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The Regulatory Function of the Renin–Angiotensin System during General Anesthesia

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Variation of plasma renin activity has been shown to occur during anesthesia, but its significance remains obscure. The recent development of a specific angiotensin II antagonist, saralasin, allows the delineation of the role of the renin–angiotensin system in blood pressure control during anesthesia. Twenty-seven rats were divided into four groups: awake, halothane (1 MAC), ketamine (125 mg/kg), and fluroxene (1 MAC). Arterial pressure was recorded continuously and plasma renin activity was determined by radioimmunoassay at the end of a two-hour awake control period, after one hour of anesthesia, and after half an hour of saralasin infusion. A similar protocol for enflurane with 1.75 vol per cent was also followed in seven anesthetized rats, but renin analysis was not performed.

Anesthesia resulted in decreases in mean arterial pressure from 123.0 ± 1.3 torr to 95.2 ± 2.2 torr for ketamine, 91.6 ± 3.9 torr for halothane, 96.9 ± 7.9 torr for fluroxene, and 84.5 ± 3.8 torr for enflurane. Renin activity did not change significantly from control (4.33 ± 0.51 ng/ml/hr). When saralasin was infused only the rats anesthetized with halothane or enflurane had significant decreases in mean blood pressure, to 75.0 ± 4.8 and 66.1 ± 3.4 torr, respectively. It is concluded that the anesthetic agents studied do not cause a detectable increase in plasma renin activity. However, through the use of a competitive inhibitor of angiotensin II, a significant role for the maintenance of blood pressure by the renin–angiotensin system during halothane and enflurane anesthesia has been demonstrated. (Keywords: Polypeptides, renin–angiotensin; Polypeptides, antagonists, saralasin; Anesthetics, volatile, halothane; Anesthetics, volatile, fluroxene; Anesthetics, volatile, enflurane; Anesthetics, intravenous, ketamine.)

THE RENIN–ANGIOTENSIN SYSTEM has been studied extensively in recent years and has been shown to play an important role in the maintenance of blood pressure, fluid volume and electrolyte balance.[1–3] Several investigators have suggested that renin release is stimulated by anesthetic agents,[4,5] but no studies comparing the effects of equipotent concentrations of various anesthetic agents on renin release have been reported.

Furthermore, the measurement of plasma renin activity is only an indirect method for estimating the amount of the potent vasoconstrictor angiotensin II that may be present in the blood. With the recent introduction of saralasin (P113), a competitive antagonist of angiotensin II, the importance of the renin–angiotensin II system in blood pressure support during anesthesia can be examined quantitatively.[6]

Methods

Twenty-seven (27) male Sprague-Dawley rats (300–400 g) that had been maintained on a normal sodium intake (4 mEq sodium/24 hours, Purina Rat Chow) were briefly anesthetized with diethyl ether, and a femoral artery and vein were cannulated with PE-50 tubing. The cannulas were exteriorized through the back of the rat and flushed with a solution of heparin and 5 per cent dextrose in water. The rats were placed in restraining cages for at least two hours to recover from anesthesia. Blood pressure, monitored continuously through the arterial cannula by a Statham P23Dc pressure transducer using a Brush Mark 220 recorder, 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-minute infusion of saralasin, and a 30-minute post-infusion period. Heart rate and blood pressure were monitored continuously. Blood for determination of plasma renin activity was obtained at the end of the control period, at the end of one hour of anesthesia, and at the end of the saralasin infusion.

In order to insure a large excess of saralasin relative to angiotensin II 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 minutes. The volume of saralasin administered did not exceed 0.2 ml.

Anesthesia was established with one of the following agents: halothane, 1.26 vol per cent (n = 8); fluroxene, 4.55 vol per cent (n = 6); or ketamine 125 mg/kg, im (n = 7). An awake control group (n = 6) was treated identically but remained unanesthetized throughout. All animals breathed room air throughout the experiment. Inhaled concentrations of the volatile agents were determined at

