K^+ shifts of skeletal muscle during stepwise bicycle exercise with and without β-adrenoceptor blockade

Jostein Hallén, Lars Gullestad and Ole M. Sejersted

Department of Physiology, National Institute of Occupational Health, N-0033 Oslo and Institute for Experimental Medical Research, University of Oslo, Ullevaal Hospital, N-0407 Oslo, Norway

1. K^+ efflux rate and control of K^+ reuptake rate in exercising muscle cells was examined in six healthy female volunteers.

2. A K^+-selective electrode in the femoral vein continuously monitored K^+ concentration ($[K^+]_{fv}$) during bicycling. Power was increased stepwise 5–6 times by 30–40 W every fourth minute until exhaustion before and after I.V. administration of propranolol. Leg blood flow was measured by bolus injections of Cardiogreen.

3. $[K^+]_{fv}$ increased from about 4·3 to 6·8 mmol l^{-1} at exhaustion both before and after propranolol administration, but after drug infusion endurance was reduced from 22·2 ± 0·6 to 19·7 ± 1·1 min, so $[K^+]_{fv}$ rose more rapidly.

4. The exercise-induced efflux rate of K^+ from the muscle cells was estimated to be about 11 μmol kg^{-1} s^{-1} at exhaustion both before and after propranolol administration.

5. As an indicator of rate of net loss of K^+ from the leg, veno-arterial concentration differences ($[K^+]_{fv-a}$) during first, fourth and fifth power increments were high after 15 and 40 s, but declined toward the end of each power step. Propranolol accentuated $[K^+]_{fv-a}$ only after 15 and 40 s of the first and fourth increments.

6. The exercise-induced increase in reuptake rate of K^+ in the muscle, estimated at exhaustion, was not significantly changed by propranolol and was about 10 μmol kg^{-1} s^{-1}, corresponding to about 15 % of maximum Na^+–K^+ pump capacity in man.

7. Extracellular accumulation and loss of K^+ from muscle during bicycle exercise is due to Na^+–K^+ pump lag. The higher $[K^+]_{fv}$ during propranolol is mainly due to impaired redistribution outside the exercising muscles. In addition at low powers, β-adrenoceptor blockade caused a transiently increased net loss due to an accentuated Na^+–K^+ pump lag.

The decrease in muscle cell K^+ content and subsequent rise in extracellular K^+ concentration ($[K^+]$) during exercise has been ascribed to insufficient K^+ reuptake mediated by the Na^+–K^+ pump in the active muscle cells (Clausen & Everts, 1989; Medbø & Sejersted, 1990; Clausen, 1990). Three different possibilities exist: (1) K^+ efflux rate is so large that even maximal stimulation of the Na^+–K^+ pump would not suffice to compensate for this efflux (the insufficient Na^+–K^+ pump capacity theory; Clausen & Everts, 1988); (2) the Na^+–K^+ pump is activated slowly and the increase in extracellular $[K^+]$ within the muscle occurs before the pump is fully activated (the Na^+–K^+ pump lag theory; Woodbury, 1963; Langer, 1983; Sejersted, 1988; Medbø & Sejersted, 1990); or (3) despite accessible capacity and time for activation, efflux and reuptake rates are not completely matched. To establish the relative importance of these possible mechanisms one needs quantitative data on the efflux rate of K^+ from muscle cells associated with electrical activation, the time course and magnitude of reuptake rate and the rate of K^+ loss from the muscle to the general circulation. Such quantitative data are not available, but may be approximated in humans during exercise by use of K^+-selective electrodes inserted into the femoral vein (Hallén & Sejersted, 1993).

It is known that β-adrenoceptor blockade will cause higher levels of plasma K^+ throughout exercise, and it has been argued that this effect is due to reduced stimulation of K^+ reuptake in the exercising muscle and therefore increased loss from the muscle to the circulation (Fellenius, 1983). The argument is based on the observation that catecholamines can stimulate the Na^+–K^+ pump, probably by increasing the sensitivity of the pump to intracellular Na^+ concentration (Clausen & Flatman, 1977, 1980; Ellingsen, Sejersted, Leraand & Ilebekk, 1987; Ellingsen, Sejersted, Vengen & Ilebekk, 1989). Hence, catecholamines increase pump rate and lower intracellular Na^+. Provided

β-adrenergic blockade removes this effect during exercise, K^+ loss from the muscle and gain of intracellular Na^+ would be expected to be larger before the pump rate could catch up with the exercise-induced efflux of K^+. However, this is not the only possibility since recent data show that the effect of catecholamines on the Na^+–K^+ pump might vanish in active muscle (Everts, Retterstøl & Clausen, 1988; Rolett, Strange, Sjøgaard, Kiens & Saltin, 1990). This could mean that the β-adrenergic effect is reduced because the Na^+–K^+ pump is already stimulated by another mechanism that also increases the Na^+ sensitivity of the pump. Even if β-adrenergic blockade did not affect K^+ loss rate from exercising muscle, uptake of K^+ in muscles that do not participate in the exercise could be reduced, thus accentuating the rise in arterial K^+ concentration. Hence, a further aim of this study was to quantify K^+ fluxes in the exercising muscle before and after β-adrenoceptor blockade to try to distinguish these possibilities.

The study was carried out on healthy subjects during a stepwise exercise protocol before and after administration of propranolol. We conclude that the Na^+–K^+ pump was stimulated with a lag in exercising muscle and compensated for the K^+ efflux with a time constant of 90 s or less. The increased plasma $[K^+]$ after β-adrenoceptor blockade was partly due to increased Na^+–K^+ pump lag in the exercising muscles, but the most prominent effect of β-adrenoceptor blockade was the impaired redistribution of K^+ to tissues other than the exercising muscles.

The data have been partly presented in abstract form (Hallén, Gullestad & Sejersted, 1992, 1993).

METHODS

Subjects

Six healthy female volunteers participated (age 21 ± 0·5 years, height 168 ± 1·3 cm and weight 66 ± 2·5 kg). They were all moderately trained and gave their written consent after being fully informed of the problems and risks. The experimental protocol was approved by The Regional Ethics Committee.

Protocols

The subjects arrived in the laboratory in the morning after a light breakfast. They were instructed not to take part in any strenuous exercise training the day preceding the experiments, and alcohol, nicotine and coffee were not allowed in the previous 12 h. On the study day a polyethylene catheter (1·0 mm o.d.) was inserted into the right femoral artery and a silicon catheter into the femoral vein (2·0 mm i.d. and 3·2 mm o.d.) by percutaneous technique after subcutaneous injection of a local anaesthetic (Xylocaine, 10 mg ml^{-1}). The catheters were used for measurement of blood pressure, for blood sampling, for flow measurement using the dye-dilution technique, and for introduction of a K^+-sensitive electrode into the femoral vein (Hallén & Sejersted, 1993). The catheters were advanced in the proximal direction and located with the tip above the inguinal ligament and taped in a fixed position.

