

# Systemic Inflammation Leads to Resistance to Atracurium without Increasing Membrane Expression of Acetylcholine Receptors

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**Background:** Systemic inflammation may be associated with resistance to nondepolarizing neuromuscular blocking drugs, the mechanisms of which are, however, uncharacterized. The authors therefore investigated the pharmacodynamics of atracurium and its relation to the expression of nicotinic acetylcholine receptors and  $\alpha_1$ -acid glycoprotein in a rat model of systemic inflammation.

**Methods:** To induce a systemic inflammation, male CD rats received 56 mg/kg corynebacterium parvum intravenously. On days 2, 4, 6, 8, 10, 12, 14, or 16 after infection, neuromuscular transmission was measured. The individual effective dose of atracurium was determined, followed by an atracurium infusion at a rate to establish a steady state neuromuscular block of 50%. Total and unbound plasma concentrations of atracurium for 50% paralysis were measured using high-performance liquid chromatography. Acetylcholine receptors were quantitated using  $^{125}\text{I}$ - $\alpha$ -bungarotoxin.  $\alpha_1$ -Acid glycoprotein concentrations in the serum were measured using a competitive chemiluminescence immunoassay.

**Results:** The effective dose of atracurium was increased on days 4, 6, and 8. Total atracurium plasma concentrations at 50% neuromuscular paralysis were increased on days 4, 6, 8, and 10, with a peak at day 8 ( $8.0 \pm 1.3 \mu\text{g/ml}$ ) compared with control rats ( $4.23 \pm 0.82 \mu\text{g/ml}$ ). The  $\alpha_1$ -acid glycoprotein concentrations were increased between days 2 and 10, with a peak on day 4 ( $6.52 \pm 1.45 \text{ mg/ml}$ ), and recovered to control values ( $0.61 \pm 0.33 \text{ mg/ml}$ ) on day 12. Unbound plasma concentrations of atracurium to achieve 50% depression, as well as the expression of acetylcholine receptors, did not differ between groups.

**Conclusion:** Resistance to atracurium during corynebacterium parvum-induced systemic inflammation is due to increased drug binding to  $\alpha_1$ -acid glycoprotein and is unrelated to changes in acetylcholine receptor expression.

RESISTANCE to the neuromuscular effects of nondepolarizing neuromuscular blocking drugs has been reported following burns,<sup>1-3</sup> immobilization,<sup>4</sup> denervation,

and infectious diseases.<sup>6-9</sup> This resistance can be manifested as either a delayed onset of effect, an incomplete neuromuscular block despite effective dose, or a rapid recovery from paralysis. Many pharmacokinetic and pharmacodynamic factors, including up-regulated acetylcholine receptors (AChRs), can potentially contribute to this resistance. The up-regulation of AChRs is usually associated with changes of isoform and distribution on the muscle surface with attendant alterations in channel gating properties and ion fluxes.<sup>10,11</sup> The observed resistance to nondepolarizing relaxants could also be caused by altered plasma protein binding.<sup>12</sup> During inflammatory states, plasma concentrations of  $\alpha_1$ -acid glycoprotein ( $\alpha_1$ -AGP) increase, which bind cationic drugs that include muscle relaxants.<sup>13,14</sup>

The etiology for the resistance to neuromuscular blocking drugs during sepsis or systemic inflammatory response syndrome has not been elucidated. In the endotoxin model of repeated intraperitoneal injections of lipopolysaccharide into mice, Tomera and Martyn<sup>6</sup> reported a threefold to fivefold shift to the right of the dose-response curve of D-tubocurarine after 2 weeks. In a model of granulomatous liver inflammation in rats, we previously demonstrated a resistance to vecuronium quantified as a 25% increase of vecuronium plasma concentrations to maintain a 50% neuromuscular paralysis.<sup>7</sup> The investigators in both studies concluded that sepsis probably leads to an increase in AChR expression; however, biochemical evidence for this was not provided. In critically ill patients, evidence for an increase in AChRs has been provided either directly, by muscle biopsy obtained from deceased patients,<sup>15</sup> or indirectly, by observing hyperkalemia after succinylcholine administration.<sup>16</sup> These studies, however, reported data from patients who were in addition immobilized or had received neuromuscular relaxants during the long-lasting intensive care treatment. Thus, the effects of disease itself versus the effects of other confounding factors that may change kinetics and dynamics have not been differentiated.

