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Preclinical Pharmacology of GW280430A (AV430A) in the Rhesus Monkey and in the Cat

A Comparison with Mivacurium

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Background: **No replacement for succinylcholine is yet available. GW280430A (AV430A) is a representative of a new class of nondepolarizing neuromuscular blocking drugs called *asymmetric mixed-onium chlorofumarates*. It undergoes rapid degradation in plasma by chemical hydrolysis and inactivation by cysteine adduction, resulting in a very short duration of effect. The neuromuscular, cardiovascular, and autonomic pharmacology of GW280430A is compared herein with that of mivacurium.**

Methods: **Adult male rhesus monkeys and adult male cats were anesthetized with nitrous oxide–oxygen–halothane and chloralose–pentobarbital, respectively. The neuromuscular blocking properties of GW280430A and mivacurium were compared at a stimulation rate of 0.15 Hz in the extensor digitorum of the foot (monkey) and the tibialis anterior (cat). Sympathetic responses were assayed in the cat in the nictitating membrane preparation, and vagal effects were evaluated in the cat *via* observation of bradycardic responses after stimulation of the cervical right vagus nerve.**

Results: **GW280430A and mivacurium were equipotent in the monkey (ED_{95} was 0.06 mg/kg in each case). GW280430A was half as potent as mivacurium in the cat. The total duration of action of GW280430A was less than half that of mivacurium in the monkey; recovery slopes were more than twice as rapid. The 25–75% recovery index of GW280430A did not vary significantly after various bolus doses or infusions, averaging 1.4–1.8 min in the monkey, significantly shorter than the same time interval (4.8–5.7 min) for mivacurium. Dose ratios for autonomic *versus* neuromuscular blocking properties in the cat were greater than 25 for both GW280430A and mivacurium. The ratio ED Hist:ED_{95} Neuromuscular Block in the monkey was significantly greater (approximately 53 *vs.* 13) for GW280430A, indicating approximately four times less relative prominence of the side effects of skin flushing and decrease of blood pressure, which are associated with release of histamine.**

Conclusions: **These experiments show a much shorter neuromuscular blocking effect and much-reduced side effects in the case of GW280430A *vis-à-vis* mivacurium. These results, together with the novel chemical degradation of GW280430A, suggest further evaluation in human subjects.**

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DESPITE many well-recognized side effects that are inherent in its depolarizing mechanism, succinylcholine is still popular for obtaining rapid control of the airway, even though an occasional patient with reduced cholinesterase activity must be dealt with.[1,2] There have been many attempts to improve the profile of succinylcholine by synthesizing nondepolarizing substances with similar kinetic properties. Although often promising in preclinical evaluations, none of these substances has been successful in clinical trials. These failures have occurred either because the side effects have been more prominent in humans than in animals or because the pattern of neuromuscular blockade did not as closely resemble the time course of action of succinylcholine in humans as preclinical data might have suggested.[3–8]

In this article, we describe the preclinical pharmacology of GW280430A (AV430A) (fig. 1), a representative of a new class of nondepolarizing neuromuscular blocking drugs that we call *asymmetric mixed-onium chlorofumarates*. We consider this a new class of relaxants because unlike mivacurium and other symmetric benzylisoquinolines, the quaternary heads are different, there is halogen (chlorine) in the molecule, and the mechanism of inactivation is completely novel. In two

