FUNCTIONAL HYPERAEMIA OF SKELETAL MUSCLE

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BY

IAN JACK DONALD HAMILTON

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INSTITUTE OF PHYSIOLOGY

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ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

CONTENTS

LIST OF TABLES

LIST OF FIGURES

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SUMMARY

1. Blood flow was recorded through the cat gastrocnemius and soleus muscles at rest and during stimulation at physiological frequencies. Deficiencies in the experimental set-up are discussed and the results compared with the findings of other workers who have recorded blood flow through "fast" and "slow" muscle.

2. ATP and adenosine were shown to be vasodilators of the vascular bed of the cat gastrocnemius and soleus muscles. Potassium, hypertonic glucose and inorganic phosphate were not vasodilator. These findings are discussed in relation to the identity of the vasodilator agent in functional hyperaemia of skeletal muscle.

3. ATP was detected in the plasma and Krebs perfusate from the resting and stimulated cat soleus muscle. This is discussed in relation to the hypothesis that ATP could be the vasodilator agent in functional hyperaemia.

4. APT was shown to be rapidly removed from cat blood in vitro. This is discussed in relation to a possible extracellular role for ATP.

INTRODUCTION

"FAST" AND "SLOW" MUSCLE BLOOD FLOW

Hilton & Vrbova (1968) demonstrated that the cat soleus muscle possessed a high resting blood flow and did not seem to possess a significant hyperaemic response. They postulated that the predominantly "slow" soleus possessed different blood flow characteristics from the predominantly "fast" gastrocnemius.

Folkow & Halicka (1968), however, showed that the cat soleus possessed a normal hyperaemic response to stimulation and Bockman (1983) was unable to demonstrate any difference in blood flow between the cat soleus and the predominantly "fast" gracilis muscle.

In the first part of this work an attempt was made to record blood flow through the cat gastrocnemius and soleus muscles using similar techniques as other workers.

FUNCTIONAL HYPERAEMIA OF SKELETAL MUSCLE

Gaskell (1880) proposed that the increase in blood flow through exercising muscle was due to the release of a vasodilator metabolite from the muscle into the blood stream. The increase in blood flow supplied nutrient to the muscle and "washed away" the accumulating vasodilator substance.

Gaskell's hypothesis is supported by the following expermimental evidence:

1. Anrep & Von Saalfield (1935) demonstrated that venous blood from active, but not resting, muscle produced a vasodilatation when perfused through the dog hind limb.

2. Barcroft & Swan (1953) showed that functional dilatation of human forearm muscle was independent of the sympathetic innervation of the forearm blood vessels.

Folkow & Neil (1971) proposed two criteria for the vasodilator agent. Firstly, it should be present in the venous effluent from contracting muscle and, secondly, it should produce a vasodilatation when infused into the muscle. A review of the literature is presented to illustrate the techniques which have been used in an attempt to satisfy the two criteria and the evidence for and against the candidates which have been proposed for the vasodilator agent in functional hyperaemia of skeletal muscle.

THE HYDROGEN ION

Gaskell (1880) perfused lactic acid through the frog lower limbs and demonstrated an increase in flow. Mathison (1910) infused glycolic acid into cats and observed a dilatation of blood vessels. Kester, Richardson & Green (1952) perfused acid solutions through the dog hind limb and showed a decrease in the resistance to blood flow and Barcroft, Greenwood & Rutt (1963) demonstrated a decrease in the pH of venous blood from exercising human forearm muscle.

More recently, Stefan McKenzie & Haddy (1982) observed a vasodilating effect of acetate as well as increased acetate concentrations in both muscle and venous effluent from contracting dog gracilis muscle.

Gollwitzer-Meier (1950) however, did not find a significant increase in pH of venous blood from contracting dog gastrocnemius muscle and Richardson, Wasserman & Patterson (1961) were unable to demonstrate an increase in human forearm muscle blood flow after perfusing lactic acid into the muscle. Furthermore, Rigler (1932) painted monacetic acid, which inhibits lactic acid production, onto the frog hind limb and found that the post-contraction hyperaemia was not reduced.

Finally, McArdle (1951) showed that patients suffering from a congenital disease characterised by the absence of the enzyme phosphorylase, which is necessary for the breakdown of glycogen to lactic acid, still possessed a normal hyperaemic response to exercise.

HISTAMINE

Histamine is a vasodilator (Dale & Richards 1918, Burn & Dale 1926, Feldberg 1927, Duff & Whelan 1954) and Lewis & Grant (1925) proposed that histamine was the vasodilator agent in reactive hyperaemia. Other workers (Anrep & Barsoum 1935. Anrep, Barsoum & Talaat 1936, Anrep et al 1939) have detected increased histamine levels in venous blood from exercising muscle.

Emmilin, Kahlson & Wicksel (1941) however, were unable to demonstrate increased histamine levels in venous blood or plasma from tetanised dog gastrocnemius muscle or exercising human forearm muscle and Lansdowne & Thompson (1948) showed that post-ischaemic hyperaemia of the foot was not reduced by oral or intravenous histamines.

Furthermore, Duff, Patterson & Whelan (1955) infused antihistamines into human forearm muscle and this had no effect on reactive hyperaemia.

ACETYLCHOLINE

Several workers have shown that acetylcholine is a vasodilator of skeletal muscle (Folkow 1949, Duff et al 1953, Kjellmar & Odelram 1965). Erici, Folkow & Unvas (1952) detected a substance like acetylcholine in the perfusate from cat tongue muscle and Brandon et al (1966) identified a substance like acetylcholine in venous blood from exercising human forearm muscle.

Hilton (1953) however, blocked the vasodilator effect of acetylcholine by infusing atropine into the contracting cat gastrocnemius muscle and found that post-contraction hyperaemia was not reduced.

BRADYKININ

Several workers have shown that bradykinin is a vasodilator of skeletal muscle (Fox et al 1961, Paldino, Hyman & Lenthall 1962).

Hilton & Lewis (1958) however were unable to detect bradykinin in the perfusate from cat tongue muscle and Allwood & Lewis (1964) could not detect bradykinin in the venous effluent from exercising human forearm muscle.

CARBON DIOXIDE

Bayliss (1901) perfused Ringer's solution with a high carbon dioxide content through the frog hind limb and demonstrated an increase in flow.

Kontos & Patterson (1964) detected increased carbon dioxide levels in venous blood from exercising human forearm muscle.

Krogh (1922) and Fleish, Sibul & Ponomerov (1932) however, showed that carbon dioxide was a poor dilator of blood vessels and Lennox & Gibson (1932), Abramson & Ferris (1944) and Crawford, Fairchild & Guyton (1959) demonstrated that muscle blood flow in human subjects remained constant despite inhalation of air mixtures rich in carbon dioxide.

Furthermore, McArdle et al (1957) demonstrated a vasoconstriction in human forearm blood vessels during inhalation of 30% carbon dioxide in air.

HYPOXIA

Kramer & Quensel (1938) showed that functional dilatation was directly related to the oxygen consumption of the muscle during contraction.

Furthermore, Ross et al (1962) demonstrated that venous blood from exercising muscle, when re-oxygenated by being recycled through the isolated dog lung, was not vasodilator when re-perfused through the dog hind limb.

Other workers have demonstrated that arterial hypoxia can cause functional hyperaemia (Dornhorst & Whelan 1953, Guyton et al 1964). Bayliss (1901) and Verzar (1912) however, were unable to demonstrate increased blood flow through skeletal muscle when the arterial blood was made hypoxic and Lowe (1955) showed increased oxygen saturation in venous blood from exercising human forearm muscle.

Furthermore, Rudko (1966) demonstrated functional hyperaemia in muscle perfused with an elevated oxygen tension.

POTASSIUM

Dawes (1941) infused KCl into the dog hind limb and found it to be vasodilator.

Other workers have detected increased potassium levels in plasma from exercising muscle (Kjellmar 1960, Skinner 1961, Barcroft 1964, Hnik, Kriz & Vyskocil 1973, Thomson, Sweetin & Hamilton, 1975).

Glover, Roddie & Shanks (1962) however infused potassium into the human forearm in concentrations sufficient to produce pain and could only demonstrate a doubling of forearm blood flow.

Furthermore, Chen et al (1962) infused ouabain, which inhibits the vasodilator effect of potassium, into the contracting dog hind limb and found that it had a minimal effect on functional hyperaemia.

HYPEROSMOLARITY

Mellander et al (1967) infused hypertonic glucose solutions into the cat calf muscle and demonstrated an increase in blood flow, they also demonstrated increased osmolarity of the venous blood from the muscle during contraction.

Other workers have shown that increased osmolarity of the tissue fluid surrounding skeletal muscle produces an increase in blood flow (Marshall & Lundvall 1971, Gray, Lundvall & Mellander 1968), Mellander & Lundvall 1971).

Stainsby & Barclay (1971) and Scott & Radawski (1971) however could not demonstrate a significant vasodilatation when hypertonic solutions were infused into the dog hind limb and Hilton & Hudlicka (1971) were unable to demonstrate any relationship between increased osmolarity and blood flow on infusing hypertonic solutions into the cat gastrocnemius muscle.

INORGANIC PHOSPHATE

Hilton & Vrbova (1970) infused NaH_2PO_4 into the cat gastrocnemius muscle and demonstrated an increase in blood flow, they also detected increased phosphate levels in the venous effluent from the muscle during contraction.

Barcroft, Foley & McSwiney (1972) however were unable to detect a significant increase in blood flow when phosphate was infused into the brachial artery of human subjects in sufficient amounts to raise the phosphate level in the venous effluent by 400%.

ATP (ADENOSINE TRIPHOSPHATE)

ATP is a vasodilator (Folkow 1944, Duff, Patterson & Shepherd 1954) and has been identified in the effluent from contracting muscle (Forrester 1968, Forrester & Lind 1969, Forrester 1972, Forrester & Hassan 1973). Parkinson (1973) also detected increased ATP levels in the blood stream of human subjects after whole body exercise.

Brashear, Ross & Smith (1968) however were unable to detect ATP in the blood stream of human subjects after whole body exercise and Bockman, Berne & Rubio (1975) could not demonstrate any increase in ATP levels in the plasma from the contracting dog hind limb and suggested that the ATP detected by Forrester (1972) was being released from the formed elements of the blood and not contracting muscle.

ADENOSINE

Dobson, Rubio & Berne (1971) infused adenosine into the dog hind limb and demonstrated that it was a vasodilator, they also detected increased adenosine levels in the venous effluent from the muscle during contraction. Proctor & Bohlen (1981) infused adenosine deaminase into hamster cremasteric muscle and showed that this could block the vasodilatation produced by adenosine.

Deuticke & Gerlach (1966) however, were unable to detect adenosine in venous blood from exercising muscle and Honig & Frierson (1980) infused dipyridamole, which prolongs the dilatation induced by adenosine, and adenosine deaminase into the dog gracilis muscle and showed that functional hyperaemia was not affected by either of these substances.

