

Induction of Anesthesia with Halothane Increases Plasma Norepinephrine Concentrations

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In seven unstimulated, unmedicated patients given halothane/O₂ via face mask, plasma norepinephrine concentration increased 15 min after induction and returned to control at 45 to 60 min. Changes in plasma norepinephrine levels did not correlate with changes in cardiovascular variables. In 10 additional awake, unpremedicated patients, plasma norepinephrine concentration did not change during 5 min of application of 100 per cent oxygen via face mask, but rose with subsequent administration of halothane and nitrous oxide. Again, changes in plasma norepinephrine did not correlate with changes in cardiovascular variables. The authors perfused seven isolated cat spleens with a Krebs-Ringer's lactate solution. Addition of 0.01 atm halothane to the perfusate initially increased release of norepinephrine into the effluent. The authors conclude that halothane or halothane-nitrous oxide initially increases plasma norepinephrine during induction of anesthesia. This increase is not due to the placement of a face mask, but may relate to an effect of halothane at sympathetic nerve endings. (Key words: Anesthetics, volatile: halothane. Equipment: masks, anesthesia. Sympathetic nerve system: catecholamines; norepinephrine.)

HALOTHANE decreases tonic sympathetic nervous system activity in dogs¹ and rats,² as reflected by decreasing plasma norepinephrine levels. If this relationship exists in humans, it may explain a portion of the associated decreases in cardiac output, arterial pressure, and myocardial contractility.³ In the present report, we have examined the impact of induction of anesthesia with halothane on plasma norepinephrine levels and cardiovascular variables. We tested and found absent the effect of face-mask placement on sympathetic response. We also studied the isolated perfused cat spleen to delineate the effect of halothane on release of norepinephrine at nerve endings.

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Methods

PART ONE

After receiving approval from the Committee on Human Research, we studied seven ASA class I patients, 22-33 years of age, who gave informed consent. No patient took medication chronically or received preoperative medication. An intravenous catheter was inserted into a forearm vein; 15 min later, a blood sample was obtained for determination of plasma norepinephrine concentration⁴ and pH and P_{CO₂} values.⁵ Anesthesia was induced with gradually increasing concentrations of halothane applied in 100 per cent oxygen by face mask. When the second stage of anesthesia was reached, ventilation was controlled to maintain end-tidal CO₂ concentrations (measured using a Beckman LB2[®] analyzer) near control levels. A stable end-tidal halothane concentration of 1.5 per cent was established (as measured by a Beckman LB1[®] analyzer) and was maintained for 15 min. The concentration of halothane was then reduced to 1 per cent for 30 min, and then to 0.5 per cent for 15 additional minutes. Blood samples were drawn at the end of each of these steady-state periods, and cardiovascular variables were measured at each sampling period. We analyzed plasma norepinephrine concentration using a modification of the Peuler-Johnson radioenzymatic assay.⁶ A patient was eliminated from study if any venous blood pH value was not between 7.33 and 7.45, or if any venous blood P_{CO₂} value was not within a 6-mmHg range of baseline levels (these ranges were chosen arbitrarily before initiation of the study). We obtained all samples before laryngoscopy or surgical stimulation. The Newman-Keuls test⁷ and repeated-measures analysis of variance were used to determine statistical significance. Changes in cardiovascular variables and plasma norepinephrine concentration were compared using linear regression.

PART TWO

This portion of our study examined the role of face-mask placement on the plasma norepinephrine changes seen during induction. Institutional approval and informed consent were obtained, and face-mask placement was discussed with each patient. We studied ten ASA class I-II patients, 16-62 years of age, who did not take medicine chronically or receive preoperative medication.

An intravenous catheter was placed in a forearm vein to obtain blood samples for plasma catecholamine analysis and measurement of pH and P_{CO_2} . Fifteen and 18 min after intravenous catheter placement, control samples were obtained. An anesthesia face mask was then placed, and 100 per cent oxygen administered. One, 3, and 5 min later, samples were again obtained. At 5.5 min after face-mask placement, and without announcement, we administered 60 per cent nitrous oxide and gradually increasing concentrations of halothane. The inspired halothane concentration was increased until an end-tidal concentration of 1.5 per cent (measured by mass spectrometry) was achieved; this concentration was maintained for the remainder of the study. Blood samples were obtained at 0.5, 3, 6, 8, and 13 min after initiation of halothane/nitrous oxide. Ventilation was controlled, patient's results were excluded, and statistical analysis was performed as described in Part One.

