British Journal of Anaesthesia **100 (6):** 841–5 (2008)
doi:10.1093/bja/aen089 Advance Access publication April 19, 2008

BJA

Lipophilic β-adrenoceptor antagonist propranolol increases the hypnotic and anti-nociceptive effects of isoflurane in a swine model

T. Kurita*, K. Takata, K. Morita and S. Sato

Department of Anesthesiology and Intensive Care, Hamamatsu University School of Medicine, 1-20-1 Handayama, Hamamatsu 431-3192, Japan

**Corresponding author. E-mail: tadkur@hama-med.ac.jp*

Background. We have previously reported that landiolol, an ultra-short-acting β1-adrenoceptor antagonist, does not alter the anaesthetic effects of isoflurane. Here, we investigated the influence of propranolol on the electroencephalographic (EEG) effects and minimum alveolar concentration (MAC) of isoflurane.

Methods. Fourteen swine [25.0 (SD 4.0) kg] were anaesthetized by isoflurane inhalation. The inhalation concentration was decreased to 0.5% and maintained for 25 min, before being returned to 2%, and maintained for a further 25 min. End-tidal isoflurane concentrations and spectral edge frequencies were recorded. Pharmacodynamic analysis was performed using a sigmoidal inhibitory maximal effect model for spectral edge frequency *vs* effect-site concentration. After measurement of the EEG effect, MAC was determined using the dew-claw clamp technique, in which movement in response to clamping is recorded. After completion of control measurements, a propranolol 4 mg bolus followed by an infusion (2 mg h^{-1}) was started. After a 30 min stabilization period, the inhalation concentration of isoflurane was varied as in the control period and MAC was re-assessed.

Results. Propranolol shifted the concentration–effect relationship to the left and decreased the effect–site concentration that produced 50% of the maximal effect from 1.30 (0.18) to 1.13 (0.17)%. Propranolol also decreased isoflurane MAC from 1.91 (0.35) to 1.54 (0.32)%.

Conclusions. Propranolol alters both the hypnotic and anti-nociceptive effects of isoflurane. In contrast to landiolol, lipophilic β-adrenoceptor antagonists may increase the potency of inhalational anaesthetics.

Br J Anaesth 2008; **100**: 841–5

Keywords: anaesthesia, depth; anaesthetics volatile, isoflurane; potency, anaesthetic, inhalational; potency, anaesthetic, MAC; sympathetic nervous system, propranolol

Accepted for publication: February 15, 2008

β-Adrenoceptor antagonists have been proposed as cardioprotective agents because of their role in sympathetic activation in adverse perioperative cardiac outcomes.[1 2] Therefore, β-adrenoceptor antagonists are frequently administered both before and during surgery.[3–5] In addition, several studies have suggested that β-adrenoceptor antagonists have an anaesthetic-sparing effect during general anaesthesia,[6–9] and they may also attenuate EEG responses,[10–12] although this remains controversial.[13–16]

We have examined the influence of landiolol, an ultra-short-acting β1-adrenoceptor antagonist, on the EEG effect and MAC of isoflurane in a swine model and concluded that landiolol does not alter either the hypnotic or anti-nociceptive effect of isoflurane.[17 18] However, since landiolol is hydrophilic (similarly to esmolol), it does not readily cross the blood-brain barrier; therefore, our results may not apply to β-adrenoceptor antagonists, in general. In particular, lipophilic β-adrenoceptor antagonists show relatively high penetration of the blood-brain barrier, and concentrations of these compounds in the brain are higher than those of hydrophilic β-adrenoceptor antagonists,[19 20] resulting in significantly higher central nervous system side-effects compared with those of hydrophilic drugs.[21]

We conducted the present study to investigate the influence of propranolol, a lipophilic β-adrenoceptor antagonist,[20] on the EEG effects and MAC of isoflurane. We hypothesized that propranolol would alter the anaesthetic potency of isoflurane.

