1417

Regulation of Norepinephrine Release by β_2-Adrenergic Receptors during Halothane Anesthesia

Robert Deegan, M.B.B.Ch., Ph.D., F.F.A.R.C.S.(I.),* Huai Bing He, Pharm.D.,† Yuri Krivoruk, M.D.,*
Alastair J. J. Wood, M.B.Ch.B., F.R.C.P.,‡ Margaret Wood, M.B.Ch.B., F.R.C.A.§

Background: Presynaptic receptors control norepinephrine (NE) release. It has been hypothesized that epinephrine stimulates prejunctional β_2-adrenergic receptors to facilitate NE release from sympathetic nerve endings, and therefore, presynaptic receptors controlling NE release are potential therapeutic targets to limit the adverse effects of excess sympathetic stimulation during anesthesia. We have previously demonstrated β_2-adrenergic receptor–augmented release of NE in the human forearm and have shown that halothane inhibits sympathetic activity *in vivo* by decreasing the NE spillover rate into plasma. The goal of the current study was to determine the effect of halothane on β_2-adrenergic receptor–augmented NE release in a canine hind-limb experimental model.

Methods: Seven female dogs were studied awake and during halothane anesthesia (1.0 minimum alveolar concentration). A trace dosage of [^{3}H]NE (15 μCi over a 1-min period and 0.6 μCi/min thereafter) was infused into the femoral vein. Before and during femoral arterial administration of isoproterenol at two dosages (30 and 80 mg/min), hind-limb blood flow was measured by an ultrasonic flow probe and hind-limb NE spillover by an isotope dilutional technique.

Results: In awake dogs, isoproterenol significantly increased hind-limb blood flow and NE spillover into the hind limb. Halothane had no effect on baseline or isoproterenol-stimulated hind-limb blood flow (a postjunctional β_2 effect) but sig-

nificantly inhibited the isoproterenol-induced increase in hind-limb NE spillover (a prejunctional β_2 effect).

Conclusions: The isoproterenol-mediated increase in NE release is inhibited by halothane anesthesia, indicating that halothane inhibits prejunctional β_2-adrenergic receptor regulation of NE release. (Key words: Anesthetics, volatile: halothane. Receptors: β_2-adrenergic. Sympathetic nervous system, β_2-adrenergic receptor agonists: isoproterenol. Sympathetic nervous system, norepinephrine: clearance; spillover.)

ANESTHETIC agents produce cardiovascular depression in part through their effects on the central and peripheral sympathetic nervous systems. Halothane has been shown to inhibit sympathetic ganglionic transmission,[1-3] to reduce central sympathetic activity,[3,4] and to decrease plasma catecholamine concentrations.[5] Peripheral norepinephrine (NE) concentration is a poor index of NE release because plasma NE concentration depends on the NE clearance rate and on the NE release rate. A more sensitive index of NE release can be obtained by using radiotracer kinetic methods, allowing measurement of NE spillover.[6,7] NE spillover is the rate of NE entry or appearance into plasma and is a measure *in vivo* of the rate of NE release. These isotope dilutional techniques have been used by Esler *et al.* to measure regional NE kinetics, thus allowing the measurement of local NE spillover and clearance in various organs in addition to measurement of systemic spillover.[6,8,9] NE pharmacokinetic measurements overcome the confounding factor of changes in NE clearance, as may occur for example with alterations in blood flow to the major organs, in particular the lungs and hepatic mesenteric circulation, which are responsible for NE uptake and metabolism. We have recently used radiotracer NE kinetics to show that inhalational anesthetics (halothane, isoflurane, and enflurane) and the intravenous anesthetic propofol markedly decrease the rate of NE release from sympathetic terminals into the circulation in an experimental dog model.[5,10]

Figure 1 represents the sympathetic neuroeffector junction, in which basal nerve stimulation releases NE into the junctional cleft. NE is then removed by specific neuronal and extraneuronal uptake processes, and the remainder spills over into plasma. The amount of NE released with each nerve impulse is subject to inhibitory and facilitatory control by activation of prejunctional receptors. Thus, NE released from peripheral sympathetic nerve terminals is regulated by inhibitory feedback by means of prejunctional α_2-adrenergic receptors and by positive feedback by means of facilitatory prejunctional β_2-adrenergic receptors that facilitate exocytotic NE release from the sympathetic nerve endings (fig. 1). NE stimulates prejunctional α_2 receptors to inhibit the further release of NE, and stimulation of β_2 receptors, for example by the humoral circulating agonist epinephrine, has been hypothesized to stimulate or facilitate NE release.[11,12] It also has been suggested that there exist prejunctional α_1-adrenergic receptors on sympathetic nerve terminals that also mediate a negative feedback mechanism on NE release.[13,14] Furthermore, circulating epinephrine may be taken up into sympathetic nerve endings and released again later, acting by means of β_2-adrenergic receptors to provide positive feedback for NE release.[11] Indeed, epinephrine taken up by sympathetic nerve endings and its subsequent release may explain prolonged pressor responses to epinephrine infusion and may be a factor in the genesis of hypertension.[11] Other presynaptic receptors not shown in figure 1 include presynaptic prostanoid receptors[15] and angiotensin II receptors.[16] Thus, modulation of prejunctional receptor function during anesthesia may play an important role in the control of blood pressure and myocardial performance.

