

Measurement of Acetylcholine Receptor Concentration in Skeletal Muscle from a Patient with Multiple Sclerosis and Resistance to Atracurium

ROGER S. BRETT, M.D., PH.D.,* JAKOB H. SCHMIDT, PH.D.,† JOHN S. GAGE, M.D.,*
SCOTT A. SCHARTEL, D.O.,‡ PAUL J. POPPERS, M.D.§

Resistance to nondepolarizing neuromuscular blockers is a characteristic feature of several pathophysiological states, including spastic paresis following a cerebrovascular accident,¹⁻³ immobilization of skeletal muscle,⁴⁻⁷ and thermal injury.⁸⁻¹⁰ Despite speculation, the mechanisms underlying this resistance remain unknown. On the basis of analogy to animal data,^{11,12} it has been suggested that these states may be characterized by an abnormal increase in the number of skeletal muscle acetylcholine receptors, and these additional receptors play a role in the resistance to nondepolarizing blockers.^{1-5,8,10,13} We are currently investigating the association between the clinical response to neuromuscular blockers and the number and distribution of acetylcholine receptors in skeletal muscle.⁹ Here, we describe a patient with multiple sclerosis who exhibited both clinical resistance to atracurium and an abnormal elevation of the number of skeletal muscle acetylcholine receptors.

REPORT OF A CASE

A 44-yr-old woman with a 13-yr history of multiple sclerosis was brought to the operating room for closure of a sacral decubitus ulcer with a myocutaneous flap. Her neurological disease had progressed to a stage characterized by spastic paraparesis, mild impairment of upper extremity strength, and mild dysarthria, without impairment of mood or sensorium. Apart from multiple sclerosis and associated minor complications, her medical history was unremarkable. She had previously undergone both general and regional anesthesia without incident. Her usual medications included baclofen, oxybutynin (Ditropan), amantadine, and trimethoprim-sulfamethoxazole (Bactrim). On physical examination, she was alert and cooperative. The airway and cardiorespiratory system were unremarkable. The neurological examination re-

vealed dysarthria, paraparesis, and generalized hyperreflexia. The laboratory data, including serum electrolytes, were within normal limits.

General anesthesia was induced and maintained using the same protocol for the patient described above and seven other (control) patients in whom no evidence of neuromuscular disease was present. In each of these eight cases, an initial iv bolus of thiopental (0.4 mg/kg) was administered, and a satisfactory mask airway established for administration of oxygen (40%), nitrous oxide (60%), and enflurane (0-0.5%). Sufentanyl (20-50 µg iv) was administered at the discretion of the investigator during the interval between induction and tracheal intubation. Neuromuscular transmission was monitored electromyographically by administering trains of four transcutaneous stimuli (0.5 Hz) to the ulnar nerve every 20 s and recording the amplitude of the resulting compound action potentials from the thenar musculature. After measurement of the control response to ulnar nerve stimulation, atracurium (0.4 mg/kg) was administered as an iv bolus. Tracheal intubation was performed when the initial amplitude of the compound action potential had declined to 5% of its control value. Neuromuscular blockade was subsequently maintained by intermittent doses of atracurium, 0.1 mg/kg, administered each time the initial amplitude of the compound action potential returned to 10% of control. Anesthesia was maintained with 10-20-µg increments of sufentanyl iv, as required, and enflurane was discontinued or reduced. Pulmonary ventilation was adjusted to maintain the end-tidal P_{CO₂} between 35 and 40 mmHg.

In the multiple sclerosis patient described above, neuromuscular blockade developed abnormally slowly following the initial bolus of atracurium (table 1),^{15,16} and failed to reach standard criteria¹⁴ for the facilitation of tracheal intubation. Two additional doses of atracurium (0.2 mg/kg each) were subsequently administered, and tracheal intubation was eventually performed when the initial amplitude of the compound action potential had declined to 5% of control. In comparison with measurements obtained from the seven control patients, the study patient's atracurium dose requirement per unit time was markedly increased, and the dose interval shortened (table 1). At the end of the procedure, she was allowed to recover spontaneously from neuromuscular blockade. The postoperative course was routine.