This study in rats, using an established experimental model of systemic inflammation,<sup>17,18</sup> examined (1) the neuromuscular response to atracurium, a drug independent of the liver and kidney for its metabolic clearance<sup>19</sup>; (2) the relation of the time course and the duration of the inflammatory state to atracurium pharmacodynamics; and (3) the role of AChRs and  $\alpha_1$ -AGP to atracurium neuromuscular pharmacodynamics. Systemic inflammation is associated with release of cytokines and increased

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Received from the Klinik für Anaesthesiologie der Technischen Universität München, Klinikum rechts der Isar, Munich, Germany. Submitted for publication May 10, 2002. Accepted for publication July 11, 2002. Supported by a personal stipend from the Deutscher Akademischer Austauschdienst, Bonn, Germany (to Dr. Fink); a grant from GlaxoWellcome, Hamburg, Germany (to Dr. Blobner); and by grants No. GM 31569-19, GM 55082-06, and GM 611411-4 from the National Institutes of Health, Bethesda, Maryland, and Shriners Hospital for Children, Boston, Massachusetts (to Dr. Martyn).

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expression of inducible nitric oxide (NO). Therefore, the plasma concentrations of the NO metabolites  $\text{NO}_2\text{-NO}_3$  were measured as a reflector of the systemic inflammation.

## Materials and Methods

### *Animal Model and Study Design*

After we obtained governmental approval for the study (Regierung von Oberbayern, AZ 211-2531-70/97), 99 male Sprague-Dawley rats (Charles River GmbH, Kisslegg, Germany; weight, 250–300 g) were allowed to accommodate to the standard conditions of our animal facility with free access to chow and water for 14 days. Seventy-two rats received an intravenous injection of 56 mg/kg of a whole cell preparation of heat-killed corynebacterium parvum (Roche, Penzberg, Germany) in a total volume of 0.5 ml saline. The injection of the bacteria induces a granulomatous liver inflammation, with peak systemic inflammatory signs at approximately 5 days and an average length of the inflammation of 8 days.<sup>17</sup> To determine the time course of the inflammation on neuromuscular pharmacodynamics, the infected rats were randomly assigned to be examined at days 2, 4, 6, 8, 10, 12, 14, or 16 after injection (11 rats per time point). The *in vivo* and *in vitro* variables studied at these periods included atracurium pharmacodynamics, AChR concentrations on gastrocnemius, and  $\alpha_1$ -AGP, as well as nitrite-nitrate ( $\text{NO}_2\text{-NO}_3$ ) concentrations in plasma. Controls ( $n = 11$ ) received the same volume of saline with no bacterium. *In vivo* experiments of control animals were performed at day 2 after saline injection. Because of rat deaths during induction of anesthesia or during the surgical procedures, the number of rats per group decreased. In addition, rats were excluded if they were hemodynamically unstable at the beginning of the experiments or if their hemodynamic or blood gas status at the designated measuring points were not within our predetermined range. This finally led to *in vivo* experiments in 69 rats with a group split-up of 8 animals at day 2, 11 at day 4, 8 at day 6, 7 at day 8, 8 at day 10, 6 at day 12, 8 at day 14, 5 at day 16, and 8 in the control group.

### *Anesthesia and Vital Parameters*

Anesthesia was induced by inhalation of sevoflurane in a glass cylinder. After loss of consciousness, the rats were endotracheally intubated and mechanically ventilated with oxygen in nitrous oxide (ratio 1:2). Anesthesia was maintained with 4–6% sevoflurane. After cannulation of the left external jugular vein, anesthesia was switched to a continuous infusion of propofol ( $20\text{--}40 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ) and fentanyl ( $4 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ) and maintained according to cardiovascular signs of adequate anesthesia. The left carotid artery was cannulated to measure mean arterial pressure and perform blood gas analyses. Following cannulation and start of intravenous

anesthesia, all animals were allowed to stabilize over a period of 60 min to eliminate any major effects of sevoflurane on neuromuscular transmission.

