Partial neuromuscular blockade in humans enhances muscle blood flow during exercise independently of muscle oxygen uptake and acetylcholine receptor blockade

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Abstract

This study examined the role of acetylcholine for skeletal muscle blood flow during exercise by use of the competitive neuromuscular blocking agent cisatracurium in combination with the acetylcholine receptor blocker glycopyrrone. Nine healthy male subjects performed a 10-min bout of one-legged knee-extensor exercise (18W) during control conditions and with cisatracurium blockade, as well as with cisatracurium blockade with prior glycopyrrone infusion. Thigh blood flow and vascular conductance in control and with cisatracurium infusion were similar at rest and during passive movement of the leg, but higher (p<0.05) during exercise with cisatracurium than in control (3.83±0.42 vs. 2.78±0.21 L min⁻¹ and 26.9±3.4 vs. 21.8±2.0 ml min⁻¹ mmHg⁻¹ at the end of exercise). Thigh oxygen uptake was similar in control and with cisatracurium infusion both at rest and during exercise, being 354±33 and 406±34 ml min⁻¹, at the end of exercise. Combined infusion of cisatracurium and glycopyrrone caused a similar increase in blood flow as cisatracurium infusion alone. The current results demonstrate that neuromuscular blockade leads to enhanced thigh blood flow and vascular conductance during exercise, events that are not associated with either acetylcholine or an increased oxygen demand. The results do not support an essential role for acetylcholine, released from the neuromuscular junction, in exercise hyperaemia or for the enhanced blood flow during neuromuscular blockade. The enhanced exercise hyperemia during partial neuromuscular blockade may be related to a greater recruitment of fast-twitch muscle fibres.
Introduction
Regulation of blood flow to contracting muscle remains incompletely understood. Several locally formed compounds have been suggested to directly or indirectly cause relaxation of the smooth muscle of the vessels in the active muscles. Moreover, a close relationship between arterial oxygen delivery, energy demand and blood flow indicates that oxygen is a critical factor for the regulation of exercise hyperemia (11, 20). One compound that has been shown to be a potent mediator of vasodilation in skeletal muscle is acetylcholine. Acetylcholine acts on muscarinic receptors on the endothelium leading to the release of vasodilators such as nitric oxide (NO) and prostanoids (8). Acetylcholine is also one of the most reliable inducers of conducted vasodilation, a mechanism where vasodilation travels up- or downstream in the endothelial cell layer or the smooth muscle cells of the arteriolar wall via gap-junctional communication (21). Thus, it is likely that acetylcholine is involved in skeletal muscle blood flow regulation, and yet, the importance of acetylcholine for skeletal muscle vasodilation has been questioned (4). In two human studies, infusion of the acetylcholine receptor blocker atropine was shown to have either none, or a minor, effect on forearm blood flow in response to muscle contraction (7, 22). However, in one of these studies mild handgrip contractions were used with only small changes in forearm blood flow of about 100ml/min during exercise, and, therefore, flow changes due to acetylcholine receptor block may not have been detectable. In the second study (7) the role of acetylcholine was specifically examined during a single contraction.

Thus, the aim of the present study was to further assess the role of acetylcholine for skeletal muscle blood flow using the human thigh muscle as experimental model to obtain substantial hyperemia with exercise. We moreover approached the potential role of acetylcholine for muscle vasodilation by using cisatracurium to induce a partial neuromuscular blockade, mainly of slow-twitch fibers (6). The rationale behind this intervention was that partial neuromuscular blockade during exercise would result in greater release of acetylcholine in order to produce the same amount of work (6, 9, 27) resulting in elevated muscle blood flow.
Muscle blood flow and oxygen uptake were measured and the acetylcholine receptor blocker glycopyrrone was used to examine involvement of acetylcholine in the blood flow response.

