maximal  $\text{Ca}^{2+}$ -activated force was significantly increased by 0.6 mM desflurane in comparison with the control, with no added substance ( $P \leq 0.05$ ).

**Conclusion:** Desflurane induces only slight  $\text{Ca}^{2+}$  release in skinned skeletal muscle fibers. (Key words:  $\text{Ca}^{2+}$  release channel;  $\text{Ca}^{2+}$  sensitivity; fast-twitch muscle; skinned fibers.)

DESFLURANE has advantageous pharmacokinetic properties in comparison with other volatile anesthetics. Because of its low blood-gas coefficient general anesthesia can be more precisely controlled and recovery is faster.<sup>1,2</sup> Similar to other volatile anesthetics, however, it can trigger malignant hyperthermia (MH) in humans.<sup>3-5</sup> In several of these studies and reports, desflurane was the only agent that induced MH; whereas in the other four cases, the drug may have contributed to the occurrence of MH. It has been shown in two of six MH-susceptible pigs that desflurane induced no clinical signs of MH.<sup>7</sup> In addition, the clinical onset of MH induced by desflurane in MH-susceptible pigs was significantly slower than with halothane.<sup>8</sup> Desflurane, therefore, appears to be a weaker trigger of MH than does halothane. The mechanism for this lack of effect is obscure, *i.e.*, there is, to our knowledge, no study of the direct effect of desflurane on the calcium release channel in skeletal muscle.

Because we previously studied the effects of sevoflurane, isoflurane, and halothane on the calcium regulation by the sarcoplasmic reticulum (SR) in skinned skeletal muscle fibers during controlled physiologic conditions,<sup>9</sup> in the current study we investigated the effects of desflurane on (1)  $\text{Ca}^{2+}$  release from the SR, (2)  $\text{Ca}^{2+}$ -induced force, and (3) calcium sensitivity of the contractile proteins. Force transients were used to calculate the  $\text{Ca}^{2+}$  transients, based on the  $\text{Ca}^{2+}$  sensitivity of the same muscle fiber.<sup>10,11</sup>

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## Materials and Methods

After approval by the local Animal Care Committee of the University of Heidelberg, BALB/c mice were anesthetized with carbon dioxide and then killed by cervical dislocation. The fast-twitch muscle extensor digitorum longus (EDL) was isolated and single muscle fibers were prepared, as described previously.<sup>9</sup>

The concentrations of the experimental solutions (high-activating solution, high-relaxing solution, and low-relaxing solution; ionic strength: 157 mM, pH: 7.0, 22°C) were aimed to mimic the internal myoplasmic medium and were the same as those described previously with respect to adenosine triphosphate, creatine phosphate, creatine phosphate kinase, HEPES, EGTA,  $\text{Ca}^{2+}$ ,  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Mg}^{2+}$ .<sup>9</sup> The release solution consisted of the low-relaxing solution plus caffeine (30 mM), liquid desflurane, or sevoflurane. The solutions for measurements of the  $[\text{Ca}^{2+}]$ -force relation were prepared as reported previously.<sup>9</sup>

Liquid desflurane (0.5–5  $\mu\text{l}$ ) was diluted on ice, and liquid sevoflurane (0.5–5  $\mu\text{l}$ ) was diluted at room temperature in 5 ml of the corresponding solution, and both were kept in an air-tight glass syringe. Shortly before the muscle fiber was immersed in the solution, the solution with the anesthetic was put in a vial. Control measurements of the desflurane concentration were performed in a control vial with the anesthetic and then drawn out for concentration measurements. According to control measurements, the desflurane and sevoflurane concentrations were still higher than 90% of the original concentration at 3 min in the vials.