The exercise was carried out on a modified Krogh-type cycle ergometer, using a constant pedalling rate of 70 r.p.m. The subjects performed continuous bicycling, while power was increased stepwise, starting at 30–40 W for 4 min with subsequent increments of 30–40 W every 4 min until exhaustion (defined as the inability to pedal at 70 r.p.m.).

Two stepwise exercise bouts were carried out. After the first, the subjects rested for 50 min and were then given the non-selective β-adrenoceptor blocker propranolol as a bolus injection I.V. at a dose of 0·15 mg (kg body weight)$^{-1}$. A new exercise bout was carried out starting 30 min after the injection.

Before the experiments, pre-tests were carried out on two separate days to familiarize the subjects with the exercise protocols and to find the right workload. On the last of these two days the subjects performed exercise according to a protocol similar to that of the main study, but with no drugs and no measurements apart from heart rate, oxygen consumption and time to exhaustion. This was done to find out to what extent the control experiment itself influenced the time to exhaustion during the propranolol experiments. The time to exhaustion was reduced by 1–2 %, and heart rate was higher with no significant difference in oxygen consumption (Gullestad, Hallén & Sejersted, 1993).

Measurements

Limb blood flow (LBF) was measured after 3·5 min at each power step as previously described (Gullestad *et al.* 1993). Indocyanine Green (Cardiogreen; Hynson, Westcott and Dunning, Baltimore, MD, USA) was injected as a bolus into the femoral artery during continuous sampling of blood from the femoral vein over a 30 s period by means of a syringe pump (Infusion–Withdrawal pump, 2202 A, Harvard Apparatus, South Natick, MA, USA). A cuvette densitometer (DC-410 transducer, Waters, Milford, MA, USA) was used to measure changes in optical density (OD) during passage of the blood. The stable signal observed just before appearance of dye in the vein was chosen as the zero line. A monoexponential curve was fitted to the initial decaying part of the OD curve. Flow was calculated from the time integral on the basis of a calibrating procedure, in which known amounts of dye were mixed with venous blood sampled at the end of experiments and the OD was measured.

$[K^+]_{fv}$ was measured continuously by flexible K^+-selective electrodes (1·0 mm o.d.) (Hallén & Sejersted, 1993). Blood samples were drawn through the outer catheter for electrode calibration. Potassium was also measured in arterial blood samples (see below).

Signals from the cuvette densitometer, ECG amplifier and potassium electrode were continuously sampled by a 12-bit A/D converter. Sampling rate for each channel was 200 Hz. Maximal range of signals covered 9–11 bits.

Expired air was collected in Douglas bags for the last 40–120 s of each power increment during exercise. Volume was measured in a wet spirometer. Fractions of O_2 and CO_2 in the expired air were measured by CO_2 and O_2 analysers (Simrad Optronics, Oslo, Norway and 3A/I AMTEK, Pittsburg, PA, USA). O_2 uptake and CO_2 release were calculated and corrected to STPD (standard temperature and pressure, dry).

Blood samples were drawn simultaneously from the artery and vein before exercise, after 3·5 min in the first, fourth and fifth increments (steps 1, 4, and 5) and after 3·5 min of the recovery period. In addition arterial samples were drawn 15 and 40 s after the start of steps 1, 4 and 5, and two samples were drawn as rapidly as possible after cessation of the exercise for analysis of $[K^+]$. Plasma was separated immediately by centrifugation. Plasma $[K^+]$ was measured by an ion-sensitive electrode (Mikrolyte, Kone Corp., Espoo, Finland), together

Table 1. Oxygen uptake, leg blood flow, haematocrit and plasma [K^+] before, during and after a stepwise bicycle exercise without (Control) and with propranolol

	Oxygen uptake (l min^{-1})	Leg blood flow (l min^{-1})	Arterial haematocrit (%)	Arterial [K^+] (mmol l^{-1})	Venous [K^+] (mmol l^{-1})
Rest					
Control	3·0 ± 0·2		39·6 ± 1·0	4·24 ± 0·10	4·25 ± 0·12
Propranolol	3·4 ± 0·2		37·6 ± 0·9*	4·34 ± 0·08*	4·36 ± 0·10
Step 1					
Control	14·1 ± 0·5	2·1 ± 0·2	38·6 ± 1·3†	4·45 ± 0·09	4·53 ± 0·08
Propranolol	13·5 ± 0·4	1·6 ± 0·1*	37·6 ± 0·9†	4·63 ± 0·07*	4·74 ± 0·06*
Step 4					
Control	31·0 ± 1·3	4·1 ± 0·4	39·8 ± 1·2†	4·97 ± 0·14	5·04 ± 0·13
Propranolol	31·8 ± 1·3	4·0 ± 0·4	39·8 ± 0·9	5·57 ± 0·13*	5·62 ± 0·12*
Step 5					
Control	38·1 ± 1·5	4·2 ± 0·4	41·6 ± 0·9	5·54 ± 0·15	5·64 ± 0·15
Propranolol	39·5 ± 1·6	4·3 ± 0·6	40·7 ± 1·0	6·26 ± 0·15*	6·34 ± 0·14*
Exhaustion					
Control			42·5 ± 1·0	6·44 ± 0·26	6·77 ± 0·28
Propranolol			40·9 ± 0·9*	6·67 ± 0·24*	6·79 ± 0·29
Recovery					
Control			43·3 ± 1·0	3·82 ± 0·09	3·68 ± 0·08
Propranolol			41·8 ± 0·9	4·34 ± 0·13*†	4·28 ± 0·13*†

Values are means ± S.E.M., $n = 6$. * Propranolol values different from control ($P < 0·05$); except for those marked †, all exercise and recovery values were significantly different from resting values given at top of table ($P < 0·05$). Power steps amounted to 30–40 W and lasted 4 min. Samples were taken after 3·5 min at each step and 4 min after the end of the exercise. Time to exhaustion was 22·2 ± 0·6 min in the control study and 19·1 ± 1·1 min in the presence of propranolol, which was given at a dose of 0·15 mg kg^{-1}.

with [Na^+] and [Cl^-], which are not reported here. Haematocrit was determined by centrifugation in a Cellokrit (AB Lars Ljungberg & Co., Stockhom, Sweden). Lactate in plasma was analysed by an enzymatic method (Lowry & Passonneau, 1972). Plasma concentrations of adrenaline and noradrenaline were determined by a high performance liquid chromatographic 600 Multisolvent delivery system (HPLC; Waters, Milford, MA, USA) equipped with an electrochemical detector, and analysed by a 3000 series chromatographic data system (Nelson Analytical, Cupertino, CA, USA) (Hjemdahl, Daleskog & Kahan, 1979). Insulin was measured by a radio-immunoassay kit (Diagnostic Product Corporation, Los Angeles, USA).

