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The Biochemical Actions of Phentolamine and Papaverine on Rat Perfused Skeletal Muscle

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Abstract—The direct actions of the vasodilators, papaverine and phentolamine, on skeletal muscle metabolism were investigated in an isolated perfusion system. Eviscerated male rats were hemisected above the diaphragm and perfused, via the aorta, with a physiological perfusion medium containing erythrocytes. Papaverine, but not phentolamine, reduced vascular resistance throughout the 80 min study. Papaverine caused marked reductions in muscle concentrations of ATP and phosphocreatine, when compared with muscle from preparations without added vasodilators. This was accompanied by elevations in lactate concentrations. Water content of papaverine-treated muscle was also higher than values in unperfused muscle taken in-vivo. Phentolamine, in contrast, had no effect on muscle ATP, phosphocreatine, lactate or water content. The metabolism of the entire preparation was also investigated. Papaverine induced increases in lactate output while phentolamine treatment caused an initial uptake, followed by an increased output of lactate. There was no significant effect of either papaverine or phentolamine on the metabolism of K^+ and glucose. Arteriovenous differences in oxygen-saturation of haemoglobin and pH were also unaltered. Investigations on aspects of protein metabolism demonstrated that papaverine and phentolamine caused significant reductions in muscle protein synthesis when compared with control perfusions or in-vivo values. The reductions in synthesis were not due to reductions in cAMP or limitations in branched-chain amino acid supply. However, there was the suggestion that phentolamine caused a decrease in protein breakdown. The overall data indicated that papaverine and phentolamine may cause impairment of skeletal muscle metabolism. This has important implications for their therapeutic or experimental use.

Vasodilators are used in a variety of clinical and experimental situations. Although the pharmacological properties of these compounds are well documented, little knowledge exists about their actions on non-targeted organs. As skeletal muscle is the largest tissue in animals and man, it is possible that small pertubations in normal function may have profound effects on whole-body metabolism. From the literature there appears to be little or no information on the actions of vasodilators on skeletal muscle metabolism. We have been investigating their use in an isolated muscle preparation, the rat perfused hemicorpus, in an attempt to improve its performance. Papaverine and phentolamine are used in a variety of in-vitro skeletal muscle preparations for this purpose, i.e. Ruderman et al (1971), O'Donovan et al (1975). Our data suggest that skeletal muscle metabolism may be adversely affected. Their use in isolated muscle preparations is contra-indicated and illustrates the need to investigate their long-term effect in the intact organism.

Materials and Methods

Materials

Biochemicals were purchased from Sigma (Poole, Dorset, UK) and L-[U-^{14}C]tyrosine from Amersham International (Amersham, Bucks, UK). Papaverine sulphate was obtained from the Pharmacy, St. Thomas' Hospital, (London, UK) and phentolamine mesylate (Rogitine) was from Ciba Laboratories (Horsham, West Sussex, UK). All other chemicals were from BDH Ltd (Poole, Dorset, UK).

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Animals

Male Wistar rats (Charles River, Margate, Kent, UK) were obtained at an initial body weight of 70–100g, housed singly in wire-bottomed cages and fed a powdered diet (18% w/w protein, Payne & Stewart 1972) in a humidified, temperature-controlled environment on a 12 h light/12 h dark cycle. At 175–210 g rats were either subjected to operational procedures for perfusion or killed after obtaining in-vivo measurements of protein synthesis or muscle metabolites.

Perfusion medium

The perfusate was made in bicarbonate buffer, pH 7·45 (Krebs & Henseleit 1932) and contained: 45% (v/v) washed human erythrocytes (12–16 g haemoglobin per 100 mL perfusate, 5·25% (w/v) dialysed bovine serum albumin (Cohn Fraction V), 11 mM glucose, 0·043 mM pyruvate, 1 iu heparin mL^{-1}, normal plasma concentrations of amino acids as determined in similar rats in-vivo (Preedy 1981), and 25m iu mL^{-1} insulin. The vasodilators phentolamine and papaverine were added at concentrations of 5 and 100 $\mu g\ mL^{-1}$ perfusate, respectively. All aqueous solutions were filtered through membranes of pore-size 0·5 μm before use. To remove recirculating debris and emboli during perfusion, the perfusate flowed through a series of filters, decreasing in four steps from 140 to 26 μm. The medium was oxygenated with O_2/CO_2 (19:1) via a glass lung (Preedy 1981).