### Experimental Protocol

As described previously,<sup>9</sup> after permeabilization of the sarcolemma with saponin (50  $\mu\text{g}/\text{ml}$ ), the SR of the fiber was loaded at 0.41  $\mu\text{M}$   $\text{Ca}^{2+}$ . Before the fiber was exposed to the release solution that contained 30 mM caffeine, liquid desflurane, or sevoflurane, the loading was discontinued and the fiber was equilibrated. After the calcium release, maximum force ( $T_{\text{max}}$ ) was measured at 24.9  $\mu\text{M}$   $\text{Ca}^{2+}$ . Only those fibers were used that showed initial force transients reaching more than 30% of  $T_{\text{max}}$  with 30 mM caffeine. The  $[\text{Ca}^{2+}]$ -force relation was measured between 0.003 and 24.9  $\mu\text{M}$   $\text{Ca}^{2+}$  and fitted by a Hill curve, giving the  $\text{EC}_{50}$  value ( $\text{Ca}^{2+}$ -concentration for half-maximal isometric force activation) as a measure of the sensitivity and the Hill coefficient ( $h$ ).<sup>9</sup> Based on the individual  $[\text{Ca}^{2+}]$ -force relation reflecting the sensitivity of the  $\text{Ca}^{2+}$ -regulatory proteins and the

corresponding force development, the  $[\text{Ca}^{2+}]$ -force relation was used to convert force transients from the  $\text{Ca}^{2+}$ -release of the SR to apparent  $\text{Ca}^{2+}$ -transients.<sup>10,11</sup> The effects of 30 mM caffeine and sevoflurane (0.6 and 3.5 mM) were measured on the same fibers as a control.

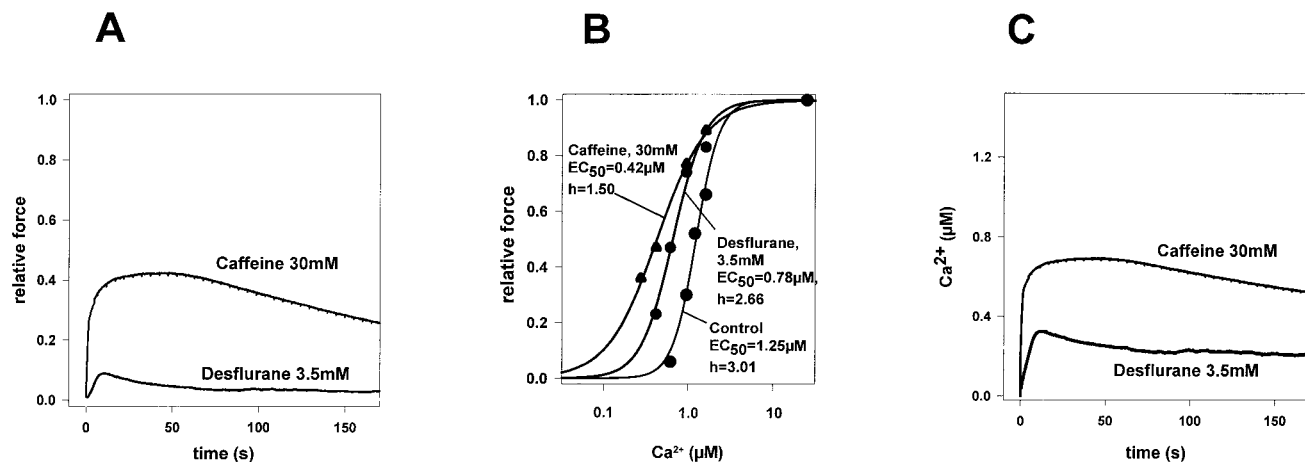
Desflurane concentrations were measured using gas chromatography-mass selective (GC-MS) analysis.<sup>9</sup> The corresponding anesthetic concentrations in volume percent at 37°C were calculated as described by Franks and Lieb<sup>12</sup> under consideration of the Ostwald water-gas partition coefficient in an aqueous solution at 37°C.<sup>13</sup>

For statistical analysis, the Kolmogorov-Smirnov test, one-way analysis of variance and the Bonferroni  $t$  test were applied, and  $P$  values less than 0.05 were defined as significant. Data are presented as mean  $\pm$  SD.

