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Halothane Selectively Attenuates α_2-Adrenoceptor Mediated Vasoconstriction, In Vivo *and* In Vitro

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The mechanism by which halothane interferes with catecholamine-induced vasoconstriction was examined, utilizing specific agonists at postjunctional α_1- and α_2-adrenoceptors on vascular smooth muscle. Stimulation of either adrenoceptor subtype normally produces vasoconstriction. Two experimental models of drug-induced vasoconstriction were used: 1) *in vivo* blood pressure response in pithed rats, and 2) *in vitro* isometric tension development in canine saphenous vein rings. These models were then utilized to examine the anti-vasoconstriction properties of halothane. *In vivo*, halothane (1 MAC) produced a significant depression in the vascular response to azepexole (an α_2-adrenoceptor agonist), but halothane did not alter vasoconstriction by phenylephrine (an α_1-adrenoceptor agonist). Halothane caused a 24% reduction of maximal response ($P < 0.0001$) to azepexole in pithed rats, and a 3.2-fold rightward shift of the log dose-response curve ($P < 0.0001$). Similarly, *in vitro*, halothane significantly attenuated α_2- but not α_1-adrenoceptor responsiveness. Halothane (4%) depressed maximal vein contraction to azepexole by 26% ($P < 0.0001$), and shifted the log concentration-response curve 2.4-fold to the right ($P < 0.0001$). The observed selective interference with α_2-mediated vasoconstriction by halothane is unlikely to represent drug antagonism at the receptor level. Our observations may suggest, indirectly, that halothane interferes with Ca^{+2} entry into vascular smooth muscle.

The phenomenon of selective anti-vasoconstriction at α_2-adrenoceptors by halothane may explain why α_1-adrenergic agonists often appear to retain their vasopressor activity during halothane anesthesia. The mechanism of halothane-induced vasodilation thus includes attenuation of α_2- but not α_1-adrenergic vasoconstriction; this further demonstrates the multifactorial nature of halothane-induced vasodilation. (Key words: Anesthetics, volatile: halothane. Pharmacology: azepexole (BHT-933). Sympathetic nervous system, alpha adrenergic receptors: alpha$_1$- and alpha$_2$-adrenergic receptors. Sympathetic nervous system, sympathomimetic agents: phenylephrine.)

HALOTHANE-INDUCED HYPOTENSION is, in part, due to vasodilation.[1,2] Halothane causes vasodilation by directly relaxing vascular smooth muscle (VSM),[3] and by indirectly reducing the magnitude of vasoconstrictor stimuli, or the effectiveness of these stimuli for inducing vasoconstriction.[4]

Because of the importance of the sympathetic nervous system in controlling vascular tone, many researchers have examined how halothane alters the activation of VSM by sympathetic stimuli.[5] These studies have revealed that the mechanism of halothane-induced vasodilation is multifactorial: reducing brain sympathetic outflow,[6] impairing ganglionic neurotransmission,[7] suppressing norepinephrine release by prejunctional sympathetic nerve terminals,[8,9] sensitizing baroreceptor activity,[10] as well as inhibiting the vasoconstrictor actions of catecholamines, *in vivo*[11-13] and *in vitro*.[14,15] However, little is known about the mechanism whereby halothane inhibits the vasoconstrictor actions of catecholamines at vascular smooth muscle, a process called anti-vasoconstriction. Recent advances in autonomic pharmacology have made further study of this question feasible.

Catecholamine vasoconstriction is mediated by post-junctional α-adrenergic receptors (adrenoceptors), which are situated on VSM cell membranes. It is now accepted that two subtypes of post-junctional α-adrenoceptors exist on VSM.[16-18] Both the α_1- and α_2-adrenoceptor subtypes produce vasoconstriction when stimulated by agonists, although each receptor acts through somewhat different activation mechanisms.[19] The contributions of both receptor subtypes to the maintenance of vascular tone have been demonstrated in tissues of many species, including humans.[20,21] *In vivo*, epinephrine and norepinephrine both activate α_1- and α_2-adrenoceptors, and are the physiologic agonists. Agonist drugs which preferentially stimulate one subtype of α-adrenoceptor, with a high degree of specificity, are now available; examples include phenylephrine (PE) which activates α_1-, and azepexole (BHT-933) which activates α_2-adrenoceptors.[22,23]

It is not known whether halothane's anti-vasoconstrictor action against catecholamines depends upon which subtype of α-adrenoceptor is activated. This information may have important implications for determining the precise mechanisms of halothane vasodilation. Therefore, we examined the interactions of halothane with the vasoconstriction produced by activation of post-junctional vascular α_1- or α_2-adrenoceptors. We performed these studies both *in vivo*, using the diastolic blood pressure responses of the pithed rat, and *in vitro*, utilizing the

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contractile response of the canine saphenous vein. Our experiments indicate that halothane, *in vivo* and *in vitro*, selectively impairs the vasoconstriction mediated by α_2-adrenergic receptors on blood vessels, but that α_1-adrenoceptor responses remain intact.

Methods and Materials

In Vivo Experiments: Pithed Rats

Experimental techniques. Male Sprague-Dawley rats (weight 384 ± 47 (SD) g, range 310–510 g) were briefly anesthetized with halothane 2% in O_2:N_2O (1:2), first in a plexiglas box, and then by scavenged facemask. A tracheotomy was performed, and controlled ventilation was initiated *via* a pressure-cycled ventilator (Analytical Specialties SAR-2, St. Louis MO) and a non-rebreathing circuit. The rats were pithed using 2 mm stainless steel rods inserted through a dorsal cervical incision, to disrupt the entire brain and spinal cord. Success of pithing was confirmed by the lack of heart rate changes in response to α-agonist-induced hypertension, and the lack of reflex movement. The absence of a central nervous system justified subsequent termination of the anesthetic. Halothane and N_2O were discontinued, inspired gases were changed to O_2:N_2 (1:2), and heparin 1000 $U \cdot kg^{-1}$ was given intravenously.