To determine whether stimulation of prejunctional β_2-adrenergic receptors alters local release of NE in humans, we recently examined the effects on NE kinetics of small doses of isoproterenol administered directly into the human brachial artery.[17] Stimulation of prejunctional β_2-adrenergic receptors by isoproterenol resulted in a marked increase in forearm NE release or spillover, indicating that β_2 receptors facilitate local release of NE in the human forearm.[17] The current study was undertaken first to determine whether NE release from sympathetic nerves in the dog hind limb is subject to modulation *in vivo* by facilitatory prejunctional β_2-adrenergic receptors and second to determine whether inhibition of presynaptic β_2 receptor–induced local NE release by halothane contributes to our previously ob-
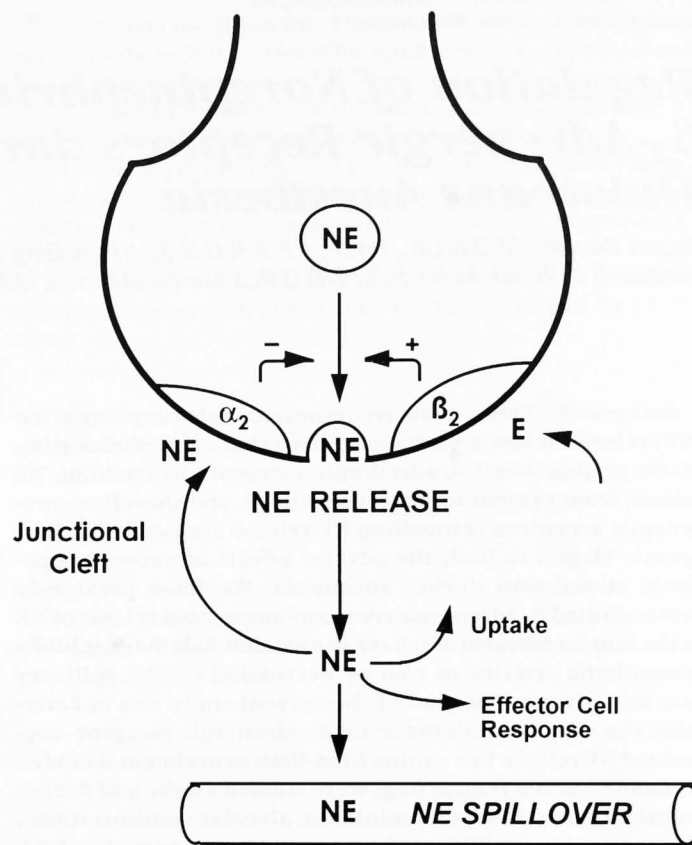

Fig. 1. Diagrammatic representation (much simplified) of the sympathetic neuroeffector junction. Norepinephrine (NE) stimulates α_1-adrenergic receptors at the postjunctional site to produce vasoconstriction. NE stimulates prejunctional α_2-adrenergic receptors to inhibit the further release of NE, while epinephrine (E) and isoproterenol stimulate prejunctional β_2-adrenergic receptors to facilitate further NE release.

served reduction of NE release during halothane anesthesia.

Materials and Methods

Approval for the study was obtained from Vanderbilt University Animal Care Committee. Seven female mongrel dogs (27 ± 1.9 kg, mean $\pm$ standard error of the mean) were studied. At least 7 days before the experiment, the animals underwent surgery for the placement of an ultrasonic flow probe (Transonic Systems, Ithaca, NY) in the left external iliac artery and insertion of intravascular catheters bilaterally in the femoral artery and vein. Catheters were placed in the right femoral artery and vein and advanced so that the catheter tips were in the distal aorta and inferior vena cava. Another right femoral venous catheter was advanced to

the level
measureme
of the left f
from those
troduced t
flow throug
be studied.
ternal iliac
surements
Anesthesia
pental (20
anesthesia

To allow
tributed to
baseline co
conscious
lasting abo
experiment
slow deep
were used a

Experime
On each
and then du
alveolar con
acted as her
NE spillover
tope dilutio
dogs as we
of measurem
sion of NE-
[^{3}H]NE of s
(New Engla
to 1 μCi/ml
ascorbic aci
15 μCi in th
thereafter. V
be a true trac
Thirty minut
steady state[
blood sampl

After 30 m
conscious, s
pressure, hea
limb blood fl
and venous
study hind li
and [^{3}H]NE
cooled ethyl
acetic acid a

the level of the intrathoracic inferior vena cava for measurement of central venous pressure. Implantation of the left femoral arterial and venous catheters differed from those on the right in that the catheters were introduced through side tributaries to preserve blood flow through the femoral vessels of the hind limb to be studied. These catheters were advanced into the external iliac vessels, but to avoid disrupting flow measurements they were left well distal to the flow probe. Anesthesia for the surgery was performed with thiopental (20–25 mg/kg) followed by maintenance of anesthesia with halothane.

To allow observed changes in NE kinetics to be attributed to the anesthetic state, it was essential that the baseline control values be measured in calm, resting, conscious dogs. In five or six training sessions, each lasting about 1 h, the dog was familiarized with the experimental room and procedures. Relaxed posture, slow deep respirations, and spontaneous eye closure were used as indicators of adequate training.

