

The Onset of Disuse-related Potassium Efflux to Succinylcholine

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Disuse atrophy of skeletal muscle produces resistance to nondepolarizing relaxants and increased potassium efflux after the administration of succinylcholine. These changes appear to be due to development of perijunctional and/or extrajunctional receptors (up-regulation). In this study, the authors searched for the earliest detectable appearance of increased potassium efflux in beagles in whom disuse atrophy was simulated. Seven beagles underwent unilateral cast immobilization of a hind limb. Between 4 and 42 days, they periodically received succinylcholine 0.25 mg/kg while anesthetized with thiamylal and nitrous oxide. Sequential bilateral femoral venous samples showed that the casted limb did not manifest potassium release greater than the upper limit of normal (1 mEq/l) until cast immobilization periods of 14 days or longer. When this occurred, the increase in the potassium concentration in the femoral venous blood of the casted limb exceeded that from the noncasted limb by at least 0.7 mEq/l ($P < 0.01$). The range for the onset of this response after casting was 14-42 days, the mean 27.2 days, and the standard deviation 9.8 days. These findings imply that up-regulation of skeletal muscle receptors, associated with exaggerated potassium efflux after administration of succinylcholine, is dependent on progressive development of extrajunctional receptors over surface membrane areas beyond the endplate. (Key words: Ions, potassium; hyperkalemia. Muscle: disuse; resistance to nondepolarizing relaxants. Neuro-muscular relaxant: succinylcholine. Receptor: up-regulation.)

DISUSE ATROPHY is associated with a two- to three-fold increase in the requirement for nondepolarizing muscle relaxants (NDMR).¹⁻³ This is comparable in magnitude to the resistance observed in the denervation lesion of an upper motor neuron defect.⁴ Disuse atrophy is likewise associated with increased potassium (K^+) efflux after succinylcholine (SCh), but this change is quantitatively less than that noted in upper motor neuron lesions.^{5,6}

Both of these responses—resistance to NDMR and increased K^+ efflux after SCh—appear to be related to an increase in the number of nicotinic receptors on skeletal muscle membranes, a phenomenon also called skeletal muscle up-regulation.^{1,2,4-7} Whereas NDMR are antagonists for these receptors, SCh is regarded as an agonist, because it initiates its paralyzing action with direct acetylcholine (ACh)-like stimulation. When the exposure of ACh receptors to ACh is reduced, receptor up-regulation begins, leading to agonist sensitivity and antagonist resistance.⁸ In disuse atrophy, the usual stimulation of muscle

by ACh is reduced, thereby leading to up-regulation. Resistance to NDMR occurs secondary to alterations or increases in receptors at and around the motor endplate area, or the neuromuscular junction,¹ whereas increased K^+ efflux involves increased numbers of extrajunctional cholinergic receptors that flux K^+ , sodium, and calcium upon exposure to cholinergic agonists.⁶ When the number of receptors is sufficiently increased, the increase in K^+ efflux is detectable through measurement of that muscle's venous K^+ concentration.⁵

We hypothesize that the receptor changes responsible for the development of resistance to NDMR should be lesser in magnitude than those necessary to result in measurable increase in K^+ efflux by SCh. Therefore, resistance to NDMR should occur earlier. Preliminary results suggest that resistance to NDMR commences less than 2 weeks after cast immobilization.³ The present study examines the onset of increased K^+ efflux to SCh during development of disuse atrophy.

Materials and Methods

The protocol was approved by the Animal Care Committee. Nineteen colony-bred beagles (1-10-yr old females, 8.9-14.3 kg) were entered into the study. Muscle disuse was produced by immobilizing the right hind leg with Scotchcast Plus casting tape over stockinette and Webril. The leg was positioned in 90° flexion at the hip, knee, and ankle joints so that weight could not be supported on the casted leg. The cast was applied during intravenous thiamylal anesthesia (25 mg/kg) and required approximately 15 min. Care was taken to avoid pressure injury. The dogs were checked daily to verify that immobility was maintained and there were no pressure points, since any source of direct muscle damage would lead to marked changes in the response to SCh.⁹⁻¹¹ To be certain that this study involved only pure disuse, without the exaggerated response to SCh noted with direct muscle damage, we took great care to exclude any animal that showed the slightest sign of pressure or ulceration under the cast.