PART THREE

We sought to determine if halothane altered plasma catecholamines by an action on nerve endings. We prepared isolated spleens of seven male cats, as described by Kopin *et al.*,⁸ and perfused the spleens at a constant rate with Krebs-Ringer's solution saturated with 95 per cent oxygen and 5 per cent carbon dioxide. The solution contained 1 g of glucose per liter. Subsequently, we used the same solution saturated with 0.01 atm halothane carried by 95 per cent oxygen and 5 per cent carbon dioxide. Splenic arterial perfusion pressure was monitored with a Statham P-23 AC® transducer. Samples of effluent were collected during 2-min intervals after a 30-min period of equilibration. The Krebs-Ringer's solution that was saturated with oxygen and carbon dioxide was used for 30 min; then the second Krebs-Ringer's solution (halothane- CO_2 - O_2) was infused by turning a stopcock. Effluent samples were collected again every 2 min during a 30-min period of equilibration. The control perfusate was reinstituted, followed by a 30-min washout period. The effluent collect from the splenic vein during each 2-min interval was immediately cooled, mixed with 0.5 ml of 60 per cent perchloric acid, and kept at 0-4°C until assayed that evening.

Aliquots of the samples were assayed for norepinephrine using the fluorometric method of Häggendal.⁹ The Newman-Keuls test⁷ and repeated-measures analysis of variance were used to determine statistical significance.

Results

PART ONE

Halothane administration initially increased plasma norepinephrine levels ($P < 0.005$). Only the value ob-

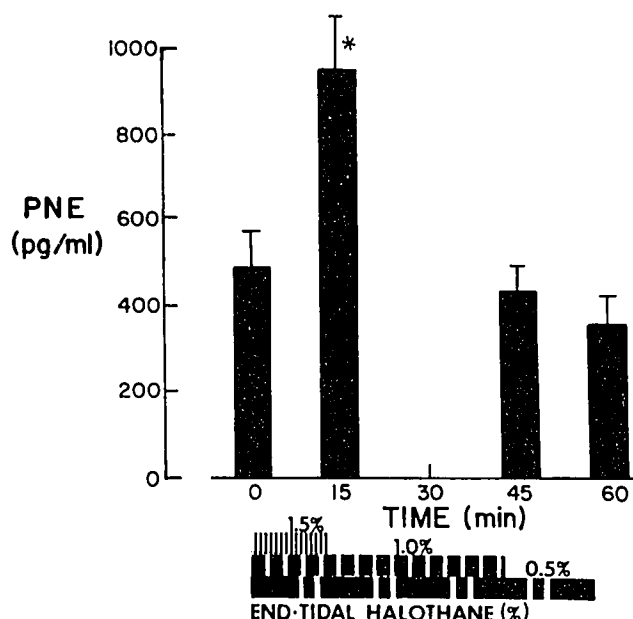


FIG. 1. Changes in plasma norepinephrine concentrations during administration of halothane over time in normal humans. Compared with control values, plasma norepinephrine levels increased significantly (* $P < 0.005$) at 15 min. Values are means \pm SEM.

tained 15 min after initiation of halothane administration was increased significantly (fig. 1., table 1). Plasma norepinephrine levels subsequently returned to baseline levels (fig. 1) upon continued administration of halothane at lower concentrations. Changes in cardiovascular variables did not correlate with changes in plasma norepinephrine.

PART TWO

Placement of a face mask and administration of 100 per cent oxygen did not affect plasma norepinephrine levels (fig. 2, table 1). However, these levels increased significantly ($P < 0.001$) when halothane and 60 per cent nitrous oxide were administered. Cardiovascular variables were stable throughout the study, except for a decreasing trend near the end as the anesthetic continued. This decrease first became statistically significant for blood pressure ($P < 0.01$) at the final sampling period, which corresponded to 13 min of halothane and nitrous oxide anesthesia. Again, changes in any cardiovascular variable between any two sampling periods failed to correlate with changes in plasma norepinephrine levels.