Monitoring was then instituted, including ECG, aortic pressure (left carotid artery), rectal temperature (maintained 36.5–38° C with a heating pad), and end-tidal concentrations of CO_2, halothane, and isoflurane by mass spectroscopy (Perkin-Elmer 1100MGA, Pamona, CA). A small-bore tube was passed through a Y-adapter in the ventilator expiratory line, terminating within the tracheotomy tube; this permitted accurate end-tidal gas sampling even at respiratory rates of $60 \cdot min^{-1}$, as documented by the close correspondence between arterial and end-tidal P_{CO_2} values (r = 0.97, n = 80, Larach D: unreported observations). The mass spectrometer was calibrated daily using gravimetric gas standards. Intravascular catheters were PE-50 tubing filled with heparinized saline, attached to Gould P231D transducers, and calibrated to a mercury column. Arterial blood samples were assayed for *p*H, P_{CO_2}, P_{O_2} (Corning 168), Na^+, K^+, and Ca^{+2} (Nova I and II), and microhematocrit. The total quantity of blood drawn was sufficiently small (1.5–2.5 ml per experiment) to make transfusion unnecessary. Maintenance fluids were $4\ ml \cdot kg^{-1} \cdot h^{-1}$ 0.9% saline iv. Ventilation was adjusted to maintain normal P_{CO_2}, P_{O_2}, and *p*H. Continuous recordings of ECG, heart rate, aortic pressure, and intermittent airway concentration waveforms for CO_2 and halothane were made on a Gould 2800 recorder.

Control group. Rats were assigned to either the control or the halothane groups. Rats in the control group (n = 15) had their initial halothane anesthetic terminated immediately following pithing. Administration of vasoactive drugs was begun only after the end-tidal [halothane] had decreased to less than 0.05% (which usually required 30–45 min. of anesthetic wash-out).

Halothane group. Animals in the halothane group (n = 15) received halothane for induction and pithing, and also during vasoactive drug administration. Vasoactive drugs were given only when end-tidal [halothane] reached a stable level equivalent to 1 MAC (1.0% ± 0.05%).[24]

Dose-response curves to the two pressor drugs were generated by administering incremental, ascending, bolus IV doses of PE (0.01–30 $\mu g \cdot kg^{-1}$) or azepexole (0.01–10 $mg \cdot kg^{-1}$) *via* a jugular venous cannula. A non-cumulative dose-response technique was used, with the blood pressure response to each drug dose having abated before the next dose was given. Only one agonist dose-response curve was constructed per rat. The volume of each dose was $0.5\ ml \cdot kg^{-1}$, followed by 0.3 ml flush. Changes in diastolic arterial pressure (DAP) were utilized as an index of drug-induced vasoconstriction.

Supplemental control group. Because the control group rats could not totally eliminate the halothane they had received prior to pithing, and because halothane may have persistent vascular actions,[25] a supplemental control group was studied. This additional control group (n = 9) was initially anesthetized with isoflurane 3.5%, which was discontinued after pithing. Pressor drug dose-response curves were later obtained after the end-tidal (isoflurane) decreased below 0.05%, using the same technique as the control group. The responses of the two control groups (initial halothane *vs.* initial isoflurane) to α_1 and α_2 agonists were compared, to study whether trace concentrations of the two anesthetics produced discernible differences in vascular reactivity.

Vasopressin studies. Since halothane decreased the baseline blood pressures prior to and during vasoactive drug administration, additional experiments were performed to examine whether the hypotension itself reduced vascular responsiveness to azepexole. A group of rats (n = 4) were studied using the protocol described above for the animals that received halothane, with one addition: following pithing and stabilization, an infusion of arginine vasopressin (AVP) was administered to these rats together with 1 MAC halothane. The AVP dose was titrated to restore the diastolic arterial pressure (DAP) to a level similar to that observed in control group rats. The azepexole-DAP dose-response relationship was then measured, during the continuous infusion of this constant dose of AVP.

In Vitro Experiments: Vein Rings

Segments of canine saphenous vein were removed from mongrel dogs anesthetized with pentobarbital, cut into

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four rings 1–3 mm wide, and immediately placed in separate thermostatically controlled (37°) tissue baths. The rings were equilibrated at 1 g tension for 1 h in HEPES buffer between stainless steel hooks which were attached to isometric tension transducers (Gould UC2). A total of 16 rings from six dogs were studied. The buffer was made fresh daily, and contained (mM): NaCl 140, KCl 5.0, $MgCl_2$ 1.0, $CaCl_2$ 1.5, HEPES 5.0, and glucose 10; *p*H was adjusted to 7.40 with NaOH, while being continuously aerated with O_2:CO_2 (97:3 v/v).

Rings were exposed to increasing concentrations (10^{-8}–10^{-3} M) of PE or azepexole to generate cumulative [agonist]-tension curves; the tension response to each agonist concentration was allowed to reach a plateau before the next dose was added.[26] Each ring acted as its own control, and was only given one agonist. The total volume of drug added to each 20 ml bath during generation of an entire dose-response curve was 400–600 μl. Rings were then washed four times over 30 min, restored to 1 g tension in the absence of agonist, and allowed to equilibrate for 1 h by bubbling each bath with gas containing halothane 4% (v/v, confirmed by mass spectroscopy). A second [agonist]-tension curve was then generated using the same agonist. Additional control rings, which received no halothane during either pressor challenge, were serially examined to detect degradation in responsiveness during the experimental period. Contraction or relaxation responses were recorded on a Gould 2800 recorder.

Statistical Analysis: Dose-response Functions

The purpose of fitting the data to mathematical dose-response functions was to estimate certain important pharmacological parameters: maximal and minimal response, ED_{50} (dose which produces 50% of maximal response), slope, and dose ratio (ED_{50} with halothane/ED_{50} control). These parameters were then used to test hypotheses concerning the mechanism of halothane vasodilation. A commonly utilized technique for data analysis involves fitting a mathematical model to each experimental curve separately, determining the pharmacological parameters for each curve alone, and then comparing the derived slopes and potencies from each curve; unfortunately, that procedure does not extract all of the information contained in the data.[27] Instead, we performed simultaneous curve-fitting of families of nonlinear dose-response curves, to determine which pharmacological parameters were shared in common. Two types of mathematical functions were utilized for this purpose: a symmetrical logistic function which fit the symmetrical shape of the data from the *in vitro* experiments, and an asymmetrical Weibull function which fit the data from the *in vivo* experiments.