During each of the eight operative procedures, two small specimens of skeletal muscle were taken from the surgical field for subsequent measurement of acetylcholine receptor concentrations. These measurements were performed *in vitro*, using ¹²⁵I-labelled α-bungarotoxin, a highly specific and essentially irreversible ligand for the nicotinic acetylcholine receptor. The toxin was purified from the venom of the Formosan Krait *Bungarus Multicinctus* (Miami Serpentarium, Miami, Florida) and labelled with ¹²⁵I-α-bungarotoxin, using the method of Wang and Schmidt.¹⁴ The specific activity of the labelled toxin ranged from 3.5-5.0 GBq/mole. Muscle tissue was homogenized in dilute phosphate buffer using a Polytron homogenizer, and a crude particulate fraction was prepared and extracted with 1% Triton X-100. The detergent extract was incubated with ¹²⁵I-α-bungarotoxin (10 nM), and receptor-bound toxin was quantified by filtration over Whatman GF/C glass fiber disks.

The results of these measurements are illustrated in figure 1.

* Assistant Professor of Anesthesiology and Physiology & Biophysics.

† Associate Professor of Biochemistry.

‡ Resident in Anesthesiology.

§ Professor and Chairman of Anesthesiology.

Received from the Department of Anesthesiology, Physiology and Biophysics, and Biochemistry, School of Medicine, Health Sciences Center, State University of New York at Stony Brook, Stony Brook, New York 11794-8480.

Address reprint requests to Dr. Brett.

Key words: Neuromuscular junction: acetylcholine receptors. Neuromuscular relaxants: atracurium.

¹ This ongoing study has received institutional approval from the Committee on Research Involving Human Subjects. Informed consent for participation was obtained from all patients described here.

TABLE 1. Comparison of Clinical EMG Parameters Exhibited by the Study Patient and Seven Others with Unrelated Illnesses

Principal Diagnosis	Muscle Specimen	Atracurium Requirement		
		Onset (Min)	Steady-state Dose Req. (mg/Min)	Mean Dose Interval (Min)
Multiple sclerosis Cholecystitis	Medial gluteus, Lateral gluteus	6.8	0.85	12
	Rectus abdominis, Internal, Oblique	2.3	0.59	23
Pancreatitis	Rectus abdominis	1.4	0.26	
Breast cancer	Pectoralis minor	1.7	0.36	19
Pancreatic cancer	Rectus abdominis	3.4	0.25	23
Lung cancer	Trapezius, Latissimus dorsi	2.9		
Hepatoma	Rectus abdominis, External, Oblique		0.34	
Colon cancer	Rectus abdominis	1.6	0.47	23

The column "onset" refers to the time, in minutes, between bolus administration of 0.4 mg/kg atracurium and 50% attenuation of the amplitude of the initial compound action potential. "Steady state dose requirement" refers to the total atracurium dose, in mg/min, required to maintain the amplitude of the initial compound action potential at or below 10% of control, using the dose protocol described in the text. "Mean dose interval" refers to the average time, in minutes, between successive maintenance doses of 0.1 mg/kg.

DISCUSSION

Multiple sclerosis is characterized by progressive demyelination of corticospinal tract neurons, typically resulting in an ascending spastic paresis of skeletal muscle. Although it is reasonable to expect, on the basis of experience with other lesions involving by upper motor neuron degeneration,¹⁻³ that patients with multiple sclerosis might exhibit resistance to nondepolarizing neuromuscular blockers, this phenomenon has not been previously reported. We urge caution, however, in generalizing our observation to all patients with multiple sclerosis, as the disease may also occasionally be associated with impaired (myasthenia-like) neuromuscular transmission.^{17.**}

It is also reasonable to expect, on the basis of animal studies of upper motor neuron denervation,¹² that the disease process of multiple sclerosis might lead to the synthesis of an abnormally large number of acetylcholine receptors and to their incorporation into extrajunctional muscle membrane. Thus, although the method used in the present study does not permit separate measurement

of junctional and extrajunctional acetylcholine receptors, it is possible that virtually all of the "extra" receptors we detected were distributed extrajunctionally. It is also possible, however, that the pathological processes (*e.g.*, upper motor neuron injury) which initiate the synthesis of new receptors simply fail to discriminate between junctional and extrajunctional sites for incorporation, leading to an increase in the number of receptors within the endplate membrane, as well as in nonsynaptic areas (*cf.* ref. 18). Can either of these alternatives plausibly account for resistance to atracurium?