Experimental Protocol

On each occasion the dogs were studied first awake and then during halothane anesthesia (1.0 minimum alveolar concentration [MAC] = 0.9%) so that each dog acted as her own control. Systemic and local hind-limb NE spillover and clearance rates were measured by isotope dilution[6] in awake and halothane-anesthetized dogs as we have previously described.[5,10] For each set of measurements a separate 50-min intravenous infusion of NE-L-[ring-2,5,6-^{3}H] ([^{3}H]NE) was performed. [^{3}H]NE of specific radioactivity 43.7–56.9 Ci/mmol (New England Nuclear, Wilmington, DE) was diluted to 1 μCi/ml in 0.9% sodium chloride with 1 mg/ml ascorbic acid and infused into the right femoral vein; 15 μCi in the 1st min was followed by 0.6 μCi/min thereafter. We have previously shown this regimen to be a true tracer dose, devoid of pharmacologic effects.[5] Thirty minutes was allowed for plasma [^{3}H]NE to reach steady state[5] before hemodynamic measurement and blood sampling procedures were performed.

After 30 min of [^{3}H]NE infusion when the dog was conscious, systolic and diastolic aortic arterial blood pressure, heart rate, central venous pressure, and hind-limb blood flow were measured. Simultaneous arterial and venous blood samples were then taken from the study hind limb (left) for measurement of endogenous and [^{3}H]NE concentrations. Blood was collected in cooled ethyleneglycol-bis-(β-aminoethyl ether) tetraacetic acid and glutathione (CAT-A-KIT blood collec-

tion tubes, Amersham, Arlington Heights, IL) and centrifuged, and the plasma was stored at −20°C until assayed. NE concentrations were measured as previously reported[18] by high-performance liquid chromatography; the effluent coinciding with the NE peak was collected and counted by liquid scintillation. This approach allowed the assay of plasma [^{3}H]NE concentration without interference from tritiated metabolites.

During the above control period, the arterial catheter was maintained patent by a continuous infusion of 0.9% sodium chloride at a rate of 60 ml/h. When all of the baseline control measurements had been taken, isoproterenol was then infused intraarterially into the study limb at two dosages, 30 and 80 ng/min. During intraarterial administration of isoproterenol, the total flow rate through the arterial catheter was maintained constant at 60 ml/h by adjustment of the sodium chloride infusion rate. Each dosage of isoproterenol was administered for 7 min; hind-limb blood flow was measured; and simultaneous arterial (10 ml) and venous (10 ml) blood samples were again drawn at isoproterenol dosages of 30 and 80 ng/min.

On completion of the awake stage, the [^{3}H]NE and isoproterenol infusions were stopped, and anesthesia was induced with thiopental 20–25 mg/kg, followed by tracheal intubation and pulmonary ventilation with halothane (1.0 MAC) in oxygen. Continuous measurement of end-tidal concentrations of carbon dioxide and halothane (254 monitor, Datex), with regular arterial blood gas analysis, allowed adjustment of respiratory rate to maintain arterial carbon dioxide tension between 30 and 40 mmHg. Throughout anesthesia, 0.9% saline was administered at 4 ml·kg^{-1}·h^{-1}. All blood samples were replaced with twice their volume of 0.9% saline.

Sixty minutes was allowed for equilibration after induction of anesthesia before the [^{3}H]NE infusion was recommenced. The study protocol was repeated with measurements made at baseline and then during two intraarterial infusion rates of isoproterenol, 30 and 80 ng/min.

Data Analysis

NE pharmacokinetic parameters were calculated as follows:

NE clearance rate

$$= \frac{[^3\text{H}]\text{NE infusion rate (DPM} \cdot \text{min}^{-1} \cdot \text{min}^{-1})}{\text{steady-state plasma } [^3\text{H}]\text{NE concentration (DPM} \cdot \text{min}^{-1} \cdot \text{ml}^{-1})}$$

but also

NE clearance rate

$$= \frac{\text{endogenous NE spillover rate (ng/min)}}{\text{endogenous plasma NE concentration (ng/ml)}}$$

Therefore,

endogenous NE spillover rate =

$$\frac{[^3H]NE \text{ infusion rate} \times \text{endogenous plasma NE concentration}}{\text{steady-state plasma } [^3H]NE \text{ concentration}}$$

Processes of NE removal and of NE release occur simultaneously in any vascular bed, so that simple measurement of the net arteriovenous increment of NE concentration underestimates regional NE spillover. A correction factor must be added to allow for the NE removal during passage through the hind limb.

$$\text{FE of } [^3H]NE \text{ in hind limb} = \frac{A^* - V^*}{A^*}$$

where FE = fractional extraction of NE by the hind limb; A^* = arterial concentration of $[^3H]NE$ (disintegrations per minute per milliliter); V^* = venous concentration of $[^3H]NE$ (disintegrations per minute per milliliter), and;

hind-limb NE spillover rate (ng/min)

$$= ([V - A] + [A \times FE])\dot{Q}$$

where A = arterial concentration of endogenous NE (ng/ml); V = venous concentration of endogenous NE (ng/ml); and $\dot{Q}$ = hind-limb plasma flow (ml/min).

$$\dot{Q} = \text{hind-limb blood flow} \times (1 - \text{hematocrit})$$

$$\text{hind-limb NE clearance} = FE \times \dot{Q}$$

where hematocrit is expressed as a fraction and hind-limb NE clearance is expressed in milliliters per minute. Plasma NE clearance from the whole body (*i.e.*, systemic clearance) was calculated at steady state as:

$$\text{systemic NE clearance} = \frac{[^3H]NE \text{ infusion rate}}{A^*}$$

where systemic NE clearance is expressed in milliliters per minute and $[^3H]NE$ infusion rate is expressed in disintegrations per minute per minute. The rate at which NE entered plasma for the whole body (*i.e.*, systemic spillover) was calculated at steady state as

systemic NE spillover rate (ng/min)

$$= \text{systemic NE clearance (ml/min)} \times A \text{ (ng/ml)}$$

Conversion of blood flow to plasma flow required determination of arterial hematocrit. Hematocrit samples were collected in capillary tubes prepared with heparin; spun for 10 min in a Micro Hematocrit centrifuge; and read on a Micro-capillary Reader (both from Damon, IEC Division, Needham Heights, MA). Measurements were made at three times: (1) at the beginning of the awake stage; (2) at the end of the awake stage, before the anesthesia stage; and (3) on completion of the study. The average of the first and second measurements was used for calculation of the hind-limb plasma flows during the awake stage, and the average of the second and third measurements was used for the anesthesia stage. Data were analyzed by repeated measures analysis of variance followed by Student's paired *t* test or Wilcoxon's signed rank test as appropriate. $P < 0.05$ was accepted as the minimal level of significance.

