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ADENOSINE MODULATES REACTIVE HYPEREMIA IN RAT GUT

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Intestinal reactive hyperemia is an abrupt blood flow increase following release from anterior mesenteric arterial occlusion. We investigated the role of adenosine in reactive hyperemia. In anesthetized rats, mesenteric arterial velocity of blood flow was determined with pulsed Doppler velocimetry and arterial pressure with a transducer. Three indices quantifying reactive hyperemias obtained following 30, 60, and 120 s arterial occlusions included duration, the volume of blood flow exceeding preocclusion blood flow, and the percentage increase in conductance. In six rat groups (half fasted and half with intrajejunal bile-oleate solutions), hyperemia parameters were determined before and after administration of either adenosine deaminase (ADA) or two adenosine receptor antagonists, namely 8-phenyltheophylline (8-PT) and 1,3-dipropyl-7-methylxanthine (DPMX). In fasted gut the three agents had variable effectiveness against reactive hyperemia, although 8-PT was the most consistent inhibitor. Instillation of intrajejunal lipid evoked a stable hyperemia and increased duration and blood flow volume after each occlusive period. ADA and 8-PT were more effective against reactive hyperemia in fed gut than in fasted gut. Our findings suggest that adenosine is a vasodilator metabolite modulating mesenteric reactive hyperemia, especially during enhanced intestinal metabolic activity.

K e y w o r d s: *adenosine, reactive hyperemia, mesenteric circulation, postprandial hyperemia, rat, micellar lipid, adenosine deaminase, 8-phenyltheophylline, 1,3-dipropyl-7-methylxanthine, adenosine receptor antagonists.*

INTRODUCTION

Blood flowing through organs is controlled mainly by metabolic, myogenic, and neurogenic factors (1, 2). In the gut there are also autoregulatory events, such as reactive hyperemia (RH), in which blood flow is increased by local mechanisms, at times in opposition to active vasoconstrictor influences (3-5). RH is a vasodilator response to release from short-term, mechanical occlusion of a mesenteric artery. It is probable that the underlying mechanisms for RH also involve local metabolic, myogenic, and peripheral neurogenic modulators.

Adenosine is a potent, naturally occurring vasodilator of the intestinal circulation (4, 6-10). Abundant evidence implicates adenosine as a local modulator of intestinal vasodilation in other autoregulatory phenomena, such as pressure-flow autoregulation and postprandial hyperemia (1-3, 5, 7, 8, 11). It has been proposed that the dilator function of this nucleoside becomes more evident when either the oxygen supply to the organ is threatened or there is an enhanced need for more oxygen (7, 12, 13). Accordingly, the role of adenosine appears more prominent in modulating intestinal hyperemias in the presence of nutrients in the intestinal lumen than in the fasted gut (7, 14-16).

The role of adenosine in modulating RH is somewhat uncertain, with conflicting results from the use of drugs which antagonize the vasodilator effects of exogenously administered adenosine (6, 7, 17). We assessed adenosine modulation of RH by using different pharmacological agents which antagonize exogenously administered adenosine mediated vasodilator responses (18) in fasted rat gut versus gut containing micellar lipid, and we varied the length of arterial occlusion in RH.

MATERIALS AND METHODS

Experiments were performed on 58 fasted male Sprague-Dawley rats (Sasco) with a mean weight of 343 ± 5 g. Animals were anesthetized with sodium pentobarbital (50 mg/kg) administred intraperitoneally. Body temperature was maintained at 37.5°C by warming rats with a heating pad, monitored by a rectal thermistor and regulator (Yellow Springs Instruments, model 74). The trachea was cannulated, and the animals were artificially ventilated (Harvard, model 683). A saline filled catheter was placed in the right jugular vein for administering required supplemental anesthetic and fluids. Mean systemic arterial pressure was monitored via another saline-filled catheter inserted into the right carotid artery and connected to a strain gauge transducer (Gould-Statham, model P-50). During each experiment mean heart rate was electronically determined from the phasic signals of the arterial blood pressure or the velocity of blood flowing (VBF) in the anterior mesenteric artery.

A midline laparotomy was performed to expose the main trunk of the anterior mesenteric artery for placement of a Doppler flow probe (1.0 mm i.d., Titronics) on the vessel. Mesenteric artery VBF was determined with a directional pulsed Doppler velocimeter (Bioengineering, University of Iowa, model 545C-4). Signals were recorded as pulsatile and mean flow velocities of a Doppler shift in kHz and were expressed in volts where a value of one volt equals a 2 kHz shift in Doppler signal, and 2.8 V would be equivalent to a flow of 14.6 ml/min in the rat mesenteric artery. This system has been shown to provide reliable measurement in comparison with electromagnetic blood flow determinations (19). Mechanical zero VBF was obtained experimentally with a stainless stell, miniature vascular clamp to occlude the anterior mesenteric artery distal to the flow probe. Continuous recordings of mean blood pressure, heart rate, and both phasic and mean VBF were made on a polygraph (Sensor Medics Dynograph, model R611). In experiments with the intrajejunal placement of a nutrient solution or saline, silicone tubes were inserted into proximal and distal parts of the jejunum for instillation or withdrawal of test solutions. Both jejunal ends were isolated surgically from the remainder of the small intestine. We have described these methods in greater detail previously (20-24).

