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MULTIFACTORIAL MEDIATION OF POST NOREPINEPHRINE INDUCED INTESTINAL HYPEREMIA

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We evaluated several potential endogenous mediators of post norepinephrine induced hyperemia in the mesenteric circulation. Hyperemia was elicited in the anesthetized rat anterior mesenteric artery after cessation of intravenous norepinephrine infusion at 0.125 to 1.0×10^{-8} M/min × 4 min. Arterial blood pressure was measured with a transducer, and the velocity of arterial blood flow was determined with pulsed Doppler velocimetry. Conductance at the height of mesenteric hyperemia, the post norepinephrine hyperemia volume, and the autoregulatory escape volume were calculated from recorded measurements. The higher doses of norepinephrine increased both the hyperemia volume and peak conductance in both control and capsaicin pretreated rats. Hyperemic parameters were significantly diminished by pretreatment with either yohimbine, propranolol, adenosine deaminase, or methylene blue. Combining adenosine deaminase with propranolol further reduced peak conductance and the hyperemia volume compared with enzyme pretreatment alone. The magnitude of hyperemia was related to the escape volume but not to the extent of norepinephrine induced vasoconstriction. We conclude that post norepinephrine induced hyperemia in the rat mesenteric circulation is modulated by alpha 2 and beta 2 adrenergic receptor activation, adenosine release, and endothelial factors.

Key words: *mesenteric circulation, adrenergic receptors, norepinephrine, adenosine, endothelium, nitric oxide, autoregulation, vasodilation*

INTRODUCTION

Post norepinephrine induced hyperemia (PNH) is an acute increase in blood flow above baseline after cessation of prolonged drug administration. PNH may restore adequate tissue oxygenation following the norepinephrine (NE) induced ischemic challenge. During NE infusion, mesenteric blood flow tends to recover towards control values because of autoregulatory escape (1). Furthermore, the increased oxygen extraction during NE infusion compensates for the oxygen deficit due to the loss of perfusion volume. Nevertheless,

cessation of NE infusion is followed by an abrupt vasodilation and a transient hyperemia.

Several mechanisms have been suggested for intestinal autoregulatory events, such as PNH, including the accumulation of metabolic products like adenosine (2, 3), beta adrenergic receptor mediated relaxation of vascular smooth muscle (4, 5), and local release of other vasodilator mediators (3, 6). We have recently demonstrated involvement of capsaicin sensitive nerves in post nerve stimulation hyperemia (7). Adenosine, capsaicin sensitive and sympathetic nerves have been implicated in post occlusive reactive hyperemia (8–10).

In the present study we examined the possible involvement of primary sensory and sympathetic nerves, alpha 2 and beta and 2 adrenergic receptors, adenosine release, and endothelial factors in NE induced PNH.

MATERIALS AND METHODS

Experiments were performed on 84 male Sprague-Dawley rats (SASCO) ranging from 275–325 g in weight. The animals were fasted for 24 hrs with free access to water prior to anesthetization with intraperitoneal sodium pentobarbital (50 mg/kg). Our experimental design, hemodynamic measurements, and administration of drugs were similar to previous descriptions (7–10). At the termination of each experiment euthanasia was performed in the deeply anesthetized rat with intravenous air embolization and monitoring of arterial pressure.

Anterior mesenteric artery blood flow was estimated by measuring the velocity of blood flow (VBF) with a sonic pulsed Doppler system (model 545C-4, Department of Bioengineering, University of Iowa) (11). This technique offers several advantages for the experimental model used in our studies:

1) only Doppler and electromagnetic blood flowmetry are well established techniques for continuous estimation of moment-by-moment freely changing mesenteric arterial blood flow (12);

2) the Doppler flow probe is smaller than the electromagnetic and, therefore, easier to implant on a rat mesenteric artery (12);

3) there is no zero flow drift with the Doppler probe (as opposed to the electromagnetic) which obviates frequent calibrations during experiments (11, 12);

4) the Doppler technique has been calibrated on rat gastrointestinal arteries and found to yield blood flow values which were in close agreement with simultaneous measurements using either electromagnetic blood flowmetry (11) or radiolabeled microspheres (13); and 5) the pulsed Doppler method has been used successfully to estimate rat gastrointestinal artery blood flows by multiple laboratories (8, 11, 13–19).

Following a midline laparotomy to expose the anterior mesenteric artery, the probe of the Doppler velocimeter was implanted near the origin of the vessel. VBF was recorded continuously in volts as a shift in Doppler signals in which 2 V corresponds to a 1 kHz shift in Doppler signal (11), and 1 V represents 5.2 ml/min blood flow in the rat anterior mesenteric artery (10). Mechanical zero VBF was obtained experimentally with a stainless steel vascular miniclamp to occlude the artery distal to the Doppler probe. Care was taken during the clamping not to compress perivascular nerve trunks. The mechanical zero calibration was performed before starting the experimental procedure, once during the experiment, and after completion of the experiment.

Arterial blood pressure was determined using a strain gauge transducer (model P-50, Gould-Statham) and recorder (model R611, Sensor Medics Dynograph). The transducer was connected to the right carotid artery *via* a heparinized, saline filled catheter (PE-50). Mean VBF and pressure recordings were obtained electronically from the phasic VBF and pressure recordings.

Conductance (C) values were calculated before NE infusion (resting C) and at the peak of VBF (peak C) approximately 30 sec following cessation of NE administration. C was calculated as the ratio of mean VBF/mean arterial pressure in units of mV/mmHg which has the advantage of not requiring conversion from kHz shift to ml/min-kg. Calculation of C rather than resistance values is also preferred under free flow and free pressure hemodynamic conditions (20).

