Laboratory Investigations

Myocardial contractility and ischaemia in the isolated perfused rat heart with propofol and thiopentone

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The effects of propofol and thiopentone on myocardial contractility and global ischaemia were evaluated using an isolated non-working perfused rat heart preparation. Contractility was assessed using a tension transducer linked to the cardiac apex, and the contractility was expressed as a ratio of the deflection size before and after infusion of the drug. Ischaemia-induced leakage of myocardial proteins and ions (potassium and magnesium) was assessed by comparing the concentrations in the effluent perfusate immediately before and after 60 min of isothermic ischaemia, in the presence of propofol, thiopentone or plain Krebs' buffer solution (control). Mean contractility ratios of 1.15 and 1.3 were obtained with control and propofol groups respectively (NS), but were reduced to 0.5 in the thiopentone group ($P < 0.001$). The magnitude of the post-ischaemic leakage of proteins and potassium was similar in each group; however, the post-ischaemic leakage of magnesium was greater in the thiopentone group than in the propofol or control groups. These data suggest that, compared with thiopentone, propofol is not a potent negative inotrope, and that it may cause less disturbance of myocardial magnesium homeostasis during myocardial ischaemia.

Les effets du propofol et du thiopentone sur la contractilité myocardique et l'ischémie globale furent évalués utilisant une préparation isolée de cœur de rat perfusé au repos. La contractilité fut évaluée utilisant un transducteur de pression lié à l'apex et la contractilité fut exprimée par le ratio de la déflection avant et après perfusion des médicaments. La fuite des protéines myocardiques et des ions (potassium et magnésium) induite par ischémie fut évaluée en comparant des concentrations à la sortie dans le liquide de perfusion immédiatement avant et après 60 minutes d'ischémie isothermique en présence de propofol, thiopentone ou une solution de Krebs (contrôle). Des ratios de contractilité moyenne de 1,15 et 1,3 furent obtenus dans les groupes contrôle et propofol respectivement (NS), mais furent diminués à 0,5 dans le groupe thiopentone ($P < 0.001$). L'étendue de la fuite post-ischémique des protéines et du potassium était similaire dans chaque groupe, cependant elle était plus grande pour le magnésium dans le groupe thiopentone comparativement aux groupes contrôle et propofol. Ces données suggèrent que comparativement au thiopentone, le propofol ne possède pas un effet inotrope négatif puissant et qu'il peut causer moins de perturbation de l'homéostasie du magnésium myocardique lors de l'ischémie myocardique.

Key words
ANAESTHETICS, INTRAVENOUS: propofol, thiopentone; HEART: contractility, ischaemia.

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Reduction of systemic arterial pressure with thiopentone may involve alteration of several cardiovascular regulatory mechanisms including a direct negative inotropic effect on the myocardium.[1] However, the mechanisms of hypotension associated with propofol may not primarily involve depression of myocardial contractility. In patients without known coronary artery disease, the major effect seems to be reduced diastolic systemic arterial pressure consequent upon diminished systemic vascular resistance.[2,3] In patients with known ischaemic heart disease, the mechanisms are less clear. It appears likely that the hypotensive response in this group is not mediated

primarily by a reduction in the systemic vascular resistance, but rather by a reduction in the cardiac index as a result of reduced left ventricular filling pressures as demonstrated by lowered pulmonary capillary wedge pressures. Furthermore, in these patients indices of myocardial contractility such as dp/dt and ejection fraction remained unchanged.[4,5] However, considerable controversy exists as to possible effects of propofol on cardiac output,[6–8] in the light of studies associating a reduction in SVR with the infusion of propofol.[2,9] In these studies haemodynamic variables were recorded in the presence of respiratory acidosis, which itself can result in reduced SVR.[6] Further clinical studies may clarify this issue.[8] These issues are important as systemic hypotension in patients with coronary artery disease could compromise an already ischaemic myocardium. We used the isolated perfused rat heart preparation as a laboratory model to compare the direct effects of propofol and thiopentone on myocardial contractility.

