The Changing Pharmacodynamics of Metocurine
Identify the Onset and Offset of Canine Gastrocnemius Disuse Atrophy

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Background: Immobilization of skeletal muscle results in disuse atrophy and resistance to nondepolarizing muscle relaxants. We studied the pharmacodynamics of metocurine (MTC) to identify the development and recovery of disuse-related resistance to MTC.

Methods: Nineteen dogs underwent cast immobilization of a hind limb for as long as 3 weeks. Before, during, and after casting, dogs were intermittently anesthetized with thiopental-N₂O-fentanyl. The blood concentration of MTC and the corresponding degree of paralysis after a brief infusion were recorded and used to characterize the pharmacokinetics and pharmacodynamics of MTC.

Results: Pharmacodynamic study of the response to MTC demonstrated resistance by the 4th day of casting. The effect-site concentration associated with 50% paralysis of twitch increased after 3 weeks from approximately 250 to 750 ng/ml. After cast removal, resistance persisted for 2 more weeks. Six weeks after cast removal, the effect-site concentration associated with 50% paralysis of twitch was normal in every dog.

Conclusions: Within the context of this study of immobilization disuse atrophy, pharmacokinetic and pharmacodynamic characterization of antagonist responses can be used to infer muscle disuse-related changes in acetylcholine receptors. (Key words: Muscle; skeletal; disuse atrophy; immobilization; Neuromuscular relaxants, nondepolarizing; metocurine. Pharmacodynamics-metocurine. Pharmacokinetics-metocurine.)

DURING neuromuscular transmission, acetylcholine (ACh) released from the motor nerve terminal interacts with postsynaptic acetylcholine receptors (AChRs). When motor activity is decreased, as in immobilization of skeletal muscle, release of ACh for interaction with these receptors is diminished, without loss of neural integrity. This change results in spread of AChRs beyond the endplate region; this spread has been demonstrated by iontophoresis and by the response to close arterial injection of ACh. This change is also manifested as resistance of skeletal muscle to competitive antagonists (nondepolarizing muscle relaxants).

We hypothesized that changes in the responses to a competitive antagonist would permit characterization of the development and recovery of the AChR changes associated with muscle disuse atrophy. We studied the change in response to metocurine (MTC) in dogs with disuse atrophy produced by immobilization of one hind limb and used the noncasted limb for comparison. MTC studies involved pharmacokinetic and pharmacodynamic determinations using data for time, concentration in blood, and degree of paralysis after a brief infusion of MTC.

Materials and Methods

These studies were approved by the Animal Care and Use Committee, and all procedures conformed to institutional and National Institutes of Health animal care guidelines. Each MTC study required 6 h of anesthesia and recovery time. If the same dogs had been scheduled for study every day or every other day, disuse atrophy could have developed in both legs because of the associated inactivity; thus serial MTC studies could not be scheduled at close intervals. Pharmacokinetic-pharmacodynamic MTC studies were scheduled as shown in table 1. Nineteen dogs underwent MTC pharmacokinetic-pharmacodynamic studies (table 1). In groups 1 and 2 the development of resistance to MTC was examined, and in group 3 the recovery from resistance to MTC was examined.
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Table 1. MTC Testing Protocol: Metocurine Concentration Response Studies during Development of and Recovery from Immobilization Disuse Atrophy

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<th>Days after Cast Application</th>
<th>Days after Cast Removal</th>
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<td>2</td>
<td>3</td>
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<td>Group (N)</td>
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<td>I (6)</td>
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<tr>
<td>II (8)</td>
<td>2</td>
<td>3</td>
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<tr>
<td>III (5)</td>
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I, II = beagles; III = mongrels; one dog died on day 7.

All dogs (weight 8–18 kg, groups 1–3) (table 1) underwent cast immobilization on day 0. Casts were applied during thiamylal sedation (10–20 mg/kg); the pelvic girdle and one hind limb were positioned in the cast with the knee and ankle at 90° and the hip flexed so that the dog could walk on three legs without dragging the casted paw. Casts were removed and reapplied every 5–7 days, and the dogs were examined for pressure sores.