**Methods**
The study encompassed a pilot study and two experimental parts of which the first (Part I) examined the effect of the neuromuscular blocker cisatracurium on muscle blood flow and oxygen uptake during one-legged knee-extensor exercise. In the second (Part II), the role of acetylcholine for the effect of cisatracurium on muscle blood flow was investigated by having subjects performing one-legged exercise without and with cisatracurium alone and with simultaneous infusion of the acetylcholine receptor blocker glycopyrrone.

**Subjects**
In Part I, nine healthy male subjects ranging in age from 20 to 29 yrs, with an average height of 181 (range: 169-185) cm, and a body mass of 81.5 (59.9-89.2) kg participated. In Part II, five healthy male subjects, ranging in age from 23 to 34 yrs, with a height of 176 (161-183) cm, and a mean body mass of 76.2 (51.7-87.6) kg participated. The subjects were untrained or recreationally active with a maximal oxygen uptake of 48.3 ml min\(^{-1}\) kg\(^{-1}\) (41.2-57.3; part I) and 49.6 ml min\(^{-1}\) kg\(^{-1}\) (42.7-58.1; part II). The subjects were fully informed of the risks and discomforts associated with the experimental procedures, and all provided written consent. The study was carried out in accordance with the guidelines contained in the Declaration of Helsinki and was approved by the Ethics Committee of Copenhagen and Frederiksberg communities.

**Exercise model**
Supine one-legged knee-extensor exercise was carried out on an ergometer that permitted the exercise to be confined to the quadriceps muscle (1, 4). Prior to the study, the subjects made several visits to the laboratory in order to become familiarized with the exercise. After at least two familiarisation sessions, subjects
performed incremental exercise in order to determine the maximal power of the knee extensors (64 [56-82] W).

**Experimental procedures**

*Subject preparation- common for Part I and Part II and pilot study*

The subjects arrived at the laboratory at 8 am after consuming a standard breakfast and rested in the supine position. Under local anesthesia, one catheter to collect arterial blood samples was placed into the femoral artery of the control left leg and one catheter was placed in the femoral artery of the experimental leg for infusion. The catheter tips were positioned approximately 2 cm proximal to the inguinal ligament. A second catheter for collection of venous blood samples was placed in the femoral vein of the experimental right leg with the tip positioned 2 cm distal to the inguinal ligament. A thermistor (Edslab, T.D. Probe, 94-030-2.5F, Baxter A/S, Allerod, Denmark) for measurement of blood temperature was advanced 8 cm beyond the tip of the venous catheter for measurement of blood temperature for the calculation of thigh blood flow.

*Experimental protocols for Part I and Part II*

The subjects initiated the exercise protocol including a 10-min warm up period at 18 W followed by 30-min of rest and a 10-min bout of exercise at 18 W (Control; CON).

**Part I (CIS):** After 45 min of rest, the 18-W exercise was carried out again but with additional infusion of cisatracurium in the femoral artery of the experimental leg before and during the warm-up period as well as during exercise.

**Part II (CIS + GLY):** The same protocol described under Part I was carried out. In addition, forty-five minutes after this procedure, acetylcholine was infused as a bolus at rest in the femoral artery of the experimental leg and blood flow was measured before and for the subsequent 3 min. After 15 min glycopyrrone was infused in the femoral artery of the experimental leg as a bolus and 5 min later acetylcholine was again infused as a bolus in the femoral artery of the experimental leg. Blood flow was measured before the acetylcholine infusion and for the subsequent 3 min. After 30 min infusion of cisatracurium and an additional bolus infusion of glycopyrrone were conducted in the
femoral artery of the experimental leg. The subjects then exercised at 18 W again. The kicking frequency was controlled by a metronome and the kicking frequency was recorded in 5-s intervals throughout exercise. The average kicking frequency during CON, CIS and CIS+GLY was 60±0, 59±1 and 59±1 kicks min⁻¹, respectively.