Results

The number of observations at each data point varies as indicated in the figures. Five dogs received both isoproterenol dosages, one only 30 ng/min, and another only 80 ng/min. In addition, arterial pressure measurements were unavailable for one dog during halothane anesthesia because of occlusion of the catheter. Table 1 shows the baseline (*i.e.*, before intraarterial infusion of isoproterenol) values for the various hemodynamic and NE pharmacokinetic parameters both awake and during 1.0 MAC halothane anesthesia. The low values during the awake period for systolic and diastolic blood pressure, heart rate, and arterial NE concentration confirm that we achieved our objective of a calm resting state in the dogs. Halothane anesthesia resulted in a significant decrease in systolic blood pressure ($P < 0.001$) without a change in diastolic blood pressure or heart rate. Consistent with our previous report,[5] arterial NE concentration was decreased by halothane because of a 38% reduction ($P < 0.01$) in systemic NE spillover accompanied by a relatively small decrease in systemic NE clearance. There was no significant difference between hind-limb blood flow awake and during halothane anesthesia, probably because of a significant decrease in hind-limb vascular resistance produced by halothane (table 1).

Table 1. Hindlimb Blood Flow, Arterial Norepinephrine Concentration, Hindlimb Norepinephrine Spillover and Clearance, Systemic Norepinephrine Spillover and Clearance, Heart Rate, and Systolic and Diastolic Blood Pressure at Baseline before Intraarterial Infusion of Isoproterenol in Awake Dogs and during Halothane (1.0 MAC) Anesthesia

Parameter	Awake	Halothane	% Change	Significance
Systolic blood pressure (mmHg)	159 ± 4	116 ± 4*	−38 ± 10.5	$P < 0.001$
Diastolic blood pressure (mmHg)	68 ± 2	62 ± 2*	−20.4 ± 13.6	NS
Heart rate (beats/min)	63 ± 3	71 ± 2	15.6 ± 8.4	NS
Hindlimb blood flow (ml/min)	184 ± 16	159 ± 9	−10.8 ± 6	NS
Hindlimb vascular resistance $(mmHg \cdot ml^{-1} \cdot min^{-1})$	0.54 ± 0.4	0.47 ± 0.3*	−28.6 ± 12.4	0.015
Arterial norepinephrine (pg/ml)	83.8 ± 15.4	59.9 ± 12.4	−28.6 ± 8.4	$P < 0.01$
Systemic norepinephrine spillover (ng/min)	147 ± 27	94 ± 23	−37.8 ± 6.4	$P < 0.005$
Systemic norepinephrine clearance (L/min)	1.75 ± 0.12	1.54 ± 0.10	−11.1 ± 5.5	$P < 0.05$
Hindlimb norepinephrine spillover (ng/min)	5.2 ± 0.8	2.0 ± 0.4	−42.8 ± 24.2	$P < 0.05$
Hindlimb norepinephrine clearance (ml/min)	53 ± 6	50 ± 2	5.6 ± 20.1	NS
Hindlimb fractional extraction	0.44 ± 0.04	0.47 ± 0.03	15.4 ± 17.9	NS
Hematocrit	0.32 ± 0.02	0.31 ± 0.02	—	NS

Values are mean ± SEM; n = 7. NS = not significant.

* n = 6.

Local intraarterial infusion of isoproterenol resulted in a significant dosage-related increase in hind-limb blood flow in all dogs (fig. 2). Because arterial blood pressure did not increase, this isoproterenol-induced increase in hind-limb blood flow resulted from reduced hind-limb vascular resistance (fig. 2) and can be attributed to postjunctional β_2-adrenergic receptor stimulation. This response was not significantly affected by halothane anesthesia (fig. 2), indicating a lack of effect of halothane on postjunctional β_2 receptor–mediated vasodilation.

Stimulation of prejunctional β_2-adrenergic receptors by intraarterial isoproterenol markedly increased local hind-limb NE spillover in awake dogs (fig. 3). Isoproterenol, 80 ng/min, increased hind-limb NE spillover 288% from an awake baseline spillover of 5.2 ± 0.8 to

13.2 ± 1.7 ng/min ($P < 0.02$). Halothane anesthesia produced a 43% decrease in hind-limb spillover compared with the awake baseline value ($P < 0.05$). Furthermore, halothane abolished isoproterenol-mediated facilitation of NE release; NE spillover after infusion of isoproterenol, 80 ng/min, was 4.8 ± 2.3 ng/min during 1.0 MAC halothane compared with 13.2 ± 1.7 ng/min in the awake state ($P < 0.05$) (fig. 3). NE clearance by the hind limb was unchanged during isoproterenol infusion and was not significantly affected by halothane anesthesia (fig. 3).

