

Mechanisms of action of hypocapnic alkalosis on limb blood vessels in man and dog

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KONTOS, HERMES A., DAVID W. RICHARDSON, A. JARRELL RAPER, ZUBAIR-UL-HASSAN, AND JOHN L. PATTERSON, JR. *Mechanisms of action of hypocapnic alkalosis on limb blood vessels in man and dog.* *Am. J. Physiol.* 223(6): 1296-1307. 1972.—The mechanisms of action of hypocapnic alkalosis on limb blood vessels were investigated in man and in the anesthetized dog. In man hypocapnic alkalosis was produced by voluntary hyperventilation or by intra-arterial administration of tromethamine, and in the dog it was produced by intra-arterial administration of tromethamine or sodium hydroxide. Hypocapnic alkalosis had a biphasic effect on limb blood vessels consisting of an initial vasodilator effect and a subsequent vasoconstrictor action. The initial vasodilation was primarily related to release of histamine, since it was accompanied by increased blood histamine concentration and since it was inhibited or reversed by antihistamines. The subsequent vasoconstriction probably represented the direct action of hypocapnic alkalosis on vascular smooth muscle. Other mechanisms, such as changes in the activity of vasomotor nerves, release of catecholamines, and changes in calcium ion activity were found to play no significant role in the response of limb blood vessels to hypocapnic alkalosis.

antihistamines; atropine; calcium ion; histamine release; local regulation of blood flow; phenoxybenzamine; skeletal muscle blood vessels; tromethamine; vascular smooth muscle

THE EFFECT OF HYPOCAPNIC ALKALOSIS on limb blood vessels has been studied extensively in both man and in anesthetized animals with variable results (1-7, 9, 10, 12-15, 17, 21, 23, 27, 33). Vasodilator and vasoconstrictor responses have been reported, but neither the mechanisms of these responses nor the reasons for the divergent results have been fully identified.

This is a systematic study of the effects of hypocapnic alkalosis on limb blood vessels in man and in the anesthetized dog. Our interest in the effects of hypocapnic alkalosis on limb blood vessels originated with study of the mechanism of the vasodilator response to voluntary hyperventilation in the human forearm. This response was of particular interest because of the consistency with which it has been obtained by previous investigators, and because it was unexpected, in view of the fact that hypercapnic acidosis is vasodilatory in the human forearm (19). Investigation was later extended to include the effects of hypocapnic alkalosis produced by intra-arterial adminis-

tration of alkaline agents in both man and in the anesthetized dog.

METHODS

Experiments were performed in 64 young healthy volunteers and in 43 dogs anesthetized with pentobarbital (25-30 mg/kg). Subjects were studied while recumbent on a table in an air-conditioned laboratory. In some subjects more than one experiment was performed. When this was the case, studies were carried out at least 2 weeks apart, and the same type of experiment was not performed more than once on the same subject.

Forearm blood flow was measured by venous occlusion plethysmography using water-filled plethysmographs whose temperature was maintained thermostatically at 34 C, except in five experiments in which blood flow was measured with the forearm above heart level, when Whitney-type mercury-in-rubber strain gauges were employed. Arterial blood pressure was measured with a Statham strain gauge connected to a Teflon catheter placed into the brachial artery at the elbow. Forearm vascular resistance was calculated as the ratio of mean arterial blood pressure divided by forearm blood flow. Deep forearm venous blood was obtained from a catheter introduced in a retrograde direction into a deep forearm vein so that its tip lay into the portion of the forearm enclosed by the plethysmograph. Blood gas tensions and pH were measured by oxygen and CO₂ electrodes (29) and by a Metrohm pH meter, respectively, at 37 C shortly after collection. Venous blood gas tension and pH were corrected to the temperature of the venous blood at the time of collection as described previously (18). In most experiments the expired air CO₂ concentration was measured with an infrared CO₂ analyzer. The subjects breathed through a mouthpiece and low-resistance valve so that recording of the expired air CO₂ concentration could be carried out. In some experiments subjects breathed 6% CO₂ administered from a Douglas bag. The CO₂ analyzer was calibrated before each experiment with at least three concentrations of carbon dioxide which straddled the expected range of CO₂ concentration in the expired air during the experiment.

Blood histamine concentrations were determined by the fluorometric method of Shore, Burkhalter, and Cohn (30), using an Aminco-Bowman spectrofluorimeter. Blood for

histamine determination was collected in dry syringes and immediately transferred into tubes containing ice-cold perchloric acid. Hypocapnic alkalosis was produced by two methods: voluntary hyperventilation and intra-arterial infusion of 0.3 M solution of tromethamine (tris(hydroxymethyl)aminomethane). The osmolality of tromethamine solution was 325–340 mOsm/liter. Tromethamine titrated to pH 7.3–7.4 with 0.15 N HCl had no effect on perfusion pressure in the perfused dog's hindlimb when infused intra-arterially at 4.9 ml/min. Subjects were instructed to hyperventilate vigorously on command. No effort was made to regulate the rate and depth of hyperventilation until expired CO_2 concentration reached a value approximately half its value during room air breathing. At this time subjects were instructed to adjust their breathing so that a constant level of end-expiratory CO_2 concentration was maintained. Tromethamine was administered into the brachial artery by means of a constant-infusion pump. Its infusion was always preceded by administration of 0.9% sodium chloride solution given at the same rate as tromethamine.

The effect of a number of blocking agents on the response to hypocapnic alkalosis was studied. The dose and mode of administration of each of these were as follows: 1) phenoxybenzamine was given into the brachial artery in a total dose of 8 mg. For this purpose phenoxybenzamine was diluted into 10–15 ml of 0.9% sodium chloride solution and administered slowly over a period of 5 min. Following the administration of phenoxybenzamine, the vasoconstrictor response which normally occurs during the post-Valsalva maneuver overshoot in arterial blood pressure was either abolished or reversed. In addition, in response to intravenous infusion of 10 $\mu\text{g}/\text{min}$ of epinephrine there was a sustained, approximately threefold, increase in blood flow in the phenoxybenzamine-treated forearm. 2) Propranolol was given intra-arterially in a dose of 0.5 mg. This dose was diluted into 10 ml of 0.9% sodium chloride solution and given slowly in the same manner as phenoxybenzamine. In these experiments following the administration of propranolol and phenoxybenzamine, the infusion of 10 $\mu\text{g}/\text{min}$ of epinephrine intravenously produced no change in forearm blood flow. 3) Atropine, 1 mg, was diluted into 10 ml of isotonic saline and given slowly over a period of 5 min into the brachial artery. The effectiveness of the blockade was demonstrated by showing that the vasodilator response to 20 μg of acetylcholine given intra-arterially was abolished. 4) The antihistamines promethazine, 12.5 mg, or diphenhydramine, 25 mg, were each diluted in 10 ml of 0.9% sodium chloride solution and given slowly into the brachial artery. The effectiveness of the blockade was checked by intra-arterial injection of 0.1 μg of histamine.

Two preparations were employed in the dog: the entire hindlimb was perfused through the external iliac artery, or the gracilis muscle was perfused through its arterial supply. Arterial blood was obtained from cannulation of one carotid artery, passed through an occlusive pulsatile pump and used to perfuse either the entire limb or the gracilis muscle. The volume output of the pump was adjusted so that perfusion pressure was approximately equal to the systemic pressure of the animal and then kept constant throughout the experiment. Hypocapnic alkalosis was produced by

intra-arterial infusion of either tromethamine or 0.15 N sodium hydroxide solution. All infusions were given upstream from the pump so that flow was not affected. The infusion of alkaline solutions was always compared to infusion of isotonic saline given at the same rate.