The symmetric logistic function. The four-parameter logistic function and its application in dose-response studies is described in detail,[27] and will only be briefly summarized here. This function is represented by:

$$Y(x) = \frac{Y_0 - Y_{max}}{1 + (x/EC_{50})^b} + Y_{max},$$

where $Y(x)$ is the magnitude of the response at agonist concentration x (with x measured on the arithmetic scale), Y_0 is the response at zero concentration, Y_{max} is the response at infinite agonist concentration, b is the slope factor, and EC_{50} is the concentration producing 50% of the maximal response. The data from the *in vitro* experiments were used to estimate the parameters of this model using a modified Gauss-Newton algorithm for nonlinear least squares. The statistical program "ALLFIT," developed by DeLean *et al.*[27] for this purpose, was modified for the IBM PC/XT microcomputer and utilized to test hypotheses about the existence of shared parameters among groups of curves. Using "ALLFIT," it was possible to test whether the introduction of halothane into a system caused a change in Y_0, Y_{max}, b, or EC_{50}. Hypothesis tests in "ALLFIT" are based on F ratio tests; the software also provides diagnostics for goodness of fit and for homoscedastic variance among curves.

The asymmetric Weibull function. The *in vivo* rat experiments produced asymmetric dose-response curves. The asymmetric shape was modeled with a Weibull response function[28] described by:

$$Y(x) = (Y_{max} - Y_0)\exp[-\theta(D_{max} - x)^{\lambda}] + Y_0,$$

where x is the $\log_{10}$ dose, $Y(x)$ is the arithmetic change in DAP response at dose x, D_{max} is the $\log_{10}$ dose which is considered maximal (input as an empirically determined constant), Y_{max} is the response at maximum dose D_{max}, Y_0 is the response at zero dose, θ is the slope parameter, and λ is the asymmetry parameter.

The parameters of this model were estimated from the data using the multivariate secant method[29] available in the SAS statistical software procedure "NLIN."¶ F-ratio tests were used to determine whether parameters were shared among families of curves. The ED_{50} was calculated from an algebraic combination of parameters from the Weibull model. The standard error of the ED_{50} was then determined from large-sample statistical properties.[30] The re-expression of the ED_{50} on the arithmetic dose scale was based on the postulate of log-normal distribution of the ED_{50} values.[31] Fieller's method was used to give a 95% confidence interval of the dose ratio on the arithmetic dose scale.[30]

¶ SAS® User's Guide: Statistics, Version 5. Cary NC 27511-8000, SAS Institute, 1985, pp. 575–606.

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TABLE 1. Laboratory Data—*In Vivo* Experiments

		n
*p*H	7.36 ± .05	172
P_{CO_2}	37.5 ± 5.9 mmHg	172
P_{O_2}	122 ± 21 mmHg	172
Na^+	143 ± 3 mEq · l^{-1}	139
K^+	4.5 ± .8 mEq · l^{-1}	139
Ca^{+2}	1.06 ± .08 mmol · l^{-1}	25
Hematocrit—initial	36 ± 3%	19
Hematocrit—final*	37 ± 5%	15

The laboratory values presented are the mean ± SD for all data obtained during each *in vivo* rat experiment, during controlled ventilation. Initial values were obtained after pithing and cannulation; therefore, initial hematocrit data do not reflect initial blood loss. Final hematocrit was obtained after dose-response measurements were completed.
n is the number of measurements reported; the number of animals was 39.
* $P > 0.2$ (ns) compared with initial hematocrit.

For both the Weibull and the logistic functions, the existence of shared parameters between curves (*e.g.*, a common EC_{50}) is represented by a single parameter estimate, because of the increased precision of a pooled statistical estimate. Specific comparisons between groups were made with *t* tests except where the standard error of the estimates were large sample approximations (*e.g.*, the standard error of the ED_{50} values), in which case z-tests were used. Values are presented as mean ± SEM, except as indicated.

MATERIALS

The drugs utilized were: arginine vasopressin (Sigma), azepexole (BHT-933 · 2HCl, 2-amino-6-ethyl-4,5,7,8-tetrahydro-6H-oxazolo[5,4-d]azepin, Boehringer Ingelheim KG, gift), halothane (Halocarbon), heparin sodium (porcine, Elkins-Sinn), HEPES (N-2-hydroxyethyl-piperazine-N′-2-ethanesulfonic acid, Sigma), 1-phenylephrine (Sigma). All other chemicals were of the highest quality obtainable, and purchased from either Fisher Scientific or Sigma Chemical Co. All drugs were dissolved in 0.9% NaCl; appropriate vehicle controls were utilized. These experiments were approved by our institutional Animal Care Committee, and conformed to the Guiding Principles in the Care and Use of Animals of the American Physiological Society.

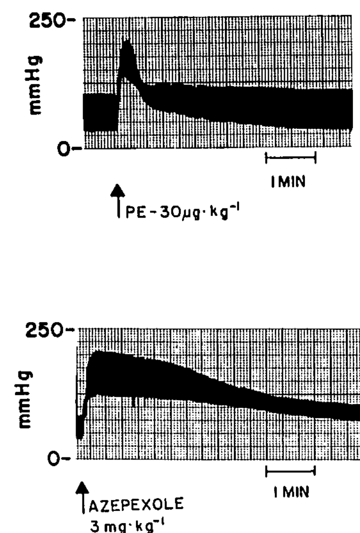

FIG. 1. Arterial pressure responses of pithed rats to large, equipotent doses of phenylephrine (PE, α_1 agonist; top panel) and azepexole (α_2 agonist, lower panel) in (−)halothane animals. Note the differences in shape and duration of action of the responses to activation of α_1- and α_2-adrenoceptors.