Calculations

Loss and restoration rates of whole muscle potassium were calculated as the product of veno-arterial differences (fv − a) of plasma concentrations and plasma flow. Arterial plasma [K^+] at the end of the exercise was extrapolated from the last blood sample obtained before and the first sample obtained after cessation of exercise.

Statistical analyses

Values are given as means ± S.E.M. unless otherwise stated. Each subject served as her own control. Differences in variables between rest and peak exercise in each test were evaluated by Student's t test; or for insulin, Wilcoxon's signed rank test was used for comparison of paired data. A three-way analysis of variance with repeated measures was performed for evaluation of differences before and after propranolol administration. If this showed significant results, Student's t test was performed on data from each stage of the protocol. Bonferroni's method was used to adjust for multiple comparisons. Differences were considered significant when $P < 0·05$ (Sokal & Rohlf, 1981).

RESULTS

Changes of the $[K^+]_{fv}$ in the control study

During exercise mean $[K^+]_{fv}$ increased from a resting value of 4·25 ± 0·12 to 6·77 ± 0·28 mmol l^{-1} at exhaustion and about 70 % of the increase occurred during the two last power steps (out of six; Table 1). When exercise started and every time exercise intensity was increased stepwise, $[K^+]_{fv}$ increased suddenly after a time lag (Fig. 1). This lag, which probably represents the time required for blood to flow from the muscle capillaries to the electrode site, was 6–8 s at step 1 and 3–5 s at steps 2–6. When $[K^+]_{fv}$ started to rise the fastest rate of rise was observed before $[K^+]_{fv}$ had risen by more than 0·05 mmol l^{-1}. This initial fast rate of rise of $[K^+]_{fv}$ was maintained almost constant for several seconds and amounted to 19 μmol l^{-1} s^{-1} in step 1 (measured over 5 s). The rate of rise was not different between subsequent steps and averaged 11 μmol l^{-1} s^{-1}, which is significantly lower than the first step ($P < 0·05$). This difference probably reflects the fact that the real power increment at the first step was higher than 37 W due to the internal work required for cycling at 70 r.p.m.

After cessation of exercise $[K^+]_{fv}$ fell after a time lag of 3–5 s. The initial rate of fall in $[K^+]_{fv}$ equalled 59·3 μmol l^{-1} s^{-1} (Fig. 2).

The pattern that $[K^+]_{fv}$ followed during each step changed significantly with power. At low intensities $[K^+]_{fv}$

Table 2. Arterial lactate, arterial pH, adrenaline, noradrenaline and insulin before, during and after a stepwise bicycle exercise, without (Control) and with propranolol

	Arterial lactate (mmol l^{-1})	Arterial pH	Adrenaline (nmol l^{-1})	Noradrenaline (nmol l^{-1})	Insulin (mU l^{-1})
Rest					
Control	1·10 ± 0·20	7·40 ± 0·01	0·7 ± 0·1	1·7 ± 0·2	19·0 ± 3·4
Propranolol	0·98 ± 0·19	7·39 ± 0·01	0·8 ± 0·1	2·1 ± 0·2*	17·0 ± 2·7
Step 1					
Control	1·12 ± 0·19				
Propranolol	0·88 ± 0·15*				
Step 4					
Control	3·63 ± 0·61	7·35 ± 0·01	1·6 ± 0·3	8·9 ± 1·1	15·0 ± 2·0
Propranolol	3·21 ± 0·55*	7·37 ± 0·01	3·8 ± 0·5*	14·2 ± 1·7*	7·5 ± 0·5*
Step 5					
Control	8·69 ± 1·12	7·30 ± 0·01			
Propranolol	7·16 ± 1·09*	7·31 ± 0·02			
Exhaustion					
Control	10·3 ± 1·69	7·23 ± 0·02	11·5 ± 1·6	56·1 ± 7·4	13·2 ± 1·6
Propranolol	8·29 ± 1·71*	7·29 ± 0·03*	15·4 ± 3·3	46·8 ± 5·4*	7·2 ± 0·4*
Recovery					
Control	11·5 ± 0·63	7·17 ± 0·07	1·0 ± 0·1	16·3 ± 1·9	35·7 ± 4·9
Propranolol	8·10 ± 1·42*	7·26 ± 0·07*	1·4 ± 0·2	12·8 ± 2·0*	19·5 ± 1·5*

Values are means ± S.E.M., $n = 6$. * Values different from control ($P < 0·05$). All exercise and recovery values were significantly different from resting values ($P < 0·05$). See Table 1 for further legends.

reached a maximal value 1·5–2·0 min after the power increment and then fell until power was increased again (4 min). During the first exercise step $[K^+]_{fv}$ increased by a maximum of 0·48 ± 0·05 and then fell by 0·13 ± 0·03 mmol l^{-1} before power was increased. During the first 2 min of the second step $[K^+]_{fv}$ increased by 0·19 mmol l^{-1} and then fell by almost the same amount so that the value at the end of this step did not differ from step 1 (Fig. 1). The decline in $[K^+]_{fv}$ from the second minute (peak value) to the fourth minute of each step became less with increasing exercise intensities and was not significant at step 4 (120–160 W). Above this power $[K^+]_{fv}$ increased throughout the 4 min period or to exhaustion (Fig. 1).

