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A ^{31}P-NMR STUDY OF THE ACUTE EFFECTS OF ALTERED β-ADRENOCEPTOR STIMULATION ON THE BIOENERGETICS OF SKELETAL MUSCLE DURING CONTRACTION

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Abstract—The effects of acute administration of a β-adrenoceptor agonist (isoprenaline) or antagonist (propranolol) on skeletal muscle contraction and metabolism in the rat have been studied *in vivo* using ^{31}P-nuclear magnetic resonance spectroscopy and conventional metabolite analysis.

In resting muscle, isoprenaline caused a three-fold increase in cyclic AMP concentration, whereas propranolol decreased cyclic AMP concentration by 40%.

Isometric contraction of gastrocnemius muscle at a frequency of 4 Hz was caused by supramaximal sciatic nerve stimulation. Altered β-adrenoceptor stimulation had no effect on contractile performance at any time during the 30 min stimulation period. During the initial stimulation period (0–4 min) intracellular pH decreased to significantly lower values in the isoprenaline-treated animals (6.24 ± 0.03) compared to either the control (6.44 ± 0.08) or propranolol-treated (6.42 ± 0.08) groups.

During the subsequent stimulation period (after 15–30 min stimulation at 4 Hz), pH recovered in all experimental groups to values >6.90 and phosphocreatine concentration achieved a constant level at 35–40% of resting values. Calculation of free ADP concentrations using ^{31}P-NMR determined metabolite concentrations and the creatine phosphokinase equilibrium showed that at similar tension development, $[ADP]_{free}$ varied between the three experimental groups; with the lowest (47 ± 4 μM) and highest (73 ± 4 μM) values being calculated for the β-adrenoceptor agonist- and antagonist-treated groups respectively.

Upon termination of stimulation, recovery of phosphocreatine concentration to pre-stimulation values was rapid and similar in all experimental groups. However, gastrocnemius muscle ATP concentration, determined by ^{31}P-NMR and analysis of freeze-clamped muscle, was lower in the isoprenaline-treated group.

This study has shown that although altered β-adrenoceptor stimulation had no effect on contractile performance, significant changes in muscle metabolism were observed *in vivo*; these effects are discussed with respect to the role of β-adrenoceptors in skeletal muscle.

Catecholamines can cause physiological and metabolic changes in skeletal muscle [1]. Increases in plasma adrenaline concentration may alter contractile force and twitch duration for a given contracting muscle [2, 3], increase glycogenolytic [4] and oxidative fluxes [5], and alter cation transport [6, 7]. However, because all of these effects of adrenoceptor stimulation can also be induced by other regulators, it has proven difficult to ascribe quantitative importance specifically to adrenoceptor-dependency. In particular, the fatigue experienced by patients during β-blocker therapy [8, 9] does not have an adequate biochemical explanation.

In the present study, we have been able to measure physiological performance of rat gastrocnemius muscle *in vivo* and to correlate this with bioenergetic changes measured in parallel using ^{31}P-nuclear magnetic resonance (NMR) spectroscopy. In this way we have investigated the acute effects of β-adrenoceptor agonist or antagonist administration on skeletal muscle metabolism at rest and during muscle contraction.

MATERIALS AND METHODS

Male Wistar rats (200–220 g) were prepared for muscle stimulation as described previously [10, 11]. Anaesthesia was caused by intraperitoneal injection of pentabarbital sodium (60 mg/kg body wt). The tail artery was exposed and cannulated. The left sciatic nerve was exposed and silver electrodes sewn into place in contact with the nerve. The wound was sutured and brass pins were forced through the knee and ankle joints and the leg immobilised in a perspex frame. The distal tendon of the gastrocnemius muscle was ligatured and attached to a force displacement transducer. The animal was placed into a perspex cradle and a three-turn, 14 mm surface coil [12] was placed over the medial head of the gastrocnemius muscle. The surface coil, which acted as transmitter

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Table 1. Effects of acute isoprenaline or propranolol administration on gastrocnemius muscle metabolites at rest