Despite a paucity of experimental evidence, the concept that pathological resistance to nondepolarizing neuromuscular blockers is causally linked to the presence of extrajunctional acetylcholine receptors has enjoyed wide acceptance. This proposal (see, for example, ref. 4) appears to have emerged largely by analogy to the phenomenon of hyperkalemia following administration of succinylcholine, which may, indeed, be plausibly explained by an increase in the number of extrajunctional acetylcholine receptors.⁴ It is not clear, however, by what mechanism increased extrajunctional receptors would be expected to

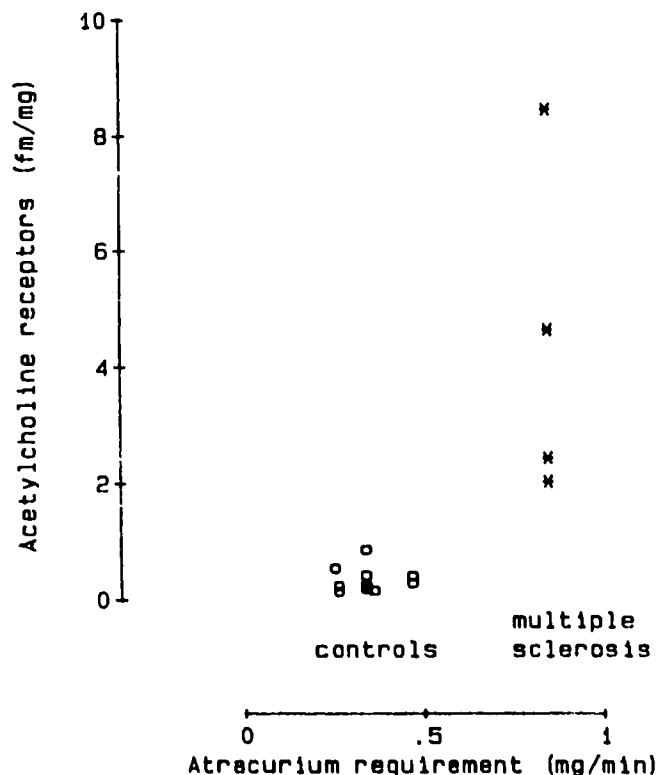


FIG. 1. Acetylcholine receptor concentration in muscle specimens from the study patient (asterisks) and controls (open circles), plotted versus steady-state atracurium requirement. Each point represents a single determination of receptor concentration. Usually, two determinations were made from each of two anatomically separate specimens from each patient.

** Hokkanen E, Pirskanen R, Bergstrom L: Simultaneous occurrence of demyelinating disease and the myasthenic syndrome. A report of 3 cases. Xth Congress of Neurology. Excerpta Med Found, Amsterdam, 1973.

cause resistance to nondepolarizing agents, as these receptors do not participate in neuromuscular transmission. Thus, although our observations support the concept that resistance is associated with an increase in the total number of receptors, we suspect that the resistance phenomenon is due either to an increase in the number of junctional acetylcholine receptors, occurring *pari passu* with the increase in extrajunctional receptors,¹⁸ or to an altogether different mechanism. We are currently investigating these alternatives.

The authors gratefully acknowledge the excellent technical assistance of Ms. Valerie Burger.