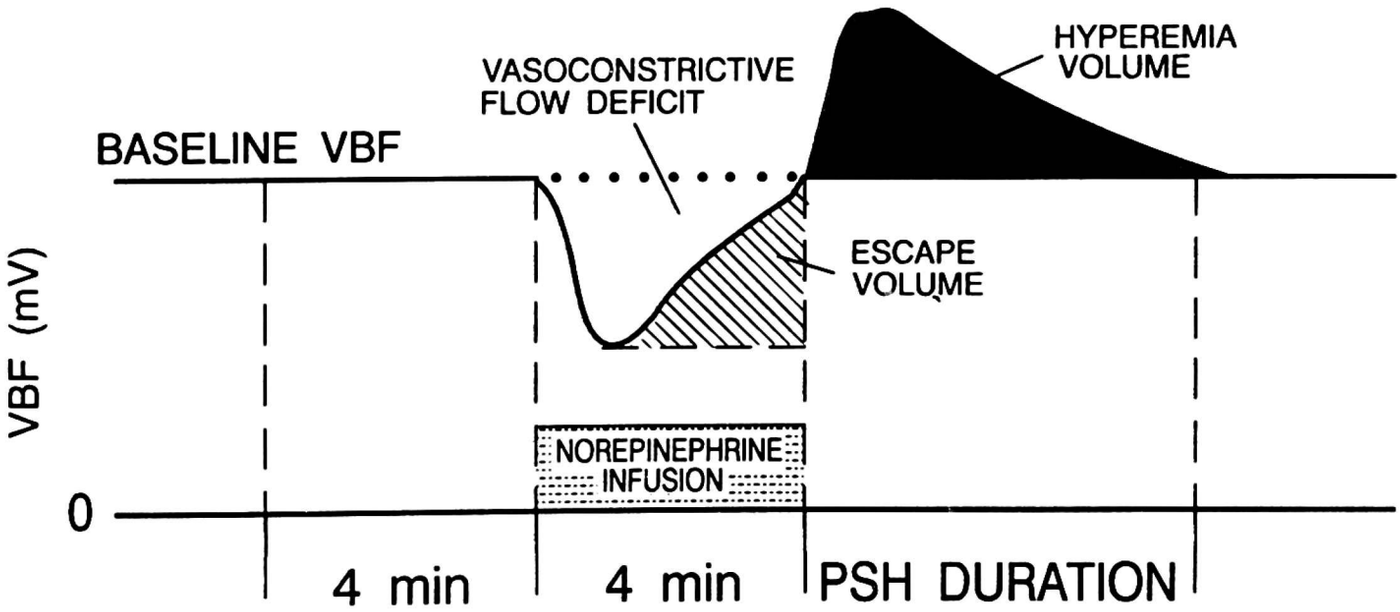

Fig. 1. Schematic presentation of a mean VBF tracing before, during, and after a 4 min NE infusion period. The vasoconstrictive flow deficit and escape volume occur during NE infusion, whereas the hyperemia volume is observed after cessation of NE infusin. The flow deficit, escape volume, and hyperemia volume are determined by electronic planimetric measurement of appropiate areas as described in the methods. The flow deficit quantifies the constrictor response to NE; the escape volume quantifies NE induced autoregulatory escape; and the hyperemia volume quantifies the PNH.

Blood flow volumes during and after NE infusion were determined by measuring the area under the curve of mean VBF recordings using a Sigma Scan measurement system (Jandel Scientific). This device incorporates a Numonics 2210 opaque digitizing tablet whose resolution was calibrated for 0.01 mm. Each measurement on the chart was performed three times and averaged for the tabulated values. Mean values were then calculated for each group. A schematic representation of blood flow volume measurements in relation to NE administration is shown in *Fig. 1.* For each series of experiments 4 min of control VBF, 4 min of VBF during NE infusion, and the full duration of PNH (at least 2.7 min) were used for volume measurements. In addition to determining the post NE hyperemia volume as an indicator of PNH, we calculated both the volume of blood flow deficit as an indicator of NE-induced vasoconstriction and the escape volume as an indicator of autoregulatory escape during NE infusion. With cessation of NE infusion, VBF increased within 3 sec to exceed the pre-NE infusion baseline value, at which point PNH was considered to have commenced.

EXPERIMENTAL GROUPS:

Group I. NE dose-response

0.125×10^{-8}, 0.25×10^{-8}, 0.5×10^{-8} and 1.0×10^{-8} M/min NE were infused successively into the left jugular vein of 11 anesthetized rats for 4 min periods each at 30 min intervals. Dose dependent changes in PNH parameters were measured and evaluated as described above.

The intravenous route of administering NE was used rather than the intra-arterial for several reasons. First, other investigations have identified no significant differences in catecholamine-induced autoregulatory escape in the gut as a function of intravenous versus intra-arterial administration (4). Second, in our rat model reproduceable intra-arterial administration is difficult to achieve and requires either occlusion of the mesenteric artery with a secured catheter or incurs loss of some of the infused drug, as during intra-aortic infusion proximal to the mesenteric arterial ostium. Finally, with continuous intra-arterial infusion of norepinephrine, some of the agent escapes tissue clearance and reaches the systemic circulation.

Group II. Neonatal capsaicin treatment

Newborn rats were treated with capsaicin according to a protocol published previously (16, 19, 21). Littermate controls were injected with the same volume of solvent for capsaicin (vehicle control). The animals were used for experiments when they had reached a weight of 275–325 g (about four months age). Effectiveness of the capsaicin treatment was assessed by the corneal sensitivity test using a 0.1% capsaicin solution and by the characteristic depressor response to 3 ug intravenous capsaicin at the end of experiments (21). NE induced PNH was registered in six sham treated littermate control rats and 13 capsaicin treated animals in the same manner as in Group I.

Group III. Hexamethonium

Sympathetic nerve modulation of PNH was tested by ganglion blockade (9). Hexamethonium (15 mg/kg) was infused into the left jugular vein over 10 min. Changes in peak C and the hyperemia volume were recorded in six rats in response to 0.5×10^{-8} M/min NE infusion before and 30 min after ganglionic blockade. This dose of NE was infused for 4 min in this and in all succeeding experimental groups to elicit a standard PNH response (see *Table 1*).

Group IV. Surgical denervation

We carefully sectioned the anterior mesenteric periarterial nerves in seven rats using an operating microscope as previously described (18). NE was infused prior to and 60 min after nerve sectioning.

Group V. Lidocaine

The effect of nonspecific, sensory nervous blockade on PNH was observed by injecting 2 mg lidocaine dissolved in 0.1 ml normal saline. The 60 sec injection into the ascending aorta utilized the arterial pressure catheter which was advanced temporarily from the right carotid artery into the aorta as described previously (14). After drug injection the cannula was pulled back to its original position in the carotid artery. PNH changes were recorded before and 10 min after lidocaine administration in five rats.