Acute myocardial infarction is characterised by myocardial tissue damage secondary to ischaemia, and in the experimental setting the extent of damage is reflected by the leakage of myocardial intracellular proteins.[10,11] Alterations in normal potassium and magnesium homeostasis are important in the genesis of cardiac dysrhythmias, especially in the presence of myocardial ischaemia.[11–13] Although the importance of these ionic changes in myocardial ischaemia during anaesthesia has received little attention to date, the frequency of systemic potassium and magnesium deficiency is increasingly recognised, particularly in those receiving diuretic therapy.[14] Therefore, we studied the effects of propofol and thiopentone on the extent of myocardial ischaemic cellular damage and on ion flux, especially magnesium and potassium leakage, following experimental myocardial ischaemia.

Most methods of intraoperative cardiac protection utilise negative cardiac inotropic effects and are thought to exert their protective effects on the reduction of ischaemic damage by reducing the consumption of high energy intra-cellular compounds such as ATP. This may lessen the extent of ischaemic damage and lead to an improvement in haemodynamic recovery following an ischaemic episode.[15,16] We were interested to see whether the relative negative inotropic effect of thiopentone, in contrast to propofol, would afford any myocardial protection against ischaemic damage, as reflected by a reduction in the ischaemia-induced leakage of myocardial potassium, magnesium, or proteins.

Methods

Following approval from the institutional ethics committee, 27 male Wistar rats (300–400 g) were studied. Each animal received 500 iu heparin intraperitoneally, approximately 30 min before a stunning dose of inhaled ether and subsequent cervical dislocation, which was followed by rapid excision of the heart. The hearts were immersed in modified iced-cooled Krebs'-Henseleit buffer medium until beating ceased. The aorta was securely attached to a plastic cannula and retrogradely perfused in the Langendorff mode[11] with Krebs'-Henseleit buffer containing the following: $NaHCO_3$ 25.0 mM, NaCl 112.5 mM, KH_2PO_4 1.2 mM, $MgSO_4$ 1.2 mM, D-Glucose 12.0 mM, $CaCl_2$ 1.38 mM. The pH was corrected to 7.4, temperature maintained at 37° C, and the solution bubbled with 95% O_2 and 5% CO_2 gas mixture. The perfusion flow rate was constant at 7.5 $ml \cdot min^{-1}$, maintaining constant preload and afterload. A constant heart rate was ensured in all preparations by the use of an electronic epicardial ventricular pacemaker (0.05 mV, square wave, 0.05 msec), at a rate of 180 $beats^{-1}$. Contractility was measured using a Washington recorder coupled to an electronic tension transducer attached to the cardiac apex and "baseline" contractility was expressed as millimeters deflection of the recording needle. Following an initial period of stabilisation, all hearts were perfused with the Krebs'-Henseleit buffer solution for five minutes.

The "baseline" contractility was measured during this time. The hearts were then perfused for 30 min with Krebs' buffer (controls), or Krebs' buffer containing either propofol (1.6 $\mu g \cdot ml^{-1}$ or thiopentone (57 $\mu g \cdot ml^{-1}$). Allocation into the three groups ($n = 9$ in each group) was in a random fashion. Contractility measurements were continued for the following 30 min in the same manner as before. Comparisons were then made with the baseline contractility. The contractility ratio was defined as the magnitude of the deflection (mm) during 30 min infusion divided by the magnitude of the deflection (mm) during the baseline period.

