Microvascular Responses to Norepinephrine and Vasopressin during Halothane Anesthesia in the Rat

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This experiment was designed to determine the microvascular responses to the two known naturally occurring vasoconstrictors, norepinephrine (NE) and vasopressin, at known levels of central vasomotor activity before, during and after halothane anesthesia. The responses to topical application of NE and vasopressin were studied in the microvasculature of the mesentery and cremaster muscle, using microscopic methods. Neural (CNS) stimulation was accomplished through electrodes chronically implanted in vasoactive sites of the forebrain and midbrain. The increase in blood pressure in response to CNS stimulation was decreased during halothane anesthesia (32.4 ± 5.4 per cent before and 24.7 ± 6.1 per cent during; P < 0.001). There was no significant change in the steady-state diameter of the microvasculature under study during or after halothane anesthesia. Marked abatement of arteriolar vasoconstriction in response to CNS stimulation was seen prior to halothane. However, the same target vessel showed increased constriction in response to topically applied NE (from 32.3 ± 4.7 to 53.2 ± 7.6 per cent; P < 0.01) during halothane anesthesia. By contrast, the response to vasopressin decreased (from 42.4 ± 5.7 to 1.0 ± 6 per cent; P < 0.001) with halothane. The precise mechanism(s) underlying the described hypersensitivity to NE and hyporesponsivity to vasopressin in the same vascular structure during halothane anesthesia remains undetermined. (Key words: Microcirculation; Anesthetics, volatile, halothane; Hormones, antidiuretic, vasopressin; Sympathetic nervous system, norepinephrine.)

Anesthetics are associated with marked changes in microvascular vasomotor activity and reactivity to drug stimuli.1-5 Such changes are known to be, in part, dependent on the animal species, the extent of neurogenic suppression and the particular anesthetics used.6 For example, in the ear chamber preparation of the unanesthetized rabbit,1,2 the omental microvasculature of the dog prepared with local procaine,2,3 and the mesappendix of the minimally sedated rat,4 arteriolar constriction and enhanced vasomotion are seen during administration of ether and cyclopropane. This pattern of heightened vascular tone and reactivity to drug stimuli (epinephrine—norepinephrine) was also found to occur in the rabbit’s ear preparation during light chloroform anesthesia,1,2 and in the mesentery microvasculature of the rat during light halothane anesthesia.4 In the bat’s wing preparation, small doses (50 mg/kg) of pentobarbital resulted in prompt increase in the rate of venous vasomotion, but marked dilation of small arteries and small veins.5 The increase in venous vasomotor tone occurred without measurable change in the diameters of small arteries and veins during thiopental anesthesia. However, the reactivity changes in the animal were restricted to only a few vasoactive substances and selected tissue microvasculatures. Whether such changes are generalized in all tissues and applicable to other known smooth-muscle agonists remains undetermined. In our preliminary report of microcirculatory studies, we described diverse responses of the same microvessels to known vasoconstrictor agents during halothane anesthesia. Aortic strips from rabbits were reported to become hyperresponsive to norepinephrine (NE) during anesthesia with cyclopropane, thiopental, nitrous oxide, and chloroform, but hyporesponsive to NE in the presence of halothane.7 In studies with rat aortic strips, cyclopropane, which induced spontaneous contractions and enhanced responses to phenylephrine, decreased the cyclic adenosine monophosphate/adenosine triphosphate (AMP/ATP) ratio.8 More recently, a difference in responses of isolated venous smooth muscle to electrical and chemical (tyramine, NE) stimuli was also seen during exposure of the strip to halothane.9 The present report is concerned with the experiments designed to determine the changes in microvessel dynamic activity and sensitivity in response to two well-known vasoactive chemicals during known levels of suppression of neural influence by halothane.