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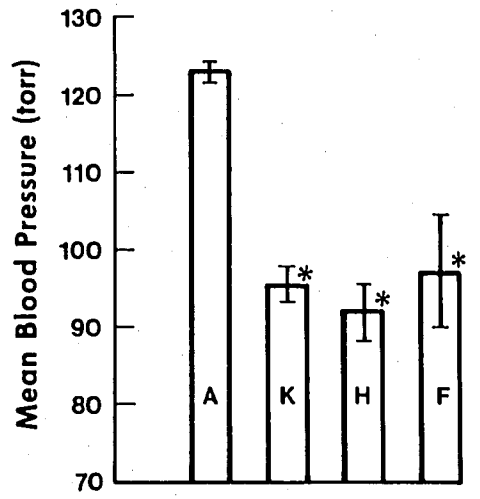

FIG. 1. Mean arterial pressure (±SEM) after an hour of stable anesthesia. A = awake, n = 27 (no anesthesia); K = ketamine, 125 mg/kg, n = 7; H = halothane, 1.26 vol per cent, n = 8; F = fluroxene, 4.55 vol per cent, n = 6. Asterisk denotes significant decrease from awake value.

15-minute 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. Since enflurane anesthesia has become widely used clinically, seven additional rats were similarly treated except that enflurane, 1.75 vol per cent, was administered and blood samples for renin analysis were not drawn. We chose 1.75 vol per cent so that mean arterial blood pressure would be comparable to those of rats that had received halothane. All animals were placed under a heating light to maintain rectal temperatures at 36–37 C.

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. A third arterial blood sample was obtained at the end of the saralasin infusion. 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 plasma by a slight modification of the procedure described by Haber *et al.*[7] The plasma was incubated for two hours to generate angiotensin I, which was estimated by radioimmunoassay and the renin activity calculated as ng/ml/hr. The plasma samples from the four groups were assayed randomly in three separate renin assays. An equal volume of a "standard" plasma was assayed with each assay and the values normalized to the standard. This correction allows assay conditions to be standardized when large numbers of samples are being assayed. Renin values calculated to be more than 50 ng/ml/hr are reported as 50 ng/ml/hr. These high renin values occurred only after administration of saralasin.

The data presented are the mean values ± 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

There was no significant difference in either heart rate or blood pressure among the four groups during the awake period. Therefore, the data for these animals were pooled. Mean blood pressure for 27 rats during the control period was 123.0 ± 1.3 torr. Plasma renin activity was 4.33 ± .51 ng/ml/hr.

With the introduction and maintenance of stable anesthesia, there were significant decreases in arterial blood pressure in the anesthetized groups. In the animals anesthetized with ketamine, mean blood pressure was 95.2 ± 2.2 torr; halothane, 91.6 ± 3.9 torr; fluroxene, 96.9 ± 7.9 torr (fig. 1). Renin activity, however, did not change significantly from the control with any of the anesthetics. Renin activity for the rats anesthetized with ketamine was 6.78 ± 1.6 ng/ml/hr; halothane, 4.79 ± .9 ng/ml/hr; fluroxene 5.66 ± 1.3 ng/ml/hr (fig. 2). In the group that received no anesthesia, neither blood pressure nor renin activity changed significantly.

After one hour of stable anesthesia, saralasin was administered. A brief (2–3 minute) initial increase in blood pressure of 15–25 torr was seen. Blood

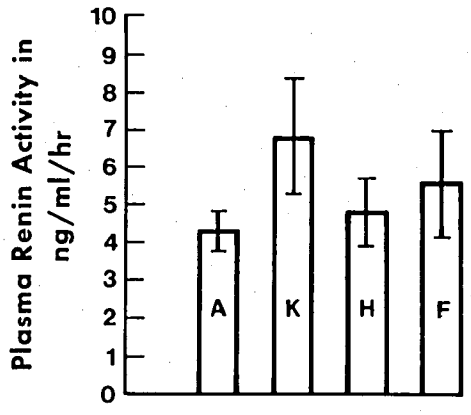

FIG. 2. Plasma renin activity after an hour of anesthesia. A = awake (no anesthesia); K = ketamine; H = halothane; F = fluroxene. Anesthesia did not alter plasma renin activity significantly.

§ Gow-Mac Model 750 Flame Ionization Detector; 1.83 meters SS column containing 20 per cent SE30 on Chromasorb W.

pressure then returned to pre-infusion levels in all the rats except those receiving halothane. A statistically significant decrease in mean arterial pressure averaging 16.6 ± 1.8 torr, was observed in the halothane-treated group. This was almost twice the decrease observed in any of the other groups (fig. 3).