During periods of immobilization ranging from 4-42 days, the response of each dog to SCh was tested. For this study an abnormal K^+ efflux to SCh was defined as an increase in femoral venous K^+ concentration greater than 1 mEq/l after the administration of SCh. (Studies in humans and dogs have demonstrated that the increase in K^+ after SCh is about 0.2-1 mEq/l and that the peak increase in normal subjects is about 1 mEq/l.¹²⁻¹⁶ Furthermore, a greater than 1 mEq/l increase has been the

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criterion in studies in humans and dogs for an abnormal response of exaggerated K⁺ release after SCh.^{13,17} Each dog was anesthetized and given SCh every 4–8 days, until the increase in K⁺ concentration exceeded 1 mEq/l in the femoral venous blood of the casted limb. During thi-amylal (27 ± 6 mg/kg, mean ± standard deviation) and nitrous oxide anesthesia, an endotracheal tube was inserted. Mechanical ventilation (Harvard Pump) and inspired oxygen concentrations were adjusted so that exhaled carbon dioxide and oxygen concentrations were, respectively, 5.6 ± 0.9 and 25 ± 4%, as measured by mass spectrometry. Esophageal temperature was maintained at 36.7 ± 0.6° C by a heating mattress. Pulse rate and blood pressure were evaluated by intermittent indirect methods.

Both femoral veins were cannulated percutaneously with 3.8-cm 18-G Teflon catheters (Angiocath). The catheters were directed distally to sample venous blood returning from the hind legs. After withdrawing control venous samples, SCh 0.25 mg/kg was given as an intravenous bolus into a forelimb vein. The canine paralytic dose of SCh is 0.25 mg/kg because of lower plasma cholinesterase concentrations in dogs.¹⁸ Simultaneous femoral vein samples (3 ml each) were obtained at 1, 1.5, 2, 2.5, 3, 5, 6, and 10 min after SCh. The samples were withdrawn into heparinized syringes at a rate of 1 ml/10 s to avoid hemolysis and contamination from the contralateral femoral vein or inferior vena cava. Each 30-s sampling period included its sampling time at its midpoint; *i.e.*, the 1-min sample began at 45 s and finished at 1 min and 15 s.

The blood samples were immediately immersed in ice. Plasma was separated after centrifugation and refrigerated until analysis by an IL Flame Photometer. Each sample was analyzed in duplicate and the average value recorded. If a 1 mEq/l or greater increase in serum K⁺ was not observed, the cast was reapplied and the SCh challenge was repeated 4–8 days later. When a 1 mEq/l increase in plasma K⁺ was observed, the dog was released from the study into the university animal research pool. The duration of SCh-mediated K⁺ efflux was not determined. Results are expressed as mean ± standard deviation. Statistical comparison of the results of the casted and non-casted legs was performed with the paired *t* test; *P* < 0.05 was deemed significant.

Results

The onset of increased K⁺ efflux after SCh ranged from 2–6 weeks (fig. 1). All dogs were tested every 4–8 days until they were released from the protocol. No dog exhibited a 1 mEq/l femoral venous plasma K⁺ increase in response to SCh until the 14th day. Seven dogs completed the study, and all seven developed a greater than 1 mEq/l increase in venous plasma K⁺ from the casted leg after