PROSTAGLANDINS

Hedwell et al (1971) and Conway & Hatton (1975) infused prostaglandins into dog gracilis muscle and demonstrated a vasodilatation in the muscle and Herbaczynska-Cedro et al (1976) detected PGE2 in the venous blood from the muscle during contraction.

Morganroth, Young & Sparks (1977), Beaty & Donald (1979) and Kilbom & Wenmalm (1976) however infused indomethacin, which blocks the effects of prostaglandins, into skeletal muscle and found that functional hyperaemia was not reduced.

COMBINATION OF FACTORS

Several workers have suggested that a combination of factors could be responsible for functional hyperaemia

1. Skinner & Costin (1967), Haddy & Scott (1968), Scott et al (1970) and Skinner & Costin (1970) have suggested that a combination of potassium, hyperosmolarity and hypoxia could fulfill the criteria for the vasodilator substance.

2. Hudlicka (1985), on the basis of different types of muscle releasing different vasodilator substances, has suggested a combination of potassium, inorganic phosphate and adenosine could meet the requirements of the vasodilator agent.

In the second part of this work potassium, hypertonic glucose solutions, inorganic phosphate, adenosine and ATP were close arterially infused into the vascular bed of the cat gastrocnemius and soleus muscles and their relative vasodilator potency assessed.

RELEASE OF ATP FROM CONTRACTING MUSCLE

Boyd & Forrester (1968) detected ATP in the bathing solution of stimulated frog sartorius muscle and postulated that ATP was released from the muscle during contraction. Jacob & Berne (1960) had previously shown that adenosine could move across the cell membrane by facilitated transport but on theoretical grounds the possibility that such a highly charged molecule as ATP could cross the cell membrane seemed unlikely (Forrester 1981). Furthermore, Dobson et al (1971) suggested that the ATP detected by Boyd & Forrester was being released from damaged cells and Forrester & Lind (1969) had to conclude that up to half of the ATP identified in the venous effluent from exercising human forearm muscle resulted from damage to platelets during the collection process.

In the third part of this work the cat soleus muscle was perfused with an oxygenated Krebs Henseleit solution, to remove blood as a source of ATP, and the perfusate from the resting and stimulated muscle assayed for ATP using the firefly luminescence technique. The results were compared with similar experiments in which ATP was assayed in plasma.

DEGRADATION OF ATP IN BLOOD

Chen & Jorgensen (1956) showed that ATP was rapidly broken down in human blood. Mills (1966) then demonstrated the presence of an enzyme ATPase capable of degrading ATP by catalysing the reaction:

$$ATP \xrightarrow{ATPase} ADP \xrightarrow{ATPase} AMP$$

Forrester (1972) found that within 2 hours of incubating 1-10 ug/ml of ATP in human plasma all the ATP had disappeared suggesting that there was a mechanism in blood for the rapid removal of ATP.

In the fourth part of this work the rate of degradation of ATP in cat blood was assessed.

THE FIREFLY LUMINESCENCE TECHNIQUE

An assay is defined as the measure of a pharamcologically active substance in tissue extract in terms of the response it elicits, the result being determined on the strength of a standard preparation of the same substance (Bowman & Rand 1980).

Strehler & McElroy (1957) devised an assay for ATP based on the linear luminescence response of firefly extracts to added ATP, the reaction being:-

$$ATP + Mg^{++} + O2 + LH2 + Luciferase = Light$$

for a more detailed description of the reaction, see McElroy & DeLuca (1983).

Fireflies in large numbers can be easily collected during the summer months in most temperate regions of the world and after drying in a vacuum desiccator over CaCl2 can be maintained indefinitely in a deep freeze.

The assay is extremely sensitive and specific for ATP and in the present experiments a modification of the method devised by Strehler (Forrester 1966) was used.

METHODS

BLOOD FLOW STUDIES

Cats of either sex weighing 1.5-5 kg. were intraperitoneally anaesthetised with sodium pentobarbitone (45 mg/kg). The femoral vessels of one hind limb were exposed in the adductor region of the thigh and all branches ligated. The two heads of gastrocnemius were divided and all vessels which did not supply the soleus muscle were tied. The soleus nerve was placed over two platinum electrodes for indirect stimulation with supramaximal pulses of 1msec. duration. The soleus tendon was detached from its insertion to the calcaneum and tied to a Grass tension transducer (Type FDO 3C) for insometric tension recording. Knee and ankle joints were firmly clamped. The jugular vein was exposed in the neck and 5000IU/kg of heparin (Pularin, Evans Medical Ltd.) was injected. The common carotid artery was dissected free from the surrounding structures and cannulated for blood pressure recording using a Pye Ether pressure transducer. The femoral vein was cannulated and the venous outflow from soleus passed through a Palmer integrating drop counter. It was then collected into a filter funnel and recycled proximally into the femoral vein in the groin using a Watson Marlowe constant flow pump. To record blood flow through the gastrocremius muscle the same technique was used except that all vessels which did not supply the medial head of the gastrocnemius were tied and the muscle prepared for stimulation and tension recording with pulses of 1msec. duration.

INFUSION OF TEST SOLUTIONS

For close arterial infusion of the test solutions the same technique was used except that a branch of the femoral artery was retrogradely cannulated and the test solutions were infused at 1 ml/min using a Sage syringe pump (Model 351).

After each experiment Naphthol Green dye was injected into the femoral artery to confirm the selective perfusion of soleus or medial head of gastrocnemius and the muscle was dissected out and weighed.

CALIBRATIONS

The drop counter was calibrated with stop clock and measuring cylinder, the tension transducer by suspending known weights from the metal spring and the pressure transducer with a mercury manometer.

MAKING UP THE TEST SOLUTIONS

The control Krebs Henseleit solution was made up from the following stock concentrations:

	STOCK CONCENTRATION g/1
NaCl	90
KCl	11.5
NaHcO3	12.9
KH2PO4	21
MgSO47H2O	18.5
CaCl126H2O	24.1

80 ml. of stock NaCL was diluted by 10 with distilled water to 800 ml., 32 ml. of stock KCl and 8 ml. of stock KH2PO4 were added. The solution was shaken thoroughly and oxygenated with 95% O2 and 5% CO2 for 15 minutes and 8 ml. of stock MgSO47H2O and 24 ml. of stock CaCl26H2O were added.

POTASSIUM: A molar solution of KCl (molecular weight 74.55) was made up in Krebs Henseleit solution and from this concentration the intermediate concentrations of 1 mM, 5 mM, 10 mM and 20 mM were made up.

HYPERTONIC GLUCOSE: A molar solution of glucose (molecular weight 180.16) was made up in Krebs Henseleit solution and from this concentration the intermediate concentrations of 20 mM, 40 mM and 80 mM were made up.

INORGANIC PHOSPHATE: A 10^{-3} molar solution of NaH2PO42H2O (molecular weight 156.01) was made up in Krebs Henseleit solution and from this concentration the intermediate concentrations of 0.1μM, 1μM, 2.5μM and 10μM were made up.

ADENOSINE: A 10^{-3} molar solution of adenosine (molecular weight 267.2), obtained from the Sigma Company, St. Louis, Missouri, was made up in Krebs Henseleit solution and from this concentration the intermediate concentrations of 0.1μM, 1μM, 2.5μM and 10μM were made up.

ATP: A 10^{-3} molar solution of ATP (disodium salt, molecular weight 551), of 99% purity, obtained from the Sigma Company, St. Louis, Missouri, was made up in Krebs Henseleit solution and from this concentration the intermediate concentrations of 0.1μM, 1μM, 2.5μM and 10μM were made up.

ASSAY OF ATP BY THE FIREFLY LUMINESCENCE TECHNIQUE

Powdered firefly lantern extract (Type FLE, Sigma Company, St. Louis, Missouri) was taken from the deep freeze and reconstituted by adding 5 ml. of distilled water. 0.2 ml. of firefly extract and 0.2 ml. of the sample were simultaneously pipetted into a glass cuvette in a darkroom illuminated with a red safety light.

As quickly as possible (within 6 seconds of mixing the sample with the firefly extract), the cuvette was placed in a photo-multiplier tube covered with a copper cannister and 1300 volts were applied across the tube. The light produced by mixing the ATP and the firefly extract was changed into an electrical impulse and recorded in millivolts on a Devices pen recorder.

After each assay a dose response curve was obtained by mixing firefly extract with know concentrations of ATP (see Figure 1).

ASSAY OF ATP IN THE EFFLUENT FROM THE CAT SOLEUS MUSCLE

PLASMA: The blood supply to soleus was isolated and the muscle prepared for indirect stimulation as previously described. Blood was sampled from the muscle at rest and during stimulation at 10 Hz for 1 minute into 1.5 ml. plastic centrifuge tubes kept in a beaker of melting ice. The test samples were then immediately centrifuged at 25000g for 1 minute (Quickfit Instr. Ltd.) and the supernatant plasma was assayed for ATP.

KREBS: The soleus muscle was perfused with an oxygenated Krebs Henseleit solution, which was heated by being passed through a heat exchanger coil at 40° C. The level of perfusion was

adjusted to give a hydrostatic pressure of 100 mmHg. Perfusate samples were collected from the muscle at rest and during stimulation at 10 Hz for 1 minute into 10 ml. siliconised glass centrifuge tubes and immediately assayed for ATP.

DEGRADATION OF ATP IN CAT BLOOD IN VITRO

In heparinised cats intraperitoneally anaesthetised with sodium pentobarbitone the right common carotid artery was cannulated and blood sampled into 10 ml. siliconised glass centrifuge tubes and placed in a water bath at 40° C. A stock solution of 10^{-4} molar solution of ATP was made up in Krebs Henseleit solution and the following concentrations of ATP were made up in cat blood.

2_{μ}M ATP:

0.02 ml. of 10^{-4} molar ATP was added to 0.98 ml. of cat blood.

5_{μ}M ATP:

0.05 ml. of 10^{-4} molar ATP was added to 0.95 ml. of cat blood.

10_{μ}M ATP:

0.1 ml. of 10^{-4} molar ATP was added to 0.9 ml. of cat blood.