Methods

Animal preparation

This study was approved by the Committee on Animal Research, Hamamatsu University School of Medicine, Hamamatsu, Japan. Fourteen pigs [body weight range: 18.4–30.8 kg, mean (SD)=25.0 (4.0) kg] were used in the study. General anaesthesia was induced by isoflurane inhalation (5%) in oxygen at 6 litre min^{-1} using a standard animal mask. After tracheostomy, the lungs were mechanically ventilated and anaesthesia was maintained with a 2% inhalation concentration of isoflurane in an oxygen–air mixture (oxygen:air=3:3 litre min^{-1}). Expiratory gases were analysed using a Capnomac Ultima (ULT-V-31-04, Datex-Ohmeda, Helsinki, Finland) throughout the study. The ventilator was adjusted to maintain the end-tidal carbon dioxide between 4.7 and 6 kPa during the preparation period, and this setting was maintained throughout the study. Lead II of an ECG was monitored using three cutaneous electrodes. A pulmonary artery catheter (5 F, 4 lumen, Nihon Kohden, Tokyo, Japan) and a central venous catheter (16 guage) were inserted via the right jugular vein, and another catheter (16 gauge) was placed in the right femoral artery. Blood temperature was measured using a pulmonary artery catheter and maintained between 39.0 and 39.5°C. After preparation, EEG monitoring was started by preparing the skin over the fronto-occipital regions bilaterally and positioning four cutaneous electrodes (Zipprep, Aspect Medical Systems, Natick, MA, USA). Four channels of the EEG were amplified and digitally recorded using an Aspect A-1000 EEG instrument with version 3.0 software (Aspect Medical Systems). The low-pass and high-pass filters were set at 2 and 70 Hz, respectively. Digitized raw EEG waveform data and processed EEG values were collected electronically at intervals of 5 s.

Experimental protocol

After completion of animal preparation, baseline measurements were made after a further 30 min, and then the inhalation isoflurane concentration was decreased from 2 to 0.5% and maintained at this level for 25 min, before being returned to 2% and maintained at this level for a further 25 min period. After EEG measurement, MAC was assessed beginning at close to a 2% end-tidal concentration. After determination of MAC under control conditions, the inhalation isoflurane concentration was returned to 2%, and 4 mg of propranolol was administered via a central venous catheter. Subsequently, propranolol was continuously administered at a rate of 2 mg h^{-1} with an infusion pump until the end of the study. After 30 min, and after confirming the stability of the EEG, the inhalation isoflurane concentration was decreased from 2 to 0.5% and maintained at this level for 25 min, before being returned to 2% and maintained at this level for a further 25 min, in a similar manner to the procedure under control conditions. After EEG measurement, MAC was assessed again beginning at close to a 2% end-tidal concentration. No other drugs were used throughout the study. Heart rate (HR), mean arterial pressure (MAP), mean pulmonary arterial pressure (MPA), central venous pressure (CVP), and cardiac output (CO) were recorded at each inhalation concentration during EEG measurements under control conditions and during propranolol administration. CO was determined with a thermodilution computer (Cardiac Output Computer, MTC6210, Nihon Kohden, Tokyo, Japan) using 5 ml of cold 5% glucose injected into the right atrium.