Table 1. Resting C values before and peak C values after 4 min of NE infusion in 9 experimental groups

Experimental Group	NE Doses ($\times 10^{-8}$ M/min)	Resting C (mV/mmHg)	Peak C (mV/mmHg)
I. Control NE dose-response	0.125	14.3±0.8	19.5±0.8
	0.25	15.2±1.2	20.6±1.6
	0.5	14.9±0.9	21.0±1.4
	1.0	15.2±1.4	23.5±0.9*
II. Neonatal capsaicin/NE dose-response	0.125	20.6±1.3	21.7±2.3
	0.25	20.0±1.3	21.5±2.5
	0.5	19.8±1.3	23.7±2.6
	1.0	19.5±1.5	29.8±3.1*
III. Control	0.5	12.7±2.2	21.7±4.8
After hexamethonium	0.5	23.1±2.8*	23.2±4.0
IV. Control	0.5	14.8±1.8	21.9±1.8
After denervation	0.5	17.7±2.1	21.8±3.9
V. Control	0.5	21.0±3.2	27.2±3.1
After lidocaine	0.5	18.6±2.6	23.2±2.8
VI. Control	0.5	14.0±2.3	18.5±2.5
After vehicle	0.5	15.3±2.9	20.7±2.7
After yohimbine + vehicle	0.5	18.1±3.5	20.1±4.3
VII. Control	0.5	15.2±1.1	19.3±1.0
After 0.015 μM propranolol	0.5	15.5±1.3	21.7±1.5
After 0.150 μM propranolol	0.5	13.2±0.8	16.3±1.9
After 1.50 μM propranolol	0.5	13.4±1.4	12.8±1.4*
VIII. Control	0.5	18.3±1.5	28.2±3.1
After adenosine deaminase	0.5	17.1±2.2	19.4±2.6*
After adenosine deaminase + propranolol	0.5	17.1±2.2	14.0±1.6*
IX. Control	0.5	16.8±1.4	29.8±3.0
After methylene blue	0.5	15.5±1.4	17.0±1.5**

* = $p < 0.05$ and ** = $p < 0.01$ of no difference compared with either the control value or the initial value in the same vertical column of that group of rats.

Group VI. Alpha 2 Adrenergic receptor blockade

Involvement of alpha 2 adrenergic receptors in PNH was studied using yohimbine (1.5 μM/kg, i.p.) in five rats (22). The effect of the yohimbine solvent was tested in four other rats. PNH was evoked by NE infusion before and 10 min after the drug or solvent administration. Because NE elicited a mesenteric vasodilator response in yohimbine pretreated rats, peak C values were calculated at 30 sec after cessation of NE infusion and were compared with control values (inasmuch as alpha 2 adrenergic blocked rats did not manifest PNH).

Group VII. Beta Adrenergic receptor blockade

Dose dependent actions of propranolol on PNH were evaluated in seven rats. NE was infused at 30 min intervals before and after 0.015, 0.15, and 1.5 uM/kg doses of intravenous propanolol. Isoproterenol 0.1 ug/min infusion was used to test the effectiveness of the propranolol doses in a separate series of five rats.

Group VIII. Adenosine deaminase alone and combined with propranolol

A solution containing adenosine deaminase (Type VII, Sigma) was prepared containing 750 U/kg in 0.5 ml normal saline and was infused into the ascending aorta of seven rats over a 5 min period following a control NE infusion. The effectiveness of adenosine deaminase was tested with intra-arterial injections of 10 ug of adenosine prior to and after administration of the enzyme. Confirming observations from another study (10), we found that the vasodilator effect of adenosine was inhibited for up to 45 min by adenosine deaminase pretreatment. After recording the effect of NE infusions on PNH before and after enzyme treatment, propranolol (1.5 uM/kg) was then injected intravenously to six of these rats. After 10 min of stabilization, a NE infusion was repeated to examine possible synergistic inhibition of combined adenosine and beta receptor activation on PNH.

Group IX. Methylene blue

Endothelial relaxating factor involvement in PNH was tested by the intravenous injection of 0.3 mg/kg methylene blue in eight rats. This agent appears to interfere with the vasodilator effects of nitric oxide by inhibiting soluble guanylate cyclase activity (23, 24).

Drugs utilized

Adenosine and adenosine deaminase type VII (Sigma); capsaicin (Fluka) dissolved in 10% ethanol, 10% Tween 80, and 80% normal saline as a 1% W/vol solution; hexamethonium (Sigma); isoproterenol (Winthrop); lidocaine (Elkins-Sinn); methylene blue (Sigma); norepinephrine (Winthrop-Breon); pentobarbital (Sigma); propranolol (Ayerst); and yohimbine (Sigma) dissolved as 2 μM/ml stock solution in ethanol and diluted in normal saline for experiments.

Statistical methods

All data presented are expressed as mean values ± SE. Statistical comparisons were performed using either analysis of variance or Student's t test for grouped or paired samples where appropriate (25). Statistical significance was accepted when the probability of no difference was less than 5%.

RESULTS

Group I. NE dose-response

Continuous intravenous infusion of NE for 4 min evoked a dose dependent vasoconstrictor response. The initial constriction was followed by a gradual return of blood flow toward or beyond the control level as previously reported (1). The lower doses of NE (0.125×10^{-8} and 0.25×10^{-8} M/min) failed to cause a blood flow deficit during NE infusion in control rats because VBF exceeded the pre-NE flow late in the escape from initial vasoconstriction. The higher NE doses (0.5×10^{-8} and 1.0×10^{-8} M/min for 4 min), however, did cause a measureable flow deficit (*Fig. 2A*). Hyperemia volumes showed a dose dependent response to NE as the dose increased from 0.25 to 1.00×10^{-8} M/min (*Fig. 2B*). A significant increase in peak C was seen only at the highest dose of NE (*Table 1*).

Group II. Neonatal capsaicin treatment

Mature rats which had been treated with capsaicin in neonatal life had significantly increased pre NE resting C values compared with control rats and reacted to NE with an exaggerated flow deficit compared with control and littermate rats (*Table 1* and *Fig. 2A*). This increased vessel reactivity was reflected by an increased flow deficit during the 4 min periods with different NE doses. However, compared with control rats there was no significant increase in the hyperemia volume (*Fig. 2B*), in peak C (*Table 1*), or in PNH durations (*Table 2*). Similarly, the escape volume values were not significantly different between neonatal capsaicin treated animals and littermate controls.

Group III. Hexamethonium

Intravenous hexamethonium caused a significant decrease in arterial pressure, no change in VBF, and a significant increase in resting C (26). Resting C nearly doubled after hexamethonium (*Table 1*). Hexamethonium had no significant effect on the NE induced peak C (*Table 1*), flow deficit and escape volume but did lower the hyperemia volume (*Fig. 3*). PNH duration was not significantly affected by ganglionic blockade (*Table 2*).

Group IV. Surgical denervation

Transection of the nerve branches surrounding the anterior mesentric artery reduced the hyperemia volume (*Fig. 3*) with a parallel decrease in PNH duration (*Table 2*). There were no significant changes in the values for flow deficit, peak C, or escape volume following perivascular denervation.

