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Halothane-induced Changes in the Release and Disposition of Norepinephrine at Adrenergic Nerve Endings in Dog Saphenous Vein

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The effect of halothane on the release and metabolism of norepinephrine during resting conditions and in response to electrical stimulation at 2 Hz was studied in isolated superfused segments of dog saphenous vein. Liquid chromatography with electrochemical detection was used to measure endogenous norepinephrine overflowing in response to electrical stimulation and the content of norepinephrine remaining in the tissue after stimulation. In other preparations, norepinephrine stores were labeled with [^{3}H]norepinephrine, and measurements were made of [^{3}H]norepinephrine and its metabolites (separated by column chromatography) in superfusates. Radiolabeled metabolites of norepinephrine produced intraneuronally (3,4-dihydroxyphenylglycol) and extraneuronally (O-methylated) were quantitated by liquid scintillation spectrometry. Electrically stimulated release and overflow of endogenous norepinephrine was decreased 11.9% at 0.75% halothane, 17.7% at 1.5% and 19.2% at 2.5% halothane. At each halothane concentration studied, the per cent of tissue NE content released in response to electrical stimulation was less. Halothane decreased the fraction of radioactivity lost during basal conditions and during stimulation. Less oxidative deamination of norepinephrine occurred in the presence of halothane. (Key words: Anesthetic, volatile: halothane. Measurement techniques: chromatography; column; liquid. Neurotransmitters. Sympathetic nervous system: adrenergic nerve endings; catecholamines, norepinephrine.)

HALOTHANE is known to cause peripheral venodilatation in anesthetized subjects.[1–3] This action by halothane has been attributed to depression of central nervous system control of sympathetic activity[4,5] and to depression of sympathetic ganglionic transmission[6–9] in *in vivo* models. Halothane also decreases the electrically induced contractions of *in vitro* segments of cutaneous vein of dog.[10] Since sympathetic ganglia have not been demonstrated in peripheral cutaneous veins,[11] the above mechanisms do not explain the halothane-depressed contractions of *in vitro* veins. Other effects of halothane on vessels include a direct effect on the smooth muscle cells[12,13] and impaired release of NE from sympathetic nerve endings located in the wall of the blood vessel.[10] Since the contractile response of an isolated blood vessel relates directly to the synaptic concentration of NE,[14] the depressed contractions occurring in the presence of halothane may be due to reduced levels of NE in synaptic clefts. That the depressed contraction is not solely a result of a direct action by halothane on the smooth muscle cell is suggested by the failure of the contraction produced by exogenously applied NE or by NE released by an indirect-acting vasopressor to be depressed by halothane.[10] Halothane-induced decrease release of NE from nerve ending in isolated blood vessels has not been demonstrated directly; nevertheless, indirect studies suggest that halothane decreases release of NE from these nerve endings.[10] Studies showing decreased catecholamine release from adrenal medullary cells in the presence of halothane[15,16] support this theory.

The present study determines the effect of halothane on the release of NE from nerve endings in an isolated cutaneous vein of dog using two experimental preparations. In one preparation, halothane-induced changes in the release of endogenous NE are measured directly; in the other, stores of NE in the vein are labeled with [^{3}H]NE and changes in release of total radioactivity induced by halothane are measured. Using this latter preparation it also has been possible to measure an effect of halothane on the biotransformation of NE.

Methods

Part One: Studies that Examined the Effect of Halothane on the Release of Endogenous NE

Vein Collection and Preparation: Segments of lateral saphenous vein were removed from dogs (15–25 kg) anesthetized with pentobarbital (30 mg/kg, iv). The perivascular tissue was dissected away, and the vein was cut into a helical strip 2–4 mm wide and 70–100 mm long.

Superfusion and Stimulation of Veins. The strips of saphenous vein were suspended immediately after preparation in a constant temperature (37°C) superfusion chamber between and in contact with two platinum wire electrodes. The vein was set to an initial tension of 2 g and then allowed to equilibrate for 45 min. Superfusion (flow rate, 2 ml/min) was with Krebs–Ringer solution[17]

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that had been aerated with 95% O_2–5% CO_2. After equilibration, a three-way stopcock was turned to Krebs–Ringer solution containing cocaine (10^{-5} M), in order to block neuronal uptake,[18,19] and corticosterone (4×10^{-5} M), to block extraneuronal uptake of NE.[20,21] This solution was used for superfusion throughout the remainder of the experiment.