Before the neuromuscular experiments, ventilation was adjusted to maintain an arterial carbon dioxide partial pressure between 36 and 40 mmHg. Whenever necessary, base excess was corrected with 1 mM sodium bicarbonate to values between  $-2 \pm 2$  mM. Arterial oxygen partial pressure, heart rate, and mean arterial pressure were continuously monitored to ensure stable hemodynamic conditions throughout the experiment. Rectal temperature was controlled between 36.8 and 37.2°C with a warming blanket and heating lamp. Blood gases and hemodynamic variables were documented at two time points: (1) directly before determination of atracurium neuromuscular pharmacodynamics and (2) during maintenance of steady state neuromuscular paralysis at 50%.

### *Neuromuscular Function*

Neuromuscular function was monitored by evoked mechanomyography (Myograph; Biometer, Copenhagen, Denmark). The sciatic nerve of the left leg was exposed at its exit from the lumbosacral plexus and stimulated using the train-of-four pattern (2 Hz for 2 s every 12 s). The knee was pinned and firmly fixed. A force transducer was connected to the Achilles' tendon, and the contraction of the gastrocnemius muscle was measured. Supramaximal stimulus and control twitch height ( $T_0$ ) were established. Baseline mechanomyographical response was stabilized over a period of 10 min before determining the individual dose-response relation of atracurium in each rat using the cumulative dose-response method described previously.<sup>20</sup> Bolus doses of atracurium were given intravenously in increments between 0.2 and 0.8 mg/kg until the first twitch of the train-of-four ( $T_1$ ) was below 5% of baseline values. Each incremental dose was given only when the previous dose had produced maximal effect, as indicated by three equal consecutive  $T_1$  twitches. After the last dose of atracurium, twitch response was allowed to recover to baseline values. The recovery interval was calculated as follows: recovery interval [s] = time at ( $T_1/T_0 = 75\%$ ) – time at ( $T_1/T_0 = 25\%$ ). Following complete recovery of  $T_1$ , a continuous infusion of atracurium was started, and the infusion rate was adjusted to achieve a constant  $T_1/T_0$  of 50%. Following 10 min of stable  $T_1/T_0 = 50\%$  at a certain infusion rate, steady state conditions were assumed. The required infusion rate was documented, and 1 ml of heparinized blood was withdrawn to determine total plasma concentrations of atracurium. The blood was immediately transferred to Eppendorff tubes containing  $20 \mu\text{l}$  1 M  $\text{H}_2\text{SO}_4$  and centrifuged (3,500 rpm, 10 min, 4°C). The supernatant was collected, and 0.2-ml portions were aliquoted into Eppendorff tubes containing 0.8 ml 15 mM  $\text{H}_2\text{SO}_4$ . The samples were immediately

frozen at  $-70^{\circ}\text{C}$ . Directly after blood sampling, both gastrocnemii muscles were dissected from of the surrounding structures and rapidly frozen in isopentane precooled in liquid nitrogen and stored at  $-70^{\circ}\text{C}$ . Following this, animals were killed by exsanguination. Blood collected during exsanguination was separated into plasma by centrifugation (3,500 rpm, 10 min,  $4^{\circ}\text{C}$ ) and immediately stored at  $-70^{\circ}\text{C}$  for later determination of  $\alpha_1$ -AGP and  $\text{NO}_2$ - $\text{NO}_3$  plasma concentrations.

#### Acetylcholine Receptor Assay

The muscle was homogenized, the protein extracted, and the amount of membrane AChRs quantified by the  $^{125}\text{I}$ - $\alpha$ -bungarotoxin binding assay as described previously.<sup>21</sup> The protein concentration of the muscle extract was assayed using the Bio-Rad DC protein assay kit (Bio-Rad Laboratories, Hercules, CA), and the content of AChRs calculated and expressed in femtomoles per milligrams of protein.

