Converting-enzyme Activity and Pressor Responses to Angiotensin I and II in the Rat Awake and during Anesthesia

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Plasma renin activity (rate of angiotensin I generation) does not increase during anesthesia with ketamine, fluroxene, halothane or enflurane in the sodium-repleted rat. However, blood pressure decreases when an angiotensin II agonist, saralasin, is administered during halothane or enflurane anesthesia, but not during ketamine or fluroxene anesthesia. Differences in the rates of conversion of angiotensin I to angiotensin II induced by various anesthetic agents could help explain these previous findings. To determine the effects of anesthetic agents on angiotensin I conversion, experiments were performed in vitro and in vivo.

The activities of rabbit pulmonary converting enzyme in the presence and absence of halothane or fluroxene were measured as rates of appearance of the dipeptide, histidyl-histidine, a product of angiotensin I hydrolysis to angiotensin II. Halothane and fluroxene did not alter conversion.

Infusions of angiotensin I and angiotensin II were given to Wistar rats to construct dose–blood pressure response curves. The animals were then anesthetized with ketamine or halothane and infusions were repeated. Angiotensin I and angiotensin II induced similar blood pressure responses in awake and anesthetized rats. However, ketamine accentuated the pressor responses to angiotensin I and angiotensin II, whereas halothane depressed the responses. With the anesthetic agents studied, there is no significant effect on conversion of angiotensin I to angiotensin II either in vitro or in vivo. (Key words: Anesthetics, intravenous; ketamine. Anesthetics, volatile; halothane; fluroxene. Blood pressure. Enzymes. Kidney: renin—angiotensin system.)

Some anesthetic agents have been shown to modulate the activity of the renin–angiotensin system in a manner that maintains homeostasis of blood pressure, while others have been found to affect blood pressure homeostatic mechanisms adversely. Studies of anesthetic effects on the renin–angiotensin system have been facilitated by the use of the competitive inhibitor of angiotensin II, saralasin. Miller et al.1 have shown that with halothane, enflurane, ketamine, and fluroxene anesthesia, plasma renin activity does not increase. However, the administration of saralasin produced a marked decrease in arterial blood pressure in animals anesthetized with halothane or enflurane, but not in animals anesthetized with fluroxene or ketamine. Since plasma renin activity measures the amount of angiotensin I generated, but not the amount of angiotensin II, changes in conversion of angiotensin I to the potent vasopressor angiotensin II could explain the differences seen in this study. This investigation was undertaken to determine at what step, if any, in the renin–angiotensin peptide cascade these anesthetic agents manifest their effect. Our initial approach was to examine the possible modulating effects of anesthetic agents on converting-enzyme activity. Studies of converting enzyme in vitro were performed with halothane and fluroxene. Experiments in awake and anesthetized rats utilizing halothane and ketamine were then undertaken to examine the effects of the anesthetics on converting-enzyme activity in vivo and on the responses of blood pressure to angiotensin I and angiotensin II.

Methods

The in-vitro assay of the activity of angiotensin-converting enzyme utilized a fluorometric determination of the amount of the dipeptide, histidyl-leucine, released as a product of angiotensin I cleavage to angiotensin II. Rabbit pulmonary converting enzyme was a purified preparation supplied by Tsai and Peach.2 The assay and the fluorometric determination employed were identical to those described by Tsai and Peach. Briefly, the enzyme was incubated at 37°C in a total volume of 250 μl. During a 5-minute preincubation period, air was bubbled through control tubes containing enzyme and phosphate buffer (pH = 7.5), and halothane, 2 per cent, or fluroxene, 4.3 per cent, was bubbled through experimental tubes with all gases delivered at 10 ml/min. The pH of the medium was unaltered by the anesthetic agents. Following this preincubation period, the reaction was initiated by addition of substrate (angiotensin I) to the mixture. At 1, 2, 4, and 6 min, a 50-μl volume of the reaction mixture was removed and the enzymatic activity was stopped with 50 μl of 10 per cent trichloroacetic acid. o-Phthalaldehydilc acid treatment of the histidyl-leucine product in these samples yielded a fluorescent condensation product, which was measured with an