After 30 min, the contractility measurements were discontinued and the effluent perfusate eluting from the hearts were then sampled.[10,11] A single 500 μl aliquot was collected and following this, global isothermic ischaemia was applied to all hearts for 60 min. This was achieved by stopping all the perfusion to the hearts whilst maintaining the preparations in an insulated double-walled glass jacket with the internal temperature held constant at 37° C. Following global ischaemia, perfusion was re-established and a single 500 μl aliquot of effluent perfusate was collected immediately. All samples were assayed for total protein concentration using the standard Lowry technique. In addition, the samples were assayed for potassium and magnesium concentrations using a Varian-Tectron AA-475® atomic spectrometer (emission at 766 nm and absorption at 285 nm respectively). The magnitudes of the differences between the concentrations of protein, potassium, and magnesium assayed before and after ischaemia were assumed to represent the degree of

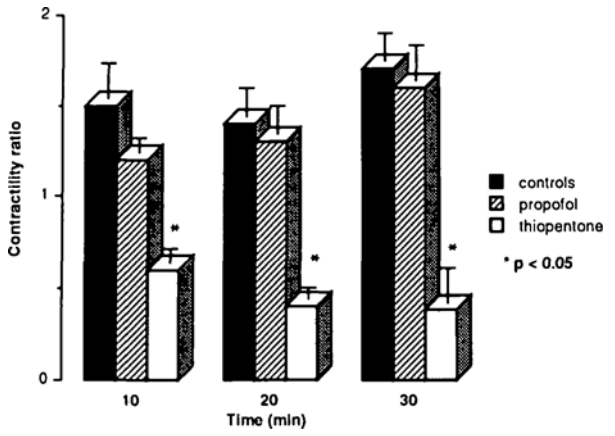

FIGURE 1 Contractility changes at 10, 20 and 30 minutes.

myocardial leakage of these substances. Statistical analysis included paired Student's t test for within-group analysis and unpaired t test for between-group analysis. All t test analyses were modified using the Bonferroni correction. Values of $P < 0.05$ were considered significant.

Results

Myocardial contractility

At ten-minutes the contractility ratios (Figure 1) for the control, propofol and thiopentone groups were 1.5 ± 0.19 (mean ± SEM), 1.3 ± 0.08 and 0.6 ± 0.08 respectively. At 20 min the contractility ratios for the control, propofol and thiopentone groups were 1.4 ± 0.16, 1.3 ± 0.16 and 0.5 ± 0.07 respectively. At 30 min the contractility ratios for the control, propofol and thiopentone groups were 1.6 ± 0.17, 1.5 ± 0.18 and 0.4 ± 0.06 respectively. At all times there was no difference between the contractility ratios in the control and propofol groups, but at every stage the contractility ratio was lower in the thiopentone group than in the control ($P < 0.05$) and propofol ($P < 0.05$) groups. Thus, there was a negative inotropic effect associated with thiopentone which was not observed with propofol.

Myocardial protein leakage

Following ischaemia, the protein concentration (Figure 2) in the effluent perfusate increased from 0.02 ± 0.01 mg% (mean ± SEM) to 0.20 ± 0.04 mg% in the control group ($P < 0.05$), from 0.02 ± 0.00 mg% to 0.20 ± 0.05 mg% in the propofol group ($P < 0.05$), and from 0.03 ± 0.00 mg% to 0.29 mg% ± 0.07 mg% in the thiopentone group ($P < 0.05$). There were no significant differences in the magnitude of the protein leakage among any of the groups.

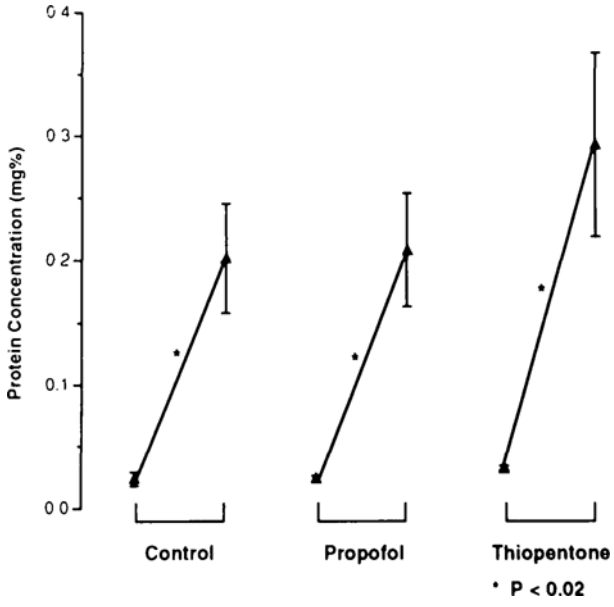

FIGURE 2 Protein concentrations before and after ischaemia (mean ± SEM).