#### Chemical Analyses

**Nitric Oxide.** Production of NO, a product of inducible NO synthase as a consequence of inflammation and release of cytokines, was assessed by measuring the stable metabolite,  $\text{NO}_2$ - $\text{NO}_3$ , concentrations in plasma. Plasma samples were deproteinized with 0.5 M NaOH and 10%  $\text{ZnSO}_4$ . Nitrate was then converted to nitrite using high-performance liquid chromatography (HPLC) on a cadmium column. Nitrite concentrations were determined spectrophotometrically at 540 nm using a method based on the Griess reaction.<sup>22</sup>

**$\alpha_1$ -Acid Glycoprotein.** To determine the  $\alpha_1$ -AGP in the rat serum, we established a competitive chemiluminescence immunoassay. Signaling was performed by applying horseradish peroxidase-conjugated streptavidin and quantifying the enzyme activity using an enhanced chemiluminometric method (Amerlite System; Ortho Clinical Diagnostics, Neckargemünd, Germany). Polyclonal antibodies against rat  $\alpha_1$ -AGP were raised in rabbits using pure commercially available rat  $\alpha_1$ -AGP (Sigma, Deisenhofen, Germany). The specific  $\alpha_1$ -AGP antibodies were coated onto the microwell surface (Dynatech Laboratories, Chantilly, VA) *via* goat antirabbit catcher antibodies (Biogenesis, Berlin, Germany). The tracer in the assay is biotinylated rat  $\alpha_1$ -AGP. Biotinylation was performed using biotin-*N*-hydroxysuccinimide ester. The biotinylated protein was purified by size-exclusion chromatography using a Sephadex G-25 column (Amersham Pharmacia, Freiburg, Germany), followed by a reverse-phase HPLC on a 5- $\mu\text{m}$   $\text{C}_{18}$  column (Machery und Nagel, Düren, Germany). The calibration curves were also produced from pure rat  $\alpha_1$ -AGP by serial dilutions in phosphate-buffered saline containing 0.5% bovine serum albumin in the range of 0.1-100 mg/ml. Lower detection limit was found to be 0.1 mg/ml in serum.

**Total Atracurium Plasma Concentrations.** Atracurium plasma concentrations were determined by HPLC. Briefly, 20  $\mu\text{g}$  verapamil as an internal standard was added to the pretreated samples (200  $\mu\text{l}$  serum + 800  $\mu\text{l}$  15 mM  $\text{H}_2\text{SO}_4$ ). Samples were deproteinized using 1 ml acetonitrile and centrifuged at 5,000g. A total of 50  $\mu\text{l}$  of the supernatant was injected into the HPLC column (RP18, LiChrospher, 5  $\mu\text{m}$ , 100 mm, 4.6 ID; Merck, Darmstadt, Germany). Atracurium was separated using a linear gradient elution system: from A to 100% B in 8 min (A [23.5 ml acetonitrile, 5 ml methanol, and 0.03 M  $\text{K}_2\text{HPO}_4$ , 57.5 ml, adjusted to pH 5], B [35.5 ml acetonitrile, 15 ml methanol, and 0.1 M  $\text{K}_2\text{HPO}_4$ , 47.5 ml, adjusted to pH 5], flow rate 1.7 ml/min, fluorescence detection at 240 nm excitation and 320 nm emission). A calibration solution was prepared from authentic substances with 5.0, 15.0, and 24.0  $\mu\text{g}/\text{ml}$  atracurium and 1.0, 2.0, and 3.0  $\mu\text{g}/\text{ml}$  laudanosine in 0.005 M  $\text{H}_2\text{SO}_4$ , pH 2.5. The plasma clearance of atracurium during steady state conditions was calculated by the equation: clearance = infusion rate/plasma concentration.