The possibility that changes in calcium ion concentration resulting from alterations in blood pH might be involved in the production of the vasodilator response to hypocapnic alkalosis was explored in 12 dogs. In six dogs the perfused hindlimb was utilized. Hypocalcemia was produced by intra-arterial infusion of sodium EDTA. Sodium EDTA was dissolved in 0.9% sodium chloride solution in concentrations ranging from 25 to 50 mg/ml. It was infused at a rate of 2.5 ml/min. Calcium concentration in femoral venous blood was measured by EDTA titration (22). In six other dogs the gracilis muscle was used and the effects of hypocalcemia, produced by intra-arterial infusion of sodium EDTA, were compared to those produced by intra-arterial infusion of sodium hydroxide. Venous blood from the gracilis muscle was collected and calcium ion activity was determined by a means of a flow-through Orion Research calcium electrode (20, 25).

RESULTS

Figure 1 shows a typical experiment illustrating the time course of mean arterial blood pressure, blood flow, and

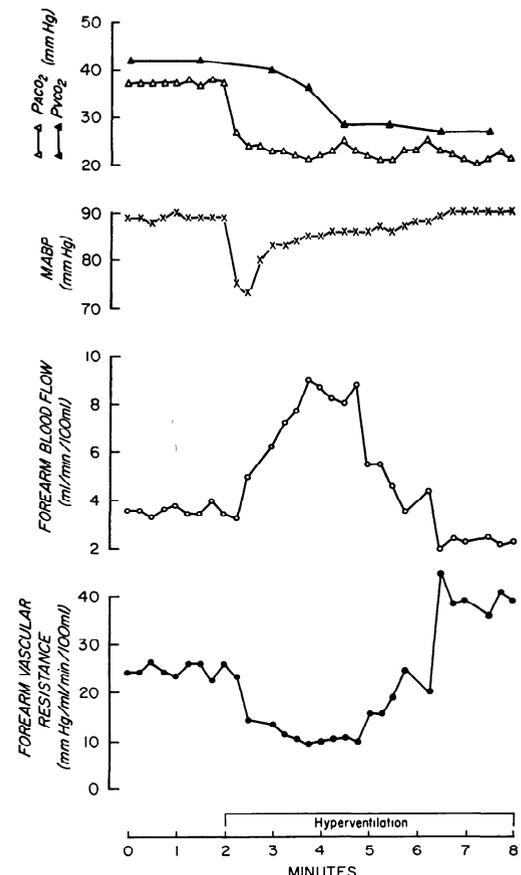


FIG. 1. Typical experiment illustrating time course of alveolar (PaCO_2) and deep forearm venous blood (PvCO_2) CO_2 tension, of mean arterial blood pressure (MABP), of forearm blood flow, and vascular resistance in a normal subject during voluntary hyperventilation.

vascular resistance in the intact forearm in relation to the changes in alveolar and deep forearm venous blood PCO_2 during voluntary hyperventilation. Alveolar PCO_2 decreased very rapidly and reached its final value within the 1st min of hyperventilation. In contrast, venous blood PCO_2 decreased much more slowly and reached a final steady-state value approximately 4 min after the onset of hyperventilation. Mean arterial blood pressure declined for a short period of time at the beginning of hyperventilation and then returned to its control value. The changes in forearm blood flow and vascular resistance were biphasic. Forearm blood flow increased and forearm vascular resistance decreased shortly after the onset of hyperventilation. In most experiments, the onset of the increase in flow and decrease in forearm vascular resistance occurred within 30 sec following the onset of hyperventilation, and in several experiments a definite change was evident within 15 sec. The decrease in forearm vascular resistance, in the face of an unchanged or decreased arterial blood pressure, indicates that the decreased resistance must have been due to decreased vascular tone. The decrease in forearm vascular resistance was transient and was replaced after 4 min by an increase in resistance, despite continuing hyperventilation and hypocapnia. This delayed increase in vascular resistance was seen in 80% of the experiments. It must be emphasized, however, that this pertains to experiments limited in duration to 6 min. It is uncertain whether or not it would have occurred in all experiments had hyperventilation continued for a longer period of time.

The effect of hyperventilation on the circulation of the intact forearm was studied in a total of 41 experiments. In these experiments, decrease in arterial blood PCO_2 from 38.2 ± 0.3 to 19.4 ± 0.6 mm Hg was associated with an increase in forearm blood flow from 3.2 ± 0.2 to a maximum of 7.5 ± 0.5 ml/min per 100 ml and with a decrease in forearm vascular resistance from 34.3 ± 2.3 to a minimum of 14.4 ± 1.0 mm Hg/ml per minute per 100 ml. The decrease in vascular resistance lasted an average of 213.5 ± 14.6 sec. Because the period of observation was limited to 6 min, the average duration of the decreased resistance given above underestimates the true value, because in six experiments the forearm vascular resistance was still below the control value at the end of 6 min. In only one subject did the forearm vascular resistance fail to decrease during hyperventilation.

In six experiments serial sampling of forearm venous blood was carried out in the course of voluntary hyperventilation. Forearm blood flow in these experiments increased from 4.2 ± 0.3 to a maximum of 9.5 ± 0.9 ml/min per 100 ml, and forearm vascular resistance decreased from 21.6 ± 1.3 to a minimum of 9.6 ± 0.9 mm Hg/ml per minute per 100 ml. At the time of occurrence of the minimum forearm vascular resistance, the arterial blood PCO_2 had decreased substantially from 37.5 ± 0.8 to 19.3 ± 1.3 mm Hg; in contrast, venous blood PCO_2 at this time had decreased only slightly from 41.5 ± 0.6 to 38.0 ± 0.7 mm Hg. At the end of 6 min of hyperventilation, forearm blood flow had declined to 3.2 ± 0.3 ml/min per 100 ml, a value significantly lower than that seen in the control period. Forearm vascular resistance at the end of hyperventilation increased significantly to 28.2 ± 2.5 mm Hg/ml per minute per 100 ml. At this time arterial blood PCO_2 was 16.8 ± 0.9 mm Hg, a

value not significantly different from what was seen at the time of the maximum effect of hyperventilation. Venous blood PCO_2 , however, had decreased further to 28.0 ± 1.6 mm Hg.

In 10 subjects the response to hyperventilation was studied following alpha-adrenergic receptor blockade of one forearm, whereas the opposite forearm served as control. The increase in forearm blood flow was not significantly different in the two forearms (Fig. 2). The decrease in forearm vascular resistance was, however, significantly less pronounced in the forearm with alpha-adrenergic receptor blockade than in the intact forearm, and the duration of the decrease in vascular resistance was significantly shorter in the forearm with alpha-adrenergic receptor blockade. Because of the phenoxybenzamine-induced marked decrease in resting vascular resistance, interpretation of these experiments was complicated. It was considered desirable to study the effect of hyperventilation in the forearm with alpha-adrenergic receptor blockade before and after beta-adrenergic receptor blockade. We expected that this would increase significantly the sensitivity of the experiment in detecting the possible effect of increased concentration of circulating catecholamines, because in the forearm with alpha-adrenergic blockade intravenous administration of epinephrine causes a threefold increase in forearm blood flow, whereas in the forearm with alpha- and beta-adrenergic receptor blockade no change in flow occurs in response to the same stimulus. It was undesirable to administer phenoxybenzamine into both brachial arteries because of