Figure 4 shows the effect of halothane anesthesia and intraarterial administration of isoproterenol on systemic NE kinetics. Systemic NE spillover did not significantly change during 30-ng/min administration of isoproterenol but increased 28 ± 10% ($P < 0.05$) when isopro-

Fig. 2. Hind-limb blood flow (milliliters per minute) and hind-limb vascular resistance (millimeters mercury per milliliter per minute) at baseline (time 0) and after intraarterial infusion of isoproterenol 30 and 80 ng/min awake (squares) and during 1.0 minimum alveolar concentration (MAC) halothane anesthesia (circles). Data are expressed as mean values ± SEM.
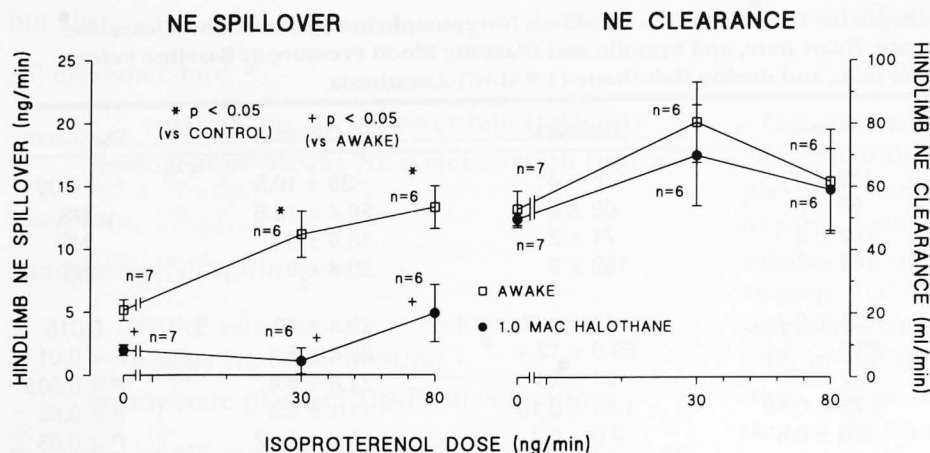
NE SPILLOVER
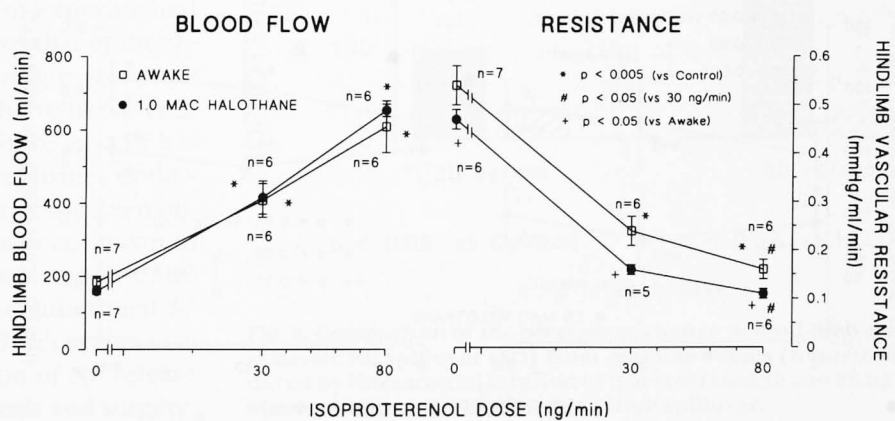
NE CLEARANCE

Fig. 3. Hind-limb norepinephrine (NE) spillover (nanograms per minute) and hind-limb NE clearance (milliliters per minute) at baseline (time 0) and after intraarterial infusion of isoproterenol 30 and 80 ng/min awake (squares) and during 1.0 minimum alveolar concentration (MAC) halothane anesthesia (circles). Data are expressed as mean values ± SEM.

terenol 80 ng/min was administered. Figure 5 shows the arterial blood pressure and heart rate measurements during the study. There was no significant change in systolic blood pressure during intraarterial isoproterenol infusion, both awake and during halothane anesthesia, but diastolic blood pressure was significantly reduced (by 9 mmHg awake and 4 mmHg during halothane) only during infusion of the greater dosage of isoproterenol (80 ng/min).

In addition to the observed increase in hind-limb spillover, there was also a smaller increase in systemic spillover (fig. 6). However, when the contribution of hind-limb NE spillover is subtracted from systemic NE spillover, the systemic NE spillover at 80-ng/min isoproterenol infusion (172 ± 22 ng/min) is not significantly different from baseline (152 ± 29 ng/min). Thus, although the increase in hind-limb spillover contributes to the increase seen in systemic spillover and may account for that increase, systemic spillover

does not contribute to the measured increase in hind-limb spillover. Thus, the increase in systemic spillover cannot account for the change seen in hind-limb spillover, because at the 30-ng/min isoproterenol dosage, hind-limb spillover increased by $98 \pm 26\%$ ($P < 0.05$), whereas there was no significant increase in systemic spillover (fig. 6). At the greater isoproterenol dosage, 80 ng/min, when significant increase in systemic spillover was seen, that increase was only $28 \pm 10\%$ in contrast to the $288 \pm 150\%$ increase in hind-limb spillover.

