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The Effect of Halothane on Norepinephrine Responsiveness in Rabbit Small Mesenteric Veins

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The effect of halothane on the response of small isolated mesenteric capacitance veins to exogenous norepinephrine and electrically induced endogenous norepinephrine release was studied. The role of extra- and intracellular Ca^{2+} in norepinephrine-induced contractions was also examined. Two-millimeter-long segments from the second-order branch of the mesenteric vein were stretched to twice their resting diameter, and the generated tension was measured with a force transducer. Dose-dependent effects of norepinephrine on generated tension were examined before and after exposure to 0.75 and 1.5% halothane. (These concentrations produced perfusate halothane concentrations of 0.31 and 0.49 mM respectively.) Norepinephrine produced an increase in the basal vessel tension along with a superimposed rhythmic oscillation in tension. Although the magnitude of the tension increase was not affected by either concentration of halothane, the amplitude of the oscillations was reduced. Ryanodine (a blocker of Ca^{2+} release from the sarcoplasmic reticulum), like halothane, decreased the amplitude of the oscillations, but did not affect overall tension development. In the Ca^{2+}-free medium the contractile response to norepinephrine was greatly attenuated as compared to control, whereas the oscillatory behavior was completely abolished. Norepinephrine release was examined indirectly by measuring the increase in tension during electric field stimulation. Response to endogenously released norepinephrine was significantly decreased by exposure to halothane 1.5% (0.49 mM) and blocked by pretreating the vessel with phentolamine. At concentrations used clinically, halothane did not affect overall developed tension in response to exogenously applied norepinephrine. However, 1.5% (0.49 mM) halothane decreased both sarcoplasmic-reticulum-dependent oscillations in tension and electrically induced release of endogenous norepinephrine. (Key words: Anesthetics, volatile: halothane. Sympathetic nervous system, catecholamines: norepinephrine. Species: rabbit. Muscle: sarcoplasmic reticulum. Muscle, smooth: mesenteric veins.)

A LARGE COMPONENT of the total blood volume in humans is contained in the venous circulation, with 25–30% of the total found in the splanchnic venous circulation.[1] Within the venous system the greatest cross-sectional area and major blood storage site is the small veins that have a diameter of less than 2 mm. Active changes in their diameter in response to baro- or chemoreflexes may provide a mechanism for venous pooling or an autotransfusion.[2] A number of studies have indicated that general anesthetic agents may produce attenuation of arterial baroreflexes in experimental animals[3,4] and in humans.[5] Preliminary evidence indicates that halothane is a potent inhibitor of reflex constriction of mesenteric veins,[6] although the level at which this inhibition occurs (*i.e.* central *vs.* peripheral) has not been fully established. Some of this effect could be due partially to a decrease in baroreflex-induced changes in sympathetic efferent nerve activity by halothane.[7] Halothane has also been shown to dilate peripheral veins and decrease the responsiveness of some venous beds to reflex stimulation.[8] It induces prolonged hyporesponsiveness of vascular smooth muscle to catecholamines,[9] and decreases the stimulation-evoked release of norepinephrine from nerve endings.[10,11]

The purpose of this study was to determine the mechanisms by which halothane affects the responses of small mesenteric veins to both exogenously applied and endogenously released norepinephrine. The role of extra- and intracellular Ca^{2+} in these responses also was examined. We used an isolated vein preparation, which allowed the study of direct effects of halothane on the veins and eliminated systemic hemodynamic responses and reflex activity.

Materials and Methods

ANIMALS

This study was approved by Animal Care Committee of the Medical College of Wisconsin. New Zealand White rabbits weighing 1.5–3.0 kg were fasted for 24 h before use. The animals were anesthetized *via* the ear vein with thiamylal sodium (25 mg/kg) and alpha-chloralose (40 mg/kg). A midline laparotomy was performed and a segment of ileum was exteriorized. The mesentery was excised and placed in a dissection dish containing oxygenated physiologic saline solution (PSS) at 4° C. The components of the PSS (in millimolar concentrations) were: Na^+ 141, K^+ 4.7, Cl^- 125, Ca^{2+} 2.5, Mg^{2+} 0.72, $H_2PO_4^-$ 1.7, HCO_3^- 25, and glucose 11. Ca^{2+} was omitted from the solution for Ca^{2+}-free experiments.