## Results

Desflurane was evaluated at the concentration of 0.6 (corresponding to 7.63 vol% desflurane at 37°C<sup>12</sup>) and 3.5 mM to evaluate the extent and direction of the effects. Following a standardized  $\text{Ca}^{2+}$  loading procedure by the SR in our preparations,<sup>9</sup> force transients induced by  $\text{Ca}^{2+}$  release from the SR were monitored.  $\text{EC}_{50}$  values and Hill coefficients ( $h$ ) of the  $[\text{Ca}^{2+}]$ -force relation were used to calculate directly the  $\text{Ca}^{2+}$  transient from each force transient; *i.e.*, each  $[\text{Ca}^{2+}]$ -force relation served as a  $\text{Ca}^{2+}$ -indicator and allowed reversal of each point of the force transient into the corresponding free  $\text{Ca}^{2+}$  level.<sup>10,11</sup>

A representative  $\text{Ca}^{2+}$ -induced force-transient,  $[\text{Ca}^{2+}]$ -force relation and the derived SR  $\text{Ca}^{2+}$  transient for desflurane 3.5 mM is shown in figure 1. This recording shows that the peak force transient was much lower than the standard transient of 30 mM caffeine (fig. 1A). The individual  $[\text{Ca}^{2+}]$ -force relation showed a higher  $\text{EC}_{50}$  value for desflurane when compared with 30 mM caffeine and showed a lower  $\text{EC}_{50}$  value when compared with the control, suggesting that desflurane has a  $\text{Ca}^{2+}$ -sensitizing effect on the thin filament system. The Hill coefficient of desflurane was greater than that of caffeine, resulting in a steeper  $\text{Ca}^{2+}$  dependence of isometric force (fig. 1B). The calculated peak  $\text{Ca}^{2+}$  transient, which was derived from the force curves, was, again, lower with desflurane than that induced by caffeine (fig. 1C); however, to a lesser degree. This can be explained by the comparison with the desflurane higher calcium sensitivity of the contractile proteins induced by the presence of 30 mM caffeine (fig. 1B).



**Fig. 1.** (A) Representative recording of the effect of 3.5 mM desflurane on a muscle fiber bundle, showing a calcium induced force transient normalized to maximal force obtained in the presence of  $24.9\ \mu M\ Ca^{2+}$ . As a control, the caffeine transient (30 mM) of the same fiber is shown. (B)  $[Ca^{2+}]$ -force relation of 3.5 mM desflurane in comparison with 30 mM caffeine and the control with no added substance. (C) The calculated  $Ca^{2+}$  transient is derived from the force transient based on the relation between  $[Ca^{2+}]$  and force of the same fiber.

The mean peak values of isometric force transients induced by desflurane in comparison with equimolar sevoflurane and 30 mM caffeine are shown in figure 2A. Peak isometric force transients induced by caffeine were significantly higher than those induced by desflurane or sevoflurane. At 3.5 mM, the effect induced by desflurane was significantly reduced in comparison with that of equimolar sevoflurane. The corresponding calculated calcium transients revealed lower peak values for desflurane in comparison with caffeine and a significant difference between 3.5 and 0.6 mM desflurane (fig. 2B).

The  $[Ca^{2+}]$ -force relation was fitted to the Hill equation with mean correlation coefficients ( $r$  values) more than 0.995 (SD < 0.05). Mean  $EC_{50}$  values after addition of desflurane or caffeine in comparison with the control, with no added substances, are summarized in figure 2C. No differences of Hill coefficients ( $h$ ) were observed between desflurane and the control. However, for 30 mM caffeine,  $h$  was reduced significantly in comparison with the control value or with desflurane.