Discussion

This study was designed to test the hypothesis that prejunctional β_2-adrenergic receptors facilitate neuronal NE release in response to stimulation by isoproterenol and that this effect is abolished by halothane anesthesia. We have clearly shown that intraarterial
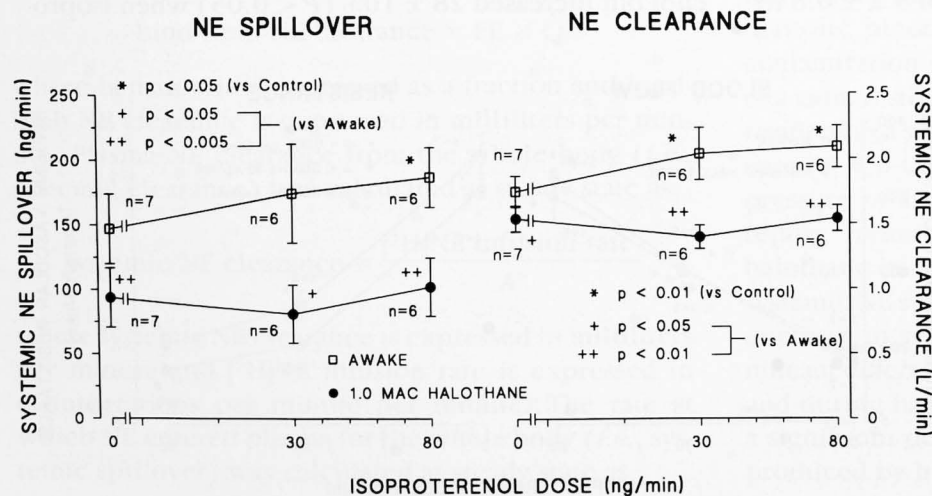
NE SPILLOVER

NE CLEARANCE

Fig. 4. Systemic norepinephrine (NE) spillover (nanograms per minute) and systemic NE clearance (liters per minute) at baseline (time 0) and after intraarterial infusion of isoproterenol 30 and 80 ng/min awake (squares) and during 1.0 minimum alveolar concentration (MAC) halothane anesthesia (circles). Data are expressed as mean values ± SEM.

BLOOD PRESSURE   HEART RATE

Fig. 5. Systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate at baseline (time 0) and after intraarterial infusion of isoproterenol 30 and 80 ng/min awake (squares) and during 1.0 minimum alveolar concentration (MAC) halothane anesthesia (circles). BP = blood pressure. Data are expressed as mean values ± SEM.
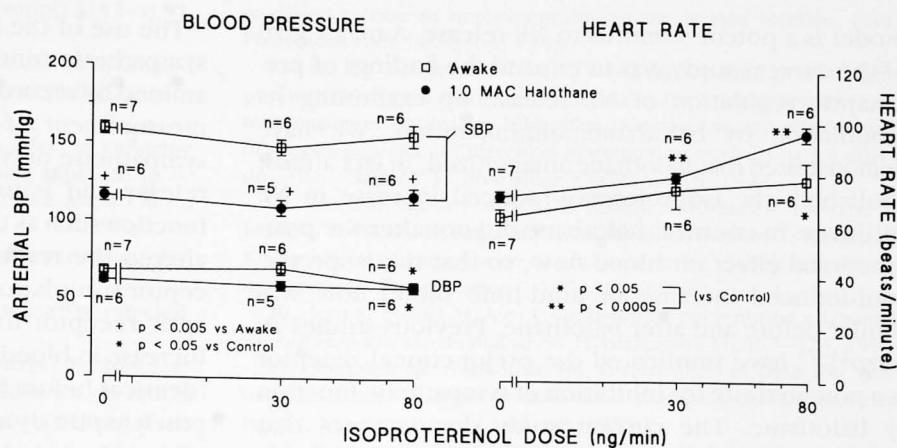
isoproterenol administration is a potent stimulus for NE release and that halothane markedly impairs the prejunctional β_2 receptor–induced increase in NE release but has no effect on postsynaptic vasodilation.

Anesthetic agents produce cardiovascular depression in part through their effects on the sympathetic nervous system. We have previously shown that inhalational and intravenous anesthetics decrease the rate of NE release into the circulation.[5,10] Prejunctional receptors modulate NE release; they may be targets for blood-borne humoral agents such as epinephrine, for example, and hence they act as a link in presynaptic feedback control of NE release. There is now a large body of evidence that epinephrine can augment NE release in humans.[11,19,20] Small, repetitive increases in circulating epinephrine from the adrenal medulla in response to stress for example, can augment NE release by stimulating prejunctional β_2-adrenergic receptors that facilitate NE release from sympathetic nerve endings. Sustained indirect aftereffects of epinephrine also may occur by the neuronal uptake of epinephrine and later release as a cotransmitter when autofacilitation of NE release by NE may result. Several lines of experimental evidence have thus led to the hypothesis that epinephrine stimulates prejunctional β_2-adrenergic receptors that facilitate NE release from sympathetic nerve endings and that epinephrine augments NE release in humans.[11,19,20] Systemic infusion of epinephrine, in dosages that result in epinephrine concentrations seen under physiological stress conditions, has been shown to produce a delayed or protracted pressor response, indicating the functional importance of prejunctional β_2-adrenergic receptors.[19] Prejunctional β_2 receptors may play an important role in the regulation of NE release *in vivo* and in particular during anesthesia and surgery.