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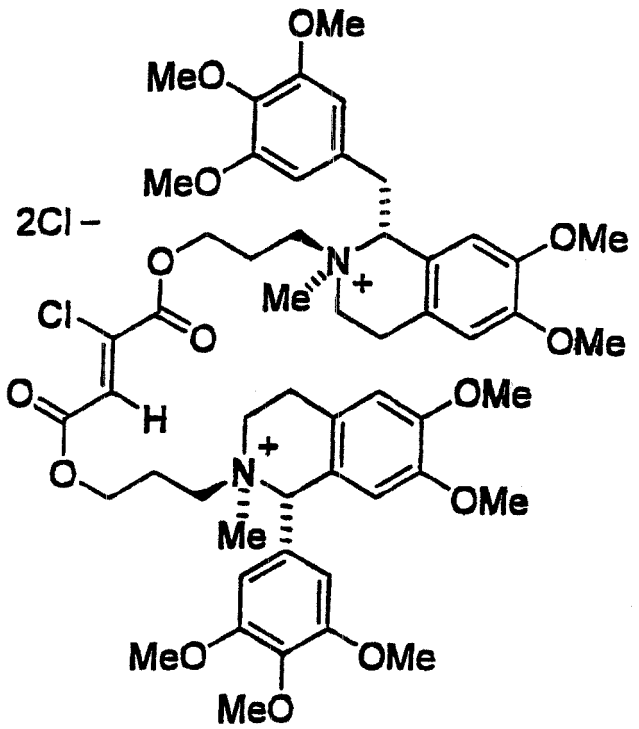

Fig. 1. Chemical formula of GW280430A, an asymmetric mixed-onium chlorofumarate. See text for description of the asymmetries in the molecule.

pertinent animal models, the cat and the rhesus monkey, the new compound's blocking characteristics and autonomic and cardiovascular side effects are compared with the properties of mivacurium to facilitate future clinical evaluation.

Materials and Methods

All experiments were approved by the Institutional Animal Care and Use Committees of Weill Medical College of Cornell University (New York, New York) and of Albany Medical College (Albany, New York).

Evaluation in Anesthetized Rhesus Monkeys

GW280430A. Evaluation of GW280430A was performed in a colony of eight male adult rhesus monkeys weighing 9–15 kg. Each animal was anesthetized and studied on several occasions, and all occasions were separated from each other by at least 3 weeks. The animals were fed standard monkey chow supplemented with fruits, vegetables, and vitamins and were housed for the entire period of the study under veterinary supervision (D. L. C.). Clinical laboratory tests (complete blood counts, urinalysis, chemistries) were followed semiannually. Poststudy care was under the direction of the veterinarian and included analgesics and antibiotics if necessary.

Anesthesia. On the morning of each study, each monkey received 5 mg/kg intramuscular ketamine for heavy sedation–light anesthesia. If necessary, 5 mg/kg thiopental was then given intravenously to deepen anesthesia. The trachea was sprayed with 1–1.5 ml lidocaine (2%, 2 mg/kg), and intubation of the trachea was performed without a muscle relaxant. Anesthesia was maintained with nitrous oxide–oxygen–halothane (2 l–1 l–1% end-tidal) in a pediatric circle system. Ventilation was controlled at 200 ml $\cdot$ kg^{-1} $\cdot$ min^{-1} to keep end-tidal carbon dioxide in the range 25 $\pm$ 5 mmHg to ensure no spontaneous respiratory efforts. Esophageal and peripheral skin temperatures were monitored and kept in the ranges of 37°–38° and 34°–35°C, respectively, using thermal blankets. The electrocardiogram (lead II) was monitored continuously. Peripheral oxygen saturation was kept at 98–100%, as indicated by pulse oximetry.

Experimental Setup. A 20-gauge peripheral intravenous cannula was placed percutaneously, and lactated Ringer's solution was infused at a rate of 15–20 ml $\cdot$ kg^{-1} $\cdot$ h^{-1}. During longer experiments ($>$ 4 h), the bladder was catheterized. A 20-gauge catheter was also placed percutaneously in the superficial tibial artery between the ankle and the knee of either leg. Arterial pressure was recorded *via* a Statham P23D transducer. Heart rate was monitored by a Grass 7P44A tachometer (Grass Instruments, Quincy, MA) triggered by the arterial pulse wave.