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## Table 1. Effects of Halothane and Fluoxetine on Isolated Pulmonary Converting-enzyme Activity

<table>
<thead>
<tr>
<th>Time</th>
<th>Angiotensin I, 2.8 x 10^-8 M</th>
<th>Angiotensin I, 1.1 x 10^-8 M</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (Air)</td>
<td>Halothane (2.0 per cent)</td>
</tr>
<tr>
<td>1 min</td>
<td>28.5 ± 6</td>
<td>22.6 ± 6</td>
</tr>
<tr>
<td>2 min</td>
<td>51.5 ± 8</td>
<td>33.7 ± 8</td>
</tr>
<tr>
<td>4 min</td>
<td>81.5 ± 13</td>
<td>58.5 ± 12</td>
</tr>
<tr>
<td>6 min</td>
<td>140.3 ± 20</td>
<td>80.4 ± 13*</td>
</tr>
<tr>
<td>Rate in</td>
<td>22.8 ± 4</td>
<td>11.7 ± 2*</td>
</tr>
</tbody>
</table>

Concentrations of product (histidyl-leucine) in nanomoles liter\(^{-1}\) generated at 37°C. \(N = 8\) for substrate concentration (angiotensin I) 2.8 x 10\(^{-8}\) M with both halothane and control. \(N = 6\) for 1.1 x 10\(^{-8}\) M substrate with both halothane and control. Fluoxetine was delivered with a substrate concentration of 1.1 x 10\(^{-8}\) M (\(N = 7\)).

* Significant difference from corresponding control value, \(P < 0.05\).

Amino-Bowman spectrofluorometer set at 365 nm for excitation and 495 nm for emission.

The studies in vivo used 18 male Wistar rats (300–400 g), which were maintained on a normal-sodium diet. The animals were prepared for blood pressure monitoring using a procedure identical to that described by Miller et al. A femoral artery and vein were cannulated with PE-50 Intramedic\(^{\circ}\) tubing while the animals were anesthetized with diethyl ether. The cannulas were exteriorized through the dorsal surface of the rat and flushed with a small volume of heparin and physiologic saline solution. The rats were placed in restraining cages for at least an hour to recover from anesthesia and to achieve a stable baseline blood pressure. Mean arterial blood pressure was monitored continuously via the arterial cannula by a Statham P-23Db transducer and a Brush Mark 260 recorder.

The conscious (control) and anesthetized rats received 2-min infusions of small volumes of angiotensin I and angiotensin II (\(<70 \, \mu l\)). Orders of the infusions were randomized. The drugs were dissolved in physiologic saline solution and were delivered by a Harvard automatic-infusion pump. Each drug was infused at increasing concentrations, allowing mean arterial pressure to return to a stable baseline for 10 min between doses in both control and anesthetized animals.

Halothane was administered through a small nose cone by a Dräger vaporizer at approximately 1.3 vol per cent (1 MAC). At least 90 min were allowed for establishment of a near-constant blood anesthetic concentration. Ketamine was delivered as an intramuscular injection of 125–140 mg/kg\(^{-1}\), with supplemental doses as indicated to maintain a stable mean arterial blood pressure. Usually, supplemental doses in the amount of a fourth of the initial dose were administered for the duration of the experiment. Stable anesthesia was obtained in approximately 20 min with both agents. Body temperature was maintained by heating lamps.

Angiotensin I was obtained as a lyophilized preparation from Beckman Company, Palo Alto, Cal.; angiotensin II, lyophilized, from Schwarz-Mann Company, Orangeberg, New York (both peptides were Asp\(^{\circ}\)-Ile\(^{\circ}\)); halothane from Halocarbon Laboratories, Inc., Hackensack, New Jersey; ketamine from Bristol Laboratories, Syracuse, New York; fluoxetine from Ohio Medical Products, Madison, Wis. Converting enzyme was supplied by Dr. M. J. Peach, University of Virginia Department of Pharmacology.

The data presented are the mean values ± standard errors of the mean. Statistical significance of the results was determined using the Student \(t\) test for unpaired data. \(P < 0.05\) was considered significant.