**Blood flow measurements and blood sampling procedures common for Part I and Part II**

At rest and before each of the exercises, blood flow was measured and femoral arterial and venous blood samples were obtained. For 15 s immediately prior to the onset of exercise, the leg was passively moved in order to accelerate the ergometer flywheel and ensure a constant power from the onset of exercise. Blood was drawn from the femoral artery and vein during passive exercise and frequently during the initial phase of exercise; i.e. arterial samples were obtained after 0, 5 and 10 s and venous samples were obtained after 2, 6, 9, 12 and 15 s. For the rapid initial sampling of venous blood, stop-cocks in series were used (5, 16). Further arterial and venous samples were obtained at ~0.5, 1, 1.5, 2, 3, 6 and 10 min of exercise. In order to account for the transit time of blood from the artery, through the muscle capillary bed and to the collection point at the vein (4), in the first 15 s of exercise the arterial sample was obtained approximately 10 s before the venous sample, 6 s before for the next 60 s of exercise and 5 s before for the remainder of the exercise. Blood flow was measured at rest, prior to CON, during passive exercise, from 15-30 s, 40-55, 120-135, 185-200, 370-385 s and 565-580 s. Heart rate (HR) and arterial blood pressure were measured continuously. An occlusion cuff placed below the knee was inflated (240 mmHg) 30 s prior to the exercise and remained inflated throughout exercise in order to avoid contribution of blood from the lower leg.

**Infusion of cisatracurium, glycopyrron and acetylcholine**

Cisatracurium besilate (Nimbex) was a product of GlaxoSmithKline Pharma A/S, (Brøndby, Denmark), Glycopyrrone (Robinul) was a product of Meda AB (Solna, Sweden) and Acetylcholine was a product of ALEXIS Biochemical corporation (San Diego, CA, USA). Cisatracurium was infused at a concentration of 0.2 mg/l thigh volume during the warm up and the 18 W exercise, respectively. 25% of the dose
was infused 4 min prior to the exercise, another 25% of the dose 2 min prior to the exercise and 10% of the dose after 1, 3, 5, 7 and 9 min, respectively. Glycopyrrone was administered as bolus infusions of 0.2 mg. Acetylcholine was infused at a dose of 0.14 mg/min/l thigh volume for 2 min.

**Pilot study to determine the effect of cisatracurium on fiber type recruitment**

In a pilot experiment, we tested the effect of cisatracurium administration on fibre type recruitment during moderate intensity knee-extensor exercise. Six of the subjects performed a 10-min control bout of knee-extensor exercise at 30 W followed by a 10-min exercise bout with cisatracurium infusion using a similar protocol of cisatracurium administration as described above. Biopsies were collected from the medial portion of m. vastus lateralis prior to and immediately after each of the bouts. The biopsy samples were rapidly frozen and after freeze-drying, fragments of single fibres were manually dissected and identified as ST or FT fibres. An average of 15 fibre fragments of each type were subsequently weighed on a quartz-fibre fish-pole balance and CP content was determined using luminometric analyses with the firefly luciferase method (16, 26). During CON, ST fibre CP content decreased (p<0.05, two-tailed paired t-test) from 67±2 to 42±8 mmol/kg d.w., whereas no significant change occurred for FT fibres (68±4 vs. 53±9 mmol kg d.w.). During CIS, no significant change was observed for ST fibres (65±2 vs. 53±8 mmol/kg d.w.), whereas CP content decreased (p<0.05) by 22±8 mmol/kg d.w. in FT fibres (78±4 to 56±9 mmol/kg d.w.). Hence, the ∆FT/∆ST CP ratio was 1.8 in CIS and 0.6 in CON providing evidence for that the blockade was efficient in leading to a greater FT fibre recruitment.

