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Acute Effects of Halothane Anesthesia on Arterial and Venous Concentrations of Propranolol in the Dog

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The present study determined the effect of halothane on arterial and venous propranolol concentrations during a continuous intravenous infusion of propranolol. Arterial and venous concentrations of propranolol remained constant during the experiment in the group of dogs anesthetized only with pentobarbital. However, there was a rapid increase in arterial propranolol concentration from 88 ± 10 ng/ml (mean ± SEM) prior to halothane to 116 ± 12 ng/ml ($P < 0.05$) and 130 ± 7 ng/ml ($P < 0.05$) 60 and 120 min, respectively, after starting halothane anesthesia (2.0 MAC 1.74%). The increase in venous propranolol concentration lagged substantially behind that of the arterial concentration, so that, 60 min after starting halothane, the arterial to venous (A/V) concentration ratio increased from 1.13 ± 0.05 to a maximum of 1.48 ± 0.08. In contrast to the changes following halothane, no significant change in the A/V ratio occurred following fentanyl. Both halothane and fentanyl administration produced a small but significant increase in the free fraction of propranolol ($P < 0.05$). The results from this study emphasize the importance of the choice of sampling sites in pharmacokinetic experiments, as well as excluding subtle pharmacokinetic changes during anesthesia before ascribing changes in drug effect to changes in sensitivity to the drug. (Key words: Anesthetics, intravenous: fentanyl. Anesthetics, volatile: halothane. Pharmacokinetics: arterial and venous blood sampling; propranolol. Sympathetic nervous system: propranolol; sympatholytic agents.)

INHALATIONAL ANESTHETICS, such as halothane, may influence perioperative drug disposition by two major mechanisms: 1) by acute alterations in drug distribution, such as may occur due to changes in volume of distribution, and 2) by changes in drug elimination. The volume of distribution is dependent upon a number of factors; these include the volume of the tissues into which the drug is distributed, the partition coefficient between blood and tissue, the cardiac output and blood flow to the tissues that take up drug, and the extent of both plasma and tissue binding. Halothane causes hemodynamic changes and markedly affects peripheral blood flow.[2-4] We postulated that halothane-induced changes in peripheral blood flow might alter drug distribution, with consequent increase in plasma drug concentration, which, in turn, might have important implications for the intraoperative period.

Inhibition of hepatic drug metabolism by halothane has been described in animals[5,6]†† and humans,[7] and we have recently reported the effects of halothane anesthesia on propranolol clearance in the dog, where we showed that during 2.0 MAC (1.74%)[8] halothane anesthesia, portal clearance decreased by 62%, systemic clearance decreased by 40%, and bioavailability was increased by 16%.[6]

The purpose of the present study was to define the acute effects of the administration of halothane anesthesia on drug distribution in the dog, using propranolol as a model compound. We, therefore, determined: 1) the acute effects of halothane anesthesia on plasma propranolol concentrations, 2) the effects of halothane anesthesia on tissue drug uptake by measurement of the arterio-venous concentration ratio across the leg, and 3) the effect of halothane anesthesia on propranolol plasma protein binding.

Methods

Three groups of six mongrel dogs were studied. All were anesthetized with intravenous pentobarbital (30 mg/kg), and endotracheal intubation was performed without the aid of a neuromuscular blocking agent. Ventilation was controlled mechanically (with 100% oxygen) throughout the study to maintain arterial *p*H and p_{CO_2} within the normal physiological range. Silastic cannulae were then placed in a femoral artery, an ipsilateral femoral vein, and, in addition, two separate forelimb veins. The three groups consisted of a control group that was anesthetized with pentobarbital, 60 mg iv, at hourly intervals (n = 6, wt 23.8 ± 1.0 kg) (mean ± SEM), a group anesthetized with halothane in addi-