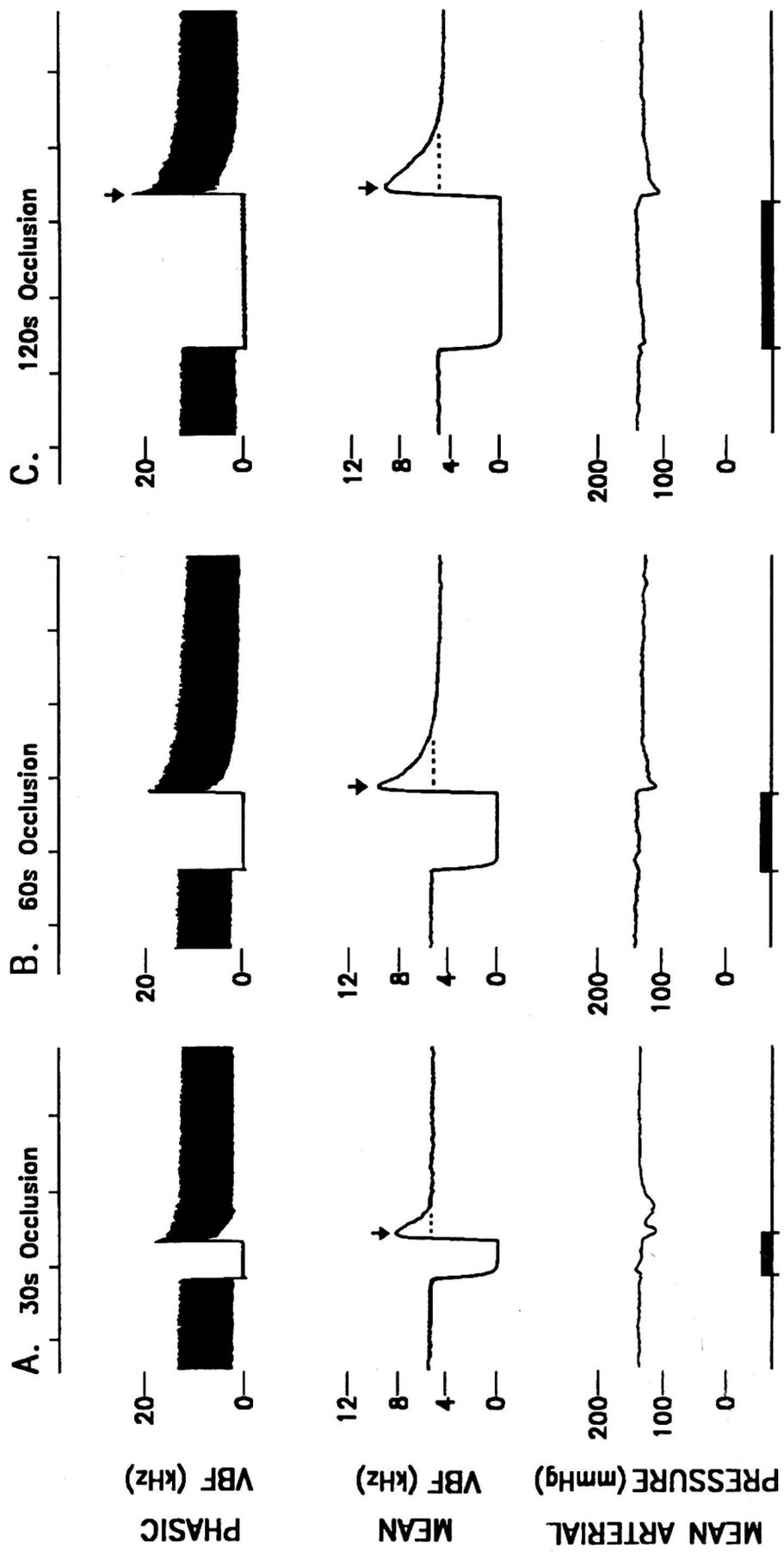

Fig. 1. Sample recordings of RH responses in a typical control experiment are shown for 30, 60, and 120 s of mesenteric artery occlusions. Note the progressive increase in VBF responses at the peak of RH (arrows) and the increase in durations of hyperemia (dashed lines) as the interval of arterial occlusion was lengthened. Also note the hypotensive responses to release from occlusion.

In six separate groups of rats we performed 30, 60, and 120 s of complete mesenteric arterial occlusion during control and experimental periods (*Fig. 1*). RH was quantified three ways. First, we calculated the percentage increase in vascular conductance at the height of VBF following release from occlusion, relative to the control (preocclusion) conductance value. This proportional increase in conductance at the highest VBF during RH was designated C_h (23). Mesenteric arterial conductances were calculated from mean VBF divided by mean systemic arterial pressure (21) and were computed just before arterial occlusion and at peak VBF during RH. Second, we measured the duration of RH in s after release from occlusion, from the point in time when VBF exceeded the preocclusion value until VBF had resumed its preocclusion value. The third measurement was that of the excess total volume of the hyperemia, designated as „Volume", which was quantified by determining the area under the postocclusive VBF curve (above the preceding control VBF value), and was expressed as ml of blood. A Jandel Scientific Sigma Scan measurement system was calibrated to a resolution of 0.01 mm and used to determine the estimated volumes in triplicate. The triplicate determinations were then averaged for the tabulated values from which mean values for each group were calculated.

Table 1. Duration (s) of the excess VBF during RH in six experimental groups wherein the three periods of occlusion were performed.

Experimental Group	n	Periods of Occlusion		
		30s	60s	120s
I. Fasted Gut	10	60±4	71±4	101±6
After ADA		37±5**	51±4**	60±8**
II. Fasted Gut	9	66±10	83±8	114±12
After 8-PT		55±5	61±4**	88±4*
III. Fasted Gut	8	73±5	84±4	127±7
After DPMX		70±3	86±4	117±5
IV. Fasted Gut	7	51±5	83±7	115±15
Fed Gut		85±13**	113±13**	169±14*
Fed Gut after ADA		59±6*	76±6*	95±5*
V. Fasted Gut	7	59±8	64±6	74±7
Fed Gut		110±10**	127±13**	139±11**
Fed Gut after 8-PT		61±6**	73±5**	111±16**
VI. Fasted Gut	7	65±3	77±5	101±11
Fed Gut		86±3**	109±6**	143±8**
Fed Gut after DPMX		71±5	93±8	116±9*

All numerical values for durations are in units of seconds. n = number of rats assessed in each experimental group.* and ** denote $p < 0.05$ and $p < 0.01$, respectively, for significant differences in mean values for the corresponding occlusion values from the immediately preceding value in the same vertical column.