Perfusion of muscle

This has previously been described in detail (Preedy & Garlick 1981, 1983; Preedy et al 1984). After pentobarbitone anaesthesia (50 mg kg^{-1}) and heparinization (5000 iu kg^{-1} body weight) blood vessels to non-muscle tissues were ligated and tissues eviscerated. The aorta was cannulated and the

preparation hemisected above the diaphragm. Rats were administered oxygen (100%) via a cranial hood during the operation. After aortic cannulation the preparation was flushed with 50 mL of non-circulating perfusate, gassed with O_2/CO_2 (19:1) at a rate of 300 mL min^{-1}. The vena cava was also cannulated for measurement of arteriovenous differences in oxygen saturation and pH (Preedy 1981). The entire preparation was then transferred to a temperature-controlled (37·5°C) cabinet and perfused with 62 mL recirculating perfusion medium. At this point perfusions were timed, (i.e. time 0 min) and L-[U-^{14}C]tyrosine in an additional 38 mL perfusate was added to the perfusate to measure protein synthesis, as described below. At 40 min the left common iliac vessels were ligated, the flow rate reduced in proportion to the amount of remaining tissue and the left leg removed. This facilitated the measurement of tissue metabolites during perfusion. At 80 min perfusions were terminated.

In-vivo measurements

In-vivo measurements were made in the same batch of rats used for perfusion studies. Concentrations of tissue metabolites were made on samples taken from rats which were subjected to the same anaesthesia, heparinization and oxygenation procedure as used for perfusion studies. However, this treatment was not applied to rats in which rates of protein synthesis were measured. Tissue processing and analysis for all in-vivo samples was carried out at the same time as in-vitro samples, to make the data comparable.

Measurement of protein synthesis in-vitro

Rates of muscle protein synthesis were estimated as described previously (Preedy & Garlick 1983) with slight modification. At the end of the washout period (corresponding to: time 0 min) 4 μCi (28 μmol) of L-[U-^{14}C]tyrosine was added via 38 mL of perfusate. The additional perfusate was identical to the recirculating medium, except for the presence of radiolabel. This procedure of introducing a high concentration of tyrosine was necessary to increase intracellular free tyrosine specific radioactivity rapidly to a value similar, but not equal to, the specific radioactivity of free tyrosine in plasma (Preedy & Garlick 1983).

Fractional rates of protein synthesis in gastrocnemius muscles (defined as the percentage of tissue protein renewed each day by synthesis i.e. k_S, %/day) were then calculated from the specific radioactivity of tyrosine in protein (S_B) and free tyrosine in muscle (S_i) during the labelling period (t days) from the equation:

$$k_S = \frac{S_B \times 100}{S_i \times t} \text{ (\%/day)}$$

We have previously described the theoretical and practical basis of these measurements (Preedy & Garlick 1983).

Measurement of protein synthesis in-vivo

The constant infusion technique of Garlick et al (1973, 1975) was used to obtain in-vivo rates of protein synthesis in gastrocnemius muscle. The same isotope used in-vitro was used for in-vivo measurements (i.e. L-[U-^{14}C]tyrosine). Fractional rates of protein synthesis were calculated from the relationship between specific radioactivities of protein-bound and tissue-free tyrosine in samples taken after 3 h infusion (Garlick et al 1973, 1975; Preedy & Garlick 1983).

Measurement of vascular resistance

Vascular resistance was estimated from an in-line pressure gauge (Preedy 1981). As perfusate flow was pulsatile, minimum and maximum values were recorded, and the mean calculated. A correction was made for resistance by the perfusion apparatus, in the absence of the hemicorpus.

Measurements of tissue and perfusate metabolites

Muscles were exposed, frozen between aluminium blocks and stored at −196°C until analysis. Frozen tissue was powdered and then precipitated in 0·9 M $HClO_4$ and the supernatants neutralized with 4 M KOH. For estimation of K^+ release, analysis was made on perfusate plasma. For measurement of lactate and glucose metabolism by the preparation, estimations were made on whole perfusate precipitated directly into ice-cold 0·9 M $HClO_4$ or 0·004 M uranyl acetate in 0·15 M NaCl, respectively. Where appropriate, i.e. glucose, lactate, K^+ and amino acid metabolism, measurements were made on the perfusate in the absence of perfused muscle to account for metabolism of erythrocytes alone (Preedy 1981). Assays were as follows: cAMP assays were carried out by Dr P. J. Reeds (Rowett Research Institute, Aberdeen, Scotland) with a kit (TRK.432, Amersham International); ATP and phosphocreatine, (Lamprecht et al 1974); lactate, (Gutman & Wahlefeld 1974); amino acids by ion exchange chromatography, (Preedy 1981); water content, by drying to constant weight; K^+ (Collins & Polkinhorne 1952); glucose by the GOD-Perid kit from Boehringer; arteriovenous difference in percentage oxygen saturation of haemoglobin and pH as described previously (Selman & Tait 1976; Preedy et al 1984).