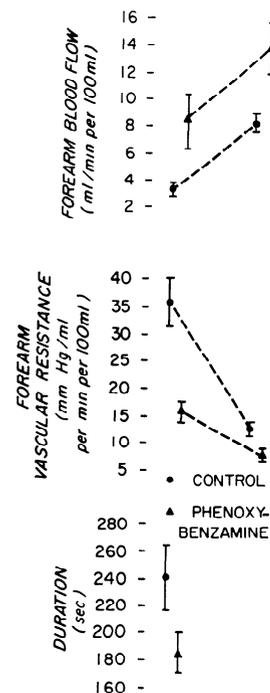


FIG. 2. Comparison of vasodilator response to hyperventilation in intact (circles) and in phenoxybenzamine-treated (triangles) forearms. Values on left were obtained during quiet breathing; values on right were obtained during maximum effect of hyperventilation. Means \pm SE from 10 experiments are shown. Arterial blood PCO_2 decreased from 38.2 ± 0.6 to 20.9 ± 0.8 mm Hg during hyperventilation. Increase in forearm blood flow was not significantly different in the 2 forearms, but decrease in forearm vascular resistance and duration of vasodilation were significantly less pronounced in phenoxybenzamine-treated forearm.

the enhanced probability of hypotension from escape of the drug into the general circulation; for this reason the experiments had to be performed in the same forearm. Before this could be carried out, it was essential to study the reproducibility of the response to hyperventilation in the forearm with alpha-adrenergic receptor blockade. In seven subjects the increase in forearm blood flow, the decrease in forearm vascular resistance, and the duration of the period of vasodilation in response to two periods of hyperventilation separated by a 20-min rest period were not significantly different. In seven subjects decrease in arterial blood P_{CO_2} from 38.3 ± 0.7 to 21.1 ± 0.9 mm Hg produced an increase in blood flow in the phenoxybenzamine-treated forearm from 6.9 ± 0.5 to 11.9 ± 1.8 and a decrease in forearm vascular resistance from 13.6 ± 1.1 to 7.9 ± 1.6 mm Hg/ml per minute per 100 ml. Following the intra-arterial infusion of propranolol into the forearm with alpha-adrenergic blockade, decrease in arterial blood P_{CO_2} from 38.8 ± 1.0 to 20.0 ± 0.8 mm Hg caused an increase in forearm blood flow from 6.2 ± 1.6 to 11.6 ± 1.0 ml/min per 100 ml and a decrease in forearm vascular resistance from 15.0 ± 1.1 to 7.5 ± 0.7 mm Hg/ml per minute per 100 ml. The duration of the vasodilation was 167.1 ± 28.4 sec following phenoxybenzamine and 172.9 ± 24.6 sec following phenoxybenzamine and propranolol. There was no significant difference in either the magnitude or duration of the vasodilation produced by hyperventilation before and after the administration of propranolol.

A total of 55 experiments were carried out in which the effect of hyperventilation on the circulation of the forearm with alpha-adrenergic or with a combination of alpha- and beta-adrenergic receptor blockade was studied. In response to a decrease in arterial blood P_{CO_2} from 37.8 ± 0.3 to 19.8 ± 0.4 mm Hg, forearm blood flow increased from 6.7 ± 0.5 to a maximum of 13.7 ± 0.8 ml/min per 100 ml and forearm vascular resistance decreased from 16.5 ± 0.8

to a minimum of 7.9 ± 0.5 mm Hg/ml per minute per 100 ml. The duration of the decrease in vascular resistance averaged 184.9 ± 8.3 sec. In comparison to what was seen in the intact forearm, the decrease in forearm vascular resistance and the duration of the vasodilation in the forearm with alpha- or alpha- and beta-adrenergic receptor blockade were significantly less pronounced.

It was reported that intravenous administration of atropine inhibited the increase in cardiac output seen during voluntary hyperventilation in man (32). For this reason we studied the effect of intra-arterial atropine on the forearm vascular response to voluntary hyperventilation. Two series of experiments were carried out. In 10 subjects, atropine was administered into one brachial artery, while the other forearm served as control. Decrease in arterial blood P_{CO_2} from 37.5 ± 1.3 to 21.4 ± 2.8 mm Hg induced by hyperventilation was associated with an increase in blood flow to the intact, untreated forearm from 2.9 ± 0.4 to a maximum of 5.1 ± 0.5 ml/min per 100 ml, and with an increase from 3.1 ± 0.6 to a maximum of 5.7 ± 0.5 ml/min per 100 ml in the atropine-treated forearm. Forearm vascular resistance decreased from 33.2 ± 3.6 to a minimum of 17.4 ± 1.6 mm Hg/ml per minute per 100 ml in the intact forearm and from 34.7 ± 6.3 to 15.7 ± 2.0 in the atropine-treated forearm. The duration of vasodilation was 240.0 ± 51.1 and 222.0 ± 39.8 sec in the intact and in the atropine-treated forearms, respectively. There was no significant difference in the response of forearm blood flow, nor in forearm vascular resistance, nor in the duration of vasodilation in the two forearms. In a second series of five subjects the effect of hyperventilation was observed in the forearm with alpha-adrenergic receptor blockade before and after the administration of atropine. The results were similar to those of the first series in that atropine had no significant effect on the response to hyperventilation.

As noted above the decrease in forearm vascular resist-

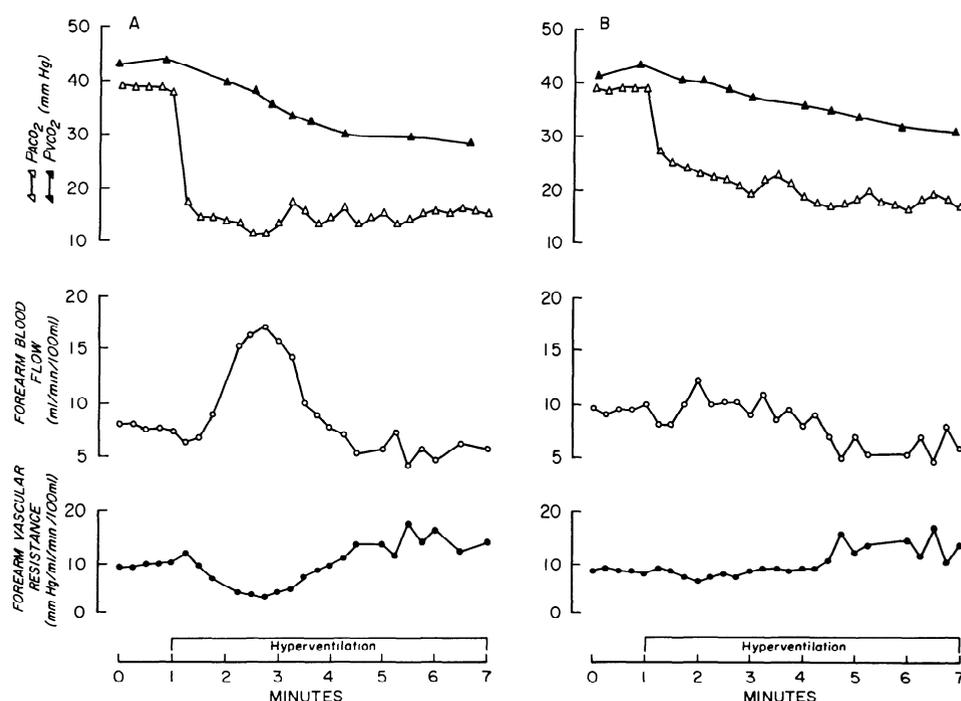


FIG. 3. Comparison of effect of rapid (A) and slower (B) reduction in CO_2 tension by hyperventilation on forearm blood flow and resistance.