**Free Plasma Concentrations of Atracurium.** The impact of altered  $\alpha_1$ -AGP concentrations on the plasma binding (or free fraction) of atracurium was evaluated *post hoc* using the plasma samples that were drawn during exsanguination. The total plasma concentrations of atracurium in these (not acidified) samples are expected to be lower than the values collected and measured during steady state 50% depression. This lower concentration is due to the time effect of later sampling (during exsanguination) and the spontaneous degradation of atracurium at pH 7.4 even though in the freezer. The binding conditions, however, are maintained. We therefore divided each plasma sample into two parts: one part was processed as it was, and the other was warmed to  $37^{\circ}\text{C}$  followed by the addition of atracurium to increase plasma concentrations to an arbitrary concentration. In both (original as well as atracurium-spiked) samples, we assessed the total (bound and unbound) plasma concentrations of atracurium and, in addition, the plasma concentrations of bound atracurium. For the assessment of the protein-bound component, atracurium was separated from the unbound fraction using a microdialyzer system (Pierce, Rockford, IL) with an appropriate dialysis membrane (molecular weight cutoff of 8 kd). A 0.08 M phosphate buffer, pH 7.2, was used as dialysis buffer. All plasma samples were dialyzed for 40 min at room temperature. The plasma concentrations of bound and total atracurium were measured using the same HPLC set-up as for total atracurium plasma concentrations. The free atracurium fraction of each sample was then calculated by the equation: 1 - bound plasma concentration/total plasma concentration, and the mean value of the two sample parts was calculated. The plasma concentrations of unbound atracurium that represent the steady state condition during the 50%



**Table 1. Signs of Systemic Inflammation on Each Experimental Day**

Group	Control	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14	Day 16	P	Power
Body weight, g	373 ± 18	365 ± 21	354 ± 9*	338 ± 9*	364 ± 7	380 ± 14	369 ± 11	388 ± 11*	444 ± 13*	0.0001	1.00
NO <sub>2</sub> /NO <sub>3</sub> , mM	33 ± 2	147 ± 63	1052 ± 244*	1632 ± 359*	389 ± 90	153 ± 35	119 ± 46	152 ± 71	77 ± 16	0.0001	1.00

\*  $P < 0.05$  versus control.

neuromuscular paralysis were calculated using the respective total plasma concentration multiplied by the free fraction of atracurium.

### Statistical Analyses

Data are presented as mean and SD. The effective doses to achieve a 50% and 95% neuromuscular paralysis (ED<sub>50</sub> and ED<sub>95</sub>) were calculated from the cumulative dose-response curve for each rat. The individual ED<sub>50</sub> and ED<sub>95</sub> values were calculated by interpolation from the linear regression of the degree of blockade in logit scale and the respective cumulative dose of atracurium in log scale.

Statistical analyses were conducted using a factorial analysis of variance. To evaluate the effect of inflammation, we tested *post hoc* with the Dunnett-T procedure comparing the values of groups day 2, 4, ..., and 16 with the values of control rats at a 5% level of significance. To address differences at the day of inflammation, the analysis of body weight included the body weight at the day of inflammation as a covariable.

## Results

### Systemic Signs of Inflammation

After injection of the corynebacterium parvum suspension, body weights of the rats were significantly decreased at days 4 and 6. At days 8, 10, and 12, the weights were stable and were not different from controls. At days 14 and 16, the weights were significantly increased compared with controls. NO<sub>2</sub>-NO<sub>3</sub> plasma concentrations were significantly elevated by day 4 and peaked at day 6 (relative to control rats). After this period, the elevated NO<sub>2</sub>-NO<sub>3</sub> concentrations did not reach statistical significance and gradually decreased over time (table 1). The α<sub>1</sub>-AGP concentrations were significantly increased at day 2, with a peak elevation at days 4-6. Thereafter, these values gradually decreased and were normalized by days 12-16 (fig. 1).

### Model Stability

Hemodynamic and metabolic variables were kept stable throughout the experiment in each rat. The values were not different between groups, and therefore the group means are reported. At the beginning of the

experiments, the mean arterial pressure was 108 ± 14 mmHg, heart rate was 344 ± 35 beats/min, arterial blood pH ranged between 7.352 and 7.440, base excess was -2.6 ± 1.2 mM, and body temperature was 37.1 ± 0.2°C. During steady state atracurium infusion, the mean arterial pressure was 98 ± 13 mmHg, heart rate was 355 ± 32 beats/min, arterial blood pH ranged between 7.344 and 7.432, base excess was -2.4 ± 1.2 mM, and body temperature was 37.1 ± 0.2°C.