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Vessels

The mesentery was pinned to a Sylgard-coated dish, and mesenteric veins were isolated, with care to avoid actually touching the vessels. Two-millimeter-long ring segments were cut from the second-order branch of the mesenteric vein. The rings were threaded onto two pieces of 22-μm tungsten wire. The wires then were stretched over the open jaws of two stainless steel rings. One ring was anchored and the other attached to a force transducer (Grass) for measurement of generated tension. The veins were continuously superfused with PSS from a water-jacketed reservoir at 37° C and equilibrated with a 95% O_2 and 5% CO_2 gas mixture to give a PO_2 of 400 mmHg, PCO_2 of 35 mmHg, and pH of 7.40 in the vessel chamber. The vessels were allowed to equilibrate for 15 min before the resting diameter was measured. They then were stretched to twice their resting diameter and allowed to equilibrate for 60–90 min before use. All preparations exhibited spontaneous oscillations in tension when contractions were elicited with high concentrations of norepinephrine. The tension at each norepinephrine concentration was measured from the zero line to the middle of the oscillatory pattern. Amplitude of the oscillations was measured from top to bottom of the tension oscillation, excluding any erratic peaks. A Grass electric stimulator was connected through a Grass isolation unit to two parallel platinum plates placed on either side of the vein. The stimulating parameters were: 40 V, 5 Hz, 2-ms pulse duration for 2 min.

Drugs

Stock solutions of 10^{-3} M norepinephrine hydrochloride (Sigma Chemical Co., St. Louis, MO), ryanodine (S.B. Penick & Co., Lyndhurst, NJ), and phentolamine (Ciba Geigy, Summit, NJ) were prepared daily. Halothane, 0.75 and 1.5%, was delivered with a Draeger® vaporizer and preequilibrated with PSS for at least 15 min prior to introduction into the vessel chamber. Flow through the vaporizer was 2 l/min. Samples of the PSS were collected from the bath and placed into sealed 1-ml vials for measurement of anesthetic concentration by gas chromatography.

Protocols

Effect of Halothane on the Response to Exogenous Norepinephrine. Cumulative dose–response curves for norepinephrine (0.01–1 μM) were performed in the absence and in the presence of 0.75 and 1.5% halothane. After anesthetic exposure, the dose–response curve was repeated, and 1 h elapsed between consecutive dose–response curves. The norepinephrine concentration was increased by adding aliquots of 10^{-3} M norepinephrine as soon as the effect of the previous concentration had reached a plateau.

Effect of Halothane on the Response to Suprathreshold Electric Field Stimulation. The response to electric field stimulation (40 V, 5 Hz, 2 ms, 2 min) was measured in the absence and presence of halothane (1.5%). Field stimulation was verified by blocking the response with 10^{-6} M phentolamine introduced 15 min prior to stimulation, and 1 h was allowed between consecutive stimulations.

The Role of Extra- and Intracellular Ca^{2+} in the Response to Exogenous Norepinephrine. To assess the contribution of extracellular Ca^{2+} influx to the norepinephrine-induced contraction, dose–response curves were performed in Ca^{2+}-free medium, preceded and followed by dose–response curves done in the presence of normal (2.5 mM) Ca^{2+}.

To assess the role of intracellular Ca^{2+} in the sarcoplasmic reticulum in the norepinephrine response, the dose–response curve for the same norepinephrine concentrations was performed in the presence of ryanodine (5×10^{-6} M). Finally, norepinephrine dose–response curves were performed in Ca^{2+}-free solution in the presence of ryanodine, a selective inhibitor of sarcoplasmic reticulum.

Statistical Analysis

One-way analysis of variance was used to analyze the effects of norepinephrine, halothane, electric stimulation, and ryanodine. Two-way analysis of variance was used to evaluate the effects of halothane, ryanodine, and zero Ca^{2+} on the response to norepinephrine or electric stimulation. The chi-squared test was used for analysis of the incidence of oscillation.

Results

The millimolar concentrations of halothane in PSS perfusing the vessels were 0.31 ± 0.03 and 0.49 ± 0.03 mM, for 0.75 and 1.5%, respectively.