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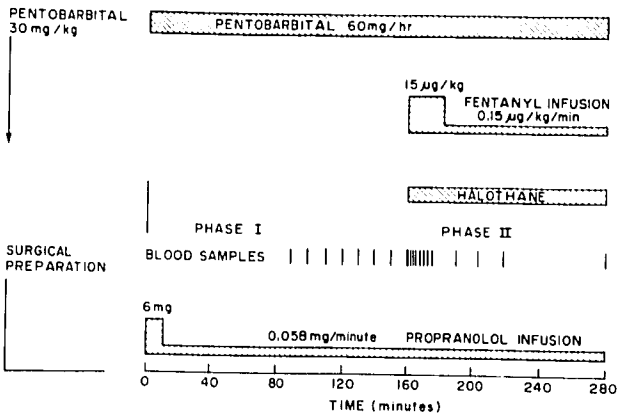

FIG. 1. Study protocol: Three groups of dogs (n = 6) received either fentanyl, halothane, or pentobarbital. Cannula insertion was carried out prior to commencing the study. All dogs received pentobarbital 30 mg/kg iv for induction, and then pentobarbital (60 mg iv) at hourly intervals. In addition, one group received halothane (2 MAC), while the other received fentanyl, 15 μg/kg for 20 min, followed by an infusion of 0.15 μg/kg/min thereafter. Blood samples were taken at the times shown by the vertical marks.

tion to pentobarbital (n = 6, wt 22.2 ± 1.9 kg), and a group that received fentanyl in addition to pentobarbital (n = 6, wt 22.6 ± 1.5 kg). Halothane anesthesia was administered to achieve an end-tidal concentration of 2.0 MAC (1.74%),[8] which was monitored using the Engstrom gas analyzer (EMMA). End-tidal halothane concentrations were also determined by gas chromatography every 20 min. The dogs in the fentanyl group received a loading dose of 15 mcg·kg^{1} over 20 min, followed by a maintenance infusion of 0.15 mcg·kg^{-1}·min^{-1} through a separate peripheral vein. These infusion rates have been shown by other workers to produce stable fentanyl concentrations.[9]

Figure 1 shows the study design. The study was divided into two parts: phase I and phase II. In phase I, after completion of surgery and a period to allow stabilization, each dog received a loading dose of 6 mg propranolol (time zero) administered over 10 min into a peripheral vein using a constant rate infusion pump. The infusion rate was then reduced to a maintenance rate of 0.058 mg/min for the duration of the study. Previous experience with this technique has demonstrated that this method of propranolol administration rapidly produces constant propranolol concentrations.[10] After 90 min of propranolol administration, femoral arterial and venous blood samples were obtained simultaneously every 10 min over a 60-min period (phase I). During phase I of the study, all dogs in the three groups were anesthetized with pentobarbital, 60 mg iv, at hourly intervals. The second part of the study (phase II) began 160 min from the start of propranolol administration when, in the halothane-and fentanyl-treated groups, halothane anesthesia or fentanyl was commenced. Further femoral arterial and venous samples were then collected simultaneously every 1 min for 5 min following the commencement of halothane or fentanyl anesthesia, every 2 min for the next 10 min, and then at 30, 45, 60, and 120 min after the start of halothane or fentanyl anesthesia, so that the study was completed 280 min from the start of propranolol administration.

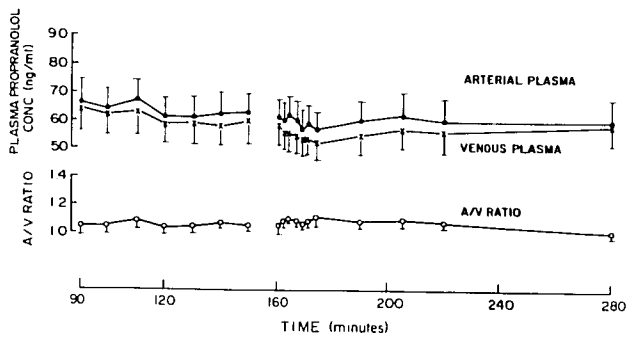

FIG. 2. Arterial and venous plasma propranolol concentrations (mean ± SEM, ng/ml) and A/V ratio (mean ± SEM) for the control group of dogs that received only pentobarbital anesthesia. No significant change occurred during the study.