Results

IN VIVO EXPERIMENTS

Laboratory data. The mean blood gas, electrolyte, and hematocrit values, which were intermittently sampled during each experiment, are presented in table 1.

Control and supplemental control data. In the control groups, no anesthetic was administered following pithing. The anesthetic agents utilized for the brief initial phase of the experiment (halothane or isoflurane) were examined to determine whether they altered the subsequent control phenylephrine (PE) dose responses; no significant difference was detected ($P > .90$) between the control group and the supplemental control group. Similarly, the azepexole dose response was not significantly altered by the choice of the induction anesthetic ($P > .90$). We were unable to detect any difference in vascular reactivity caused by trace concentrations of halothane or isoflurane. Therefore, the data from the two control groups were pooled for subsequent analyses, and are referred to as the (−)halothane experiments.

Control pressor responses. PE and azepexole both caused dose-dependent pressor responses in pithed rats. Figure 1 demonstrates that the time course of DAP elevation, in response to a large dose of azepexole, was delayed in onset and longer in duration than the pressor action of PE, at doses which caused similar blood pressure changes. In the absence of halothane, the maximal acute rise in DAP obtainable with the largest PE bolus dose was significantly greater than that achievable with azepexole (table 2, $P < .001$).

Phenylephrine. Figure 2A demonstrates the asymmetric shape of pressor log-dose response curves obtained from pithed rats. The parameter estimates obtained from fitting the data to the Weibull dose-response function are summarized in table 2. There was no significant statistical distinction between the parameter estimates for the PE data with and without halothane. The high degree of coincidence of these curves is shown in figure 2A.

Azepexole. In contrast, there was a significant statistical difference for all parameters with the azepexole data, when the responses with and without halothane were

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TABLE 2. Pithed Rats: Effect of Halothane (1 MAC) on α-adrenergic Agonist Responses

Group	Parameter Estimates from Weibull Dose-response Functions			
	Y_{max}* (mmHg)	θ (slope factor)	λ (asymmetry factor)	ED_{50} ($\mu g \cdot kg^{-1}$)
Phenylephrine†	105 ± 4.4	1.18 ± .096	1.45 ± .14	6.50 ± .89
Azepexole				
(−) halothane	85.6 ± 3.1	0.183 ± .036	2.33 ± .33	219 ± 45
(+) halothane	65.4 ± 2.1	0.503 ± .036	1.38 ± .13	696 ± 85
P-value‡	<0.0001	<0.0001	<0.001	<0.0001

See text for explanation. Values are mean ± SEM, and are derived from a total of 30 rats. Values of absolute initial baseline diastolic blood pressures are given in table 3.

* Maximal blood pressure responses Y_{max} were computed for maximal doses D_{max} as follows: Phenylephrine: D_{max} = 1.5, corresponding to $\log_{10}$ 31.6 $\mu g \cdot kg^{-1}$; Azepexole: D_{max} = 4.1, corresponding to $\log_{10}$ 12590 $\mu g \cdot kg^{-1}$. These values for D_{max} were chosen because higher drug doses were lethal. In the absence of halothane, Y_{max} with phenylephrine was significantly higher than that achieved with axepexole ($P < 0.001$).

† The two phenylephrine curves, (+) halothane and (−) halothane, both shared all four parameters ($P > 0.9$).

‡ Comparing azepexole groups: (−) halothane vs. (+) halothane, using z-test.

compared (table 2, fig. 2B). For example, the maximal response was reduced 24% by halothane. The dose ratio of 1 MAC halothane for azepexole (ED_{50} with halothane/ ED_{50} control) was 3.18 (95% confidence interval 2.05–5.54), which was significantly different from 1.00 at the α = .05 level. Thus, in the presence of halothane, azepexole potency (ED_{50}) was reduced by a factor of approximately three.

Vasopressin group. Addition of halothane 1 MAC to pithed rats significantly lowered the baseline diastolic blood pressure measured prior to injection of any pressor drugs (table 3). In a group of pithed rats receiving 1 MAC halothane, intravenous infusion of vasopressin (range 4.5–47.6 $\mu g \cdot kg^{-1} \cdot min^{-1}$) raised the baseline diastolic blood pressure to a value not significantly different from the baseline value seen in control rats receiving no anesthetic (table 3). This AVP dose was continued in each rat. Despite the abolition of baseline hypotension, figure 3 demonstrates that these rats' responsiveness to azepexole during halothane was not significantly altered by the concurrent administration of AVP ($P > .9$).

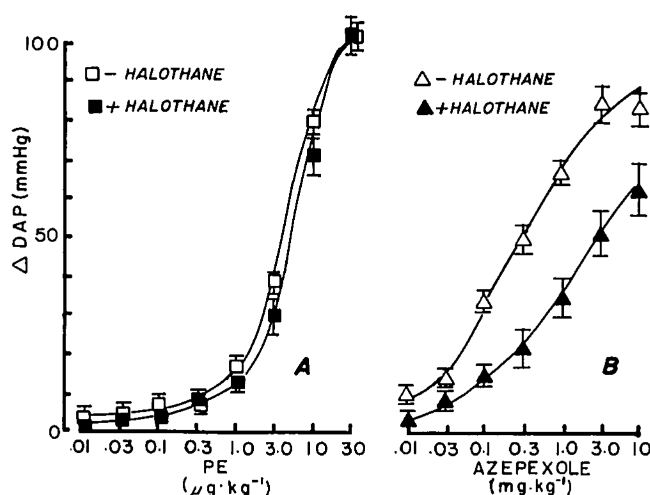

FIG. 2. Effect of halothane on diastolic arterial pressure (DAP) log-dose-responses, in pithed rats *in vivo*, to increasing doses of phenylephrine (PE; panel A, α_1 agonist) and azepexole (panel B, α_2 agonist). Addition of halothane (1 MAC) produced no change in the PE responses, but significantly altered the pressor actions of azepexole (table 2). Agonists were administered intravenously, and each rat received only one agonist. The (−)halothane curves were derived from control and supplemental control group data. See text for details. Note that the dose scale is logarithmic. Each point represents the mean ± SEM derived from four to 11 rats.