A typical norepinephrine dose–response curve for a single mesenteric vein ring before, during, and after exposure to 1.5% halothane is shown in figure 1. Addition of norepinephrine resulted in a dose-dependent increase in tension exerted by the vein. At norepinephrine concentrations greater than 5×10^{-8} M, rhythmic oscillations of tension (4–8 Hz) were observed, with their amplitudes increasing at higher norepinephrine concentration. Halothane (1.5%) reduced the amplitude of the oscillations but did not affect the magnitude of the overall tension generated by the vessels.

Figure 2 shows the average change in tension generated by mesenteric veins during cumulative dose–response curve to norepinephrine in the absence and presence of

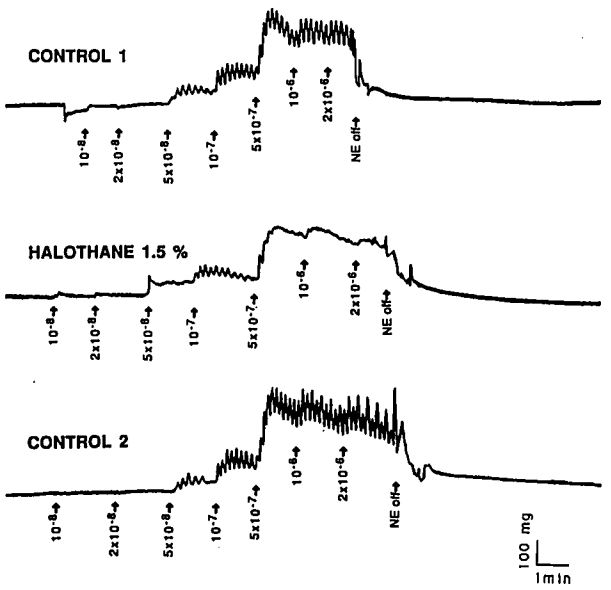

FIG. 1. Typical tracing of norepinephrine cumulative dose response curve in the rabbit isolated mesenteric vein ring. The next concentration of norepinephrine was added as soon as the response to the preceding concentration of norepinephrine reached a plateau. Halothane (1.5%) was introduced 15 min before exposure to 10^{-8} M norepinephrine. Concentration of halothane in this particular experiment was 0.48 mM as measured by gas chromatography. CONTROL 1 = control preceding halothane; CONTROL 2 = control after halothane.

1.5% (0.49 mM) halothane. Because the tension generated by mesenteric veins pre- and posthalothane was not different, we pooled the data and the mean ± SEM is shown as control in figure 2. Halothane produced neither a decrease in basal vein tension nor a reduction in the overall magnitude of the tension generated in response to norepinephrine.

Figure 3 shows the effect of halothane on oscillations produced in response to norepinephrine. Halothane 1.5% (0.49 mM) significantly decreased the amplitude but not the frequency of these oscillations; the frequency remained 4–8 Hz. Halothane 0.75% (0.31 mM) produced a smaller and nonsignificant decrease (10–15%) in the amplitude of oscillations (data not shown).

Figure 4 shows the change in tension of vein rings with suprathreshold electric field stimulation. Halothane 1.5% (0.49 mM) significantly decreased the response to electric stimulation, whereas phentolamine (10^{-6} M) abolished 80% of it. Exposure to halothane in the presence of phentolamine did not further decrease the response to electric stimulation. This suggests that the increase in tension produced by electric stimulation is mediated through an alpha-receptor response to electrically-induced release of norepinephrine, and that halothane may decrease norepinephrine release.

To evaluate the importance of mobilization of Ca^{2+} from the sarcoplasmic reticulum in the norepinephrine response, we repeated the dose–response curves during exposure to 5×10^{-6} M ryanodine, a selective inhibitor of sarcoplasmic reticulum Ca^{2+} channels. Ryanodine, like halothane, did not change the tension response to norepinephrine (fig. 5) but did significantly decrease the amplitude of oscillations at all norepinephrine concentrations. Unlike halothane, ryanodine delayed the onset of the oscillations to higher norepinephrine concentrations (fig. 6).