Statistics

Data for some perfusions were not included for analysis. These were perfusions rejected on the basis of criteria we previously described as suitable for use in hemicorpus studies (Preedy et al 1984). Rejection occurred if muscle ATP or phosphocreatine fell below 50% or if lactate content rose above 275% of the mean values for the remaining preparations in that group (Preedy et al 1984). Using this technique 1, 2 and 1 perfusions were rejected in control, papaverine and phentolamine groups, respectively. All data are presented as mean ± s.e.m., with the number of observations in parentheses. Significance was assessed by Student's *t*-test, using pooled variance and 1-tailed tests for vascular resistance (as vasodilators do not cause an increase), and 2-tailed tests for the remaining data.

Results

Table 1 shows that addition of papaverine to the perfusate caused a 20% fall in vascular resistance during the first 40 min, and a 25% fall between 40–80 min. Phentolamine had no significant effect on resistance during either time period. This may have been due to lack of sympathetic tone in the hemicorpus. In these studies it was not possible to assess directly the effects on skeletal muscle. As we have previously shown (Preedy & Garlick 1981), non-muscle tissue such as

Table 1. Vascular resistance during muscle perfusion and the effects of papaverine and phentolamine. Perfusions were prepared from male rats as described in the text. During operative procedures, preparations were hemisected so sympathetic tone was abolished. Either papaverine or phentolamine was included in the perfusate of treated preparation and vascular resistance measured with an in-line pressure gauge. The data was corrected for resistance of the perfusion circuit in the absence of muscle. The discontinuity of vascular resistance between 39 and 45 min was due to ligature of left common iliac vessels and dissection of the left leg to obtain concentrations of tissue metabolites. Other experimental details are described in the Methods section.

Perfusion time (min)	Vascular resistance (mmHg/preparation)		
	Control	+Papaverine	+Phentolamine
0	46±3 (7)	39±1 (6)*	47±3 (5)
7	48±3 (7)	40±2 (5)*	49±4 (4)
23	53±5 (4)	42±2 (5)*	49±4 (3)
39	58±3 (8)	46±2 (6)**	58±5 (5)
45	36±3 (7)	28±2 (6)*	32±3 (5)
60	50±6 (5)	35±4 (4)*	44±6 (5)
80	58±5 (8)	47±4 (6)	58±9 (5)

Differences between control and papaverine-treated perfusions; *$P<0.05$; **$P<0.01$.

skin and bone are also perfused. Nevertheless, the information on resistance is important as it pertains to peripheral metabolism. However, in all three groups there was a gradual increase in pressure throughout the perfusion, even in the presence of induced vasodilation. Possible causes for the rise during perfusion include emboli of lipid aggregates (Belzer et al 1968). The discontinuity between 39 and 45 min resulted from amputation of the left leg.

We have shown previously (Preedy et al 1984) that muscle concentrations of ATP, phosphocreatine and lactate are suitable indices of metabolic integrity in isolated perfused skeletal muscle. Table 2 shows that concentrations of ATP and water from control muscles at 40 and 80 min were similar to in-vivo values. However, whilst the concentration of phosphocreatine was maintained at 40 min, there was a small decline at 80 min. Lactate concentration was elevated at both time points, presumably because of the inability of skeletal muscle to metabolize this compound. Despite these changes, we consider the control preparations to be in a metabolically acceptable state (Preedy et al 1984). The two vasodilators had no effect on muscle ATP, phosphocreatine, lactate or water at 40 min of perfusion. Papaverine caused a significant reduction in both ATP and phosphocreatine as well as an increase in lactate at 80 min. By contrast, phentolamine had no significant effects on ATP, phosphocreatine, lactate or water at 80 min.