This study has shown that hind-limb intraarterial infusion of isoproterenol results in an increase in NE spillover into the hind limb; stimulation of prejunctional β_2-adrenergic receptors by isoproterenol facilitates NE release. This result is consistent with our recent finding of β_2-adrenergic receptor–mediated release of NE in the human forearm.[17] We have therefore demonstrated that stimulation of the prejunctional β_2 receptor in humans by isoproterenol[17] and now in a dog
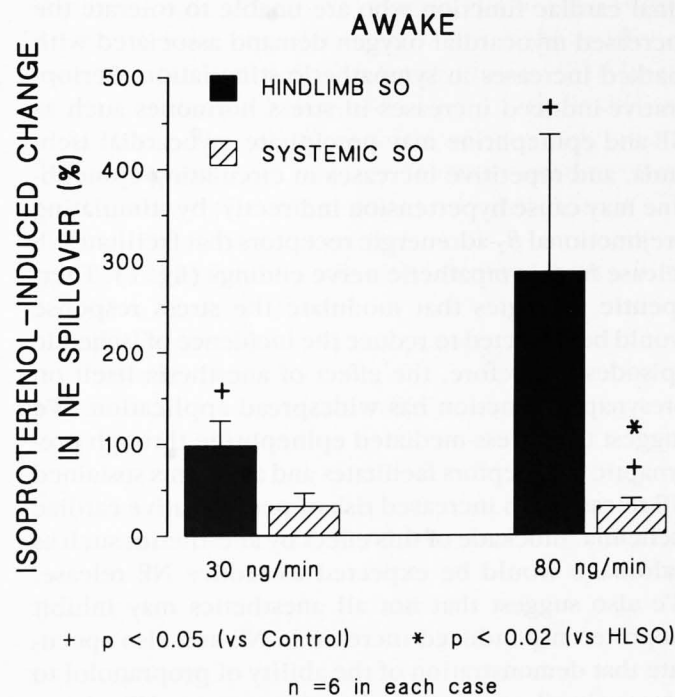
AWAKE

Fig. 6. Comparison of the percentage change in hind-limb and systemic NE spillover (SO) from baseline values (time 0) induced by intraarterial infusion of isoproterenol 30 and 80 ng/min in awake dogs. HLSO = hind-limb spillover.

model is a potent stimulus to NE release. A major goal of the current study was to expand the findings of presynaptic regulation of NE release by examining its modulation by halothane administration. We have demonstrated that halothane antagonized, in fact almost abolished, the isoproterenol-induced increase in NE spillover. In contrast, halothane did not alter the postjunctional effect on blood flow, so that the isoproterenol-induced increase in hind-limb blood flow was similar before and after halothane. Previous studies *in vitro*[21-23] have implicated the prejunctional receptor as a potential site for inhibition of sympathetic function by halothane. The current study demonstrates that halothane affects presynaptic regulation of NE release *in vivo*. We have thus identified a new site of action for halothane-induced sympathetic inhibition, in addition to its well-recognized actions on baroreceptor function,[3] central sympathetic control,[3,4] and ganglionic transmission.[1-3]

Perioperative myocardial ischemia is a major cause of morbidity after anesthesia and surgery. Intraoperative risk factors include hypertension, tachycardia and dysrhythmias. Anesthetic implications of the response to surgical stress induced changes in catecholamines and other hormones are important for patients with marginal cardiac function who are unable to tolerate the increased myocardial oxygen demand associated with marked increases in sympathetic stimulation. Perioperative-induced increases in stress hormones such as NE and epinephrine may precipitate myocardial ischemia, and repetitive increases in circulating epinephrine may cause hypertension indirectly, by stimulating prejunctional β_2-adrenergic receptors that facilitate NE release from sympathetic nerve endings (fig. 1). Therapeutic strategies that modulate the stress response would be expected to reduce the incidence of ischemic episodes. Therefore, the effect of anesthesia itself on presynaptic function has widespread application. We suggest that stress-mediated epinephrine through presynaptic β_2 receptors facilitates and augments sustained NE release with increased risk of perioperative cardiac ischemia. Blockade of this effect by anesthetics such as halothane would be expected to reduce NE release. We also suggest that not all anesthetics may inhibit isoproterenol-induced increase in NE and also speculate that demonstration of the ability of propranolol to antagonize β_2 receptor–induced increase in NE release would be of therapeutic importance as it would provide a scientific rationale for the development of selective β_2-adrenergic receptor antagonists as premedicants.

The use of the isotope dilution technique examines sympathetic function at a different site from that examined by recording sympathetic nerve traffic.[24,25] The measurement of sympathetic nerve traffic measures sympathetic nerve activity proximal to the site of NE release and is unaffected by changes in presynaptic function such as those in this study. Although halothane altered the response to presynaptic β_2-adrenergic receptor stimulation, there was no effect on the postsynaptic receptor that mediates increases in blood flow. Increase in blood flow in response to isoproterenol was identical before and after halothane, implying that the postsynaptic β_2-receptor stimulation response was unaffected by halothane and that differences in blood flow before and after halothane in response to isoproterenol could not explain the altered NE spillover shown in this study.

In summary, we have demonstrated that stimulation of prejunctional β_2-adrenergic receptors has a profound effect on NE release and that halothane acts directly at the peripheral sympathetic nerve terminal to inhibit β_2-adrenergic receptor–mediated NE release. We also have confirmed our previous finding that halothane decreases systemic spillover and clearance of NE and have identified the prejunctional β_2-adrenergic receptor as an important site of action for halothane.

