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Exercise-induced ^{31}P -NMR metabolic response of human wrist flexor muscles during partial neuromuscular blockade

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Mizuno, Masao, Allan Horn, Niels H. Secher, and Bjørn Quistorff. Exercise-induced ^{31}P -NMR metabolic response of human wrist flexor muscles during partial neuromuscular blockade. *Am. J. Physiol.* 267 (Regulatory Integrative Comp. Physiol. 36): R408–R414, 1994.—The effects of a depolarizing (decamethonium, DECA) and a nondepolarizing neuromuscular blocking agent (vecuronium, VECU) on the phosphorus-31 nuclear magnetic resonance (^{31}P -NMR)-detected metabolic response to muscle contractions were studied separately in six healthy untrained males. Subjects who showed splitting of the P_i peak during graded rhythmic forearm exercise without the drugs were selected. It was found that both drugs abolished the P_i peak splitting during exercise. Despite a similar reduction in phosphocreatine (PCr) during exercise with each drug, a smaller increase in P_i was observed with DECA than with VECU ($P < 0.05$). End-exercise muscle pH was higher with DECA (6.93 ± 0.07) than with VECU (6.79 ± 0.11) ($P < 0.05$). The PCr and P_i recovery was two- and threefold faster with DECA than with VECU, respectively ($P < 0.05$). On the basis of the concept that depolarizing and nondepolarizing agents have a preferential effect on fast- and slow-twitch muscle fibers, respectively, the present results support the hypothesis that the NMR-observed splitting of the P_i peak reflects the metabolic differences between the two major fiber types of human skeletal muscle.

decamethonium; muscle fiber types; forearm exercise; inorganic phosphate; muscle pH; phosphocreatine; vecuronium

NONDEPOLARIZING and depolarizing neuromuscular blocking agents affect preferentially red and white skeletal muscle, respectively, as demonstrated among various muscles in several animal species (11, 21, 32). Human skeletal muscle is heterogeneous with respect to muscle fiber types, often classified as slow- (ST; type I or oxidative) and fast-twitch (FT; type II or glycolytic) fibers (15, 22). Typically, an equal proportion of the two major fiber types is present, although a large variation exists among muscles and subjects (22). However, the preference of the two types of neuromuscular blocking agents also appears to be valid for human skeletal muscle. The nondepolarizing agent tubocurarine affects a head lift more than a handgrip (12), whereas the opposite is true for the depolarizing agent decamethonium (DECA) (13). Within a single muscle group, a preference of the two types of neuromuscular blocking agents for a specific fiber type is indicated by an opposite effect on fatigue (14, 18). Furthermore, DECA affects rate of rise in tension more than endurance, whereas the reversed effect is seen with tubocurarine (23, 25, 27), as also reflected in the electromyographic response (14, 23).

An alternative approach has been made by histochemical evaluation of muscle-biopsy samples. A correlation is

demonstrated between the degree of neuromuscular blockade by tubocurarine and the relative occurrence of ST fibers in the soleus, gastrocnemius, and vastus lateralis muscles (26). On the basis of the glycogen depletion pattern, ST fibers are affected to a greater extent than FT fibers in thigh muscles during cycling with tubocurarine (3). In contrast, the depolarizing agent suxamethonium has a preferential effect on glycogen depletion of FT fibers (24). Thus energy metabolism during exercise under partial neuromuscular blockade depends on which type of muscle fiber remains relatively unaffected.

The use of phosphorus-31 nuclear magnetic resonance (^{31}P -NMR) spectroscopy allows continuous monitoring of relative changes in intramuscular concentrations of several metabolites, including P_i , phosphocreatine (PCr), and ATP. A noninvasive determination of intracellular pH in human skeletal muscle can be performed as well. A wide variation exists even among untrained individuals with respect to response pattern of these energy metabolites during forearm exercise and recovery (2, 17, 20). This metabolic variation has been related to contractile and metabolic characteristics of specific fiber types (1, 15, 17, 20, 31) rather than to different proportion of active and inactive fibers and/or muscle groups within the wrist and finger flexors (7, 8, 10, 30). Experiments combining ^{31}P -NMR and muscle-biopsy technique have shown that muscle pH compartmentation in the form of splitting of the P_i peak, differentiating high- and low-pH P_i peaks during exercise observed in some individuals (1, 20, 31), reflects the metabolic characteristics of ST and FT fibers (17). Furthermore, an abolished splitting of the P_i peak has been reported during forearm rhythmic exercise with partial curarization (16).