Under sterile conditions, a surgical cutdown was performed on a tendon of the extensor digitorum through a 1-cm incision on the dorsum of the foot opposite the site of the arterial cannula. The tendon was split longitudinally, and a thin strip was then tied to a Grass FT10 transducer (Grass Instruments) at baseline tension of 50 g for recording of twitch and train-of-four (TOF; 2 Hz for 2 s) responses. Sterile conditions were maintained for the duration of the experiment. The common peroneal nerve was stimulated at the knee at a rate of 0.15 Hz with supramaximal square-wave pulses of 0.2 ms in duration *via* 23-gauge steel needle electrodes placed percutaneously. The stimuli were generated by a Grass S-88 laboratory stimulator and SIU5 isolation unit (Grass Instruments). TOF stimulation was interposed during the experiment whenever appropriate. Recordings of neuromuscular and cardiovascular responses were made simultaneously on a Grass model 7B polygraph (Grass Instruments).

At the end of each experiment, all surgical wounds were closed in sterile fashion. Animals were awakened from anesthesia, placed back in their cages, and attended until sitting, walking, and climbing. All animals received appropriate postanesthesia and postoperative care, including antibiotics and analgesics if these were believed to be indicated by the primatologist. All animals maintained normal body weight, remained clinically healthy, and maintained normal clinical laboratory values (CBC, chemistries, urinalysis) throughout the study period (6