20_{μ}M ATP:

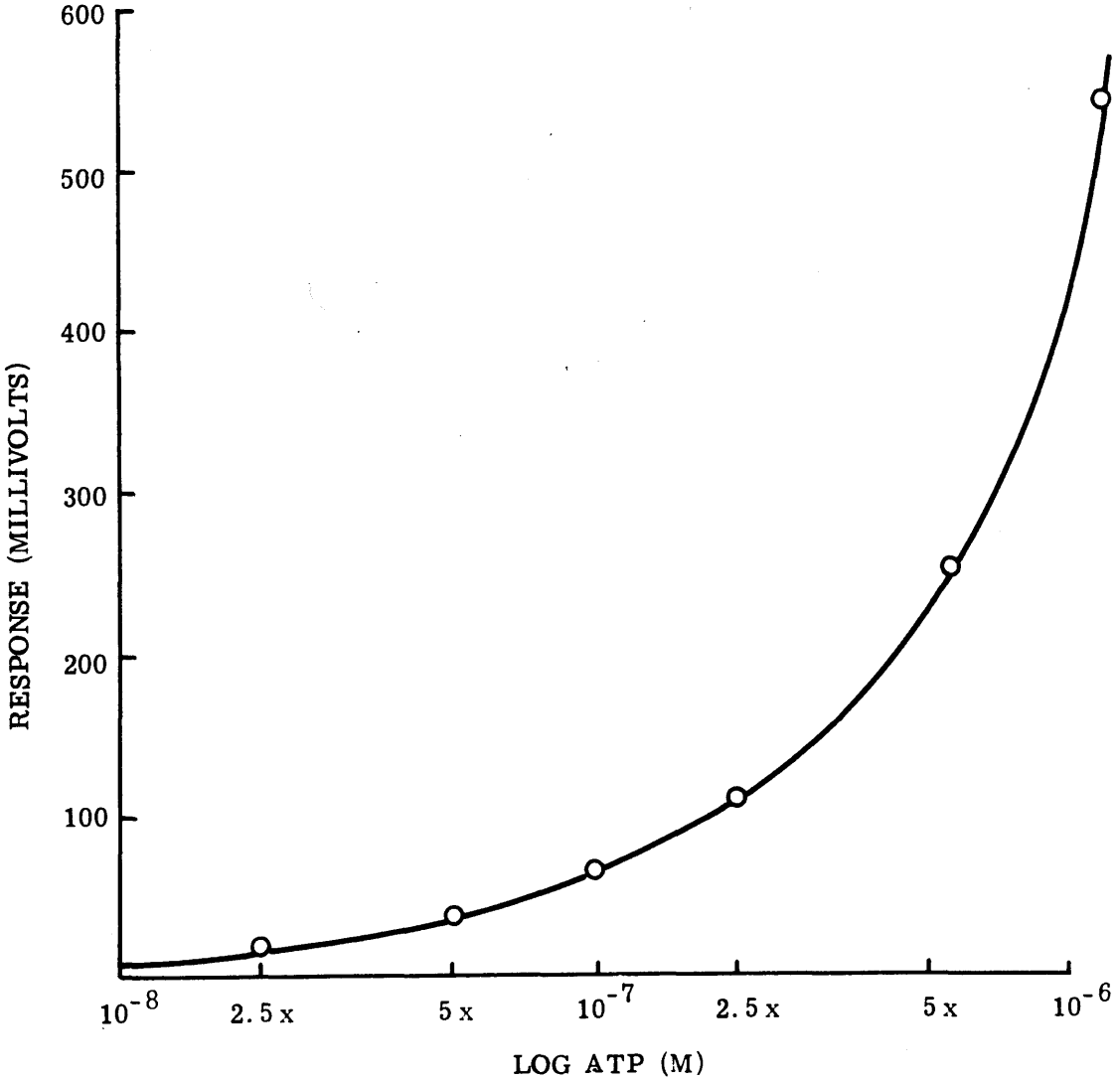
0.2 ml. of 10^{-4} molar ATP was added to 0.8 ml. of cat blood.

The test solutions were incubated at 40° C. for periods of 2, 4, 6 and 8 minutes in 1.5 ml. plastic centrifuge tubes. They were then centrifuged at 25000g for 1 minute and the supernatant plasma assayed for ATP using the firefly luminescence technique.

FIGURE 1:

Dose response curve for the assay of ATP by the firefly luminescence technique. The abscissa represents the concentration of ATP mixed with firefly lantern extract and the ordinate is the light signal response recorded in millivolts.

TYPICAL DOSE RESPONSE CURVE FOR ATP



RESULTS

BLOOD FLOW STUDIES

GASTROCNEMIUS

Figure 2 represents a typical experiment. The top trace shows the arterial blood pressure (mmHg) falling steadily from 130-110 mmHg and the middle trace the blood flow (ml/min) through the medial head of gastrocnemius at rest and during stimulation at 1, 5, 10 and 20 Hz for periods of 1 minute. The maximum increase in blood flow was approximately twice the resting value. The bottom trace shows the tension in grams developed by the medial head of gastrocnemius during stimulation at 1, 5, 10 and 20 Hz.

Discreet twitches of approximately 40 g. were recorded at 1 Hz, a subtetanic twitch in the region of 100 g. at 5 Hz, the muscle was tetanised at 320 g. at 10 Hz and the tetanic tension was 400 g. at 20 Hz.

Results of a series of experiments are presented in table form. (See Table 1).

FIGURE 2:

Typical experiment to show blood flow through the cat gastrocnemius muscle in response to graded indirect stimulation.

(a) Arterial blood pressure recording.

(b) Muscle blood flow

(c) Tension developed during stimulation at 1, 5, 10 and 20 Hz.

Periods of stimulation are for 1 minute

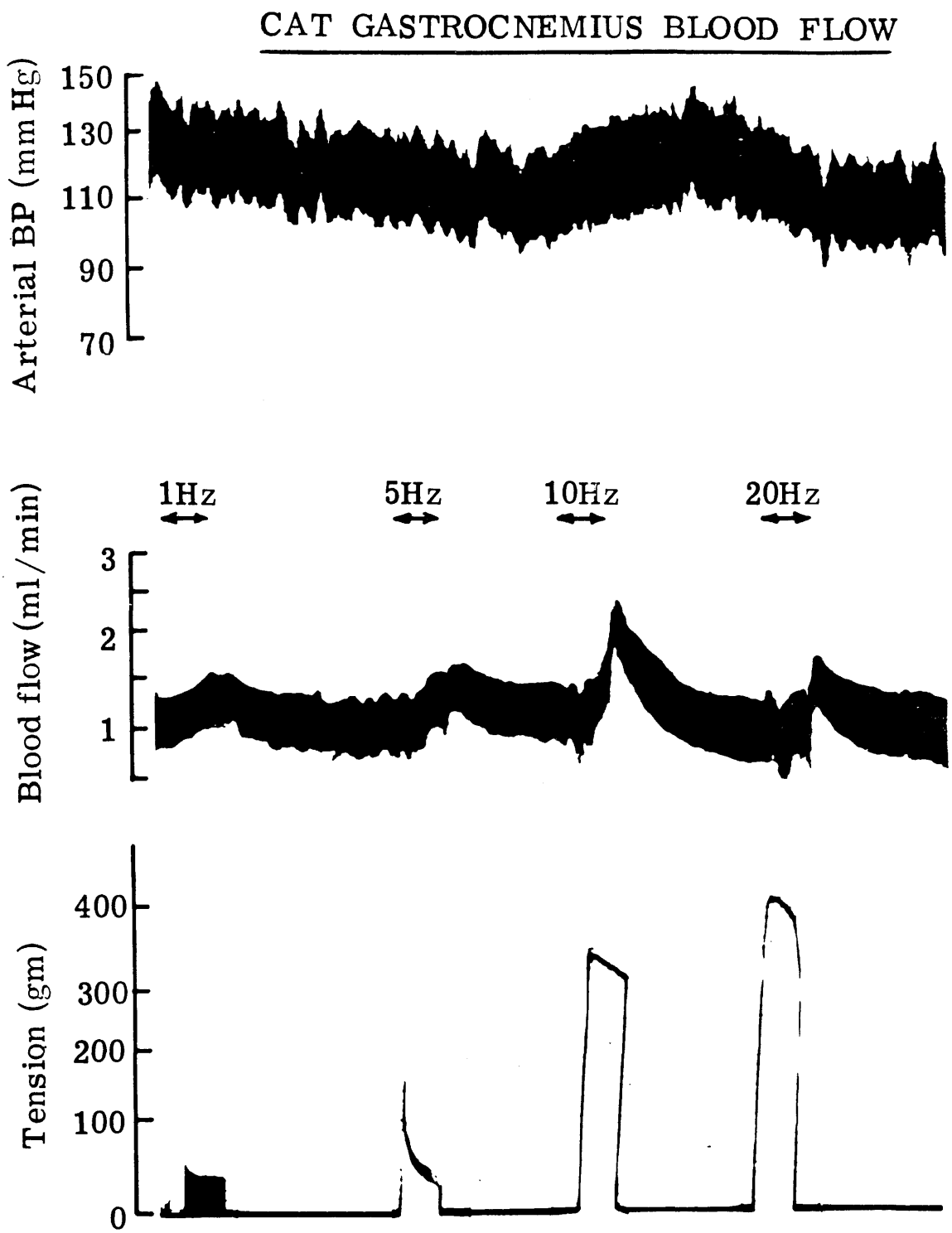

CAT GASTROCNEMIUS BLOOD FLOW
Arterial BP (mm Hg)
150
130
110
90
70
1Hz
5Hz
10Hz
20Hz
Blood flow (ml/min)
3
2
1
Tension (gm)
400
300
200
100
0

TABLE I - Means (± S.E.M.) of the blood flow through the cat gastrocnemius muscle in response to stimulation at 1, 5, 10 and 20 Hz for 1 minute periods. Tension recorded in grams, blood flow expressed in ml/100g. min. and "n" indicates the number of experimental runs.

	RESTING	1 Hz	5 Hz	10 Hz	20 Hz
Blood Flow	18	19	23	28	26
(n=10)	(±2.9)	(±2.8)	(±4.2)	(±6.3)	(±3.7)
Tension		78	96	172	312
(n=6)		(±6)	(±12)	(±22)	(±48)

COMMENT: Resting blood flow through gastrocnemius was 18 ml/100g.min. and the maximum dilatation in the muscle in response to stimulation at 10 Hz for a period of 1 minute was 28 ml/100g. min. Tensions recorded ranged from individual muscle twitches of 78g at 1 Hz to a tetanus of 300g at 20 Hz.

SOLEUS

Figure 3 represents a typical experiment. The top trace shows the arterial blood pressure (mmHg) remaining steady at 135 mmHg and the middle trace, the blood flow (ml/min) through the soleus muscle at rest and during stimulation at 1, 10 and 20 Hz for periods of 1 minute. The maximum increase in blood flow was approximately twice the resting value. The bottom trace shows the tension recorded in the soleus muscle during stimulation at 1, 10 and 20 Hz.

Discreet twitches of approximately 60 g. were recorded at 1 Hz, a sustained tetanic twitch of 300 g at 10 Hz and a tetanus of 420 g. at 20 Hz.

Results of a series of experiments are presented in table form. (See Table II)

FIGURE 3:

Typical experiment to show the blood flow through the cat soleus muscle in response to graded indirect stimulation.

(a) Muscle blood flow

(b) Arterial blood pressure recording

(c) Tension developed during stimulation at 1, 10 and 20 Hz.