Six beagles (group 1) were anesthetized 3–4 weeks before cast application to determine normal values for the effect-site concentration of MTC that would result in 50% paralysis (IC50). IC50 was determined again 2 days after casting in all six dogs and again after 21 days. Group 2 beagles (n = 8) were examined for onset of resistance to MTC 2–5 days after cast immobilization. Group 3 mongrels (n = 5) were examined for changes in response to MTC after casting for 6, 13, and 20 days. The casts were removed, and MTC IC50 was serially determined through the 60-day period after cast removal and resumption of activity.

Pharmacokinetic-Pharmacodynamic Studies

Kinetic and dynamic data were obtained during 60% N2O-thiamyl (10–20 mg/kg)-fentanyl (0.1–0.4 mg) endotracheal anesthesia. Controlled ventilation (pump, model 613, Harvard, South Natick, MA) and gas mixtures were adjusted to provide an arterial CO2 tension of 37–40 mmHg (end-expired CO2 32–36 mmHg) and arterial O2 tension greater than 100 mmHg. Other baseline parameters included pH 7.35–7.50, hematocrit greater than 30%, pulse oximeter O2 saturation greater than 95%, esophageal temperature 36.8–37.6°C, arterial blood pressure 120/80–160/100 mmHg, and heart rate 60–140 beats/min; lactated Ringer’s solution was infused intravenously at 5 ml·kg⁻¹·h⁻¹. Percutaneous cannulations included two peripheral veins (one for thiamylal and maintenance fluids and one for MTC infusion) and one femoral artery (for blood samples for MTC and blood gas analyses).

For measurements of skeletal muscle function, the dog was placed supine in a specially designed, padded, rigid frame with both hind limbs flexed 90° at the hip and knee. A light metal stirrup was fastened to each distal paw and connected to transducers (FT10, Grass) that were secured to the frame. Stimulating needles were placed bilaterally into the popliteal fossae and positioned to evoke a maximal plantar twitch response. At supramaximal voltage, the length of the muscles was adjusted until the amplitude of the twitch was maximal. The stimulation pattern involved four twitches at 2 Hz (train-of-four), duration 0.1 ms, every 30 s (stimulator S11, one output channel for each leg, Grass, Quincy, MA). The transducer outputs were digitized by an analogue-to-digital board (2820, Data Translation, Marlboro, MA) in a personal computer, analyzed, and displayed in real time every 30 s by a program written in the SYST language. Each train-of-four was analyzed to determine the amplitude of the first twitch (T1), its value compared with control as paralysis progressed, and the ratio of the fourth to the first twitch (T4/T1).

A recorder (model 2800S, Gould, Valley View, OH) continuously displayed muscle tension and blood pressure. After stabilization of the twitch response, a dilute solution of MTC (0.15–0.5 mg/ml) was infused intravenously (syringe pump, Harvard) for 4–8 min to achieve 80–90% blockade of the casted side. The train-of-four response was measured and displayed until recovery from MTC was complete, as judged by return of T1 amplitude to control and recovery of fade of T4/T1. Three-milliliter arterial blood samples for MTC concentrations were taken every 1.5 min from 0 to 18 min and at 20, 25, 30, 45, 60, 90, 120, 150, 180, and 240 min. Plasma MTC was measured by radioimmunoassay (RIA) (mongrels) or high-pressure liquid chromatography (HPLC) (beagles).

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Pharmacokinetic-Pharmacodynamic Analysis

Three curves were obtained in each study: one related plasma MTC concentration to time, and two related paralysis of each hind limb to time. The MTC concentration versus time data were fit to a two-compartment model for pharmacokinetic values: central volume, distribution half-life, elimination half-life, and clearance. These data provided the basis for the pharmacodynamic analysis, which yielded the half-time equilibrium value between the effect compartment and plasma, the effect-compartment concentration associated with 50% paralysis (IC₅₀), and the slope factor of the sigmoid dose-response curve. This modeling was performed on a microcomputer running the MS-DOS platform (Microsoft, Redmond, WA) with MKMODEL, an extended least-squares regression program.⁷

Data are reported as means ± SE. Statistical comparisons included one-way analysis of variance and paired (Bonferroni's correction) and unpaired t tests; P < 0.05 was considered to indicate statistical significance. Findings in the groups estimating development of resistance (groups 1 and 2) were compared with values before casting (pooled IC₅₀ values for both legs before casting in group 1) to establish differences from control values. Findings in the recovery group (group 3) were compared with control values (pooled values for both legs), which were obtained on day 61 after cast removal. IC₅₀ values for casted and noncasted legs were compared within treatment groups throughout the study. For between-group comparisons, groups 1 and 2 were combined because they were the same species and involved the same MTC assay technique.