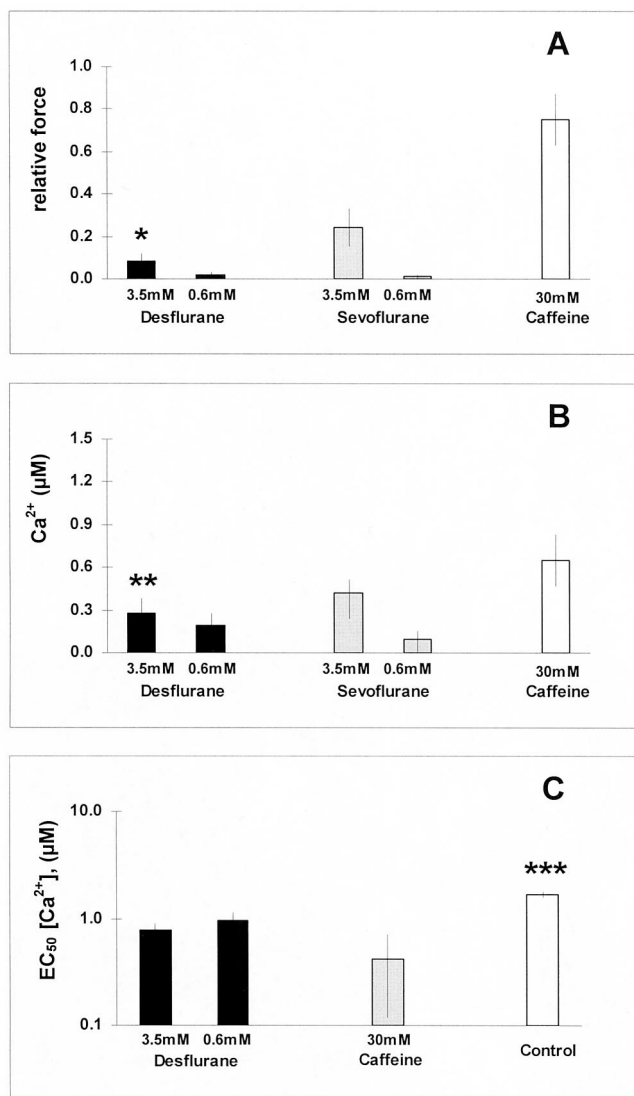
Compared with the control, the maximal  $Ca^{2+}$ -activated force in the presence of desflurane showed significantly increased (by 8.4%) maximal values at 0.6 mM desflurane, whereas no significant difference was found with 3.5 mM desflurane.

## Discussion

Volatile general anesthetics have an effect on calcium release mainly *via* the calcium release channel in skeletal

muscle fibers. This has been shown to differ quantitatively among halothane, isoflurane, and sevoflurane (halothane > isoflurane > sevoflurane).<sup>9</sup> Interestingly, we found in the current investigation that desflurane induces only minor  $Ca^{2+}$ -induced force transients. Mean maximal force values induced by 3.5 mM desflurane were 3 times smaller than those for equimolar sevoflurane of the same fiber ( $P < 0.05$ ) and 4 to 5 times smaller than previously shown mean maximal force values of isoflurane and halothane.<sup>9</sup> Therefore, our results show a possible pathophysiologic background for the observation that desflurane seems to be a weak trigger for MH. However, effects of volatile anesthetics on calcium release from the SR may vary in subjects with different types of calcium release channel mutations.

The skinned fiber model is one of the few preparations in which the direct effects of volatile anesthetics on intracellular organelles or proteins can be investigated during controlled conditions. In many preparations, experiments evaluate anesthetic effects on the entire cells, including the channels and receptors of the cell membrane, thereby complicating interpretation of intracellular signal transduction. One intracellular effect is  $Ca^{2+}$  sensitization of the contractile proteins. In addition to its application for transformation of force transients into calculated  $Ca^{2+}$  transients, two further aspects of  $Ca^{2+}$  sensitivity and volatile anesthetics are of importance. First, desflurane induced a leftward shift of the  $[Ca^{2+}]$ -force relation, the degree of which is comparable to that of previously reported effects of isoflurane, halothane, and

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**Fig. 2.** (A)  $\text{Ca}^{2+}$  release-induced peak force transients. The relative force induced by 3.5 mM desflurane is normalized to maximal force obtained in the presence of  $24.0 \mu\text{M}$   $\text{Ca}^{2+}$ . \*Significant difference ( $P < 0.05$ ) in comparison with all other groups. (B) Calculated peak  $\text{Ca}^{2+}$  transients derived from force transients based on the  $[\text{Ca}^{2+}]$ -force relation. \*\*Significant difference ( $P < 0.05$ ) when compared with desflurane 0.6 and 30 mM caffeine. (C)  $\text{EC}_{50}$  values of the  $[\text{Ca}^{2+}]$ -force relation (corresponding to the  $\text{Ca}^{2+}$  concentration at half-maximal force). \*\*\*Significant difference ( $P < 0.05$ ) when compared with 3.5 mM desflurane and 30 mM caffeine. Results are mean  $\pm$  SD,  $n = 5-8$ .