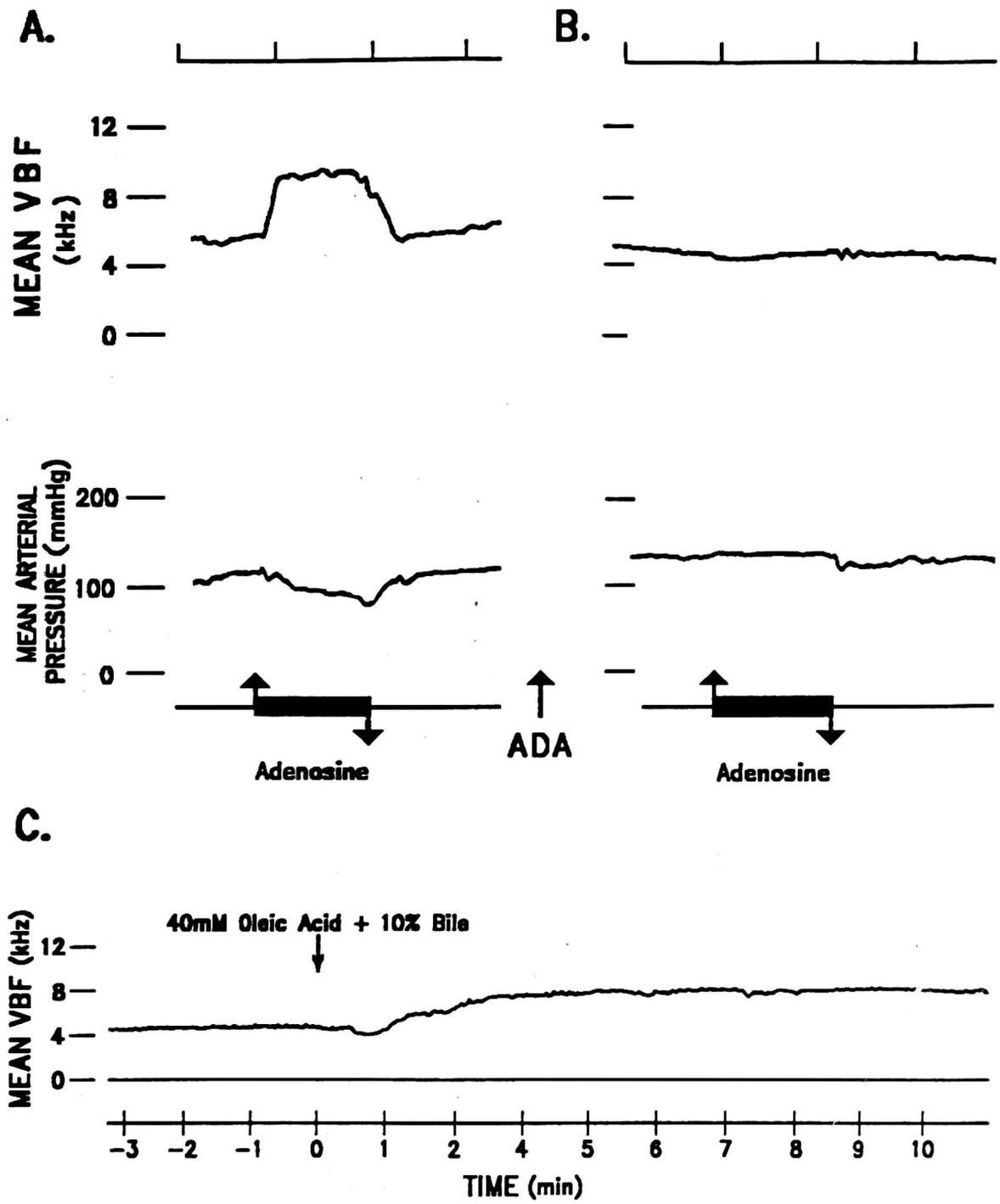

Fig. 2. Effects of ADA on the vasodilator response to exogenous adenosine in fasted gut. In A, the vasodilator response to an intravenous adenosine test dose of 500 U./0.5 ml is shown. In B, the same adenosine test dose was administered after treatment of the same rat with ADA and demonstrates abolition of both VBF and pressure responses to adenosine. In C intrajejunal instillation of a bile-oleate solution elicited a sustained increase in VBF in one rat, thereby providing the control situation for fed gut.

After the surgical preparation was completed, hemodynamic parameters were allowed to stabilize for 20-30 min before initiating one of the seven experimental protocols (*Tables 1-3*).

In Group I rats, RH responses following 30, 60, and 120 s arterial occlusions were examined before and after administration of adenosine deaminase (ADA) in a dose of 500 U/rat. ADA, type VIII (lyophilized, 200 U/mg protein, Sigma) was prepared in a final concentration of 500 U/0.5 ml. The effectiveness of adenosine degradation in this group and in Groups IV and VII was tested by administering two i.v. injections of vasodilatory doses of adenosine 20 min apart, the first dose before and the second dose after administration of ADA (*Fig. 2 A & B, Table 2*). The two

vasodilator responses to adenosine were compared. Adenosine (Sigma) was dissolved in warm physiological saline and injected i.v. in a dose of 0.5 mg/kg. In the doses administered ADA and 8-phenyltheophylline were effective inhibitors of the VBF response to exogenous adenosine for 50 min. Our experimental protocols were completed in each rat well within 45 min after administering the adenosine inhibitors.

Table 2. Inhibitory effect of ADA, 8-PT, and DPMX on the mesenteric hyperemic response to infusing exogenous adenosine (0.5 mg/kg).

Group	Agents	n	% Reduction in adenosine elicited hyperemia
I	ADA	7	89±12%
II	8-PT	9	65±5%
III	DPMX (4 mg/kg)	8	45±8%
	(5 mg/kg)	5	42±10%
IV	Bile Oleate then ADA	5	78±10%
V	Bile Oleate then 8-PT	7	64±9%
VI	Bile Oleate then DPMX (4 mg/kg)	7	42±8%
VII	ADA	6	61±14%
	ADA then Bile Oleate	8	78±5%

The effect is expressed as a percentage reduction in VBF to an adenosine test dose which was caused by each antagonist, and is compared with the response before giving each of the three inhibitory agents. In every case treatment effects produced significant ($p < 0.01$) reductions in the exogenous adenosine test dose responses.

In Group II rats, the three sequential durations of arterial occlusions were performed before and after antagonism of adenosine A_1 and A_2 subtype receptors with 8-phenyltheophylline (8-PT, Sigma). 8-PT was dissolved in warm saline at a pH of 12 and was administered in two doses of 2 mg/kg each. The first dose was injected i.v. as a bolus, and was followed by the second dose, administered as an i.v. infusion at a rate of 0.1 mg/kg-min for 20 min. Arterial occlusions were performed during the infusion of 8-PT. The effectiveness of adenosine receptor antagonism was tested in this group and in rats from groups III-VI with exogenous adenosine in the same manner as in Group I (*Table 2*).

In Group III rats, RH responses to the three durations of arterial occlusion were recorded before and after antagonism of adenosine A_2 receptors with 1,3-dipropyl-7-methylxanthine (DPMX, Research Biochemicals). DPMX was diluted in normal saline and administered i.v. in two doses of 2 mg/kg each as described for 8-PT above and administered to eight rats. A higher dose (5 mg/kg) of DPMX was also tested against exogenous adenosine vasodilation in an additional five rats from this group (*Table 2*).

In Group IV rats, we compared the hyperemic response following release from arterial occlusions in the presence of normal saline (termed fasted gut) versus micellar lipid in the jejunal lumen (termed fed gut). The volume of administered saline was 1.5 ml at 37°C. After performing

the three occlusions with saline in the gut, luminal saline was replaced with 1.5 ml of 40 mM oleic acid in 10% bile. The solution was prepared by combining one part rabbit's gallbladder bile to nine parts isoosmotic saline solution and then adding the oleic acid (99% pure, Sigma) to its final concentration of 40 mM. When the bile-oleate- induced hyperemia reached a stable level (Fig. 2C), the 30, 60, and 120 s arterial occlusions were performed, before and after i.v. ADA (250 U/rat).

In Group V rats, the three successive periods of arterial occlusions were performed in fasted gut and in fed gut as described in group IV. In group V, RH responses to the three periods of occlusion were examined in the presence of the bile-oleate solution in the intestinal lumen before and after 8-PT (administered as in Group II).

In Group VI rats, the three successive periods of arterial occlusion were performed in fasted and fed gut experiments as described in group IV. In group VI, RH responses to the three periods of occlusion and release were observed in the presence of intrajejunal bile-oleate before and after DPMX, administered as in Group III.

In Group VII rats, we determined the effects of ADA on bile-oleate-induced intestinal hyperemia. ADA (250 U/rat) was administered as in previous groups. Following ADA injection, 1.5 ml of the bile-oleate solution was instilled into the jejunal lumen. No arterial occlusions were performed in this group. Peak hyperemic flow and conductance values in response to the intrajejunal bile-oleate solution in Group VII rats were compared with pooled control responses from Groups IV, V, and VI, since the individual means for these groups were not different.

In all groups, and nearly all animals, the adenosine test dose (0.5 mg/kg) was injected i.v. before and after either ADA, 8-PT, or DPMX to assess the effectiveness of either adenosine degradation or the antagonism of its receptors. In all experimental groups at least five min were allowed for the ADA, 8-PT, and DPMX to equilibrate with the tissues before the next experimental intervention. Thus, in rat groups I-VI the following events occurred in each experimental protocol: 1. stabilization period after experimental preparation, 2. three RH periods, 3. VBF responses to the adenosine test dose, 4. administration of either ADA, 8-PT or DPMX and stabilization period, 5. repetition of three RH periods, and 6. repetition of adenosine test dose. In rats with fed gut (Groups IV-VI), the luminal bile-oleate solution was administered intrajejunally between event 3 and event 4 listed above and was followed shortly by another repetition of the three RH periods.

All data are presented as mean $\pm$ SE values. Statistical analysis was performed using the two tailed student's test for either paired or group data, with significance inferred at a confidence level less than 5%. Analysis of variance with multiple comparison test was used to assess treatment effects (drug and/or bile oleate administration) for the three arterial occlusions in each experimental group.