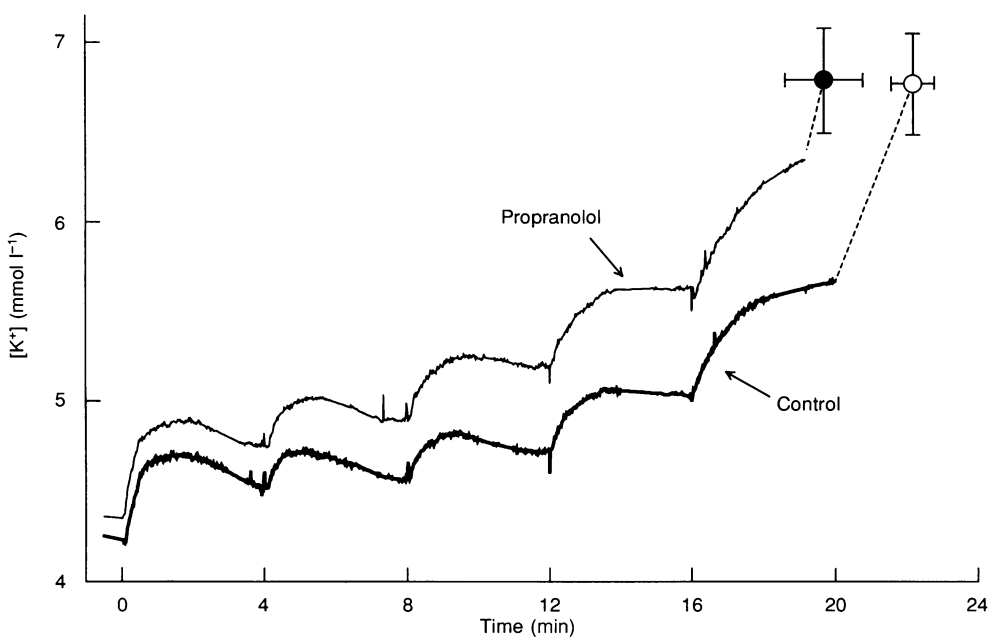

Figure 1. Continuous recording of plasma $[K^+]$ by an electrode inserted into the femoral vein of six healthy subjects during a stepwise bicycle exercise protocol carried out until exhaustion
Power was increased by 30–40 W every fourth minute. Curves are means obtained during a control exercise test and following intravenous administration of propranolol (0·15 mg (kg body weight)$^{-1}$ I.V.).

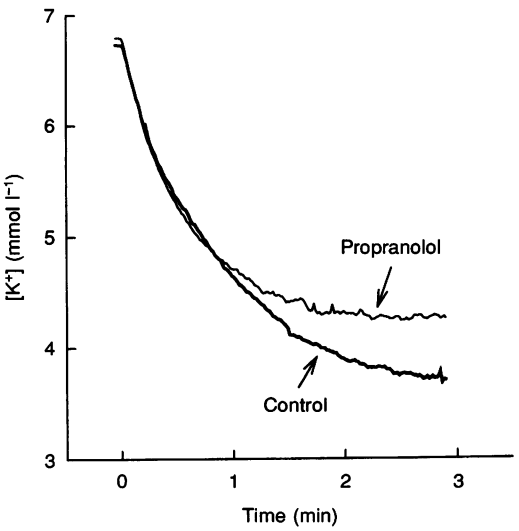

Figure 2. Decline of femoral venous plasma K^+ after cessation of a stepwise bicycle exercise protocol carried out until exhaustion
K^+ was monitored by a K^+-selective electrode inserted into the femoral vein. Curves are means obtained during a control exercise test and following intravenous administration of propranolol (0·15 mg (kg body weight)$^{-1}$ I.V.).

Changes of $[K^+]_{fv}$ during propranolol

Propranolol reduced time to exhaustion from 22·2 ± 0·6 (range 20·8–25·3) to 19·7 ± 1·1 min (range 16·1–24·1) ($P < 0·05$). Resting $[K^+]_{fv}$ during propranolol was not significantly different from the control. During exercise, however, the overall rate of rise of $[K^+]_{fv}$ was significantly faster, so that the peak $[K^+]_{fv}$ at exhaustion equalled 6·79 ± 0·29 mmol l^{-1}, which was not different from the control (Fig. 1, Table 1). Qualitatively the rise pattern of $[K^+]_{fv}$ was the same during the propranolol study as in the control study, but quantitatively there were important differences. The initial rate of rise in $[K^+]_{fv}$ at the start of each step was on average 13 % higher with propranolol ($P < 0·05$), and the maximum increment during each power step was also significantly higher. After the end of exercise the initial rate of decline of $[K^+]_{fv}$ amounted to 62·7 μmol l^{-1} s^{-1}, which was not significantly different from the control (Fig. 2).

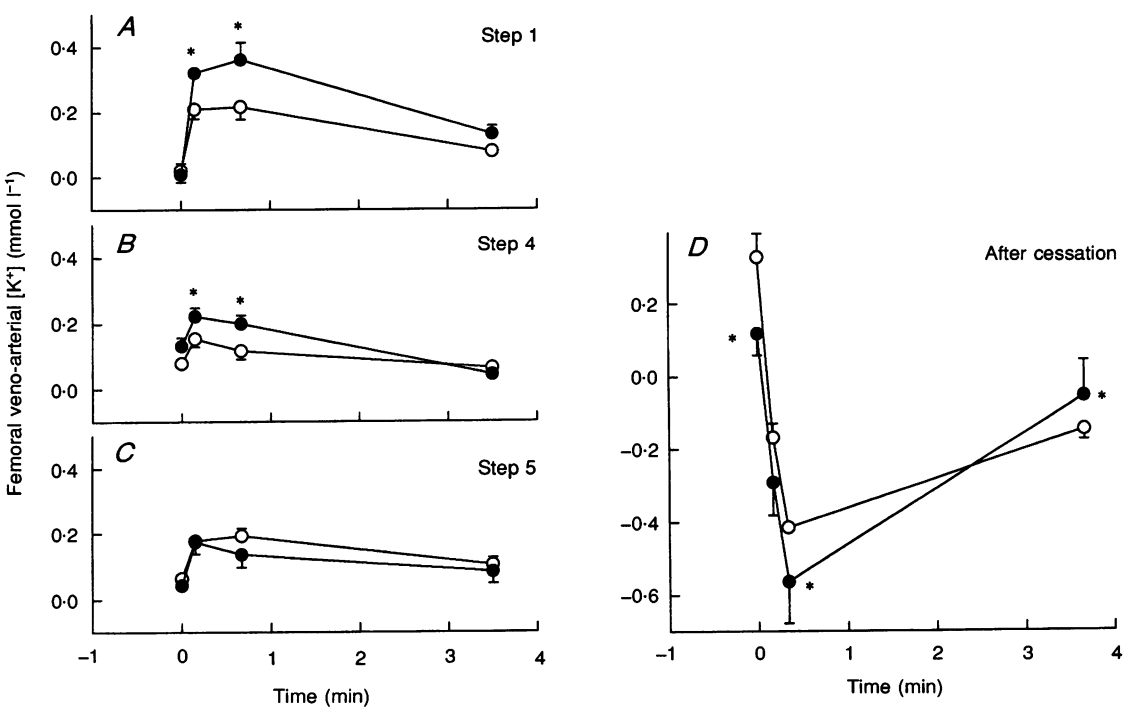

Figure 3. Concentration differences between femoral venous and arterial plasma potassium ($[K^+]_{fv-a}$) at three different power steps and after cessation of exercise during a stepwise bicycle protocol carried out until exhaustion
The exercise test was performed before and after administration of propranolol (0·15 mg (kg body weight)$^{-1}$ I.V.) and the power steps equalled 30–40 W. ○, control; ●, propanolol. *Propranolol values different from control.