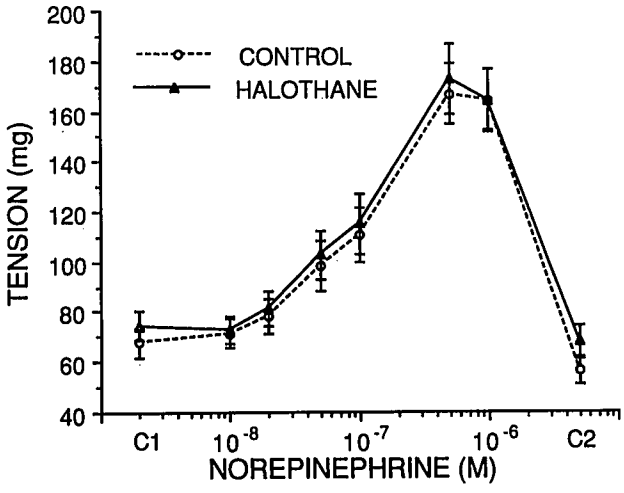

FIG. 2. Effects of halothane (1.5%) on the cumulative norepinephrine dose-response curve. The CONTROL curve represents pooled data for the control preceding and following exposure to halothane. C1 and C2 represent tension before exposure to norepinephrine and after norepinephrine was washed out, respectively. Data from 25 vessels are presented as mean ± SEM.

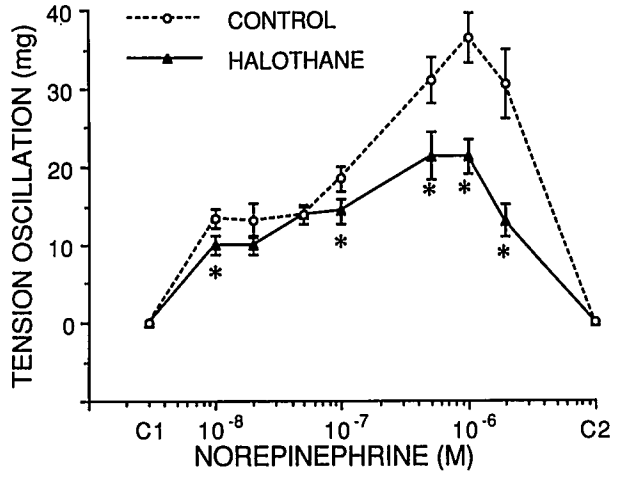

FIG. 3. Effects of exposure to halothane 1.5% on the oscillatory response induced by norepinephrine in isolated mesenteric vein rings. Halothane decreased the average amplitude of the oscillation compared to averaged precontrols and postcontrols. Data from 25 vessels are presented as mean ± SEM. *$P < 0.05$ *versus* CONTROL.

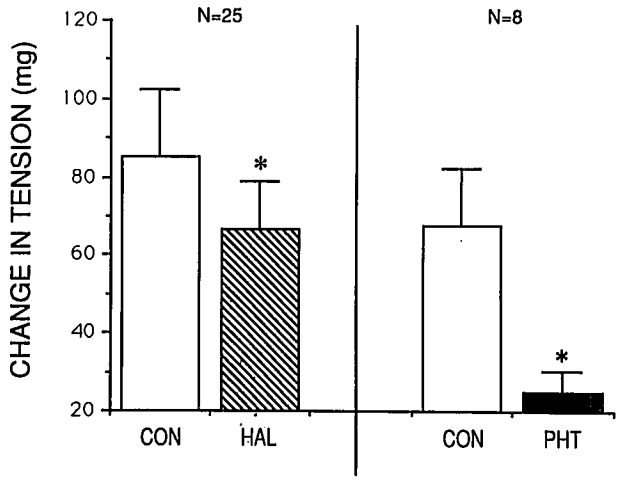

FIG. 4. Effects of HAL (1.5%) and phentolamine (PHT) on vessel tension generated in response to electrical field stimulation. Vessels were stimulated for 2 min, and maximum tension was recorded. Halothane and PHT significantly decreased response to stimulation. Data from 25 vessels pretreated with halothane and 8 vessels pretreated with PHT are presented as mean ± SEM. $*P < 0.05$ *versus* CONTROL.

