Blood Pressure Support during General Anesthesia in a Renin-dependent State in the Rat

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Previous work had shown that halothane and enflurane at 1 MAC and ketamine, 125 mg/kg, did not increase plasma renin activity (PRA) in the normal sodium-replete rat. To investigate the renin–angiotensin system with increased PRA, 25 rats were fed a low-sodium diet for five to seven days and divided into four groups: awake; halothane, 1.26 vol per cent; enflurane, 1.75 vol per cent; ketamine, 125 mg/kg, intramuscularly. The protocol consisted of a two-hour awake period, then an hour of stable anesthesia, followed by 30 min infusion of saralasin, an angiotensin II competitive inhibitor. An additional 18 rats had PRA measured by radioimmunoassay before and after an hour of stable anesthesia. Stable anesthesia decreased mean arterial pressure from 122 ± 2 to 99 ± 4 torr for the halothane group, 70 ± 3 torr for the enflurane group, and 103 ± 7 torr for the ketamine group. When saralasin was infused for 30 min, blood pressure decreased to 100 ± 3 torr for the awake group, 40 ± 1 torr for the halothane group, 44 ± 2 torr for the enflurane group, and 73 ± 3 torr for the ketamine group. PRA increased from 4.8 ± 0.5 ng/ml/hr for sodium-replete rats to 12.3 ± 1.7 ng/ml/hr for sodium-depleted rats. After an hour of stable anesthesia, PRA increased in all the anesthetized groups. The authors conclude that the anesthetic agents studied increase renin release in the sodium-depleted rat. The initial renin level may be important in determining whether changes in renin release occur with anesthetic agents. (Key words: Polypeptides, renin–angiotensin; Polypeptides, antagonists, saralasin; Anesthesiology, volatile, halothane; Anesthesiology, intravenous, ketamine; Ions, sodium.)

The renin–angiotensin system is known to play an important role in support of the blood pressure and regulation of total-body fluid volume and electrolyte balance.1-3 Previous work from our laboratory has shown that halothane and fluoroxene at minimum anesthetic concentrations (MAC) and ketamine, 125 mg/kg, do not increase plasma renin activity (PRA) in the normal rat.4 However, resting PRA may be increased by sodium restriction,5 hemorrhage,6 or congestive heart failure.7 In order to study the effects of anesthesia on the renin–angiotensin system when PRA is increased, sodium-restricted rats were anesthetized with halothane, enflurane, or ketamine and blood pressure and PRA responses measured.

The measurement of plasma renin activity is only an indirect method for estimating the amount of the potent vasoconstrictor angiotensin II present in the blood. Therefore, saralasin, a competitive antagonist of angiotensin II, was infused to determine the importance of the renin–angiotensin II system in blood pressure support in such a renin-dependent state.

Methods

To determine the effects of a low-sodium diet on blood volume, six male Wistar rats (250–350 g) were fed a low-sodium diet (<4 μEq/g sodium chow) for five to seven days. Eight control rats were fed a normal-sodium diet (4 μEq sodium/24 hours, Purina Rat Chow). The rats were anesthetized with diethyl ether, a femoral vein exposed and Evans blue dye, 50 μl of 0.5% per cent, was injected rapidly into the vein by a Hamilton syringe. Exactly 5 min later, 2–4 ml of blood were rapidly drawn by cardiac puncture into a heparinized syringe. Duplicate samples were obtained for determination of packed cell volume by the capillary method. Capillary tubes were centrifuged for 5 min in an International Microcapillary centrifuge. The percentage erythrocyte value and the percentage plasma value were determined from the average of two readings.

To determine total blood volume, the method of Bruckner-Kardoss and Wostmann was used.6 Briefly, it consists of centrifuging the remaining blood, isolating the dye-stained plasma, and reading the amount of dye present on a spectrophotometer at 610 μM. A standard curve is prepared for comparison with the unknown plasma. Plasma sodium and potassium concentrations were also measured in four animals in each group.