Changes of $[K^+]_a$ and loss of K^+ to the general circulation

Neither in the control experiments nor during β-adrenoceptor blockade did $[K^+]_a$ change significantly during the first 15 s of any power step. Hence, $[K^+]_{fv-a}$ rapidly became positive and remained positive during the rest of the power step (Fig. 3). However, $[K^+]_{fv-a}$ never amounted to more than about one-tenth of $[K^+]_{fv}$, which means that $[K^+]_{fv}$ closely followed $[K^+]_a$ (Table 1).

During step 1, $[K^+]_a$ did not change significantly from the sample taken after 40 s compared with the sample taken after 3·5 min, despite continued loss of K^+ from the leg. During steps 4 and 5, $[K^+]_a$ increased significantly by $0{\cdot}13 \pm 0{\cdot}03$ and $0{\cdot}44 \pm 0{\cdot}06$ mmol l^{-1} respectively, over the same period.

The highest $[K^+]_{fv-a}$ was observed after 15 or 40 s and the 3·5 min value was significantly lower at the three power steps where it was measured (steps 1, 4 and 5) ($P < 0{\cdot}05$) (Fig. 3). After propranolol $[K^+]_{v-a}$ was significantly increased at 15 and 40 s of power steps 1 and 4, but not at step 5 (Fig. 3).

Since flow was measured after 3·5 min at each power step, net rate of loss of K^+ from the leg could be quantified at this point using matching haematocrit and $[K^+]_{fv-a}$ data. Flow increased almost linearly with power up to the third step, but no further increment could be observed from the fourth to the fifth step (Table 1). The loss rate of K^+ to the blood stream was significant at step 1 and was not detectably higher at power step 4, but was doubled at step 5 ($P < 0{\cdot}05$) (Fig. 4).

Propranolol significantly reduced flow at low, but not at high powers (Table 1). At the first power step, reduced flow and increased $[K^+]_{fv-a}$ cancelled so that the rate of K^+ loss from the leg did not change significantly with propranolol (Fig. 4). At the fifth step none of these variables changed significantly with propranolol (Table 1).

Restoration of muscle K^+ during recovery

After cessation of exercise, $[K^+]_{fv}$ fell to a minimum value after 3·5 min, which in the control situation was significantly lower compared with the pre-exercise $[K^+]_{fv}$ value (Table 1). In contrast, after propranolol administration $[K^+]_{fv}$ did not fall below pre-exercise concentration (Fig. 2).

About 5–10 s after cessation of exercise $[K^+]_{fv-a}$ became negative (Fig. 3*D*) showing net reuptake in the recovering muscles. Due to the significantly higher $[K^+]_a$ after propranolol administration, $[K^+]_{fv-a}$ was larger (more negative) than in the control over the first 20 s of recovery. After 3·5 min of recovery there was still a significant negative $[K^+]_{fv-a}$ difference in the control condition, but not with propranolol present (Fig. 3*D*).

Lactate, pH, haematocrit and hormonal changes

Arterial lactate concentration increased and pH decreased with increasing power (Table 2). Propranolol did not affect pre-exercise arterial lactate concentrations, but slightly lower concentrations were observed during submaximal exercise ($P < 0{\cdot}05$). Owing to reduced endurance this difference was accentuated at exhaustion.

Haematocrit was not significantly changed during the first four power steps, but was higher than resting level at the fifth step and at exhaustion (Table 1). Propranolol caused a slight decline in resting haematocrit as compared with the control, but propranolol did not change haematocrit during exercise except at exhaustion.

Arterial adrenaline and noradrenaline concentrations increased with power. However, the increment from the fourth power step until exhaustion was 5–10 times higher than the increment from rest until the fourth power step (Table 2). Hence, the relationship between arterial concentrations and power fitted exponential curves, as described by Häggendal (1970). After exercise adrenaline was almost normalized at 4 min, whereas noradrenaline was reduced by two-thirds.

During administration of propranolol the rise of adrenaline and noradrenaline concentrations were accentuated so that higher concentrations were reached at power step 4 (Table 2). At exhaustion propranolol had different effects on the two hormones. As was the case for lactate, the exhaustion level for noradrenaline was lower than in the control experiment, conceivably due to reduced

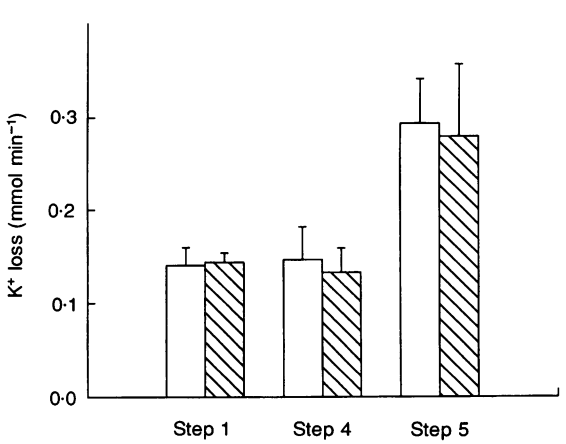

Figure 4. Loss rate of potassium from one exercising leg at three different power steps during a stepwise bicycle exercise protocol before and after administration of propranolol (0·15 mg (kg body weight)$^{-1}$
Power was increased by 30–40 W every fourth minute and the loss rate was calculated 3·5 min later as the product of plasma flow and the difference between femoral venous and arterial $[K^+]$. □, control; ▨, propranolol.

endurance. Adrenaline, on the other hand, was slightly, but not significantly, higher. Propranolol had no significant effect on adrenaline 4 min after the end of exercise, while the noradrenaline concentration was significantly lower.

Insulin concentration fell during exercise from resting levels that were not different before and after administration of propranolol (Table 2). However, during exercise propranolol caused significantly lower insulin concentration than in the control experiment. In the recovery period insulin rose abruptly in the control experiment, and this rise was significantly blunted by propranolol (Table 2).

DISCUSSION

The present study shows that with stepwise increments of power during bicycle exercise, $[K^+]_{fv}$ increased transiently at the low power steps, but above a power level of 120–160 W, $[K^+]_{fv}$ was no longer well 'controlled' in the sense that it continued to rise throughout the exercise. Since $[K^+]_a$ also increased more rapidly toward the end of exercise, it is clearly a power limit above which the rate of loss of K^+ from the exercising muscle exceeds the rate of redistribution and uptake in other tissues. Acute administration of β-adrenoceptor blocker caused a slight increment of initial rate of rise of $[K^+]_{fv}$ as well as higher $[K^+]_{fv-a}$ at the start of every power step, and $[K^+]_{fv}$ and $[K^+]_a$ were higher throughout the exercise. In accordance with previous investigations, exhaustion was reached earlier than in the control, but at almost identical $[K^+]_{fv}$ (Gullestad, Dolva, Nordby, Skaaraas, Larsen & Kjeskshus, 1989). Since K^+ reuptake in cells is directly or indirectly connected to the pumping rate of the Na^+–K^+ pump, the data indicate that the rate of pump-mediated reuptake is at least transiently insufficient to keep up with the exercise-induced efflux rate. The data are compatible with a time course for pump activation that is independent of intensity of exercise.