**Measurements and analyses**

*Thigh blood flow, heart rate and blood pressure*

Femoral venous blood flow (i.e. thigh blood flow) was measured by the constant infusion thermodilution technique (2) as modified by Gonzalez-Alonzo et al. (13). Briefly, venous and infusate temperatures were measured continuously before and during ice-cold saline infusion (10-15 s) at 120 ml min⁻¹. This resulted in a drop in
venous blood temperature of ~0.6-2°C. Resting blood flow measurements were made with an infusion rate of ~30 ml min⁻¹ for 30–45 s. Infusate temperature (0-4°C) was measured at the site of entry to the catheter (Edslab flow-through thermistor). Venous blood temperature and saline infusate temperatures were recorded at 400 Hz analog-to-digital sampling rate (Powerlab 16s data acquisition system, Chart v4.13 software, AD Instruments, Sydney, Australia). Heart rate was obtained from an electrocardiogram, while arterial blood pressure was monitored with transducers positioned at the level of the heart (Pressure Monitoring Kit, Baxter).

**Blood analyses**

All arterial and venous blood samples were immediately analysed for pO₂, O₂ saturation and hemoglobin (ABL510, Radiometer, Copenhagen, Denmark) from which O₂ content was calculated. For the determination of blood lactate and glucose (YSI 2300, Yellow Spring Instruments, Yellow Springs, OH, USA), 200 µl of whole blood was hemolysed within 10 s of sampling by adding to 200 µl buffer (YSI; 0.5% Triton X-100). Potassium was determined by a flame photometer (FLM3, Radiometer, Copenhagen, Denmark) with lithium as internal standard.

**Thigh volume and quadriceps muscle mass**

The thigh volume and the mass of the quadriceps femoris muscle of the experimental leg were estimated anthropometrically, using measurements of thigh length, circumference and skin fold thickness (14), and corrected based on a comparison between MR-scan and anthropometric determinations (16). The quadriceps muscle mass of the experimental leg was 2.4±0.3 kg for the subjects in part I and 2.3±0.5 kg for the subjects in part II.

**Calculations**

Thigh O₂ uptake and lactate release were calculated by multiplying blood flow with arterial-venous O₂ difference and venous-arterial lactate difference, respectively. A continuous blood flow curve was constructed for each subject by linear interpolation of the measured blood flow data points to obtain time-matched values of blood flow with the blood variables.
Statistics
All data were analyzed using a two-factor (condition x time) repeated measures analysis of variance (ANOVA), with significance set at P<0.05. Significant interactions and main effects were subsequently analyzed using a Newman-Keuls post-hoc test. Data are presented as means±standard error of the mean (SEM), unless otherwise stated.
Results

Experimental part I

Thigh blood flow
Thigh blood flow was the same in CON and CIS at rest and during passive exercise, but after 68 s of exercise blood flow was higher (P<0.05) in CIS compared to CON (3.20±0.30 vs. 2.6±0.2 L min⁻¹) and remained higher (P<0.05) throughout exercise, reaching 3.8±0.4 and 2.8±0.2 L min⁻¹ at the end of exercise in CON and CIS, respectively (Fig. 1a).

Mean arterial pressure and heart rate
Mean arterial pressure (MAP) at rest was similar in CON and CIS (135±7.2 vs. 133±6.6 mmHg) and during passive movement and onset of exercise (Fig. 1b). At 90 sec of exercise, MAP was higher (P<0.05) in CIS than in CON (163±6.9 vs. 150±6.6 mmHg) and a ~10% higher level was sustained throughout the exercise bout. Heart rate at rest was 63.4±3.8 and similar for CIS and CON. Heart rate during exercise was higher (P<0.05) in CIS than in CON (115±7 vs.90±3 bpm at 10 min).

Vascular conductance
At rest vascular conductance was similar in CON and CIS (4.5± 2.0 vs. 4.1±0.5 l min⁻¹ mmHg⁻¹) and during passive movement of the leg and onset of exercise (Fig. 1c). At 90 s of exercise vascular conductance was higher (P<0.05) in CIS than in CON (20.7±1.7 vs. 15.9±1.2 ml min⁻¹ mmHg⁻¹). Conductance was also ~20% higher (P<0.05) at 30, 60 and 540 s of exercise in CIS than in CON.