Measurements of cardiovascular function and renin levels were made in 25 rats fed a low-sodium diet for five to seven days. They were briefly anesthetized with diethyl ether and had a femoral artery and vein cannulated with PE-50 tubing. The cannulas were exteriorized through the skin over the back and flushed with a solution of heparin and dextrose, 5 per cent, in water. The rats were placed in restraining...

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Received from the Department of Anesthesiology and Pharmacology, University of Virginia School of Medicine, Charlottesville, Virginia 22901. Accepted for publication September 1977. Supported in part by a grant from the American Society of Anesthesiologists (Parker B. Francis Foundation Awards Program). Presented in part at the Annual Meeting of The American Society of Anesthesiologists, New Orleans, Louisiana, October 1977.

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0003-3022/78/0500—0404$00.75 © The American Society of Anesthesiologists, Inc.

404
cages for at least two hours to recover from anesthesia. Blood pressure was monitored continuously through the arterial cannula by a Statham P23Db pressure transducer using a Brush Mark 260 recorder, and had stabilized well before the end of the recovery period.

The protocol consisted of a two-hour control period, a one-hour period of stable anesthesia (one group had no anesthesia), a 30-min infusion of saralasin, and a 30-min post-infusion period. Blood pressure was monitored continuously. To insure a large excess of saralasin relative to angiotensin 11 at the angiotensin binding sites, a loading dose of saralasin, 100 µg/kg, dissolved in dextrose, 5 per cent, in water, was infused intravenously and the infusion of saralasin was maintained at 10 µg/kg/min for the next 30 min. The volume of this infusion did not exceed 0.2 ml.

Anesthesia was established with one of the following agents: halothane, 1.26 vol per cent (n = 6); enflurane, 1.75 vol per cent (n = 6); or ketamine, 125 mg/kg, intramuscularly (n = 6). A control group (n = 7) was treated identically but remained unanesthetized throughout. All animals breathed room air throughout the experiment. Inhaled concentrations of the volatile agents were determined at 15-min intervals by gas chromatography. The inhaled concentrations represent approximately 1 MAC values for the volatile agents in young rats. Ketamine was supplemented with half of the initial anesthetic dose as indicated to prevent purposeful movements of the rats during the experimental protocol. All animals were placed under a heating light to maintain rectal temperatures at 36–37 °C.

Eighteen additional rats were similarly fed a low-sodium diet, divided into three groups, and plasma renin activity determined before and after an hour of stable anesthesia with each of the three anesthetics. These additional rats were not given saralasin.

Arterial blood (0.5 ml) was drawn immediately prior to the anesthetic period and again after one hour of anesthesia for determination of plasma renin activity. An equal volume of saline solution (0.9 per cent) was administered intravenously to replace the shed blood. Plasma renin activity was estimated using 0.2 ml of plasma by a slight modification of the procedure described by Haber et al. The plasma was incubated for two hours to generate angiotensin 1, which was estimated by radioimmunoassay and the renin activity calculated as ng/ml/hr. The plasma samples from the three groups were assayed randomly in four separate renin assays.

The data presented are the mean values ± the standard error of the mean. Statistical significance of the results was determined using one-way analysis of variance among groups and Student's t test for paired data. P < 0.05 was considered significant.

Results

Blood volume determinations using Evans blue dye showed that rats receiving a low-sodium diet for five to seven days had a blood volume of 8.0 ± 0.6 ml/100 g. Rats fed a normal diet had a blood volume of 8.0 ± 0.3 ml/100 g. Plasma sodium and potassium values were similar in the two groups of animals.

There was no significant difference in either heart rate or blood pressure among the four groups during the control period. Therefore, the data were pooled. Mean blood pressure for 43 rats during the control period was 122 ± 1 torr. Plasma renin activity for the 18 rats studied during the control period was 12.5 ± 1.6 ng/ml/hr.