ance and the duration of the vasodilation in response to voluntary hyperventilation were less pronounced in the forearm following alpha-adrenergic receptor blockade. This could have been due to one or more of the following: 1) withdrawal of vasoconstrictor tone might be important in the response; 2) it could be merely due to the production of vasodilation by the administration of phenoxybenzamine, or 3) to another effect of phenoxybenzamine unrelated to its alpha-adrenergic receptor blocking effect, such as its antihistamine effect (33). To examine the effect of changes in resting vascular resistance on the response to hyperventilation, in five experiments in which forearm was pre-treated with phenoxybenzamine and propranolol the effect of voluntary hyperventilation was studied first with the forearm at heart level when blood flow was high and then with the forearm elevated above heart level so that flow decreased to levels which were normally seen in the intact forearm. In these experiments forearm blood flow was measured with Whitney mercury-in-rubber strain gauges. In the elevated forearm reduction in the blood P_{CO_2} from 36.4 ± 1.4 to 19.5 ± 1.9 mm Hg was associated with an increase in forearm blood flow from 2.5 ± 0.4 to a maximum of 7.7 ± 1.4 ml/min per 100 ml and with a decrease of forearm vascular resistance from 37.2 ± 4.6 to a minimum of 12.3 ± 2.4 mm Hg/ml per minute per 100 ml. The duration of the vasodilation averaged 245.0 ± 24.4 sec. In the forearm at heart level reduction in arterial blood P_{CO_2} from 36.8 ± 1.3 to a 18.8 ± 0.8 mm Hg caused an increase in forearm blood flow from 6.3 ± 1.5 to 15.3 ± 4.3 ml/min per 100 ml and a decrease in forearm vascular resistance from 18.3 ± 3.0 to 8.4 ± 2.3 mm Hg/ml per minute per 100 ml. The duration of the vasodilation was 149.0 ± 18.2 sec. The increase in forearm blood flow was not significantly different in the two experiments; however, the decrease in forearm vascular resistance was significantly less pronounced and the duration of vasodilation was significantly shorter in the forearm at heart level than in the elevated forearm. It should be noted that, although one might reasonably have expected an enhanced fall in resistance in response to a given stimulus when the resting vascular resistance was high (26), there is no a priori reason why the duration of the vasodilation would be longer when vascular resistance was high. It was previously reported that the response to hypercapnia in the human forearm under circumstances similar to those of the present experiments is biphasic consisting of an initial vasoconstrictor response and a delayed vasodilator response (19). Similar findings were reported by others (8) in the dog. Evidence was presented that the initial vasoconstrictor response in the human forearm was dependent on an increased arterial blood P_{CO_2} , whereas the delayed vasodilator response was related to increase in P_{CO_2} at some unspecified site within the tissues of the forearm (19). It was furthermore shown that an increase in flow accelerated the development of the delayed vasodilator response to hypercapnia and diminished the magnitude and duration of the initial vasoconstrictor response. The biphasic nature of the response to hyperventilation and the fact that the decrease in vascular resistance followed closely the decline in alveolar (and arterial) P_{CO_2} , whereas the delayed increase in vascular resistance was associated with the decline in venous blood P_{CO_2} , and presumably depletion of

tissues of CO_2 and decline in tissue P_{CO_2} suggested that similar factors might be operative in the case of hypocapnia. To examine this possibility, two series of experiments were carried out. In the first series of experiments, the effect of rapid reduction in arterial blood P_{CO_2} as carried out in the usual manner was studied in the phenoxybenzamine- and propranolol-treated forearm and compared to that seen following more gradual reduction in blood P_{CO_2} induced by hyperventilation which was less intense. It was reasoned that the more gradual hypocapnia might allow more extensive depletion of the tissues of CO_2 , whereas the arterial blood P_{CO_2} was maintained at a relatively high level. As illustrated in Fig. 3, in an experiment typical of a total of eight such experiments, the response to fast hyperventilation was the one usually seen, whereas in response to more gradual reduction in P_{CO_2} there was no initial decrease in forearm vascular resistance and no increase in forearm blood flow. However, the delayed increase in vascular resistance persisted. This occurred despite the fact that the final levels of arterial and venous blood P_{CO_2} were similar in the two types of hyperventilation. In the second series of experiments, five subjects hyperventilated at constant depth and rate, and periodically 6% CO_2 was substituted for room air as the inspired gas mixture. A typical experiment is shown in Fig. 4. It is seen that with each reduction in alveolar P_{CO_2} there was an increase in forearm blood flow and a decrease in forearm vascular resistance. When CO_2 was introduced into the inspired gas, forearm blood flow de-

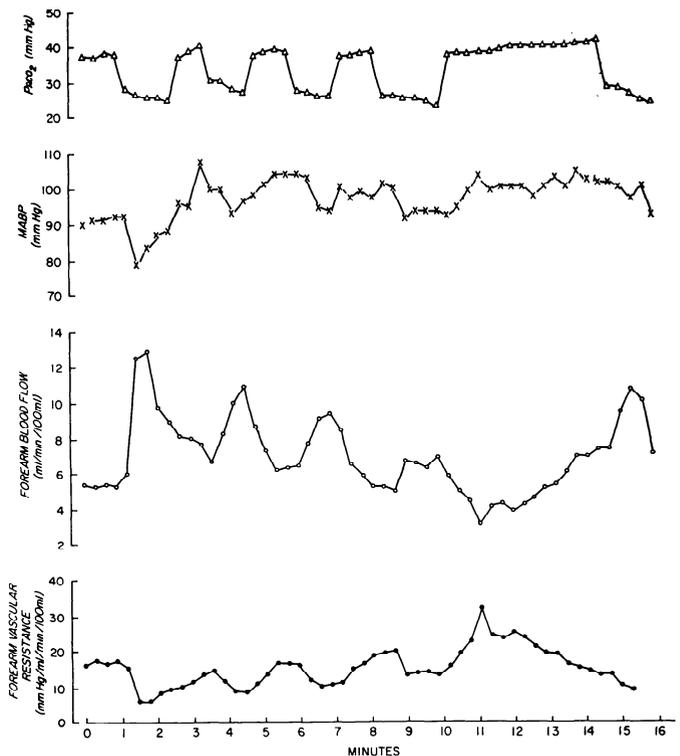


FIG. 4. Effect of changes in CO_2 tension during sustained hyperventilation on forearm blood flow and vascular resistance. Hyperventilation began at the end of 1st min and was maintained at same level thereafter. Increases in CO_2 tension were achieved by substituting 6% CO_2 in air for room air as inspired gas mixture. Deep forearm venous blood P_{CO_2} were as follows: at time 0, 42.3 mm Hg; at time 3, 37.1 mm Hg; at time 7, 32 mm Hg; at time 11, 28.7 mm Hg.

clined and forearm vascular resistance increased. As the venous blood PCO₂ declined to lower levels, rise in alveolar PCO₂ to its control value was associated with flow values less than control and vascular resistance higher than its control value. Note that when CO₂ inhalation was continued for 2–3 min the vasodilator response to a subsequent period of hypocapnia was restored.