Methods and Materials

Data were obtained from 12 male rats of Wistar strain (Carworth Farms) weighing between 90 and 199 g. The animals had monopolar electrodes chroni-
cally implanted in the brain by procedures previously described. In essence, stainless steel or Nichrome wire (250 μm) electrodes with a common ground, insulated with Formvar enamel except for 0.5 mm at the tip, were stereotaxically implanted, according to the coordinates of Sherwood and Timiras for 39-day-old rats, in midbrain and forebrain sites during methoxylurane anesthesia. Except for one animal studied 32 days after implantation of electrodes, in all others three to ten days later a tracheostomy was performed, using pentobarbital, 25 mg/kg, intramuscularly, for anesthesia. A carotid artery was canulated for continuous recording of arterial blood pressure. For microscopic study of the mesenteric microvasculature, the cecum was exteriorized, the mesoappendix spread, and the preparation maintained by standard methods. The procedure for the cremaster muscle (striated) preparation has also been reported. From the moment of exposure, the tissues were continuously irrigated with mammalian Ringer's gelatin solution of the following composition, in mM/l: NaCl 153.3; KCl 5.63; CaCl₂ 2.16; with gelatin 10 g/l, adjusted to pH 7.4 with NaHCO₃. Dextrose, 1 g/l, was added to this solution in open cremaster preparations. The temperatures of irrigating solutions were maintained at 37.0 ± 0.5 C for mesentery, and 34.0 ± 0.5 C for striated muscle. An acceptable preparation had a brisk capillary flow with occasional intermittency, and absence of petechial hemorrhage or leukocytic adherence to venular walls. Preparations that did not fulfill these criteria were discarded. Microvessel lumen diameter was measured by the image-splitting method via TV microscope and graphic recording. The vasoactive drugs used as stimulants were NE (levartenol bitartrate, U.S.P., Winthrop), diluted in distilled water as stock solution of 10 μg/ml, and vasopressin, U.S.P. (Parke-Davis), diluted in stock solution of 1.0 IU/ml in distilled water. Further dilutions were made as needed with Ringer's solution just before topical application. Midbrain and forebrain sites were electrically stimulated with a Grass stimulator through the chronically implanted electrodes with 10-sec trains of 100-μsec pulses at 5–100 Hz, delivered at intervals of at least 8 min, at currents of 0.02–1.0 ma. We aimed for subcortical regions in which stimulation would result in microvascular response, and studied only those sites that were effective. After termination of the
Fig. 2. Microvascular hypersensitivity to exogenous norepinephrine during halothane (0.8 per cent) anesthesia in the rat. Top, carotid arterial blood pressure. Second line, electrical stimulation (0.7 ma, 10-sec trains of 100-sec pulses, 100 Hz) of a vasoactive site in the hypothalamus. The records shown: before halothane, arteriolar luminal decreases of 40 and 54 per cent, respectively, in response to topical NE (1.0 µg/ml, 0.05 ml vol) and CNS stimulation; during halothane, marked abatement of blood pressure and suppression of arteriolar response to CNS electrical stimulation. Note the marked prolonged constriction (80 per cent) of the target arteriole in response to the standard dose of topical NE. Time marker 10 sec.

experiment, all electrode tracks were marked by passing direct current (1 ma for 5–10 sec) through the electrode. All brains were perfused with formalin, 10 per cent, removed, and fixed in paraffin; 15-µm sections were cut and stained with cresyl violet and luxol fast blue; localization of electrode tips were then determined (fig. 1).

Halothane–oxygen mixtures were administered via a Fluotec vaporizer from the anesthesia machine with a Harvard animal respirator through the tracheostomy cannula. End-expiratory halothane was analyzed by gas chromatography (Perkin-Elmer). Appropriate microvessels (third- or fourth-order arterioles or metarterioles) were selected as target vessels and the doses of NE and vasopressin solutions necessary to produce 40–50 per cent constriction were determined. Electrical stimulation of the CNS sufficient to cause 40–50 per cent constriction was also determined by appropriate changes in the electrical current and frequency. The animal, which had been lightly anesthetized with pentobarbital, was now exposed to a mixture of a low halothane concentration and oxygen. The initial halothane concentration was then increased gradually at 10–15-min intervals until the animal ceased to respond to tail clamping. End-expiratory halothane concentrations were measured by gas chromatographic technique for the determination of minimal alveolar concentration (MAC).15 After attaining the MAC for halothane,
the control electrical stimulus and NE and vasopressin at the predetermined concentrations were reapplied, and the changes in vessel response recorded. After 20–25 min, halothane administration was discontinued. When the end-expiratory concentration approached zero, the response of the target vessel to the stimuli was tested again. All data were analyzed statistically and significances calculated by two-tailed t test for paired data.