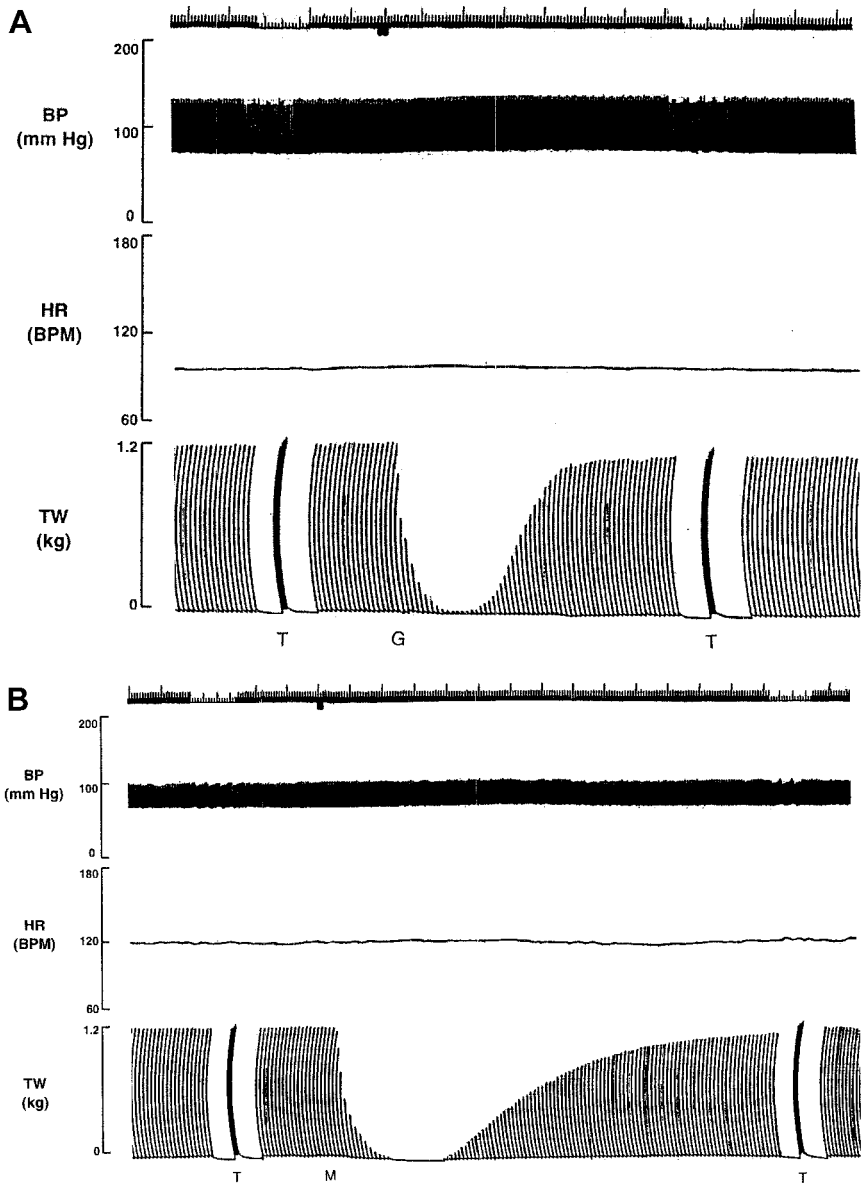

Fig. 2. Comparative neuromuscular blocking properties after rapid bolus injections of GW280430A (*A*) and mivacurium (*B*) in the anesthetized rhesus monkey. The twitch (TW) of the extensor digitorum of the foot (*bottom record*) was elicited at 0.15 Hz. Blood pressure (BP, *top record*) and heart rate (HR, *middle record*) were monitored continuously. (*A*) GW280430A, 0.08 mg/kg, was given intravenously at *G*. (*B*) Mivacurium, 0.08 mg/kg, was given intravenously at *M*. Time scale (minutes) is at the *top*, and calibrations are at the *left*. At *T*, train-of-four stimulation (2 Hz for 2 s) was interposed. BPM = beats/min.

months) and thereafter until the present (approximately 5 years' time).

Mivacurium. A comparative series of experiments (n = 6) was performed with mivacurium in the same animals on different occasions during the same period, under the same experimental conditions.

Conduct of Experiments in Monkeys.

Dose–response curves for GW280430A (n = 8). Stable baseline recordings of twitch, TOF, blood pressure, and heart rate were obtained for at least 20 min. Each animal then received successive doses of GW280430A at 0.03, 0.05, 0.08, 0.20, 0.40, 0.80, 1.60, and 3.20 mg/kg. Subsequent doses were given 15 min after TOF responses had returned to normal after previous doses (normal TOF ratio in the rhesus monkey is 1.10–1.20; for example, see fig. 2). Dose–response data for neuromuscular blockade, and changes in blood pressure and heart rate were generated. The skin of the face was carefully observed for evidence of flushing (erythema).

Dose–response curves for mivacurium (n = 6). In a separate series of experiments in the same animals, mivacurium was given in doses of 0.02, 0.04, 0.08, 0.20, 0.40, 0.80, and 1.60 mg/kg, and dose–response data were generated for neuromuscular blockade and for cardiovascular responses. Doses of 0.02–0.20 mg/kg were given as separate boluses 15 min after TOF recovery from the previous dose. The large doses of 0.4, 0.8, and 1.6 mg/kg (total = 2.8 mg/kg) were given separately in escalating cumulative fashion, 15 min apart, beginning with 0.4 mg/kg, which was given 15 min after recovery from the 0.2-mg/kg dose. Baseline heart rate and blood pressure were reestablished before each dose. Full spontaneous recovery of neuromuscular function to TOF of 100% or greater was achieved after the final (1.6-mg/kg) dose.

Continuous infusions and antagonism. In another separate series of experiments performed in the same animals on other occasions, to evaluate lack of cumulation and ease of maintenance of blockade, four animals received continuous infusions of GW280430A. After bolus doses of 0.08 or 0.10 mg/kg to induce full paralysis, infusion of GW280430A was initiated at 50 $\mu g \cdot kg^{-1} \cdot min^{-1}$. The rate was adjusted downward until a dosage level was established where a constant rate of infusion was achieved that maintained 95 ± 4% block for 60 min. Slope/speed of recovery after discontinuation of infusion was compared with slopes of recovery of individual bolus doses observed during spontaneous recovery. These slopes were also compared with the speed of recovery after antagonism with edrophonium (0.5 mg/kg, given at 25% twitch height).