PART THREE

Perfusion of cat spleens with halothane initially increased the spontaneous release of norepinephrine ($P < 0.005$) from the nerve ending (fig. 3, table 1). Continued exposure to halothane, however, decreased the

TABLE 1. Norepinephrine (NE) and Cardiovascular Variables in Three Experiments

Experiment	Time Period (min)	Anesthetic	Plasma NE Concentration (±SEM, pg/ml)	Blood Pressure (±SD, mmHg)	Heart Rate (±SD, beats/min)
Part 1 (n = 7)	Control		497 ± 74	118 ± 11	69 ± 7
	15	1.5 per cent halothane	953 ± 126*	89 ± 9*	65 ± 8
	45	1.0 per cent halothane	418 ± 76	98 ± 9*	66 ± 4
	60	0.5 per cent halothane	358 ± 44	99 ± 10*	65 ± 5
Part 2 (n = 10)	Control		133 ± 20.9	125 ± 16	71 ± 13
	1	Face mask-O ₂ only	129 ± 19.6	127 ± 17	69 ± 12
	3		135 ± 24	124 ± 15	71 ± 13
	5		147 ± 31	122 ± 18	71 ± 13
	6		161 ± 32	121 ± 20	71 ± 13
	8.5	Halothane-60 per cent-N ₂ O-38 per cent O ₂	170 ± 27	121 ± 20	71 ± 13
	8.5		170 ± 27	117 ± 23	77 ± 15
	11.5		229 ± 32†	110 ± 25	74 ± 19
	13.5		287 ± 35†	106 ± 26	68 ± 17
	18.5	357 ± 42†	102 ± 24†	64 ± 15	
	Time		NE (ng)/2 min (±SEM)		
Part 3 (n = 7)	Before halothane		6.0 ± 1.6		
	1st 2 min of halothane		7.5 ± 0.98†		
	1st 2 min without halothane		6.8 ± 1.3		

* Different from control ($P < 0.01$).† Different from control ($P < 0.05$).‡ Different from control ($P < 0.005$).

norepinephrine content of the effluent; the first 2-min sample after halothane exposure was the only one that showed an increase. When halothane was discontinued, norepinephrine content of the splenic effluent was not different from baseline levels.

Discussion

The interaction of halothane and the sympathetic nervous system in humans has not been clearly defined. Reports before 1970 suggested that halothane caused no significant changes in plasma norepinephrine, epinephrine or total catecholamines in dogs or humans.¹⁰⁻¹² More recent investigations, however, reported that halothane decreased plasma catecholamines in both dogs¹ and

rats.² The earlier reports probably give an incorrect impression because they used the trihydroxy-indole method to analyze catecholamines. The sensitivity of this older method (reported to be 3,000 pg/ml of plasma¹⁰) is insufficient to detect small changes in plasma catecholamine levels. The radioenzymatic assay used in the present study has a sensitivity of 6 pg/ml of plasma and is capable of detecting small changes in catecholamine concentration. In addition, differences in the experimental procedures of the earlier studies, such as induction with thiopental, stimulation from intubation and surgery, and the addition of nitrous oxide, may have contributed to the failure to detect a change in catecholamines.

We chose plasma norepinephrine concentration as our index of adrenergic responsiveness because 1) plasma norepinephrine concentration is the most reliable *in vivo* index of adrenergic activity in humans (it is accurate and measures small changes), and 2) it responds rapidly to changing stimuli (norepinephrine has a half-life of less than 3 min).¹³ This rapid change allows us to focus on relatively constant stimuli, *i.e.*, placing the face mask and inducing anesthesia, unaffected by preceding stimuli that might affect other indices of neurohormonal response having longer half-lives (*e.g.*, cortisol, ADH). We realize, however, that other indices of sympathetic activity exist and that factors other than sympathetic activity also may govern plasma concentration of norepinephrine and epinephrine.¹⁴

Previous reports have not suggested that halothane significantly increases plasma norepinephrine content in unstimulated humans or animals. In contrast, the present report does suggest such an increase and further suggests