Pharmacodynamic analysis of the hypnotic effect

The hypnotic effect of isoflurane was characterized by examining the influence of isoflurane on the spectral edge frequency (SEF: the 95th percentile of the power distribution), as described previously.[17] The SEF was related to the effect-site concentration (C_e), which was derived from the classic first-order decay of the end-tidal isoflurane concentration (E_{Iso}): $dC_e/dt=k_{e0}$ ($E_{Iso}-C_e$), where k_{e0} is the elimination constant from the effect-site and determines the equilibrium between E_{Iso} and C_e. The k_{e0} value was calculated for each animal using the non-linear least-squares fitting method in Microsoft Excel (Microsoft Excel 2000, Microsoft Corporation, Redmond, WA, USA). Optimization of k_{e0} was accomplished using the Solver tool in Excel, by minimizing the area bounded by the hysteresis loop plotted between the SEF values every 10 s and the E_{Iso} values at the respective times. Because plots of the concentration–EEG effect relationship were sigmoidal, an inhibitory sigmoid E_{max} equation (Hill equation)[22] was used to model the relationship parametrically. The equation $E=E_0-(E_0-E_{max})\times[C_e^{\gamma}/(C_e^{\gamma}+EC_{50}^{\gamma})]$, where E is the predicted effect, E_0 is the baseline effect, E_{max} is the maximal effect, EC_{50} is the effect-site concentration that produces 50% of the maximal effect, and γ is a measure of curve steepness, was used to fit the equation to data for an individual animal. The parameters in the model were estimated using non-linear least squares fitting in Excel, through optimization with the Solver tool to minimize the sum of squares between the estimated and measured SEF values. We also report the coefficient of determination (R^2) as an objective function,[23] as described previously.[17]

Determination of MAC

Minimum alveolar concentration was assessed under control conditions and with propranolol administration in each animal. A supramaximal pain stimulus was created using a clamp to the dew claw for 60 s, and the presence or absence of a withdrawal reaction during the 60 s period was recorded. The detailed method has been described previously.[18]

Statistical analysis

Data are expressed as mean (SD). Differences in HR, MAP, MPA, CVP, and CO at each inhalation concentration during EEG measurements, differences in pharmacodynamic parameters, and MAC between control conditions and propranolol administration were analysed using a paired *t*-test. Haemodynamic variables for each condition were analysed using repeated-measures one-way analysis of variance (ANOVA). If the ANOVA indicated significance, a Scheffe *F*-test for multiple comparisons was performed. *P*-values of <0.05 were considered to be statistically significant.

Results

Averaged haemodynamic variables at each isoflurane concentration during EEG measurements under control conditions and with propranolol administration are shown in Table 1. Propranolol significantly decreased HR and CO, but did not change any other haemodynamic variable. Compared with the isoflurane 2% baseline, all haemodynamic variables (with the exception of CVP) significantly increased when isoflurane was reduced to 0.5% and returned to baseline values with restoration of 2%.

A plot of SEF against E_{Iso} showed hysteresis in all animals, as reported previously.[17] The hysteresis was collapsed by estimating the elimination constant from the effect-site (k_{e0}), resulting in the effect-site concentration–SEF effect relationship for isoflurane. The individual curves for all animals in both conditions are shown in Figure 1. Correlations of SEF to the effect-site concentration were good in both conditions: the correlation coefficients (R^2) were 0.91 (0.05) under control conditions and 0.91 (0.09) with propranolol. Propranolol significantly shifted the concentration–effect relationship to the left. The EC_{50} (effect-site concentration that produces 50% of the maximal spectral edge effect) values were 1.30 (0.18) under control conditions and 1.13 (0.17) with propranolol ($P<0.01$ *vs* control). The pharmacodynamic parameters are presented in Table 2. The k_{e0} and EC_{50} values decreased with propranolol administration. Other pharmacodynamic variables did not change.

Minimum alveolar concentration values for both conditions in all animals are shown in Figure 2. It decreased with propranolol administration in all but four animals. The mean MAC values were 1.91 (0.35)% under control conditions and 1.54 (0.32)% with propranolol ($P<0.01$ *vs* control), indicating that propranolol significantly decreases the MAC of isoflurane.