We also examined indices of metabolic integrity in the whole preparation, using parameters previously shown to be useful in assessing muscle function (Preedy et al 1984; Ruderman et al 1971; Rennie & Holloszy 1977; Hillgartner et al 1982; Ward & Buttery 1979). Table 3 shows that papaverine increased lactate output in both perfusion periods, to 4–17 times the rate in control perfusions. In contrast, phentolamine induced a net uptake from the perfusate in the first period, and subsequently a net output in the second period. The differences between these two time periods was statistically significant; $P<0.001$. There was no significant effect of vasodilators on glucose uptake in either period (Table 3). The response in K^+ release, between 7–40 min, was similar to the trend in lactate output, but was not significant when compared with control perfusions at the corresponding time points. However, in phentolamine-treated perfusions, K^+ release and glucose uptake in the 7–40 min period were significantly different to values in the 40–80 min period; $P<0.001$ and $P<0.01$, respectively. There were no significant effects of vasodilators on arteriovenous differences in oxygen saturation of haemoglobin and pH (Table 3).

Table 4 shows that rates of protein synthesis in muscle of control perfusions, were comparable with in-vivo values. However, synthesis rates were reduced by both papaverine and phentolamine. Between 0 and 40 min of labelling, papaverine and phentolamine inhibited synthesis by 30 and 28%, respectively. In the subsequent period the decrease was less pronounced, i.e. 14 and 25%, respectively, and was only significant for phentolamine. Table 5 also shows that although the vasodilators reduced the concentrations of cAMP, by small amounts, the differences were not statistically significant.

Table 2. Effect of papaverine and phentolamine on concentrations of ATP, phosphocreatine, lactate and water content in muscle in-vivo and in-vitro. In-vivo data were obtained from the same batch of rats as used for perfusion studies, but muscles were freeze-clamped immediately after anaesthesia and heparinization. In-vitro data were obtained from perfused muscle as described in the Methods section. Muscle concentrations of ATP, phosphocreatine and lactate were estimated in quadriceps. Muscle water was measured in tibialis anterior. Intermediate values were obtained from removal of the left leg, whilst values at 80 min were from muscles at the end of perfusion.

Treatment		Content (μmol g^{-1} wet weight)			
		ATP	Phosphocreatine	Lactate	Water (% w/w)
In-vivo		6·1±0·4 (7)	9·6±0·7 (7)	4·2±0·3 (8)	76·9±0·2 (4)
In-vitro	Min				
Control perfusion	40	5·8±0·3 (6)	8·7±0·6 (7)	14·8±2·0 (8)***	76·4±0·3 (8)
	80	6·5±0·3 (8)	5·5±0·5 (8)***	24·2±3·0 (7)***	77·4±0·2 (8)
+Papaverine	40	6·1±0·4 (6)	7·3±0·8 (6)	15·0±0·5 (6)***	76·7±0·6 (6)
	80	5·7±0·2 (6)†	3·6±0·5 (6)***†	34·2±3·9 (6)***†	77·9±0·1 (6)**
+Phentolamine	40	6·7±0·4 (6)	10·0±0·5 (5)	11·8±0·9 (5)***	76·6±0·4 (5)
	80	7·3±0·7 (5)	5·4±0·3 (5)***	25·5±2·1 (5)***	77·3±0·3 (5)

Differences between in-vivo and in-vitro data; **$P<0.01$; ***$P<0.001$. Differences between control perfusions and vasodilator treated perfusions at the corresponding time point, †$P<0.05$.

Table 3. Effect of papaverine and phentolamine on lactate, potassium and glucose metabolism and arterio-venous differences in pH and oxygen saturation of haemoglobin. Control and treated perfusions were prepared as described in the Methods section. Rates of metabolism are calculated in terms of the amount of skeletal muscle in each preparation. Corrections were made for metabolism of glucose, lactate and K^+ by the perfusate in absence of the isolated preparation. The negative prefix for lactate metabolism in phentolamine-treated perfusions at 7–40 min indicates a net uptake.