Periods of stimulation are for 1 minute

CAT SOLEUS BLOOD FLOW

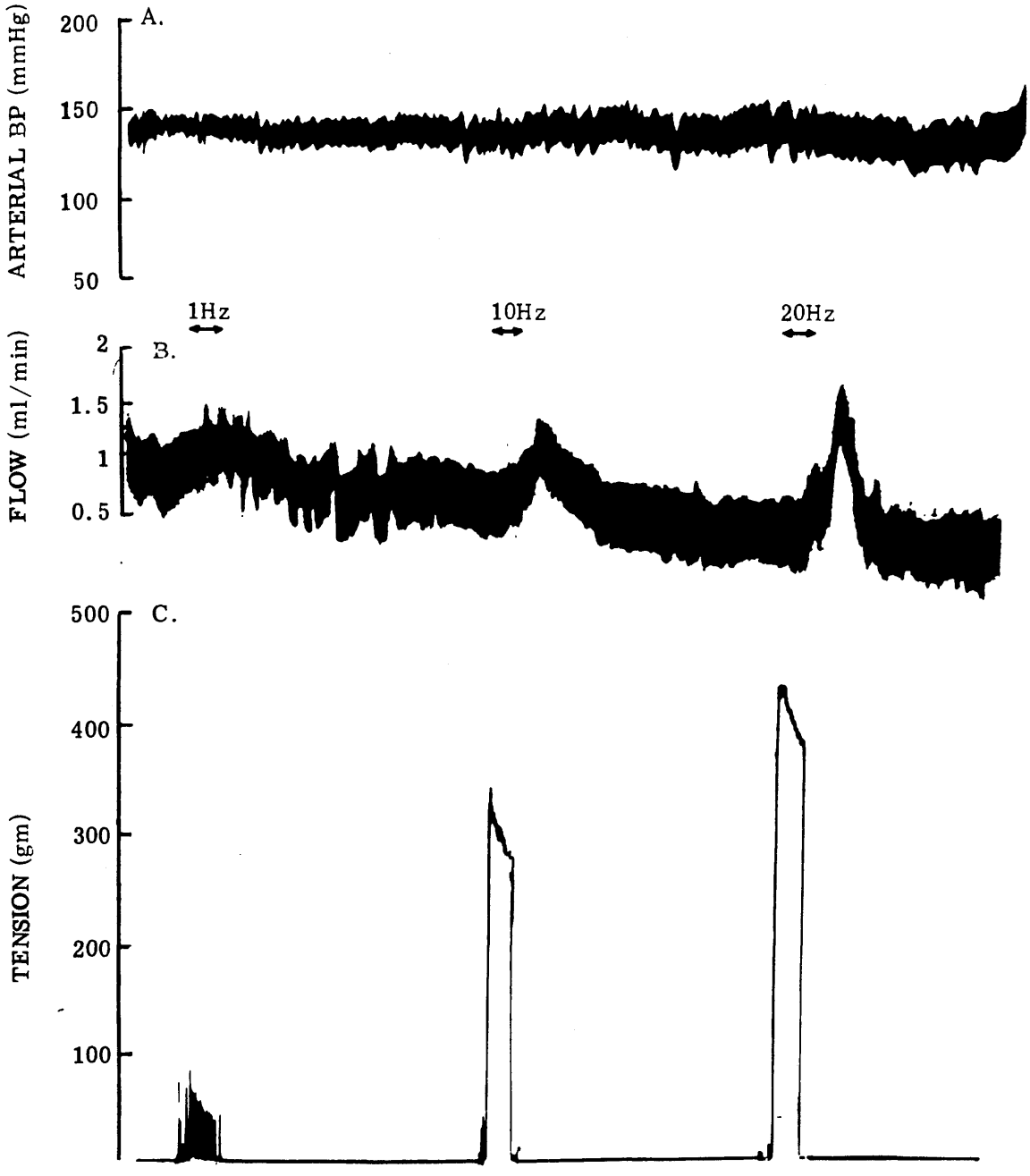


TABLE II - Means (± S.E.M.) of the blood flow through the cat soleus muscle in response to stimulation at 1, 5, 10 and 20 Hz for 1 minute periods. Tensions recorded in grams, blood flow expressed in ml/100g. min. and "n" indicates the number of experimental runs.

	RESTING	1 Hz	5 Hz	10 Hz	20 Hz
Blood Flow	34	38	39	45	57
(n=6)	(±3.8)	(±3.7)	(±2)	(±3.7)	(±6.6)
Tension		57	220	365	310
(n=6)		(±12)	(±44)	(±50)	(±65)

COMMENT: Resting blood flow through soleus was 34 ml/100g. min. and the maximum dilatation in the muscle in response to stimulation at 20 Hz for a period of 1 minute was 57 ml/100g. min. Tensions recorded ranged from individual muscle twitches of approximately 50g at 1 Hz to a tetanus 300g at 20 Hz.

POTASSIUM

Figure 4 represents two typical experiments.

The top trace shows the arterial blood pressure (mmHg) rising gradually from 90-95 mmHg in gastrocnemius and falling slowly from 140-130 mmHg in soleus.

The bottom trace shows the blood flow (ml/min) in response to close arterial infusion of control Krebs, 1, 5, 10 and 20 mM KCl at 1 ml/min.

No dilatation in either gastrocnemius or soleus was recorded.

Results of a series of experiments are presented in table form. (See Table III).

FIGURE 4:

Typical experiment to illustrate the effect of close arterial infusion of KCl into the cat gastrocnemius and soleus muscles.

(a) Arterial blood pressure recording

(b) Muscle blood flow in response to infusing control Krebs, 1mM, 5mM, 10mM and 20mM KCl at the rate of 1 ml/min over a 1 minute period.

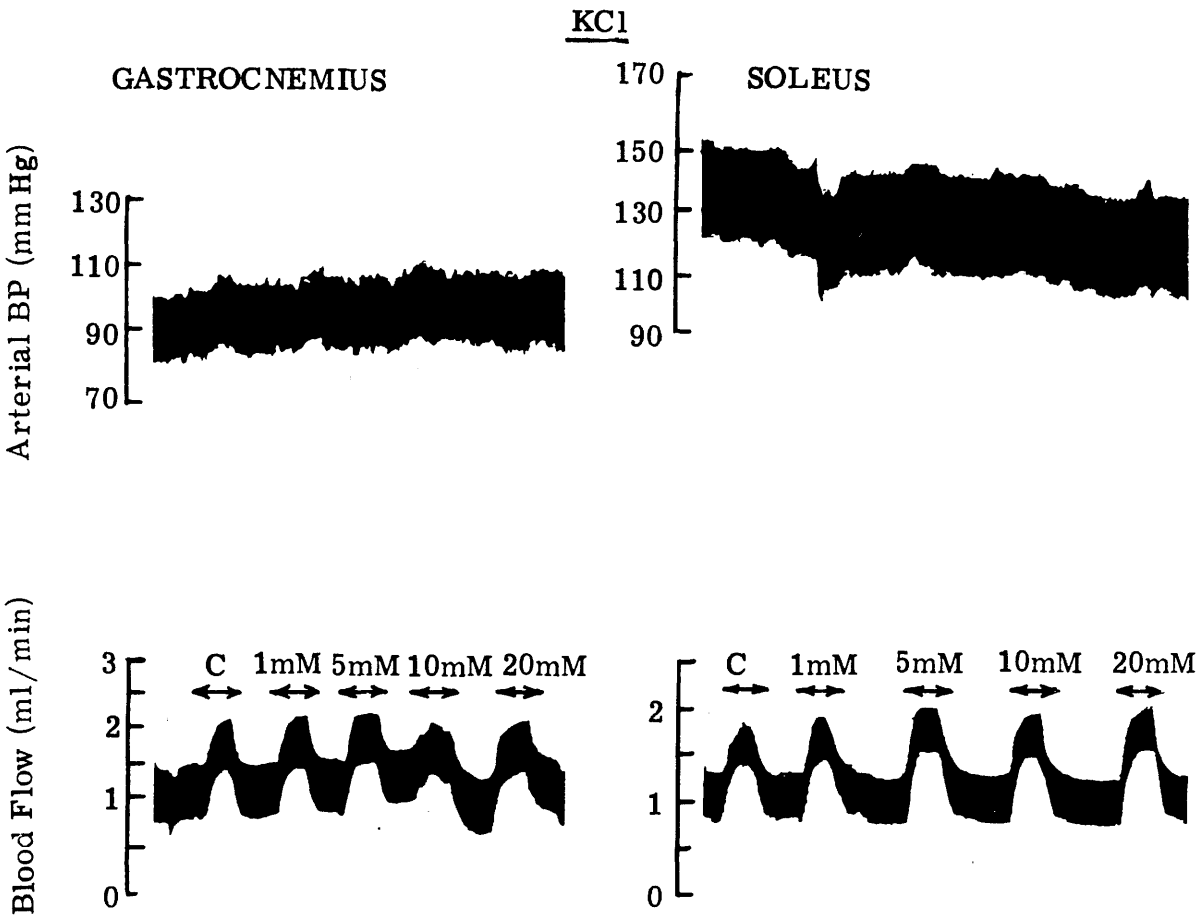

KCl
GASTROCNEMIUS
SOLEUS
Arterial BP (mm Hg)
130
110
90
70
170
150
130
110
90
Blood Flow (ml/min)
3
2
1
0
C
1mM
5mM
10mM
20mM
C
1mM
5mM
10mM
20mM
2
1
0

TABLE III - Means (± S.E.M.) of the blood flow through the cat gastrocnemius and soleus muscles in response to close arterial infusion of 1 mM, 5 mM, 10 mM and 20 mM KCl. Blood flow expressed in ml/100g. min and "n" indicates the number of experimental runs. P values refer to the difference between the control Krebs and 20 mM KCl. N.S. means not significant at the 5% level.

	RESTING	CONTROL	1 mM	5 mM	10 mM	20 mM
Gastrocnemius	23	29	30	30	30	29
(n=11)	(±1.6)	(±2.7)	(±2.7)	(±2.3)	(±2.3)	(±2.3)

P> 0.05 N.S.

Soleus	45	62	60	70	69	70
(n=6)	(±11.8)	(±10.4)	(±7.9)	(±11.7)	(±11.3)	(±10.9)

P> 0.05 N.S.

COMMENT: No significant increase in blood flow through gastrocnemius or soleus in response to close arterial infusion of KCl in concentrations ranging from 1-20 mM.

HYPERTONIC GLUCOSE

Figure 5 represents two typical experiments.

The top trace shows the arterial blood pressure remaining steady at 90 mmHg in gastrocnemius and falling gradually from 120-105 mmHg in soleus.

The bottom trace shows the blood flow (ml/min) in response to close arterial infusion of control Krebs, 20, 40 and 80 mM glucose at 1 ml/min. No dilatation in either gastrocnemius or soleus was recorded.

Results of a series of experiments are presented in table form. (See Table IV).

FIGURE 5:

Typical experiment to illustrate the effect of close arterial infusion of hypertonic glucose solutions into the cat gastrocnemius and soleus muscles.

(a) Arterial blood pressure recording.

(b) Muscle blood flow in response to infusing control Krebs, 20mM, 40mM and 80mM glucose at the rate of 1 ml/min over a period of 1 minute.