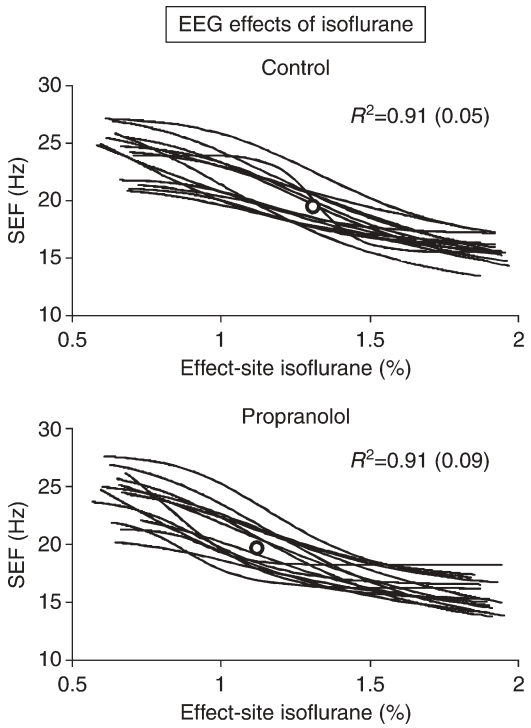

Fig 1 Individual relationships between spectral edge frequency and effect-site isoflurane concentration under control and propranolol-treated conditions. Open circles are mean EC_{50} values.

Table 1 Haemodynamic variables. CO, cardiac output; CVP, central venous pressure; HR, heart rate; MAP, mean arterial pressure; MPA, mean pulmonary arterial pressure; CO 2%, CVP 2%, HR 2%, MAP 2%, and MPA 2%, CO, CVP, HR, MAP, and MPA at the first isoflurane concentration of 2%, respectively; CO 0.5%, CVP 0.5%, HR 0.5%, MAP 0.5%, and MPA 0.5%, CO, CVP, HR, MAP, and MPA at an isoflurane concentration of 0.5%, respectively; CO $2\%_2$, CVP $2\%_2$, HR $2\%_2$, MAP $2\%_2$, and MPA $2\%_2$, CO, CVP, HR, MAP, and MPA at the second isoflurane concentration of 2%, respectively; *significant difference compared with control; †significant differences compared with both 2% states

	Control	Propranolol
HR 2% (beats min^{-1})	118 (14)	104 (8)*
HR 0.5% (beats min^{-1})	192 (32)†	121 (11)*†
HR $2\%_2$ (beats min^{-1})	127 (14)	105 (10)*
MAP 2% (mm Hg)	62 (7)	64 (9)
MAP 0.5% (mm Hg)	93 (10)†	94 (9)†
MAP $2\%_2$ (mm Hg)	63 (6)	64 (7)
MPA 2% (mm Hg)	16 (2)	17 (3)
MPA 0.5% (mm Hg)	19 (4)†	21 (3)†
MPA $2\%_2$ (mm Hg)	16 (3)	17 (2)
CVP 2% (mm Hg)	4 (2)	4 (1)
CVP 0.5% (mm Hg)	3 (1)	3 (1)†
CVP $2\%_2$ (mm Hg)	3 (1)	4 (1)
CO 2% (litre min^{-1})	3.2 (0.4)	2.6 (0.3)*
CO 0.5% (litre min^{-1})	4.1 (0.6)†	3.2 (0.4)*†
CO $2\%_2$ (litre min^{-1})	3.2 (0.4)	2.7 (0.3)*

Table 2 Pharmacodynamic variables. E_0, baseline level spectral edge effect; E_{max}, maximal effect spectral edge; EC_{50}, effect-site concentration that produces 50% of the maximal spectral edge effect; γ, a measure of curve steepness; k_{e0}, elimination constant from the effect site; *significant difference compared with control

	Control	Propranolol
k_{e0} (min^{-1})	0.81 (0.48)	0.50 (0.30)*
E_0 (Hz)	25.1 (2.8)	25.1 (2.8)
E_{max} (Hz)	13.6 (3.3)	14.3 (2.3)
γ	5.7 (4.1)	5.6 (2.9)
EC_{50} (%)	1.30 (0.18)	1.13 (0.17)*

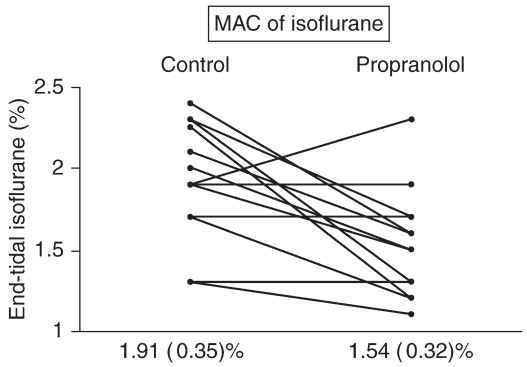

Fig 2 Change in minimum alveolar isoflurane concentrations after propranolol administration in each animal.