K^+ electrode measurements

To sort out the mechanisms for control of K^+ balance during exercise one needs quantitative data on K^+ efflux and reuptake rates across the muscle cell membrane. To the extent that these two do not match, K^+ content of the interstitial space will change and K^+ will be transferred between the interstitial space and plasma that perfuses the muscle. The following equation relates these flux rates:

$$K^+_{efflux} - K^+_{reuptake} = \Delta K^+_{int}/\Delta t + [K^+]_{fv-a}PF, \quad (1)$$

where K^+_{efflux} is the exercise-induced K^+ efflux rate from the exercising cell, $K^+_{reuptake}$ is exercise induced K^+ reuptake rate, $\Delta K^+_{int}/\Delta t$ is rate of change of interstitial K^+ content and $[K^+]_{fv-a}PF$ is rate of loss of K^+ (PF is plasma flow). These rates can be expressed in micromoles per second per kilogram active muscle. The use of K^+-sensitive electrodes in the femoral vein allows us to approximate the quantities in this equation based on some conditions which have been discussed previously (Hallén & Sejersted, 1993). We will here elaborate a few points.

K^+_{efflux} and $K^+_{reuptake}$

Exercise-induced K^+ efflux rate, related to repolarization of the action potential, increases with each power step in a step-like fashion. Likewise, at cessation of exercise this outward current is suddenly turned off. This is corroborated by electromyographic recordings showing that the signal is rapidly turned on and is maintained quite constant with submaximal dynamic exercise not lasting more than 4 min (Gerdle, Hedberg, Jonsson & Fugl-Meyer, 1987). Provided the Na^+–K^+ pump is activated gradually with some lag, K^+_{efflux} and $K^+_{reuptake}$ can be distinguished with the electrode technique. Both the present and previous data show that K^+ reuptake rate is increased with a lag (Woodbury, 1963; Langer, 1983; Vøllestad, Hallén & Sejersted, 1993). However, we cannot exclude the possibility that part of the increment in reuptake of K^+ may be instantaneous (Blum, Nioka & Johnson, 1990). Furthermore, we cannot exclude the possibility that K^+ currents not associated with the action potential occur during and after exercise. With these provisos, we assume that at the moment of commencement of exercise, $K^+_{reuptake}$ is zero, and at the moment of cessation of exercise, K^+_{efflux} becomes zero.

$\Delta K^+_{int}/\Delta t$ and $[K^+]_{fv-a}PF$

$[K^+]_{fv}$ closely reflects interstitial K^+ concentration. Endothelial permeability to K^+ is high and equilibration between interstitial space and capillary plasma is rapid so that, with the rates of $[K^+]_{fv}$ changes presently observed, interstitial K^+ concentration is probably only slightly underestimated (Renkin, 1959; Sheehan & Renkin, 1972; Hallén & Sejersted, 1993). Transformation from rate of rise of $[K^+]_{fv}$ to $\Delta K^+_{int}/\Delta t$ can then be made by multiplication with the interstitial volume in human muscle amounting to approximately 15% of the muscle volume (Ahlborg, Bergström, Ekelund & Hultman, 1967; Sjøgaard & Saltin, 1982). $[K^+]_{fv-a}PF$ is zero at the onset of exercise since $[K^+]_{fv-a}$ is zero. After onset of exercise rate of loss of K^+ increases and peaks after 1–2 min (Juel, Bangsbo, Graham & Saltin, 1990). Toward the end of each power step $[K^+]_{fv-a}$ is small and becomes zero again shortly after cessation of exercise before it turns negative.

From this background we conclude that the initial rate of rise of $[K^+]_{fv}$ at the onset of exercise reflects the exercise- induced K^+ efflux from the muscle cells associated with repolarization, since we assume that $K^+_{reuptake}$ and $[K^+]_{fv-a}PF$ are both zero. When power was increased at subsequent steps the initial rate of rise of $[K^+]_{fv}$ again reflects the increase in K^+_{efflux}. By similar reasoning the initial rate of fall of $[K^+]_{fv}$ at the end of

exercise will reflect exercise-induced stimulation of K^+ reuptake inside the muscle, since we assume that K^+_{efflux} and again $[K^+]_{fv-a}PF$ are both zero.

Exercise-induced K^+ efflux

Since initial rates of rise of $[K^+]_{fv}$ were 19 and 11 μmol l^{-1} s^{-1}, exercise-induced K^+_{efflux} can be estimated from eqn (1) to be 2·9 μmol s^{-1} (kg muscle)$^{-1}$ at step 1 and 1·7 μmol s^{-1} (kg muscle)$^{-1}$ at subsequent steps. When the efflux rates for each power step are added, one gets an approximation of the accumulated or total exercise-induced K^+ efflux rate, reaching 11 μmol s^{-1} (kg muscle)$^{-1}$ at exhaustion. This is slightly more than one-third of the maximum K^+ efflux rate observed by Vøllestad *et al.* (1993) at the onset of bicycling at almost 500 W in healthy men. The maximum power in the present study was about 225 W and the two efflux rate estimates are therefore comparable. The accumulated K^+ efflux rates are given in Table 3 for power steps 1, 4 and 5 and at exhaustion.

We are not aware of any estimate of firing frequency during bicycling, but firing rate approaches 30 Hz in humans, at which level the force–frequency relationship has reached a maximum (Thomas, Bigland-Ritchie & Johansson, 1991). Assuming this firing rate in trains of 200 ms per pedal cycle (Gregor, Komi & Järvinan, 1987) (i.e. on average 6 action potentials (APs) per second) at maximal exercise intensity (240 W), the efflux per action potential will be in the order of 2 μmol AP^{-1} kg^{-1}. This is the same as the theoretical minimum value calculated from a K^+ efflux per AP of 4 pmol cm^{-2} and a cell diameter of 80 μm. Reported values of K^+ efflux in the rat range from 4–9 μmol AP^{-1} kg^{-1} (Clausen & Everts, 1988). Since the diameter and hence the surface-to-cell volume ratio of rat muscle cells is half that of human muscle cells, the expected K^+ efflux in human muscle cells should also be 50 % of the rat. Hence, there is correspondence between our estimate of K^+ efflux associated with action potentials and a best estimate of the minimum value calculated from measurements of cell capacitance (4 pF cm^{-2}) and 100 mV repolarization.