Electrical stimulation (ES) used to stimulate adrenergic nerve endings consisted of rectangular wave impulses (9 V, 2 ms duration, 2 Hz) provided by a DC power supply and a switching transistor triggered by a model SD9 Grass® stimulator. Previous studies have shown this stimulation to be supramaximal.[16] Superfusate was collected continuously during the 19-min interval of ES.

In half of the experiments selected randomly, halothane (0.75, 1.5, or 2.5%) was added to the system, and 30 min was allowed for equilibration prior to ES. Following the 19 min of ES, the halothane was discontinued promptly and after a 30-min halothane-free rest interval, ES was resumed for an identical interval of stimulation. In the other half of the experiments, the same protocol was followed except the control stimulation was carried out first, followed by the addition of halothane and the second stimulation. Superfusate was collected for analysis for endogenous NE throughout the period of ES. To the volume of superfusate collected each minute (2 ml), the following were added to prevent oxidation of NE: 2 N HCl, 0.066 ml, and 1% sodium metabisulfite, 0.033 ml.

The Content of NE Remaining in Saphenous Vein at the End of Study: In addition to measuring the amount of NE released into the superfusion fluid during stimulation, the amount of NE remaining in the tissues after superfusion and stimulation also was determined. Correlations therefore could be made between reduced NE overflow in the presence of halothane and the concentration of NE remaining in the tissue at the end of study.

At the end of each experiment, the vein was blotted dry, weighed, and the NE remaining in the tissue was extracted with 1 N acetic acid containing 0.4 ml 1% ascorbic acid and 0.3 ml 1% sodium metabisulfite.[22]

Method for Measurement of Endogenous Norepinephrine Concentration in Saphenous Vein and Superfusate: NE was isolated from the extracts of vein and from the superfusate by the method of Valori *et al.*[23] This method involves adsorption of catechols on alumina at *p*H 8.4 followed by elution with dilute (0.05 N) perchloric acid. Catecholamines are separated from catechol acids in the perchloric acid eluate by chromatography on small columns (0.3 cm diameter, 3 cm height) of the cation exchange resin Amberlite® C650. Elution from the resin is accomplished with 1.0 ml 2/3 M boric acid. The concentration of the NE in the extract was determined by liquid chromatography with electrochemical detection (LCEC) using an 18 μ Bondapak column.[17] A mobile phase solvent consisted of 0.07 M NH_2PO_4, 0.2 mM ethylenediaminetetraacetic acid (EDTA), 1 mM heptane sulfonate, and 4% methanol at *p*H 4.8. The electrochemical detector was set at 0.65 mV against a silver/silver chloride reference electrode.

The average recovery of 10 ng of NE added to extracts was 69.29 ± 1.58% (SEM; n = 29).

Part Two: Studies that Examined the Effect of Halothane on Release of Total Radioactivity and on the Metabolism of [^{3}H]NE

This portion of our study examined the effect of halothane on the release of NE as well as its effect on the biotransformation of the NE that is released into synaptic clefts. By continuously following the release of total radioactivity and the production of metabolites of NE that are produced following the neuronal and extraneuronal uptake of NE, it is possible to draw inferences on the effect of halothane on the release and on the metabolism of NE.