	Control	+Isoprenaline	+Propranolol
ATP	6.53 ± 0.21	6.68 ± 0.39	6.80 ± 0.17
ADP	1.17 ± 0.04	1.08 ± 0.05	1.16 ± 0.05
AMP	0.04 ± 0.004	0.04 ± 0.003	0.04 ± 0.005
IMP	0.12 ± 0.017	0.21 ± 0.048	0.07 ± 0.014†
Phosphocreatine	20.2 ± 1.1	21.2 ± 1.2	20.1 ± 0.9
Creatine	7.7 ± 0.4	7.2 ± 0.4	7.9 ± 1.3
Lactate	1.36 ± 0.14	2.71 ± 0.13*	1.37 ± 0.13‡
Glycogen[a]	33.5 ± 1.4	30.2 ± 1.7	31.3 ± 1.8
Cyclic AMP[b]	380 ± 18	1093 ± 92*	224 ± 22‡§
n	8	9	6

Gastrocnemius muscles were freeze-clamped 5 min after completion of isoprenaline or propranolol infusion. Metabolite concentrations are given as μmol/g of tissue, except [a]μmol glucosyl equiv./g of tissue and [b]pmol/g of tissue. Statistical significance is shown as: *$P < 0.001$ for isoprenaline-treated versus controls; †$P < 0.05$, ‡$P < 0.001$ for isoprenaline-treated versus propranolol-treated; and §$P < 0.01$ for propranolol-treated versus controls.

and receiver, was tuned to the ^{31}P-resonance frequency and the whole apparatus was placed into the vertical bore of a wide bore 4.3T magnet. Anaesthesia was maintained by delivery of 0.5–1% halothane in N_2O:O_2 (1:1) via a face mask. Animal temperature was monitored using a rectal thermistor probe and maintained at 37° by a stream of warm air.

Magnetic field homogeneity was adjusted whilst observing the proton signal of tissue water. At the start of each experiment the muscle was adjusted to optimise the maximal isometric contraction [10, 13]. ^{31}P-NMR spectra were collected and quantified as described previously [11, 14] and the pH was calculated from the chemical shift of inorganic phosphate (P_i) relative to phosphocreatine (PCr) [15].

An initial resting spectrum was collected with a pulse recycle time of 10 sec (fully-relaxed acquisition), followed by spectra with a pulse repetition time of 2 sec. The β-adrenoceptor agonist (isoprenaline; 500 μg/kg) or antagonist (propranolol; 2.5 mg/kg) dissolved in 0.9% NaCl, 1 mg/ml ascorbate were infused slowly over 3–5 min into the tail artery, three further resting spectra were collected (2 sec recycle time) and then sciatic nerve stimulation (50 μsec pulse, 40 V, 4 Hz) was commenced and spectra (2 min time resolution) were collected for 30 min; stimulation was terminated and recovery spectra collected. After 20 min recovery a second spectrum was collected under fully-relaxed conditions and the animal was removed from the magnet and gastrocnemius muscles from stimulated and contralateral limbs freeze-clamped for metabolite analysis. Adenine nucleotides, IMP, creatine and phosphocreatine were determined using the HPLC method of [16]. Lactate was determined spectrophotometrically [17], glycogen was enzymically degraded to glucose using amyloglucosidase and the glucose concentration determined [18]. Cyclic AMP was assayed using a competitive binding protein assay [19].

Data were analysed where appropriate by one-way analysis of variance and paired or unpaired Student's *t*-test. All results are reported as means ± SE and statistical significance between values was defined as a value of $P < 0.05$.

Materials. (−)-Isoprenaline and DL-propranolol were obtained from Sigma. [2,8-^{3}H]cyclic AMP was obtained from Amersham International.

RESULTS

Resting muscle

Intra-arterial infusion of isoprenaline increased heart-rate and decreased arterial blood pressure [20], whereas propranolol had no effect on resting heart-rate or arterial blood pressure [11]. The effects of β-adrenoceptor agonist or antagonist infusion on resting gastrocnemius muscle metabolites are shown in Table 1. Isoprenaline caused a 3-fold increase in cyclic AMP concentration and caused a significant increase in muscle lactate concentration, whereas propranolol reduced cyclic AMP concentration by 40%. ATP and phosphocreatine concentrations were not affected by altered β-adrenoceptor stimulation in resting muscle. Analysis of resting muscle ^{31}P-NMR spectra confirmed that phosphocreatine concentration was not affected and showed no effect of either isoprenaline or propranolol on muscle pH or free ADP concentration (Table 2). Table 2 also shows that isoprenaline infusion caused an insignificant 40% decrease in NMR-visible P_i; however, if each spectral series was analysed separately it was found that for each animal studied isoprenaline caused a significant decrease in P_i in resting muscle (before infusion: 2.4 ± 0.3 μmol/g; 5 min after completion of infusion: 1.2 ± 0.3 μmol/g; $P < 0.05$ for paired *t*-test). Furthermore, the decrease in P_i concentration temporally coincided with the appearance of a broad peak at 6.8 ppm in the ^{31}P-NMR spectrum, which has previously been assigned as a sugar phosphate resonance [21]. In a separate series of experiments, we have shown that intra-arterial or intraperitoneal administration of isoprenaline (500 μg/kg body wt) or adrenaline (100 μg/kg body wt) caused a similar decrease in intracellular P_i concentration and a prolonged elevation of a peak at 6.8 ppm, with