RESULTS

Resting mean VBF and arterial pressure values in Group I were 2.8 ± 0.1 V and 114 ± 5 mmHg, respectively. VBF and blood pressure changed maximally within a few s following release from occlusion. *Figure 1* shows the recording from typical 30, 60, and 120 s occlusions. The duration of the control RH responses to release from each period of mesenteric arterial occlusion are shown in *Table 1*. In Group I experiments, i.v. injection of ADA *per se* did not significantly change either VBF or arterial pressure. In seven rats before ADA administration, an adenosine test dose increased VBF by $52 \pm 9\%$ (*e.g., Fig. 2*). This hyperemic response due to adenosine was significantly reduced by

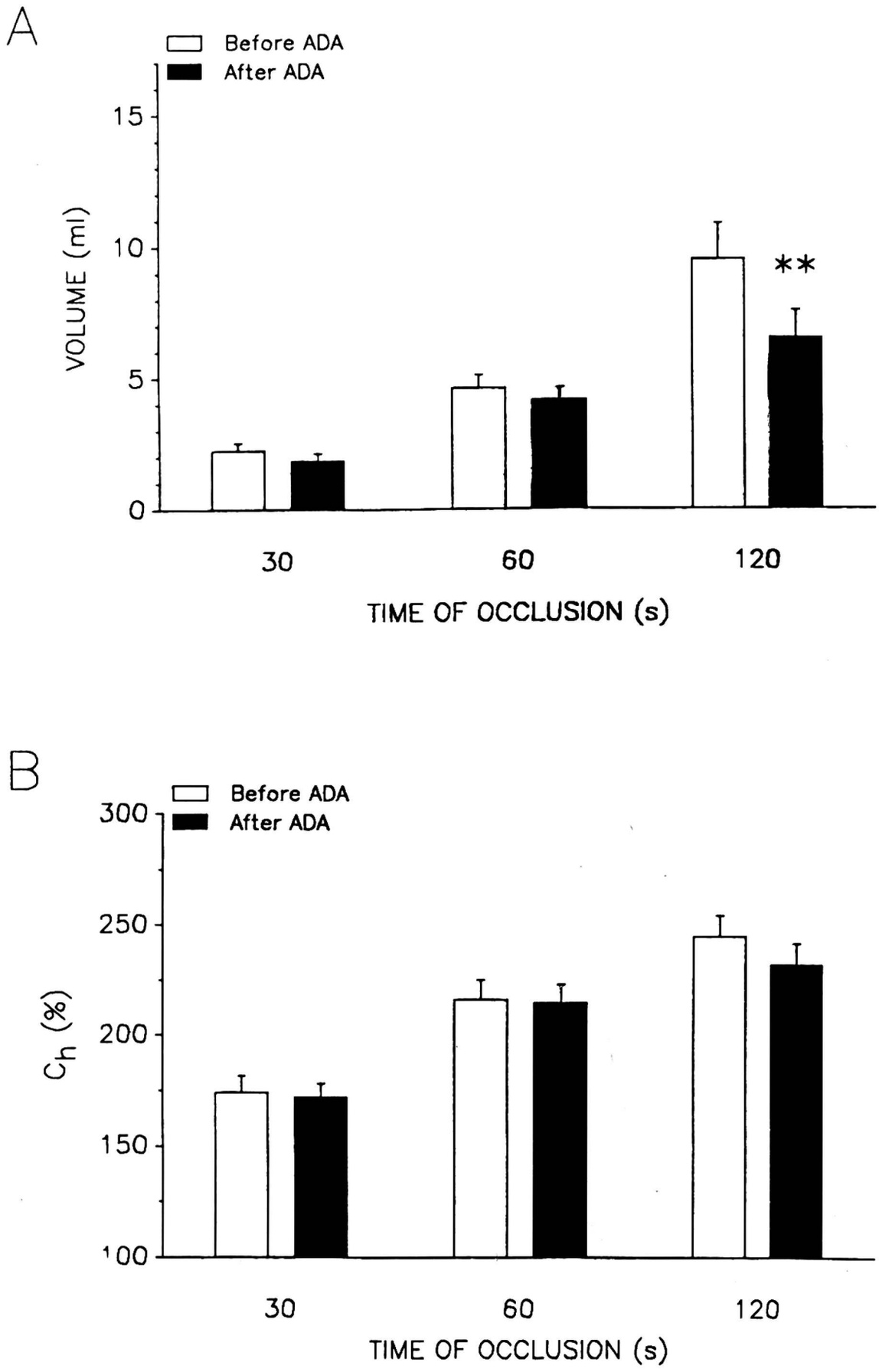

Fig. 3. Effect of ADA on RH parameters in fasted gut. A. ADA significantly attenuated the Volume response only to release from 120 s occlusion. B. ADA did not significantly inhibit C_h after any period of occlusion. ** indicates $p < 0.01$.

89±12% (*Table 2*), with inhibition persisting for more than 50 min after ADA was injected. Changes in Volume and C_h as the period of occlusion was prolonged from 30 to 120 s before and after administering ADA appear in *Figure 3A and B*. Occlusions lasting 30, 60, and 120 s evoked C_h increases of 174±7%, 216±9%, and 245±10%, respectively *(Fig. 3B)*. Proportionately similar increases in Volume and duration were noted as the period of occlusion was lengthened from 30 to 120 s (*Table 1 and Fig. 3A*). Furthermore, the enhancement of RH responses as a function of the length of occlusion appears in all experimental groups. After ADA, a response pattern similar to that of control was obtained. Only the 120 s Volume measurement was reduced after ADA, and no C_h value was significantly attenuated by ADA administration (*Fig. 3A and B*). However, ADA significantly reduced the duration of RH responses to 30, 60, and 120 s of arterial occlusion (*Table 1*). The dose of ADA used in these experiments significantly reduced the hyperemia evoked by exogenous adenosine (*Table 2*).

In Group II, control VBF and arterial pressure values were 2.6±0.1V and 118±2 mmHg, respectively. Control RH responses to 30, 60, and 120 s arterial occlusions were similar to those observed in Group I (*Table 1, Fig. 4*). 8-PT administered intravenously did not influence either resting VBF or arterial pressure. Before 8-PT treatment the adenosine test dose increased VBF by 69±11%. After 8-PT treatment there was a 65±5% reduction in the hyperemic response to the exogenous adenosine test dose (*Table 2*). Following 8-PT receptor blockade significant reductions occurred in Volume values after each occlusion period and significant decreases occurred in C_h after the 30 and 120 s occlusion periods (*Fig. 4*). Duration values following occlusions of 60 and 120 s were significantly reduced after 8-PT (*Table 1*).

In Group III, resting VBF and arterial pressure values were 2.5±0.2V and 110±4 mmHg, respectively. Control RH responses to 30, 60, and 120 s arterial occlusions were similar to those observed in Groups I and II (*Table 1, Fig. 5A and B*). DPMX administered i.v. did not significantly alter resting VBF or arterial pressure. The DPMX treatment significantly reduced the hyperemic response to exogenously administered adenosine by 45±8% (*Table 2*). Increasing the dose of DPMX to 5 mg/kg failed to enhance the inhibitory effect on exogenous adenosine in five animals (*Table 2*). After DPMX treatment there was no significant reduction in the Volume or duration of RH following any occlusion period (*Table 1; Fig. 5A*). However, a significant reduction in C_h was noted for all three arterial occlusions following DPMX treatment (*Fig. 5B*).