In 10 experiments the effect of hyperventilation was studied following the intra-arterial administration of promethazine into one brachial artery. The opposite forearm served as control. Promethazine caused a transient vasodilation which subsided within a few minutes. The response to hyperventilation in the promethazine-treated forearm was modified so that the magnitude and duration of the vasodilation were reduced significantly (Fig. 5). The vasodilator response to hyperventilation was, however, not abolished. It is worth noting, however, that it was diminished to the same extent as the response to intra-arterial histamine. It is likely that antihistamines have other nonspecific vascular effects, besides their antihistamine action, which might have accounted for the modification of the response to hyperventilation. Of interest is the fact that promethazine

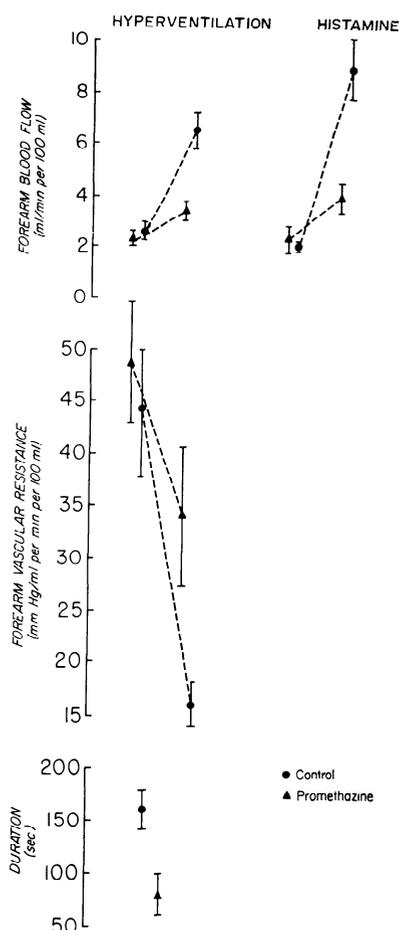


FIG. 5. Comparison of response to hyperventilation (left) and to intra-arterial histamine (right) in untreated control forearm (circles) and in promethazine-treated forearm (triangles). Values on left in each panel represent control values, whereas values on right were obtained either during hyperventilation or during administration of histamine. Increase in forearm blood flow, decrease in forearm vascular resistance, and duration of vasodilation were significantly less pronounced in promethazine-treated forearm (*t* test).

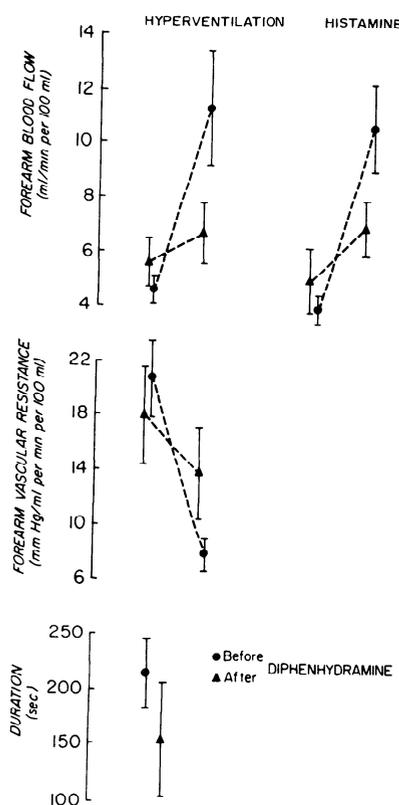


FIG. 6. Comparison of circulatory response to hyperventilation (left) or to intra-arterial histamine (right) before (circles) or after (triangles) diphenhydramine. Values on left in each panel represent control values; values on right were obtained during hyperventilation or during intra-arterial administration of histamine. Increase in forearm blood flow, decrease in forearm vascular resistance and duration of vasodilation were significantly less pronounced following diphenhydramine than before.

and other antihistamines might inhibit the reuptake of norepinephrine by the sympathetic nerve endings (11, 16). It was thought possible that if activation of vasoconstrictor nerve fibers occurred during hyperventilation, inhibition of the reuptake of norepinephrine and consequent increased vasoconstriction might have accounted for the diminished vasodilator response to hyperventilation following promethazine administration. For this reason, the response to hyperventilation was studied in five subjects in the forearm treated with phenoxybenzamine. The intra-arterial administration of 0.1–0.5 μ g of norepinephrine, after such treatment, produced vasodilation. As shown in Fig. 6, the intra-arterial administration of the antihistamine diphenhydramine in these subjects inhibited the vasodilator response to voluntary hyperventilation.

Table 1 summarizes the results of six experiments in which arterial and forearm venous blood were obtained before and during voluntary hyperventilation for blood histamine determinations. There were significant increases in both arterial and forearm venous blood histamine concentration. However, the arteriovenous difference of histamine across the forearm did not change significantly. The increase in arterial blood histamine concentrations suggested release of histamine from sources other than the forearm. It is possible that the increase in venous blood histamine was secondary to the increased arterial blood histamine, and the possibility

of additional release of histamine locally in the forearm could not be ascertained from these experiments. In another series of seven experiments, only arterial blood histamine was sampled in the course of voluntary hyperventilation to examine more precisely the time course of the changes in blood histamine concentration. There was a significant increase in arterial blood histamine concentration in the first sample 1.5 min following the onset of hyperventilation which persisted at approximately the same level for the entire duration of hyperventilation (Fig. 7).

TABLE 1. Effect of voluntary hyperventilation histamine concentration in arterial and deep forearm venous blood and on arteriovenous difference of histamine across the forearm

	Control	Hyperventilation	
		1 min	3 min
A	0.085 ± 0.014	0.055 ± 0.015*	0.104 ± 0.015*
V	0.090 ± 0.010	0.104 ± 0.014*	0.103 ± 0.016*
A-V	-0.005 ± 0.006	-0.004 ± 0.005	+0.001 ± 0.005
FBF	3.7 ± 0.6	8.8 ± 1.8*	4.9 ± 1.1*
PaCO ₂	38.6 ± 0.9	22.7 ± 1.9*	20.2 ± 1.6*

All values are means ± SE obtained from six experiments. A: arterial blood histamine concentration in µg/ml; V: histamine concentration in deep forearm venous blood in µg/ml; A-V: arteriovenous blood histamine concentration difference across the forearm in µg/ml; FBF: forearm blood flow in ml/min per 100 ml; PaCO₂: end-tidal CO₂ tension in mm Hg. * Values whose difference from control is significantly different from zero.

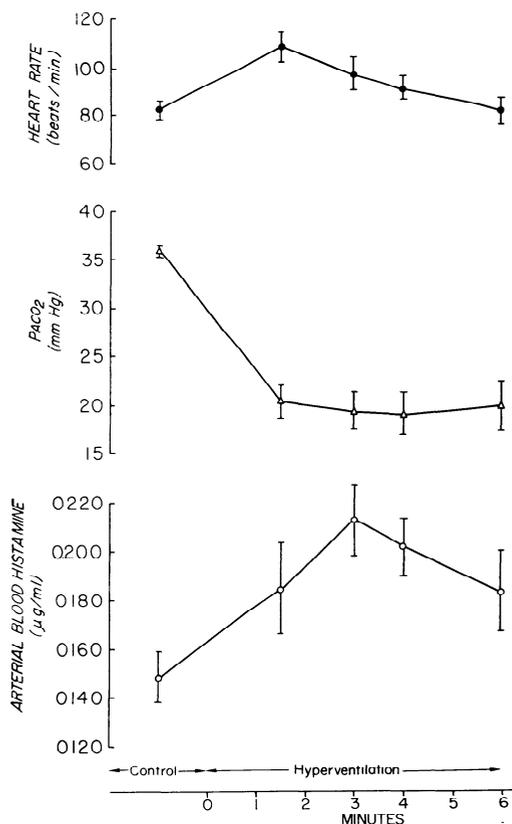


FIG. 7. Time course of heart rate, alveolar PCO₂, and arterial blood histamine concentration during voluntary hyperventilation. Means ± SE are shown.