In Vitro EXPERIMENTS

Control data. The canine saphenous vein data (fig. 4) showed many similarities to the pithed rat results. Both agonist drugs caused a concentration-dependent contraction of the vessels. In the absence of halothane, the maximal tension generated by PE was 7.6 ± .46 g, and by azepexole 5.6 ± .86 g ($P < .05$). At every agonist concentration, the generated tension for each ring in the presence of halothane was compared with the maximal tension obtained in that ring prior to halothane addition;

TABLE 3. Use of Vasopressin to Reverse Halothane-induced Baseline Hypotension

Group	Halothane	Vasopressin	Baseline Diastolic Blood Pressure (mmHg)	n
I	−	−	38 ± 3	20
II	+	−	32 ± 1	15
III	+	+	41 ± 1	4

Blood pressure data are presented for control and 1 MAC halothane-treated rats, as well as a group of rats receiving both halothane and an arginine vasopressin infusion. Details are provided in the text. The baseline blood pressure was measured at equivalent times in each group after pithing, cannulation, and stabilization, but prior to the administration of any α-adrenergic drugs. Statistical significance: groups I *vs.* II: $P < 0.0001$; groups II *vs.* III: $P < 0.0001$; groups I *vs.* III: $P > 0.07$ (ns).

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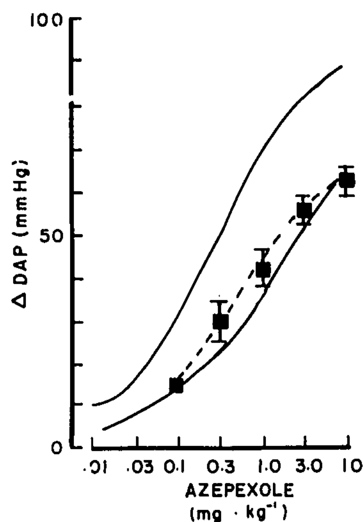

FIG. 3. Dashed line: Arginine vasopressin (AVP) infusion during halothane (1 MAC) administration does not significantly alter the pressor responsiveness of azepexole in pithed rats [$P > 0.9$ compared to (−)halothane (−)AVP controls by Weibull analysis]. AVP dose was titrated to restore baseline DAP of (+)halothane rats to level seen in (−)halothane rats. Control and halothane (1 MAC) curves from figure 2, without AVP, are included for comparison (solid lines). Each point represents the mean ± SEM derived from one to four rats.

this ratio was expressed as a percentage for figure 4 and for subsequent analysis. Halothane-free control rings showed minimal degradation in responsiveness by serial challenges with PE and azepexole during the time course of the halothane experiments. Only two out of 12 of these control rings tested with three serial challenges showed a decrease in maximal response in the third trial relative to the maximal response in the first trial.

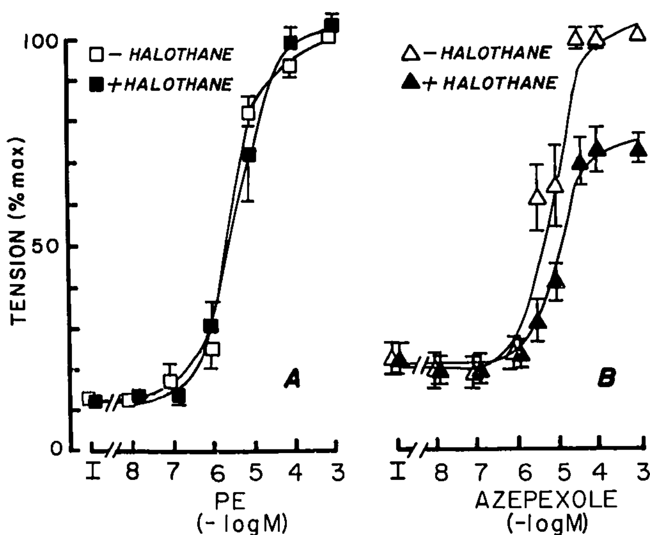

FIG. 4. Effect of halothane on tension generated in canine saphenous vein rings *in vitro*, in response to increasing concentrations of phenylephrine (PE; panel *A*, α_1 agonist) and azepexole (panel *B*, α_2 agonist). Addition of halothane (4%) to the bath produced no change in the PE responses, but significantly reduced the maximum tension and EC_{50} of the pressor dose-response to azepexole (table 4). Tension is presented as the percent of the maximal total tension obtained with each agonist in the absence of halothane [the (−) halothane curves]. Each ring was its own control. See text for details. Note that the dose scale is logarithmic. Each point represents the mean ± SEM derived from four to eight rings. I = initial value without α agonist.

Figure 4 demonstrates the symmetric concentration-response curves uniformly obtained in the experiments with canine saphenous veins. The four-parameter logistic response function was fit to the data, and the parameter estimates obtained during different experimental conditions are summarized in table 4.

Phenylephrine. The parameters of the two PE curves (one with and one without halothane) were not statistically distinct (table 4). The lack of halothane effect on PE responsiveness in canine saphenous vein is shown in figure 4A.

Azepexole. The two curves for azepexole demonstrate that halothane caused statistically significant reductions of the maximal tension response (a 26% reduction) and EC_{50} (table 4, fig. 4B). The azepexole log concentration-response curve was shifted rightward by halothane 2.43-fold (the dose ratio), with 95% confidence interval 1.59–3.27. This *in vitro* halothane-azepexole dose ratio was not significantly different from the dose ratio obtained in the *in vivo* experiment.