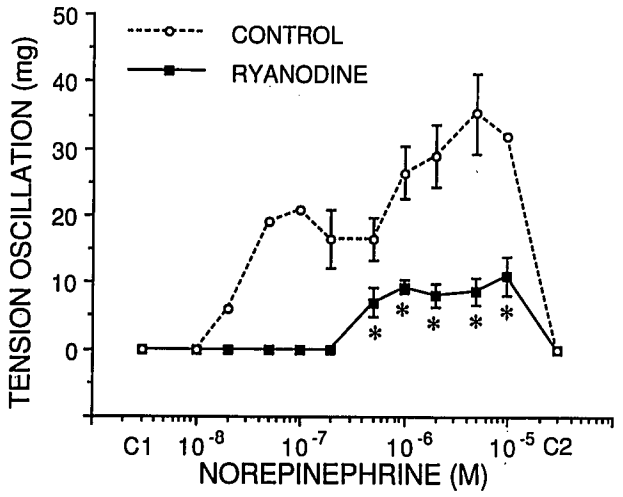

FIG. 6. Effects of ryanodine on oscillations in tension during cumulative dose-response curve to norepinephrine. Ryanodine decreased the amplitude of oscillations and also shifted the onset to higher norepinephrine concentrations. However, the frequency was not affected. Data from nine vessels are presented as mean ± SEM. $*P < 0.05$ *versus* CONTROL.

When Ca^{2+}-free medium was substituted for normal PSS solution, the contractile response to norepinephrine was greatly attenuated (fig. 7). At the same time, the oscillatory behavior was completely abolished.

Discussion

The venous side of the peripheral circulation acts as a large reservoir of blood that can be stored or mobilized in response to reflex stimulation.[12] Mobilization of this reservoir is of paramount importance in the event of hemorrhage and hypotension, which may occur in surgical procedures performed under general anesthesia.

Simultaneous *in situ* pressure and diameter measurements in rabbit small mesenteric veins showed that these vessels actively constrict or dilate in response to reflex stimuli.[13] Sympathetic efferent innervation to these mesenteric veins was documented by a frequency-dependent venoconstriction in response to celiac ganglion stimulation. This venoconstriction could be completely abolished by local application of tetrodotoxin.[13] Additional studies in our laboratory have further demonstrated that clinically

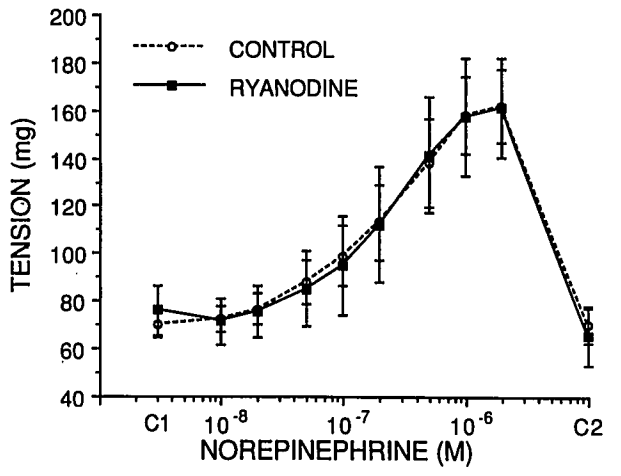

FIG. 5. Effect of 5×10^{-6} M ryanodine on tension response of isolated mesenteric vein rings. Ryanodine did not affect the increase in tension. Data from nine vessels are presented as mean ± SEM.

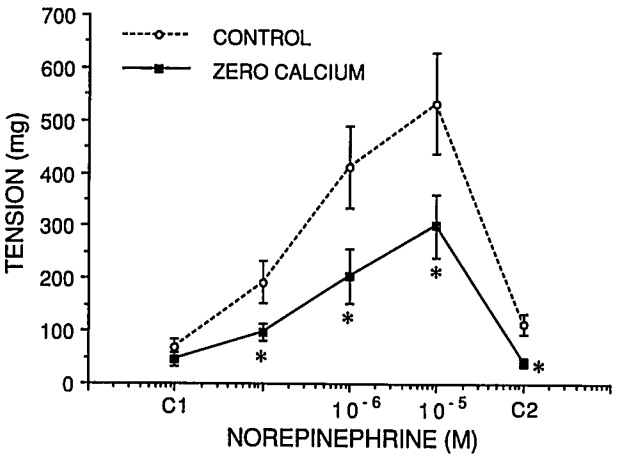

FIG. 7. Effect of extracellular Ca^{2+} on the norepinephrine response. Zero Ca^{2+} solution was introduced 15 min before the first concentration of norepinephrine. Absence of Ca^{2+} in extracellular space dramatically reduced increase in contraction to norepinephrine. Data from eight vessels are presented as mean ± SEM. $*P < 0.05$ *versus* CONTROL.

