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Exercise Produces Sensitivity to Metocurine

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Chronic muscle disuse decreases the sensitivity of skeletal muscle to nondepolarizing relaxants, such as metocurine (MTC). In this study, the authors determined whether chronic conditioning would produce the opposite effect and increase the sensitivity of skeletal muscle to MTC. Five dogs were exercised by daily running over a period of 5 weeks. At the conclusion of this training period, a pharmacokinetic and pharmacodynamic study of the MTC dose-response relationship was performed. The same analysis was performed on four dogs housed in the same kennel who did not undergo conditioning. Neuromuscular blockade was measured and recorded bilaterally in both gastrocnemius muscles while the animal was anesthetized with nitrous oxide and pentobarbital, 30 ml · kg^{-1}. Plasma concentrations of MTC were measured by radioimmunoassay. The MTC concentration estimated in the effect compartment which produced 50% paralysis was 0.114 ± 0.008 μg · ml^{-1} (mean ± SD) in exercised dogs and 0.189 ± 0.038 μg · ml^{-1} in nonexercised dogs, which was significant at $P < 0.005$. The MTC concentration *versus* response curves were parallel. This supports the authors' hypothesis that exercise increases sensitivity to the nondepolarizing muscle relaxant metocurine. (Key words: muscle, skeletal: disuse; exercise. neuromuscular relaxants: metocurine. neuromuscular transmission, receptors.)

DIMINISHED MUSCLE ACTIVITY, or disuse, modestly increases sensitivity to depolarizing muscle relaxants, as measured by succinylcholine-induced potassium release.[1] Conversely, disuse reduces sensitivity to nondepolarizing muscle relaxants. This resistance varies from twofold during simulated clinical non-steady-state conditions[2] to eightfold in pseudo-steady-state conditions, using a bolus-infusion method for stepwise increases in plasma concentration.[3] The sigmoidal concentration response curve is thus shifted to the right.[2,3] These findings led us to examine the opposite possibility: if disuse atrophy reduces sensitivity to nondepolarizing relaxants, would chronic conditioning exercise, *i.e.*, running, result in greater sensitivity? If conditioning increases sensitivity to nondepolarizing relaxants, this might influence the dosing and antagonism of these drugs in athletes.

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Methods

Five mongrel dogs were exercised with the aid of a nonmotorized free-wheeling carousel. Four nonexercised dogs in the same kennel environment served as controls. Conditioning was performed 5 days per week for 4.5–5 weeks. The dogs ran twice each day, for 45-min periods. Their sustained velocity increased each week. When they achieved conditioning, they ran at an estimated speed of 19–24 kph. This study was approved by the institutional review board and care followed institutional guidelines.

After the 5-week period of exercise, the response to the nondepolarizing relaxant metocurine (MTC) was determined; at this time, the exercised dogs weighed 16.1 ± 1.8 kg (mean ± SD) and the control or nonexercised dogs 15.6 ± 4.6 kg. The dogs were anesthetized with intravenous (forelimb vein) pentobarbital sodium (30 mg · kg^{-1}). Following tracheal intubation, their lungs were ventilated with a mixture of nitrous oxide (65–75%) and oxygen (25–35%). Gas mixtures and ventilation were adjusted to maintain an arterial oxygen tension of 120–180 mmHg and an arterial carbon dioxide tension of 38–42 mmHg. Temperature (mid-esophagus) was maintained at 36.8–37.2° C using heat lamps and heating pads. Arterial blood pressure was transduced *via* a percutaneous femoral catheter; blood pressure, pulse rate, and temperature were regularly monitored.