Myocardial potassium leakage

Following ischaemia, the concentration of potassium (Figure 3) in the effluent perfusate increased from 4.0 ± 0.05 mM (mean ± SEM) to 5.8 ± 0.34 mM in the control group ($P < 0.01$), from 3.9 ± 0.08 mM to 6.4 ± 0.43 mM in the propofol group ($P < 0.01$), and from 4.0 ± 0.04 mM to 6.5 ± 0.27 mM in the thiopentone group ($P < 0.01$). There were no statistically significant differences in the magnitude of the potassium leakages among the three groups.

Myocardial magnesium leakage

Following ischaemia, the concentration of magnesium (Figure 4) in the effluent perfusate increased from 1.2 ± 0.01 mM (mean ± SEM) to 1.6 ± 0.08 mM in the control group ($P < 0.05$), from 1.2 ± 0.01 mM to 1.4 ± 0.08 mM in the propofol group ($P < 0.05$), and from 1.2 ± 0.02 mM to 1.7 ± 0.07 mM in the thiopentone group ($P < 0.01$). There was no significant difference in the magnitude of the magnesium leakage between the control and propofol groups but there was a greater leakage of magnesium in the thiopentone group than in the control ($P < 0.05$) and propofol ($P < 0.05$) groups.

Discussion

At the concentrations used, thiopentone resulted in profound negative inotropic effects whereas propofol did not. These data support clinical studies suggesting that propofol does not affect myocardial contractility.[1-5,17]

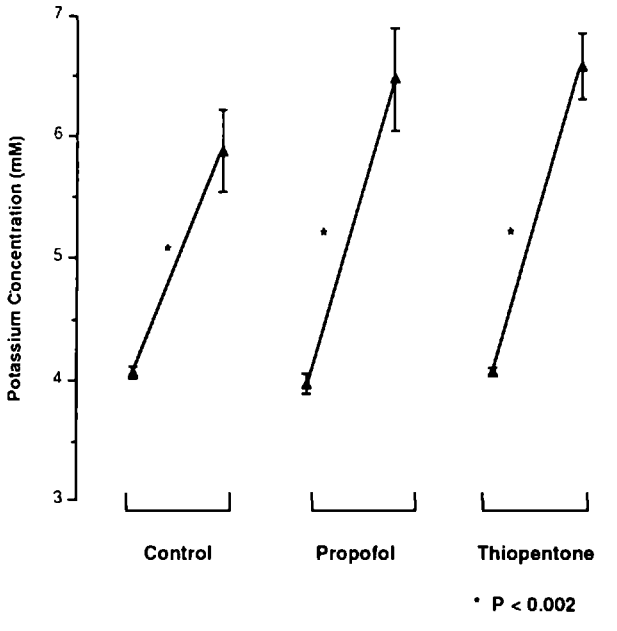

FIGURE 3 Potassium contractions before and after ischaemia (mean ± SEM).