Table 2. Resting metabolite concentrations in rat gastrocnemius muscle *in vivo* determined by ^{31}P-NMR

	Control	+Isoprenaline	+Propranolol
Total creatine[a]	27.9 ± 1.4	28.4 ± 0.9	28.0 ± 2.1
ATP[a]	6.53 ± 0.21	6.68 ± 0.39	6.80 ± 0.17
Phosphocreatine	24.9 ± 1.4	25.3 ± 1.8	25.0 ± 1.4
P_i	2.3 ± 0.4	1.4 ± 0.3	2.4 ± 0.5
pH	7.03 ± 0.02	7.01 ± 0.02	7.00 ± 0.01
ADP_{free}[b]	7.6 ± 2.3	7.5 ± 0.9	7.3 ± 2.7
N	6	6	6

[a]Values are taken from freeze-clamping studies (see Table 1). ^{31}P-NMR data are obtained from spectra assuming that total tissue ATP is NMR-visible. All values are expressed as μmol/g of tissue except [b] which is expressed as μM assuming a cell water content of 0.67 ml/g of tissue.

Table 3. Changes in gastrocnemius muscle twitch-tension during stimulation at 4 Hz in animals treated with isoprenaline or propranolol prior to initiation of stimulation

Time (min)	Control	+Isoprenaline	+Propranolol
Initial	1.83 ± 0.08	1.93 ± 0.14	1.75 ± 0.11
Peak	2.77 ± 0.10	2.96 ± 0.23	2.59 ± 0.17
10	1.33 ± 0.05	1.52 ± 0.14	1.33 ± 0.10
20	1.23 ± 0.05	1.17 ± 0.11	1.12 ± 0.06
30	1.15 ± 0.06	1.15 ± 0.15	1.07 ± 0.09

Twitch-tension (mean ± SE) is expressed as g tension developed per g of body wt. for at least 6 experiments in each group. Peak tension was developed for all groups approximately 90 sec after initiation of stimulation at 4 Hz. Final steady-state values were 63 ± 2, 60 ± 3 and 61 ± 2% of initial twitch-tension for control, isoprenaline- and propranolol-treated groups respectively.

no change in intracellular pH (Challiss and Hayes, unpublished results).

Muscle stimulation at 4 Hz

Twitch-tensions for gastrocnemius muscles of control and antagonist- or agonist-treated animals are shown in Table 3. No significant effects of altered β-adrenoceptor stimulation were observed throughout the stimulation period.

^{31}P-NMR revealed that PCr concentration decreased markedly during the first 4 min of stimulation in all experimental groups (Fig. 1), with a stoichiometric increase in P_i concentration observed for control and propranolol-treated animals. For animals treated with isoprenaline prior to muscle stimulation, the stoichiometric relationship between PCr decrease and P_i increase was not observed; during the initial 2 min of stimulation, the area of the peak at 6–7 ppm increased and only about 90% of phosphate liberated from PCr breakdown was quantified in the P_i peak of the ^{31}P-NMR spectrum. The peak at 6–7 ppm was broad and heterogeneous making it difficult to obtain quantitative information on the concentrations of metabolites which compose this region of the spectrum; however, a prominent resonance at 6.3 ppm was observed, as well as the resonance at 6.8 ppm observed at rest. After 4 min