In Group IV, resting VBF and arterial pressure values were 2.9±0.1V and 118±6.0 mmHg, respectively. Placement of 1.5 ml normal saline into the jejunal lumen (fasted gut) did not alter either VBF or pressure. The control RH response in this group had characteristics similar to previous groups (*Table 1 and Fig. 6A and B*). Placement of the bile-oleate solution in the gut (fed gut)

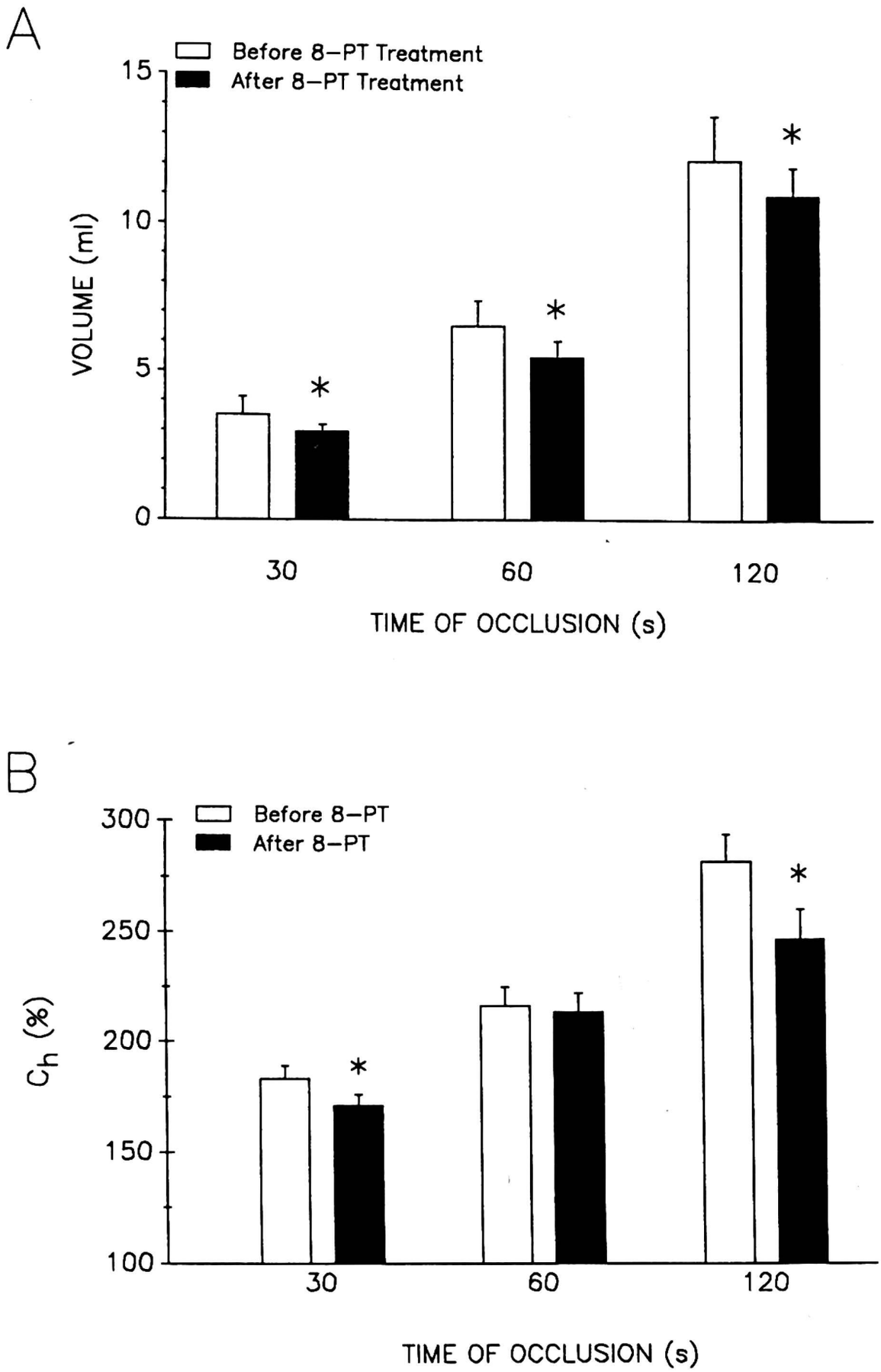

Fig. 4. Effect of 8-PT on RH parameters in fasted gut. A. 8-PT significantly attenuated RH Volumes after all three periods of occlusion, although the magnitude of inhibitions was small. B. 8-PT significantly inhibited C_h values for 30 and 120 s occlusions. * indicates $p < 0.05$.

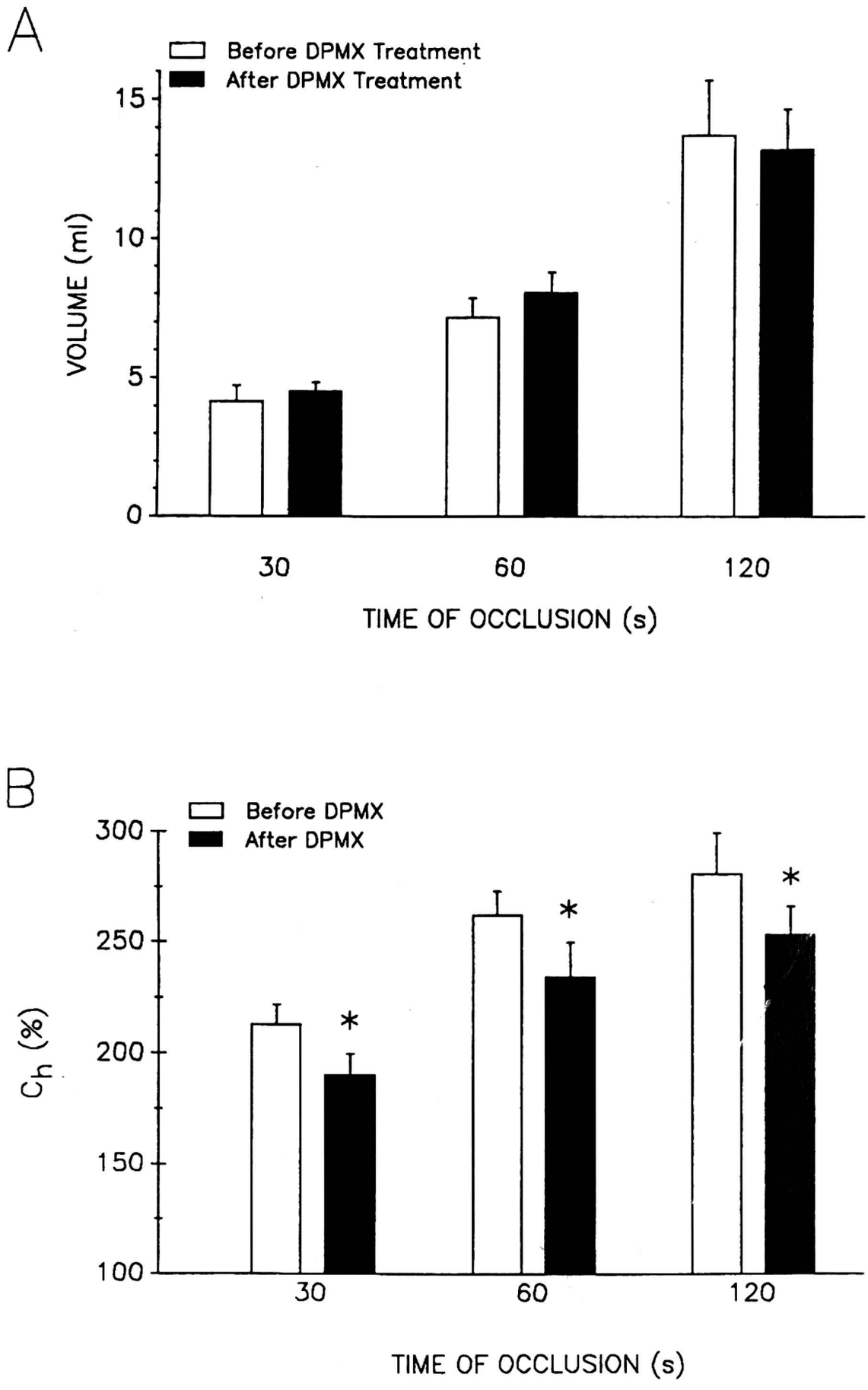

Fig. 5. Effect of DPMX on RH parameters in fasted gut. A. DPMX did not attenuate Volume responses to arterial occlusions. B. DPMX elicited a small but significant reduction in C_h after each period of occlusion. * indicates $p < 0.05$.