The dog was supine, with careful fixation by clamps of the knees, hips, and ankles at right angles. The force of contraction of the gastrocnemius muscles was measured bilaterally, using calibrated transducers and pen-paper recorder. A thin light rectangular bar, about 3 × 12 cm, was taped to the bottom of each hind foot and the tip of each bar was wired to a transducer to measure the force of plantar flexion by the gastrocnemius muscle. Needle electrodes for tibial nerve stimulation were placed at each popliteal fossa and localized for the strongest twitch response. The nerves were simultaneously stimulated with a Grass stimulator at 0.1-ms duration, using supramaximal voltage (a 20% greater voltage than that at which twitch amplitude no longer increased). The muscles were stimulated at 2 Hz for 4 pulses (train-of-four). The tension generated by the muscle in response to the pulse was measured as the absolute difference between the resting muscle tension and the stimulated muscle tension. Only the tension of the first twitch (T1) was used in the analysis. Prior to the administration of MTC, the length of the gastrocnemius muscle of the anesthetized animal was ad-

justed by flexion/extension of the paw until maximum force was generated in response to the first pulse. This provided the baseline tension and the control T1 response. MTC-induced neuromuscular blockade was determined by the percent attenuation of the T1 response (1-[T1 measured/T1 baseline]) × 100, and is expressed as percent block.

MTC was given as a constant-rate infusion to achieve 95% depression of the T1 in a period of about 7 min. Exercised dogs received 0.080 $mg \cdot kg^{-1}$ in 5 min (one dog), 0.098 $mg \cdot kg^{-1}$ in 6.5 min (one dog), and 0.090 $mg \cdot kg^{-1}$ in 6 min (three dogs). Nonexercised dogs received 0.137 $mg \cdot kg^{-1}$ in 7.2 min, 0.154 $mg \cdot kg^{-1}$ in 6.17 min, 0.163 $mg \cdot kg^{-1}$ in 6.5 min, and 0.281 $mg \cdot kg^{-1}$ in 7.5 min (one dog each dose). After the infusion was begun, the stimulation pattern was performed every 1.5–2 min for 15 min; arterial blood was sampled each 1.5 min for 15 min. After 15 min, both were performed simultaneously, at 20, 25, 30, 45, 60, 90, 120, 150, 180, and 240 min, or until spontaneous recovery from paralysis was complete. Blood samples were separated for plasma and analyzed by radioimmunoassay using a modification of the technique for analyzing d-tubocurare.[4] The concentration of MTC that inhibits antigen-antibody binding by 50% is 2.5 $ng \cdot ml^{-1}$. The maximum variation of the assay is ±5% at all concentrations. The animals appeared to be satisfactorily anesthetized throughout the study, as foot movement with stimulation was the only observed response.

Pharmacokinetic and Pharmacodynamic Analysis

Three curves were obtained in each dog: one curve related the plasma MTC concentration to time, and the other two curves (one for each hind limb) related the percent neuromuscular block to time. The plasma MTC concentration rose and fell more briskly than did the neuromuscular blockade. This delay, called hysteresis, is more clearly represented when both neuromuscular blockade and blood levels are plotted against time (fig. 1A).

To understand the relationship between MTC concentration and neuromuscular blockade, it is necessary to perform separate pharmacokinetic and pharmacodynamic analysis steps, as described by Sheiner *et al.*[5] In the pharmacokinetic step, the plasma MTC *versus* time curve is fit to a compartmental model (fig. 1B). In the pharmacodynamic analysis, the plasma MTC concentrations predicted by the pharmacokinetic model are used to estimate the parameters of the pharmacodynamic model. The pharmacodynamic model assumes that the drug must first transfer from the central compartment into a site where the drug exerts its effect, a new compartment, called the effect compartment. The effect compartment is strictly a mathematical concept, but it presumably corresponds to the site at which acetylcholine interacts with its receptors. The concentration of drug in the effect compartment (Ce) cannot be measured directly. However, the rate of transfer of the drug into the effect compartment can be described by a rate constant, K_{eo}. The half-time of equilibration between plasma and the effect compartment ($t_{1/2}$ K_{eo}) is calculated as $\ln(2)/K_{eo}$. K_{eo} was simultaneously estimated with the parameters of the pharmacodynamic model.