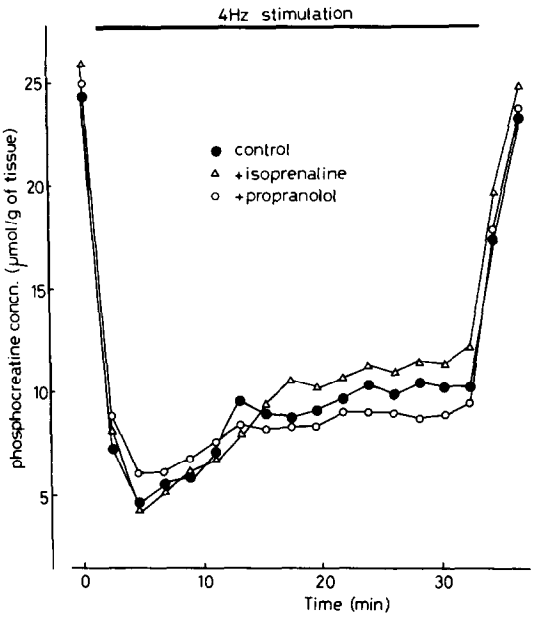

Fig. 1. Changes in phosphocreatine concentration in gastrocnemius muscle during stimulation at 4 Hz and during recovery for control, isoprenaline-treated and propranolol-treated animals. Mean values for at least 5 experiments in each group are shown. Error bars are omitted for clarity.

muscle stimulation, the peak area at 6.8 ppm had decreased, such that quantitation became impossible due to signal:noise considerations.

Intracellular pH decreased precipitously upon stimulation, such that pH_i was 6.42 ± 0.06 (6) after 4 min stimulation in gastrocnemius muscle of control animals (Fig. 2). A similar decrease in pH was observed in propranolol-treated animals; however, isoprenaline treatment exaggerated the initial fall in pH, causing pH_i to decrease to 6.27 ± 0.04 (5) after 4 min muscle stimulation ($P < 0.05$, compared to control and propranolol-treated values).

In a parallel series of experiments to complement ^{31}P-NMR data, gastrocnemius muscle of animals receiving isoprenaline, propranolol or saline vehicle were stimulated unilaterally at 4 Hz for 5 min, at

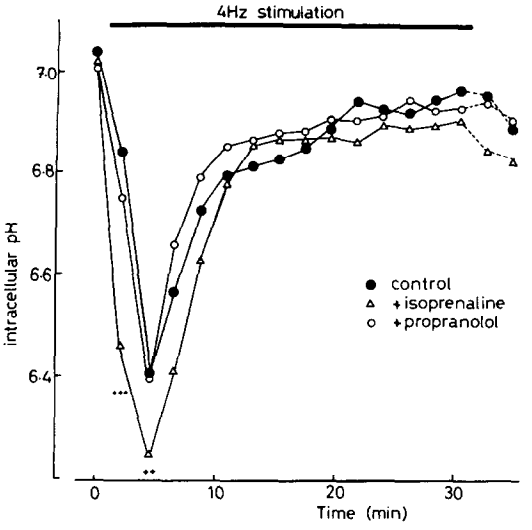

Fig. 2. Changes in intracellular pH in gastrocnemius muscle during stimulation at 4 Hz and during recovery for control, isoprenaline-treated and propranolol-treated animals. Mean values for at least 5 experiments in each group are shown. Error bars are omitted for clarity. Statistical significance (Student's *t*-test, for unpaired observations) for isoprenaline-treated versus control values is indicated as $^{++}P < 0.01$, $^{+++}P < 0.001$.

which time the stimulated and contralateral gastrocnemius muscles were freeze-clamped. The effects of stimulation on muscle metabolite concentrations are presented in Table 4. After 5 min stimulation at 4 Hz, phosphocreatine and ATP concentrations had decreased by 70% and 20% respectively for all experimental groups. Comparison of the β-agonist and β-antagonist treatment groups revealed a number of differences. The concentrations of AMP and IMP in stimulated muscle were significantly higher in isoprenaline-infused compared to propranolol-infused animals (Table 4). The increase in IMP concentration may be due to the accentuated acidosis associated with the initial phase of stimulation in this experimental group (Fig. 2). It has previously been shown that IMP appears at about 6.3 ppm in the ^{31}P-NMR spectrum, thus IMP may contribute to the broad peak observed in the ^{31}P-NMR spectrum upfield of inorganic phosphate.