Thigh oxygen uptake
At rest, during passive exercise and in the initial phase of exercise oxygen extraction (a-νdiff O₂) was not different in CON and CIS, but after 14 s of exercise (79±8 vs. 66±4 ml min⁻¹) and throughout the exercise the a-νdiff O₂ was higher in CON compared to CIS with the difference at the end of exercise being 19% (130±6 vs. 109±5 ml min⁻¹; Fig. 2a).
Thigh VO$_2$ was similar in CON and CIS both at rest and during exercise being $328\pm34$ and $333\pm23$ ml min$^{-1}$, respectively, after 3 min of exercise, and $354\pm33$ and $406\pm34$ ml min$^{-1}$, respectively, at the end of the exercise (Fig. 2b). There was no difference in blood pO$_2$ between CON and CIS.

**Blood variables**

Blood lactate release increased (P<0.05) similarly from rest to exercise in CON and CIS (from $0.0\pm0.0$ to $1.3\pm0.4$ mmol min$^{-1}$ and from $0.0\pm0.1$ to $2.0\pm0.5$ mmol min$^{-1}$, respectively; Fig. 3).

Arterial and venous blood pH levels at rest and during exercise were similar in CON (venous pH: $7.36\pm0.01$ to $7.30\pm0.01$ at 10 min) and CIS (venous pH: $7.36\pm0.01$ to $7.29\pm0.01$ at 10 min).

Arterial and venous blood HCO$_3^-$ levels at rest and during exercise were similar for CON (venous HCO$_3^-$: $26.6\pm0.6$ to $29.1\pm1.3$ mmol l$^{-1}$ at 10 min) and CIS ($24.3\pm0.7$ to $25.4\pm0.7$ mmol l$^{-1}$ at 10 min).

Venous plasma potassium levels in CON increased from $4.1\pm0.1$ to $4.3\pm0.1$ mM from rest to exercise and similarly after CIS treatment ($3.8\pm0.1$ to $4.1\pm0.1$ mM).

**Experimental part II**

*Resting blood flow and acetylcholine receptor blockade*

Prior to the infusion of acetylcholine thigh blood flow was $0.30\pm0.09$ L min$^{-1}$. After 45 and 135 s of acetylcholine infusion the level had increased 3- and 5-fold, respectively (Fig. 4). Infusion of glycopyrron prior to acetylcholine abolished the effect of acetylcholine ($0.44\pm0.10$ control flow vs. $0.57\pm0.10$ L min$^{-1}$ at 135 s of acetylcholine infusion).

*Combined neuromuscular and acetylcholine receptor blockade*

No differences were observed in resting basal muscle blood flow, oxygen extraction or oxygen uptake between CON, CIS and CIS+GLY. The thigh blood flow response to exercise in CIS was higher (P<0.05) than in CON and similar to that in CIS+GLY (Fig. 5a). Oxygen extraction during exercise in CIS+GLY was similar to that in CIS, but both levels were lower (P<0.05) than in CON. Thigh oxygen uptake in CIS+GLY was not different from either CON or CIS during exercise and recovery (Fig. 5b).
**Discussion**

The main finding of the present investigation was that the administration of a competitive neuromuscular blocking reagent enhanced thigh blood flow during exercise by \( \sim 30\% \) and thigh vascular conductance by \( \sim 20\% \), without an effect on oxygen uptake. Blood flow and vascular conductance were not altered by inhibition of muscarinic receptors with glycopyrrone. The data suggest that endogenous acetylcholine released from neuromuscular junctions is not essential for exercise hyperemia and, moreover, show that the elevation in blood flow and vascular conductance with neuromuscular blockade are due to factors other than acetylcholine and enhanced oxygen demand.