Results

Pharmacodynamics

Effects on the First Twitch in the Train-of-four (T₁, Comparison with Control and IC₅₀). When resistance to MTC was present, it was immediately evident during the infusion of MTC. T₁ of the noncasted limb became paralyzed sooner and to a greater degree than did T₁ of the casted limb. Differences in IC₅₀ between the two limbs were significant by the 4th day of immobilization (paired t test) (fig. 1). This difference was pronounced by day 20 or 21, when the cast was removed. Values from day 20 in figure 1 for IC₅₀ represent pooled data for day 20 (mongrels, 713 ± 122 ng/ml) and day 21 (beagles, 749 ± 110). Upon emergence from anesthesia on day 20, group 3 dogs were immediately ambulatory and appeared to have normal walking and running patterns. Two days after removal of the cast, resistance to MTC had diminished considerably. However, the casted leg was still significantly resistant in comparison with the noncasted leg, and this difference persisted for 14 days after cast removal (paired t test).

Differences from normal MTC sensitivity (i.e., IC₅₀) in active dogs before casting were statistically evident.
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on days 4, 6, 13, and 20 or 21 (fig. 1). During recovery the casted limb IC50 differed from control IC50 (day 61 after cast removal) through 2 weeks after cast removal. IC50 values for the noncasted limb were significantly greater than normal (+15 ± 135 ng/ml) only on days 20 and 21.

Other Effects. Differences between the casted and noncasted limbs were erratic for the T1/T0 ratio, and significant resistance was noted in the casted limb only on days 20 and 21. This prejunctional response did not appear to be easily altered by postjunctional disorders. The equilibration value between the effect compartment and plasma and the slope factor were not consistently altered with prolonged immobilization or the postimmobilization exercise period. Mean values for the equilibration value between the effect compartment and plasma ranged from 0.2 to 0.46 min⁻¹ throughout the study. Mean values for the slope factor ranged from 1.6 to 8.

Pharmacokinetics

The central volume was larger in mongrel dogs, and mean values ranged from 69–166 ml/kg, with no discernible effect of immobilization or resumption of activity after immobilization. Mean values for the central volume in beagles ranged from 39 to 86 ml/kg and, again, disuse and recovery during the study did not alter these. Other pharmacokinetic values (data not shown) were similar to those described in a study of exercise and did not change during disuse and recovery.

Additional Parameters

Blood pressure, pulse, temperature, hematocrit, and blood gases were well maintained and within normal ranges in all studies (data not shown). Anesthesia appeared satisfactory in all groups: foot movement was the only observed response. One dog from group 5 was found dead in its kennel on the 7th day of immobilization (table 1).

Method of Metocurine Studies

The definition of MTC resistance depends on the accuracy of the control values. The IC50 before casting was 262 ± 30 ng/ml, and the IC50 after 4 days of immobilization was 429 ± 36. At 4 days, the IC50 for the noncasted leg was 338 ± 32 ng/ml. The IC50 for the casted leg after 4 days of immobilization was significantly different from the IC50 before casting and from the 4-day IC50 for the noncasted leg (P < 0.05). We believe that the onset of MTC resistance occurred by 4 days after cast removal. After cast removal, the IC50 of approximatively 750 ng/ml diminished within 2 days, to 341 ± 107 ng/ml, but for 14 days after cast removal it was significantly greater than the value for the noncasted leg and the control value (day 61 after cast removal).

The current studies were performed in two breeds with two MTC assays: beagles and HPLC. and mongrels and RIA. We believe that data from these two groups are comparable. First, mongrel IC50 RIA normal values for MTC from a study of exercise, at 189 ± 19 ng/ml are similar to those for the dogs in the current study, at 209 ± 12 ng/ml (pooled control and days 4 and 61 after cast removal). Second, the two assays provided comparable results on the same samples for a wide range of values (as discussed in Materials and Methods). Third, most of the comparisons in the current study involved changes in the responses of the casted leg to those of the noncasted leg, so the assay and the breed for each comparison were the same. Fourth, the degree of MTC resistance in the casted leg at 3 weeks was the same in mongrels (20 days, RIA assay) and beagles (21 days, HPLC assay): 713 ± 122 and 749 ± 110 ng/ml, respectively.