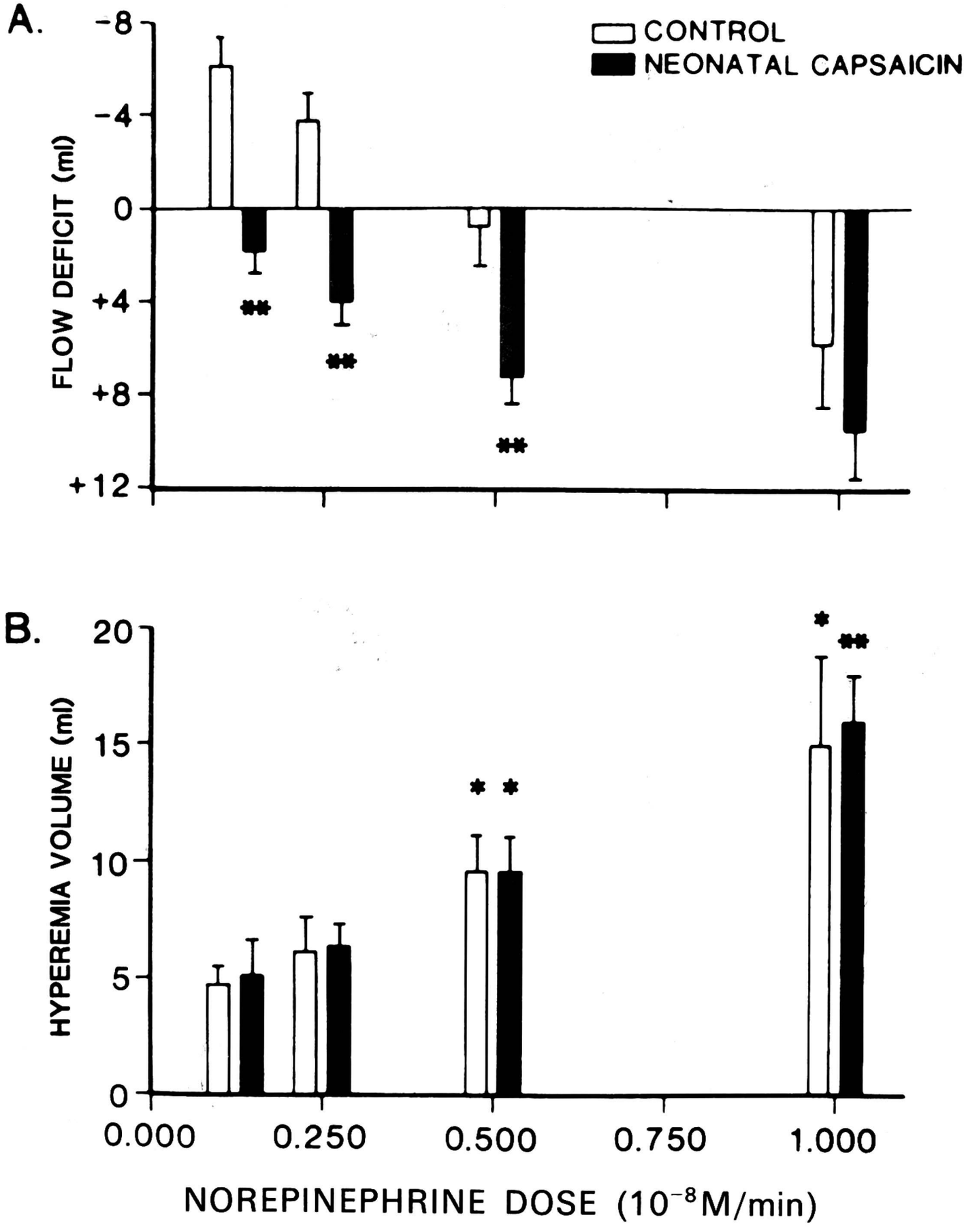

Fig. 2. Comparison of NE induced responses in littermate control and neonatally capsaicin treated rats, gauged by flow deficit (2A) and hyperemia volume (2B) values. A negative flow deficit value (bar above the zero line) indicates an increase in VBF above the pre NE control value. * = $p < 0.05$ of no difference from the pre NE hyperemia volume and ** = $p < 0.01$ of no difference from either the pre NE hyperemia volume or from the pre NE flow deficit value.

Table 2. Durations of PNH in min determined after 4 min of NE infusion in 9 experimental groups.

Experimental Group	NE Infusion Rate ($\times 10^{-8}$ M/min)			
	0.125	0.250	0.50	1.00
I. Control dose-response	3.0±0.4	3.0±0.3	4.6±0.4	6.0±1.2
II. Neonatal capsaicin	3.6±0.5	3.7±0.3	4.4±0.5	6.4±0.7
III. Control After hexamethonium			8.8±1.2 7.0±0.9	
IV. Control After surgical denervation			7.6±1.0 4.4±0.6*	
V. Control After lidocaine			4.5±0.2 3.5±0.5	
VI. Control After yohimbine			12.3±1.2 2.8±0.2**	
VII. Control After 0.015 μM/kg propranolol After 0.150 μM/kg propranolol After 1.50 μM/kg propranolol			6.3±0.6 4.5±0.3* 3.2±0.6* 2.7±0.4**	
VIII. Control After adenosine deaminase After adenosine deaminase and propranolol			5.2±0.3 4.8±0.6 4.0±0.6	
IX. Control After methylene blue			7.4±1.2 4.0±0.5*	

* = $p < 0.05$ and ** = $p < 0.01$ of no significant difference from the value immediately above in the same vertical column of that group of rats. All numerical values are in units of min.

Group V. Lidocaine

Administration of intra-arterial lidocaine had no significant effect on either the NE induced flow deficit and escape volume or PNH volume, peak C, and duration values.