Partial neuromuscular blockade with CIS specifically blocks slow twitch fibres in animals (18) and in humans (6) and such fiber type specific blockade would require enhanced recruitment of fast twitch (FT) fibers to accomplish a given workload. Thus, the hypothesis of the present study was that partial neuromuscular blockade leads to a greater recruitment of muscle fibres for the same amount of work performed, which would enhance the concentration of acetylcholine in the interstitium (27) and thereby blood flow. To provide evidence for the efficacy of the neuromuscular blockade with cisatracurium, measurements of creatine phosphate in single muscle fibers were conducted in a pilot study in which eight of the subjects in the current study were included. Muscle biopsies were obtained from m. vastus lateralis before and immediately after a bout of 30-W exercise without and with intravenous infusion of cisatracurium. The net CP breakdown in muscle was similar for CON and CIS whereas, in CIS, the drop in average CP content was larger (\( p<0.05 \)) for FT than for ST fibres (11.7±3.7 mmol/kg d.w.), which was not the case in CON (-5.1±4.4 mmol/kg d.w.; unpublished data). These data suggest that the cisatracurium blockade was effective and that ST fibers were more effectively blocked than FT fibers.

On the assumption that acetylcholine released from the neuromuscular junctions is involved in the regulation of muscle blood flow (21), the enhanced
concentration of acetylcholine due to activation of more fibres would be expected to result in greater vasodilation. The results of the first part of this study support this hypothesis since blockade by cisatracurium induced an elevation in both blood flow and vascular conductance during exercise, compared to exercise in the control situation. However, upon block of acetylcholine receptors with glycopyrrone prior to cisatracurium infusion, no reduction in flow during exercise was observed which suggests that the enhancement in blood flow with cisatracurium was not due to enhanced acetylcholine release. The lack of effect of glycopyrrone was not likely to be due to insufficient inhibition of the acetylcholine receptors, as it was effective in blocking the hyperemic response to acetylcholine by about 90%. Thus, the data show that acetylcholine is a potent vasodilator, but do not support that endogenous release of acetylcholine from the neuromuscular junction is essential for the control of exercise hyperemia in the human leg.

Previous experiments performed on arterioles of hamster cheek pouch have shown that the acetylcholine analog methacholine is equally effective in causing vasodilation when applied luminally or abuminally (19), thus acetylcholine released from the neuromuscular junction could mediate vasodilation by acting on either receptor location. In the current experimental design, glycopyrrone was infused intraarterially, and it was assumed that the drug reached both luminal and abluminal muscarinic receptors on the endothelial cells. However, it cannot be fully excluded that glycopyrrone mainly blocked the luminal muscarinic receptors and that the efficacy in blocking acetylcholine from the abluminal side of the arterioles was lower than the 90% determined by acetylcholine infusion. In a study on arterioles of hamster retractor muscle atropine was applied abuminally and was found to reduce the contraction induced vasodilation, which could suggest that extravascular application of a blocker could be more effective (25). Such an approach would, however, be difficult to apply to humans.

Infusion of cisatracurium did not alter blood pressure at rest, during passive movement of the leg, or in the first minute of exercise but, during the remaining part of the exercise period, blood pressure was approximately 10% higher in CIS compared to CON. This effect corresponds well with the increase observed in previous studies with neuromuscular blockade in humans during exercise (17, 23).
and may be explained by enhanced central command and reflex neural mechanisms due to the greater voluntary effort needed to perform the exercise in this condition (10, 17).