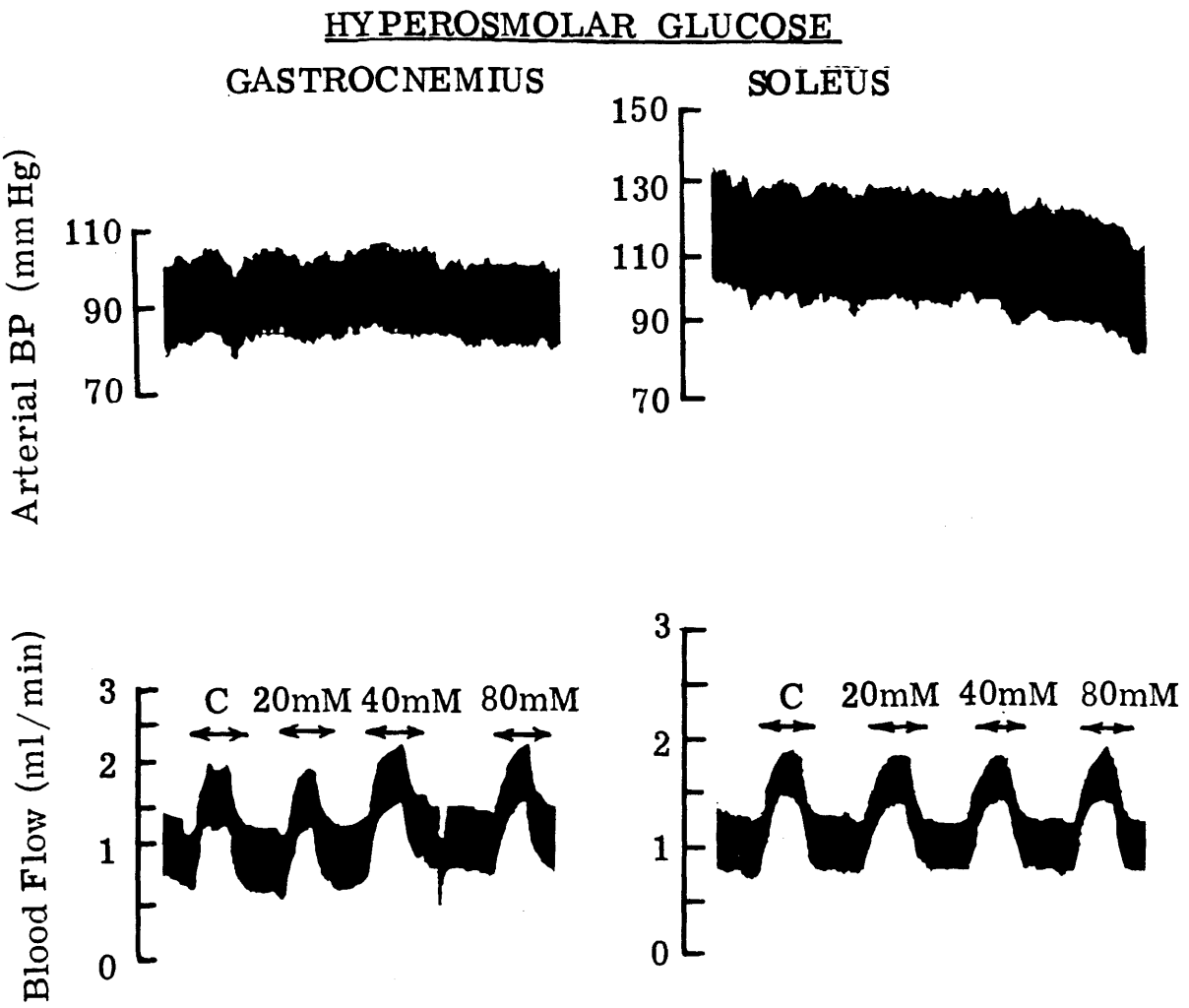

HYPEROSMOLAR GLUCOSE
GASTROCNEMIUS
SOLEUS
Arterial BP (mm Hg)
110
90
70
150
130
110
90
70
Blood Flow (ml/min)
3
2
1
0
C
20mM
40mM
80mM
C
20mM
40mM
80mM
3
2
1
0

TABLE IV - Means (± S.E.M.) of the blood flow through the cat gastrocnemius and soleus muscles in response to close arterial infusion of 20, 40 and 80 mM glucose. Blood flow expressed in ml/100g. min and "n" indicates the number of experimental runs. P values refer to the difference between the control Krebs and 80 mM glucose. N.S. means not significant at the 5% level.

	RESTING	CONTROL	20 mM	40 mM	80 mM
Gastrocnemius	24	33	33	34	32
(n=12)	(±3)	(±4.5)	(±4.5)	(±4.2)	(±2.6)

P> 0.05 N.S.

Soleus	35	51	61	61	65
(n=6)	(±8.3)	(±7.3)	(±11.4)	(±12)	(±12)

P> 0.05 N.S.

COMMENT: No significant increase in blood flow through gastrocnemius or soleus in response to close arterial infusion of hypertonic glucose solutions in concentrations ranging from 20-80 mM.

INORGANIC PHOSPHATE

Figure 6 represents two typical experiments.

The top trace shows arterial blood pressure (mmHg) remaining steady at 150 mmHg in gastrocnemius and falling from 170-150 mmHg in soleus.

The bottom trace shows the blood flow (ml/min) in response to close arterial infusion of control Krebs, 0.1μM, 1μm, 2.5μM and 10μM NaH_2PO_4 at 1 ml/min.

No dilatation in either gastrocnemius or soleus was recorded.

Results of a series of experiments are presented in table form. (See Table V).

FIGURE 6:

Typical experiment to illustrate the effect of close arterial infusion of NaH_2PO_4 into the cat gastrocnemius and soleus muscles.

(a) Arterial blood pressure recording.

(b) Muscle blood flow in response to infusing control Krebs, 0.1μM, 1μM, 2.5μM, 5μM and 10μM NaH_2PO_4 at the rate of 1 ml/min over a 1 minute period.

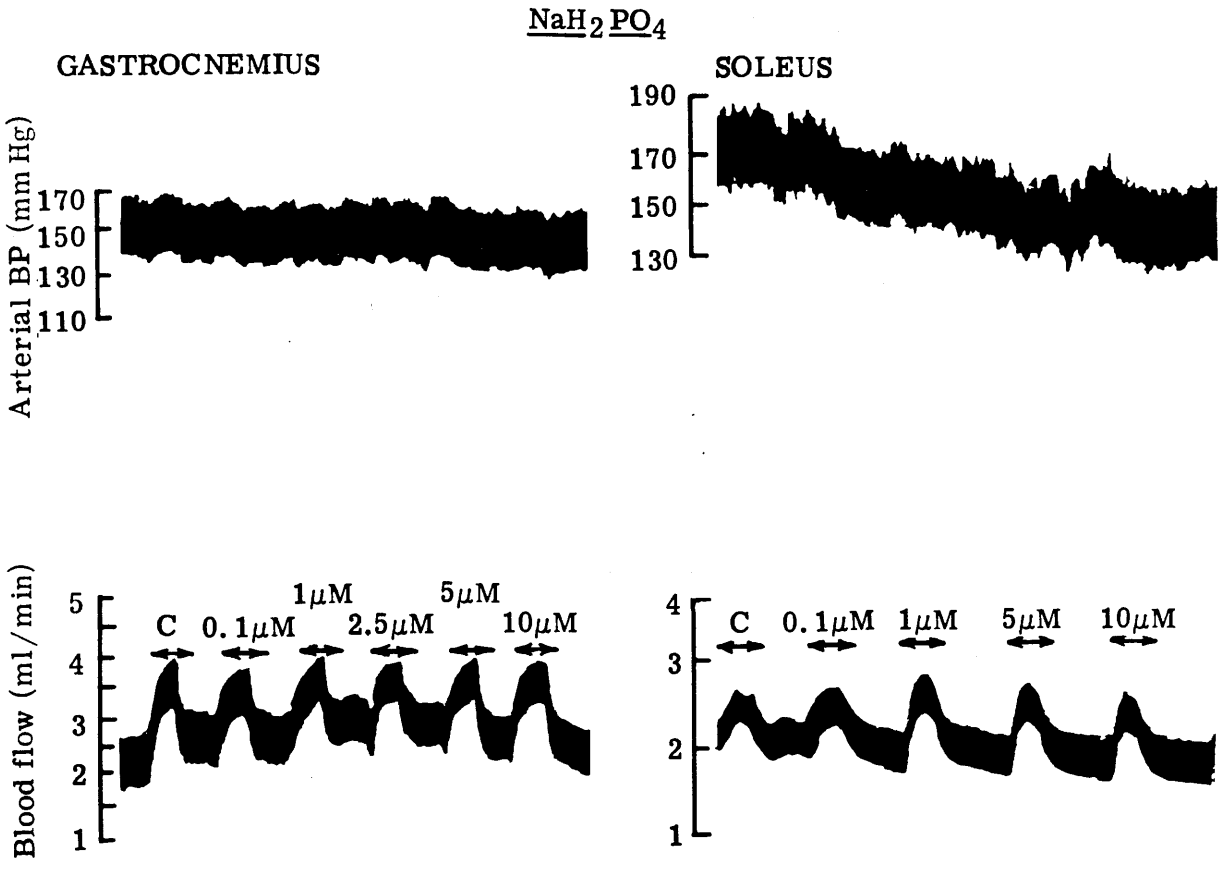

NaH2PO4
GASTROCNEMIUS
SOLEUS
Arterial BP (mm Hg)
170
150
130
110
190
170
150
130
Blood flow (ml/min)
5
4
3
2
1
C
0.1μM
1μM
2.5μM
5μM
10μM
4
3
2
1
C
0.1μM
1μM
5μM
10μM

TABLE V - Means (± S.E.M.) of the blood flow through the cat gastrocnemius and soleus muscles in response to close arterial infusion of 0.1μM, 1μM, 2.5μM, 5μM and 10μM NaH_2PO_4. Blood flow expressed in ml/100g. min and "n" indicates the number of experimental runs. P values refer to the difference between the control Krebs and 10μM NaH_2PO_4. N.S. means not significant at the 5% level.

	RESTING	CONTROL	0.1μM	1μM	2.5μM	5μM	10μM
Gastrocnemius	26	36	33	36	33	32	32
(n=12)	(±6.2)	(±9.3)	(±7.9)	(±10.1)	(±7.7)	(±7.6)	(±7.4)

P> 0.05 N.S.

Soleus	32	57	62	64	60	63	58
(n=6)	(±2)	(±1.9)	(±2.3)	(±2.2)	(±2)	(±4.3)	(±2.3)

P> 0.05 N.S.

COMMENT: No significant increase in blood flow through gastrocnemius or soleus in response to close arterial infusion of NaH_2PO_4 in concentrations ranging from 0.1-10μM.

ADENOSINE

Figure 7 represents two typical experiments.

The top trace shows the arterial blood pressure (mmHg) falling steadily from 85-80 mmHg in gastrocnemius and 150-130 mmHg in soleus.