Group VI. Alpha 2 Adrenergic receptor blockade

NE evoked a stable vasodilator response in yohimbine treated rats, which was not followed by further increase in VBF after cessation of NE. Peak C values were similar before and after yohimbine, but the duration (*Table 2*) and the hyperemia volume (*Fig. 4*) were considerably reduced by alpha

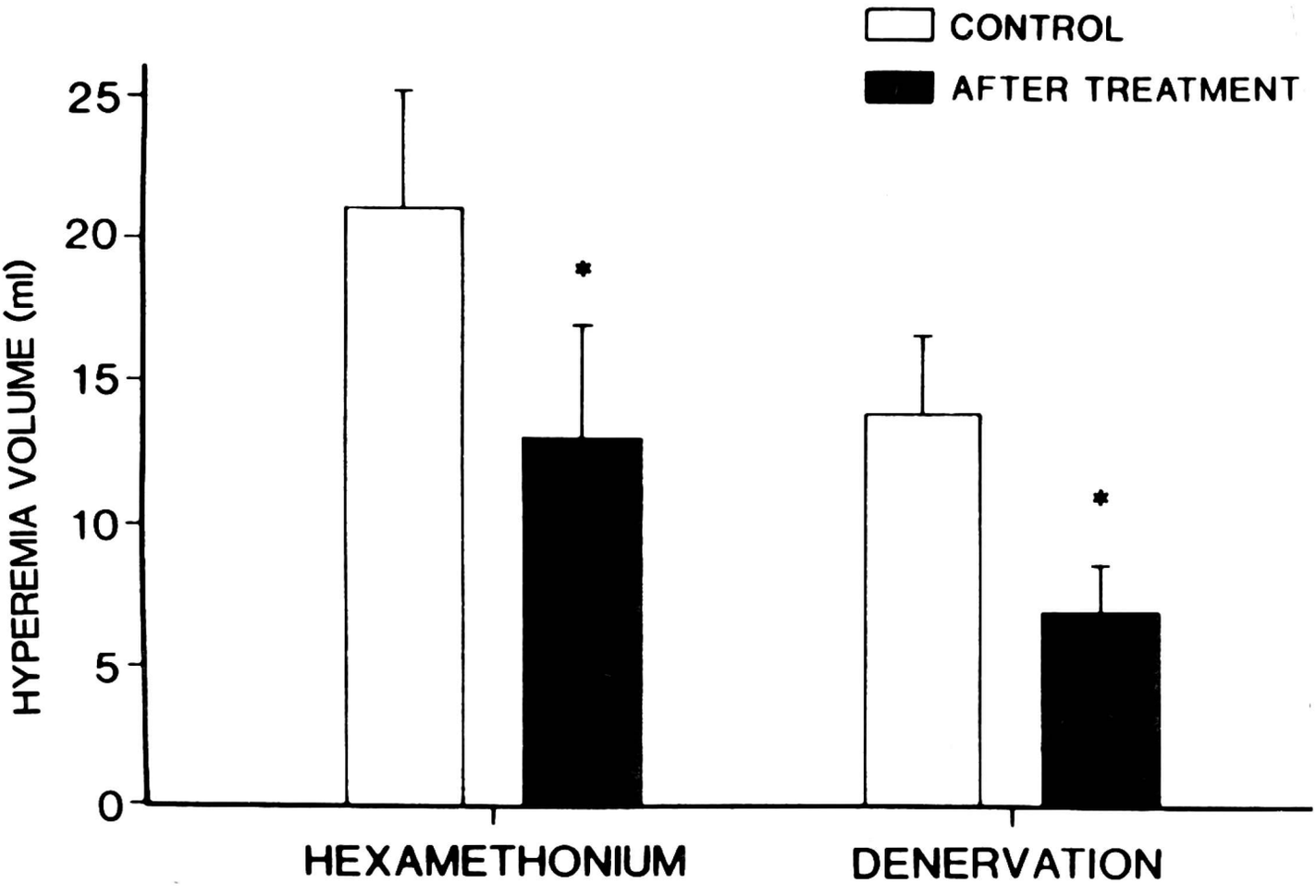

Fig. 3. Reductions in the hyperemia volume observed after hexamethonium and acute surgical denervation. * = $p < 0.05$ of no difference from control.