Discussion

There are a variety of situations, including use of drugs such as anticonvulsant medications, induction of enzyme pathways important in metabolism, changes in muscle membrane-receptor phenomena related to neurologic dysfunction, and forced inactivity such as immobilization, that result in resistance to a skeletal muscle competitive antagonist. In the current study the first three factors were avoided, and the sole abnormality that could have altered muscle responses was immobilization. The dogs were active in a kennel environment before the study, and cast immobilization of one hind limb was the sole change introduced into their lifestyle. Between anesthetics, the dogs were active and walked on three legs.

Our findings confirm previous observations of resistance to a competitive antagonist after significant disuse atrophy has occurred. Our study examined the development and recovery associated with this response and demonstrated that MTC resistance was apparent by the 4th day of immobilization as an increase in IC50. This onset is similar to that observed in rat hind-limb soleus immobilization. By 20 or 21 days after casting,
IC₅₀ was increased by threefold in groups 1 and 3. At this time, the noncasted leg also showed a modest resistance to MTC, presumably related to volitional decrease over all activity. Such resistance of the contralateral limb has been seen in another model.¹

The period of recovery from disuse-induced resistance to a competitive antagonist has not previously been reported. Fischbach and Robbins¹ and Solandt et al.,² using iontophoretic and close arterial administration of ACh, respectively, for determination of receptor spread in rat soleus muscle, showed a beginning return toward normality during the period of disuse.¹,² This observation does not appear to apply to the current study in dogs for the following reasons. The response to MTC relates to an interaction between MTC and postnaptic AChRs (junctional and extra-junctional) that are exposed to ACh released by the motor nerve terminal, whereas the AChR data of Fischbach and Robbins and of Solandt et al. measure a different aspect. The Fischbach and Robbins¹ method involves iontophoretic pulsed release of ACh by a micropipette placed on the muscle membrane physically near but not at the junction; the pulsed ACh depolarizes the membrane when sufficient nonendplate AChRs are present, and the resulting current is detected by a microelectrode placed some distance away on the muscle membrane. The Solandt et al.² technique of close arterial injection of ACh estimates the size of the muscle membrane with increased cholinergic sensitivity (attributable to increased numbers of AChRs). The findings for both these techniques relate to peripheral or additional receptors that are more widely spread from the endplate area and most of which are likely beyond the reach of nerve-released ACh. Thus, resistance to MTC during disuse is directly related to increased numbers or different types of postnaptic receptors that are exposed to ACh diffusing to the endplate from the nerve terminal and is based on the relation between the response of the extrajunctional AChRs in disuse and a competitive antagonist.¹¹

In group 3, 2 days after cast removal, the IC₅₀ of the casted leg decreased dramatically but was still greater than the IC₅₀ of the contralateral leg (which had returned to the normal range) and the control value on day 61 after cast removal. The IC₅₀ value for the casted limb did not return to the normal range until 2 weeks after cast removal and resumption of normal activity.

We do not have direct measurements of AChRs during the development and recovery of resistance during this period of disuse atrophy, and our correlation with resistance to MTC assumes a relationship with extra-junctional AChRs. Receptor data from several sources help to confirm this correlation. Newly developed AChRs have been measured in other situations, such as the long-term use of anticonvulsant agents and use in the intensive care unit.¹² The former is not an example of disuse but has reactions parallel to those of disuse (as discussed below); the latter involves disuse directly. Our own pilot study in the intensive care of three dogs that were sedated (without use of competitive antagonists) and the lungs of which were mechanically ventilated for 3 weeks demonstrated markedly increased numbers of intercostal muscle peripheral AChRs, from a normal value of 0.2–0.4 pmol/g tissue to 0.63, 1.27, and 2.29 pmol/g, assayed by a high-titer polyclonal antiserum. These findings were associated with marked resistance to MTC in the gastrocnemius: IC₅₀ increased from 227 ± 43 to 1,297 ± 194 ng/ml. In addition, patients in the intensive care unit whose lungs are ventilated and who are given nondepolarizing muscle relaxants have an increased number of AChRs, according to findings of rectus abdominis biopsy.¹² A nondepolarizing relaxant can increase the number of AChRs through motor nerve blockade, but the associated disuse is still a factor affecting skeletal muscle receptors.¹¹

