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Reduction of Exercise Dilation by Theophylline[1] (39611)

HAROLD M. A. TABAIE, JERRY B. SCOTT, AND FRANCIS J. HADDY

Department of Physiology, Michigan State University, East Lansing, Michigan 48824

Previous studies, utilizing bioassay and chemical techniques, suggest that adenosine (ADO) and adenosine monophosphate (AMP) participate in the genesis of exercise hyperemia (1-5). Other studies show that theophylline (Theo) competitively inhibits the coronary vasodilation produced by ADO and the adenine nucleotides (6) and that it also blocks the renal vascular responses to ADO and AMP (7; unpublished observation). It occurred to us that Theo might be used as a tool to further investigate the possible roles of ADO and AMP in exercise hyperemia. High concentrations of Theo cannot be used in the heart because they are complicated by changes in rate and contractility. This is not the case in skeletal muscle. Thus, this line of approach seemed promising in this tissue.

Methods. The right gracilis muscle of mongrel dogs was exposed and freed from connective tissue. All blood vessels communicating with the gracilis, except the major artery and vein, were ligated (Fig. 1). The origin and insertion of the muscle were tied with heavy ligatures to further ensure that blood entered only through the major artery and left only via the major vein. The gracilis nerve was severed and distally fixed on stimulating electrodes. The muscle was perfused at constant flow by diverting femoral artery blood through a pulsatile pump into the gracilis artery. Flow was adjusted to a level that produced a perfusion pressure similar to systemic arterial pressure and averaged 9.5 ml/min. Perfusion pressure and aortic pressure were continuously measured with low volume pressure transducers coupled to oscillograph recorders (paper speed 0.25 mm/sec in all cases).

In some experiments, contractile force and venous blood oxygen tension were also measured. Force was measured with a transducer attached to the distal end of the muscle (severed from its insertion) with a ligature. The proximal end of the muscle was fixed in a clamp to prevent sliding. Venous oxygen tension was measured with an oxygen-sensitive probe interposed in a cannula which led gracilis venous blood to a small reservoir (the blood was pumped back into the animal via the contralateral femoral vein). Both variables were continuously recorded on the oscillograph; oxygen tension was also read intermittently from a digital meter.

The basic experimental protocol is illustrated in Fig. 2. After perfusion pressure reached a steady state, dose-response relationships were established for ADO, AMP, and adenosine triphosphate (ATP) by injecting 0.25, 0.5, 1.0, 5.0, 10.0, and 20.0 μg of each (0.1 ml in all cases) into the arterial line upstream to the pump. Acetylcholine (Ach), 0.5 μg (0.1 ml) and 1.0 μg (0.1 ml), was also injected. The gracilis muscle was then exercised by stimulating the gracilis nerve once per sec (6 V, 1.6 msec) for 30 sec and then six times per sec (6 V, 1.6 msec) for 30 sec.

A solution of Theo (180 mg/100 ml = 10^{-2} *M*) was infused upstream to the pump at 0.76 ml/min, producing a calculated blood concentration of $\simeq 10^{-3}$ *M*. This decreased resistance. Since an altered response to exercise is difficult to interpret when initial resistance is not the same, an infusion of norepinephrine (NE) was superimposed at a rate which returned resistance to the original value. The gracilis nerve was then again stimulated for 30 sec at both frequencies using the same voltage and pulse duration. When the response to high frequency stimulation had subsided, all doses of all drugs were again injected.

This basic protocol (B in Tables I and II) was modified in four ways. To provide a time control, Theo and NE administration was deleted and exercise was repeated 20 to

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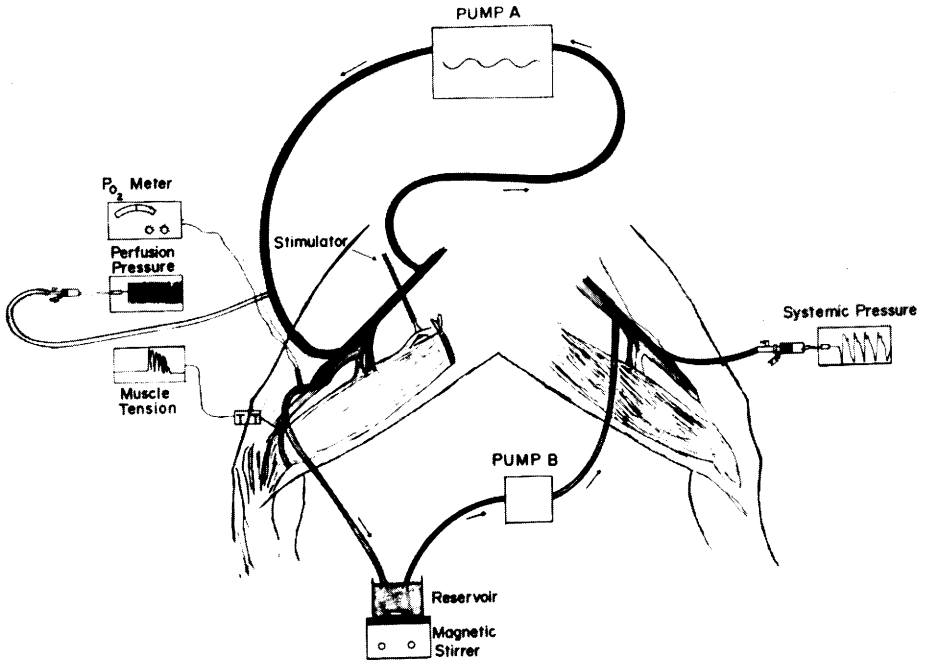