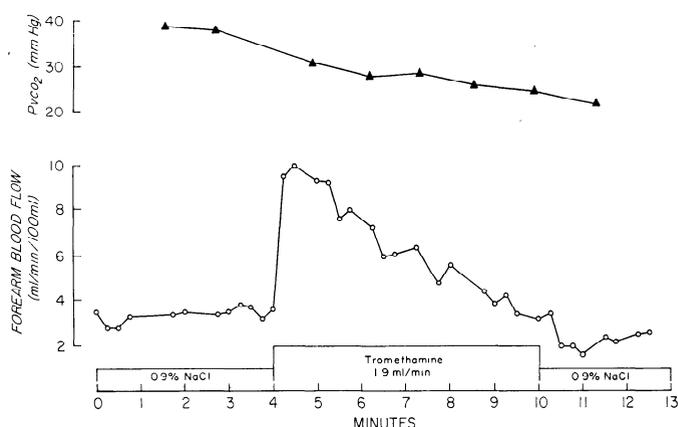


FIG. 8. Time course of deep forearm venous blood PCO₂ and forearm blood flow during intra-arterial infusion of tromethamine.

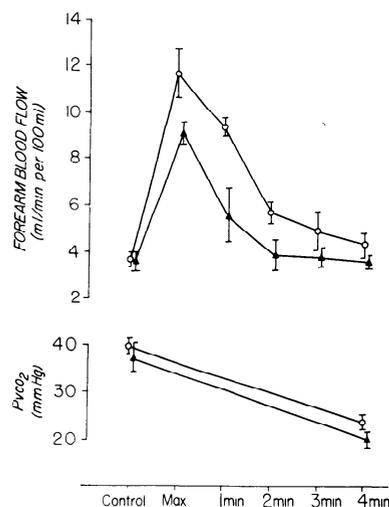


FIG. 9. Circulatory response to tromethamine infusion in intact human forearm before (open circles) and after (triangles) intra-arterial administration of promethazine. Values are means ± SE from 5 experiments. Control value, maximum value during infusion, and values at 1, 2, 3, and 4 min during infusion of tromethamine are shown. Increases in forearm blood flow were less pronounced at each time during infusion following promethazine than before.

In three experiments we attempted to determine whether or not the forearm vasodilation during the hyperventilation was entirely due to release of a vasodilator agent, such as histamine, elsewhere, or whether or not a local component was also present. In these experiments during voluntary hyperventilation, isotonic sodium chloride solution equilibrated with 100% CO₂ was infused into the brachial artery to maintain forearm venous blood PCO₂ at a high level. This attempt was unsuccessful, as a substantial decrease in venous blood PCO₂ occurred in all three experiments.

The effects of localized hypocapnic alkalosis of the human forearm, without change in blood PCO₂ or pH elsewhere in the body, were studied during the intra-arterial infusion of tromethamine. As illustrated in Fig. 8, the time course of forearm blood flow during tromethamine infusion was similar to that seen during voluntary hyperventilation. There was an initial increase in forearm blood flow which lasted for several minutes and was sometimes replaced by a delayed decrease in forearm blood flow below the control level. The delayed decrease in forearm blood flow and in-

crease in forearm vascular resistance were less consistent during tromethamine infusion than during voluntary hyperventilation.

Tromethamine was infused intra-arterially at rates of 1.9–4.9 ml/min in a total of 36 experiments. In these experiments tromethamine caused an increase in forearm blood flow from 3.8 ± 0.2 to a maximum of 12.9 ± 0.5 ml/min per 100 ml. By the end of the infusion forearm blood flow had decreased to 5.2 ± 0.4 ml/min per 100 ml, a value significantly higher than control. Venous blood P_{CO_2} at the end of the infusion decreased from 38.1 ± 0.7 to 26.4 ± 0.8 mm Hg. A delayed decrease in forearm blood flow below the control level was seen in 18 out of 36 experi-

ments within the 6 min of observation period. In seven experiments the maximum increases in forearm blood flow and the duration of vasodilation produced by two infusions of tromethamine at a rate of 4.9 ml/min separated by a 20- to 30-min rest period were not significantly different. The response to tromethamine was therefore reproducible.

It must be noted, however, that in these experiments sufficient time was allowed for the effects of the first infusion to subside almost completely. In other experiments in which a second infusion followed the first one too closely, before the hypocapnic alkalosis associated with the first infusion subsided, the vasodilation was less pronounced. Figure 9 shows that in five experiments promethazine reduced both the magnitude of the increase in flow and the duration of the hyperemia in response to intra-arterial administration of tromethamine. Table 2 summarizes experiments in eight subjects in which the concentration of histamine in arterial and deep forearm venous blood was determined before and during intra-arterial tromethamine administration and during the intra-arterial infusion of histamine. There was a significant increase in venous blood histamine concentration during tromethamine infusion, whereas arterial histamine concentration at the end of the infusion remained unchanged from the control value. During the intra-arterial infusion of histamine in a dose which produced an increase in forearm blood flow slightly higher than the maximum increase in the flow produced by tromethamine, there was an increase in venous blood histamine concentration comparable to that seen during tromethamine infusion.

The effects of intra-arterial NaOH or tromethamine infusion in the perfused hindlimb or gracilis muscle were similar to those seen in the human forearm with tromethamine infusion. Hypocapnic alkalosis was associated with a reduction in perfusion pressure which was maximal during the 1st min of the infusion and gradually declined over the next several minutes (Fig. 10). A delayed increase in perfusion pressure above its control value was only rarely seen within the period of observation.

Table 3 summarizes the results of nine experiments in

TABLE 2. Effect of intra-arterial tromethamine infusion on histamine concentration in arterial and deep forearm venous blood and on arteriovenous difference of histamine concentration across the human forearm

	Control	Tromethamine Infusion	
		1 min	4 min
<i>I) Tromethamine infusion, 4.9 ml/min</i>			
A	0.092 ± 0.011		0.091 ± 0.010
V	0.098 ± 0.013	$0.111 \pm 0.010^*$	$0.115 \pm 0.012^*$
A-V	-0.005 ± 0.004		$-0.023 \pm 0.004^*$
FBF	3.7 ± 0.3	$10.8 \pm 1.0^*$	$6.0 \pm 0.8^*$
	Control	Histamine	Difference
<i>II) Histamine infusion</i>			
V	0.098 ± 0.009	$0.120 \pm 0.011^*$	0.022 ± 0.010
FBF	3.2 ± 0.3	$17.8 \pm 2.1^*$	$14.5 \pm 1.8^*$

All values are means \pm SE obtained from eight experiments in Part I and from six experiments in Part II. A, V, and A-V refer to the arterial deep forearm venous and arteriovenous differences of blood histamine concentration in $\mu\text{g/ml}$. Rate of histamine infusion was adjusted to give a steady-state flow approximately equal to maximum flow during tromethamine infusion. The latter averaged 14.3 ± 1.0 ml/min per 100 ml. * Values whose difference from control is significantly different from zero.