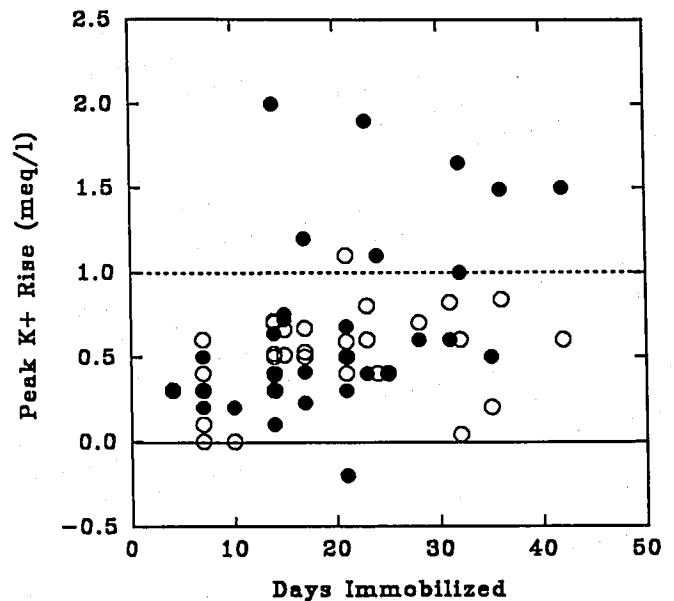


FIG. 1. Peak increase in femoral venous K⁺ (vertical axis) after administration of succinylcholine 0.25 mg/kg in 19 dogs with cast immobilization ranging for periods of 4–42 days (horizontal axis). Solid circles = casted; open circles = uncasted. Note that the increase in K⁺ concentration from the casted leg is ≥ 1 mEq/l by 14 days, but not sooner. There are overlapping points on days 7 and 14: day 7 has 16 values between 0 and 0.6 mEq/l, and day 14 has 13 values between 0.1 and 0.7 mEq/l. There are many more points below the dotted line than above it, because all dogs were challenged with succinylcholine every 4–8 days until they developed an increase > 1 mEq/l. At that point, they were eliminated from the protocol, because increased K⁺ efflux after succinylcholine had been demonstrated; one dog was inadvertently retested after the initial ≥ 1 mEq/l increase, and therefore this figure has 8 such points.

a period of immobilization ranging from 14–42 days, or 27.2 ± 9.8 days. One dog demonstrated this exaggerated increase on two occasions, and therefore figure 1 has eight points showing an increase greater than 1 mEq/l. When this exaggerated increase occurred, the increase in venous K⁺ in the casted leg exceeded that in the noncasted leg by at least 0.7 mEq/l (*P* < 0.01). The remaining 12 dogs were released from the protocol for technical reasons, such as ulceration under the cast.

When an increase in plasma K⁺ greater than 1 mEq/l occurred, it was transient, lasting less than 5 min. One dog had a 1.1-mEq/l increase in plasma K⁺ in the non-casted extremity after 3 weeks of immobilization. Temperature, pulse rate, and blood pressure were normal in all dogs (data not reported).

The mean increase in K⁺ for all noncasted legs was 0.5 mEq/l (standard deviation 0.24 mEq/l). The mean increase plus two standard deviations approximates 1 mEq/l. Statistically, this includes 75% of all values, with 95% confidence limits. In fact, as stated above, only one increase from the noncasted legs exceeded 1 mEq/l.

Discussion

This study estimated the time of onset of increased K^+ efflux by SCh during muscle disuse. This change—an efflux just greater than the upper limit of normal—was detected in all seven dogs that completed the protocol and occurred 14 or more days after the cast was applied.

The increase in K^+ release from the casted leg must be detected against the background variability of “normal” (noncasted) K^+ release. Because of this problem of signal *versus* noise, the time of onset depends on the definition of “abnormal increase in K^+ release.” The standard deviation for post-SCh K^+ concentration from the noncasted leg has led us to believe that a 1 mEq/l increase is a reasonable criterion. Too small a criterion would result in “false positive” on the noncasted side (too sensitive). Too large a criterion would lengthen the observed onset times (too insensitive). Because our observed variability in K^+ increase from the noncasted leg is similar to that of others’ observations in whole-body experiments,¹²⁻¹⁷ we believe that our criteria are valid. Similar considerations must apply to analysis of data examining resistance to NDMR. A comparable study in dogs with a denervation lesion could lead to a more definite and specific determination of onset of increased K^+ efflux after SCh; this may be an appropriate future study. However, denervation-induced changes occur rapidly, and it might then be difficult to distinguish clearly between the onset of resistance to NDMR and the onset of an exaggerated SCh-mediated K^+ efflux.