Discussion

Our results show that propranolol alters the EEG effect and MAC of isoflurane, suggesting that this β-adrenoceptor antagonist influences the hypnotic and anti-nociceptive effects of isoflurane. In addition to these pharmacodynamic changes, pharmacokinetic modification of co-administered drugs induced by the significant decrease in CO, with administration of a β-adrenoceptor antagonist, may also contribute to an anaesthetic-sparing effect during general anaesthesia.

We have previously examined the influence of landiolol, an ultra-short-acting β1-adrenoceptor antagonist, on the EEG effect and MAC of isoflurane in a swine model, and concluded that short-acting β1-adrenoceptor antagonists do not alter the anaesthetic effects of isoflurane.[17 18] In contrast to these results, Zhao and colleagues[24] recently demonstrated that intrathecal landiolol dose-dependently decreases pain-related behaviour in a mouse formalin test, and also demonstrated that intrathecal landiolol reduces c-Fos expression in the dorsal horn of the spinal cord after formalin injection. The discrepancy among these landiolol studies could be because of the drug administration site, as landiolol does not readily cross the blood-brain barrier as a result of its hydrophilicity. In contrast, propranolol is a first-generation β-adrenoceptor antagonist that is non-selective for β1- and β2-receptors and has high lipophilicity.[20] Several reports have indicated that brain concentrations of lipophilic β-adrenoceptor antagonists are 10–20 times higher than those of hydrophilic drugs, and central nervous system side-effects of lipophilic drugs, such as drowsiness, fatigue, lethargy, sleep disorders, nightmares, depressive moods, and hallucinations, are significantly higher than those with hydrophilic drugs.[20 21] We therefore speculate that β-adrenoceptor antagonists are likely to have an effect on the potency of anaesthetics if a significant concentration is maintained in the brain.

The increase in potency of an inhalation anaesthetic may occur as follows. The locus coeruleus (LC)-noradrenergic system is one of the ascending neuromodulatory systems implicated in the regulation of behavioural and forebrain neuronal activity.[25] The enhancement of LC neuronal discharge rates elicits bilateral activation of the forebrain, as measured by EEG,[26] and conversely the suppression of LC discharge activity elicits bilateral depression of the forebrain EEG in the anaesthetized rat.[27] Intravenous pretreatment with clonidine (an α2-adrenoceptor agonist) and intracerebroventricular pretreatment with propranolol prevents the EEG activation typically observed after LC activation,[26] indicating that α2- and β-receptors participate in LC-dependent activation of the forebrain. Therefore, propranolol is likely to suppress LC discharge activity, resulting in an increase in the EEG effect of isoflurane. Hageluken and colleagues[28] have demonstrated that β-adrenoceptor antagonists directly activate Gi-proteins, with an effect that is closely correlated with the lipophilicity of β-adrenoceptor antagonists.[28] Therefore, Gi-protein activation induced by β-adrenoceptor antagonists might contribute to inhibition of nociceptive signal transduction in the spinal cord. However, they also suggested that it is unlikely that Gi-protein activation induced by β-adrenoceptor antagonists contributes to clinical effects, because the concentration required to activate Gi-proteins is higher than that obtainable *in vivo*. Therefore, the mechanism underlying the increased anti-nociceptive effect is less clear than that for the hypnotic effect, and further investigations are needed.