With the introduction and maintenance of stable anesthesia there were significant decreases in arterial blood pressure in the anesthetized groups (fig. 1). In the group anesthetized with halothane the mean blood pressure was 69 ± 4 torr; in the group anesthetized with enflurane, 70 ± 3 torr; in the group anesthetized with ketamine, 103 ± 7 torr.

Renin activity increased significantly from control with all of the anesthetics (fig. 2). Renin activity for the group anesthetized with halothane was 32.4 ± 8.2 ng/ml/hr; enflurane, 22.4 ± 4.6 ng/ml/hr; ketamine, 64.5 ± 10.6 ng/ml/hr. By analysis of variance, the greatest increase in plasma renin activity among the groups was that in those animals receiving ketamine.

During saralasin administration, a brief (2–3 min) initial increase of 15–25 torr in blood pressure was seen in most animals. Blood pressure then decreased significantly in all groups (fig. 3). The awake group had a mean blood pressure of 100 ± 3 torr (Δ 30 ± 3 torr) at the end of saralasin therapy. The value for the group anesthetized with halothane was 40 ± 1 torr (Δ 31 ± 3 torr); enflurane, 34 ± 2 torr (Δ 26 ± 2 torr); ketamine, 72 ± 3 torr (Δ 31 ± 3 torr). One animal in the group receiving halothane died 15 min into the saralasin infusion.

Following termination of saralasin infusion, blood pressure during the next 30 min returned toward pre-infusion levels except in the group anesthetized with halothane. The awake group had a mean arterial pressure of 124 ± 5 torr; halothane, 46 ± 3 torr; enflurane, 71 ± 4 torr; ketamine, 93 ± 13 torr. A significant difference (P < .001) from pre-infusion to post-infusion was seen only in the group receiving halothane.
Fig. 1. Mean arterial pressure (±SEM) after an hour of stable anesthesia. A = awake, n = 43 (no anesthesia). H = halothane, 1.26 vol per cent, n = 6. E = enflurane, 1.75 vol per cent, n = 6. K = ketamine, 125 mg/kg, n = 6. *Denotes significant decrease from awake value.


Discussion

Restriction of sodium intake increases plasma renin activity in rat, dog and man. Our data are consistent with these findings, and showed that after only five to seven days of sodium restriction, plasma renin activity increased from 4.3 ± 0.5 mg/ml/hr (n = 27), as we reported previously, to 12.5 ± 1.6 mg/ml/hr (n = 18). This change occurred even though mean blood pressures were the same for the normal rats (123 ± 1 torr) and the sodium-restricted rats (122 ± 1 torr).

Since blood volume changes could influence the effects of different anesthetic agents, we investigated the effects of short-term sodium restriction on blood volume. Our data show that short-term restriction does not result in a significant change in blood volume. Our blood volume determinations are in accord with those of Brucher-Kardoss and Wostmann, who found a value of 7.51 ± 0.15 ml/100 g for rats of similar size and species. Similarly, plasma sodium and potassium values were comparable in the two groups, which supports the data from the more extensive study of sodium deprivation in the rat by Sarstedt et al. These investigators showed that a rat whose sodium intake is severely restricted maintained normal serum sodium and potassium concentrations.

With the introduction and maintenance of stable anesthesia, blood pressure decreased significantly in all groups. In contrast to what was observed in the normal animals, blood pressure decreased much more with halothane and enflurane. In normal animals halothane and enflurane anesthesia resulted in mean blood pressures of 92 ± 4 torr and 85 ± 4 torr, whereas in the present study the mean values were 69 ± 4 torr and 70 ± 3 torr, respectively. Ketamine anesthesia, however, resulted in similar decreases in blood pressure in the two groups (95 ± 2 torr in the normal group; 103 ± 7 torr in the sodium-depleted group).

Why the sodium-depleted animals had greater decreases in blood pressure with halothane or enflurane anesthesia is not explained by our study. There is some evidence that vascular smooth muscle responsiveness is decreased in salt-depleted subjects. However, results of other studies do not support this. Other explanations not examined by our experiments would be alterations of the carotid sinus reflex, vascular responsiveness to catecholamines, or release of endogenous catecholamines in the sodium-depleted group.