After the initial decreases in phosphocreatine concentration and intracellular pH, a rapid recovery occurred (Figs 1 and 2), such that after 15 min stimulation at 4 Hz, the phosphocreatine concentration was approximately constant for each experimental group. During this period (16–32 min of stimulation at 4 Hz), it was observed that PCr achieved a significantly higher steady-state concentration in isoprenaline-treated compared to propranolol-treated animals ($P < 0.05$), with the control group occupying an intermediate position. For all experimental groups pH_i was >6.90 for the 16–32 min period of gastrocnemius muscle stimulation (Fig. 2; Table 5), suggesting that energetic requirements of contracting muscle are satisfied by aerobic pathways.

It is known from NMR measurements that ATP

Table 4. Effects of isoprenaline on propranolol administration on changes in gastrocnemius muscle metabolites during supramaximal isometric contraction at 4 Hz

	Control		+Isoprenaline		+Propranolol	
	Non-stimulated	Stimulated	Non-stimulated	Stimulated	Non-stimulated	Stimulated
ATP	6.67 ± 0.41	5.37 ± 0.34*	6.51 ± 0.24	5.00 ± 0.32†	6.85 ± 0.31	5.67 ± 0.42
AMP	0.03 ± 0.002	0.12 ± 0.033‡	0.05 ± 0.008	0.21 ± 0.037‡	0.04 ± 0.003	0.09 ± 0.011†‖
IMP	0.09 ± 0.03	0.97 ± 0.23‡	0.19 ± 0.06	1.84 ± 0.42‡	0.08 ± 0.01	0.78 ± 0.17‡‖
Phosphocreatine	21.9 ± 2.1	6.2 ± 0.7‡	23.4 ± 1.5	5.8 ± 1.0‡	21.9 ± 1.9	7.1 ± 0.4‡
Creatine	7.0 ± 0.6	19.8 ± 1.7‡	6.3 ± 0.4	19.4 ± 2.8‡	7.3 ± 0.2	20.0 ± 1.8‡
Cyclic AMP[a]	421 ± 71	329 ± 29	591 ± 70	487 ± 45§	245 ± 24§	233 ± 31§¶
N	5	5	6	6	5	5

Gastrocnemius muscles were freeze-clamped 5 min after commencement of unilateral muscle stimulation at 4 Hz. Metabolite concentrations are expressed as μmol/g of tissue, except [a]pmol/g of tissue. Statistical significance is denoted by: *$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$ for stimulated versus non-stimulated conditions, §$P < 0.05$ for drug-treatment versus control, and ‖$P < 0.05$, ¶$P < 0.01$ for propranolol-treated versus isoprenaline-treated groups.

Table 5. Concentrations of phosphorus-containing metabolites in gastrocnemius muscle *in vivo* after 25 min stimulation at 4 Hz determined by ^{31}P-NMR

	Control	+Isoprenaline	+Propranolol
Phosphocreatine	9.9 ± 0.9 (5)	11.2 ± 1.2 (5)	8.8 ± 1.3 (6)
ATP	6.47 ± 0.20 (5)	6.34 ± 0.24 (5)	6.61 ± 0.19 (6)
pH	6.92 ± 0.02 (5)	6.90 ± 0.02 (5)	6.92 ± 0.01 (6)
ADP_{free}[a]	59 ± 5 (5)	47 ± 4 (5)	73 ± 4 (6)*

Total creatine values are assumed to be identical to those determined in gastrocnemius muscle at rest (see Table 1). All values are expressed as μmol/g of tissue except [a] which is expressed as μM assuming a cell water content of 0.67 ml/g of tissue. Statistical significance is shown as: *$P < 0.01$ for propranolol- versus isoprenaline-treated.

and pH_i are constant and similar for each group (Table 5), also from determinations made on freeze-clamped muscle it is known that total creatine (creatine + PCr) concentration is not affected by the stimulation protocol; therefore it follows from the creatine phosphokinase equilibrium:

$$[ADP] = \frac{[Creatine][ATP]}{[PCr][H^+] \cdot K_{eq}}$$

where K_{eq} is the equilibrium constant for creatine phosphokinase (see Ref. 22), that at similar twitch-tension development, the steady-state free ADP concentration is lower in gastrocnemius muscle of isoprenaline-treated animals than in propranolol-treated animals (Table 5).

Recovery after muscle stimulation

No differences in rates of PCr resynthesis were observed between the experimental groups (Fig. 1). The transient acidosis associated with initial PCr resynthesis [22] was greater in isoprenaline-treated animals; however, this difference was not statistically significant (Fig. 2).