Superfusion of Veins with NE Stores Labeled with [^{3}H]NE. The vein strips used in these studies were prepared as were those used in the studies of endogenous NE release. After preparation the strips were incubated for 1 h in Krebs–Ringer solution containing L-[^{3}H]NE (5.47×10^{-9} M), EDTA (0.27 mM), and ascorbic acid (0.56 mM), while being aerated continuously with 95% O_2–5% CO_2. Strips then were mounted in a prewarmed (37°C) enclosed superfusion chamber between and in contact with two platinum wire electrodes. The vein was set to an initial tension of 2 g and then allowed to equilibrate for 90 min. During this period of equilibration and afterward, the vein was superfused at a rate of 2 ml/min with aerated (95% O_2–5% CO_2) Krebs–Ringer solution (no uptake blockers added) maintained at 37°C. The superfusion chamber itself also was exposed to a constant source of 95% O_2–5% CO_2. Samples of superfusate were collected in 6-min intervals as follows. Immediately following the 90-min equilibration period, a 6-min basal sample was collected (sample 1); 30 min after collection of the first sample, another 6-min basal sample was collected (sample 2). ES was started immediately following the collection of sample 2 and continued for 18 min, during which samples 3–5 were collected. Sample collection was continued for 24 min following ES (samples 6–9). Using this protocol, changes in the efflux of [^{3}H]NE and its metabolites with time were studied in four groups of saphenous veins: 1) controls, 2) veins exposed to 1.5% halothane, 3) veins exposed to 3.0% halothane, and 4) veins exposed throughout to superfusate containing clorgyline (2×10^{-5} M) to block intraneuronal monoamine oxidase (MAO).[21] To the volume of superfusate collected each minute, the following were added: 2 N HCl, 0.066 ml; 1% sodium metabisulfite, 0.033 ml; and 0.017 ml of a solution contain-

ing 0.1 mg/ml each of NE, normetanephrine (NMN), 3,4-dihydroxymandelic acid (DOMA), 3,4-dihydroxyphenylglycol (DOPEG), 3-methoxy-4-hydroxyphenylglycol (MOPEG), and 3-methoxy-4-hydroxymandelic acid (VMA) as mixed carriers.

In experiments using radiolabeled tissues in which halothane was used, delivery of superfusate equilibrated with the desired halothane concentration was started immediately upon completion of collection of the first basal sample and was continued until the end of the experiment. Thirty minutes were thus allowed for the equilibration of halothane with the tissue prior to collection of samples for study. At the time superfusate-containing halothane began to flow over the strip of vein, the identical concentration of halothane was added to the O_2–CO_2 mixture flowing into the superfusion chamber.

At the end of the experiment, the tissues were blotted dry and weighed, and NE and its metabolites remaining in the vein strips were extracted with 1 N acetic acid containing 0.4 ml of 1% ascorbic acid, 0.3 ml of 1% sodium metabisulfite, and 0.1 ml of each of the mixed carrier solutions.[19,22]

Column Chromatography Method Used to Separate NE and the Metabolites of NE: Total tritium was determined by removing an aliquot (1.0 ml) from each sample of superfusate or vein extract for scintillation counting prior to separation of [^{3}H]NE from its metabolites. [^{3}H]NE was separated from its metabolites in the samples of superfusate by the method described by Graefe *et al.*[24] Corrections were made for crossover contamination. Superfusate samples and the effluents and eluates of the chromatographic separations were counted in duplicate 1.0-ml aliquots added to 10 ml of Safety-Solve® (Research Products International Corp.). Corrections for quenching were made with an external standard. The counting efficiency was 32–38%. The samples were counted for 10 min or long enough to reach 10,000 counts. The average recovery of 20 ng [^{3}H]NE added to extracts of superfusate or arteries was 85.17 ± 1.23% (n = 6).

Calculation of Fractional Release of Radioactivity: Fractional release of radioactivity was obtained by dividing the absolute rate of tritium loss at any given time by the amount of tritium present in the vein at the same time. The amount of radioactivity in the tissue at the start of each collection period was calculated by adding the total tritium present in each preceding sample to the amount of tritium present in the vein extract at the end of the experiment.[25]

Halothane Delivery to Superfusate: Halothane was delivered from a copper kettle vaporizer. In experiments measuring release of endogenous NE, delivered halothane concentrations in the O_2–CO_2 mixture preaerating the Krebs–Ringer superfusate and flowing through the superfusion chamber were 0.75, 1.50, and 2.5%. These values resulted in superfusate concentrations of 0.74 ± 0.008, 1.43 ± 0.031, and 2.24 ± 0.045% (means ± SEM, n = 5) as measured by gas chromatography (Barber–Colman System 5000).[26] In experiments using radiolabeled tissues, concentrations of 1.5% and 3% in the O_2–CO_2 mixture were used for preaerating the Krebs–Ringer solution and for flowing through the superfusion chamber. These values resulted in superfusate concentrations of halothane of 1.21 ± 0.047% and 2.41 ± 0.025% (means ± SEM, n = 6). The concentration of halothane in the gas mixture was monitored continuously throughout each experiment by an infrared halothane analyzer (LB-II®, Beckman), which was calibrated prior to each experiment with a known concentration of halothane.