The bottom trace shows the blood flow (ml/min) in response to close arterial infusion of control Krebs 0.1μM, 1μM, 2.5μM, 5μM and 10μM adenosine at 1 ml/min.

A graded increase in blood flow in both gastrocnemius and soleus was recorded.

Results of a series of experiments are presented in table form. (See Table VI).

FIGURE 7

Typical experiment to illustrate the effect of close arterial infusion of adenosine into the cat gastrocnemius and soleus muscles.

(a) Arterial blood pressure recording

(b) Muscle blood flow in response to close arterial infusion of control Krebs, 0.1μM, 1μM, 2.5μM, 5μM and 10μM adenosine at the rate of 1 ml/min over a 1 minute period.

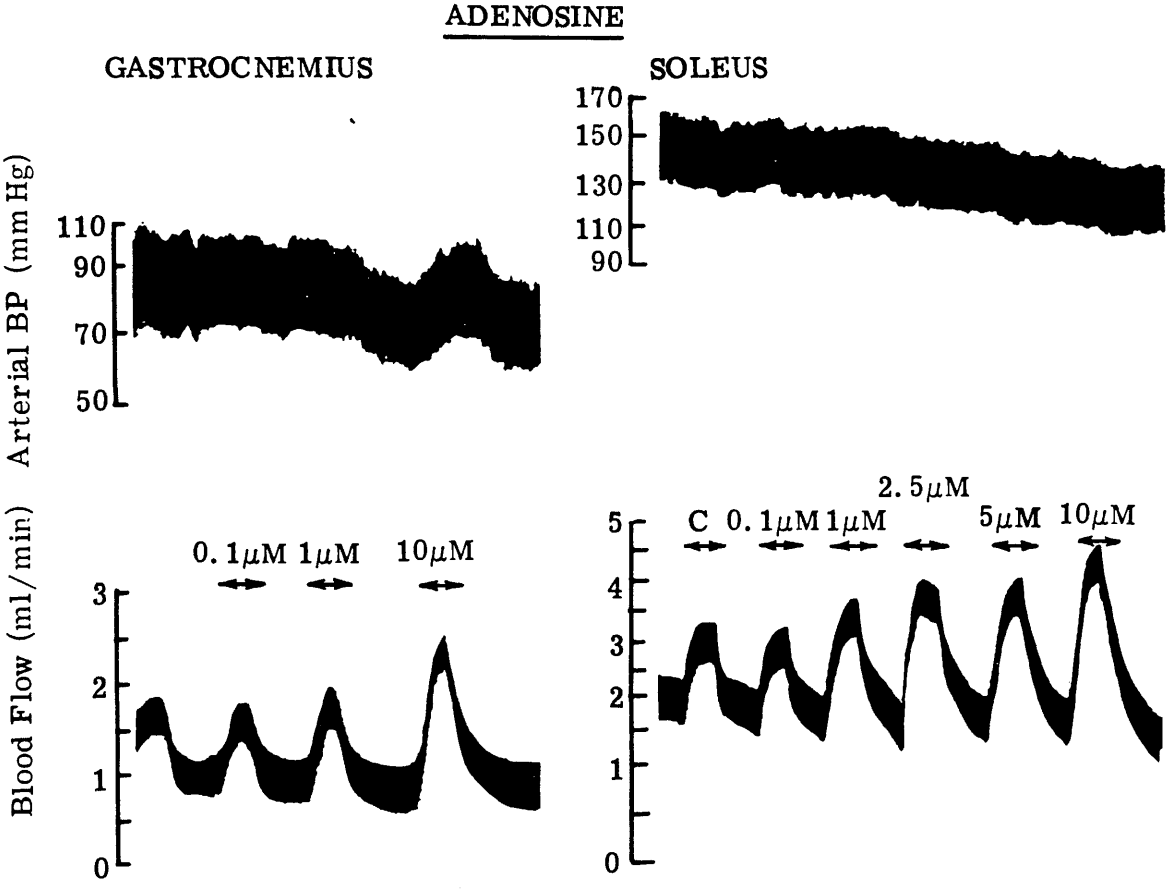

ADENOSINE
GASTROCNEMIUS
SOLEUS
Arterial BP (mm Hg)
110
90
70
50
170
150
130
110
90
Blood Flow (ml/min)
0.1μM 1μM 10μM
3
2
1
0
C 0.1μM 1μM 2.5μM 5μM 10μM
5
4
3
2
1
0

TABLE VI - Means (± S.E.M.) of the blood flow through the cat gastrocnemius and soleus muscles in response to close arterial infusion of 0.1μM, 1μM, 2.5μM, 5μM and 10μM adenosine. Blood flow expressed in ml/100g. min and "n" indicates the number of experimental runs. P values refer to the difference between the control Krebs and 10μM adenosine.

	RESTING	CONTROL	0.1μM	1μM	2.5μM	5μM	10μM
Gastrocnemius	28	40	39	45	45	47	52
(n=10)	(±4.2)	(±4.2)	(±4.9)	(±5.8)	(±5.5)	(±6)	(±7.3)

P< 0.05

Soleus	25	52	50	57	70	79	60
(n=6)	(±2.6)	(±3.8)	(±4.7)	(±6.3)	(±9.9)	(±3.3)	(±3.8)

P< 0.05

COMMENT: A significant increase in blood flow through gastrocnemius and soleus in response to close arterial infusion of adenosine in concentrations ranging from 0.1-10μM.

ATP

Figure 8 represents two typical experiments.

The top trace shows the arterial blood pressure (mmHg) remaining steady at 100 mmHg in gastrocnemius and falling from 100-90 mmHg in soleus.

The bottom trace shows the blood flow (ml/min) in response to close arterial infusion of control Krebs, 0.1μM, 1μM, 2.5μM, 5μM and 10μM ATP at 1 ml/min.

A pronounced but irregular increase in blood flow through gastrocnemius and a graded increase in blood flow through soleus was recorded.

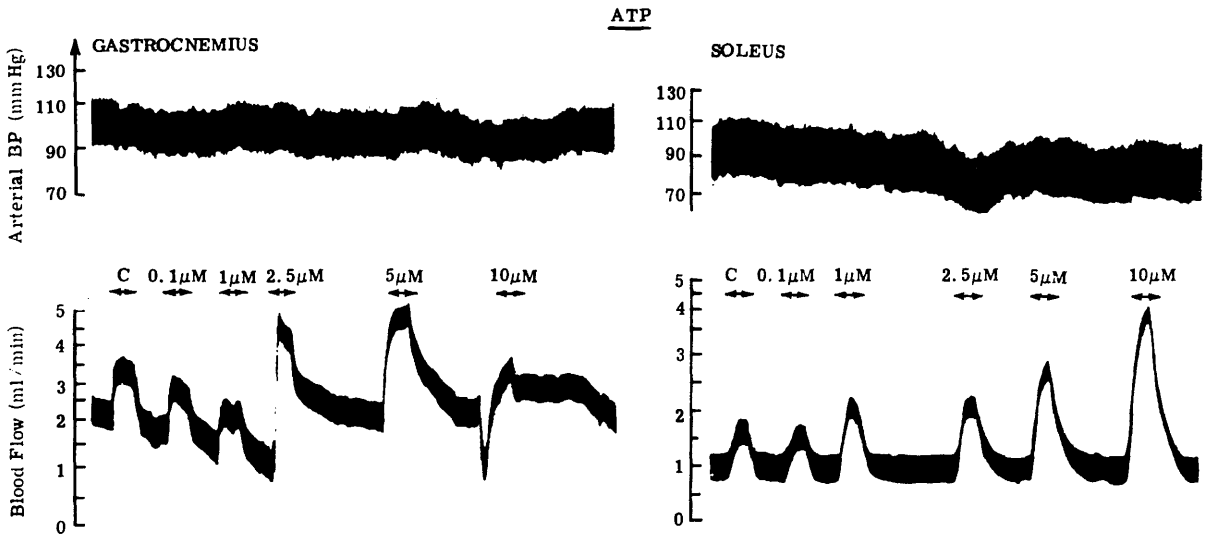
Results of a series of experiments are presented in table form. (See Table VII).

FIGURE 8:

Typical experiment to illustrate the effect of close arterial infusion of ATP into the cat gastrocnemius and soleus muscles.

(a) Arterial blood pressure recording.

(b) Muscle blood flow in response to close arterial infusion of control Krebs, 0.1μM, 1μM, 2.5μM, 5μM and 10μM ATP at the rate of 1 ml/min over a 1 minute period.


ATP
GASTROCNEMIUS
SOLEUS
Arterial BP (mm Hg)
130
110
90
70
Blood Flow (ml / min)
5
4
3
2
1
0
C
0.1μM
1μM
2.5μM
5μM
10μM

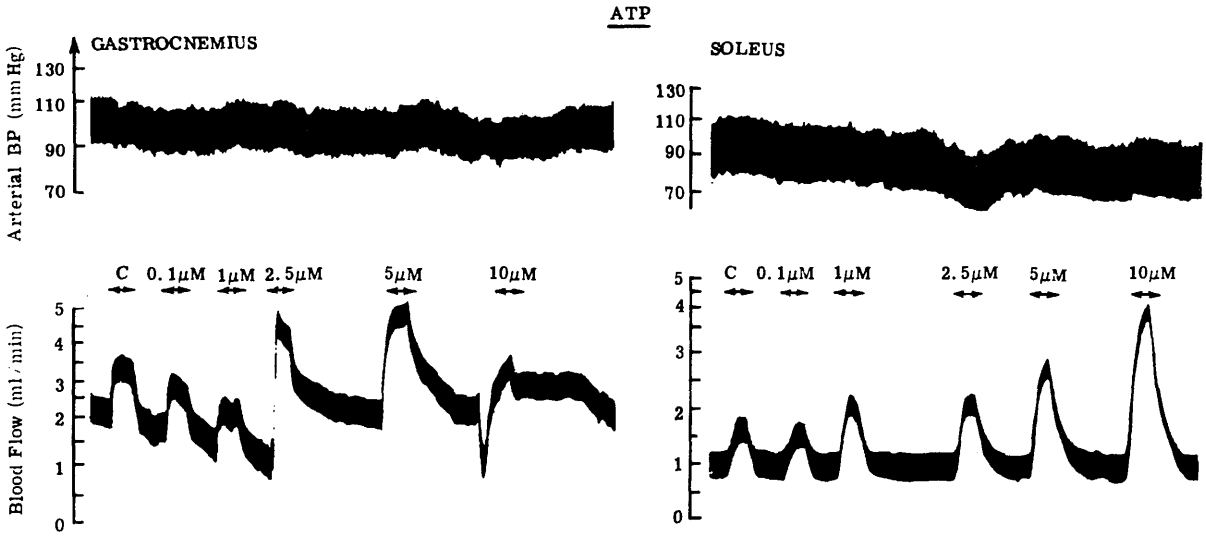

ATP
GASTROCNEMIUS
SOLEUS
Arterial BP (mm Hg)
130
110
90
70
Blood Flow (ml/min)
5
4
3
2
1
0
C
0.1μM
1μM
2.5μM
5μM
10μM

TABLE VII - Means (± S.E.M.) of the blood flow through the cat gastrocnemius and soleus muscles in response to close arterial infusion of 0.1μM, 1μM, 2.5μM, 5μM and 10μM ATP. Blood flow expressed in ml/100g. min and "n" indicates the number of experimental runs. P values refer to the difference between the control Krebs and 10μM ATP. N.S. means not significant at the 5% level.