Muscle relaxant pharmacodynamics were modeled using the Hill equation:

$$\text{paralysis} = (1.0 \cdot Ce^{\gamma})/(IC50^{\gamma} + Ce^{\gamma}),$$

where 1.0 indicates that the maximum possible effect is 100% paralysis, Ce is the estimated MTC concentration in the effect compartment, IC50 is the MTC concentration in the effect compartment which produces 50% paralysis, and γ is the slope factor of the sigmoidal concentration-response curve (fig. 1C).[3]

The pharmacokinetic and pharmacodynamic modeling were performed on an 80386 microcomputer running MS-DOS using MKMODEL,[6] an extended least squares regression program. Data are reported as mean ± SD. Pharmacokinetic and pharmacodynamic parameters were compared using the unpaired Student's *t* test at the $P < 0.05$ level for significance.

Results

A two-compartment model satisfactorily described the pharmacokinetics of each animal. Table 1 summarizes the pharmacokinetic results for the exercised and nonexercised dogs. Four significant differences were observed in the pharmacokinetics between the two groups. The central volumes (Vc) of the exercised dogs were uniformly smaller than the central volumes of the nonexercised dogs. This resulted in higher initial MTC concentrations in the exercised dogs, as demonstrated in figure 1D. The exercised dogs all had higher normalized initial MTC concentrations than did the nonexercised dogs.

The exercised dogs also had a more rapid initial distributional half-life than the nonexercised dogs. Thus, there was initially a more rapid decrease in the plasma concentration in the exercised dogs.

The exercised dogs had smaller steady-state volumes of distribution (Vdss) than the nonexercised dogs, and slower central (presumably renal) clearances. These differences tend to cancel, resulting in nearly identical terminal elimination half-lives. Physiological values, *e.g.*, temperature, blood pressure, etc, were stable and within the normal canine ranges for general anesthesia or the ranges described in Methods.