Fig. 3. Average decrease in mean arterial blood pressure (torr) after 30 minutes of saralasin infusion during stable anesthesia. A = awake (no anesthesia), n = 7. H = halothane, n = 6. E = enflurane, n = 6. K = ketamine, n = 6. *Denotes significant decrease in MAP compared with pre-infusion value.
RENIN ACTIVITY WITH ANESTHETIC AGENTS

407
deleated animal. Further studies are in progress to examine these possibilities.

In contrast to results in our previous study, plasma renin activity increased in all of the anesthetized rats that had been sodium-depleted. The increase seen in the present study may have been due in part to the greater decrease in blood pressure, but this is not the complete explanation. The group that received ketamine had the smallest decrease in blood pressure but the greatest increase in plasma renin activity, implying a direct effect of the altered sodium itself on renin-releasing mechanisms.

These data are consistent with the findings of Blaine et al., who showed that with either hemorrhage or suprarenal aortic constriction, which are known to cause renin release, the greatest change in plasma renin activity occurred in those animals with the largest control PRA. It would appear that sodium balance is an important factor in determining whether plasma renin activity will be altered by anesthetic agents. Fray and associates have shown that in healthy, chronically trained dogs anesthetized with pentobarbital PRA does not increase even though blood pressure decreases, whereas in the sodium-depleted dog a similar decrease in mean arterial pressure does result in an increase in plasma renin activity. Perhaps the conflicting data of Pettinger and co-workers, suggesting that various anesthetic agents increase PRA, can be explained by this mechanism, i.e., for an equal stimulus for renin release, the response will be altered depending on the initial state of sodium balance and resting PRA.

The second portion of the study examined the physiologic importance during anesthesia of the increased PRA by the use of a competitive inhibitor of angiotensin II, saralasin. The dose of saralasin we used was chosen to conform to the suggestions of Ishikawa and Hollenberg, so that not only the systemic vascular response to angiotensin II but also the compensatory increase in cardiac output was blocked. Like Ishikawa and Hollenberg, we saw a transient blood pressure increase within 3 min of the infusion of saralasin, which had dissipated within 5–8 min. This may reflect angiotensin II receptors' being stimulated initially by the antagonist.

These experiments demonstrate the dependence of mean arterial blood pressure on the renin–angiotensin system in a sodium-depleted awake animal. This work is substantiated by studies in dog and man. Furthermore, the blood pressure decrease with saralasin is not accentuated or attenuated by these anesthetic agents. The mean decrease in blood pressure either awake or anesthetized was about 30 torr. This study also demonstrates the value of using angio-

tensin inhibitors in determining the importance of PRA. Ketamine, which induced the greatest increase in PRA, produced a blood pressure decrease similar to those obtained with the other agents.

In the group receiving halothane, blood pressure did not return to the pre-infusion level once saralasin was terminated. Perhaps the low pressure (40 ± 1 torr) for 30 min produced a shock-like state, thereby preventing compensatory responses. The rats receiving enflurane had a similar low pressure (44 ± 2 torr) but were able to compensate. From the data of Brown and Crout, halothane, 1.26 vol per cent, and enflurane, 1.75 vol per cent, seem to be comparable inspired concentrations, and therefore the group receiving halothane should not have been more depressed than the enflurane-treated group. The cause of this difference in responses to saralasin is not known.

In summary, in the sodium-depleted rat three commonly employed anesthetics cause renin release not observed in rats fed normally. These anesthetic agents do not alter the importance of the renin–angiotensin system in blood pressure support during anesthesia. The initial plasma renin activity is an important determinant in the renin release seen after stimulation of the renin–angiotensin system.

The authors are grateful for the able technical assistance of Mr. Gregory Rose and Mrs. Nancy Ragland. They are also grateful for the advice and encouragement of this work of Drs. A. C. Barger and R. M. Epstein.

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