FIG. 1. Schematic drawing of the isolated gracilis muscle preparation.

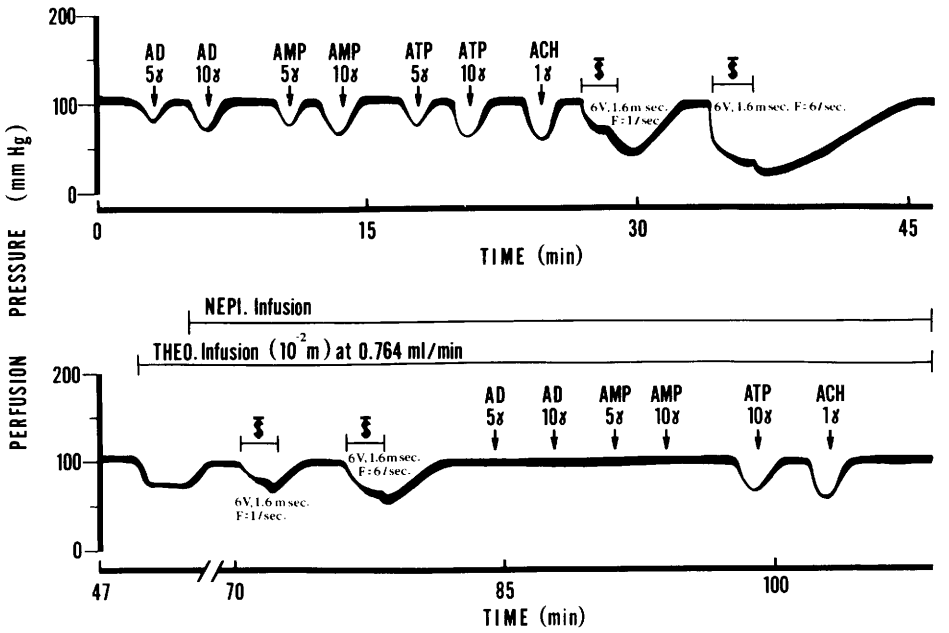

FIG. 2. Diagrammatic illustration of basic protocol.

25 min later (A in Tables I and II). To determine whether the changed exercise responses seen in series B were peculiar to NE administration, an entirely different type of vasoconstriction, vasopression (ADH), was substituted for NE (D in Tables I and II). To determine whether the findings were peculiar to the vasodilator activity of theophyl-

TABLE I. LOW FREQUENCY EXERCISE.[a]

Series	Initial slope (mm Hg/sec)		Maximal decrease (mm Hg)		Response duration[t] (sec)		Area (cm^2)		Muscle tension (g)	
	C	E	C	E	C	E	C	E	C	E
A Time Control	2.9	2.7	44.7	47.1	96.0	98.0	1.6	1.6	1259.0	1218.0
$N = 7$	±1.8	±1.9	±28.3	±29.5	±40.1	±37.9	±1.0	±0.9	±436.0	±429.0
B Theo + NE	2.9	2.1	38.9	22.8	99.0	53.3	1.4	0.5	990.0	1064.0
$N = 8$	±1.5	±1.1**	±23.1	±12.5**	±30.0	±7.5**	±0.8	±0.1**	±570.0	±617.0
C Theo + NE	1.5	0.8	17.0	9.1	78.6	48.9	0.8	0.4		
$N = 7$	±0.4	±0.3**	±5.2	±4.4**	±21.3	±12.0**	±0.2	±0.3**		
D Theo + ADH	3.0	1.9	48.1	29.6	104.5	61.1	1.8	0.8	897.0	895.0
$N = 7$	±1.8	±0.7*	±20.1	±14.1**	±30.6	±17.5*	±0.8	±0.2**	±573.0	±613.0
E Ach + NE	2.8	3.0	48.8	46.1	106.4	109.6	1.9	2.1	1272.0	1231.0
$N = 9$	±1.5	±1.5	±32.7	±23.8	±43.0	±45.4	±1.4	±1.3	±401.0	±298.0

[a] All headings except the last refer to perfusion pressure. Abbreviations used: C = control period during which drugs were not administered; E = experimental period during which drugs were administered except in the case of series A; [t] = time to 75% recovery of perfusion pressure. These parameters (6 V, 1.6 msec, 1 pulse/sec) were used for all experiments except those in series C, where 4 V, 0.5 msec, 1 pulse/sec were used.