The aim of this study was to evaluate the hypothesis that the NMR-observed splitting of the P_i peak during rhythmic forearm exercise reflects the metabolic differences between the two major fiber types of skeletal muscle. Inasmuch as the preferential effects of the two types of neuromuscular blocking agents on ST and FT fibers have been well established, the above hypothesis predicts that administration of the depolarizing agent DECA suppresses primarily the low-pH component of the P_i peak during exercise, whereas the nondepolarizing agent vecuronium (VECU) mainly affects the high-pH P_i peak.

METHODS

Subjects. The nondominant arm of six healthy untrained males (age 21–25 yr, weight 60–70 kg, height 175–183 cm) was studied. Subjects were fully informed about the nature and possible risks of the experiments before they gave written

consent to participate in the study, which was approved by the Local Ethics Committee.

All subjects had participated in previous NMR experiments (17) and herein demonstrated muscle pH compartmentation in the form of high- and low-pH P_i peaks during graded rhythmic forearm exercise. Forearm circumference, maximal voluntary contraction force (MVC), and maximal rhythmic exercise capacity were 25.5 ± 1.1 (SD) cm, 27.0 ± 3.3 N·m, and 1.6 ± 0.3 W, respectively. Four subjects participated in the muscle-biopsy procedure and showed an even proportion of the two major fiber types in the flexor carpi radialis muscle ($47 \pm 5\%$ ST, $24 \pm 14\%$ FT_a, and $29 \pm 17\%$ FT_b fibers) (17).

NMR spectroscopy. A spectrometer (Vivospec, Otsuka Electronics) and an 80-cm-long 2.9-T horizontal superconducting magnet with a 26-cm bore (Magnex Scientific) were used. A 4-cm-diam two-turn surface coil was placed over the wrist flexor muscles centered at a point corresponding to one-fourth the distance between the olecranon and the styloid process. Thus the coil was expected to cover the flexor carpi radialis muscle and also portions of the palmaris longus and the flexor carpi ulnaris, as well as the flexor digitorum superficialis and the flexor digitorum profundus muscle.

Homogeneity of the magnetic field was optimized by shimming on the proton signal. Line width before experiments was <0.5 parts per million (ppm). ³¹P spectra were obtained at 49.83 MHz by single 90° pulse excitations with pulse-power and -width optimized by the height of the PCr peak. Data were collected in 2K points over 205 ms at 6 s of interpulse delay. Data acquisition was performed for 1.5 min at rest, during exercise, and continuously in blocks of 30 s at the end of exercise and during the first 2 min of recovery, followed by blocks of 1 min during the remaining 13 min of recovery (Fig. 1). In ³¹P spectra (16 scans) obtained at rest, the signal-to-noise ratio ranged from 12 to 16.

Spectral analysis. All spectra were analyzed by the same operator without knowledge of the type of experiments. After application of exponential line broadening of 5 Hz to each spectrum, muscle pH was calculated according to Taylor et al. (30). The chemical shift value at the maximum height(s) of P_i resonance(s) was used for this calculation.

The peak area of P_i , PCr, and β -ATP was measured by a curve-fitting program of the Vivospec spectrometer software with assumption of Lorentzian line shape. Baseline correction was performed semi-automatically by manual choosing of eight points at the same chemical shifts in all spectra. The program subsequently identified the peaks of phosphomonoester (PME), P_i , PCr, and ATP. When splitting of the P_i peak was

not identified by the program, the signal was considered as a single peak even when the P_i peak was broadened.