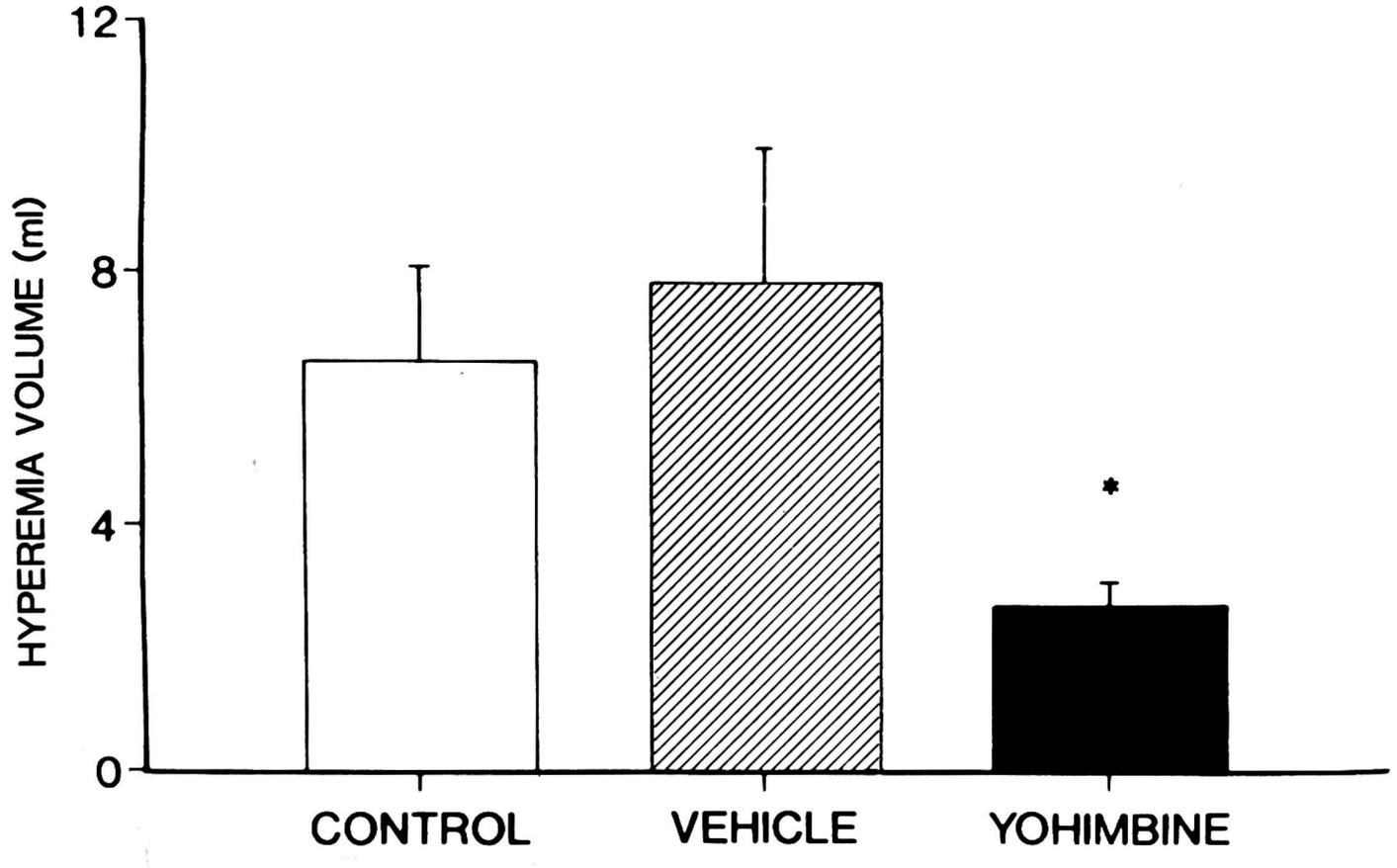

Fig. 4. Yohimbine induced decrease in the hyperemia volume during PNH. * = $p < 0.05$ of no difference from control.

2 receptor blockade. Treatment with the vehicle for yohimbine had no significant effect on the peak C, hyperemia volume, flow deficit, escape volume, or duration compared to untreated control values.

Group VII. Beta Adrenergic receptor blockade

Increasing doses of propranolol evoked progressively greater reductions in the hyperemia volume after NE infusion (*Fig. 5*). This dose dependent reduction was also reflected in the decreasing PNH duration with the 0.015, 0.15 and 1.5 μM/kg doses of propranolol (*Table 2*). The highest dose of propranolol caused a significant decrease in peak C without a change in the

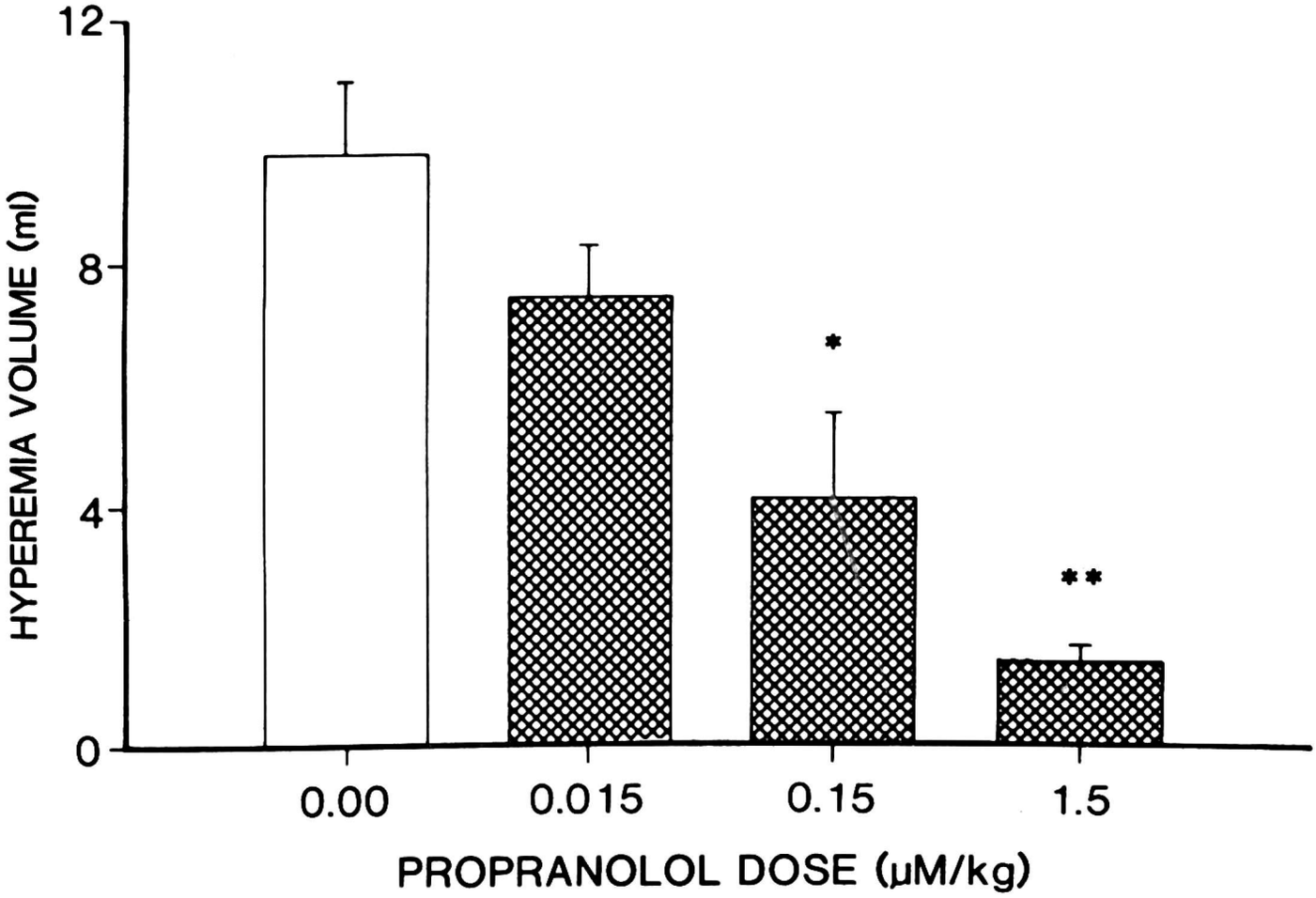

Fig. 5. Propranolol dose dependent inhibition of the hyperemia volume during PNH, * = $p < 0.05$ and ** = $p < 0.01$ of no differences from the control hyperemia volume response to cessation of NE.

resting C between measurements (*Table 1*). Beta receptor blockade had no effect on the flow deficit but with the 0.15 and 1.5 μM/kg propranolol doses there were reduced escape volume values.