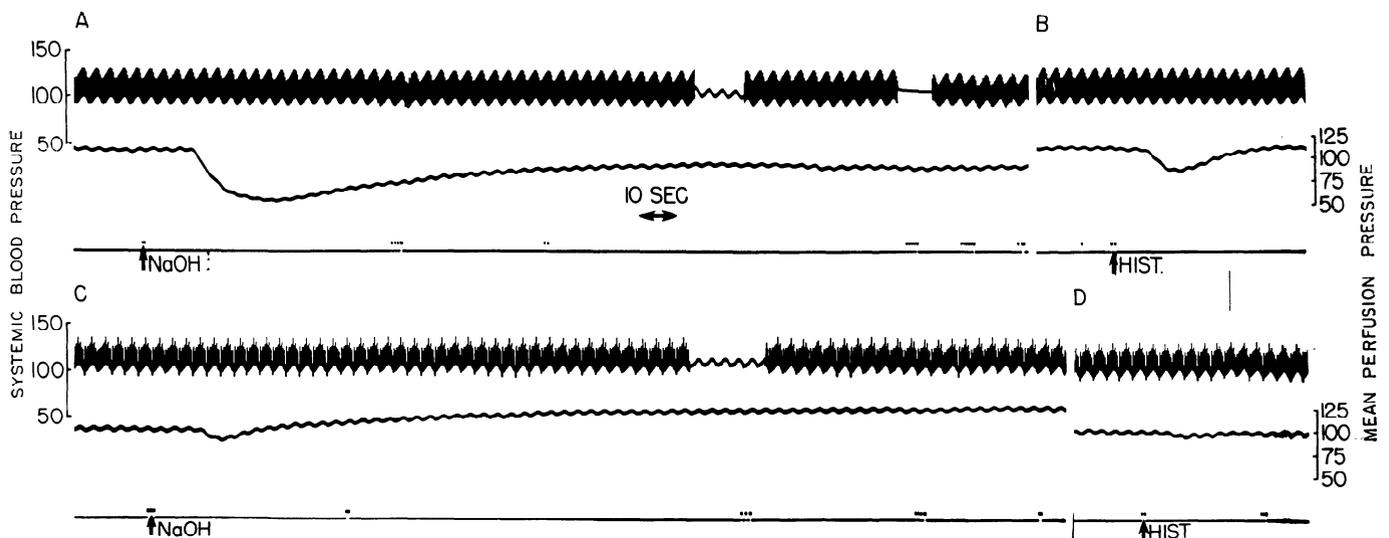


FIG. 10. Reproduction of original record illustrating effect of antihistamine promethazine on vasodilator response to intra-arterial administration of NaOH in perfused gracilis muscle of the dog. A: effect

of NaOH infusion before promethazine. B: effect of histamine injection (0.25 μg) before promethazine. C: NaOH infusion after promethazine. D: histamine injection after promethazine.

TABLE 3. Effect of antihistamine promethazine on vasodilator response to intra-arterial infusion of tromethamine in perfused hindlimb of dogs

	Control	Tromethamine Infusion		
		Maximum	1 min	4 min
PP				
A	132.0±8.0	68.2±2.9	73.4±3.4	97.9±5.7
B	134.9±5.1	98.1±6.9*	112.9±8.7*	139.3±6.0*
SP				
A	127.7±8.1	128.2±8.3	129.1±8.5	131.4±9.2
B	123.3±9.3	123.3±9.4	123.4±9.3	123.7±9.4
P _{aCO₂}				
A	36.4±1.7			35.3±1.7
B	35.6±1.7			35.0±1.6
P _{vCO₂}				
A	41.9±1.5			26.7±1.2
B	41.7±2.0			26.3±2.5
PaH				
A	7.41±0.03			7.42±0.03
B	7.40±0.03			7.40±0.03
PvH				
A	7.37±0.07			7.55±0.03
B	7.35±0.03			7.53±0.04

All values are means ± SE obtained from nine experiments. PP: mean perfusion pressure in mm Hg; SP: mean systemic arterial pressure in mm Hg. P_{vCO₂} and PvH were measured in femoral venous blood. A and B: before and after promethazine, respectively. Promethazine was given intra-arterially in a dose of 5 mg/kg body wt. * Values whose difference from their counterpart in A is significantly different from zero.

which the effect of promethazine on the response to intra-arterial administration to tromethamine was studied in the perfused hindlimb. The maximum decrease in perfusion pressure produced by tromethamine was significantly reduced following the administration of promethazine, and the duration of the vasodilation was significantly abbreviated so that 4 min following the onset of the infusion, perfusion pressure had risen above its control value in seven of the nine experiments. Table 4 shows the results of five experiments in which the effect of promethazine on the response to intra-arterial NaOH infusion was studied in the isolated gracilis muscle. The effect of promethazine was similar to that seen in the whole limb, except that it was much more pronounced in magnitude. Following promethazine administration there was no significant change in perfusion pressure during the initial phase of the infusion of NaOH, and shortly thereafter perfusion pressure rose significantly above its control value and was maintained at the high level throughout the rest of the infusion (Table 4 and Fig. 10).

Table 5 summarizes six experiments in which arterial and venous blood from the gracilis muscle were obtained during intra-arterial administration of sodium hydroxide. There was an increase in venous blood histamine concentration and an increase in venoarterial histamine concentration difference across the gracilis muscle during the infusion of sodium hydroxide. Similarly, when histamine was infused intra-arterially in a dose which was sufficient to reproduce the vasodilation produced by sodium hydroxide, there was an increase in venous blood histamine concentration comparable to that seen during sodium hydroxide infusion.

Table 6 summarizes the effect of hypocalcemia on limb

TABLE 4. Effect of antihistamine promethazine on vasodilator responses to intra-arterial administration of NaOH and histamine in perfused gracilis muscle of the dog

	Control	NaOH Infusion		
		Maximum	1 min	4 min
I) NaOH infusion 1.2 ml/min				
PP				
A	151.8±10.5	91.4±12.8	110.0±14.9	129.4±13.7
B	158.8±15.4	154.4±17.9*	176.6±10.0*	192.2±11.6*
SP				
A	138.2±10.8	138.4±10.4	137.6±10.2	138.6±10.1
B	136.2±9.9	136.2±9.9	136.2±9.9	136.0±9.8
P _{vCO₂}				
A	35.5±1.0			11.5±0.5
B	35.2±1.1			12.6±1.2
PvH				
A	7.41±0.01			7.80±0.04
B	7.40±0.01			7.80±0.03
		Control	Histamine	
II) Histamine injection, 0.25 µg				
PP				
A	153.8±11.2		117.8±7.7	
B	161.2±13.6		145.0±13.2*	

All values are means ± SE obtained from five experiments. Abbreviations and units as in Table 3. Statistical analysis as in Table 3. Promethazine was given intra-arterially in a dose of 5 mg/kg body wt.

TABLE 5. Effect of hypocapnic alkalosis produced by intra-arterial infusion of sodium hydroxide on blood histamine concentrations in perfused gracilis muscle of the dog

	Control	Sodium Hydroxide Infusion		
		1 min	4 min	
I) Sodium hydroxide infusion, 1.2 ml/min				
A	0.099 ± 0.015		0.102 ± 0.015	
V	0.096 ± 0.017	0.107 ± 0.019*	0.112 ± 0.018*	
A-V	0.003 ± 0.003		-0.010 ± 0.002*	
		Control	Histamine	
II) Histamine infusion, 1.2 µg/min				
V	0.099 ± 0.025	0.125 ± 0.025*		

All values are means ± SE obtained from six experiments. A, V, and A-V refer to arterial, venous, and arteriovenous difference of blood histamine concentration in µg/ml. * Values whose difference from control is significantly different from zero.

perfusion pressure. The infusion of Ca-EDTA had no effect on perfusion pressure, demonstrating that EDTA has no inherent vasoactivity. Hypocalcemia produced vasodilation, but a substantial reduction in calcium concentration was required to produce large decreases in perfusion pressure. The same was true in the gracilis muscle where substantial reductions in calcium ion activity could be produced with only minor changes in perfusion pressure (Table 7). In contrast, in the gracilis muscle the infusion of sodium hydroxide produced substantial decreases in perfusion pressure with little change in calcium ion activity of the venous blood.