Results

Vasoactive sites stimulated were in the amygdala (no. 10), thalamus (no. 6), subthalamus (no. 8), pretectal area (no. 4), and dorsal midbrain reticular formation (nos. 1, 2, 3, 5, 7, 9). Histological verification of the stimulation sites for nos. 1–8 is indicated (fig. 1). The results of a typical experiment are shown in figure 2). In the control period, the target arteriole (i.d. 18.5 μm) showed 49.8 and 34.0 per cent lumen constriction, respectively, in response to topical application of NE and CNS stimulation. Electrical stimulation of a vasoactive site in the anterior hypothalamus (with 10-sec trains, 100 μsec pulses of 0.7 ma) resulted in a marked biphasic blood pressure response. During the 20-min halothane (0.8 per cent) administration, the same (0.7 ma) or greater (0.9 ma) stimulus intensity resulted in a marked decrease in the blood pressure changes and a near-absence of response of the target arteriole. The application of NE in the same amount resulted in a pronounced and prolonged vasoconstriction of 81.3 per cent, 4 min in duration. The changes in blood pressure and the extent of vasoconstrictor responses to NE (not shown in the figure) returned toward control levels in the recovery period. In three experiments the vessels showed little or no change in response to NE during anesthesia. In general, a marked ($P < 0.001$) attenuation of the vasoconstrictive response to electrical stimulation of the CNS occurred during halothane anesthesia (table 1). During halothane anesthesia, the target arterioles showed a significant ($P < 0.01$) increase in response to NE. In all animals, the changes in both blood pressure and microvascular responses returned

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**Fig. 3.** Microvascular hypersensitization to NE and hyposensitization to vasopressin during halothane anesthesia. Upper line, changes in vessel diameter in response to electrical stimulation of the CNS, to topical NE, and to topical vasopressin. Second line, systemic blood pressure (el-stim = electrical stimulation). The numbers at the bottom are the percentages of luminal narrowing before, and during halothane anesthesia, and after recovery. (Note 1) the 100 per cent increase in arteriolar constriction in response to NE; (2) failure of the same target vessel to respond to vasopressin during halothane anesthesia; (3) the total suppression of the vascular response to electrical stimulation of a vasoactive site in the CNS.
**Table 2. Arteriolar Constrictor Responses to Topical Application of Norepinephrine and Vasopressin before, during, and after Halothane Anesthesia**

<table>
<thead>
<tr>
<th></th>
<th>Arteriolar Constrictor Response (mm Hg)</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Norepinephrine (n = 10)</td>
<td></td>
</tr>
<tr>
<td>Before halothane</td>
<td>17.0 ± 1.5</td>
</tr>
<tr>
<td>During halothane</td>
<td>17.6 ± 1.4</td>
</tr>
<tr>
<td>Recovery</td>
<td>16.6 ± 1.5</td>
</tr>
<tr>
<td>Vasopressin (n = 7)</td>
<td></td>
</tr>
<tr>
<td>Before halothane</td>
<td>17.1 ± 1.8</td>
</tr>
<tr>
<td>During halothane</td>
<td>16.1 ± 2.2</td>
</tr>
<tr>
<td>Recovery</td>
<td>15.4 ± 1.7</td>
</tr>
</tbody>
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* Halothane in end-expired air = 0.78 ± 0.04 per cent.
† Significant difference, P < 0.01.
‡ Significant difference, P < 0.001.

*Note: The marked increase in vasoconstrictor response with norepinephrine and the marked abatement with vasopressin during halothane anesthesia.*

toward control values in the recovery period (without halothane). Lack of vessel hypersensitization to NE during administration of halothane in three of the rats occurred with a complete suppression of the response of the same vessels to CNS stimulation. During halothane anesthesia (0.87 per cent), with suppression of the neural influence and hypotension, the selected vessels became hyperresponsive to exogenous NE, although the same vessels failed to constrict in response to the polypeptide vasopressin (fig. 3). The inhibition of the microvascular constrictive response to vasopressin during halothane anesthesia was marked (table 2). In two of the experiments the vessels that became hyperresponsive to vasopressin were the same vessels that showed hyperresponsiveness to NE during halothane anesthesia. In contrast to the almost twofold increase in constrictor response to norepinephrine (P < 0.01), the response of the same vessels to vasopressin was practically abolished (P < 0.001) (fig. 4).

**Discussion**

Using standard electrical stimulation of vasoactive sites in the CNS or stimulation by topical application of vasoconstrictor drugs, a reproducible microvessel response can be obtained. The present results show that halothane anesthesia modifies these responses. Vascular hypersensitivity to epinephrine and NE during general anesthesia both in *vivo* and *in vitro* has been abundantly described. However, the decrease in intensity of the vasoconstrictor response of vasculature of the skin to vasopressin was found to be greater than decreases in responses to norepinephrine, angiotensin, or sympathetic neural stimulation during halothane anesthesia. We present quantitative data showing differences in responses of the same vessels in vivo to vasoactive drugs at an anesthetic level sufficient to block the effect of central nervous system stimulation. Indeed, hypersensitivity to NE but hyporesponsiveness to vasopressin was found in all trials. In seven of ten experiments, the hyperresponsiveness of mesenteric and cremasteric arterioles to NE was manifested by either greater decrease in lumen size or longer duration of effect, or both. The change in response occurred concomitantly with: a) a marked abatement or suppression of the response of the vessel to electrical stimulation of vasoactive sites in the CNS; b) a moderate but persistent decrease in blood pressure. The arteriolar responses to NE tended to return to control levels in the recovery period with the restoration of the central neural influence and normotension.