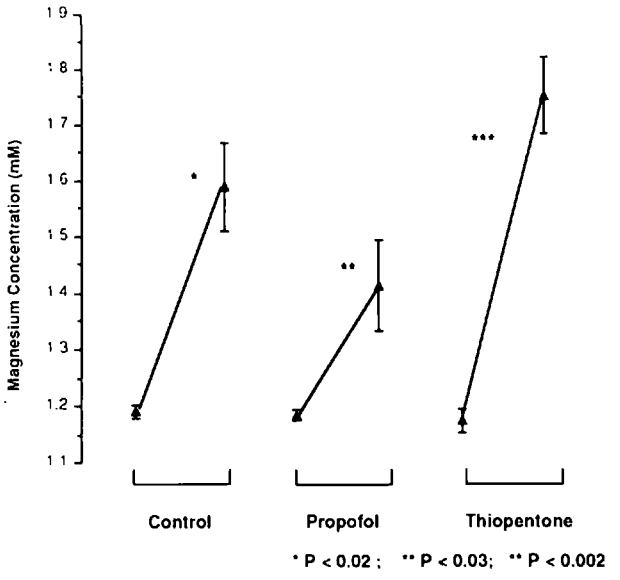

FIGURE 4 Magnesium concentrations before and after ischaemia (mean ± SEM).

The free concentrations of propofol and thiopentone used in these experiments were 1.6 $\mu g \cdot ml^{-1}$ and 57 $\mu g \cdot ml^{-1}$. The mean free plasma concentration of thiopentone necessary for anaesthesia was found to be approximately 6.0 $\mu g \cdot ml^{-1}$.[18] The whole blood EC_{50} for propofol is approximately 1.6 $\mu g \cdot ml^{-1}$,[19] and the plasma protein binding of propofol is approximately 97%.[20] Thus, the free plasma EC_{50} is approximately 0.05 $\mu g \cdot ml^{-1}$. In these experiments therefore, we have used approximately 32 times the EC_{50} of propofol and 9.5 times the mean anaesthetizing concentration of thiopentone. Despite the bias of free drug concentrations resulting in a lower relative concentration of thiopentone, the negative inotropy and increased myocardial leakage of magnesium ion were observed with thiopentone and not with propofol.

Cardiovascular regulatory mechanisms have recently been studied using experimental dogs suggesting that maintenance of cardiac output and systemic arterial pressure during propofol anaesthesia is primarily dependant upon adequate cardiac filling pressures.[17] However, this is an area of considerable controversy, as some of the clinical studies claiming that propofol-induced hypotension occurs as a result of reduced systemic vascular resistance[2,9] were confounded by the presence of an appreciable degree of respiratory acidosis.[6] The potential molecular mechanisms of anaesthetic-induced negative inotropism have recently been reviewed,[21] and it is possible that the differences in myocardial contractility observed with thiopentone and propofol may reflect a differential regulation of myocardial intra-cellular calcium control mechanisms. The calcium antagonist properties of magnesium are well recognised, and have been the subject of much discussion.[12] Perhaps, therefore, the different effects of thiopentone compared with propofol on the post-ischaemic myocardial loss of magnesium may be related to the differential effects on myocardial contractility, at least in part, through a magnesium-mediated alteration in intra-cellular myocardial calcium regulation.

Throughout the initial 30 min, the contractility ratios for the control and propofol groups were greater than unity, reflecting an increase in contractility over that period. This may reflect delayed recovery from hypothermic dissection, late release of endogenous catecholamines,[22] or perhaps a spontaneous alteration in the apparatus gain. Despite this, we feel that the comparisons between the groups are valid because each preparation functioned as its own control. A separate control group was included in the experiments, and all preparations were subjected to identical experimental protocols.

The size or extent of a myocardial infarct is usually estimated by the degree of elevation of specific myocardial proteins in the plasma. In experimental settings where the heart is perfused by a crystalloid solution, the leakage of total protein or myoglobin reflects the extent of ischaemic myocardial damage.[10,11] Several studies have suggested that pre-treatment with negative inotropic

agents prior to the onset of ischaemia may render the myocardium less susceptible to ischaemic damage. Studies have examined the protective effects of β-adrenergic receptor blockade,[10] hypothermia,[16] verapamil,[23] and elevated concentrations of magnesium.[15] One proposed mechanism for these modes of cardiac protection suggests that high intracellular compounds are conserved because of the decreased cardiac work, induced by the negative inotropism. Thus, these compounds are available to minimize hypoxic tissue damage and to improve post-ischaemic myocardial recovery.[15,23]

However, in our study we found that despite the negative inotropic effect of thiopentone, there was no decrease in the extent of ischaemic damage as reflected by the leakage of myocardial proteins. A decrease in the postischaemic leakage of myocardial proteins may have indicated some degree of myocardial protection.