Some limitations of the study should be addressed. The same doses of propranolol were given to all animals, but the effect-site concentrations (in plasma, brain, or both) may have differed among the animals, which might have influenced the extent of the pharmacodynamic changes. Indeed, four swine in which MAC did not decrease showed minimal alterations in EC_{50}, and it is possible that the brain concentrations in these animals might not have increased sufficiently to induce pharmacodynamic changes. In addition, the distribution of β-adrenoceptors in swine may differ from that in other animals and humans. In a study of the effect of propranolol on halothane MAC in dogs, MAC did not change significantly with either acute (2 and 10 mg kg^{-1} i.v. administration) or chronic (200 mg day^{-1} orally for 10 days) propranolol administration.[29] In acute administration, propranolol was given after isoproterenol (β-adrenoceptor agonist) administration to confirm the dose required for abolition of HR increase in response to isoproterenol infusion. The extent of penetration of isoproterenol into the central nervous system is unclear, but an influence of isoproterenol on the results cannot be excluded. However, with chronic administration of propranolol without isoproterenol, there was no significant change in MAC, and HR did not decrease despite the use of larger propranolol doses than those in our swine experiments. Therefore, β-adrenoceptor antagonists may not necessarily produce the same effects in swine and other animals, and further investigation of the effects of propranolol in humans is necessary. However, the present study suggests that lipophilic β-adrenoceptor antagonists can increase the potency of inhalation anaesthetics.

Funding

Support was provided solely from institutional, departmental sources or both.

References

1 Fleisher LA, Beckman JA, Brown KA, *et al*. ACC/AHA 2006 guideline update on perioperative cardiovascular evaluation for noncardiac surgery: focused update on perioperative beta-blocker therapy—a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Update the 2002 Guidelines on Perioperative Cardiovascular Evaluation for Noncardiac Surgery). *Anesth Analg* 2007; **104**: 15–26

2 Chassot PG, Delabays A, Spahn DR. Preoperative evaluation of patients with, or at risk of, coronary artery disease undergoing non-cardiac surgery. *Br J Anaesth* 2002; **89**: 747–59

3 ten Broecke PW, De Hert SG, Mertens E, Adriaensen HF. Effect of preoperative beta-blockade on perioperative mortality in coronary surgery. *Br J Anaesth* 2003; **90**: 27–31

4 Wiesbauer F, Schlager O, Domanovits H, *et al*. Perioperative β-blockers for preventing surgery-related mortality and morbidity: a systematic review and meta-analysis. *Anesth Analg* 2007; **104**: 27–41

5 Zaugg M, Schaub MC, Pasch T, Spahn DR. Modulation of β-adrenergic receptor subtype activities in perioperative medicine: mechanisms and sites of action. *Br J Anaesth* 2002; **88**: 101–23

6 Chia YY, Chan MH, Ko NH, Liu K. Role of beta-blockade in anaesthesia and postoperative pain management after hysterectomy. *Br J Anaesth* 2004; **93**: 799–805

7 Johansen JW, Flaishon R, Sebel PS. Esmolol reduces anesthetic requirement for skin incision during propofol/nitrous oxide/morphine anesthesia. *Anesthesiology* 1997; **86**: 364–71

8 Johansen JW, Schneider G, Windsor AM, Sebel PS. Esmolol potentiates reduction of minimum alveolar isoflurane concentration by alfentanil. *Anesth Analg* 1998; **87**: 671–6

9 Zaugg M, Tagliente T, Silverstein JH, Lucchinetti E. Atenolol may not modify anesthetic depth indicators in elderly patients—a second look at the data. *Can J Anaesth* 2003; **50**: 638–42

10 Johansen JW. Esmolol promotes electroencephalographic burst suppression during propofol/alfentanil anesthesia. *Anesth Analg* 2001; **93**: 1526–31

11 Menigaux C, Guignard F, Adam F, Sessler DI, Joly V, Chauvin M. Esmolol prevents movement and attenuates the BIS response to orotracheal intubation. *Br J Anaesth* 2002; **89**: 857–62