Group VIII. Adenosine deaminase alone and combined with propranolol

Enzymatic degradation of endogenous adenosine evoked a significant reduction in both peak C (*Table 1*) and the hyperemia volume (*Fig. 6*). Resting VBF and C values and the NE induced flow deficit were not significantly

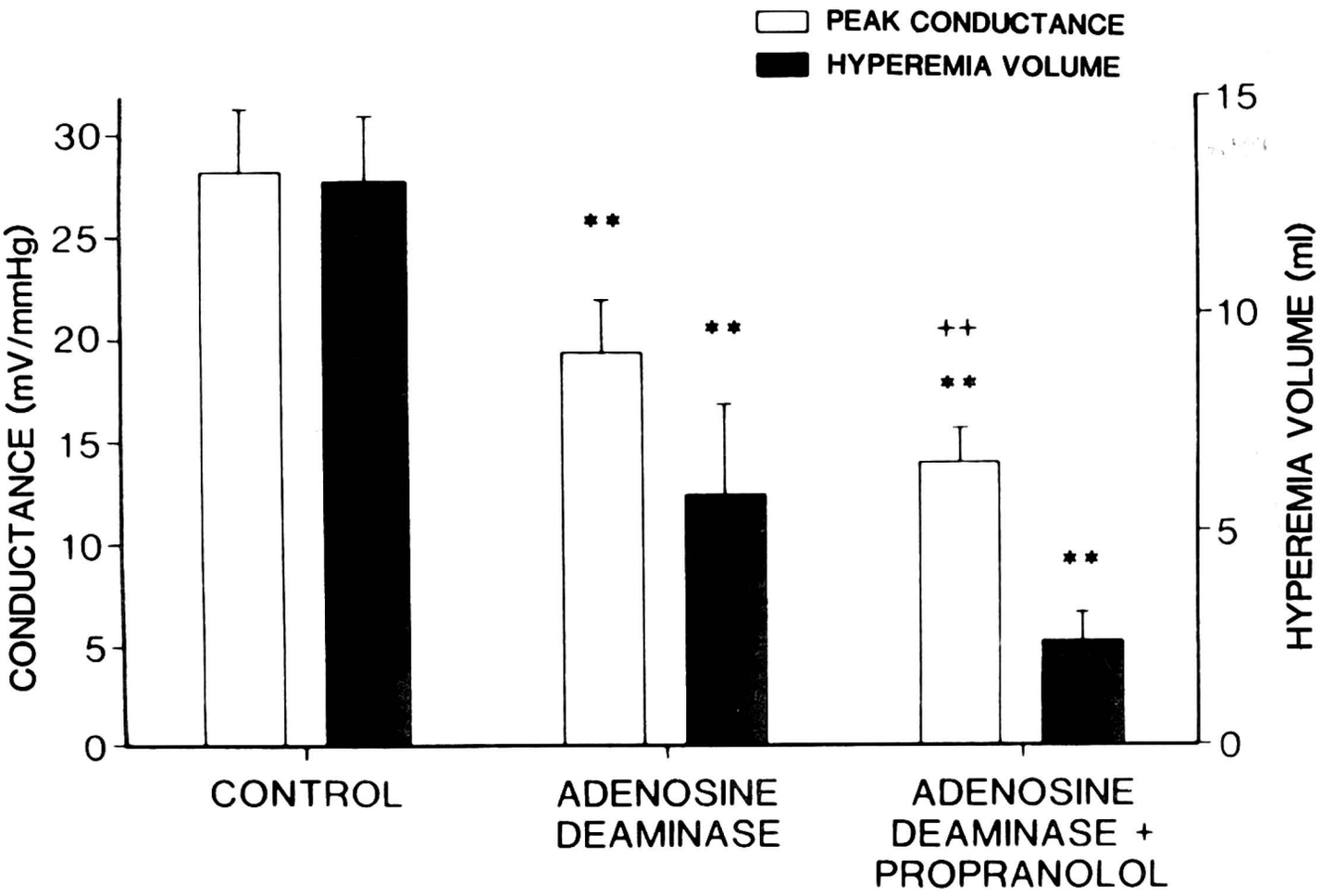

Fig. 6. Both adenosine deaminase and the combined effect of the enzyme + propranolol reduced peak C and the hyperemia volume following cessation of NE infusion. Adenosine deaminase and propranolol together further decreased peak C compared with enzyme treatment alone and nearly abolished the hyperemia volume. ** = $p<0.01$ of no difference from the control responses. ++ = $p<0.01$ of no difference from enzyme treatment alone.

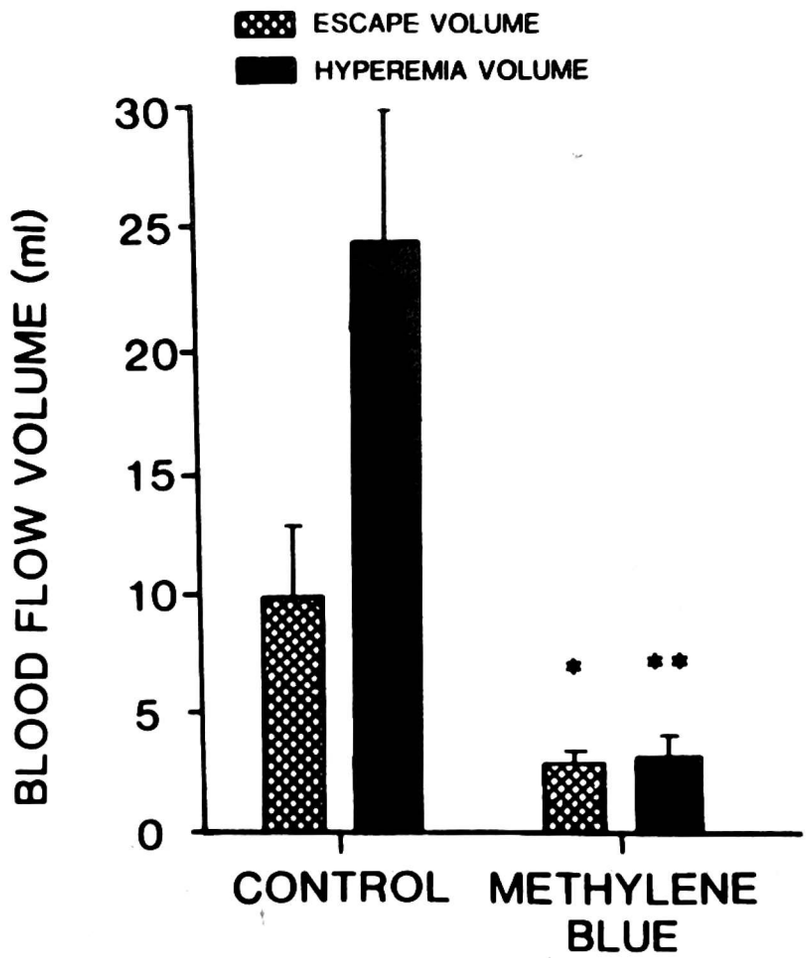

Fig. 7. Methylene blue administration reduced both NE induced auto-regulatory escape and PNH. Compared with control responses to NE, animals pretreated with methylene blue showed reduced escape and hyperemia volumes following NE. * = $p<0.05$ and ** = $p<0.01$ of no differences from respective control values.

altered by adenosine deaminase pretreatment, but there was a decrease in the escape volume from 6.9 ± 0.8 ml to 4.8 ± 0.9 ml after enzyme administration. Addition of propranolol to rats pretreated with the enzyme nearly abolished the hyperemia volume and also further reduced the peak C value after NE infusion (*Fig. 6*). There was, additionally, a significant decrease in the escape volume from 4.8 ± 0.9 ml (adenosine deaminase alone) to 1.95 ± 0.4 ml (enzyme + propranolol).