Discussion

Our results demonstrate that halothane selectively interferes with α_2-adrenoceptor-mediated vasoconstriction, while keeping α_1-mediated vascular responses essentially unchanged. These qualitative findings are seen with both *in vivo* and *in vitro* techniques. The concept of vasodilation is inherently related to that of anti-vasoconstriction, since it is impossible to vasodilate without first having some vasoconstrictive tone present. To the extent that basal α-adrenergic tone exists, our demonstration of halothane-induced anti-vasoconstriction related to α_2-adrenoceptors helps to define, in part, the mechanism of halothane vasodilation at the end-organ level (vascular smooth muscle). Since catecholamine-induced vasoconstriction *in vivo* is a combination of α_1 and α_2 adrenoceptor stimulation, these results may help explain halothane's vascular actions in the intact animal. Further examination of halothane-α_2-adrenoceptor interactions, in intact animals, are needed to define the role of α_2 anti-vasoconstriction in mediating halothane vasodilation.

Interactions of halothane with vascular α_2-adrenoceptors have not been previously described, and little is known about anesthetic actions at α-adrenoceptor subtypes in any tissues. In the myocardium, both α_1- and α_2-adrenoceptors mediate the elevation in cyclic GMP caused by halothane.[32] In the brain, stimulation of α_2-adrenoceptors by clonidine produces withdrawal of sympathetic tone (a useful anti-hypertensive effect), sedation, and reduction of halothane MAC in dogs.[33] On pre-junctional sympathetic nerve terminals, stimulation of inhibitory α_2-adrenoceptors reduce neuronal norepinephrine release. Halothane is known to inhibit prejunctional norepineph-

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TABLE 4. Canine Saphenous Vein: Effect of Halothane (4%) on α-adrenergic Agonist Responses

	Parameter Estimates from the Logistic Dose-response Function			
Group	Y_{max} (%)	Y_0 (%)	EC_{50} ($\mu mol \cdot l^{-1}$)	b (slope factor)
Phenylephrine*	100 ± 0.2	13.0 ± 1.2	2.9 ± 0.59	1.14 ± 0.18
Azepexole†				
(−) halothane	100 ± 1.5	19.3 ± 0.8	5.16 ± 0.44	1.80 ± 0.18
(+) halothane	73.5 ± 1.8		12.4 ± 1.7	
P-value‡	<0.0001	>0.7 (ns)	<0.0001	>0.09 (ns)

See text for explanation. Values are means ± SEM. Note that the values of Y_0 and Y_{max} in the table are given as percent of the maximal absolute tension, and include the 1 g resting tension.

* The two phenylephrine curves, (+) halothane and (−) halothane, both shared all four parameters ($P > 0.9$, ns). The corresponding values of absolute tension in the absence of halothane are: $Y_{max} = 7.6 \pm 0.46$ g; $Y_0 = 0.96 \pm 0.005$ g.

† The corresponding values of absolute tension in the absence of halothane are: $Y_{max} = 5.6 \pm 0.86$; $Y_0 = 0.98 \pm 0.006$ g. Y_0 and b were shared between the two curves, and are shown as single estimates.

‡ Comparing azepexole groups: (−) halothane *vs.* (+) halothane; ns = not significant.

rine release by stimulating muscarinic cholinergic receptors,[34] but we did not study pre-junctional α_2 receptors.

IN VIVO EXPERIMENTS

During intact-animal studies, drug- and anesthetic-induced changes in autonomic tone can alter the background level of activation of vascular postjunctional α-adrenoceptors. In an attempt to eliminate such variations in autonomic tone during the course of our experiments, and to permit discontinuation of all anesthetics in the control groups, all rats were pithed to destroy the entire central nervous system.

Diastolic blood pressure measurements in the pithed rat have been used successfully to dissociate postjunctional vascular α_1- and α_2-adrenoceptor effects from each other, and from prejunctional adrenoceptor actions, by employing specific agonists and antagonists.[23,35] These studies have amply demonstrated that PE and azepexole possess a high degree of α-adrenoceptor subtype specificity, through the use of specific antagonists, making the addition of antagonists unnecessary in our studies. Others have reported time courses of α-adrenergic subtype responses similar to ours.[35] Timmermans *et al.* reported ED_{50} values in pithed rats as follows: PE, 7.1 $\mu g \cdot kg^{-1}$, and azepexole, 350 $\mu g \cdot kg^{-1}$; our results in (−)halothane animals are similar, and the small differences may be related to their use of hexobarbital anesthesia and a different strain of rats.[36]

While investigations with isolated perfused rat hindquarters have shown clearly that α_1 and α_2 agonists produce resistance-vessel vasoconstriction,[37] DAP measurements in pithed rats do not provide an entirely specific indicator for alterations in vascular tone. Indeed, studies with pithed rats have demonstrated that both cardiac output and vascular resistance rise together with DAP in response to α_1 and α_2 agonists. However, increased cardiac output with α agonists appeared to be primarily due to venous constriction, which elevated the cardiac preload.[38] DAP change in the pithed rat has nonetheless proved to be a valuable technique for separating α_1 and α_2 receptor actions. The parallel between our *in vivo* and *in vitro* data further supports the use of the pithed rat as a model of anesthetic vasodilation. Thus, the pressor responses by α agonists in our experiments are inferred to be predominantly vascular actions in arteries and veins. Halothane's observed anti-α_2-vasoconstriction action was not an artifact caused by non-specific effect of halothane-induced hypotension,[39] since: 1) restoration of the blood pressure with vasopressin did not alter the α_2 inhibitory effect of halothane in rats; 2) the α_2-selective effect was duplicated *in vitro;* and 3) PE responsiveness was not altered by halothane. Since acid-base disturbances may exert a differential effect upon α_1- and α_2-adrenoceptor responses,[40] the rats' metabolic state was controlled within narrow limits (table 1).