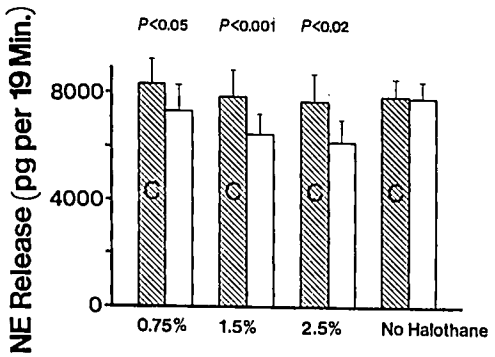

FIG. 1. Effect of halothane on release of endogenous norepinephrine from superfused dog saphenous veins stimulated electrically at 2 Hz. NE = norepinephrine. Superfusate contained cocaine (10^{-5} M) and corticosterone (4×10^{-5} M). Means ± SEM, n = 6–10 veins from different dogs. *P* values are for differences from paired controls (shaded bar marked "C"). The unshaded bar of each group represents the halothane-treated veins. The halothane concentration to which the veins were exposed is given beneath the respective groups.

Analysis of Data: Statistical analyses of the data for the control and treatment groups were done with the two-way analysis of variance. Differences between equivalent time periods of controls and treatment groups and between treatment groups were done using a Student's *t* test and were considered significant if $P < 0.05$.

Materials

L-[^{3}H]NE (ring labeled) with a specific activity of 45 Ci/mmol, obtained from New England Nuclear (NET 678), was used in the experiments.

Results

Part One: Studies of the Release of Endogenous NE

Effects of Halothane on Evoked Release of Endogenous NE: Release of NE (measured under conditions where removal of NE from the synaptic cleft was prevented by blockers of neuronal and extraneuronal uptakes) evoked

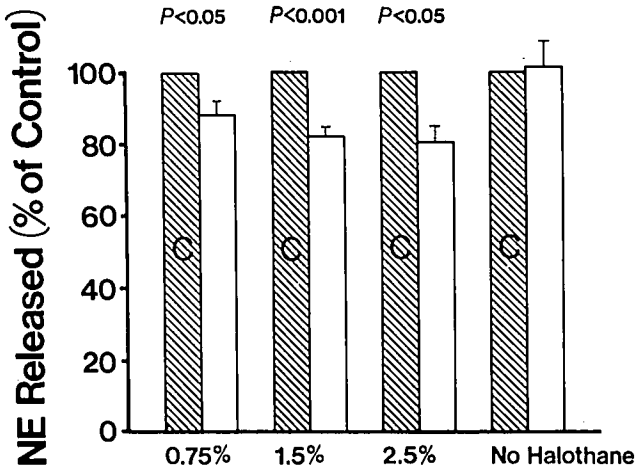

FIG. 2. Effect of halothane on total norepinephrine released (expressed as per cent of release from paired controls [shaded bar marked "C"]) from superfused dog saphenous veins stimulated electrically at 2 Hz. NE = norepinephrine. Superfusate contained cocaine (10^{-5} M) and corticosterone (4×10^{-5} M). Means ± SEM, n = 6–10 veins from different dogs. *P* values are for differences from paired controls (C). The unshaded bar of each group represents the halothane-treated veins. The halothane concentration to which the veins were exposed is given beneath the respective groups.

by ES was decreased significantly in tissues exposed to halothane (fig. 1). Expressed as a per cent of the NE released from paired controls, the release decreased 11.9 ± 3.5% (mean ± SEM) with 0.75% halothane, 17.7 ± 2.5 with 1.5%, and 19.2 ± 4.1% with 2.5% halothane (fig. 2).

NE Content in Saphenous Veins after Superfusion and Exposure to Halothane: At the end of superfusion and stimulation, there were no differences in the concentrations of NE in control veins and in veins exposed to halothane (table 1).