Propranolol concentrations in arterial and venous plasma were measured by high-performance liquid chromatography, as previously described.[11] Plasma samples were also obtained at 160 min (*i.e.*, immediately preceding the start of halothane or fentanyl) and at 280 min for the measurement of propranolol binding in plasma by equilibrium dialysis as previously described.[12]

The mean concentration of propranolol prior to treatment was calculated for each dog. The per cent change from this baseline concentration was calculated for each dog at each measurement time after the onset of treatment. Linear regression was then used to model these changes against time. A separate linear regression was performed for each dog. The slope estimates for each dog were then analyzed by a Kruskal-Wallis one-way analysis of variance.[13] When such an analysis showed a significant difference between the response to the three treatments, then pair-wise comparisons between the treatments were made using Wilcoxon rank sum tests to assess the level of significance at each time point. $P \leq 0.05$ was the minimal level of significance accepted.

Results

Figure 2 shows the mean arterial and venous plasma propranolol concentrations for the control dogs that were anesthetized with pentobarbital alone. Arterial and venous plasma propranolol levels did not change in the control dogs throughout the study in either phase I

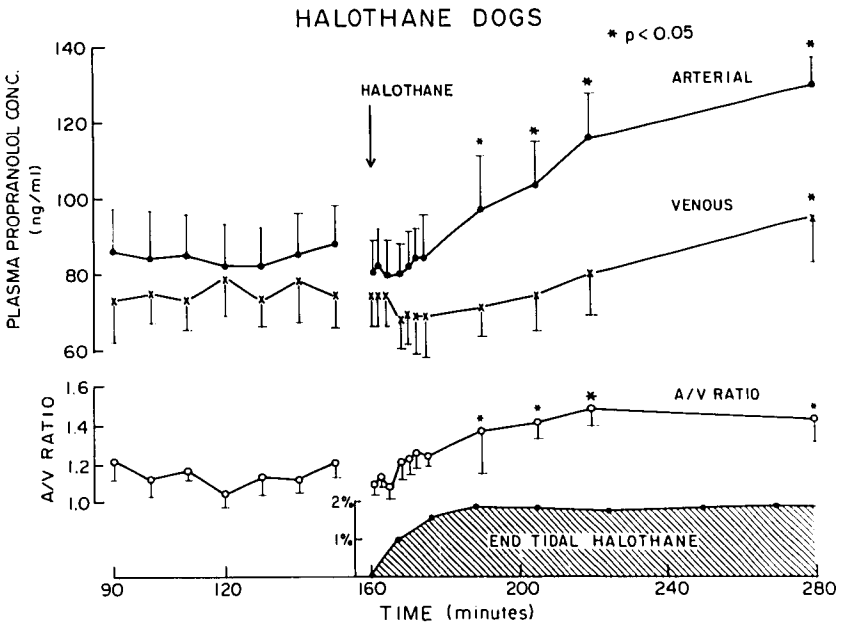

FIG. 3. Arterial and venous plasma propranolol concentrations (mean ± SEM ng/ml), and A/V ratio (mean ± SEM) for the group of dogs anesthetized with halothane. Halothane was begun at 160 min (*$P < 0.05$ compared to each dog's average value during phase I prior to halothane).

or phase II, with the result that the arterio-venous ratio (A/V ratio) remained constant.

The results for the group of dogs that were anesthetized with halothane are shown in figure 3. The mean plasma arterial and venous propranolol concentrations remained constant throughout the first period of the study (phase I). However, after halothane anesthesia was commenced, the arterial plasma propranolol concentrations rose rapidly, accompanied by a slower rise in the venous propranolol levels. The time taken to reach an end tidal concentration of 2.0 MAC was 19.3 ± 2 min. The mean arterial propranolol concentration rose from 88 ± 10 ng · ml^{-1} prior to halothane to 116 ± 12 ng · ml^{-1} ($P < 0.05$) at 220 min, and to 130 ± 7 ng · ml^{-1} ($P < 0.05$) at 280 min (fig. 3) (120 min following the commencement of halothane anesthesia). The A/V ratio increased from 1.13 ± 0.05 before the commencement of halothane anesthesia to a maximum of 1.48 ± 0.08, 60 min after the start of halothane anesthesia (220 min, fig. 3) ($P < 0.05$), and did not change further as venous propranolol concentrations started to rise. The changes in propranolol concentrations for the group of dogs that received fentanyl were less striking (fig. 4), with a small but significant rise in mean arterial