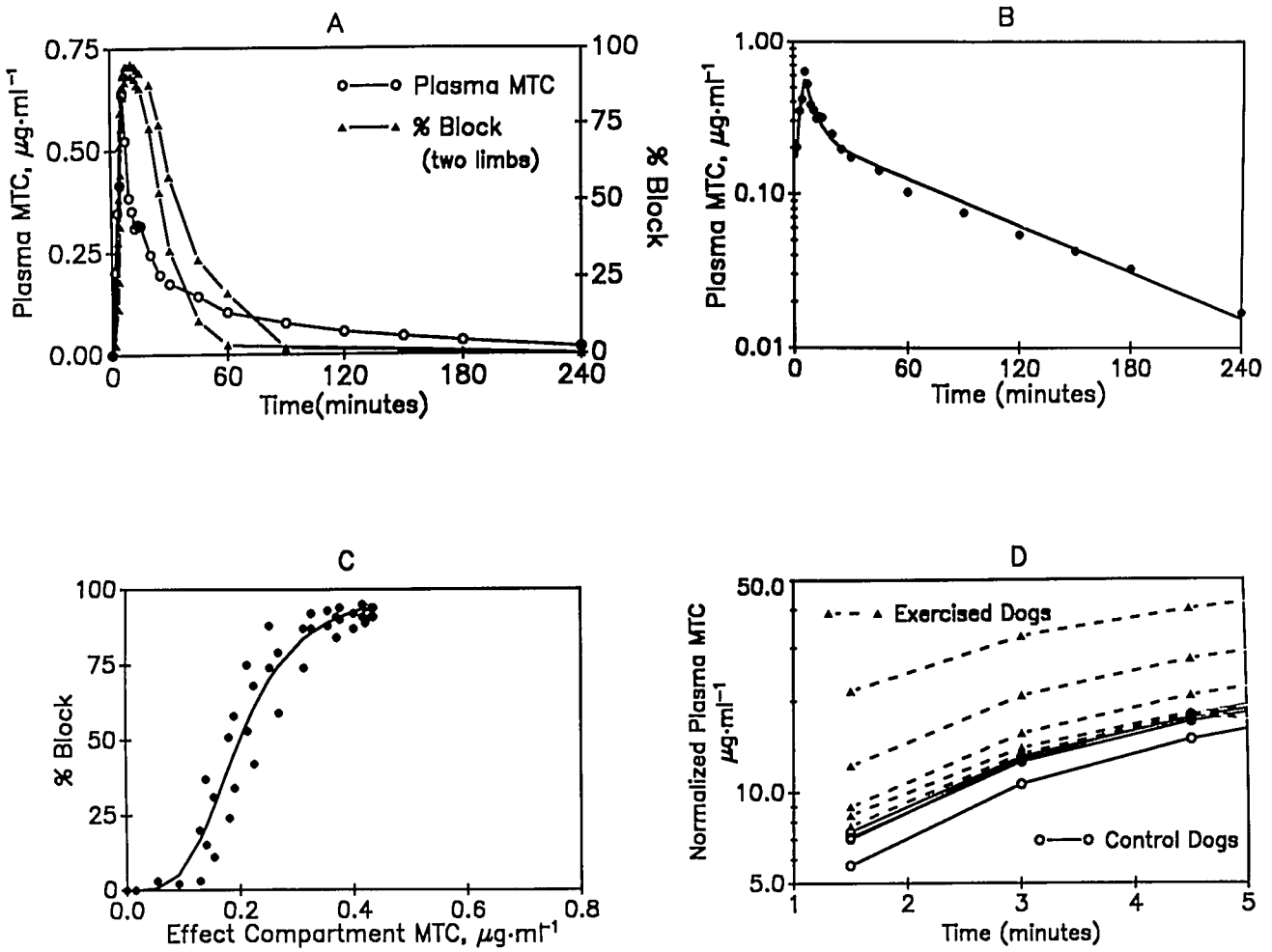

FIG. 1. *A.* Plasma metocurine (MTC) level (left vertical axis) and % block (right vertical axis) *versus* time, demonstrating hysteresis. See text for details. *B.* Typical pharmacokinetic curve in an individual dog, relating plasma metocurine (MTC) concentration to time. *C.* Typical pharmacodynamic curve in an individual dog relating % block to effect compartment concentration of metocurine (MTC). *D.* Normalized blood levels of metocurine (MTC) *versus* time during the initial 5 min of infusion. For this figure, the measured blood levels have been divided by the MTC infusion rate to normalize for the different infusion rates administered to each animal. The early portions of these curves can be directly compared because each dog was infused for at least 5 min. Blood levels are higher in exercised dogs. See text for details.

Figure 2 and table 2 illustrate the differences between the muscle single twitch pharmacodynamics of the exercised and nonexercised dogs. The two hind limb relaxant responses for each dog showed a high degree of correlation. This correlation mandated simultaneous fitting with the pharmacokinetic data. Thus, the individual curves in figure 2 represent the pooled responses of both hind limbs of each dog. The concentration-response is shifted to the left for the exercised dogs, *i.e.*, the gastrocnemius muscle of these dogs was more sensitive to MTC than that of the nonexercised dogs. The IC50 is 0.114 ± 0.008 μg/ml for exercised dogs and 0.189 ± 0.038 for nonexercised dogs, with a ratio of 1.7. The slopes are not different, *i.e.*, the curves are parallel. The half-time for MTC equilibration between the plasma and the effect compartment ($t_{1/2}$ K_{eo}) was 2.3 ± 4.9 min for the exercised dogs, and 2.2 ± 5.0 min for the nonexercised dogs (N.S.).