sevoflurane.<sup>9</sup> This similar effect of the four volatile anesthetics is in contrast to the differential effects on calcium release that we observed. Hence, volatile anesthetics seem to have similar affinities to hypothetical binding sites on the thin-filament system (actin-troponin-tropomyosin complex) in contrast to different affinities to hypothetical

binding sites at or near the  $\text{Ca}^{2+}$  release channel. Further studies with use of optical isomers of the anesthetics may provide more insight regarding the specificity of these cytosolic binding sites. Second,  $\text{Ca}^{2+}$  sensitivity of the thin-filament system may play an important role in the pathophysiology of MH after it has been induced by a triggering agent. Increased  $\text{Ca}^{2+}$  sensitivity lowers the activation threshold for actin-myosin interaction and results in increased force at submaximal  $\text{Ca}^{2+}$  concentrations. Increased force is the result of increased adenosine triphosphate turnover in the cross-bridge cycle, leading to an aggravation of adenosine triphosphate depletion and heat production. Desflurane (0.6 mM) increases force not only at submaximal  $\text{Ca}^{2+}$  concentrations, but also at maximal  $\text{Ca}^{2+}$  concentrations ( $24.9 \mu\text{M}$ ), which would also lead to increased adenosine triphosphate depletion if MH were already induced by a different trigger in the anesthetic regimen (e.g., suxamethonium). Because the leftward shift of the  $[\text{Ca}^{2+}]$ -force relation is comparable to that induced by the other volatile anesthetics, as reported previously,<sup>9</sup> all four volatile anesthetics may similarly aggravate existing MH. Nevertheless, it needs to be considered that the type of muscle may play a role in the effects of volatile anesthetics on calcium sensitivity of the contractile proteins. This has been shown when the effect of halothane was investigated in fast-twitch muscle (which we investigated) in comparison with slow-twitch muscle of human skinned striated fibers.<sup>14</sup>

Interestingly, our results show that maximal  $[\text{Ca}^{2+}]$ -induced force was increased with the lower concentration (0.6 mM) of desflurane, whereas there was no statistical difference in comparison with the control after exposure to 3.5 mM desflurane. This result implies that with exposure to higher concentrations of desflurane, the contraction-depressing effect may become more pronounced. Because membrane effects in our skinned fiber model can be excluded, the observed contraction-depressing effect should be a result of direct interaction with proteins that are directly involved in contraction (actin or myosin) or interaction with proteins that modulate contraction (e.g., troponin complex,<sup>15</sup> C protein,<sup>16,17</sup> or phospholamban<sup>15,18</sup>). The possible interactions of volatile anesthetics with intracellular proteins involved in contraction regulation are of interest for further investigations.

Many different isoforms of the ryanodine receptor exist in tissues, including the brain.<sup>19</sup> Our reported effects of volatile anesthetics on calcium release in muscle as a model system may, therefore, mirror effects of volatile anesthetics on intracellular signal transduction *via*  $\text{Ca}^{2+}$

in the brain. This hypothesis is supported by the observation that volatile anesthetics increase intracellular  $\text{Ca}^{2+}$  concentration in isolated neurons and in brain slices.<sup>20</sup> This intracellular  $\text{Ca}^{2+}$  increase is caused by release from intracellular stores, and is reduced significantly by the water-soluble ryanodine receptor blocker azumolene.<sup>20</sup>

In conclusion, our findings that desflurane induces only small calcium release in comparison with equimolar sevoflurane *in vitro* support the clinical observation that desflurane is a weak MH trigger. Intracellular effects include calcium sensitization at submaximal  $[\text{Ca}^{2+}]$  and increased maximal  $[\text{Ca}^{2+}]$  activated force at 0.6 mM desflurane.