The peak areas were corrected for partial saturation. Saturation factors, determined as the ratio of the peak area in spectra obtained with 6- and 30-s interpulse delays, were 1.44 for P_i , 1.41 for PCr, and 1.15 for β -ATP. During the course of the experiments, the total ³¹P signal (PME + P_i + PCr + β -ATP) was constant within 10%. The coefficient of variation in double determinations of the peak area was 1.6% for high-pH P_i , 1.3% for low-pH P_i , 2.8% for PCr, and 1.8% for β -ATP. In all spectra the total P_i peak area was used for calculating the P_i -to-PCr ratio (P_i /PCr). The area of PCr and β -ATP was expressed as percentage of the resting value, and fold change was used for P_i . The kinetics of PCr resynthesis and P_i decrease during recovery was evaluated by fitting data to a monoexponential function with application of least-square regression. The half time ($t_{1/2}$) of recovery was calculated from the fitted curve.

Exercise protocol. The nonmagnetic forearm ergometer and the protocol for determination of maximal intensity of forearm rhythmic exercise (one contraction every 6 s) have been described (17). The subject lay face down on a stretcher with the nondominant arm in the magnet. The elbow joint was slightly bent, and the flexor carpi radialis muscle was centered over the surface coil. Before the drug experiments, graded forearm exercise was conducted with 40% maximal intensity for 3 min followed by 80% for 2 min (Fig. 1). After 30 min of recovery, the same absolute intensity was repeated for 4 min without neuromuscular blockade (control) and then with either DECA or VECU, followed by 15 min of recovery. Three experiments on the same subject were performed, with ≥ 1 wk interval between them.

To quantitate the reduction in muscle strength caused by the neuromuscular blockade, MVC was determined in the same arm ergometer attached inside the magnet at the end of 30 min of recovery after graded exercise. Thereafter, a bolus containing 0.01 mg VECU (Organon) or 0.02 mg DECA (Burroughs-Wellcome) per kilogram body weight, followed by 20 ml saline, was administered intravenously through a catheter placed in a vein of the nonexercising arm. Small supplementary doses were given at 3- to 5-min intervals as required until the 50% MVC level was reached. It has been well documented that the effect of partial neuromuscular blockade imposed by either type of agent lasts ≥ 5 min, as evaluated by repeated measurements of MVC in limb skeletal muscle (3, 12, 13, 18, 25, 27). In all experiments an Ambu-E resuscitator apparatus, neostigmine, and atropine were available but never needed.

Fig. 1. Schematic experimental protocol. Three series of experiments were performed consisting of graded forearm exercise at 2 intensities and repeated exercise with 40% intensity without (CONT) or with partial neuromuscular blockade imposed by either decamethonium (DECA) or vecuronium (VECU).

Statistics. Values are expressed as means $\pm$ SD. Comparisons between related observations and between independent samples were performed by Friedman's two-way analysis of variance by rank and by the Kruskal-Wallis one-way analysis of variance by rank, respectively (28). When significant differences were detected, these were located by the Wilcoxon's matched-pairs signed-rank test or the Mann-Whitney *U* test. A 5% level was considered significant for two-tailed tests.

RESULTS

Control. Metabolic response to graded forearm exercise did not differ on three separate days (Figs. 2 and 3). The mean values of P_i/PCr and muscle pH at rest were 0.11 ± 0.02 and 7.03 ± 0.04 , respectively. A line width of 18 ± 4 Hz was observed in the P_i peak at rest. During 40% maximal intensity, two components of the P_i peak were evident, differentiating a high-pH and a low-pH peak ($P < 0.05$). At this exercise intensity the high-pH P_i was larger than the low-pH P_i ($P < 0.05$) and

remained constant throughout exercise, whereas the low-pH P_i further increased at 80% intensity ($P < 0.05$). Similarly, the line width of the high-pH P_i peak, 30 ± 3 Hz, was larger than that of the low-pH P_i peak, 18 ± 3 Hz, at 40% exercise intensity ($P < 0.05$). At 80% intensity, the line width of the high-pH P_i peak remained unchanged (31 ± 8 Hz), whereas that of the low-pH P_i peak further increased (23 ± 3 Hz) ($P < 0.05$) but was still lower than the value for the high-pH P_i peak ($P < 0.05$). There was a difference in end-exercise muscle pH between the two components (0.48 ± 0.12). During 40 and 80% exercise intensity, PCr decreased to 48.2 ± 6.9 and $44.8 \pm 6.3\%$ of the resting value, respectively ($P < 0.05$). The ATP also decreased slightly, to $92 \pm 5\%$ during 40% intensity and to $86 \pm 5\%$ of the resting value during 80% intensity ($P < 0.05$).