significantly increased VBF within a few min, and the hyperemia was sustained throughout the period that lipid was in the lumen (*Fig. 2C*). After the postprandial hyperemia was stable, the three subsequent periods of arterial occlusion were performed prior to and post administration of ADA. Under these conditions the hyperemic response induced by exogenous adenosine was significantly reduced by 78±10% following treatment with ADA (*Table 2*). C_h responses following 30, 60, or 120 s of occlusion in fed gut were similar to those observed in fasted gut (compare *Figs. 6B and 3B*). However, bile-oleate significantly prolonged the duration of RH post 30, 60 and 120 s occlusions compared with durations observed in the fasted gut (*Table 1*). Intralumenal micellar lipid also increased Volume values following each period of occlusion (compare *Figs. 6A and 3A*). Treatment with ADA prompted significant inhibition of duration (*Table 1*), Volume (*Fig. 6A*) and C_h (*Fig. 6B*) values in fed gut after release from all periods of occlusion. Intrajejunal micellar lipid induced increases in duration and Volume parameters were essentially reversed by ADA to RH values observed in the fasted gut. ADA reduced fed gut C_h values below fasted gut C_h levels (prior to ADA).

In Group V resting VBF and arterial pressure values were 2.7±0.1 V and 130±44 mmHg, respectively. Placement of the saline solution into the jejunal lumen did not significantly alter either parameter, and fasted gut RH duration, Volume, and C_h values resembled fasted gut values observed in previous groups (*Table 1, Figs. 3-5*). Instillation of bile-oleate in the lumen of the jejunum significantly increased duration, Volume, and C_h responses to occlusions of 30, 60, and 120 s. Following treatment with 8-PT, postocclusive Volume, C_h, and duration values in fed gut were significantly reduced in comparison with RH responses prior to 8-PT in fed gut (*Table 1, Fig. 7A and B*). 8-PT reduced the exogenous adenosine effect in fed gut by 64±9%, approximating the 8-PT effect in fasted Group II rats (*Table 2*).

In Group VI, resting VBF and arterial pressure values were 2.6±0.2 V and 113±mmHg, respectively. Fested gut duration responses in this group were similar to those observed in prior groups, that is the longer the period of occlusion, the greater the duration of RH (*Table 1*). After intrajejunal instillation of bile-oleate, the three arterial occlusions were performed prior to and following the administration of DPMX. In the presence of DPMX, the fed gut hyperemia induced by exogenous adenosine was reduced by 42±8%, corresponding to that which was observed in the fasted gut (*Table 2*). Following DPMX in fed gut, Volume was not significantly altered for any period of occlusion — a result similar to the observation with DPMX in fasted gut (*Fig. 8A*). But similar to results in the fasted gut, there was a small but consistent reduction in C_h following DPMX in the fed gut (*Fig. 8B*).

In Group VII adenosine deaminase pretreatment significantly reduced the exogenous adenosine induced vasodilation both before and after bile-oleate

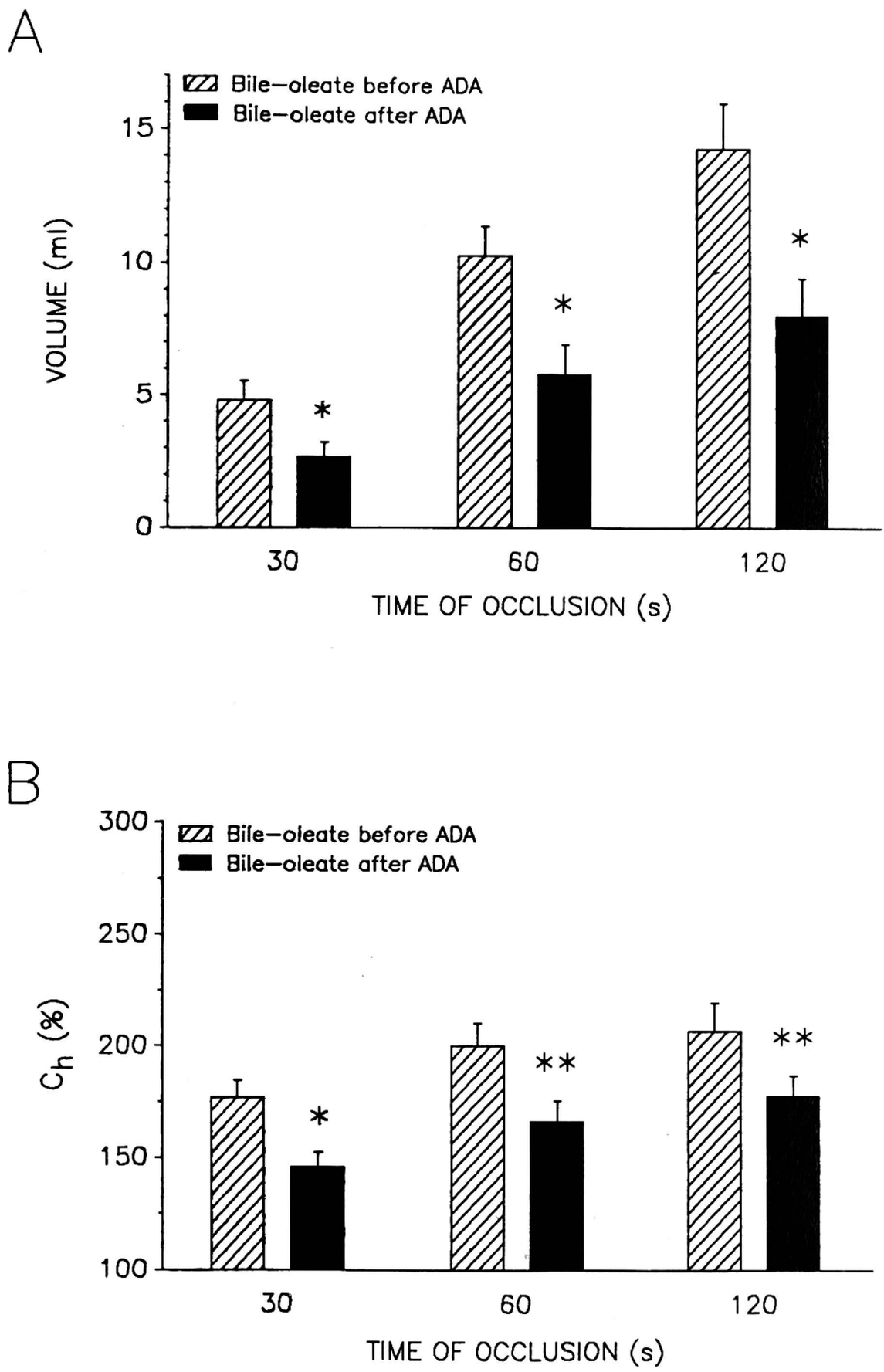

Fig. 6. Effect of ADA on RH parameters in fed gut. A. ADA significantly attenuated Volume responses to each period of occlusion. B. ADA significantly attenuated C_h responses to each period of occlusion. For both Valume and C_h ADA was a more effective inhibitor in fed gut than in fasted gut (compare results with *Fig. 3*). *indicates $p < 0.05$, **indicates $p < 0.01$.