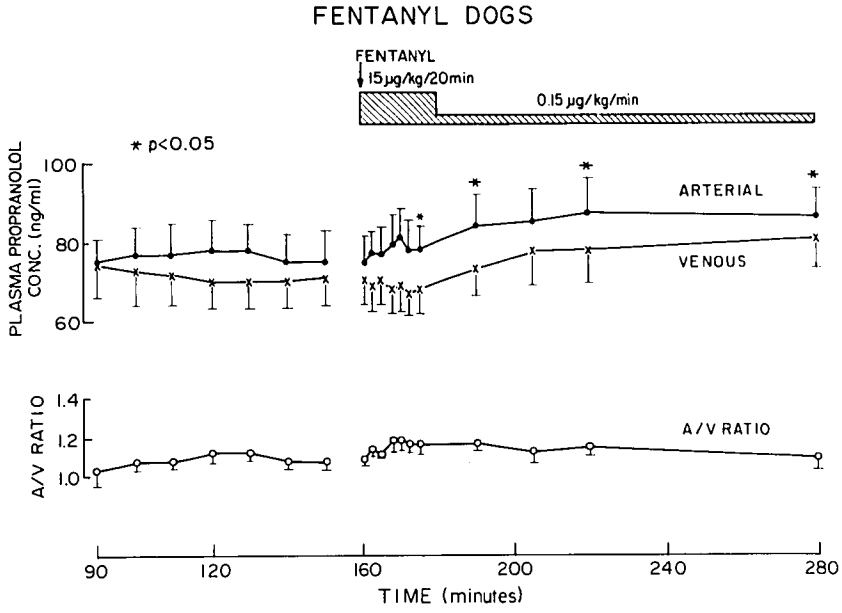

FIG. 4. Arterial and venous plasma propranolol concentrations (mean ± SEM, ng/ml) and A/V ratio (mean ± SEM) for the group of dogs which received fentanyl. Fentanyl was begun at 160 min (*$P < 0.05$ compared to each dog's average values during phase I prior to fentanyl).

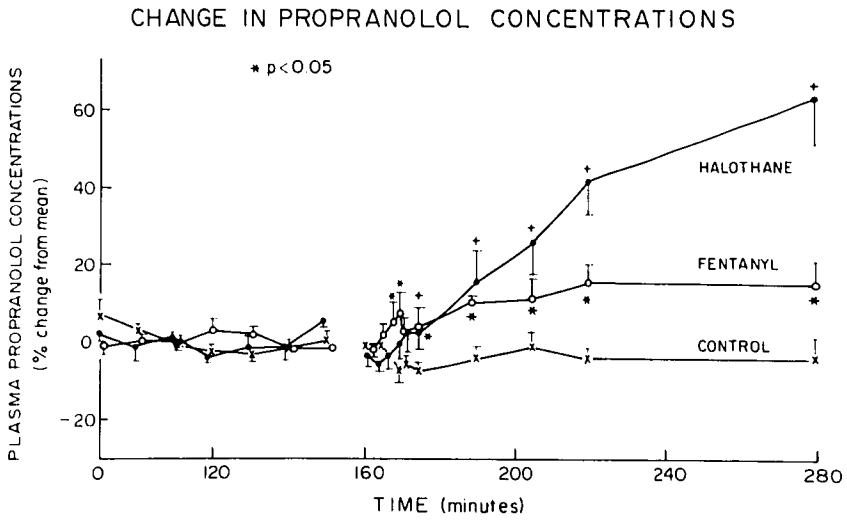

FIG. 5. Change in arterial propranolol concentrations (% change from mean values for phase I) for all three groups (mean ± SEM). The overall change in propranolol concentrations in the fentanyl and halothane treated differed significantly ($P < 0.005$ ANOVA) from those seen in the controls who only received pentobarbital. *The significance ($P < 0.05$) of the difference between fentanyl (*) and control and halothane (†) and control is shown at each time point.

plasma propranolol levels. As the mean venous propranolol concentrations also rose ($P < 0.05$) at the same rate, there was no significant change in the A/V ratio (fig. 4).