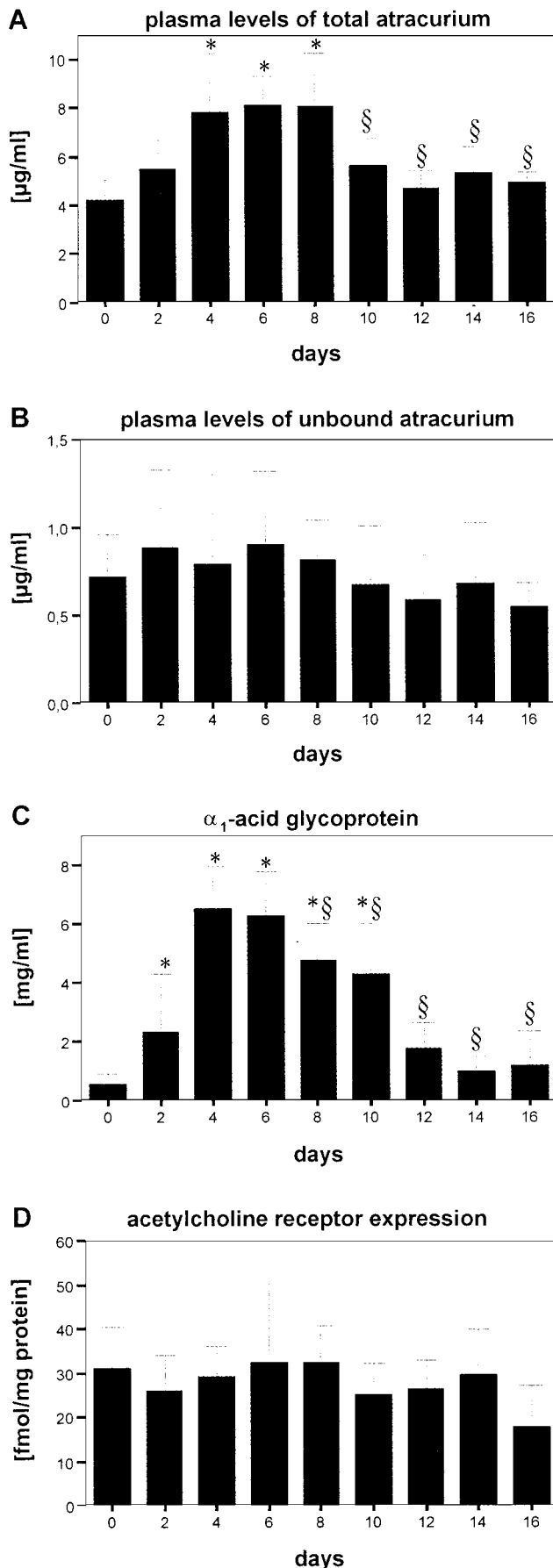
### Atracurium Pharmacodynamics, Pharmacokinetics, and Acetylcholine Receptor Expression

The ED<sub>50</sub> and ED<sub>95</sub> were increased in the experimental group compared with control rats starting at day 4. The increased ED values persisted until day 8 and returned to normal at later time points. The recovery from T1 = 25% to 75% did not differ between groups (table 2).

The infusion rate necessary to maintain a steady state 50% neuromuscular paralysis was significantly increased in the experimental groups at days 4, 6, 8, and 10 compared with control rats; the α<sub>1</sub>-AGP concentrations were also the highest at the same periods. The total plasma concentrations of atracurium to maintain a steady state 50% paralysis were significantly increased at days 4, 6, and 8 compared with control rats. The plasma concentrations of unbound (free) atracurium to achieve 50% neuromuscular paralysis, however, did not differ significantly between groups. The higher atracurium requirement paralleled the higher concentrations of α<sub>1</sub>-AGP at the same periods (fig. 1). The plasma clearance of atracurium did not differ between groups (table 2). Expression of nicotinic AChRs in the gastrocnemius muscle did not differ between groups (fig. 1).