REFERENCES

1. Graham HG: Monitoring neuromuscular block may be unreliable in patients with upper-motor-neuron lesions. *ANESTHESIOLOGY* 52:74-75, 1980
2. Moorthy SS, Hilgenberg JC: Resistance to nondepolarizing muscle relaxants in paretic upper extremities of patients with residual hemiplegia. *Anesth Analg* 59:624-627, 1980
3. Shayevitz JR, Matteo RS: Decreased sensitivity to metocurine in patients with upper motoneuron disease. *Anesth Analg* 64:767-772, 1985
4. Gronert GA: Disuse atrophy with resistance to pancuronium. *ANESTHESIOLOGY* 55:547-549, 1981
5. Matteo RS, Diaz J: Resistance to d-tubocurarine in cats following 72 hours of continuous paralysis. *ANESTHESIOLOGY* 61:A310, 1984
6. Waud BE, Amaki Y, Waud DR: Disuse and d-tubocurarine sensitivity in isolated muscles. *Anesth Analg* 64:1178-1182, 1985
7. Waud BE, Waud DR: Tubocurarine sensitivity of the diaphragm after limb immobilization. *Anesth Analg* 65:493-495, 1986
8. Martyn JAJ, Szyfelbein SK, Ali HH, Matteo RS, Savarese JJ: Increased d-tubocurarine requirement following major thermal injury. *ANESTHESIOLOGY* 52:352-355, 1980
9. Mills A, Martyn JAJ, Szyfelbein SK: Evaluation of atracurium neuromuscular blockage in patients with thermal injury. *ANESTHESIOLOGY* 63:A339, 1985
10. Dwersteg JF, Pavlin EG, Heimbach D: Patients with burns are resistant to atracurium. *ANESTHESIOLOGY* 65:517-520, 1985
11. Fischbach GD, Robbins N: Effect of chronic disuse of rat soleus neuromuscular junctions on postsynaptic membrane. *J Neurophysiol* 34:562-569, 1971
12. Fambrough DM: Control of acetylcholine receptors in skeletal muscle. *Physiol Rev* 59:165-227, 1979
13. Goudsouzian NG, Standaert FG: The infant and the myoneural junction. *Anesth Analg* 65:1208-1217, 1986
14. Wang GK, Schmidt J: Primary structure and binding properties of iodinated derivatives of α -bungarotoxin. *J Biol Chem* 255:11156-11162, 1980
15. Basta SJ, Ali HH, Savarese JJ, Sunder N, Gionfriddo M, Cloutier G, Lineberry C, Cato AE: Clinical pharmacology of atracurium besylate (BW 33A): A new non-depolarizing muscle relaxant. *Anesth Analg* 61:723-729, 1982
16. Gergis SD, Sokoll MD, Mehta M, Kemmotsu O, Rudd GD: Intubation conditions after atracurium and suxamethonium. *Br J Anaesth* 55(suppl 1):83-86, 1983
17. Cendrowski W: Multiple sclerosis associated with defective neuromuscular transmission. *J Neurol* 209:297-299, 1975
18. Brett RS, Younkin SG, Konieczkowski M, Slugg RM: Accelerated degradation of junctional acetylcholine receptor- α -bungarotoxin complexes in denervated rat diaphragm. *Brain Res* 233:133-142, 1982

Anesthesiology
66:839-842, 1987

The Effect of Propofol on Adrenocortical Steroidogenesis: A Comparative Study with Etomidate and Thiopental

ROBERT J. FRAGEN, M.D.,* HOWARD W. WEISS, M.D.,† AGOSTINO MOLTENI, M.D., PH.D.‡

Propofol (Diprivan®) is a sterically hindered phenol with intravenous hypnotic properties. It is currently under in-

vestigation in the United States as an anesthetic induction agent. A large volume of distribution and short elimination half-life give propofol potential advantages for induction of anesthesia in outpatients and as a maintenance hypnotic agent by iv infusion or multiple injection.^{1,2} The effects of induction doses of propofol on steroidogenesis are unknown.

Etomidate inhibits adrenal steroidogenesis by a concentration-dependent block of both cholesterol side chain cleavage enzyme and 11- β -hydroxylase.³⁻⁵ This occurs with doses as low as the recommended induction dose of etomidate, 0.3 mg/kg.^{3,5} Prolonged intravenous infusions of etomidate, given for sedation in intensive care units to multiple trauma patients not receiving steroids, were associated with low plasma cortisol concentrations and increased mortality due to infection.^{6,7} Induction doses of thiopental do not suppress the adrenal cortex.⁴

* Professor of Clinical Anesthesia.

† Resident in Anesthesia.

‡ Professor of Pathology.

Received from the Northwestern University Medical School, Department of Anesthesia, 303 E. Superior Street, Passavant Pavilion—Room 360, Chicago, Illinois 60611. Accepted for publication January 27, 1987. Work was done in the Departments of Anesthesia and Pathology, Northwestern University, Chicago, Illinois. Supported in part by a grant from Stuart Pharmaceuticals, a Division of ICI Americas, Inc. Presented at the 1986 meeting of the American Society of Anesthesiologists, Las Vegas, Nevada.

Address reprint requests to Dr. Fragen.

Key words: ACTH: stimulation. Adrenocortical hormones: aldosterone: cortisol. Anesthetics, intravenous: etomidate; propofol; thiopental.