All of our rats, including those in the control groups, were initially anesthetized, however briefly, with a volatile anesthetic. Although rats in the control group were not studied until alveolar anesthetic levels were unmeasurable by our techniques, it has been reported that prolonged hyporesponsiveness of VSM may follow halothane anesthesia in rabbits, perhaps due to an anesthetic metabolite.[25] It is not possible to exclude such an effect from our control group rat data, but this is unlikely given the lack of detectable differences between the control group rats initially anesthetized with halothane and the supplemental control group rats initially given isoflurane (which undergoes much less metabolism than halothane). Additionally, the *in vitro* control experiments were entirely free of volatile anesthetic, and their similarity to the rat results also support the use of our rat control groups. Finally, any depression of vascular responsiveness due to residual anesthetic in our control rats should have made it more difficult to detect a significant anti-vasoconstrictor effect of 1 MAC halothane; therefore, exclusion of volatile an-

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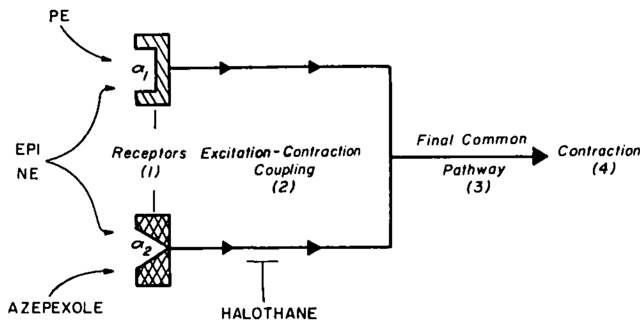

FIG. 5. Schematic representation of the sequence of events believed to link the activation of vascular α-adrenoceptors with their functional response, smooth muscle contraction. After a specific adrenergic agonist drug or hormone binds to its receptor (1), the process of excitation-contraction coupling (2) is initiated; the specific pathway used is dependent upon the receptor subtype which was stimulated. The different receptor-specific excitation-coupling pathways eventually converge on a single final common pathway (3), generally thought to be an elevation of the intracellular free Ca^{+2} concentration. This $[Ca^{+2}]$ elevation then activates the muscle contractile apparatus (4). It is hypothesized that halothane interferes with the α_2-adrenoceptor-specific excitation-contraction coupling mechanism (indicated), without substantially affecting the other elements of the vascular smooth muscle activation. See text for details. EPI = epinephrine; NE = norepinephrine; PE = phenylephrine.

esthetics from the initial phases of the experiment would have been unlikely to alter our results.

In Vitro Experiments

No *in vivo* experiment can control as many variables as can be controlled with *in vitro* techniques. Therefore, isolated blood vessel studies were also performed, using canine saphenous vein rings. The isometric contractile response of canine saphenous vein *in vitro* has been used to examine drug effects at α-adrenoceptor subtypes. Using canine saphenous vein in HEPES buffer, Matthews *et al.* reported the EC_{50} of phenylephrine as 3.4 μM, similar to our observations (table 4).[41] Reports of azepexole use in canine saphenous vein could not be found. Adequate time was provided for halothane to reach a steady state with buffer and with canine saphenous vein.[8,42]

The explanation for the higher apparent anti-vasoconstrictor potency of halothane *in vivo* than *in vitro* is not known, but may relate to differences between the responsiveness of small resistance vessels and of large capacitance vessels, or may be species related.[43] While the halothane concentration utilized in the *in vitro* canine saphenous vein experiments was higher than that used clinically, it is interesting that halothane's lack of effect upon α_1-adrenoceptor responsiveness was preserved even at a very high halothane concentration.

Sprague *et al.*[44] reported that halothane causes a dose-dependent reduction in the PE-induced contraction of rat aortic strips *in vitro.* Our results, both in the rat and in canine saphenous vein, demonstrate no effect of halothane on PE-induced vasoconstriction, and appear to conflict with their findings. Methodologic differences exist, and, also, responses may vary depending upon species, and the type of vessel used: they examined a large conducting artery, whereas we primarily studied arterial resistance vessels, and veins.[43] The significance of these factors requires further study.

Using canine saphenous vein strips, Muldoon *et al.* found that halothane (1–3%) significantly reduced electrically induced vascular contraction, but did not alter exogenous norepinephrine-induced responses.[8] Those authors concluded that halothane venous dilation was mediated primarily by a reduction in norepinephrine release by prejunctional nerve terminals. Possible explanations for their lack of halothane response to exogenous norepinephrine include experimental methodology, and their use of a nonspecific α_1- plus α_2-adrenoceptor agonist, as opposed to our specific agonists. It remains to be determined whether the magnitude of the anti-α_2-adrenergic effect of halothane which we observed using specific agonists, is sufficient to alter the vascular responsiveness to nonspecific α agonists in each species. In other experiments, also using exogenous norepinephrine, but with rabbit aorta strips, halothane did demonstrate an anti-vasoconstrictive action.[15] Further investigation of the role of these and other factors in anesthetic vasodilation is warranted.

Mechanisms of Halothane Vasodilation: Implications and Inferences

It is highly unlikely that halothane, in our preparations, acted directly at α_2-adrenoceptor binding sites, because: 1) the depression of maximum vasoconstriction by halothane would imply non-competitive inhibition[45] (although this was not examined kinetically); 2) halothane is relatively nonreactive, and, therefore, is not likely to covalently modify a receptor; and 3) halothane is an uncharged lipophilic molecule which is incompatible with known α-adrenergic drug structure-activity relationships. Thus, mechanisms involved in α-adrenergic vasoconstriction which lie distal to the receptor may help explain our findings.

Calcium ions, and α-adrenergic activation mechanisms. Adrenergic excitation-contraction coupling in VSM is believed to occur in several defined steps (fig. 5): activation of α_1 or α_2 surface receptors (1) causes transduction of signals to internal cell structures by separate second messenger pathways, resulting in an elevation of intracellular free Ca^{+2} levels (2). The rise in free Ca^{+2} concentration is believed to be the "final common pathway" of excitation-contraction coupling (3), which stimulates the actin-myosin contractile system by activating various regulatory proteins (4).[19]

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It may be possible to infer some of the cellular mechanisms by which a drug induces vasodilation, if that drug causes differential inhibition of α_1- or α_2-mediated contraction of VSM. Van Meel *et al.* first reported in pithed rats that inhibiting Ca^{+2} entry into VSM could diminish α_2- but not α_1-adrenoceptor-mediated vasoconstriction.[46] Our experimental findings, in the same pithed rat model, indicate a similar pattern of selective α_2-mediated inhibition with halothane. The van Meel group theorized that post-junctional α_2-adrenoceptor-mediated vasoconstriction requires entry of extracellular Ca^{+2}; but postjunctional α_1 vasoconstriction is much less dependent upon Ca^{+2} influx, utilizing other means for increasing the cytoplasmic Ca^{+2} concentration (*e.g.*, release of intracellular Ca^{+2} stores). Using resistance-vessel preparations from rats, rabbits, cats, and dogs, and using canine saphenous vein, a number of groups have published reports confirming and expanding these observations (see citations in ref. 47).[41,48] These investigations utilized calcium blockers (organic and inorganic) and Ca^{+2}-free bathing media to demonstrate that α_2 agonist responses uniformly were highly sensitive to inhibition of Ca^{+2} entry in VSM, and that α_1 agonist effects (by full agonist drugs, such as PE) were relatively resistant to depression by Ca^{+2} entry blockade.