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## References

1. Eger EI II: Physicochemical properties and pharmacodynamics of desflurane. *Anaesthesia* 1995; 50:3-8
2. Eger EI II, Gong D, Koblin DD, Bowland T, Ionescu P, Laster MJ, Weiskopf RB: The effect of anesthetic duration on kinetic and recovery characteristics of desflurane versus sevoflurane, and on the kinetic characteristics of compound A, in volunteers. *Anesth Analg* 1998; 86:414-21
3. Fu E, Scharf J, Mangar D, Miller W: Malignant hyperthermia involving the administration of desflurane. *Can J Anaesth* 1996; 43:687-90
4. Michalek-Sauberer A, Fricker R, Gradwohl I, Gilly H: A case of suspected malignant hyperthermia during desflurane administration. *Anesth Analg* 1997; 85:461-2
5. Garrido S, Fraga M, Martin MJ, Belda J: Malignant hyperthermia during desflurane-succinylcholine anesthesia for orthopedic surgery. *ANESTHESIOLOGY* 1999; 90:1208-9
6. Allen GC, Brubaker CL: Human malignant hyperthermia associated with desflurane anesthesia. *Anesth Analg* 1998; 86:1328-31
7. Wedel D, Iaizzo P, Milde J: Desflurane is a trigger of malignant hyperthermia in susceptible swine. *ANESTHESIOLOGY* 1991; 74:508-12
8. Wedel D, Gammel S, Milde J, Iaizzo P: Delayed onset of malignant hyperthermia induced by isoflurane and desflurane compared with halothane in susceptible swine. *ANESTHESIOLOGY* 1993; 78:1138-44
9. Kunst G, Graf BM, Schreiner R, Martin E, Fink RHA: Differential effects of sevoflurane, isoflurane, and halothane on  $\text{Ca}^{2+}$  release from the sarcoplasmic reticulum of skeletal muscle. *ANESTHESIOLOGY* 1999; 91:179-86
10. Makabe M, Werner O, Fink RHA: The contribution of the sarcoplasmic reticulum  $\text{Ca}^{2+}$ -transport ATPase to caffeine-induced  $\text{Ca}^{2+}$  transients of murine skinned skeletal muscle fibres. *Pflugers Arch* 1996; 432:717-26
11. Uttenweiler D, Weber C, Fink RHA: Mathematical modelling and fluorescence imaging to study the  $\text{Ca}^{2+}$ -turnover in skinned muscle fibers. *Biophys J* 1998; 74:1640-53
12. Franks NP, Lieb WR: Selective actions of volatile general anesthetics at molecular and cellular levels. *Br J Anaesth* 1993; 71:65-76
13. Hönemann CW, Washington J, Hönemann MC, Nietgen GW, Durieux ME: Partition coefficients of volatile anesthetics in aqueous electrolyte solutions at various temperatures. *ANESTHESIOLOGY* 1998; 89:1032-5
14. Tavernier BM, Haddad E, Adnet PJ, Etchrivi TS, Lacroix D, Reyford H: Isoform-dependent effects of halothane in human skinned striated fibers. *ANESTHESIOLOGY* 1996; 84:1138-47
15. Garvey JL, Kranias EG, Solaro RJ: Phosphorylation of C-protein, troponin I and phospholamban in isolated rabbit hearts. *Biochem J* 1988; 249:709-14
16. Gautel M, Zuffardi O, Freiburg A, Labeit S: Phosphorylation switches specific for the cardiac isoform of myosin binding protein-C: A modulator of cardiac contraction? *EMBO J* 1995; 14:1952-60
17. Kunst G, Kress KR, Gruen M, Uttenweiler D, Gautel M, Fink RHA: Myosin binding protein C, a phosphorylation-dependent force regulator in muscle that controls the attachment of myosin heads by its interaction with myosin S2. *Circ Res* 2000; 86:51-8
18. Kogler H, Ruegg JC: Cardiac contractility: Modulation of myofibrillar calcium sensitivity by beta-adrenergic stimulation. *Isr J Med Sci* 1997; 33:1-7
19. Sutko JL, Airey: Ryanodine receptor  $\text{Ca}^{2+}$  release channels: Does diversity in form equal diversity in function? *Physiol Rev* 1996; 76:1027-71
20. Kindler CH, Eilers H, Donohoe P, Ozer S, Bickler PE: Volatile anesthetics increase intracellular calcium in cerebrocortical and hippocampal neurons. *ANESTHESIOLOGY* 1999; 90:1137-45