In order to clarify the relative magnitude of these changes, the change in arterial propranolol concentration expressed as a percentage change from mean concentration during phase I (prior to 160 min) for the three groups of dogs is presented in figure 5. There was no change in propranolol concentration in the control group of dogs that received pentobarbital alone, whereas, in the group of dogs that received halothane anesthesia, there was an approximately 60% rise in propranolol concentration. The overall rise in arterial concentrations for each dog was measured by the slope estimate of the appropriate linear regression equation. The slope estimates from the dogs who received either halothane or fentanyl were significantly greater than those who received pentobarbital ($P < 0.005$). The per cent rise in arterial propranolol concentration in the halothane group was significantly greater ($P < 0.05$) than the control group at 15 min after the start of both halothane and fentanyl, and remained significantly higher until the end of the study. The maximum increase in propranolol concentration was only 15% for the group of dogs that received fentanyl.

The change in A/V ratio, again expressed as a percentage change from mean during phase I, is shown in figure 6 for the three groups. There was no significant change in A/V ratio for the groups of dogs that received either pentobarbital or fentanyl, but the A/V ratio for the dogs anesthetized with halothane rose by approximately 35% ($P < 0.05$). The change in the A/V ratio produced by halothane was significantly different from that seen in the fentanyl-treated ($P < 0.01$) and control ($P < 0.005$) dogs.

There was a small rise ($P < 0.05$) in the free fraction of propranolol during both halothane and fentanyl treatment compared to pre-anesthesia values, from 15.6 ± 1.7% to 18.1 ± 1.8% following halothane, and

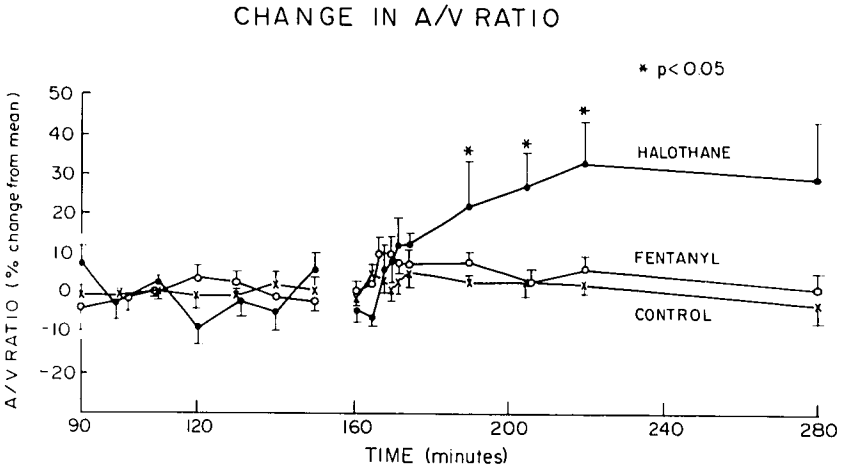

FIG. 6. Change in A/V ratio (% change from mean for phase I) for all three groups. During phase II the A/V ratio increased significantly (ANOVA) more in the halothane treated than in either the controls ($P < 0.005$) or those which received fentanyl ($P < 0.01$). In addition, the significance (*$P < 0.05$) of the difference between halothane (*) and control is shown at the individual time points.

from 11.7 ± 1.0% to 12.6 ± 1.1% following fentanyl treatment.

Discussion

Halothane has previously been shown to reduce the rate of elimination of a wide variety of drugs, including fentanyl, [14]‡‡ propranolol,[6] lidocaine,[7] meperidine,[15] and verapamil,[16] and, in some cases, this has been shown to be due to inhibition of drug metabolism *in vivo*[5,6]. This study has shown that halothane has additional effects, which result in an altered extraction of drug across the hind limb of the dog. During phase I of the study (prior to administration of either fentanyl or halothane), the arterial plasma propranolol concentrations were slightly higher than the venous concentrations, resulting in a mean A/V ratio for phase I of the study of 1.13 ± 0.05 for the dogs that subsequently received halothane and 1.08 ± 0.02 for the dogs that subsequently received fentanyl. Following halothane administration, two changes may be recognized: 1) there was a substantial rise in arterial propranolol concentrations from 88 ± 10 ng/ml prior to halothane to 116 ± 12 ng/ml after 1 h of halothane administration and 130 ± 7 ng/ml after 2 h of halothane anesthesia; and 2) venous concentrations showed no change from baseline for the first hour, and then rose over the second hour. This difference between the rise in arterial and venous concentrations resulted in a striking increase in the A/V ratio.