Discussion

The smaller volumes seen in the exercised dogs is an intriguing pharmacokinetic result for which we have no direct explanation. It may represent differences in the cardiovascular response of exercised animals to anesthesia, or differences in extracellular fluid volume in the two groups. Similarly, the decreased elimination clearance in the exercised dogs is surprising. However, the exercised dogs were sampled for just 120 min, which is only twice

TABLE 1. Pharmacokinetics

	Volumes (ml·kg^{-1})		Half-lives (min)		Clearances (ml·kg^{-1}·min^{-1})	
	Vc	Vdss	α	β	Central	Distribution
Control						
Dog 1	185	517	2.75	51.1	7.94	26.4
Dog 2	195	488	3.79	59.5	6.44	18.9
Dog 3	241	643	4.56	79.6	6.33	20.3
Dog 4	176	578	2.85	54.1	8.72	25.3
Mean	199	557	3.49	61.1	7.36	22.7
SD	29	69	0.85	12.8	1.17	3.7
Exercised						
Dog 1	100	308	1.74	58.9	3.89	25.2
Dog 2	142	487	1.50	96.9	3.63	44.5
Dog 3	163	436	1.93	49.7	6.55	33.9
Dog 4	47	203	0.94	37.4	4.16	24.0
Dog 5	143	477	2.43	59.7	6.23	25.4
Mean	119	382	1.71	60.5	4.89	30.6
SD	46	123	0.55	22.2	1.39	8.7
P value	0.02	0.05	0.01	ns	0.05	ns

Vc = central volume; Vdss = volume at steady-state; α = distribution half-life; β = elimination half-life.

TABLE 2. Pharmacodynamics

	t1/2 K_{eo} (min)	IC 50 (μg·ml^{-1})	γ
Control			
Dog 1	2.66	0.183	2.4
Dog 2	1.89	0.202	3.7
Dog 3	1.43	0.230	1.2
Dog 4	4.25	0.140	3.3
Mean	2.17	0.189	2.6
SD	5.01	0.038	1.1
Exercised			
Dog 1	4.33	0.106	1.3
Dog 2	1.47	0.109	2.7
Dog 3	1.77	0.123	3.0
Dog 4	4.30	0.108	1.3
Dog 5	2.04	0.122	1.6
Mean	2.27	0.114	2.0
SD	4.95	0.008	0.8
P value	ns	0.005	ns

t1/2 K_{eo} = half-time equilibration for the effect compartment; IC 50 = estimated effect compartment level of metocurine at 50% twitch paralysis; γ = slope factor (steepness) for the sigmoidal concentration response curve.

the elimination half-life. Possibly, longer sampling times, *i.e.*, 4–5 half-lives, might have produced different estimates for elimination clearance in the exercised group. The nonexercised dogs were sampled for 240 min, which should be sufficiently long to reliably characterize clearance during the elimination phase of the kinetics.

The pharmacodynamic results suggest that exercise increases the sensitivity of the gastrocnemius muscle to metocurine. This is an effect opposite to that of muscle disuse, which diminishes sensitivity to MTC.[2,3] Can hypothetical mechanisms relating to disuse be applied conversely for overactivity? Resistance to nondepolarizing relaxants with disuse is theoretically due to terminal nerve sprouting,[7] modestly increased numbers of extrajunctional receptors in the perijunctional area,[8] and decreased muscle cholinesterase activity,[9,10] all of which tend to increase the "potency" of acetylcholine.[11] There is evidence supporting the reverse of this hypothesis with regard to exercise: 1) exercise increases muscle cholinesterase activity,[9] and, 2) the number of functioning receptors at the endplate may be directly related to degree of activity.[11] Finally, use/disuse patterns and their related energy needs may comprise the major determinant of a fiber's metabolic enzyme profile. The profile with prolonged chronic stimulation includes alterations in composition and mass of mitochondria, increases in oxidative enzymes, decreases in glycolytic enzymes, and transformation of fiber types in the direction from fast toward slow twitch.[12] Thus, with disuse, extrajunctional receptors develop, in association with decreased activity of muscle cholinesterase. With exercise, receptors may be more "concentrated" at the endplate in conjunction with increased muscle cholinesterase activity, and variations in mitochondria, various enzyme activities, and fiber types.