Comparison of ^{31}P-NMR spectra obtained after full-recovery from muscle stimulation showed no significant differences from those acquired before commencement of muscle stimulation for control and propranolol-treated animals. However, a significant reduction in ATP concentration was determined from integration of recovery NMR spectra and from ATP determination in freeze-clamped muscle after completion of the stimulation protocol, for gastrocnemius muscle of isoprenaline-treated animals (−11 ± 4% compared to pre-stimulation NMR spectra, and −8 ± 3% compared to ATP determination in contralateral gastrocnemius muscles; $P < 0.05$ for paired Student's *t*-test).

DISCUSSION

The presence of β-adrenoceptors in skeletal muscle [1, 23, 24] and the effects of altered β-adrenoceptor stimulation on a range of metabolic and physiological processes [2–7, 24, 25] are well established. However, the degree to which direct β-adrenoceptor stimulation of skeletal muscle modulates muscle physiology during contraction remains controversial. These effects on skeletal muscle may have clinical implications. For example, treatment with β-adrenoceptor agonists may cause muscle tremor which can have dose-limiting consequences [26], whereas treatment with β-adrenoceptor antagonists often cause a sensation of muscle fatigue in patients [8, 9].

In the present investigation, we have used ^{31}P-NMR spectroscopy, in combination with conventional metabolite analyses, to study the acute effects of a β-adrenoceptor agonist and antagonist on muscle metabolism and physiology during contraction *in vivo*.

Infusion of isoprenaline caused a 3-fold increase in cyclic AMP concentration in gastrocnemius muscle at rest; it has been shown by other workers that the activities of phosphorylase$_a$ and glycogen synthase$_b$ increase under similar conditions [27, 28]. ^{31}P-NMR spectra acquired during and after isoprenaline infusion showed that concentrations of phosphocreatine, ATP and pH did not change; however, P_i concentration decreased by 40% (to 1.2 μmol/g of tissue). These values are consistent with previous studies using NMR [29, 30] and biopsy analysis [28, 31] which have suggested that such a low concentration of P_i in resting muscle may limit glycogenolytic flux. Infusion of propranolol decreased cyclic AMP concentration in gastrocnemius muscle by 41% suggesting that under the experimental conditions endogenous β-adrenoceptor agonists exert an action in skeletal muscle.

Altered β-adrenoceptor stimulation had no effect on gastrocnemius muscle twitch-tension development during isometric contraction at 4 Hz caused by supramaximal sciatic nerve stimulation. However, some changes in cellular bioenergetics were evident. Intracellular pH decreased to a greater extent in the isoprenaline treatment group. This may be explained by the enhanced rate of glycogenolysis caused by increased β-adrenoceptor stimulation [27, 28]. In addition, a more marked increased in IMP concentration was observed; it is known that AMP deaminase is activated in skeletal muscle by intracellular acidosis [32] and the increase in IMP concentration may lead to an increased loss of nucleotides from muscle causing the decrease in ATP concentration observed in muscle of isoprenaline-treated animals at the end of the stimulation protocol.

Within 15 min of commencement of muscle stimulation at 4 Hz, intracellular pH had recovered to close to resting levels, PCr concentration was constant and twitch-tension development was similar for

all experimental groups. During this period it was observed that the PCr achieved a significantly higher steady-state concentration in isoprenaline-treated compared to propranolol-treated animals, with the control group occupying an intermediate position. Furthermore, assuming that creatine phosphokinase catalyses a near-equilibrium reaction and that the Mg^{2+} concentration is similar for each experimental group, it can be demonstrated that altered β-adrenoceptor stimulation affects the steady-state free ADP concentration. This may be a β-adrenoceptor mediated effect directly on skeletal muscle, or it may be brought about by a less direct effect of β-adrenoceptor stimulation, e.g. altered skeletal muscle bloodflow [20], increased insulin secretion [33] or altered cation metabolism [6, 7].

Upon termination of muscle stimulation, recovery rates for PCr were similar for all experimental groups (at least as judged within the time-resolution of the present experimental protocol), despite lower intracellular pH and apparent ATP depletion during the recovery period in the isoprenaline-treated group. This contrasts with the report of Taylor *et al.* [34] who found that ATP depletion during an exercise protocol in human forearm had profound effects on PCr, P_i and pH_i recovery rates. However, in this study the ATP loss was much more modest compared to the changes reported in the human study [34].

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