12 Oda Y, Nishikawa K, Hase I, Asada A. The short-acting beta 1-adrenoceptor antagonists esmolol and landiolol suppress the bispectral index response to tracheal intubation during sevoflurane anesthesia. *Anesth Analg* 2005; **100**: 733–7

13 Orme R, Leslie K, Umranikar A, Ugoni A. Esmolol and anesthetic requirement for loss of responsiveness during propofol anesthesia. *Anesth Analg* 2002; **93**: 112–6

14 Wilson ES, McKinlay S, Crawford JM, Robb HM. The influence of esmolol on the dose of propofol required for induction of anaesthesia. *Anaesthesia* 2004; **59**: 122–6

15 Berkenstadt H, Loebstein R, Faibishenko I, Halkin H, Keidan I, Perel A. Effect of a single dose of esmolol on the bispectral index scale (BIS) during propofol/fentanyl anaesthesia. *Br J Anaesth* 2002; **89**: 509–11

16 Haidar SH, Moreton JE, Liang Z, Hoke JF, Muir KT, Eddington ND. The pharmacokinetics and electroencephalogram response of remifentanil alone and in combination with esmolol in the rat. *Pharm Res* 1997; **14**: 1817–23

17 Kurita T, Morita K, Fukuda K, *et al*. Landiolol, an ultra-short-acting beta 1-adrenoceptor antagonist, does not alter the electroencephalographic effect of isoflurane in swine model. *Br J Anaesth* 2006; **96**: 602–7

18 Kurita T, Takata K, Uraoka M, Morita K, Sato S. Landiolol, an ultra-short-acting β1-adrenoceptor antagonist, does not alter the minimum alveolar anesthetic concentration of isoflurane in a swine model. *Anesth Analg* 2007; **105**: 656–60

19 Neil-Dwyer G, Bartlett J, McAinsh J, Cruickshank JM. Beta-adrenoceptor blockers and the blood-brain barrier. *Br J Clin Pharmacol* 1981; **11**: 549–53

20 Cruickshank JM, Neil-Dwyer G. β-blocker brain concentrations in man. *Eur J Clin Pharmacol* 1985; **28**: 21–3

21 Westerlund A. Central nervous system side-effects with hydrophilic and lipophilic β-blockers. *Eur J Clin Pharmacol* 1985; **28**: 73–6

22 Hill AV. The possible effect of the aggregation of the molecules of hemoglobin on its dissociation curves. *J Physiol* 1910; **40**: iv–vii

23 Bowerman B, O'Connell R. *Simple Coefficients of Determination and Correlation, Linear Statistical Models (an Applied Approach)*. Boston: PWS-KENT, 1990; 174–83

24 Zhao H, Sugawara T, Miura S, Iijima T, Kashimoto S. Intrathecal landiolol inhibits nociception and spinal c-Fos expression in the mouse formalin test. *Can J Anesth* 2007; **54**: 201–7

25 Foote SL, Bloom FE, Aston-Jones G. The nucleus locus coeruleus: new evidence of anatomical and physiological specificity. *Physiol Rev* 1983; **63**: 844–914

26 Berridge CW, Foote SL. Effects of locus coeruleus activation on electroencephalographic activity in neocortex and hippocampus. *J Neurosci* 1991; **11**: 3135–45

27 Berridge CW, Page M, Valentino R, Foote SL. Effects of locus coeruleus inactivation on forebrain electroencephalographic activity. *Neuroscience* 1993; **55**: 381–93

28 Hageluken A, Grunbaum L, Nurnberg B, Harhammer R, Schunack W, Seifert R. Lipophilic β-adrenoceptor antagonists and local anesthetics are effective direct activators of G-proteins. *Biochem Pharmacol* 1994; **47**: 1789–95

29 Tanifuji Y, Eger EI, II. Effect of isoproterenol and propranolol on halothane MAC in dogs. *Anesth Analg* 1976; **55**: 383–7