### Results

The in-vitro studies with converting enzyme were undertaken initially using a substrate concentration of 2.8 x 10\(^{-8}\) M angiotensin I. Converting-enzyme activity was inhibited by halothane, 2 per cent, at 6 min, compared with bubbled-air controls (table 1). The rate of conversion was significantly depressed by halothane. At a fourfold higher substrate concentration, 1.1 x 10\(^{-4}\) M angiotensin I, no inhibition was observed with this concentration of halothane or with fluoxetine.

The reaction was linear to 6 min, as previously reported for this enzyme preparation. In summary, the direct effects of halothane and fluoxetine in vitro on isolated pulmonary converting enzyme were judged to be slight in our experimental situation.

The studies in vivo demonstrated that angiotensin I and angiotensin II produced equivalent pressor responses at any given rate of infusion. Comparison of angiotensin I with angiotensin II shows that the dose—
response curves for the two were not significantly different either for the control rats or for animals anesthetized with halothane or ketamine (figs. 1 and 2). Therefore, since angiotensin I lacks pressor activity, in-vivo converting-enzyme activity was considered to be unaffected by either halothane or ketamine.

Compared with the blood pressure response in the awake control rats, responses to the angiotensins were enhanced by ketamine anesthesia (fig. 1). At the maximum dose of each peptide, mean arterial pressure increased 69 ± 6 torr in response to angiotensin I and 63 ± 7 torr in response to angiotensin II during ketamine anesthesia. Increases of only 36 ± 4 torr in response to angiotensin I and 38 ± 5 torr in response to angiotensin II were obtained in the awake control rats. Blood pressure increases were obtained from a baseline mean arterial pressure during ketamine anesthesia of 86 ± 2 torr and a control baseline of 122 ± 1 torr.

In contrast, the pressure response to the angiotensins during halothane anesthesia was found to be blunted compared with controls (fig. 2). Infusions of 2.3 μg·kg⁻¹ yielded blood pressure increases of only 31 ± 7 torr in response to angiotensin I and 30 ± 2 torr in response to angiotensin II, compared with control increases of 47 ± 8 and 51 ± 2 torr, respectively. The baselines for halothane and control groups were 67 ± 3 and 111 ± 3 torr, respectively.

**Discussion**

The importance of the renin-angiotensin system in blood pressure support during anesthesia and the site at which anesthetics affect the renin-angiotensin system vary from agent to agent. Possible effects of halothane and fluroxene on isolated pulmonary converting enzyme were first examined. Slight inhibition of activity was found after 6 min of halothane treatment with a substrate concentration of 2.8 × 10⁻⁵ M angiotensin I. This was apparent in the rate of conversion, which decreased from 22.8 ± 4 nmol/min in the control condition to 11.7 ± 2 nmol/min in the halothane-treated rats. There was no inhibition of conversion with either halothane or fluroxene at the fourfold higher substrate concentration (1.1 × 10⁻⁴ M). Since the slight inhibitory effect observed with halothane at the 6-min sampling time was abolished by
Fig. 2. Doses of angiotensin I and angiotensin II in μg·kg⁻¹ represent total amounts of drug administered at the end of two minutes of continuous infusion. The increase in blood pressure (torr) represents the maximum sustained increase above baseline mean arterial pressure recorded over each two-minute infusion. Awake control rats are represented by solid lines and symbols (n = 9). Rats anesthetized with halothane (1.3 vol per cent) are represented by dotted lines and open symbols (n = 7). (* ) denotes a significant difference, \( P < 0.05 \), from the corresponding control value. Control and halothane-treated baseline arterial blood pressures were 111 ± 5 and 67 ± 3 torr, respectively.

Increasing the substrate concentration, any effect of halothane on converting enzyme would seem to be of the competitive type.

The *in-vivo* studies necessitated infusion of the angiotensins in pharmacologic concentrations. The validity of such manipulations is supported by Oparil *et al.*, who have reported that normal converting enzyme activity *in vivo* is sufficient to accommodate such concentrations of angiotensin I. These investigators found in the dog lung *in vivo* that the pulmonary capillary bed could convert 10⁴ times the physiologic concentration of angiotensin I to angiotensin II in a single passage across the lung. In our studies *in vivo*, angiotensin I and angiotensin II evoked the same blood pressure response for any given infusion rate. From the equivalent responsiveness to angiotensin I and angiotensin II, we infer that converting-enzyme activity was not impaired by anesthesia with ketamine or halothane.