At the end of 30 min of recovery after graded exercise, P_i/PCr and muscle pH had returned to the resting levels

Fig. 2. Muscle pH at rest, during graded forearm exercise with 40 and 80% intensity, and during repeated exercise with same absolute intensity (40%) followed by 15 min of recovery without or with partial neuromuscular blockade imposed by either decamethonium or vecuronium. Open symbols, low-pH P_i ; closed symbols, high-pH P_i . Only a single P_i peak was evident during exercise with blocking agents. An estimated preferential effect of each blocking agent is noted. Values are means $\pm$ SD ($n = 6$).

Fig. 3. Fold changes in P_i peak relative to individual resting values during control exercise with 40 and 80% intensity and repeated exercise with same absolute intensity (40%) followed by 15 min of recovery without or with partial neuromuscular blockade imposed by either decamethonium or vecuronium. (See legend to Fig. 2).

(Table 1, Fig. 2). When the subjects subsequently performed forearm exercise with 40% maximal intensity in the control experiments, splitting of the P_i peak was again distinct (Figs. 2-4). The line width of the high-pH P_i peak was 31 ± 7 Hz, larger than that of the low-pH P_i peak, 19 ± 7 Hz. Neither the muscle pH nor P_i peak response in the two components was significantly differ-

ent from those obtained at 40% intensity during graded exercise. PCr and ATP decreased by 48.5 ± 6.9 and $87.2 \pm 6.4\%$ of resting values, respectively ($P < 0.05$), which was the same decrease as that observed at 40% intensity during graded exercise. The $t_{1/2}$ of recovery for the high-pH P_i peak was twice as fast as that for the low-pH P_i peak ($P < 0.05$; Table 1). After 15 min of recovery,

Table 1. PCr and P_i /PCr at rest, during rhythmic exercise with 40% intensity, and after 15 min of recovery as well as half-time of recovery for P_i and PCr determined in control, decamethonium, and vecuronium experiments

	PCr, %			P_i /PCr			Half-Time of Recovery, min		
	Rest	Exercise	Recovery	Rest	Exercise	Recovery	High-pH P_i	Low-pH P_i	PCr
Control	100	48.5 ± 6.9	94.4 ± 5.6	0.11 ± 0.03	1.69 ± 0.54	0.11 ± 0.03	0.35 ± 0.12	1.18 ± 0.46	1.29 ± 0.53
DECA	100	54.7 ± 7.8	98.5 ± 3.4	0.11 ± 0.02	$1.00 \pm 0.24^*$	0.09 ± 0.03	0.52 ± 0.15		$0.82 \pm 0.32^*$
VECU	100	51.5 ± 11.2	$92.7 \pm 3.2^\dagger$	0.10 ± 0.02	$1.50 \pm 0.49^\dagger$	0.10 ± 0.05	$1.39 \pm 0.74^\dagger$		$1.50 \pm 0.48^\dagger$

Values are means $\pm$ SD of 6 subjects. PCr, phosphocreatine; DECA, decamethonium; VECU, vecuronium. * Significant difference from control ($P < 0.05$); † significant difference from DECA ($P < 0.05$).

Fig. 4. Phosphorus-31 nuclear magnetic resonance (³¹P-NMR) spectra obtained from wrist flexor muscles in 1 subj at rest and during forearm exercise with 40% intensity without and with partial neuromuscular blockade imposed by either decamethonium or vecuronium. Both agents prevented splitting of the P_i peak. Exponential line broadening of 5 Hz was used for each spectrum (the sum of 16 scans).

muscle pH and P_i returned to resting values, whereas PCr showed a slightly lower value, $94.4 \pm 5.6\%$ of the resting value ($P < 0.05$).