References

1. Christ D: Effects of halothane on ganglionic discharges. J Pharmacol Exp Ther 200:336–342, 1977

2. Bosnjak ZJ, Seagard JL, Wu A, Kampine JP: The effects of halothane on sympathetic ganglionic transmission. ANESTHESIOLOGY 57:473–479, 1982

3. Seagard JL, Hoop FA, Donegan JH, Kalbfleisch JH, Kampine JP: Halothane and the carotid sinus reflex: Evidence for multiple sites of action. ANESTHESIOLOGY 57:191–202, 1982

4. Millar RA, Warden JC, Cooperman LH, Price LH: Central sympathetic discharge and mean arterial pressure during halothane anesthesia. Br J Anaesth 41:918–928, 1969

5. Deegan R, He HB, Wood AJJ, Wood M: Effects of anesthesia on norepinephrine kinetics: Comparison of propofol and halothane anesthesia in dogs. ANESTHESIOLOGY 75:481–488, 1991

6. Esler M, Jennings G, Korner P, Willett I, Dudley F, Hasking G, Anderson W, Lambert G: Assessment of human sympathetic nervous system activity from measurements of norepinephrine turnover. Hypertension 11:3–20, 1988

7. Linares OA, Jacquez AJ, Zech LA, Smith MJ, Sanfield JA, Morrow LA, Rosen SG, Halter JB: Norepinephrine metabolism in humans. J Clin Invest 80:1332–1341, 1987

8. Esler M, Leonard P, O'Dea K, Jackman G, Jennings G, Korner P: Biochemical quantification of sympathetic nervous activity in humans using radiotracer methodology: Fallibility of plasma noradren-

aline measur
1982
9. Esler M
P: Measurem
in humans. A
10. Deega
and isoflurar
sessment of
77:49–54, 1
11. Floras
pertension 1
12. Lange
amines. Phar
13. Guth
Adrenergic re
dog: Evidenc
Res Cardiol 8
14. Guth
cardial effect
in dogs. Circ
15. Molde
Modulation o
the human sa
and DP recep
16. Elems
Nussberger J,
itation of nore
93:684–691,
17. Rein N

aline measurements. J Cardiovasc Pharmacol 4(suppl):S152–S157, 1982

9. Esler M, Jennings G, Korner P, Blombery P, Sacharias N, Leonard P: Measurement of total and organ-specific norepinephrine kinetics in humans. Am J Physiol 247:E21–E28, 1984

10. Deegan R, Huai BH, Wood AJJ, Wood M: Effect of enflurane and isoflurane on norepinephrine kinetics: A new approach to assessment of sympathetic function during anesthesia. Anesth Analg 77:49–54, 1993

11. Floras JS: Epinephrine and the genesis of hypertension. Hypertension 19:1–18, 1992

12. Langer SZ: Presynaptic regulation of the release of catecholamines. Pharmacol Rev 32:337–362, 1981

13. Guth BD, Thaulow E, Heusch G, Seitelberger R, Ross J: α-Adrenergic regulation of myocardial performance in the exercising dog: Evidence for both presynaptic α_1- and α_2-adrenoceptors. Basic Res Cardiol 85(suppl 1):131–141, 1990

14. Guth BD, Thaulow E, Heusch G, Seitelberger R, Ross J: Myocardial effects of selective α-adrenoceptor blockade during exercise in dogs. Circ Res 66:1703–1712, 1990

15. Molderings GJ, Colling E, Likungu J, Jakschik J, Gothert M: Modulation of noradrenaline release from the sympathetic nerves of the human saphenous vein and pulmonary artery by presynaptic EP_3- and DP-receptors. Br J Pharmacol 111:733–738, 1994

16. Clemson B, Gaul L, Gubin SS, Campsey DM, McConville J, Nussberger J, Zelis R: Prejunctional angiotensin II receptors: Facilitation of norepinephrine release in the human forearm. J Clin Invest 93:684–691, 1994

17. Stein M, Deegan R, He HB, Wood AJJ: β-Adrenergic receptor-mediated release of norepinephrine in the human forearm. Clin Pharmacol Ther 54:58–64, 1993

18. He HB, Deegan R, Wood M, Wood AJJ: Optimization of high-performance liquid chromatographic assay for catecholamines: Determination of optimal mobile phase composition and elimination of species-dependent differences in extraction recovery of 3,4-dihydroxybenzylamine. J Chromatogr 574:213–218, 1992

19. Nezu M, Miura Y, Adachi M, Adachi M, Kimura S, Toriyabe S, Ishizuka Y, Ohashi H, Sugawara T, Takahashi M, Noshiro T, Yoshinaga K: The effects of epinephrine on norepinephrine release in essential hypertension. Hypertension 7:187–195, 1985

20. Jern S, Pilhall M, Jern C: Infusion of epinephrine augments pressor responses to mental stress. Hypertension 18:467–474, 1991

21. Muldoon SM, Vanhoutte PM, Lorenz RR, Van Dyke RA: Venomotor changes caused by halothane acting on the sympathetic nerves. ANESTHESIOLOGY 43:41–48, 1975

22. Roizen MF, Thoa NB, Moss J, Kopin IJ: Inhibition by halothane of release of norepinephrine, but not of dopamine β-hydroxylase, from guinea-pig vas deferens. Eur J Pharmacol 31:313–318, 1975

23. Lunn JJ, Rorie DK: Halothane-induced changes in the release and disposition of norepinephrine at adrenergic nerve endings in dog saphenous vein. ANESTHESIOLOGY 61:377–384, 1984

24. Ebert TJ, Kampine JP: Nitrous oxide augments sympathetic outflow: Direct evidence from human peroneal nerve recordings. Anesth Analg 69:444–449, 1989

25. Ebert TJ: Differential effects of nitrous oxide on baroreflex control of heart rate and peripheral sympathetic nerve activity in humans. ANESTHESIOLOGY 72:16–22, 1990