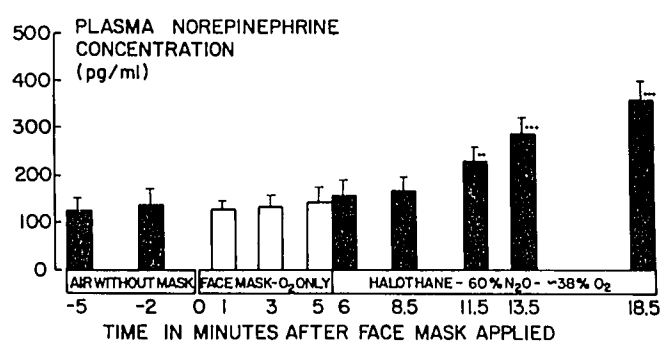


FIG. 2. Plasma norepinephrine levels during control (subjects breathing air without face mask), placement of a face mask and administration of halothane-nitrous oxide. Values are means \pm SEM. ** $P < 0.05$: Increase in plasma norepinephrine compared with control and face-mask placement. *** $P < 0.001$: Increase in plasma norepinephrine compared with control and face-mask placement.

that this increase is not simply due to application of a face mask. If changes in plasma norepinephrine levels reflect sympathetic nervous system activity, it would appear that halothane alone and in combination with nitrous oxide initially activates the sympathetic nervous system. In studies using the insensitive trihydroxyl-indole method to analyze catecholamines, Smith *et al.*¹⁵ demonstrated that addition of nitrous oxide increases plasma catecholamine levels in patients already anesthetized with halothane. However, we found that administration of halothane plus nitrous oxide and oxygen (Part Two) did not cause changes in plasma norepinephrine levels that differed qualitatively from those occurring when only halothane in oxygen (Part One) were given. We do not know why baseline norepinephrine values of patients in the two studies differed. The studies were done in different patient populations; also, the studies and their assays were done in different years. Nevertheless, each patient served as his/her own control so that the differences from baseline, rather than the absolute baseline values, were the important variables.

The implication that halothane may initially cause sympathetic activation is supported by clinical observations of second-stage anesthesia during induction of anesthesia with halothane and oxygen: pupillary dilatation, hypertension, tachycardia, excitement, and involuntary activity. The increases in plasma norepinephrine concentration that occurred during the second stage of anesthesia in this study, however, persisted after the patient had entered the third stage of anesthesia. After one hour of halothane administration, the plasma norepinephrine level decreased to control values. Since animal studies suggest that prolonged exposures to halothane decrease catecholamine levels,^{1,2} continued halothane exposure in our patients might have significantly decreased norepinephrine levels. In rats, Roizen *et al.*² demonstrated a dose-dependent decrease in plasma catecholamines with halothane; however, blood sampling did not occur until after 90 min of halothane anesthesia. In dogs, Perry *et al.*¹ demonstrated plasma catecholamine decreases, but, again, sampling did not occur until at least 30 min after induction. In both studies, catecholamines were not measured during induction; consequently, the differences in our data for humans and those for animals may reflect differences in sampling periods during the time course of norepinephrine changes rather than differences due to species.

Other possible causes for the increases in plasma norepinephrine concentration include two uncontrolled stimuli, movement, and the smell of halothane. Although halothane lacks the pungent odor associated with enflurane and isoflurane, the presence of a new stimulus (smell) that was not present during control sampling may have affected the results. Involuntary movements during the second stage of anesthesia may also have increased plasma norepinephrine levels. However, similar plasma