Parameter	Time	Control	+Papaverine	+Phentolamine
Lactate output	7–40	0·013±0·019 (8)	0·230±0·023 (6)***	−0·063±0·007 (4)*
(μmol g^{-1} min^{-1})	40–80	0·075±0·022 (8)	0·192±0·023 (6)**	0·138±0·017 (5)
K^+ release	7–40	0·021±0·010 (7)	0·038±0·018 (6)	0·006±0·006 (5)
(μmol g^{-1} min^{-1})	40–80	0·038±0·008 (7)	0·035±0·016 (6)	0·038±0·007 (5)
Glucose uptake	7–40	0·31 ±0·02 (8)	0·30 ±0·03 (6)	0·32 ±0·02 (5)
(μmol g^{-1} min^{-1})	40–80	0·24 ±0·04 (8)	0·24 ±0·01 (6)	0·15 ±0·03 (5)
AV diff	5	0·037±0·013 (4)	0·089±0·029 (4)	0·038±0·002 (4)
(pH)	40	0·052±0·012 (4)	0·047±0·006 (4)	0·041±0·012 (4)
	80	0·044±0·005 (4)	0·054±0·020 (4)	0·062±0·062 (4)
AV diff	5	45·5 ±4·4 (4)	36·3 ±5·8 (4)	54·6 ±2·4 (4)
(% saturation	40	52·2 ±5·2 (4)	48·7 ±3·8 (4)	56·6 ±3·7 (4)
of haemoglobin)	80	54·4 ±6·5 (4)	52·4 ±6·7 (4)	59·8 ±4·7 (4)

Differences between control and treated perfusions at corresponding time points; *$P<0·05$; **$P<0·01$; ***$P<0·001$.

Table 4. Effects of vasodilators on muscle protein synthesis rates in-vivo and in control and treated muscle preparations in-vitro. Fractional rates of protein synthesis (defined as the percentage of tissue protein renewed each day by synthesis, %/day) were calculated from the relationship between the specific radioactivities of free L-[U-^{14}C]tyrosine in muscle homogenates or protein-incorporated L-[U-^{14}C]tyrosine in muscle hydrolysates. Rates of protein synthesis were measured in gastrocnemius muscles after either 3 h constant infusion of isotope (in-vivo) or 40 and 80 mins perfusion (in-vitro).

Treatment	Period of measurement (min)	Fractional synthesis rate (%/day)	cAMP (pmol g^{-1} wet weight)
In-vivo		12·4±0·6 (5)	—
In-vitro			
Control perfusion	0–40	15·9±0·9 (7)*	93·6±9·5 (5)
	0–80	12·8±1·6 (8)	—
+Papaverine	0–40	11·5±0·6 (5)††	78·5 (2)
	0–80	11·0±0·8 (5)	—
+Phentolamine	0–40	11·2±0·8 (5)††	86·0±6·0 (3)
	0–80	9·5±0·4 (5)†	—

Differences between in-vivo and in-vitro measurements, *$P<0·05$. Differences between control or treated perfusions at corresponding time points; †$P<0·05$, ††$P<0·01$.

Concentrations of phenylalanine and the branched chain amino acids in perfusate and muscle at 80 min of perfusion are displayed in Table 5. In-vivo and in-vitro studies have demonstrated that branched chain amino acids can influence muscle protein synthesis (eg Li & Jefferson 1978; Preedy & Garlick 1987). Furthermore, as phenylalanine is neither synthesized nor degraded by the hemicorpus, changes in its concentration will reflect the net balance between protein synthesis and breakdown (Jefferson et al 1977). Table 5 shows that papaverine increased the concentration of all four free amino acids, in muscle and perfusate. Increases in the branched chain amino acids therefore suggest that they were not responsible for mediating the fall in protein synthesis. The increase in phenylalanine indicates that protein breakdown may be increased and/or unchanged. In either situation, papaverine markedly perturbed protein balance in-vitro. Phentolamine had no effect on free phenylalanine and branched chain amino acids. As protein synthesis was reduced, it is possible that maintenance of free phenylalanine levels reflects a reduction in protein breakdown.

Table 5. Effect of vasodilators on free amino acid levels in muscle and perfusate. Free amino acids were measured in acid-soluble fractions of quadriceps muscles and whole perfusate at the end of perfusion, as described in the Methods section.

	Control	+Papaverine	+Phentolamine
Muscle (nmol g^{-1} wet weight):			
Valine	138±5 (7)	186± 6 (6)***	132± 9 (4)
Isoleucine	44±4 (7)	70± 7 (6)**	38± 5 (4)
Leucine	99±6 (7)	146± 9 (6)**	99±11 (4)
Phenylalanine	75±3 (7)	99±11 (6)**	75± 3 (4)
Whole perfusate (nmol mL^{-1}):			
Valine	133±4 (6)	147± 5 (6)*	134± 8 (5)
Isoleucine	36±3 (6)	45± 1 (6)*	33± 2 (5)
Leucine	99±4 (6)	110± 3 (6)	92± 5 (5)
Phenylalanine	75±2 (6)	79± 2 (6)	73± 6 (5)

Differences between control and vasodilator treated perfusions: *$P<0·05$; **$P<0·01$; ***$P<0·001$.