	RESTING	CONTROL	0.1μM	1μM	2.5μM	5μM	10μM
Gastrocnemius	33	50	47	45	50	61	73
(n=9)	(±5.5)	(±7.2)	(±6.7)	(±6)	(±9)	(±11.2)	(±13.8)

P> 0.05 N.S.

Soleus	33	56	59	79	75	91	104
(n=8)	(±4.9)	(±5.6)	(±4.5)	(±11.7)	(±7.8)	(±8.3)	(±7.7)

P< 0.05

COMMENT: Significant increase in blood flow through soleus in response to close arterial infusion of 10μM ATP. The increase in blood flow through gastrocnemius was not significant due to the prolonged vasodilator effect of ATP resulting in an increase in resting blood flow following infusion of the test solutions.

ASSAY OF ATP IN THE EFFLUENT FROM THE CAT SOLEUS MUSCLE

The cat soleus muscle was perfused with blood (8 experiments) and an oxygenated Krebs Henseleit solution (12 experiments) and ATP was assayed in the perfusate from the resting and stimulated (10 Hz for 1 minute) muscle by the firefly luminescence technique.

Results of a series of experiments are presented in table form. (See Table VIII)

TABLE VIII - Means (± S.E.M.) of ATP levels in plasma and Krebs perfusate from the cat soleus muscle at rest and during stimulation at 10 Hz for 1 minute. "n" indicates the number of experimental runs and P values refer to the difference between the resting and stimulated muscle. N.S. means not significant at the 5% level.

	RESTING	DURING STIMULATION
Plasma	0.07μM	0.3μM
(n=8)	(±0.04)	(±0.05)

P< 0.05

Krebs	0.9μM	1.8μM
(n=12)	(±0.3)	(±0.5)

P> 0.05 N.S.

COMMENT: ATP was present in both plasma and Krebs perfusate from the resting muscle. Increase ATP levels were detected in the plasma but not the Krebs perfusate during muscle stimulation.

DEGRADATION OF ATP IN CAT BLOOD IN VITRO

Figure 9 shows the results of a series of experiments. The ordinate is the ATP concentration on a logarithmic scale and the abscissa is the time in minutes of incubation of ATP in cat blood. Each point represents the mean of 6 samples ($\pm$ S.E.M.).

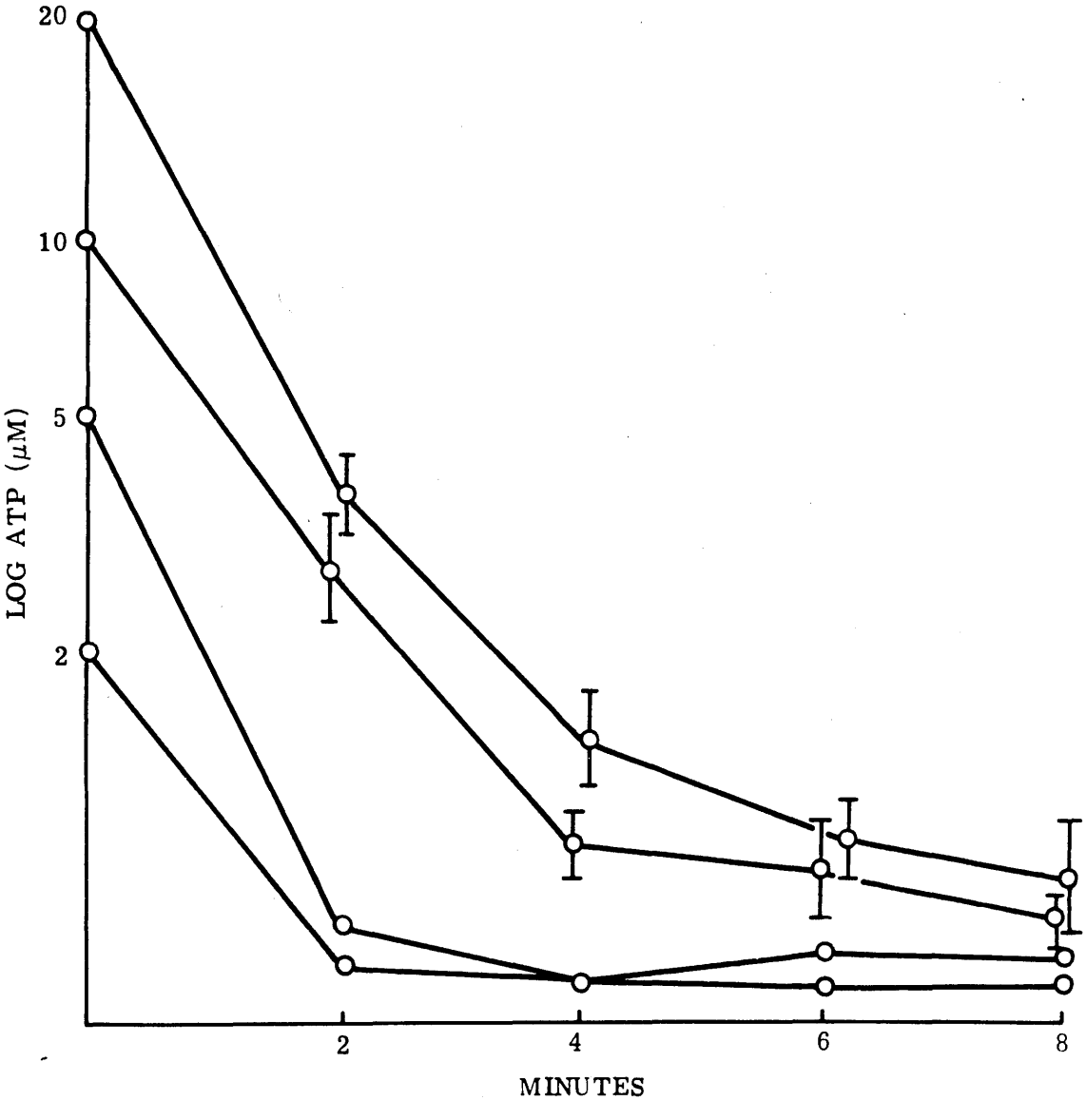
COMMENT: Within two minutes of incubation 2 and 5μM ATP had disappeared from the cat blood and at four minutes 10 and 20μM ATP had been reduced to less than 2μM.

FIGURE 9:

The rate of degradation of 2, 5, 10 and $20_{\mu}M$ ATP incubated in cat blood at 37° C. for 2, 4, 6 and 8 minute periods.

Points on the graph are the mean of 6 samples;

I bars are $\pm$ S.E.M.


20
10
5
2
LOG ATP (μM)
2
4
6
8
MINUTES

PARALLEL PROJECT

A joint research project was also performed with Dr. W. H. S. Thomson and Mr. J. C. Sweetin, The Research Laboratory, Knightswood Hospital, Glasgow.

The authors' contribution was to devise the preparation, obtain the electron micrographs and assist in the analysis of the data.

The results were subsequently published in a paper entitled "ATP and muscle enzyme efflux after physical exertion", Thomson W. H. S., Sweetin J.C. & Hamilton I.J.D., Clin. Chim. Acta (1975), 59, 241-245.

In summary, the release of the muscle enzyme creatinine phosphokinase and potassium into the venous effluent of the chronically stimulated cat hind limb was demonstrated.

The main conclusion was that adequate intracellular ATP levels, as evidenced by the depletion of muscle glycogen stores on electron microscopy, were necessary for enzyme protein retention.

Other experiments to demonstrate the release of ATP from the stimulated muscle were unsuccessful due to platelet contamination.

DISCUSSION

DEFECTS IN THE EXPERIMENTAL SET-UP

Several valid criticisms of the experimental set-up must be acknowledged (Harper and Hudlicka, personal communications). In the typical experiment to record blood flow through the cat gastrocnemius muscle, the blood pressure fell by 20 mmHg during the 10 minute recording period, suggesting that the cat was deteriorating during this period.

Furthermore, the blood pressure values should also have been included in Tables I and II.

Tensions recorded in both gastrocnemius and soleus were less than expected. Hilton et al (1970) in similar experiments recorded tensions of up to 3 kg in gastrocnemius with individual muscle twitches instead of tetanus when the muscle was stimulated at 10 Hz. This would suggest that the muscle was suffering from fatigue, perhaps due to an inadequate blood supply.

In retrospect, there should have been a greater effort made to maintain the cats in better physiological condition. This could have included intubation and administration of an oxygen rich air mixture, the assessment of the level of anaesthesia, monitoring of rectal temperature and perhaps the regular estimation of blood gas concentration.

Despite these criticisms of the preparation, it is of interest to compare the findings with results reported by other workers.

"FAST" AND "SLOW" MUSCLE BLOOD FLOW

In the present experiments, resting blood flow through gastrocnemius and soleus was 18 and 34 ml/100g. min., increasing after tetanic stimulation to 28 and 57 ml/100g. min.

Using the same method Folkow & Halicka (1968) reported resting blood flows of 9 and 20 ml/100g. min in the two muscles, increasing after tetanic stimulation to 35.9 and 118 ml/100g. min.

Corresponding blood flows reported by Hilton et al (1970) were 13.9 and 51.9 ml/100g. min and 46 and 62.7 ml/100g. min.

More recently, Bockman (1983) has reported resting blood flows of 6.1 ml/100g. min. in the predominantly "fast" cat gracilis and 6.3 ml/100g. min. in soleus increasing after muscle contractions to 35 and 40 ml/100g. min.

Using a labelled microsphere method to record blood flow, Bonde-Peterson (1981) reported resting flows of 7 and 5 ml/100g. min. in the cat gastrocnemius and soleus increasing after isometric exercise to 28 and 57 ml/100g. min.

Laughlin and Armstrong (1983) using the same method in unanaesthetised rats reported resting blood flows in gastrocnemius and soleus of 18 and 138 ml/100g. min. increasing after treadmill exercise to 39 and 224 ml/100g. min.

The results reveal a wide range of blood flow values, particularly for the soleus muscle and the reason for this is not known. Hudlicka (1985) has proposed that blood flow in the conscious animal is much higher than in the anaesthetised preparation since Laughlin & Armstrong's figures for blood flow are much higher than other workers.

Shepherd (1984) on the other hand, has suggested that the different blood flows which have been reported for soleus could be a reflection of the different methods which have been used to measure blood flow, rather than any special characteristics of the muscle itself.

Before an attempt is made to explain these conflicting results, it is necessary to discuss the development of the concept that red and white muscle could possess different blood flow characteristics.