TABLE 6. *Effect of intra-arterial infusion of Na₂-EDTA and Ca-EDTA on perfusion pressure and venous blood, calcium concentration in the dog's hindlimb*

Infusion Rate, mg/min	Perfusion Pressure, mm Hg	Venous Blood Calcium Conc'n, mM
<i>I) Na₂-EDTA infusion</i>		
0	134.2 ± 5.2	3.9 ± 0.08
25	121.7 ± 4.6	2.2 ± 0.16
37.5	88.7 ± 4.3	0.9 ± 0.08
50	54.7 ± 2.2	0
<i>II) Ca-EDTA infusion</i>		
0	136.5 ± 6.9	
50	137.5 ± 7.0	

Values are means ± SE obtained from six experiments in *part I* and from four experiments in *part II*.

TABLE 7. *Effect of intra-arterial infusion of Na₂-EDTA and of NaOH on perfusion pressure and on venous blood calcium ion activity in perfused gracilis muscle of the dog*

Infusion Rate, ml/min	Perfusion Pressure, mm Hg	Calcium Ion Activity, mM
<i>I) Na₂-EDTA infusion, 15 mg/ml</i>		
0	126 ± 7.1	1.25 ± 0.03
0.2	123 ± 8.6	1.21 ± 0.02
0.4	120 ± 6.1	1.11 ± 0.03
1.0	109 ± 9.1	0.81 ± 0.12
1.9	74 ± 5.9	0.38 ± 0.14
<i>II) NaOH infusion, 0.15 N</i>		
0	114 ± 8.2	1.28 ± 0.03
0.5	78 ± 7.7	1.27 ± 0.03
1.2	57 ± 5.2	1.24 ± 0.02

All values are means ± SE obtained from six experiments.

DISCUSSION

Hypocapnic alkalosis has a biphasic effect on limb blood vessels, consisting of an initial decrease in resistance followed by subsequent increase in resistance, which is less constant but becomes much more regular following the administration of antihistamines. The increase in blood histamine concentration and the reversal of the initial vasodilation by the administration of antihistamines suggest that this response is related to release of histamine. The subsequent increase in resistance is probably due to vasoconstriction, which most likely represents the direct effect of hypocapnic alkalosis on vascular smooth muscle. Because of the close similarity between the responses to hypocapnic alkalosis produced by intra-arterial administration of alkaline agents and that produced by voluntary hyperventilation in man, we suggest that both responses are basically governed by the same mechanisms. Since the amount of alkali infused was too small to produce a change in Pco₂ or pH elsewhere in the body and there was no change in arterial blood histamine concentration, despite such increase in venous blood concentration, it is clear that the release of histamine in this instance must have been local. In the case of voluntary hyperventilation, the increase in arterial blood histamine suggests release of histamine from tissues other than those of the limb under study. Although it is likely, in view of the results of the intra-arterial infusion of alkaline agents, that additional histamine was released from the limb tissues

during hyperventilation, this could not be proved beyond question because of the well-known difficulty in interpreting results based on arteriovenous difference determinations in the face of an unsteady state due to changing blood flow during the period of hyperventilation (34).

Since the response to alkali infusion in canine muscle was the same as in the whole limb and the same as that seen in the human forearm in response to hyperventilation, the present findings support earlier views (9, 27) that the vasodilation in response to hypocapnic alkalosis occurs primarily in skeletal muscle. The possibility that the response was influenced by opposite changes in skin vessels cannot, however, be excluded. Most studies of the effect of hypocapnic alkalosis, in response to hyperventilation or in response to intra-arterial infusion of alkaline agents, on skin vessels have found vasoconstriction. Deal and Green (9) found that in the dog hypocapnic alkalosis, induced by intra-arterial infusion of alkali, produces vasoconstriction. The effect of voluntary hyperventilation on blood flow in forearm skin is somewhat controversial. Indirect evidence based on venous blood oxygen saturation studies suggests vasoconstriction (27), whereas estimates of flow based on thermoelectric measurements show variable changes (15). In the hand, voluntary hyperventilation produces vasoconstriction (5, 15, 27).

Although the present studies do not identify with finality the origin of the histamine released during hypocapnic alkalosis, it is reasonable to suggest that it originates in mast cells. All the strong histamine releasing agents are alkaline and a large number of organic amines are known to release histamine (28). Furthermore, a change in blood pH markedly influences the histamine releasing capability of a variety of substances (24), although change in pH by itself in *in vitro* experiments does not release histamine (24). It would appear, therefore, that the most reasonable explanation is that hypocapnic alkalosis enhances the histamine releasing capability of a natural ingredient of the blood or tissues, which in turn induces release of histamine from mast cells.

Our results provide an excellent explanation for the contradictory results obtained by other investigators who studied the effect of hypocapnic alkalosis on limb blood vessels. One would reasonably expect, on the basis of the present findings, that those investigators who chose to study steady-state effects of hypocapnic alkalosis found mainly an increase or no change in vascular resistance. The likelihood of obtaining a decrease in resistance is enhanced by studying the earlier effects of hypocapnic alkalosis. Furthermore, the initial vasodilator response to hypocapnic alkalosis is strongly influenced by the prevailing conditions, such as the level of blood flow, the initial blood Pco₂ or pH, and the rapidity with which hypocapnic alkalosis is produced. These characteristics probably contributed to the divergence of results obtained by various investigators.

It is clear that the vascular response to hypocapnic alkalosis is a complicated reaction. It is pertinent to ask, therefore, whether or not other mechanisms, unrelated to release of histamine or direct effects on vascular smooth muscle, play a role in its production. The fact that the response to intra-arterial infusion of alkaline agents is substantially the same as that produced by voluntary hyperventilation suggests that neurogenic mechanisms or humoral effects are at

best of secondary importance. This was largely confirmed by the use of various pharmacological agents which showed that neither activation of cholinergic nerve fibers nor inhibition of vasoconstrictor fibers was involved in the production of the response to hyperventilation. Our results in this respect agree with those of previous investigators (4, 10, 27). Similarly, changes in serum calcium ion activity do not contribute substantially to the vasodilator response to hypocapnic alkalosis. Our findings showed that activation of beta receptors by increased blood concentration of catecholamines was not a factor in the production of the vasodilator response to hyperventilation. This finding is not in agreement with the results of two previous studies in which the use of beta-adrenergic blocking agents reduced the vasodilation in response to hypocapnic alkalosis induced by hyperventilation (3, 6). In one of these studies (6) the second period of hyperventilation following the administration of propranolol was carried out after an unspecified interval, and it is possible that it was influenced by the preceding period of hyperventilation. In the other study (3) the oppo-

site arm was used as control and this could not have been a factor. Consonant with the results of these two studies is the finding that catecholamine concentration in the blood may increase during hypocapnic alkalosis (31). We have no explanation for these differences, except to suggest that since our subjects were trained in performing voluntary hyperventilation, it is possible that the release of catecholamines is not a constant phenomenon during hypocapnic alkalosis but perhaps occurs only in inexperienced subjects.

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