## Discussion

In this study, we have demonstrated that systemic inflammation results in resistance to the neuromuscular effect of the nondepolarizing neuromuscular relaxant, atracurium. This resistance manifested itself as early as 4 days after corynebacterium parvum injection, persisted until day 8, and returned to normal at and beyond day 10. The resistance to atracurium was evidenced as increase in ED, an increase in continuous infusion rate requirement, and an increase in plasma concentration



requirement for 50% paralysis. As indicated by figures 1A and 1C, the changes in plasma concentrations of atracurium to maintain a steady state 50% paralysis paralleled the changes in  $\alpha_1$ -AGP. The similar plasma concentrations of unbound atracurium to achieve 50% neuromuscular paralysis (fig. 1B) suggest that target organ sensitivity is not changed. That target organ sensitivity is unchanged is consistent with the finding of unaltered AChR number on the muscle membrane at periods of atracurium resistance. Together, these findings suggest that increased binding of atracurium by  $\alpha_1$ -AGP caused the increased atracurium demand. The systemic inflammatory response to corynebacterium parvum, as demonstrated previously,<sup>7</sup> is self-limiting. Our study confirms that with decrease of inflammatory response, evidenced as normalization or near-normalization of  $\alpha_1$ -AGP and NO<sub>2</sub>-NO<sub>3</sub> concentrations, the resistance to atracurium dissipates.

In a previous study, we demonstrated resistance to vecuronium during systemic inflammation, evidenced as increased plasma concentration requirement of vecuronium during a steady state neuromuscular paralysis. In addition, a prolonged duration of action of vecuronium was observed.<sup>7</sup> Decreased metabolism and clearance of vecuronium by the liver explained the prolonged recovery time. Atracurium, however, is a quaternary ammonium compound degraded primarily *via* Hoffmann elimination.<sup>23</sup> Uniform distribution and degradation of atracurium were attempted by maintaining hemodynamic variables, acid-base balance, and temperature constant, resulting in comparable plasma clearance among groups. We are therefore confident that the observed differences in the effective doses and plasma concentrations of atracurium are unrelated to altered metabolism of atracurium.

The excessive production of cytokines is a characteristic feature of inflammation. A key effect of these cytokines is the increased expression of inducible NO synthase with release of NO.<sup>24</sup> Recent findings have shown that endogenous NO decreases submaximal force by modulating excitation-contraction coupling<sup>25</sup> and therefore promotes relaxation of the skeletal muscle through the cyclic guanosine monophosphate pathway.<sup>26</sup> NO also takes part in regulating acetylcholinesterase expression in the neuromuscular junction and ion channel properties by activating cyclic guanosine mono-

**Fig. 1.** Plasma concentrations of total atracurium (A), unbound atracurium (B), and  $\alpha_1$ -acid glycoprotein ( $\alpha_1$ -AGP; C) and expression of acetylcholine receptor (AChR; D) at varying periods after infection. Plasma concentrations of total atracurium to maintain 50% neuromuscular paralysis were significantly higher at days 4, 6, and 8 after infection. This requirement returned to normal by days 10–16. The  $\alpha_1$ -AGP increased by day 2, peaked at day 4, and gradually returned to normal from days 10 to 16. Expression of AChRs and plasma concentrations of unbound atracurium were unaltered throughout the observation period. \**P* < 0.05 versus day 0.

**Table 2. Atracurium-induced Neuromuscular Paralysis**

Group	Control	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14	Day 16	P	Power
Effective dose of atracurium for inducing the neuromuscular paralysis and its recovery											
ED <sub>50</sub> , mg/kg	0.85 ± 0.13	1.04 ± 0.24	1.18 ± 0.22*	1.71 ± 0.39*	1.20 ± 0.19*	0.95 ± 0.20	0.79 ± 0.19	1.03 ± 0.26	0.92 ± 0.26	0.0001	1.00
ED <sub>95</sub> , mg/kg	1.45 ± 0.09	1.82 ± 0.24	2.04 ± 0.16*	3.43 ± 0.31*	2.19 ± 0.14*	1.80 ± 0.17	1.27 ± 0.11	1.85 ± 0.20	1.88 ± 0.34	0.0001	1.00
Recovery interval, s	97 ± 9	102 ± 6	89 ± 5	81 ± 9	77 ± 7	79 ± 6	90 ± 8	93 ± 5	118 ± 10	0.014	1.00
Parameters taken during steady state 50% neuromuscular paralysis											
Infusion rate, † mg · kg <sup>-1</sup> · min <sup>-1</sup>	147 ± 12	155 ± 9	211 ± 18*	265 ± 25*	206 ± 15*	214 ± 8*	171 ± 15	163 ± 11	122 ± 12	0.0001	1.00
Plasma clearance, ‡ ml/min	37 ± 5	29 ± 2	28 ± 2	32 ± 4	27 ± 8	39 ± 3	37 ± 3	31 ± 2	25 ± 2	NS	0.79