Effect of neuromuscular blockade. Administration of 1.7 ± 0.3 mg DECA or 1.0 ± 0.02 mg VECU reduced forearm MVC to 52 ± 3 and $51 \pm 3\%$ of control values, respectively. No difference was found in the time necessary to establish this force reduction between DECA, 10 ± 5 min, and VECU, 10 ± 2 min. During rhythmic forearm exercise with the same absolute intensity, no splitting of the P_i peak was observed with either DECA or VECU (Figs. 2–4). End-exercise muscle pH with DECA was 6.93 ± 0.07 , slightly but significantly higher than with VECU, 6.79 ± 0.11 ($P < 0.05$). Despite a similar reduction in PCr during exercise between the two neuromuscular blocking agents, a smaller increase in P_i was observed with DECA than with VECU ($P < 0.05$; Table 1, Fig. 3). Thus P_i/PCr during exercise was significantly lower with DECA than with VECU ($P < 0.05$). The line width of the P_i peak during exercise was similar between DECA, 34 ± 7 Hz, and VECU, 35 ± 3 Hz. At the end of exercise ATP was $90 \pm 6\%$ of the resting value with DECA and $91 \pm 5\%$ with VECU.

Compared with the control data, end-exercise muscle pH with DECA was similar to the high-pH P_i peak, whereas the value with VECU was lower than the high-pH but higher than the low-pH P_i peak ($P < 0.05$; Fig. 2). Because the highest increase in P_i was observed during exercise with VECU (Fig. 3), P_i/PCr during exercise was lower with DECA than in the control experiment ($P < 0.05$), but no difference was demonstrated between VECU and the control experiments (Table 1). The line width of the P_i peak during exercise with DECA and VECU was higher than that of the low-pH P_i peak ($P < 0.05$) but similar to that of the high-pH P_i peak observed in the control experiments. The reduction in PCr and ATP during exercise with DECA or VECU did not differ significantly from the control value.

Muscle pH returned to the resting value after 3 min of recovery with DECA and after 5 min with VECU (Fig. 2). The $t_{1/2}$ of recovery for PCr and P_i after exercise was two- and threefold faster with DECA than with VECU ($P < 0.05$; Table 1). At 15 min of recovery after exercise with each agent, muscle pH and P_i had returned to resting values, whereas a slightly higher value of PCr was observed with DECA than with VECU ($P < 0.05$). ATP returned to the resting level by 1 min of recovery, with no difference between DECA ($101 \pm 7\%$) and VECU ($104 \pm 8\%$).

Compared with the control values, the $t_{1/2}$ of recovery for P_i after exercise with DECA was similar to the value obtained in the high-pH P_i peak, and the value with VECU corresponded to that observed in the low-pH P_i peak (Table 1). The $t_{1/2}$ for PCr after exercise with DECA was faster than the control value ($P < 0.05$), whereas no difference was found in the $t_{1/2}$ between VECU and the control experiments.

DISCUSSION

NMR response. In the present study, it was hypothesized that the NMR-detected splitting of the P_i peak during exercise reflects the metabolic differences between ST and FT fibers in working muscles, as proposed previously (20). This hypothesis was tested by applying the two types of neuromuscular blocking agents to impose partial neuromuscular blockade during exercise. The depolarizing agent DECA is predicted to affect primarily FT fibers, and consequently the NMR response during exercise will reflect mainly metabolic characteristics of ST fibers possessing relatively high oxidative potential. Conversely, VECU is expected to block selectively ST fibers, and thus the NMR response will be a reflection of unaffected FT fibers with high glycolytic capacity. The present NMR data demonstrating an abolishment of P_i peak splitting during partial

neuromuscular blockade induced by each drug as well as different dynamics in muscle pH, P_i , and PCr during rhythmic forearm exercise and recovery between the two drugs support the hypothesis.

Muscle pH compartmentation in the form of P_i peak splitting during rhythmic forearm exercise is associated with an even proportion of two major fiber types in working muscles (17). As observed during graded exercise in the present subjects, the kinetics of the high- and the low-pH P_i peak during and after exercise corresponds well to the metabolic characteristics of ST and FT fibers, respectively (1, 15, 17, 20, 31). When the subjects subsequently repeated rhythmic forearm exercise during partial neuromuscular blockade, only a single P_i peak was seen (Fig. 4).