The enhanced blood pressure after neuromuscular blockade in the current study had some effect on leg blood flow during exercise, but could not explain more than part of the difference as evidenced by the greater vascular conductance in the CIS trial during exercise. Central and femoral venous pressure were not determined in the current study, however, only very small alterations in central venous pressure occur during exercise (11) and although it is not possible to exclude that cisatracurium affected venous pressure, it is unlikely that it would have caused an alteration in venous pressure, sufficiently large to explain the observed difference in vascular conductance and blood flow. It could be speculated that cisatracurium may have a direct vasodilatatory effect leading to the increase in blood flow. However, although this possibility cannot be excluded, cisatracurium did not elevate either resting blood flow, flow during passive leg movement, or flow in the first phase of exercise. Therefore, it is more probable that it was the differentiated effect of cisatracurium of FT and ST muscle fibers rather than a direct vasoactive effect that was responsible for the enhanced blood flow. If indeed there is an association between a larger recruitment of FT fibers and the greater hyperemia during cisatracurium administration, this effect is unlikely to be due to an enhanced energy demand, since muscle oxygen uptake was similar in the control and the neuromuscular blockade condition.

Potassium has been suggested to play a role in the control of muscle blood flow (15), but the finding of slightly lower femoral venous potassium concentrations in CIS compared to CON, suggests that potassium was unlikely to be the cause of the elevated blood flow during exercise with neuromuscular blockade.

In summary, the present study demonstrates that partial neuromuscular blockade with cisatracurium raises blood flow and vascular conductance during exercise without an associated increase in oxygen uptake. The effect was not influenced by acetylcholine receptor blockade suggesting that acetylcholine is not essential for exercise hyperemia and that the elevated blood flow during neuromuscular blockade is not caused by a larger release of acetylcholine. Although the current data are not
conclusive on this point, we suggest that exercise hyperemia is greater during greater recruitment of FT as compared to ST muscle fibers.

Perspectives and significance
The results of this study suggest that acetylcholine is not involved in the control of exercise hyperemia or, alternatively, that there may be other vasodilator systems taking over when the action of acetylcholine is impaired by receptor blockade. Studies investigating the effect of acetylcholine receptor blockade in combination with block of other vasodilator systems would therefore be important. A significant finding in the current study is also that partial neuromuscular blockade enhances exercise hyperemia. This finding is important as it provides evidence for that muscle fiber recruitment and, maybe, in particular enhanced FT fiber recruitment, influences the magnitude of blood flow during exercise. Further studies are needed to explore this possibility.

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Figure legends
Fig. 1. a) Thigh blood flow b) Mean arterial blood pressure (MAP) and c) vascular conductance during 10 min of knee–extension exercise at 18 W with (CIS) and without (CON) neuromuscular blockade. Values are means±SEM (n=8). * CIS; P<0.05 different from CON.
**Fig 2.** a). Thigh oxygen extraction, b) oxygen uptake and c) Blood PO₂ during 10 min of knee–extension exercise at 18 W with (CIS) and without (CON) neuromuscular blockade. Values are means±SEM (n=8). * CIS; P<0.05 different from CON.

**Fig 3.** Thigh lactate release during 10 min of knee–extension exercise at 18W with (CIS) and without (CON) neuromuscular blockade. Values are means±SEM (n=8).

**Fig 4.** Resting thigh blood flow before and during arterial infusion of acetylcholine (ACT) and combined injections of glycopyrrrone and acetylcholine (GLY+ACT). Values are means±SEM (n=3). * ACT; P<0.05 different from baseline.

**Fig 5.** a) Thigh blood flow and b) thigh oxygen uptake during 10 min of one-legged knee–extension exercise at 18 W without arterial injections of saline (CON), with arterial injections of cisatracurium (CIS) and combined injection of cisatracurium and glycopyrrrone (CIS+GLY). Values are means±SEM (n=5). * CIS; P<0.05 different from CON. Blood flows in CIS+GLY were not significantly different from CIS.
References


Fig. 4

- **ACT**
- **ACT+GLY**

[Graph showing blood flow (L/min) over time (s) for ACT and ACT+GLY conditions.]
Fig. 5A

Blood flow (L min⁻¹)

Time (min)

CON
GS
OS+GLY