TABLE 1. Relationship between NE Overflow and Tissue Content in Saphenous Vein Exposed to Halothane

Group	n	μg NE/g Tissue	NE Released (% of Tissue Content)	*P* (From Paired Control)
Control	8		2.18 ± 0.27	
0.75% hal	8	3.80 ± 0.34	1.93 ± 0.26	0.02
Control	8		2.36 ± 0.28	
1.50% hal	8	3.68 ± 0.52	1.95 ± 0.25	0.01
Control	6		2.24 ± 0.32	
2.50% hal	6	3.39 ± 0.33	1.79 ± 0.22	0.05
Control	6		2.17 ± 0.29	
No hal	6	3.88 ± 0.71	2.13 ± 0.20	

Values are means ± SEM. NE = norepinephrine; hal = halothane. NE overflow expressed as per cent of tissue content: (NE overflow in 19 min of stimulation) ÷ (NE content in tissue after superfusion) × 100. *P* values are for differences from paired controls.

In controls, the total NE that overflowed throughout the experiment represented only 2.2–2.4% of the NE content in the tissue (table 1). These values are not significantly different. At each halothane concentration studied, the per cent of tissue NE content released in response to ES was less than that released from paired controls receiving the same stimulation (table 1).

PART TWO: STUDIES OF THE RELEASE OF TOTAL RADIOACTIVITY AND OF THE METABOLISM OF [^{3}H]NE

Effect of Halothane on Fractional Release of Total Radioactivity: The fraction of the radioactivity in veins, prelabeled with [^{3}H]NE, that was lost into the superfusate during each 6-min sample collection interval was increased markedly by ES (fig. 3). The addition of halothane significantly decreased the loss of total tritium during basal conditions and during ES; the loss of tritium, however, was not different in tissues exposed to 1.5 and to 3.0% halothane.

Effect of Halothane on Release and Overflow of [^{3}H]NE and its Metabolites: The increase in total tritium with ES (fig. 3) represents not only the release and subsequent overflow of [^{3}H]NE but also the [^{3}H]NE that has been converted to the ^{3}H metabolites of NE by the NE metabolizing enzymes MAO and catechol methyltransferase. In order to examine for changes in the metabolism of NE by the tissues, attributable to halothane, column chromatography was used to separate the tritium in samples of superfusate and in the tissue after study into unmetabolized NE and into the metabolites of NE (fig. 4).

Because halothane was found to reduce the total tritium overflow, both during basal conditions and during ES (fig. 3), the superfusate was collected throughout the entire experiment and analyzed so that halothane-induced changes with time in NE dynamics could be identified more accurately and quantitated.

Effects of Halothane during Basal Conditions: In control veins, [^{3}H]NE accounted for only 6.8% of the total radioactivity of the 0–6 min basal sample (fig. 4*A*). The major metabolite in the basal state was DOPEG, followed by OMDA (O-methylated, deaminated compounds MOPEG and VMA that are not separated by the column chromatography method), DOMA, and NMN, respectively (fig. 4*A*). No differences were detected in the proportions of [^{3}H]NE and of any radiolabeled metabolite of NE between the basal sample collected at 0–6 min (T1) and at 36–42 min (T2).

The addition of 1.5 or 3.0% halothane reduced significantly the amount of DOPEG entering the superfusate in the T2 basal sample (figs. 4*B* and *C*) but had no effect on any of the other metabolites of NE.