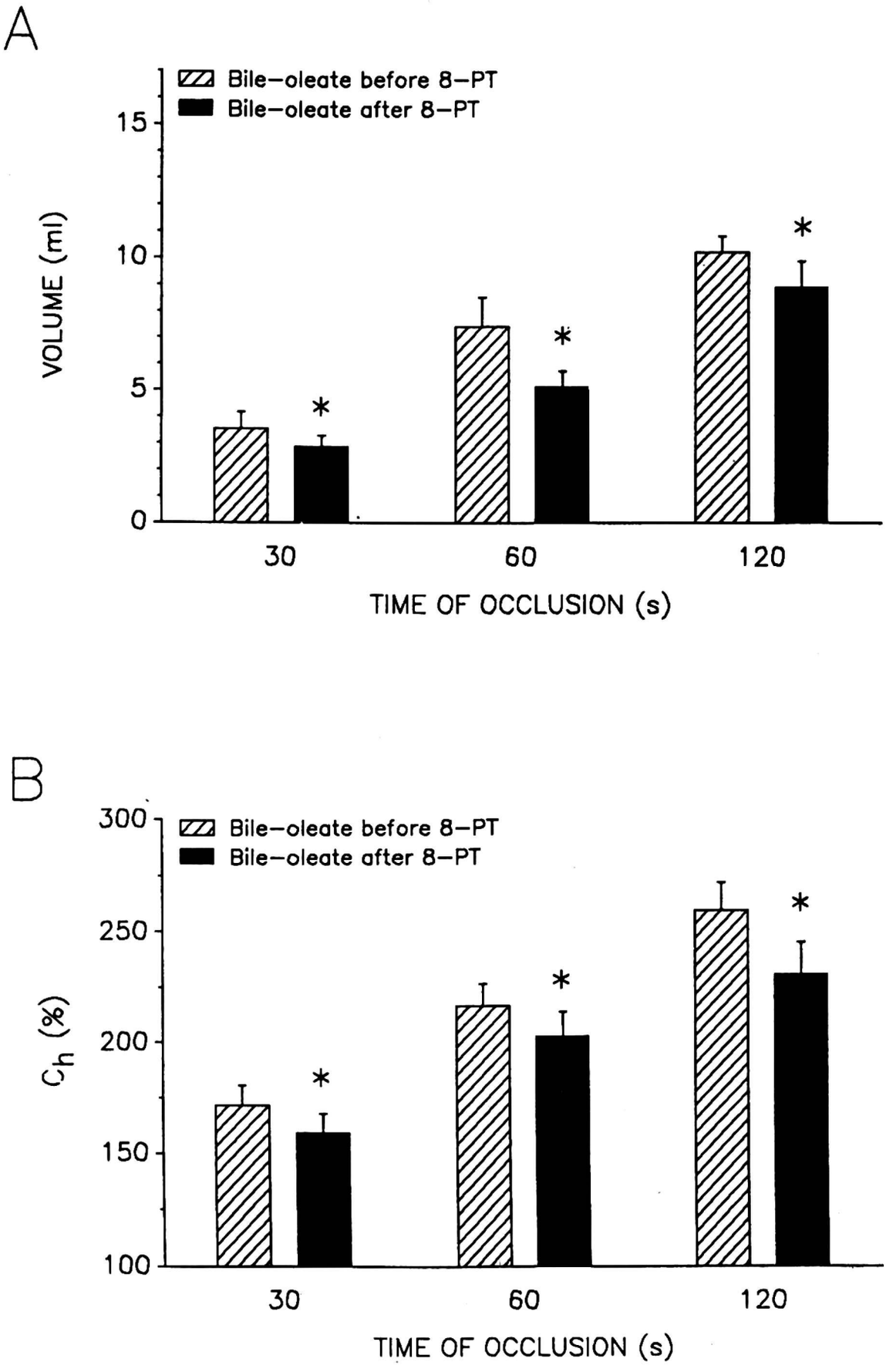

Fig. 7. Effect of 8-PT on RH parameters in fed gut. A. 8-PT significantly reduced RH Volume responses after each period of occlusion. B. 8-PT reduced C_h responses to each arterial occlusion. Attenuations in fed gut after 8-PT were more consistent than in fasted gut (compare results with *Fig. 4*). * indicates $p < 0.05$