Gronert and Theye showed that the administration of SCh results in increased K^+ efflux from disuse muscle that had been immobilized for 29–44 days.⁵ In the present study, increase of venous plasma K^+ greater than 1.0 mEq/l was observed as early as 14 days, and as late as 42 days. The reasons for this variability in onset probably include measurement error, variations in immobility, and biologic variation in the rate at which the response develops. Variations in immobility may occur because of looseness of casts, a possible result of three factors: 1) webbril is compressed with time; 2) beagles have short, tapered legs; and 3) the muscle mass of the casted leg decreases with time. Cast conditions and fit were checked each day. The cast was removed every 4–8 days to test the dog for SCh K^+ efflux. One dog developed a 1.1 mEq/l increase in K^+ in the noncasted leg on the 21st day, but restriction of use of that extremity by the cast on the contralateral leg and pelvis may have contributed to this unexpected response. Resistance to NDMR in a nonimmobilized limb has been reported, and appears to relate, at least in part, to voluntary restriction of activity once a different limb has been restricted by a cast.² However, the nondisuse leg served as an appropriate paired control: as noted above in Results, the increase in K^+ concentration

in blood from the contralateral nondisuse leg was at least 0.7 mEq/l less than the increase in venous blood from the casted leg in all seven dogs.

A decrease in muscle blood flow in atrophied muscle exposed to SCh, with no change in K^+ release, would produce the change we observed—namely, a greater increase in K^+ in the venous blood from the casted leg, as compared to the noncasted leg. This would be only an apparent exaggerated efflux, due to the concentrating effect of a decrease in blood flow. However, a prior study of muscle blood flow during disuse and SCh relaxation did not support such a mechanism, since muscle blood flow increased.⁵ It is unlikely that blood flow in the present study would have been altered in the direction opposite of that of the prior study. Furthermore, this finding suggests that the change in K^+ efflux was even greater than that measured; *i.e.*, the likely increase in muscle blood flow attenuated the measured increase in K^+ .

We doubt that hemolysis during sampling or centrifugation caused spurious changes in plasma K^+ levels. Even if this occurred despite our precautions, it should not increase plasma K^+ concentrations, because canine red-cell K^+ concentration, in contrast to that in humans, is similar to plasma concentration.¹⁹

Cross-sampling—that is, withdrawing femoral vein blood so rapidly that the venous return from the leg is exceeded—would contaminate the sample with blood from the inferior vena cava or from the opposite femoral vein. This was avoided in the present study by simultaneous sampling of both legs at a constant rate of 1 ml/10 seconds. Blood flow from disuse gastrocnemius muscle has been estimated to be $12 \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ g}^{-1}$, or 4.7 ml/min (for a muscle weight of 39 g).⁵ These gastrocnemius blood flow measurements were made in slightly larger dogs, but it is likely that total femoral vein blood flow from an entire leg is more than the 6-ml/min sampling rate that we used.

Observations of ACh receptor distributions in disuse and denervation experiments suggest that resistance to NDMR occurs before disuse-related K^+ efflux.^{2,6,9,20-23} The early increase in the perijunctional population of ACh receptors would still be accessible to ACh, thus leading to resistance. They would not, however, be expected to result in additional K^+ efflux until they developed over more widespread areas of the surface of the muscle membrane. Preliminary results suggest that the onset of detectable disuse-related resistance to NDMR occurs in less than 2 weeks.³

Other observations, of clinical disorders, suggest that resistance to NDMR appears to be achieved more easily than SCh alterations in K^+ efflux: cerebral palsy is an upper motor neuron lesion associated with resistance to NDMR²⁴ but not with SCh-induced hyperkalemia.²⁵ Disuse atrophy is associated with resistance to NDMR, but

it barely accentuated K⁺ efflux by SCh.^{1-3,5,6} Thus, resistance to NDMR can develop with conditions of lesser neurologic activity, but without total loss of motor nerve function.

Several precautions should be taken in extrapolating these results. 1) The study was done in dogs; patients may respond differently. Currently, there is no study comparing the onset of resistance with the onset of SCh-related K⁺ efflux in patients. 2) The study intervention was immobility; disuse atrophy may be only part of the explanation for resistance or sensitivity in other conditions. For example, there is evidence that burn injury may have effects on muscle remote from the site of burn⁷ and that disuse plays a small role, if any, in animal studies of thermal trauma.²⁶ In addition, the time course and intensity of SCh-related K⁺ efflux may be different in other conditions, such as denervation. It is likely that with denervation there is a faster, more consistent, and more intense change in response.^{5,6,18,27} 3) We have identified the earliest detectable appearance of increased K⁺ efflux with canine disuse, but not the onset of clinically important hyperkalemia.

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