In this study, the leakage of myocardial potassium following ischaemia closely paralleled the protein leakage in all three groups. This is similar to the findings in other studies which used comparable experimental models of myocardial infarction.[10,11] Previous studies which examined both protein and potassium leakages following experimental myocardial infarction found that cardioprotective regimens affected leakage of potassium to the same extent as protein leakage.[10,11] No differences in the degree of potassium leakage following ischaemia were observed among the three groups, suggesting that neither propofol nor thiopentone exerts a specific effect on myocardial potassium following an ischaemic episode.

There was leakage of myocardial magnesium following ischaemia in all the groups. However, thiopentone appears to have a selective effect on myocardial magnesium loss following infarction. Whilst the magnitude of leakage of protein and potassium were similar in all three groups, the leakage of magnesium was greater in the thiopentone group than in either the control or propofol groups. Furthermore, there was no difference in the degree of magnesium leakage between the control and propofol groups. As magnesium levels are important in the genesis and maintenance of cardiac rhythms,[10,11,13] this differential effect on myocardial magnesium loss following ischaemia may have important implications in the development of dysrhythmias in the intraoperative state. Magnesium protects against ischaemic injury.[24] Magnesium is known to have a pivotal role in several aspects of myocardial cellular membrane functions including modulation of potassium and calcium flux,[13] stabilisation of the sodium/potassium ATP-ase and calcium ATP-ase pump systems, attenuation of specific cardiac myocyte potassium currents,[25] and is central to the optimal regulation of myocyte contractility-tension relationships.[26] Magnesium deficiency is increasingly recognised in patients,[27] is known to increase susceptibility to ventricular dysrhythmias, and increases resistance to correction of potentially serious potassium deficiency.[14,27]

There are well described intraspecies variations in susceptibility to tissue injury, drug effect, and drug toxicity. This has been documented in the differential effects of elevated magnesium on cardiac ischaemic injury in rats and rabbits.[15] Therefore, caution must be exercised before extrapolating these results to the human situation.

Numerous difficulties exist in planning dosage regimens and protocols. The ED_{50} of propofol, whilst known for humans,[19] is unknown for rats. Furthermore, neither the plasma-tissue partition coefficient nor the dose–response characteristics for the cardiac effects of propofol are yet known.

Whilst it is not yet possible to extrapolate these data from the experimental setting to the anaesthetic care of surgical patients, they point towards the importance of further work in the area of myocardial contractility and the cellular effects of general anaesthetic agents.

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References

1 *Patrick MR, Blair IJ, Feneck RO, Sebel PS.* A comparison of the haemodynamic effects of propofol and thiopentone in patients with coronary artery disease. Postgrad Med J 1985; 62 (Suppl): 23–7.

2 *Clayes MA, Gepts E, Camu F.* Haemodynamic changes during anaesthesia induced and maintained with propofol. Br J Anaesth 1988; 60: 3–9.

3 *Coates DP, Monk CR, Prys-Roberts C, Turtle M.* Haemodynamic effects of infusion of the emulsion formulation of propofol during nitrous oxide anaesthesia in humans. Anesth Analg 1987; 66: 64–70.

4 *Pinaud M, LePage JY, Juge C, Helias J, Cozian A, Souron R.* Joint isotope and haemodynamic studies of the effects of propofol on left ventricular function in the patient suffering from coronary heart disease. Ann Fr Anesth Reanim 1987; 6: 243–6.

5 *Kling D, Bachmann B, Moosdorf R, Hempelmann G.* The haemodynamic effects of propofol with midazolam: a study in patients undergoing coronary surgery. Der Anesthesist 1987; 36: 640–5.