Renin activity measured at the end of the saralasin infusion increased significantly in every group. By analysis of variance, however, there was no difference in plasma renin activities among the groups. The greatest increase in plasma renin activity was seen in the rats receiving halothane, in which blood pressure had decreased most with saralasin (fig. 4). Following termination of the saralasin infusion, blood pressure increased toward the pre-infusion level over a 30-minute period.

The rats that received enflurane had a mean blood pressure of 84.5 ± 3.8 torr, which does not differ significantly from values for the other anesthetized groups. When saralasin was administered to the rats receiving enflurane, arterial pressure decreased to 66.1 ± 3.4 torr. These results are statistically similar to those obtained in animals that had received halothane anesthesia.

Discussion

Pettinger and co-workers have suggested that anesthetic agents cause renin release. In their studies in rats, diethyl ether, sodium pentobarbital, ketamine, morphine, urethane, urethane–chloralose, methoxyflurane, cyclopropane, and halothane caused very large increases, as much as twentyfold, in serum renin activity.[4,5,8] In these studies anesthetic depth for each agent was quantified when the righting reflex and pain reflexes were lost. Studies in man do not show such increases.[9–11] In order to resolve this discrepancy, we performed similar experiments with careful attention to the time of blood sampling, anesthetic concentration, and stability of depth of anesthesia. Another difference is that each animal served as its own control in our study.

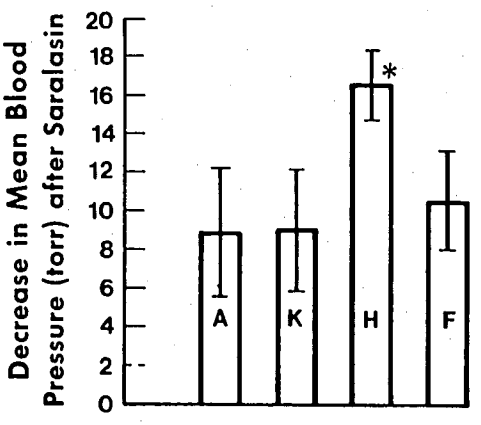

FIG. 3. Average decrease in mean arterial blood pressure (torr) after 30 minutes of saralasin (P113) infusion during stable anesthesia. A = awake (no anesthesia); K = ketamine; H = halothane; F = fluroxene. Asterisk denotes significant decrease compared with the pre-infusion value.

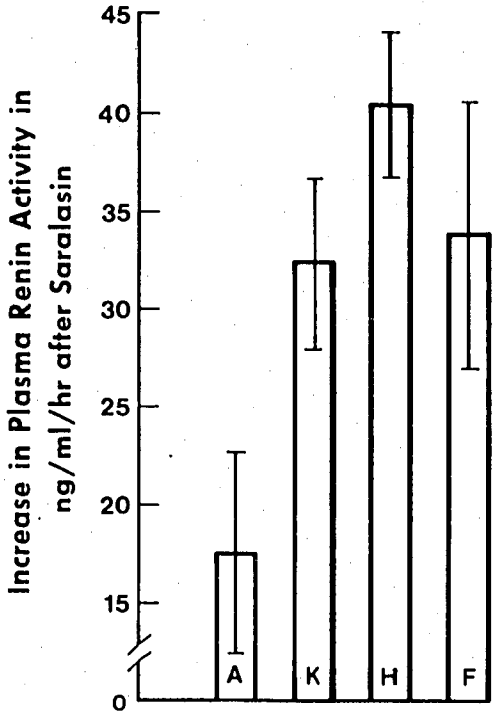

FIG. 4. Increase in plasma renin activity after 30 minutes of saralasin (P113) infusion during stable anesthesia. A = awake (no anesthesia); K = ketamine; H = halothane; F = fluroxene. Significant increases from the pre-infusion value occurred in all groups, but there was no difference among the groups by analysis of variance.