* Significant at $P < 0.05$ relative to C.

** Significant at $P < 0.005$ relative to C.

TABLE II. HIGH FREQUENCY EXERCISE.[a]

Series	Initial slope (mm Hg/sec)		Maximal decrease (mm Hg)		Response duration[t] (sec)		Area (cm^2)		Muscle tension (g)	
	C	E	C	E	C	E	C	E	C	E
A Time control	6.7	7.5	68.1	74.0	369.7	334.8	9.9	9.6	1190.0	1165.0
$N = 7$	±3.3	±4.5	±20.7	±21.3	±243.8	±175.0	±6.1	±5.7	±380.0	±379.0
B Theo + NE	7.4	4.8	72.8	44.2	441.6	90.1	8.0	1.3	898.0	896.0
$N = 8$	±4.7	±2.4*	±30.1	±18.1**	±197.9	±35.8**	±5.8	±0.6**	±490.0	±484.4
C Theo + NE	4.5	2.8	47.3	30.7	256.6	81.8	3.9	1.4		
$N = 7$	±1.7	±0.8**	±13.3	±7.7**	±81.0	±23.9**	±1.3	±0.3**		
D Theo + ADH	7.0	3.6	74.9	59.7	468.0	114.0	7.9	1.9	857.3	743.0
$N = 7$	±3.4	±3.1**	±12.3	±19.3*	±110.9	±62.3**	±3.6	±0.7**	±371.0	±315.0
E Ach + NE	6.1	6.2	74.6	71.1	346.5	311.4	8.0	8.0	1092.0	987.0
$N = 9$	±3.5	±3.6	±25.7	±19.9	±248.2	±208.6	±5.3	±5.4	±563.0	±436.0

[a] These parameters (6 V, 1.6 msec, 6 pulses/sec) were used in all experiments except those in series C, where 4 V, 0.5 msec, 6 pulses/sec were used. See footnotes to Table I for explanation of other symbols.

line, an unrelated vasodilator, Ach was substituted for Theo (E in Tables I and II). Finally, the voltage and duration of the stimulus were reduced in order to explore a wider range of stimulation parameters (C in Tables I and II).

Five parameters were used to evaluate the exercise response: Area of the perfusion pressure response in square centimeters (area enclosed by the trace and a line drawn from the beginning to the end of the response), maximal fall in perfusion pressure in millimeters of mercury (mm Hg); initial slope of perfusion pressure response in mm Hg per second; time to 75% recovery of the perfusion pressure response in seconds; and developed muscle tension in grams. Significance of difference between means during the control and experimental periods were evaluated by Student's paired *t* test.

Results. In 11 preliminary experiments (8) not shown in Tables I and II, the basic protocol was followed, but muscle tension was not measured. In these experiments Theo + NE administration completely blocked the responses to all doses of ADO and AMP but was without effect on the responses to all doses of ATP and Ach. It also significantly ($P < 0.005$) reduced the slope of the initial fall in pressure (6.5 to 3.9 mm Hg/sec), the maximal fall in pressure (74 to 51 mm Hg), the duration of the response (575 to 178 sec), and the area of the response (14.9 to 3.2 cm^2) during high frequency stimulation, and maximal fall (45 to 23 mm Hg), duration (140 to 66 sec), and area (2.3 to 0.9 cm^2) during slow frequency stimulation.

Since it seemed possible that the reduction in exercise dilation resulted simply from decreased exercise, the experiments were repeated while measuring muscle developed

tension and, in some cases, venous P_{O_2}. It is apparent (series B in Tables I and II) that all regularly measured parameters decreased relative to control during infusion of Theo + NE except developed tension. Venous P_{O_2}, measured in five of the eight experiments (not shown in Tables I and II), fell to the same extent during exercise before and during infusion of Theo + NE (the infusion itself had no effect on venous P_{O_2}). The time control series (A in Tables I and II) showd that the changes did not result from spontaneous change in the preparation with time since no parameter during the "experimental" period differed significantly from that in the control period. This was also shown in another way. In three of the experiments in Series B, the muscle was exercised a third time at both the low and high frequencies 20 to 25 min after stopping the infusion of Theo + NE and the responses elicited were not different from those in the control period (the responses were attenuated during Theo + NE infusion in each of the three animals).