The suppression of neurogenic control and hypotension were also accompanied by an unexpected hyporesponsitization of the arterioles to a previously determined effective dose of vasopressin (fig. 3 and table 2) in all trials. Most striking was the observed hyporesponsiveness of a given vessel to vasopressin and the hyperresponsiveness of that vessel to NE under the same conditions. The hyporesponsiveness to vasopressin also developed in three of the experiments in which the same vessel failed to develop hypersensitivity to NE. In contrast to vascular hypersensitivity seen following surgical denervation, the vascular effects of halothane were always reversible. The present observation would seem to warrant further consideration.

It has been suggested that vascular hyperresponsiveness to NE during neural blockade and hypotension might be related to a reversible alteration in the physical equilibrium of the vessel wall tension. Steady tension in a vessel wall is a primary function of the elastic and collagenous fibers located in the fabric of the wall, and it is postulated that the realignment or slackening resulting from hypotension or decreased transmural pressure would allow a greater freedom of action by the effector cell in response to a stimulus. Our unpublished data indicate that the hypersensitivity to NE during spinal anesthesia in the rat is prevented when normotension is maintained by blood, plasma, or dextran infusion. It is of interest in this context that observed morphologic alterations in structural realignment of muscle cells (i.e., increased cell contact; nuxuses) in the vas deferens of the rat treated with reserpine are also associated with hyporesponsitivity to catecholamines. No information is presently available to indicate whether such structural
changes are associated with hyposensitization to vasopressin, or whether such structural realignment occurs in microvessel walls during halothane-induced hypotension.

Vascular smooth muscle hypersensitization to various known agonists following surgical denervation is a well-documented phenomenon. The vascular sensitivity change is of long duration and the underlying mechanism(s) remains obscure. Obviously, following surgical denervation, effector smooth-muscle cells in the vessel wall are relieved of the influence of neurotransmitter. Since in the present study CNS influence was effectively suppressed in a reversible manner during halothane anesthesia, one is tempted to relate the above-described microvascular smooth-muscle hypersensitization to NE to a possibly attendant absence of transmitter release. However, studies in vitro with other tissues, i.e., rat brain and heart, have demonstrated that release, uptake and biosynthesis of neurotransmitter remained unaltered by the exposure of such tissues to halothane.

Hypersensitivity to catecholamines occurs in vascular structures exposed to reserpine in the presence of modified calcium binding. It is generally agreed that contraction of blood vessels is triggered by a supply of ionized calcium to the contractile protein. Further, it is known that the vascular smooth-muscle contractile responses to NE and vasopressin are equally calcium-dependent. Therefore, it is possible to contemplate that a modification in calcium binding in microvascular smooth-muscle cells by halothane may underlie the observed changes in vascular responses. However, evidence concerning calcium binding during halothane anesthesia is limited to that of erythrocyte ghosts in which, in contrast to other anesthetic agents studied, i.e., alcohol, acetone, and ether, halothane failed to affect calcium-binding ability of cell membranes.

Studies have shown an increase in the level of cyclic AMP in association with the inhibition of responses of rat uterine muscle to acetylcholine during halothane anesthesia. Also, the inhibition of responses of rat aorta strips to phenylephrine during either isoflurane or halothane anesthesia was seen to be accompanied by an increase in cyclic AMP. However, a decrease in cyclic AMP was seen during potentiation of phenylephrine-induced contraction of the rat aortic strip exposed to cyclopropane. Although an increase in cyclic AMP might have been occurring in the present in vivo study during halothane anesthesia and suppression of neurotransmitter, it is difficult to explain mediation by the same shift in cyclic AMP of clear-cut opposite microvascular smooth-muscle responses, i.e., a hyperresponse to NE and a hyposresponse to vasopressin. More germane to the present result might be observations that a number of anesthetic agents can loosen cell membrane components, a process called "membrane fluidization," and especially that halothane in anesthetic concentrations can reversibly expand human erythrocyte membrane. It has been suggested that such cell expansion and/or fluidization may cause conformational modification of the receptor components in the cell membrane so that the response to one drug may be stimulation and the response to another inhibition. Whether similar reversible membrane alterations' take place in the smooth-muscle cells in the walls of the microvessels during halothane anesthesia and lead to
modification(s) of some steady chemo-mechanical coupling process of the contractile protein, resulting in hypersensitization of the vessel to NE and hypersensitization to vasopressin, remains undetermined.

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References

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