Discussion

Our primary purpose was to investigate whether vasodilators can improve the performance of a perfused isolated system, without having adverse effects on tissue metabolism. Numerous studies routinely include vasodilators in the perfusate to improve the metabolic integrity of the isolated muscle. None of these studies have systematically investigated their use, especially with respect to protein metabolism. We have previously demonstrated the suitability of the isolated perfused muscle system to investigate protein and intermediary metabolism and have shown that it possesses features common to skeletal muscle in-vivo (Preedy & Garlick 1983; Preedy et al 1984). We are aware of the fact that the concentrations of vasodilators were above the levels prescribed for therapeutic purposes. However, it is important to make a number of points. The concentrations of phentolamine and papaverine were similar to those used in other perfusion studies. As we were testing the hypothesis that these vasodilators improve the performance of perfused

tissue, it was imperative that the concentrations were comparable. Another point is that the degradation rates of the vasodilators were unknown. It was not possible to speculate on the final concentrations of papaverine and phentolamine because of the artificial conditions in-vitro. For example, in previous studies we ascertained that hormones such as glucagon were subjected to very high losses (>98%) in the hemicorpus preparation (Preedy, V. R. & Garlick, unpublished). Nevertheless, the concentrations used here were also similar to other in-vitro studies where, for example, inhibition of phosphodiesterase occurred in skeletal muscle, i.e. Triner et al (1970). See also Rushakoff et al (1978).

The data demonstrated that papaverine could reduce vascular resistance in a system where sympathetic tone was absent, (the preparation was hemisected above the diaphragm before aortic perfusion). However, interpretation of these data requires caution, as blood vessels in non-muscle tissue also influence vascular resistance (Nyhof et al 1978). The data also showed that papaverine induced impairment of skeletal muscle metabolism, as judged by measurement of various parameters (ATP, phosphocreatine, lactate and water content and lactate efflux), including a reduction in muscle protein synthesis. Although phentolamine did not cause similar alterations in tissue integrity, it also caused reductions in protein synthesis and possibly a decrease in breakdown.

The exact mechanism whereby papaverine exerts its effects is unclear. Although it is known that it acts as a phosphodiesterase inhibitor in skeletal muscle (Triner et al 1970), this does not explain its action on protein synthesis. Beatty & Bocek (1970) suggested that elevations in cAMP may reduce rates of protein synthesis in-vitro, but preliminary analysis showed no large differences in levels of cAMP in control and papaverine-treated muscles. In fact cAMP levels in quadriceps muscles from control perfusions at 40 min were 16% higher than in vasodilator-treated perfusion at the corresponding time point. This is consistent with the observations of Garber et al (1978), which suggest that inhibitors of phosphodiesterases have minimal effect on muscle (as measured by alanine and glutamine release) when added alone, but instead, amplify the effects of other agents which increase levels of cyclic nucleotides. The effects of papaverine on synthesis, therefore, probably occur via some other mechanism. For example, Sheppard et al (1979) reported that although papaverine was thought by others to block electron transport and oxygen uptake, in fact it was able to reduce cellular ATP levels by enhancing intracellular phosphohydrolyase activity. This would perhaps account for papaverine having no effect on hemicorpus oxygen uptake whilst reducing ATP and phosphocreatine levels. Whether the changes in energy status with papaverine were directly responsible for the decrease in protein synthesis remains to be determined.

There is no concise information on the mode of action of phentolamine. The possibility exists, however, that it may also inhibit phosphodiesterase activity. Rushakoff et al (1978) have shown that the inclusion of this vasodilator in perfused hindquarters increases cAMP production from the preparation, by just over two-fold. As with papaverine, analysis of cAMP levels in the muscles of phentolamine-treated preparations of the present experiments showed no difference when compared with controls. The lack of effect of phentolamine on ATP, phosphocreatine and lactate suggests that it may have inhibited muscle protein synthesis by some other mechanism which is unrelated to energy status or muscle.

In conclusion, our data have important implications for the therapeutic and experimental use of papaverine and phentolamine, as both of these vasodilators detrimentally alter the metabolism of skeletal muscle in-vitro. This may have profound effects in the whole body, as muscle comprises 40% of whole body weight and 25% of whole body protein synthesis in man and laboratory animals. The acute or chronic use of these substances therefore requires further investigation to see if they have the same effect on skeletal muscle in the whole body as they do in the perfused rat hemicorpus.

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