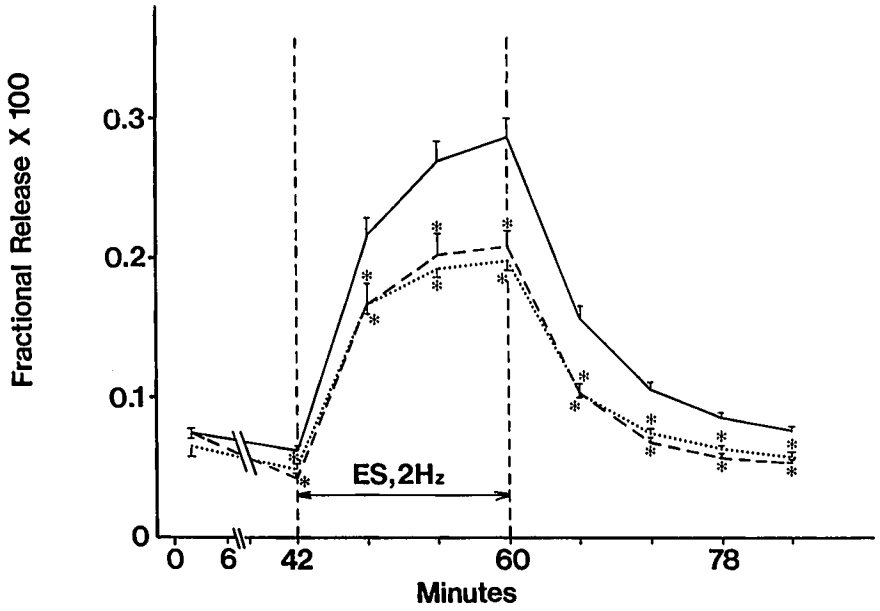

FIG. 3. Effects of halothane on the fractional loss of radiolabeled compounds with time from strips of dog saphenous vein prelabeled with [^{3}H]NE. Electrical stimulation (ES) at 2 Hz was applied between the 42nd and the 60th min. Means ± SEM, veins from five dogs. *$P < 0.05$ for difference from control vein. Control vein (——); vein exposed to 1.5% halothane (– – –); vein exposed to 3% halothane (• • •).

The Effects of Halothane during Stimulated and Poststimulated Conditions: ES increased the overflow of [^{3}H]NE and its metabolites in all vessels studied (fig. 4). Exposure of veins to 1.5% halothane caused the following changes in overflow: decreased the overflow of DOPEG and of DOMA during ES and during one or more of the poststimulation sample collection intervals, and increased NE overflow in the poststimulation periods when compared with the same intervals of control experiments. Similar effects were seen in the veins exposed to 3.0% halothane (fig. 4*C*).

Effect of Inhibition of Monoamine Oxidase on Overflow of [^{3}H]NE and the Metabolites of NE. Because of decreased formation of DOPEG by veins exposed to halothane