The reduced vasodilator responses to exercise were not peculiar to NE administration since they also occurred when ADH was substituted for NE (series D in Tables I and II). Again, developed tension ($n = 7$) and fall in venous P_{O_2} ($n = 6$) in response to exercise were unaffected by the infusion. When the stimulations were repeated a third time after stopping the infusion ($n = 5$), most measures of exercise dilation were not significantly different from those in the control period. The exceptions were response duration and area during slow frequency exercise and response duration during fast frequency exercise which remained significantly reduced (however, in the case of fast exercise, all parameters on the third test were significantly greater than on the second). Neither were the reduced vasodilator responses to exercise peculiar to the administration of a vasodilator in conjunction with NE, since they did not occur when Ach was substituted for Theo (series E in Tables I and II). Finally, the changes also occurred during Theo + NE administration when stimulation voltage and duration were reduced (series C in Tables I and II).

Discussion. In our 1965 study (1), we showed that hindlimb exercise in the dog is associated with the release of a substance or substances into femoral venous blood which has the bioassay characteristics of ADO and/or AMP. This finding was confirmed in 1971 utilizing an improved bioassay system (9). That same year, Dobson *et al.* (2) and Berne *et al.* (3) reported that ischemic exercise of rat and dog skeletal muscle is associated with increased tissue levels of AMP and ADO, but that only ADO appears in the venous effluent. Increased tissue levels of ADO and AMP in rat skeletal muscle were again reported by Rubio *et al.* (4) in 1973. In this paper, they also point out that the adenosine hypothesis for regulating skeletal muscle blood flow is inconsistent with the fact that in skeletal muscle adenine nucleotide degradation occurs primarily via inosinic acid (IMP) formation, with only a small production of ADO. However, they point out that in skeletal muscle from rats and guinea pigs, 5′-nucleotidase (the enzyme that degrades AMP to ADO) activity is found in endothelium and in localized skeletal muscle cells in close proximity to blood vessels. This strategic position with respect to the blood vessels is consistent with the adenosine hypothesis and could explain the observation that only small amounts of ADO are found in whole tissue and effluent during ischemic exercise. Recently Collingsworth *et al.* (10) reported that AMP does not appear in the effluent from exercising canine gracilis muscle (perfused at constant flow with modified Ringer's solution) unless the stimulation parameters are such as to activate the sympathetic nerves. Since ADO is a potent dilator of the skeletal muscle vascular bed, all of these observations suggest that this agent may well participate in the vasodilation seen in skeletal muscle during exercise at constant flow or under ischemic conditions.

In this study we have brought to bear on the problem an entirely different approach. We have taken advantage of the fact that Theo inhibits ADO and AMP vasodilation. We were able to administer much greater amounts that can be given to heart and, consequently, completely (rather than partially) block the vasodilator response to exogenous ADO and AMP. Under this condi-

tion we found, in preliminary experiments (8), a surprisingly large attenuation of exercise dilation. This led us to conduct a number of additional experiments in search of artifacts. Measurements of contractile force and venous oxygen tension indicated that the attenuation of exercise dilation does not result from decreased muscle activity. Experiments in the absence of drug administration showed that it does not result from a spontaneous decline in dilator capacity. Other findings indicate that the attenuated response is unrelated to NE administration; the vascular bed dilated in response to injected Ach or ATP during Theo + NE administration as well as in its absence and attenuated exercise dilation was also seen during Theo + ADH administration. Attenuation was not seen during Ach + NE administration, indicating that Theo is the essential ingredient. Attenuation of the exercise response was also seen during Theo + NE administration when the stimulation parameters were reduced to levels which do not cause AMP efflux (10). Finally, Theo did not block ATP vasodilation. These findings suggest that reduction of exercise dilation by Theo administration in fact results from blockade of the vasodilator action of ADO. Thus, it appears that ADO plays an important role in the exercise dilation seen at constant flow in the gracilis muscle of the dog. Additional experiments must be conducted to determine whether this is also the case at natural flow.

Other observations also indicate a need for more study. Both chemical (2, 3) and bioassay (1, 9) analysis of the venous effluent from the muscle mass of the canine hindlimb indicate that the blood contains adenosine only during severe or ischemic exercise. Furthermore, venous blood from the canine gracilis muscle (perfused at constant flow) develops enhanced vasodilator activity only during moderate to high frequency exercise (11) and this enhanced vasodilator activity can be completely abolished by correcting the fall in blood P_{O_2} and pH (12). These findings are not incompatible with the adenosine hypothesis because the adenosine may not always reach the sampling site or bioassay organ in sufficient concentrations for analysis. They do, however, emphasize the need for critical evaluation of the contribution of adenosine to the hyperemia seen under more natural conditions of exercise.

Summary. The canine gracilis muscle was exercised at constant flow before and during administration of theophylline at a rate which completely blocked the vasodilator response produced by injected adenosine. Exercise dilation was significantly attenuated during infusion of theophylline. This suggests a role for adenosine in the exercise dilation seen in the canine gracilis muscle during constant flow perfusion.

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