\*  $P < 0.05$  versus control. † To achieve a steady state 50% neuromuscular paralysis. ‡ Of atracurium during a steady state 50% neuromuscular paralysis. NS = not significant.

phosphate.<sup>27</sup> NO donors have been shown to inhibit the evoked release of acetylcholine, leading to a reduction of the end-plate potential.<sup>28</sup> Taken together, one can assume from these findings that the elevated NO concentrations in our model would have made the muscle more susceptible to paralysis rather than enhancing resistance.

Previous studies have documented a relation between up-regulation of AChRs and resistance to nondepolarizing neuromuscular relaxants. The resistance is related to both quantitative increases and qualitative (isoform) changes in the receptor, as well as electrophysiologic properties and differences in ligand-specific sensitivity or affinity between the two receptor subtypes.<sup>11</sup> Reports of hyperkalemic responses following administration of succinylcholine in patients with prolonged severe inflammation or sepsis support the idea that sepsis, just as burns, leads to an up-regulation of AChRs. In our studies, a single insult produced by injection of corynebacterium parvum producing an inflammatory response for a few days does not up-regulate AChR. One might pose the question of whether changes in isoform (from mature to immature) may explain the resistance. This possibility is highly unlikely. In all pathologic or acquired disease states where isoform changes occur, there is concomitant up-regulation of AChR. That is, isoform changes occur *paripassu* with up-regulation. AChR numbers were unchanged throughout the experiment, and therefore the thesis that changes in AChR from mature to immature isoform caused the resistance is untenable. There is also no evidence in the literature that either isoform of the AChR can exist in high- or low-affinity states when binding to muscle relaxants. In addition, since atracurium shows no differences in potency of inhibition between the two subtypes of receptor,<sup>29</sup> an influence of sensitivity differences or binding affinity for our results (resistance to atracurium) can be excluded.

In our model, which served as a paradigm for a systemic inflammatory response, the inflammation was self-limiting, evidenced by lack of loss in body weight and

decreasing NO<sub>2</sub>-NO<sub>3</sub> and  $\alpha_1$ -AGP at or after 8 days after infection. Other factors that induce up-regulation of AChRs include immobilization and concomitant prolonged muscle relaxant therapy. These variables, however, did not confound this study. Changes in AChRs may have occurred if the inflammation had persisted for a longer period. This could be achieved by injecting a second dose of corynebacterium parvum at 4–6 days after the initial dose or by producing a more severe form of prolonged systemic inflammatory response.

Two important drug-binding proteins have been identified: albumin and  $\alpha_1$ -AGP. Albumin binds predominantly to anionic drugs (e.g., barbiturates, benzodiazepines).  $\alpha_1$ -AGP is a component of globulin and binds cationic drugs, including nondepolarizing relaxants.<sup>30</sup> During many diseases, such as burns, malignancy, chronic inflammation, and sepsis, plasma concentrations of acute-phase reactant proteins, such as  $\alpha_1$ -AGP, are increased.<sup>13,30</sup> In this model of a systemic inflammation, we could demonstrate that  $\alpha_1$ -AGP concentrations were increased starting at day 2, reached the highest concentrations at days 4 and 8, and returned to normal concentrations by day 12. During a target controlled infusion of atracurium, the plasma concentrations of total atracurium to achieve 50% neuromuscular paralysis paralleled the changes in  $\alpha_1$ -AGP, while the plasma concentrations of unbound atracurium were not altered. As indicated previously, these findings confirm the lack of change in target organ sensitivity and the role and importance of binding of atracurium to  $\alpha_1$ -AGP in serum.

In summary, in our model of systemic inflammation, the resistance to neuromuscular blocking drugs is not due to altered metabolism or AChR expression, but represents an increase in drug binding to  $\alpha_1$ -AGP. The resistance is self-limiting and dissipates parallel with the inflammatory response. The triggering mechanisms, mediators, and duration of infection or inflammation necessary to induce an up-regulation of AChR needs further study.

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