relevant concentrations of halothane blunt baroreflex-induced changes of the *in situ* mesenteric vein diameter in rabbits.[6] However, the same concentrations of halothane (0.5 and 1%) administered to the whole animal *via* inhalation or administered by local superfusion of the veins with Krebs solution equilibrated with up to 5% halothane (1.97 mM) did not alter the response to celiac ganglion stimulation.[6] These results indicate that systemically administered halothane very effectively impaired reflexly mediated vasoconstriction in mesenteric capacitance vessels and that this inhibition appeared to act proximal to the postganglionic neurons located in the celiac ganglion. Since the neuroeffector junction was less sensitive to the effects of halothane, it was important to examine how these capacitance vessels responded to exogenous and endogenous vasoactive substances, like norepinephrine, and if these responses are altered in the presence of halothane.

The data obtained from the isolated rabbit mesenteric vein preparation used in this study indicate that halothane did not affect the magnitude of tension generated by isolated rabbit mesenteric veins exposed to exogenous norepinephrine. However, exposure to halothane did result in a decrease in tension generated in response to electric field stimulation. The contractile response to electric field stimulation was abolished if the veins were pretreated with phentolamine. These data suggest that the increase in tension produced by electric stimulation was mediated through alpha-receptor responses to electrically induced release of norepinephrine. The magnitude of this tension increase may be modified through norepinephrine uptake by neuronal and extraneuronal mechanisms, which have been shown not to be affected by halothane.[14] Since halothane decreases the splanchnic nerve sympathetic efferent activity,[7] this effect would potentiate the decrease in norepinephrine release seen in our study.

Halothane also decreased the amplitude of oscillations seen at higher norepinephrine concentrations. Rhythmical oscillations in tension or diameter have been described in a number of arterial and venous preparations.[15,16] They frequently appear during extrinsic activation with pharmacologic agonists such as norepinephrine[15] and may be caused by rhythmic changes in intracellular Ca^{2+} concentration resulting from influx or release from internal stores.[16] These rhythmic oscillations may play an important role in fine regulation of blood flow through the small vessels.[17]

The response of vascular smooth muscle to norepinephrine is complex. It involves Ca^{2+} influx from the extracellular space through activation of receptor- and voltage-operated channels, release of Ca^{2+} from intracellular stores, and activation of second-messenger systems such as inositol triphosphate.[18] Extracellular Ca^{2+} appears to be important for both the contractile and oscillatory behavior of isolated rabbit mesenteric veins exposed to norepinephrine, since contractions were reduced and oscillations were abolished in the absence of extracellular Ca^{2+}.

Intracellular Ca^{2+} appears to have a role only in the oscillatory behavior of the mesenteric veins. In the presence of ryanodine, the oscillatory response was decreased, but the magnitude of the total tension generated by norepinephrine was not affected. Ryanodine has been shown to be useful in determining the importance of sarcoplasmic reticulum in excitation–contraction coupling in vascular smooth muscle.[19,20] These data suggest that the sarcoplasmic reticulum does not play an important role in the regulation of the overall tension generated in response to norepinephrine, although it may be important in the regulation of oscillatory contractions. Similarities between the effects of halothane and ryanodine would suggest that at least part of the inhibitory effect of halothane on oscillatory contractions is due to inhibition of the sarcoplasmic reticulum. The possibility of halothane's effect on sarcoplasmic reticulum in smooth muscle was recently suggested by Su and Zhang.[20]

Norepinephrine can open voltage-gated channels through a second-messenger system or through membrane depolarization brought about by the opening of receptor-operated channels. Norepinephrine increases the probability of opening L- and T-type channels in vascular smooth muscle. The functional importance of voltage-gated channels varies from one particular vascular smooth muscle to another.[21]

It has been reported that halothane, in concentrations similar to those used in our study, significantly decreased Ca^{2+} influx through voltage-operated channels in heart muscle.[22] However, the absence of any change in the norepinephrine tension response in mesenteric veins exposed to halothane would suggest either that voltage-sensitive Ca^{2+} channels do not play an important role in the norepinephrine response or that the channels in the smooth muscle sarcolemma are structurally different from those in cardiac tissue and are not affected by halothane.

In summary, halothane may reduce the ability of the splanchnic bed to respond to reflexly induced changes in diameter and capacitance. However, the venous side of this bed does retain the ability to respond to exogenously administered vasoactive agents such as catecholamines.

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