K^+ reuptake rate and loss to the blood

The loss rate of K^+ from the muscle to the blood stream was measured after 3·5 min during steps 1, 4 and 5, and averaged 0·14, 0·15 and 0·29 mmol min^{-1}, respectively (Fig. 4). From eqn (1) it is evident that provided $[K^+]_{fv}$ is stable (i.e. interstitial $[K^+]$ is stable and $\Delta K^+_{int}/\Delta t$ is zero, which is the case at the end of step 4), this loss rate directly reflects the difference between K^+ efflux rate and K^+ reuptake rate at the cell level. However, when $[K^+]_{fv}$ is falling ($\Delta K^+_{int}/\Delta t$ is negative as seen late in step 1), part of the loss rate is due to wash-out of interstitial K^+. Therefore, the unexpected unchanged rate of loss of K^+ from step 1 to step 4 can be explained by the significant contribution of wash-out (negative rate of accumulation) to the loss rate at step 1 (Table 3). When $[K^+]_{fv}$ is increasing ($\Delta K^+_{int}/\Delta t$ is positive as during step 5), loss and reuptake rates are not able to keep up with the efflux rate and K^+ will accumulate in the interstitium. The product of $\Delta K^+_{int}/\Delta t$, approximated from the slope of the $[K^+]_{fv}$ curve, and an intramuscular distribution volume is tabulated as 'accumulation' in Table 3.

Based on the estimated values for K^+ efflux, accumulation and loss rates, K^+ reuptake rate was calculated according to eqn (1) (Table 3). Interestingly, the calculated K^+ reuptake rate at the highest power (9·4 μmol kg^{-1} s^{-1}) was very close to the rate of reuptake estimated from the initial rate of decline of $[K^+]_{fv}$ when

Table 3. Potassium fluxes (μmol kg^{-1} s^{-1}) at 3·5 min into the first, fourth and fifth power steps, at exhaustion and 10 s after cessation of exercise

	Efflux		Accumulation		Loss		Reuptake	
	Control	Prop	Control	Prop	Control	Prop	Control	Prop
Step 1	2·9	3·5	−0·2	−0·2	0·5	0·8	2·6	2·9
Step 4	7·8	9·2	0·0	0·0	0·5	0·4	7·3	8·8
Step 5	9·5	11·1	0·1	0·3	0·8	0·8	8·6	10·0
Exhaustion	11·4	11·4	1·6	0·4	0·4	0·2	9·4	10·8
After cessation	0·0	0·0	−8·9	−9·2	0·0	−0·2	8·9	9·4

Prop, propranolol. The efflux rate is calculated from the initial increases in $[K^+]_{fv}$ after the increase of power; the accumulation rate (within the extracellular space of the exercising muscle) is calculated from the slope of the potassium curve after 3·5 min; and the loss rate is calculated from $[K^+]_{fv-a}$ and plasma flow. The efflux rate and accumulation rate have been transformed from the measured changes in plasma concentration to rates per kilogram muscle by assuming an extracellular distribution volume within the muscle of 15 % of muscle volume (Ahlborg *et al.* 1967; Sjøgaard, 1990). Loss rate has been normalized to an active muscle mass of one leg of 5 kg (the quadriceps muscle amounts to approximately 2–3 kg (Andersen & Saltin, 1985), and during bicycling additional muscles drained by the femoral vein are active). The reuptake rate is calculated as the difference between the efflux rate and the sum of accumulation and loss rates. Note that the loss rate is small and even a large error in muscle mass estimate will have little consequence for the calculated reuptake rate.

exercise was discontinued (8·9 μmol kg^{-1} s^{-1}) (Table 3). Although caution should be applied when comparing these numbers, since interstitial volume may have changed, the close correspondence of these two independent estimates of K^+ reuptake rate strongly supports the idea that increments in efflux and reuptake rates associated with a Na^+–K^+ pump lag can be quantified by the present approach.

Two important points can be derived from Table 3. First, these data show that 3·5 min after a power increment, about 90 % of the exercise-induced K^+ efflux was immediately returned to the muscle. Provided the Na^+–K^+ pump rate approached K^+_{efflux} according to a monoexponential activation curve the time constant would be about 90 s. However, the activation of the pump could well occur faster, so we conclude that K^+ reuptake probably is activated with a time constant of 90 s or less. This reasoning does not exclude the possibility that a sustained insufficient stimulation of the Na^+–K^+ pump will leave a small regulatory imbalance between K^+_{efflux} and $K^+_{reuptake}$. However, Vøllestad *et al.* (1994) have recently shown that $[K^+]_{fv-a}$ becomes not significantly different from zero after 6 min of bicycling at least at submaximal powers. With other exercise protocols a sustained loss of K^+ from exercising muscle has been reported (Sahlin & Broberg, 1989; Rolett *et al.* 1990; Byström & Sjøgaard, 1991).

Second, to evaluate the hypothesis that insufficient Na^+–K^+ pump capacity is responsible for muscle K^+ loss during exercise, the reuptake rates should be compared with available data on the Na^+–K^+ pump in human muscle. Based on measurement of ouabain binding capacity and maximum *in vitro* ATP turnover, a pump capacity of 75 μmol kg^{-1} s^{-1} can be calculated (Nørgaard, Kjeldsen & Clausen, 1984). Similar estimates in rat and sheep Purkinje fibres have corresponded well to directly measured maximum pump rates for Na^+ (Clausen, Everts & Kjeldsen, 1987; Sejersted, Wasserstrom & Fozzard, 1988). Hence, at exhaustion the increase in K^+ reuptake rate above resting level would require about 15 % of the pump capacity provided the whole reuptake were carried by the pump itself. K^+ reuptake in resting muscle has been estimated to be less than 10 % of maximum pumping capacity in rat soleus muscle (Clausen *et al.* 1987). On the other hand, it is not known how much of the K^+ reuptake is actually carried by the Na^+–K^+ pump. Therefore, we suggest that during exercise on the bicycle ergometer, the Na^+–K^+ pump rate is increased 2–3 times above pump rates in resting muscle. Even so, no more than about 25 % of the maximum pumping capacity is utilized. This estimate fits with data given by Clausen & Everts (1989) in rat muscles stimulated at 6 Hz.

Even though the present data provide only an approximation of the K^+ reuptake rate, we conclude that with this kind of exercise there is a significant reserve capacity of the Na^+–K^+ pump. The K^+ loss from the exercising muscle to the circulation must primarily therefore be ascribed to a lag in pump activation.