Finally, whereas canine disuse atrophy is associated with resistance to the nondepolarizing competitive antagonist MTC,³ its physiologic counterpart, conditioning exercise, is associated with sensitivity to MTC.⁴ The sensitivity to MTC with exercise does not by itself indicate that a change in AChRs is responsible, and AChR data related to exercise are not available. However, these opposing physiologic changes are associated with opposing responses to the competitive antagonist. Because one physiologic change (disuse) is associated with increased postnaptic AChRs, we speculate that the other physiologic change (conditioning exercise) may in some as yet unknown way invoke a decrease in the quantity or density of postnaptic AChRs.

The current study attempted to examine the ACh receptor system of skeletal muscle during disuse atrophy. Receptor systems have been accurately characterized by use of appropriate antagonists, without direct measurement of the properties, structure, or numbers of receptors. An example is the study by Mandema et al.,¹¹ which the anticonvulsant action of carbamazepine was used to quantitate the nicotinic-pine receptor function in conscious, receptor binding, and pharmacodynamic analyses of a rat model. The anesthetic, receptor binding, and pharmacodynamic analyses revealed a close correlation of receptor density and affinity for the anticonvulsant drug and potentiation of its effect by unoccupied receptor binding sites.

This study was performed in a manner in which the receptor complex was specifically measured, and the receptor complex was specifically measured. The receptor complex was specifically measured in a manner in which the receptor complex was specifically measured.


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in which the anticonvulsant activity of four benzodiazepines was used to quantify γ-aminobutyric acid-benzodiazepine receptor function. Pharmacokinetic and pharmacodynamic analyses of unbound drug concentration, receptor binding, and electroencephalographic activation revealed a close correlation between potency and receptor affinity for four drugs with varying plasma protein binding and potencies. The ratio of the 50% effective unbound concentration to receptor affinity was essentially constant for the four drugs. Furthermore, slope analysis suggested a single binding site at the receptor complex. Thus, receptor function was accurately determined through action of the appropriate antagonist on seizure activity, when coupled with measurements of receptor binding.

In a similar study using this approach, we attempted to characterize AChRs of skeletal muscle indirectly by use of IC_{50} for MTC in six species varying in size: rat, cat, dog, horse, pig, and sheep. The AChR is strongly conserved across species, and our hypothesis had been that this homology would result in similar IC_{50}s, that is, a similar equilibrium effect-size concentration for MTC regardless of species size (a size-independent IC_{50}). This hypothesis presumed that other factors affecting overall AChR binding reactions were not different. Our hypothesis was supported only in part: in three species IC_{50} showed size independence (rat, pig, and sheep; IC_{50} 50–70 ng/ml), and in four IC_{50} demonstrated size dependence (rat, cat, dog, horse; IC_{50} 50–500 ng/ml). The rat appears in both categories because it presents comparison data for the smallest size. We do not have a direct explanation for these species differences but speculate that densities of AChRs may differ as a result of varying degrees of athleticism as reflected over centuries of selection and behavior.

Immobilization disuse atrophy does not directly interfere with motor nerve function. Although the nerve remains intact, there is a profound decrease in motor nerve activity (i.e., lessened ACh release over time), resulting in atrophy. This atrophy may be more marked in animals because they reportedly cease to use parts rendered useless. Long-term disuse thereby enlarges the functional endplate area and is associated with resistance to competitive antagonists. Other clinical conditions that mimic this situation include cerebral palsy, myelomeningocele, and long-term use of anticonvulsant agents. In these situations, nicotinic cholinergic receptor changes have been characterized as mild upregulation, that is, competitive relaxant resistance in the absence of marked hyperkalemia after succinylcholine.

In summary, immobilization disuse atrophy produced by casting led to the development of resistance to MTC, with recovery delayed after immediate removal of the cast and resumption of exercise. This study demonstrated that in the proper context the time course of changes in the ACh receptor system of skeletal muscle may be characterized by the responses to competitive antagonists.

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