6 *Van Aken H, Brussel T.* Propofol causes cardiovascular depression II (Letter). Anesthesiology 1990; 72: 394–5.
7 *Sebel PS, Lowden JD.* Propofol: a new intravenous anesthetic. Anesthesiology 1989; 71: 260–77.
8 *Sebel PS, Lowden JD.* (Letter). Anesthesiology 1990; 72: 396.
9 *Monk CR, Coates DP, Prys-Roberts C, Turtle MJ, Spelina K.* Haemodynamic effects of a prolonged infusion of propofol as a supplement to nitrous oxide anaesthesia. Br J Anaesth 1987; 54: 954–60.
10 *Garvey E, Counihan TB, Ryan MP.* Investigation of cardioprotection by β-adrenoreceptor antagonists in clinical and experimental myocardial infarction. Br J Pharmacol 1983; 80: 698–9.
11 *Nayler WG.* Beta-blockers in experimental myocardial infarction. Acta Med Scand 1981; 651 (Suppl): 139–45.
12 *Iseri LT, French JH.* Magnesium: nature's physiological calcium channel blocker. Am Heart J 1984; 108: 188–93.
13 *Sheehan JP, Seelig MS.* Interactions of magnesium and potassium in the pathogenesis of cardiovascular diseases. Magnesium 1984; 3: 301–14.
14 *Ryan MP.* Diuretics and potassium/magnesium depletion. Directions for treatment. Am J Med 1987, 82: 38–47.
15 *Bersohn MM, Shine KI, Sterman WD.* Effects of increased magnesium on recovery from ischaemia in rat and rabbit hearts. Am J Physiol 1982; 242: H89–H93.
16 *Hearst DJ, Stewart DA, Braimbridge MV.* Myocardial protection during ischemic cardiac arrest. J Thorac Cardiovasc Surg 1978; 75: 877–85.
17 *Goodchild CS, Serrao JM.* Cardiovascular effects of propofol in the anaesthetized dog. Br J Anaesth 1989; 63: 87–92.
18 *Becker KE.* Plasma levels of thiopental necessary for anesthesia. Anesthesiology 1978; 49: 192–6.
19 *Spelina KR, Coates DP, Monk CR, Prys-Roberts C, Norley I, Turtle MJ.* Dose requirements of propofol by infusion during nitrous oxide anaesthesia in man. Br J Anaesth 1984; 58: 1080–4.
20 *Servin F, Desmonts JM, Haberer JP, Cockshott ID, Plummer GF, Farinotti R.* Pharmacokinetics and protein binding of propofol in patients with cirrhosis. Anesthesiology 1988; 69: 887–91.
21 *Rusy BF, Komai H.* Anesthetic depression of myocardial contractility: a review of possible mechanisms. Anesthesiology 1987; 67: 87–92.
22 *Hirche HJ, Franz L, Bos R, Bissig RL, Schamm M.* Myocardial extracellular hydrogen ion and potassium increase and noradrenaline release as a possible cause of arrhythmias following acute coronary occlusion in pigs. J Mol Cell Cardiol 1980; 12: 579–93.
23 *Watts JA, Koch CD, LaNone KF.* Effects of calcium on energy metabolism: calcium and heart function after ischaemia. Am J Physiol 1980; 238: H909– H916.
24 *Borchgrevink PC, Bergan AS, Bakoy OE, Jynge P.* Magnesium and reperfusion of ischemic rat heart as assessed by P-31 N.M.R. Am J Physiol 1989; 225: H1–H8.
25 *Matsuda H.* Open-state substructure of inwardly rectifying potassium channels revealed by magnesium block in guinea pig heart cells. J Physiol 1988; 397: 237–58.
26 *Kerrick WGL, Donaldson SDB.* Effects of magnesium on submaximal calcium activated tension in skinned fibres of frog skeletal muscle. Biochim Biophys Acta 1972; 4: 367–74.
27 *Turlapaty PD, Altura BD.* Magnesium deficiency produces spasm of the coronary arteries: relationships to etiology of sudden death in ischemic heart disease. Science 1980; 208: 198–200.