HISTORICAL BACKGROUND TO THE CONCEPT OF RED AND WHITE SKELETAL MUSCLE

Ranvier (1874) demonstrated that the difference in the appearance of red and white skeletal muscle was the result of a higher density of capillaries in the vascular bed of red muscle.

Cooper & Eccles (1930) then showed that red muscle had a slower time course of contraction than white muscle and Vrbova (1963) demonstrated that the slow-twitching cat soleus was tonically active, to maintain posture, whereas the fast-twitching gastrocnemius was only active intermittently in short bursts of activity.

Beatty & Peterson (1963) then demonstrated that the oxygen consumption of red muscle was greater than that of white muscle. They suggested that the two muscles could have different rates of metabolism and this was confirmed by Romanul in 1965, who showed that red muscle had a higher succinic acid dehydrogenase activity than white muscle. Furthermore, he also proposed that red muscle was using mainly oxidative pathways, whereas white muscle was mainly dependent on anaerobic metabolism.

Romanul also suggested that red and white muscle could have different blood flow properties in keeping with their different functions.

This was confirmed by Folkow & Halicka (1968) using a drop counter technique to record blood flow through the mainly red soleus and the mainly white gastrocnemius muscles in the cat.

Hilton, Jeffries & Vrbova (1970) however, whilst agreeing with Folkow & Halicka that soleus had a high resting blood flow, were unable to demonstrate a significant hyperaemia in the muscle in response to nerve stimulation. They, therefore, postulated that the tonically active soleus could be continuously releasing a vasodilator substance in order to maintain its high resting flow and insignificant hyperaemia.

The suggestion that soleus might be continuously releasing a vasodilator substance made it an interesting muscle for further study since at the time, the author was investigating the possible role of ATP as the vasodilator agent in Gaskell's metabolic hypothesis (Gaskell 1880).

Before trying to identify ATP in the effluent from soleus however, it was necessary to confirm the previous findings regarding blood flow through the two types of muscle, using the same technique as previous workers.

The discussion of the results, however, necessitates a consideration of the limitations of the drop counter technique since the present results were not in agreement with the previous workers.

DEFECT OF THE DROP COUNTER TECHNIQUE

The drop counter technique involves tying off all branches of the femoral vessels except those supplying the muscle under investigation and recording its venous outflow.

In the present experiments, the resting and stimulated blood flows through soleus and medial head of gastrocnemius expressed in ml/min were similar. However, when the flows are expressed in ml/100g. min., then soleus would appear to have twice the flow of gastrocnemius since medial head of gastrocnemius is approximately twice the weight of soleus.

This calculation however may not be valid for the following reasons. Firstly, by channelling blood directly from the femoral artery into the soleus and gastrocnemius vascular bed, the tone in the small arterioles would inevitably be changed from their resting level. Secondly, the necessary trauma to the muscle and its blood supply during the dissection would also adversly affect vascular tone.

The present results would therefore suggest that the reported difference in blood flow through red and white skeletal muscle could be due to an artefact in the drop counter technique rather than the result of any difference in their physical properties.

Support for this view comes from the work of Bockman (1963) who used the same technique to record blood flow through the cat soleus and gracilis, which is a white muscle of similar weight and found that their blood flows were almost identical.

In the present experiments there was no significant difference demonstrated in either the rate of contraction or the tension developed by soleus and medial head of gastrocnemius.

Both muscles were tetanised at 5Hz and developed tensions in the region of 300 gm. which is comparable with the findings of Hilton et al (1970) in similar experiments on the cat soleus and tibialis anterior.

The present experiments would therefore not support the hypothesis, at least in the cat, that skeletal muscle could possess different speeds of contraction in relation to its different function.

Further experiments however would be necessary using a more physiological preparation to prove this assertion.

In the present experiments a two fold increase in blood flow was demonstrated in soleus and gastrocnemius in response to nerve stimulation. This would not be sufficient to "wash away" the accumulating vasodilator metabolites of Gaskell's metabolic hypothesis.

Another hypotheses proposed by Khaytin in 1968 could, however, explain the present results. He suggested that the contraction of the individual muscle fibres produced a fall in the intraluminal pressure of the arterioles and that this, not the release of a vasodilator substance, was the cause of the increase in muscle blood flow during exercise.

Khaytin's hypothesis would also explain why in the present experiments, the increase in blood flow through gastrocnemius and soleus occurred immediately after, and not during, muscle contraction when the intraluminal pressure would be at its lowest.

STATISTICAL ANALYSIS OF THE INFUSION EXPERIMENTS

The most appropriate statistical test for a multi-dose response in which several measurements are made on the same muscle is the repeated measures anova (analysis of variance) F ratio with a correction factor (Box 1954). This would then be followed by a paired t-test if any of the means were shown to be significantly different.

To apply this test however, all measurements have to be shown and the only values available to the author at the present time are the means of the blood flows and their standard errors.

In the present experiments, a normal t-test was used. However, its use can be justified using the following argument.

The normal t-test is a more conservative test than the paired t-test (Snedcor & Cochran 1973). In other words, the normal t-test will err on the safe side, therefore any difference which is signficant using the normal t-test would be more significant had the correct method been used.

This means that the conclusions which are drawn regarding the significance of any difference in blood flow between the control and the maximum concentration of the test solutions will still be valid.

VASODILATOR POTENCY OF THE TEST SOLUTION

The present results show that KCl infused into the cat gastrocnemius and soleus muscles in concentrations ranging from 1-20 mM did not significantly increase blood flow. This compares with a doubling of forearm blood flow demonstrated by Glover et al (1962) in response to infusing 25 mM KCl into the brachial artery of human subjects.

In another series of experiments (Thomson et al 1975), the author was able to demonstrate an increase in plasma potassium of 2-5 mM in the venous effluent from the stimulated cat soleus muscle which would compare with an increase of 2-7.5 mM demontrated by Hnik et al (1973).

Finally, Kjellmar (1960) had to increase the plasma potassium level in the venous effluent from cat calf muscle to 20 mM, which is greater than would be possible in vivo, in order to demonstrate a significant dilatation. This would suggest that potassium was not a major contributor to functional hyperaemia in skeletal muscle.

In the present experiments, glucose in concentrations ranging from 20-80 mM/1 was dissolved in Krebs Henseleit solution (osmolarity 300 mosm/1). The osmolarity of the test solutions was therefore 320-380 mosm/1. These concentrations are greater than the glucose solutions infused by Mellander et al (1967) into the cat hind limb and no dilatation was demonstrated in either gastrocnemius or soleus.

The present findings would therefore not support the hypothesis that hyperosmolarity was an important factor in functional hyperaemia.

Hilton & Vrbova (1970) infused NaH_2PO_4 in concentrations ranging from 1-10 μM/ml into the cat gastrocnemius muscle and were able to demonstrate a significant dilatation.

In the present experiments 0.1-10 μM NaH_2PO_4 was infused into the cat gastrocnemius and soleus and no dilatation was observed.

More recently, Hilton (1977) infused a mixture of NaH_2PO_4 and Na_2HPO_4 into the muscle and showed that it was not vasodilator. He suggested that inorganic phosphate was only active in the acid form and perhaps this could explain why NaH_2PO_4 was not vasodilator in the present experiments.

The present results show that both adenosine and ATP produced a significant dilatation when infused into the cat gastrocnemius and soleus muscles. This is in agreement with the findings of other workers (Duff et al 1965, Kjellmar & Odelram 1965, Dobson et al 1971) and would confirm that adenosine and ATP are potent vasodilators of skeletal muscle.

RELEASE OF ATP FROM SKELETAL MUSCLE

In the present experiments an attempt was made to remove blood as a contaminating source of ATP by perfusing the cat soleus muscle with an oxygenated Krebs Henseleit solution.

In other experiments ATP was assayed in plasma from the resting and stimulated muscle and strict precautions were taken to prevent damage to platelets by careful handling of samples and the use of non-wettable containers. The results were inconclusive.

In the Krebs perfused muscle, the difference between the resting and stimulated ATP levels (0.9-2.8μM) was significant; corresponding results from the blood perfused muscle (0.07-0.3μM) were not significant. Other workers have also reported conflicting results in ATP experiments.

Chen et al (1972) was able to demonstrate a significant increase in plasma ATP levels from the contracting dog hind limb, whereas Bockman et al (1975) could not demonstrate any relationship between resting and stimulated plasma ATP levels in a similar preparation.

There remain two valid criticisms of any experiment to test the hypothesis that ATP could be released from muscle during contraction.

Firstly, in muscles perfused with a physiological saline any ATP detected in the perfusate might be the result of muscle damage or hypoxia and secondly, any ATP detected in plasma from blood perfused muscles could be due to the release of ATP from platelets and not contracting muscle.

AN EXTRACELLULAR ROLE FOR ATP?

The present results show that ATP was rapidly removed from cat blood. Within 4 minutes of incubating 20μM ATP in cat blood almost all the ATP had disappeared. Other workers, however, have reported different findings.

Forrester (1969) incubated 10μg/ml of ATP in human plasma and found that it took almost 2 hours for all the ATP to disappear, whereas Parkinson (1971) was able to demonstrate a rise of 1.5 μg/ml in plasma ATP levels 5 minutes after whole body exercise in human subjects.

Furthermore, Bockman et al (1976) was able to detect 2.97 nmoles/ml of ATP in plasma from the dog hind limb one hour after muscle contraction. The release of ATP from platelets might explain the presence of ATP in plasma one hour after muscle contraction in Bockman et al's experiments, but would not explain Parkinson's results since damage to platelets would be unlikely after normal physical exercise. Species variation might possibly account for the different results reported by Forrester.

The present experiments have demonstrated that ATP is rapidly removed from blood and the reason could be that ATP has an important extracellular role. It is important therefore to summarise the findings of other workers regarding the profound effects of ATP on the cardiovascular system.

Emmilin & Feldberg (1948) and Davies, Gropper & Schroeder (1956) injected ATP intravenously into cats and humans respectively and observed a marked fall in blood pressure and Wolfe & Berne (1956) and Moir and Downs (1972) were able to demonstrate that ATP was a potent dilator of the coronary vessels in open chest dogs.

Gaddum & Holtz (1933) and Green & Stoner (1950) showed that ATP was a vasoconstrictor of pulmonary vessels in the dog and cat lung and De Waele & De Velde (1945) infused ATP intravenously into the dog and rabbit and observed a dilatation of the splanchnic vessels.

Forrester et al (1979) infused ATP into the carotid artery of the baboon and demonstrated a marked increase in cerebral blood flow and finally Bennet & Drury (1931) perfused adenosine compounds into the dog renal artery and demonstrated a vasoconstriction of the kidney vessels.

GENERAL CONCLUSIONS

On the basis of its vasodilator potency, ATP would seem to be a good candidate for the vasodilator metabolite in functional hyperaemia of skeletal muscle.

However, the fundamental question still remains to be answered: "Can ATP cross the intact cell membrane?"

Forrester (1981) has proposed that ATP could be released from muscle during the phase of membrane depolarisation either coming through or off the cell membrane. Hopefully, future research will provide the answer to this question.

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