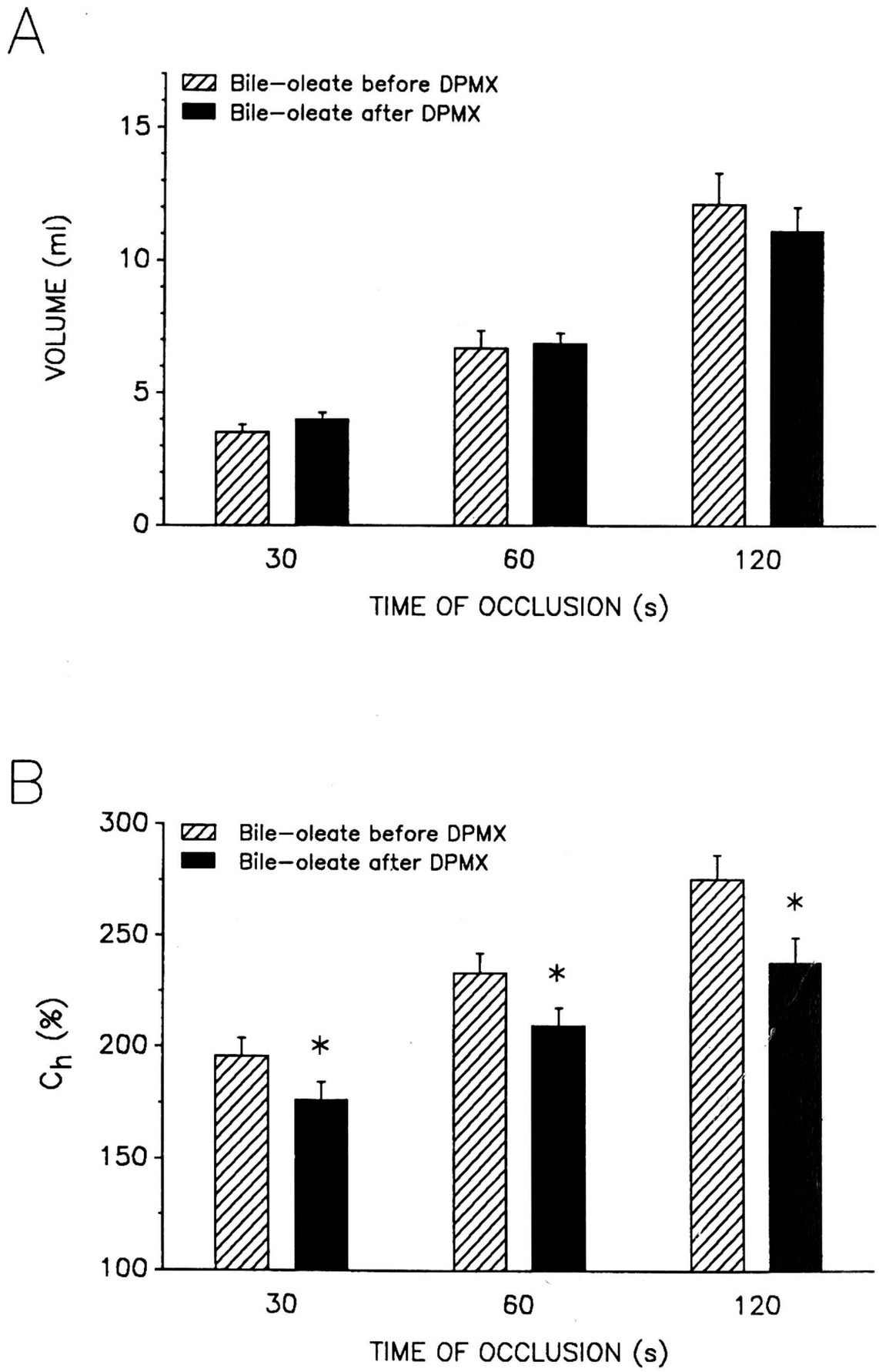

Fig. 8. Effect of DPMX on RH parameters in fed gut. A. DPMX failed to significantly attenuate RH Volume responses. B. DPMX significantly attenuated C_h responses after each period of occlusion. Results in fed gut approximated findings in fasted gut with DPMX (see *Fig. 5*). *indicates $p < 0.05$.

administration, resembling observations in Group I and IV rats (*Table 2*). However, this effective blockade of exogenous adenosine was not matched by ADA against the bile-oleate induced hyperemia as measured by the percentage increase in either VBF or conductance over baseline values compared with pooled control responses obtained in Groups IV, V, and VI (*Table 3*). However, there was a significant reduction in the time needed to reach the peak VBF value for the bile-oleate induced hyperemia noted in the ADA pretreatment group (*Table 3*).

Table 3. Effect of ADA on bile-oleate induced hyperemia in rat gut.

Group	n	Increase in peak VBF (% fasted gut value)	Increase in conductance (% fasted gut value)	Time to peak of hyperemia (s)
Control (pooled Groups IV, V, VI)	21	34.8±3.3	40.3±3.9	4.5±0.5
ADA pretreated (Group VII)	10	35.8±3.8	34.6±6.2	2.2±0.2*

Values are mean ± SE; n = number of animals. * indicates a significant ($p < 0.05$) difference in time to peak hyperemia induced by bile-oleate administration between control (untreated) and ADA (pretreated) groups.

DISCUSSION

Our findings suggest that adenosine plays a modulatory role in RH of the rat mesenteric circulation. In fasted gut RH was diminished but not abolished by doses of 8-PT and ADA that also attenuated the intestinal VBF response to exogenously administered adenosine by 65-89%. The inhibitory effects of 8-PT and ADA on RH parameters were more marked in rat gut which had been administered micellar lipid than in fasted gut. The extent of the hyperemia, following release from occlusion of the anterior mesenteric artery, was prolonged in the fed gut compared with the fasted condition, and was also prolonged by the length of the occlusion period. The three agents employed to antagonize endogenously released adenosine differed in their effectiveness, as gauged by different RH parameters.

Introduction of a bile-oleate solution into the intestinal lumen initiated a stable postprandial hyperemia, which has been reported previously in the rat and the dog (14-16, 25). The duration of RH and the inhibition of RH parameters were significantly enhanced in fed gut compared with findings in

fasted gut in most rat groups. Thus, for example, in Group IV the durations of RH in fasted gut following 30, 60, and 120 s of occlusion were 51 ± 5, 83 ± 7 and 115 ± 15 s, respectively, compared with 85 ± 13, 113 ± 13, and 169 ± 14 s, respectively, after the same occlusions in fed gut (*Table 1*). Similarly, significant attenuation of RH parameters by the three inhibitory agents following the different periods of occlusion occurred 57% more frequently in fed gut groups than in fasted rats. The effectiveness of ADA especially was more evident in the gut containing micellar lipid than in the fasted gut (compare *Figs. 3 and 6*).

These findings are consistent with the hypothesis that adenosine is one of the physiological modulators of RH and that release of adenosine is enhanced by the increased demand for oxygen imposed by the presence of absorbable nutrients in the intestinal lumen (1-3, 7, 14-16). From the results of our experiments, adenosine appears to play a minimal role in regulating RH in the fasted gut. However, adenosine becomes a more prominent modulator of the additional RH component, which is elicited by absorbable nutrient in the gut lumen, presumeably because of an enhanced mucosal metabolism.

The length of occlusion preceding RH affected the magnitude of the hyperemia in either fasted or fed gut and was evident by any parameter which we employed. Thus, for example, in Group I rats, there were 1.5-to fourfold increases in the duration, Volume, and C_h, values between the 30 s occlusion and the 120 s occlusion periods. This enhancement of RH responses by longer periods of occlusion suggests that prolonging the ischemic period augments release of dilator metabolites.

The third variable influencing our results was the pharmacological mechanism underlying antagonism of adenosine induced vasodilation. 8-PT and ADA were more effective antagonists against exogenously administered adenosine than was the selective A_2 receptor antagonist DPMX. Similarly, 8-PT and ADA were more effective inhibitors of RH parameters than was DPMX, and this disparity in effectiveness was apparent in both the empty and the fed gut. This difference between the effectiveness of adenosine inhibitors suggests that the intestinal vasculature has a greater density of A_1 receptors. It is also possible that DPMX was less able than the other two agents to penetrate the membranes separating the blood from arteriolar smooth muscle receptors.

Neither of the three RH parameters (duration, Volume, or C_h) emerged as the most reliable estimator of the postocclusive vasodilation in both the fed and fasted gut. Furthermore, there was no invariable correlation between responses with one parameter versus those with another parameter. Accordingly, DPMX failed to significantly attenuate the Volume responses after any of the three occlusions in either fed or fasted gut, whereas DPMX significantly attenuated C_h after all three occlusions in both fed and fasted gut.

Finally, in a separate examination of the role of endogenous adenosine in the bile-oleate induced hyperemia in the rat small bowel (*Table 3*), we found

that ADA failed to significantly alter the increased conductance and VBF of the fed gut. These findings suggest that additional endogenous dilator mediators play a role in postprandial hyperemia *per se* and are consistent with other reports identifying such mediators (1, 3-5, 9, 12, 20, 25).

Results of the present investigation support the concept of a multifactorial regulation of intestinal blood flow in RH. In other recent studies we have found that neuroregulators temper the magnitude of the hyperemia which follows release from occlusion of the anterior mesenteric artery in the empty rat gut (21, 23). Sympathetic nerve stimulation and depletion of primary sensory dilator neuropeptides reduced the magnitude of RH in fasted gut, and the inhibition was more pronounced than that in our present findings with adenosine antagonists in the empty intestine. In the gut containing absorbable nutrients and a more active metabolism, adenosine would be expected to contribute much more to the hyperemia. Our finding that pharmacological antagonism of adenosine was more apparent in the fed gut supports the foregoing expectation. Our findings also support the view that adenosine is one of several interacting modulators of RH.

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