Not all published research has supported the theory of van Meel *et al.*[49] The order of drug administration can be relevant: a Ca^{+2} blocker given after an α_1- or α_2-agonist modifies the sustained, tonic phase of vasoconstriction in spinal dogs.[50] In our studies, halothane was begun prior to agonist delivery, and thus we examined primarily halothane effects on initial phasic vasoconstriction. Likewise, the studies showing responses to azepexole but not to PE to be sensitive to Ca^{+2} entry inhibition (cited above) used protocols similar to ours. In other studies, phenoxybenzamine, an irreversible inhibitor of α receptors, caused responses to full α_1 agonists to become susceptible to inhibition by Ca^{+2} entry blockade.[51] This has been interpreted as evidence that "spare" α_1 receptors exist on VSM, thereby making α_1 responses less prone to inhibition by various nonspecific inhibitors of vasoconstriction.[52,53] Those authors submit that α_2 adrenoceptor populations on VSM do not contain "spare" receptors, and that the available α_2 agonist drugs are of limited efficacy in stimulating α_2 adrenoceptors. Certain vasodilator drugs also have been shown to inhibit α_2 more than α_1 vasoconstriction.[52] Van Meel *et al.* have defended their theory by showing, for example, that non-α-receptor-mediated properties of phenoxybenzamine may account for many of the results attributed to spare receptors.[47,54]

The controversy over the van Meel *et al.* theory of differential Ca^{+2} utilization pathways by α_1- and α_2-adrenoceptors remains unresolved, although some of the differences are likely related to species, tissue type, and experimental techniques.[40,43] If that theory is not valid, then one cannot infer how halothane affects VSM Ca^{+2} utilization from our data. However, our finding that halothane exerts its anti-vasoconstrictor action selectively through α_2-mediated processes remains valid, whatever mechanisms may underlie the processes of α-adrenoceptor subtype activation.

Halothane and vascular calcium entry. Assuming the validity of the theory of van Meel and colleagues for vascular smooth muscle, then the mechanism by which α agonists elevate intracellular Ca^{+2} and cause vasoconstriction will depend upon the subtype of α-receptor which is activated (step 2, fig. 5). Azepexole (α_2) vasoconstriction would primarily utilize extracellular Ca^{+2}, but PE (α_1) vasoconstriction would depend on both release of stored intracellular Ca^{+2} and entry of external Ca^{+2}. Although the precise molecular mechanism whereby halothane interferes with α_2-mediated vasoconstriction cannot be deduced from our experiments, certain inferences could be made. Application of this theory to our experimental models would indicate that halothane interferes with α_2 excitation-contraction coupling by limiting the passage of extracellular Ca^{+2} through calcium channels into VSM cells (step 2, bottom, fig. 5). Our observations of unimpaired α_1-mediated vasoconstriction in the presence of halothane would imply that α_1 excitation-contraction coupling remains intact, and that neither the release of intracellular Ca^{+2} stores (step 2, top, fig. 5) nor the final common pathway for contraction (step 3, fig. 5) were substantially impaired by halothane under the specific conditions of the experiments. These inferences remain to be proved by direct experimentation.

It is not surprising that halothane may act to decrease Ca^{+2} influx through Ca^{+2} channels in blood vessels. In heart, halothane depresses the myocardial slow inward current[55] which is carried primarily by Ca^{+2}, and in the adrenal medulla halothane selectively interferes with nicotinic-receptor-linked Ca^{+2} channel function.[56] It is unlikely that halothane blocks Ca^{+2} channels by binding to specific drug receptors in the fashion of conventional calcium blocker drugs.[57] Instead, perturbations by halothane of the specific Ca^{+2} channel regulatory proteins or its lipid environment could achieve a reduction in Ca^{+2} channel function.[58]

Regardless of the specific intracellular mechanisms which are activated by α_1- and α_2-adrenoceptors, our data clearly indicate that halothane interferes with the vasoconstriction produced by α_2-adrenoceptor stimulation. Because endogenous epinephrine and norepinephrine are agonists at both α_1 and α_2 receptors, the anti-α_2-adrenergic action of halothane which we report may contribute to halothane vasodilation *in vivo*. Since calcium blockers also attenuate α_2-adrenergic vasoconstriction, our results may help explain vascular interactions between halothane

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and calcium blockers. Also, our observation of preserved α_1-adrenoceptor-mediated vasoconstriction in the presence of halothane may explain why phenylephrine is frequently effective in reversing halothane-induced hypotension, clinically.

Further research is needed to define the quantitative role of α_2-adrenoceptors in mediating the vascular actions of anesthetics, as well as their cellular mechanisms of action in VSM. The methods and experimental approaches utilized in this research could be of importance in dissecting the adrenergic actions of various anesthetics in vascular and other tissues.

In conclusion, halothane, both *in vivo* and *in vitro*, has been shown to selectively attenuate α_2-adrenergic vasoconstriction, while preserving α_1-adrenoceptor responsiveness in vascular smooth muscle. This effect may have implications concerning the molecular actions of halothane on excitable tissues. The selective α_2-anti-vasoconstrictor action of halothane further demonstrates the multifactorial nature of halothane-induced vasodilation.

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