Further evidence for this conclusion was obtained from the $[K^+]_{fv-a}$ differences obtained early in each power step. Clearly, $[K^+]_{fv-a}$ was transiently high. Walløe & Wesche (1988) have shown that flow measured by the Doppler technique increased to 75 % of its steady-state level in the course of 11 s after the start of exercise, which would mean that the high $[K^+]_{fv-a}$ observed after 40 s probably represent a significantly higher rate of loss than that actually measured after 3·5 min. This is also in agreement with data from Juel *et al.* (1990) during knee extension exercise.

K^+ redistribution

About 10 s after $[K^+]_{fv}$ started to rise, $[K^+]_a$ rose at about the same rate, probably because of the loss from the exercising muscles. Importantly, only part of the plasma volume is available to the lost K^+ during the first 10–15 s since some of the blood volume is located in tissues with low flow. Since $[K^+]_a$ did not change significantly from 40 s to 3·5 min during power step 1, despite an accumulated loss of K^+ of at least 1 mmol from the two exercising legs during this period, K^+ is clearly redistributed to other tissues (Figs 3 and 4). However, with increasing power, redistribution becomes less efficient. This is evident considering the significant rise of $[K^+]_a$ at step 4 in spite of a K^+ loss rate not significantly different from the loss rate at step 1. Furthermore, during step 5 K^+ loss rate after 3·5 min was double that of step 4, but $[K^+]_a$ increased 3 times more than during the previous step. Taken together these data support the conclusion of Vøllestad *et al.* (1994) that the exponential rise in $[K^+]_a$ with increasing power is due to a combination of increased loss rate from the exercising muscle and less efficient redistribution. $[K^+]_{fv-a}$ was always small compared with the rise of $[K^+]_a$, which means that the main determinant for $[K^+]_{fv}$ is $[K^+]_a$, and hence redistribution of K^+ also controls the extracellular $[K^+]$ within the exercising muscle.

K^+ homeostasis during β-adrenoceptor blockade

Giving a β-blocker will reduce the working capacity and cause more rapid increase in plasma $[K^+]_{fv}$ (Gullestad *et al.* 1989; Gullestad, Birkeland, Nordby, Larsen & Kjekshus, 1991). It is not clear if the reduced working capacity is a consequence of the impaired ability to regulate K^+, but it is an interesting finding that exhaustion takes place at the same $[K^+]_{fv}$ in the propranolol study as in the control condition.

Why did plasma $[K^+]$ increase faster after β-adrenoceptor blockade? Two possibilities exist: K^+ loss rate from the exercising muscles was increased, and/or redistribution of K^+ to other tissues was impaired.

The initial rate of rise in $[K^+]_{fv}$ was significantly increased after propranolol administration as compared with control, but precautions should be taken when estimating change in efflux rate from these results, since plasma flow rate and interstitial volume may also have changed. However, reduction in haematocrit after propranolol as compared with the control experiment indicates an increased extracellular volume, which should lower the rate of rise in $[K^+]_{fv}$ rather than increase it. Furthermore, as shown earlier, a change in plasma flow rate is not very effective in changing the rate of rise in $[K^+]_{fv}$ (Hallén & Sejersted, 1993). The similar initial rate of rise of $[K^+]_{fv}$ at different power steps where flow is very different supports this conclusion. Thus, this data analysis indicates that exercise-induced K^+ efflux rate was probably higher after propranolol administration than in the control.

At low power steps the increased rate of rise of $[K^+]_{fv}$ after propranolol administation was associated with a transiently larger K^+ loss rate since $[K^+]_{fv-a}$ was significantly higher over the first 40 s (Fig. 3). However, after 3·5 min at either step K^+ loss rate was not increased (Fig. 4). We interpret this as a significant increase in Na^+–K^+ pump lag caused by propranolol at the lower power steps.

The largest difference in rise of $[K^+]_a$ between control and propranolol experiments was at step 5 where $[K^+]_{fv-a}$ was not significantly different at any time, and flow was not different, so that loss of K^+ from the exercising muscles to the circulation was the same. Hence, we conclude that at this power step the most important effect of β-adrenoceptor blockade was impaired redistribution of K^+. This coincided with very high plasma concentrations of catecholamines. At low powers propranolol transiently increased the rate of loss of K^+ in addition to reducing the redistribution. Interestingly, the effect of catecholamines within the exercising muscle occured in spite of very low plasma concentrations, but it is well known that local noradrenaline concentration is not necessarily reflected in the plasma (Hjemdahl, 1993). The impairment of the redistribution could be caused by reduced blood flow to non-exercising tissues, and/or reduced Na^+–K^+ pump-mediated intracellular uptake in these tissues.

Rate of fall of $[K^+]_{fv}$ after cessation was unchanged after propranolol administration, indicating no or little effect of β-adrenoceptor blockade on the rate of K^+ reuptake at this point. However, since Na^+–K^+ pump lag was increased, it means that β-adrenoceptor blockade caused a prolongation of the time constant for pump activation without affecting the final pump rate. Exhaustion occurred at a lower power than in the control. Therefore, K^+ reuptake rate relative to power was actually higher during β-adrenoceptor blockade (Table 3). This matches the higher K^+ efflux rate observed for a given increment in power and explains why rate of K^+ loss was unchanged.

Although the initial rate of fall in $[K^+]_{fv}$ was unchanged after propranolol, the curve levelled off faster and, in contrast to the control situation, neither $[K^+]_{fv}$ nor $[K^+]_a$ went below pre-exercise levels. This indicates that the decline in rate of reuptake has two components and that the slower component is impaired after propranolol. The lack of veno-arterial differences (fv − a) for K^+ 3·5 min postexercise during β-adrenoceptor blockade supports this hypothesis. Since insulin concentration after propranolol were lower compared with the control it is also possible that insulin-dependent stimulation of the Na^+–K^+ pump was reduced.

In conclusion, this study has revealed that exercise caused an immediate increase in K^+ efflux rate from the active muscle cells that was rapidly and to 90% compensated for by a gradual increase in reuptake rate of K^+ within 3·5 min. The loss of K^+ from the exercising muscles was primarily due to the lag of increase in reuptake (Na^+–K^+ pump lag theory). Insufficient Na^+–K^+ pump capacity can be ruled out with this type of exercise. The exponential increase in $[K^+]_a$ with increasing exercise intensity is mainly due to reduced efficiency of redistribution of K^+ to non-exercising tissues with increasing power. Acute administration of propranolol impaired the redistribution of K^+, accentuating the rise in plasma $[K^+]$ as power increased. In addition propranolol increased the transient K^+ loss from the muscles after the start of exercise at low powers. We conclude that there is a catecholamine-dependent component of the stimulation of K^+ reuptake evidenced as an increased Na^+–K^+ pump lag during β-adrenoceptor blockade. The β-adrenergic component is at least partly responsible for the hypokalaemia seen post exercise in the control situation, as suggested by Medbø & Sejersted (1990).

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