Although muscle force was reduced to the same extent (by ~50% MVC) by each agent, metabolic differences between DECA and VECU were obtained during and after rhythmic forearm exercise. Exercise-induced acidosis in the wrist flexor muscles was slightly but significantly less pronounced with DECA than with VECU (Fig. 2). Both PCr and P_i showed a faster recovery after exercise with DECA than with VECU (Fig. 3). The $t_{1/2}$ recovery values of P_i after exercise with DECA and VECU correspond well to those of the high- and the low-pH P_i peak values obtained without partial neuromuscular blockade, respectively (Table 1). Furthermore, these NMR-detected differences in muscle energetics between the two types of neuromuscular blocking agents correspond to those previously described for subjects with a predominance of either ST or FT fibers in the wrist flexor muscles (17). It is noteworthy that the experimental protocol of this study was aimed at evaluating the reproducibility of NMR data obtained on three separate days. No significant difference was found in metabolic response to graded rhythmic forearm exercise before experiments with partial neuromuscular blockade.

Selectivity of neuromuscular blocking agents. On the basis of NMR-detected metabolic response to exercise with VECU, a degree of selectivity of the nondepolarizing agent VECU for ST fibers may be questioned. The line width of the single P_i peak during exercise with VECU was expected to be larger than the value with DECA, because 1.6-fold larger line width was found in the high- than the low-pH P_i peak during control exercise without neuromuscular blockade (Fig. 4). Whereas the line width obtained with DECA was similar to that of the high-pH P_i peak in the control, the value with VECU was larger than that of the low-pH P_i peak and was similar to the value with DECA. Similarly, after exercise muscle pH with VECU (pH 6.79) was lower than that of low-pH P_i at 40% intensity during graded exercise without neuromuscular blockade (pH 6.93), whereas rhythmic forearm exercise with DECA resulted in a similar level of acidosis observed in the low-pH P_i (pH 6.93; Fig. 2). Furthermore, the reduction and resynthesis of PCr with VECU did not differ significantly from those with DECA or the control experiment (Table 1). These results were obtained despite the fact that subjects performed at the same

absolute exercise intensity both with and without partial neuromuscular blockade.

In the study in which the glycogen depletion pattern in the two major fiber types of thigh muscles was evaluated during cycling followed by administration of the nondepolarizing agent tubocurarine, ~60% of ST and 40% of FT fibers were estimated to be affected (3). Compared with this selectivity of tubocurarine to ST fibers, the depolarizing agent suxamethonium shows a relatively higher degree of preference to FT fibers, i.e., ~90% of FT fibers and 30% of ST fibers are estimated to be affected on the basis of the glycogen depletion pattern (24). Thus NMR-detected metabolic response with VECU may indicate that ST and FT fibers were blocked to a similar extent. This was not, however, reflected in the chemical shift of P_i pools, because a single P_i peak was observed throughout exercise with VECU. Alternatively, the nondepolarizing agent VECU may have an inhibitory effect on glycolysis.

Mechanism of preferential effects. What is the mechanism underlying the preferential effects of nondepolarizing and depolarizing neuromuscular agents on skeletal muscle? One explanation could be sought in a relationship between morphological as well as electrophysiological characteristics of motor end plates and the amount of a blocking agent reaching a neuromuscular junction. In animal skeletal muscle, ample evidence is available demonstrating that the size of motor end plates is two to three times smaller in ST than in FT muscles (4, 5, 19, 29). Furthermore, ST muscles also show two- to three-fold lower activity of acetylcholinesterase and a different distribution of its molecular forms compared with FT muscles (9). In humans a large variation in acetylcholine content at neuromuscular junctions of intercostal muscles has been observed after nerve stimulation (6). With the assumption that ST fibers receive the smallest amount of acetylcholine, the competitive blockade developed by a nondepolarizing neuromuscular blocking agent would cause a more pronounced inhibition primarily in these fibers. On the other hand, the smallest rise in acetylcholine concentration may be necessary for FT fibers to be depolarized, and thus these fibers are the first to be blocked by acetylcholine-type (depolarizing) neuromuscular blocking agents.

In conclusion, the results demonstrate a substantial difference in muscle pH, P_i , and PCr response during and after contractions from partial neuromuscular blockade between those imposed by VECU and those by DECA. On the basis of the concept that the nondepolarizing and depolarizing neuromuscular agents have a preferential effect on ST and FT fibers, respectively, the present results support the hypothesis that the NMR-observed splitting of the P_i peak reflects the two major fiber types of skeletal muscle.

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