Group IX. Methylene blue

There was a significant reduction in the post NE induced peak C (*Table 1*), PNH duration (*Table 2*), hyperemia volume and the NE induced escape volume (*Fig. 7*) after administration of methylene blue.

DISCUSSION

The transient hyperemia following cessation of NE infusion is a prelude to the reestablishment of systemic and local perfusion parameters to the prestimulation level. The range of NE infusion used in our experiments was chosen to simulate physiological changes in circulating NE concentration and to elicit a vasoconstrictor response corresponding to 2–15 Hz sympathetic nerve stimulation (9, 18, 22). Several mechanisms have been proposed to explain PNH (2, 3, 5, 6), and our experiments were designed to test the more likely processes.

The extent of NE induced vasoconstriction, gauged by determining the blood flow deficit during NE infusion, failed to show correlation with the post NE induced hyperemia, gauged by determining the hyperemia volume, duration, and peak C values. Accordingly, despite the increased constrictor reactivity to NE in rats pretreated during neonatal life with capsaicin, peak C and hyperemia volume values were unaffected by capsaicin treatment (compared with littermate controls). Similarly, at the low dose of NE in the control group, where the sum of the flow deficit and escape volume values exceeded the pre-NE volume, the cessation of NE administration still evoked a hyperemic response, indicating that a significant vasoconstrictive blood flow deficit is not a prerequisite for PNH.

It has been proposed that the mechanism causing autoregulatory escape involves different mesenteric microvascular elements than the mechanism underlying other autoregulatory events (26). Our hypothesis is that mesenteric autoregulatory phenomena, such as NE induced escape or PNH, involve multiple adrenergic, peptidergic, and purinergic modulatory mechanisms, both at the smooth muscle and endothelial levels (3, 7–10, 14, 16, 18, 19).

Comparison of escape and PNH responses in rat gut indicate several agents which influence both autoregulatory events. However, hexamethonium had no significant effect on either the escape volume, the duration, or peak C values, whereas this agent decreased the hyperemia volume and increased resting C values. Transection of periarterial nerves also resulted in a divergence of effects, namely a reduction in the hyperemia volume and no change in the escape volume. In these experiments the reduction in PNH volume appeared to result from an earlier return of VBF to baseline due to elevated resting C values.

Yohibine eliminated NE induced vasoconstriction and escape as well as inhibiting PNH. The calculated hyperemia volume in yohimbine treated rats may represent a passive return of vessel tone toward the baseline after cessation of the NE related beta adrenergic effect. Since circulating NE acts on both vascular smooth muscle and endothelial alpha 2 receptors in the peripheral circulation (27–29), it was expected that antagonism of these receptors would also affect PNH. Reduction of the hyperemia volume by yohimbine is probably caused by loss of the alpha 2 adrenocepter potentiating effect of adenosine-induced vasodilation (30). In addition, alpha 2 adrenoceptor antagonism would reduce endothelial cell release of vasodilator substances (31). Furthermore, the cessation of NE infusion in yohimbine treated rats did not evoke any additional increase in peak C, which suggests that initial alpha 2 receptor stimulation by NE in PNH was the major factor causing the subsequent hyperemia.

Involvement of beta receptors in NE induced escape is controversial (5, 20, 32). Our experiments, however, showed a dose dependent role for beta receptor activation during NE induced escape, as well as in PNH. In preliminary studies we have observed that the beta agonist properties of NE during alpha 2 or nonselective alpha receptor blockade, namely a mesenteric vasodilation, were antagonized by propranolol. Cessation of beta receptor activity after NE may occur well beyond the end of alpha adrenergic vasoconstriction. Beta receptor stimulation of endothelial cells would release nitric oxide (33). Ischemia invoked release of adenosine appears to interact synergistically with the beta receptor related vasodilation. The combined effects of propranolol and adenosine deaminase suggest that beta receptors and adenosine are major mediators of PNH. Although propranolol is known to decrease adenosine release during tissue hypoxia (34), the combination of propranolol and adenosine deaminase inhibited PNH volume to a greater extent than either antagonist alone (*Figs. 5* and *6*).

A putative role for adenosine in PNH is also controversial (20, 35). NE administration either directly through adrenergic stimulation or indirectly via tissue hypoxia can increase adenosine production in the mesenteric circulation. Blocking the vasodilator effect of adenosine by adenosine deaminase caused

a reduction in both escape and hyperemia volumes. The C values remained below the prestimulation level after cessation of NE stimulation when both the beta adrenoceptor and adenosine effects were blocked by antagonists. Furthermore, blockade of the post stimulation hyperemia was preceded by abolition of autoregulatory escape, again suggesting that PNH depends on the same mechanisms as the NE induced escape volume.

There is increasing evidence for the physiological involvement of an endothelium-derived relaxing factor, such as nitric oxide, in escape from vasoconstrictor stimuli (36). Our findings with methylene blue suggest a significant role for endothelial mediators in PNH. Autoregulatory escape and PNH showed parallel changes in these experiments as well. The presence of beta 2 and adenosine receptors on the endothelial cell membrane (36, 37) implies that the active vasodilation during autoregulatory escape and PNH is probably mediated by both an indirect endothelial mechanism and direct smooth muscle relaxation. Endothelial dilator mediators may be responsible for approximately half of the total VBF recovery during escape and PNH. By contrast, primary sensory nerves did not appear to be influential in PNH despite their known modulation of resting vascular tone in rat gut (16).

In the current study, PNH parameters for a given dose of NE were not directly dependent on the actual deficit in flow during NE administration. Primary sensory and sympathetic nerves modify resting C values and the extent of NE induced vasoconstriction but do not influence PNH.

In conclusion, PNH appears to depend on alpha 2 and beta receptors, adenosine, and endothelial factors. Adenosine release and beta adrenoceptor activation effects on vascular smooth muscle and endothelial cells may be the main mechanisms underlying active vasodilation during PNH.

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