Fentanyl and pentobarbital anesthesia did not produce any change in the A/V ratio during these studies. Fentanyl caused a rise in the arterial concentration of propranolol, but this change was considerably smaller than that associated with halothane anesthesia, and was accompanied by an increase in venous concentration.

The explanation for the changes seen in this study is unclear; however, it it likely that more than one mechanism is required to explain the findings. The elevation in arterial propranolol concentration may be partially due to inhibition of drug metabolism and reduced drug clearance which we have previously shown to occur with halothane.[6] However, although a fentanyl nitrous oxide-relaxant anesthetic which we used in our study has also been shown to inhibit drug metabolism,[17] there was no change in the A/V ratio in the dogs that were anesthetized with fentanyl.

Alterations in both plasma and tissue binding have been postulated to account for changes in drug distribution. Thus, it is possible that the rapid rise in arterial propranolol concentration may also reflect decreased tissue binding of drug in some tissue other than those of the hind limb, resulting in a decreased volume of distribution and increase in plasma concentrations. Changes in drug binding in plasma would also affect the distribution of drug outside the plasma space. Halothane did produce a small but statistically significant increase in the free fraction of propranolol, as shown in our previous study.[6] The direction of this change would be expected to increase the movement of drug from blood to tissue, with resultant increase in the A/V ratio. The magnitude of the measured change in plasma binding in our studies is too small to account for the observed increase in the A/V ratio.

However, altered drug metabolism or reduced tissue binding does not fully explain the increased A/V ratio following halothane anesthesia. The increased A/V ratio implies an altered uptake or removal of propranolol from plasma as it passes through the hindlimb, possibly due to an alteration in the tissue to blood partition coefficient, since, if the tissue/blood partition coefficient is altered by halothane in favor of tissue, this would result in greater drug entry into tissue and greater extraction of drug from the blood.

Another possible explanation for the increased femoral arterio-venous propranolol ratio might be that halothane caused a reduction in myocardial function and/or altered regional blood flow. A fall in peripheral blood flow would result in an increased A/V ratio across the leg, even if the total mass of drug being extracted by the tissues of the leg remained constant. It has previously been shown that congestive cardiac failure is associated with decreased volume of distribution of a number of drugs, including lidocaine[18] and quinidine.[19] This phenomenon is probably secondary to the hemodynamic changes induced by cardiac failure. Further studies are required that measure drug uptake or extraction across various tissue and organ beds in conjunction with simultaneous blood flow and clearance measurements.

Our findings of an increased femoral arterio-venous propranolol ratio also raise issues for the design of future research protocols. If venous sampling alone had been carried out, the extent of the changes in arterial concentration would have been missed and, therefore, changes in drug effect would have been assumed to be pharmacodynamic in origin, when, in fact, they were pharmacokinetic. The differing effect of fentanyl and halothane anesthesia on drug distribution emphasize this potential problem. Previous studies with griseofulvin in the dog[20] have shown that, depending on whether arterial or venous samples were used, different values were obtained for both the volume of distribution of

‡‡ Borel JD, Bentley JB, Nenadc RE Jr, Gillespie TJ: The influence of halothane on fentanyl pharmacokinetics (abstract). ANESTHESIOLOGY 57:A239, 1982

the central compartment (V_1) and the volume of distribution at steady state (Vd_{ss}).

In summary, this study has shown that halothane anesthesia markedly increases arterial propranolol concentration and, in addition, produces an increase in the ratio of propranolol concentration in the artery to those in the vein. The explanation for this change is, as yet, unclear; however, it emphasizes the importance of giving careful thought to the choice of sampling sites in pharmacokinetic experiments, and, also, to the importance of excluding subtle pharmacokinetic changes during anesthesia before ascribing changes in drug effect to changes in drug sensitivity.

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