In contrast to the findings of Pettinger *et al.*, plasma renin activity during anesthesia did not increase significantly from that in the awake rats in our study. The apparent difference may result from the timing of sampling for renin. In our study, a steady state of anesthesia had been achieved. Whether Pettinger and associates examined renin release shortly following the excitement phase of anesthesia cannot be ascertained from his reports. We are confident from our studies, as well as a great number of reports in the medical literature, that the rat model adequately reflects the response of the renin–angiotensin system in man when careful attention is paid to time of sampling, sampling size, and electrolyte status.

The use of an angiotensin II antagonist such as saralasin has proven to be a powerful tool in understanding the role of angiotensin II in a variety of physiologic and pathologic states.[12–14] The dose of

saralasin we used was chosen to conform to the suggestion of Ishikawa and others, so that not only the systemic vascular response to angiotensin II but also the compensatory increase in cardiac output was blocked.[15] Like Ishikawa *et al.*, within 3 minutes of the infusion of saralasin, we saw a transient blood pressure increase that dissipated within 5–8 minutes, which may reflect angiotensin II receptors being stimulated initially by the antagonist.

Our study clearly shows the importance of using inhibitors to define the role of angiotensin II in blood pressure support. While values of plasma renin activity obtained by the biochemical measurement showed no change among the four groups of animals, the infusion of saralasin resulted in significant decreases in blood pressure only in the groups receiving halothane and enflurane. It became apparent during the study that measurement of plasma renin activity did not adequately reflect the importance of angiotensin II in blood pressure support and, therefore, enflurane anesthesia without plasma renin determination was studied using saralasin alone.

Plasma renin activity increased after the saralasin infusion in every group studied. Renin release is influenced by several factors. When blood pressure is lowered by as little as 5 torr, renin release occurs.[16] Second, angiotensin II exerts a negative-feedback control on renin release.[17] In these studies, angiotensin II was competitively inhibited by saralasin, which resulted in the increases of plasma renin activity in all of the groups studied (fig. 4). Interestingly, in the rats receiving halothane and saralasin, blood pressure decreased and plasma renin activity was the highest. Our data are consistent with the concept that when blood pressure is lowered and angiotensin II is interfered with, plasma renin activity will increase.

Are the results of our study consistent with the known properties of the four anesthetics studied? Since cardiac output determinations are difficult in such small animals, information from man and other animals is the only source of such values. Ketamine causes an increase in cardiac output and has little effect on peripheral vascular resistance.[18,19] Fluroxene, while no longer a clinical anesthetic, was chosen as a pharmacologic tool because it is known to stimulate the sympathetic nervous system.[20] Fluroxene has little effect on cardiac output or peripheral vascular resistance at 1 MAC in man. Halothane and enflurane, in contrast, are thought to be depressants of the sympathetic nervous system. At 1 MAC in man, there is a 25 per cent decrease in cardiac output, and peripheral vascular resistance is decreased slightly.[21] Merin and co-workers have shown in trained, chronically implanted dogs that enflurane appears to be at least as depressant to the dog heart as halothane.[22] While we used slightly less than 1 MAC enflurane in the rat, the blood pressures for rats anesthetized with enflurane and halothane were similar.

These studies demonstrate that with comparable levels of plasma renin activity, the effects of inhibiting angiotensin II may differ under varying circumstances. In those animals anesthetized with agents thought to stimulate the sympathetic nervous system, such as fluroxene or ketamine, angiotensin II does not appear to play an important role in blood pressure support. On the other hand, when the anesthetic agent depresses the sympathetic nervous system, as halothane and enflurane do, the importance of angiotensin II in blood pressure support becomes apparent. The exact mechanism of these differences cannot be determined from these studies.

The regulatory function of the renin–angiotensin system has not been determined in altered renin-level states. In this study, the animals were receiving normal-sodium diets, and presumably blood volumes were not altered. It is known that hemorrhage, hypovolemia, congestive heart failure, and sodium depletion all increase resting renin values.[17] Whether this has clinical importance, and whether there are potential differences in interactions with different anesthetic agents, can be determined only after further study.

In summary, renin release as measured by plasma renin activity does not increase in normal animals during moderate depths of anesthesia achieved with several anesthetics. Through the use of a competitive inhibitor of angiotensin II, a significant role for the maintenance of blood pressure by the renin–angiotensin system during halothane and enflurane anesthesia has been demonstrated.

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