Variable alterations in plasma renin activity during anesthesia have been reported. Pettinger *et al.*, have found enhancement of renin release in the rat by a wide variety of anesthetic agents, including ketamine, halothane, sodium pentobarbital and morphine. Alternatively, Miller *et al.* and Bailey *et al.* have shown that in the normal, sodium-replete rat and in man, increases in plasma renin activity do not occur with agents such as ketamine, halothane, fluroxene, and enflurane. An unstable anesthetic plane during the time of sampling for renin assay is cited as a potential explanation for the disparity in results by Miller *et al.* However, alterations in the conversion of angiotensin I to angiotensin II occurring because of the anesthetic agents could have significant effects on plasma renin activity and/or on the amount of angiotensin II that would interact with vascular smooth muscle. Therefore, studies such as those here reported are essential to show that measurement of plasma renin activity during anesthesia (angiotensin I generated) is a reliable technique for estimating the amount of the potent vasopressor angiotensin II present. Conversion of angiotensin I to angiotensin II was not interfered with either *in vitro* or *in vivo* by the anesthetics studied by us.

In comparison with awake control rats, rats anesthetized with ketamine had significantly enhanced pressor responses to both angiotensin I and angiotensin II. This increased responsiveness might be expected, since mean arterial pressure is decreased during ketamine anesthesia in the rat. Angiotensin II
elicits an increased pressor response as blood pressure is lowered. The exact mechanism of action of ketamine is unknown, and the reported central autonomic effects, actions on the baroreceptors, and actions on endogenous catecholamine release cannot be distinguished in this model. Therefore, further investigations are needed to separate these effects on the interaction of ketamine and angiotensin II.

In contrast, rats anesthetized with halothane, 1.3 per cent, had blunted pressor responses to infusions of angiotensin I and angiotensin II. That halothane exerts direct effects on the peripheral vasculature and alters the response of the smooth muscle to several agents has already been proposed. As a result of a study using cross-perfusion techniques, Wang and associates concluded that halothane depresses cardiovascular function by acting at a peripheral rather than a central site. In studies of rabbit aortic strips, Price and Price demonstrated that halothane attenuated responses to norepinephrine in vascular smooth muscle. Ngai and others demonstrated that halothane may have a direct relaxing action on vascular smooth muscle in studies of the cyclic-AMP system in the rat aorta. Alternatively, many of the effects of halothane may be attributed to its interaction with the sympathetic nervous system. Roizen et al. report depression of plasma catecholamine concentrations during halothane anesthesia. Decreases in circulating catecholamine concentrations induced by halothane have also been reported by Perry et al.

Whether the difference in blood pressure responses to the angiotensin infusions seen in rats anesthetized with halothane and those receiving ketamine can be explained by one or a combination of the mechanisms cited above cannot be determined from this model. This study does not support the concept that changes in vascular reactivity to angiotensin II are responsible for the difference seen in our previous study. We showed that with similar plasma renin activity, rats anesthetized with halothane were more dependent on angiotensin II for blood pressure support than those anesthetized with ketamine. If changes in "sensitivity" to angiotensin II were responsible for the difference, then animals anesthetized with ketamine would show a greater decrease in blood pressure when angiotensin II was inhibited.

In conclusion, we have found no significant effect of halothane or fluoroxyne on converting-enzyme activity. During ketamine anesthesia, angiotensin I- and angiotensin II-induced pressor responses were enhanced, while with halothane, pressor responses were diminished. However, during anesthesia with either halothane or ketamine, comparable responses were obtained whether angiotensin I or angiotensin II was infused. Therefore, no effect of the agents on conversion of one to the other could be demonstrated either in vitro or in vivo. Plasma renin activity is a reliable surrogate for estimating the amount of the potent vasopressor, angiotensin II, present.

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References