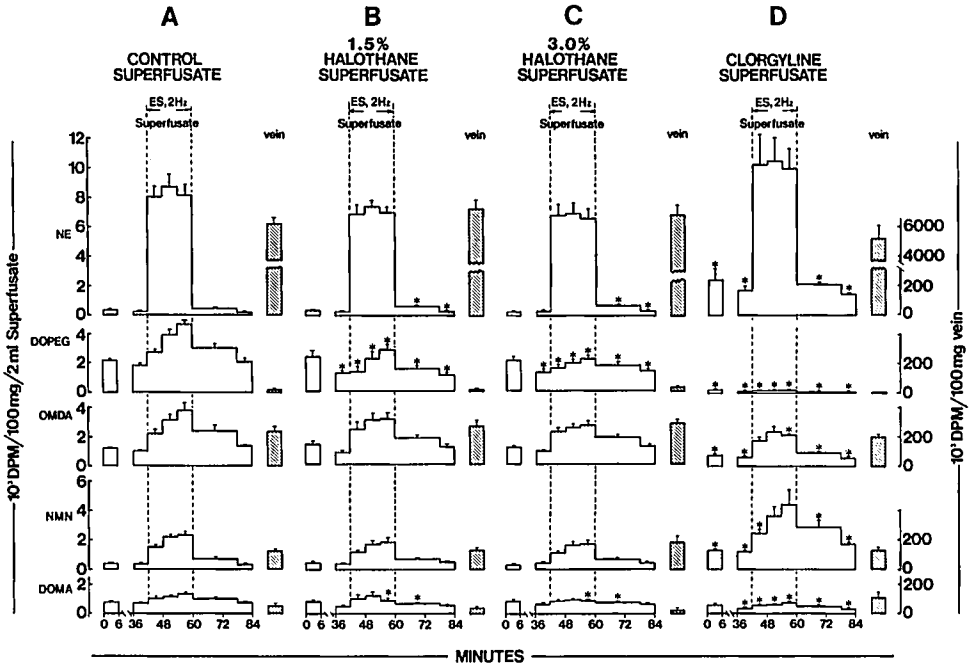

FIG. 4. Changes in the efflux of [3H]NE and its metabolites before, during, and after electrical stimulation (ES) at 2 Hz of strips of saphenous vein of dogs superfused with Krebs–Ringer in *A:* veins from five control dogs; *B:* veins from five dogs exposed to 1.5% halothane; *C:* veins from five dogs exposed to 3% halothane; and *D:* veins from three dogs exposed to the monoamine oxidase inhibitor clorgyline (2×10^{-5} M). Clear areas represent superfusate, shaded tissue analysis after completion of superfusion. ES applied between the 6th and 24th min. NE = norepinephrine; DOPEG = 3,4-dihydroxyphenylglycol; OMDA = a mixture of 3-methoxy-4-hydroxyphenylglycol and 3-methoxy-4-hydroxymandelic acid; NMN = normetanephrine; DOMA = 3,4-dihydroxymandelic acid. Values are means ± SEM, veins from five dogs. *$P < 0.05$ for differences from comparable time intervals of control group.

(figs. 4*B* and *C*), inhibition of intraneuronal MAO by halothane was suspected. Comparisons therefore were made in DOPEG formation in tissues exposed to halothane and to a MAO inhibitor. Veins were exposed to clorgyline in a concentration known to inhibit intraneuronal MAO in this tissue (fig. 4*D*). In the basal state, clorgyline produced the following changes in overflow of [^{3}H]NE and its metabolites: decreased the overflow of DOPEG, DOMA, and OMDA, and increased the overflow of NE and NMN compared with controls. During ES and afterwards, changes in [^{3}H]NE overflow and in NE metabolite profile included the following: decreased DOPEG, OMDA, and DOMA formation and increased NMN formation and NE overflow.

Content of [^{3}H]NE and its Metabolites in Vein after Study: The content of [^{3}H]NE or its metabolites remaining in tissue at the completion of experiments was not different in controls from that in any treatment group (fig. 4).

Discussion

The present data indicate that in the isolated dog cutaneous vein preparation halothane decreases the release of endogenous NE and total tritium in [^{3}H] labeled tissues (figs. 1 and 2; table 1) and reduces the oxidative deamination of NE (fig. 4).

An important point in understanding our interpretation of these data is a precise definition of overflow of NE and of release of NE. Overflow of NE is that part of the NE released into the synaptic cleft that is not taken up by neuronal or by extraneuronal uptakes and thus escapes from the synaptic cleft. Release of NE refers to all NE entering the synaptic cleft from the nerve ending. It includes that which is taken back up into the nerve ending, that which enters the extraneuronal tissues, and that which overflows from the synaptic cleft. Thus, if neuronal and extraneuronal uptakes are operative, overflow of NE would be less than release of NE. If neuronal and extraneuronal uptakes were rendered inoperative by using blockers of these uptakes, overflow of NE should approximate release of NE. In the experiments in which release of endogenous NE was measured, blockers of neuronal and extraneuronal uptakes[21] were used throughout. The overflow of NE under these conditions therefore is referred to as release of NE. It is assumed that these blockers were effective during basal conditions and during ES and that nonspecific effects of the drugs were minimal. In previous studies, these assumptions have been found to be valid in this tissue by our group and by other investigators.[18,20,21,27]

Effects of Halothane on Release of NE and its Subsequent Disposition

Results of the present study indicate that halothane decreases the total release of transmitter under basal conditions and during ES, as evidenced by a significant decrease in the fractional loss of tritiated compounds. It is of interest, however, that when the tritium overflowing is separated into [^{3}H]NE and into the radiolabeled metabolites of NE, the amount of tritium present as unmetabolized NE is not different in controls and in halothane-treated veins (figs. 4 *A–C*). At first appearance, this might be considered to not support the studies showing a clear decrease in release of endogenous NE in the presence of halothane. However, previous studies from this laboratory have demonstrated that contractile tension more closely follows total radioactivity in superfusate than does [^{3}H]NE.[19] In addition, studies by Langer have proposed that the metabolism of NE that has been released in response to stimulation is most likely a step subsequent to activation of postsynaptic receptors.[28] Hence, it is important to include metabolites as well as NE when calculating the actual amount of transmitter released. A similar sequence of events has been proposed by Bevan to occur to NE released in tissues with narrow synaptic clefts.[29] Together, these observations suggest that total tritium overflow more closely reflects the amount of transmitter interacting with receptors than does [^{3}H]NE overflow. Thus, the results of our [^{3}H]NE-labeled experiments are considered to support the findings in the endogenous NE studies.