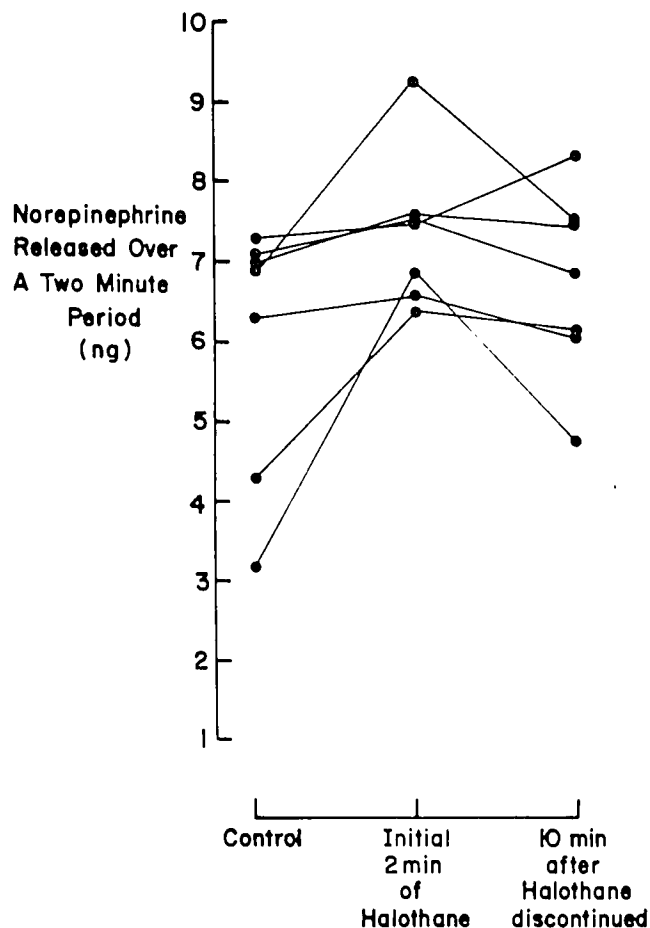


FIG. 3. Change in norepinephrine concentration in splenic effluent with perfusion and subsequent discontinuance of halothane. Norepinephrine increased significantly ($P < 0.005$) after initial perfusion with halothane. Values are means \pm SEM.

norepinephrine increases occurred in patients who had minimal or no such movements.

The absence of a correlation between cardiovascular variables and plasma norepinephrine may relate to the fact that other factors influence these variables. Although sympathetic activation and increased plasma catecholamine levels may produce hypertension and tachycardia, increased catecholamine levels may also result from reflex response to hypotension and bradycardia. An example of the latter has been demonstrated in patients rising rapidly from the supine position¹³ and in patients receiving nitroprusside.¹⁶ The decrease in cardiovascular variables in this study may relate to a direct depression of the myocardial muscle or sinoatrial node by halothane, rather than to changes in endogenous catecholamine levels caused by halothane.

Although plasma norepinephrine concentration is probably the best currently available *in vivo* index of sympathetic activity in humans, it measures only a fraction of the total amount of norepinephrine released at the nerve ending. Since most of the norepinephrine re-

leased at the nerve ending undergoes reuptake by nerve endings or metabolism by enzymes, any change in the concentration of norepinephrine in the plasma may reflect changes in reuptake, postsynaptic binding, or metabolism rather than changes in the amount released (*i.e.*, sympathetic nervous activity). A previous report suggested that halothane initially decreased reuptake of radioactive norepinephrine in the rat myocardium, but that with continued exposure, norepinephrine reuptake equalled that of controls.¹⁷ However, radioactive norepinephrine was first injected after one hour of halothane anesthesia. Other studies have shown that halothane has no effect on uptake of norepinephrine in cat ventricular slices¹⁸ or in guinea-pig atria.¹⁹ Furthermore, Ngai *et al.*²⁰ has demonstrated that halothane does not affect the biosynthesis of norepinephrine *in vitro*. Metabolism of amines by monamine oxidase (MAO) is unaffected by halothane.^{19,21} We have found (unpublished observations) that halothane does not inhibit *in vitro* metabolism of catecholamines by MAO and catechol-o-methyl transferase. Results from these studies indicate that the change in plasma norepinephrine levels probably is related to changes in release of norepinephrine from the nerve ending rather than to changes in reuptake or metabolism of norepinephrine.

Our finding of a transient increase in norepinephrine in cat spleen effluent when halothane was perfused implies that halothane directly augments release of norepinephrine at the nerve ending. Whether this is so in *in vivo* sympathetic nerve endings, in nerve endings of other areas of the body, or in other species remains to be determined. Other *in vitro* studies^{22,23} have demonstrated that halothane causes a dose-dependent decrease in nerve-stimulated release of norepinephrine; however, the initial effluent was discarded in each study. These *in vitro* results correlate well with the changes in plasma norepinephrine that occurred in part one of this study. This correlation supports the hypothesis that the effect of halothane on norepinephrine concentration in the plasma is related to the effect of halothane on the release of norepinephrine at the nerve ending.

Explanations for the initial sympathetic activation with halothane other than halothane's effect on isolated nerves are possible. The mechanism could be direct stimulation or greater depression of inhibitory synapses than excitatory synapses. Perhaps more than one mechanism accounts for the increase in plasma norepinephrine during induction of halothane anesthesia. The increase is not due to placement of a face mask, but (at least in part) to an action of halothane at sympathetic nerve endings.

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