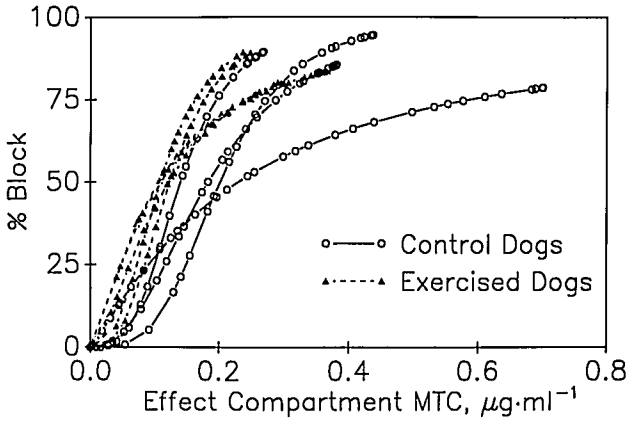

FIG. 2. Percent block *versus* effect compartment concentration of metocurine (MTC) with twitch stimulation of the gastrocnemius muscle in dogs with exercise (running) and normal (kennel) activity. Muscle in exercised dogs is more sensitive to the effects of MTC, $P < 0.05$.

Our pilot studies (not reported) indicated that smaller doses of MTC would be required to achieve the same degree of neuromuscular blockade in the exercised dogs; therefore, lower infusion rates were used in that group. These results confirm the reduction in dose requirement in the exercised dogs, and provide both pharmacokinetic and pharmacodynamic differences that contribute to this reduction. The exercised dogs have a smaller initial volume, and a smaller volume of distribution, which result

in higher initial plasma concentrations for the same dose of drug. Their more rapid elimination clearance neutralizes the difference in blood level. Lastly, the exercised dogs have increased sensitivity to MTC. These factors contribute to a greatly reduced dose requirement in the exercised dogs.

Clinical considerations relate to relaxant use in situations involving disuse and muscle conditioning. With disuse, succinylcholine is unlikely to cause hazardous acute systemic hyperkalemia (arbitrarily a value greater than 7 meq/l), unless virtually all of the musculature of the body is involved. This might occur with complete bed rest, whole body spicas, etc. Relaxant resistance, in addition to its association with disuse, is observed in patients with thermal trauma,[13] upper motor neuron lesions,[14] and anticonvulsant therapy.[15,16] In those situations in which only a portion of muscle is affected, it is important to monitor relaxation in other muscle groups. If most muscle in the body becomes resistant, it may be that the usual difference in sensitivity between ventilatory and other muscles may be lost. Therefore, monitoring of return of function is vitally important; partial recovery could be associated with inadequate protection of the airway and an inadequate cough reflex.

In someone who is physically conditioned, the potentially increased response to nondepolarizing muscle relaxants may be associated with initially higher blood levels and, therefore, profound paralysis following an ordinary relaxant dose. In this situation, the usual difference in sensitivity between the diaphragm and skeletal muscles would likely be increased. Furthermore, monitoring of blockade of conditioned muscles need not necessarily reflect the surgical needs for a particular procedure. Finally, patient variability in relaxant responses is a well-recognized and poorly understood phenomenon. It may, in part, be due to differences in conditioning, and to differing use and disuse of particular muscles throughout the body in an individual patient. The muscle that the anesthesiologist selects for neuromuscular monitoring may not necessarily reflect the status of that patient's muscle in general. Clinical data at present are lacking that could define these situations more definitively.

This study suggests that the normal endplate region undergoes continuing regulation of the function of its receptors, depending in part upon the degree of muscle activity, in addition to regular receptor turnover.[11] Furthermore, these regulatory changes may involve the number and quality of receptors, their location, alteration in distal nerve terminals, altered enzyme and mitochondrial functions, and variations in muscle cholinesterase activity.

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