In unstimulated and in stimulated veins, halothane caused a significant decrease in DOPEG overflow (fig. 4). The decrease tends to be greater at 3.0% than at 1.5% halothane and to affect DOPEG formation to a greater extent during ES than during basal conditions. DOPEG is formed by two sequences of events: 1) under basal conditions DOPEG is formed as a result of a slow efflux of NE from vesicles into neuroplasm where mitochondrial MAO catalyzes the oxidative deamination of this NE; 2) during the exocytotic release of NE, DOPEG is formed subsequent to neuronal reuptake and oxidative deamination of the released NE. The decrease in DOPEG formation during resting conditions could result from the following: 1) an inhibition of contact between NE and MAO, 2) decreased vesicular efflux of NE, or 3) decreased intrinsic MAO activity.

Inhibition of contact between MAO and NE could result from halothane-induced blockade of neuronal uptake or from increased vesicular uptake of NE. We are aware of no studies that have tested for facilitated vesicular uptake of NE induced by halothane. Several studies, however, have failed to show that concentrations of halothane used here impair neuronal uptake.[30–32] The present data support these studies and provide some information that suggests that neuronal uptake is, in fact, operative in the presence of halothane. The failure to see compensatory increases in the metabolites of NE of extraneuronal origin (NMN and OMDA) and in NE overflow when DOPEG formation was decreased markedly (figs. 4*B* and *C*) suggests that an operative

neuronal uptake system accumulated NE in the intraneuronal space but that oxidative deamination of the NE was impaired.

Impaired oxidative deamination of NE could result from several processes. Work by some investigators suggests that halothane may function as an inhibitor of MAO,[10] yet preliminary data by Richter and Murphy[33] suggest that halothane, rather than affecting the intrinsic activity of MAO, may cause some redistribution of mitochondrial MAO, resulting in a physical barrier between MAO and NE.

Impaired deamination of NE also could result from reduced efflux of NE from the vesicle into the neuroplasm. It seems unlikely that expansion of biologic membrane, attributed to general anesthetics,[34,35] could have selectively affected the vesicular or mitochondrial membranes sufficient to reduce efflux of NE into the neuroplasm or entry of NE into the mitochondria and explain the present findings. Other causes of reduced efflux of NE from the vesicle are obviously possible. While our preparation does not allow us to define precisely the mechanism by which halothane reduces DOPEG formation, a comparison of DOPEG formation during halothane and during the addition of clorgyline, a known inhibitor of MAO, was made. At the clorgyline concentration used, two differences were noted: 1) clorgyline was far more potent than halothane in inhibiting intraneuronal MAO both during basal conditions and during ES, as evidenced by marked decreases in DOPEG formation; and 2) clorgyline inhibited nonneuronal MAO, as evidenced by decreases in OMDA and in DOMA. Thus, if halothane is an inhibitor of MAO, its potency is not great at the concentrations likely to be used clinically.

Content of [^{3}H]NE and its Metabolites in Vein after Study

As in the experiments measuring release of endogenous NE, halothane treatment had no effect on the tissue content of NE or metabolites of NE remaining in the vein after study.

In summary, these studies indicate that halothane inhibits release of NE from postganglionic sympathetic nerve endings located in the wall of saphenous vein of dogs. The decreased NE release results in reduced contraction of smooth muscle cells, causing venodilatation. In addition, evidence is presented that halothane alters the biotransformation of NE by reducing its oxidative deamination. These studies serve to emphasize the wide variety of actions that halothane may have on physiologic processes. To the previously reported causes of vasodilatation attributed to halothane—depressant effects on central sympathetic centers, depression of ganglionic transmission, and direct action on vascular smooth muscle cells—it now is necessary to add inhibition of NE release from nerve endings. Additional studies now are needed to define the precise mechanisms by which halothane impairs release of NE from nerve endings and alters the biotransformation of NE. It also would be of interest to know whether other halogenated general anesthetic agents share with halothane this multiplicity of actions on NE dynamics in vascular tissue.

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