Acute Experiments in Anesthetized Cats

Anesthesia and Experimental Setup. All studies were approved by the Institutional Animal Care and Use Committees. Eight adult male cats weighing 2.9–5.2 kg were anesthetized with α-chloralose (80 mg/kg) and sodium pentobarbital (7 mg/kg) intraperitoneally. Ventilation and monitoring were performed as described in the experiments in monkeys. The left femoral artery and vein were cannulated. The tendon of the right tibialis anterior was separated from its insertion and tied to a Grass FT-10 transducer at baseline tension of 50 g. Twitch was elicited *via* the peroneal nerve at 0.15 Hz. TOF or tetanic stimulation (50 Hz for 5 s) was interposed where appropriate.

The right vagus nerve and sympathetic trunk were exposed in the neck and cut centrally. The distal ends of the nerves were placed on a platinum electrode and stimulated every 3–5 min with 10-s trains of pulses (0.5-ms duration at 20 Hz). The elicited bradycardic responses (vagal response) were recorded. The elicited contractions of the right nictitating membrane (sympathetic response) were measured using a Grass FT-03 transducer at a baseline tension of 5 g.

Recordings of autonomic responses, arterial pressure, heart rate, and neuromuscular responses were made simultaneously on a Grass model 7B polygraph (Grass Instruments) using the same methodology and equipment as described in the section on monkeys (Evaluation in Anesthetized Rhesus Monkeys).

Cats were killed humanely at the end of each experiment by administration of an overdose of anesthetic (400 mg/kg intravenous pentobarbital).

Conduct of Experiments in Cats. Dose–response data for GW280430A-induced inhibition of neuromuscular and autonomic responses were generated in a subset of four animals. GW280430A was given in bolus doses of 0.02–6.4 mg/kg successively. The experimental protocol was generally similar to the procedure described for experiments in monkeys. Further details of the methods used in experiments in cats have been described previously.[9]

Comparative dose–response data were similarly generated for mivacurium in another subset of four different animals. Mivacurium was given in doses from 0.02 to 5.12 mg/kg.

Data, Calculations and Statistical Analysis

Sigmaplot (RockWare, Inc., Golden, CO) was used to calculate dose–response data including ED_{50} and ED_{95} for neuromuscular blockade and ED_{50} for autonomic blockade (sympathetic, vagus). This package transforms data to log/probit format. ED Hist, the dose producing a cardiovascular/cutaneous response suggestive of histamine release, was defined as the bolus dose producing more than 10% decrease in mean arterial pressure or more than 10% increase in heart rate, plus flushing of the skin of the face. Ratios of vagal and sympathetic ED_{50} and ED Hist *versus* ED_{95} for neuromuscular blockade were calculated. The statistical packages JMP (SAS Institute, Cary, NC) or Excel (Microsoft Inc., Redmond, WA) were used to perform the Student *t* test or analysis of variance, as appropriate. A *P* value of less than 0.05 was considered significant.

Results

Neuromuscular Blockade

Potency and Duration of Action in the Monkey. Data comparing GW280430A with mivacurium are listed in table 1. The potencies of the two compounds in the rhesus monkey are identical. The ED_{95} was 0.06 mg/kg for each compound. The onset, duration, and recovery slopes of GW280430A, however, were all significantly faster or shorter (compare figs. 2A and B) than the corresponding values for mivacurium ($P < 0.05$). The total duration of action (injection to 95% twitch recovery) of GW280430A was approximately one half to one third that of mivacurium at equipotent dosage (table 1). For example, at 0.2 mg/kg or approximately 3 × ED_{95}, the comparative durations to 95% twitch recovery were 8.5 ± 0.5 *versus* 22.0 ± 2.6 min, respectively. Figure 2 shows typical recordings of the neuromuscular blocking properties of GW280430A and mivacurium after doses of 0.08 mg/kg or approximately 1.3 × ED_{95}.

As the dosage of GW280430A was doubled, the total duration of effect lengthened by only 1.5–3 min (table 1), indicating that time interval as an elimination half-life. The slopes of recovery remained parallel (fig. 3) after all doses up to and including approximately 50 × ED_{95} (3.2 mg/kg), indicating a lack of cumulative effect.

Continuous Infusions. After a few initial adjustments, final infusion rates of 17–38 $\mu g \cdot kg^{-1} \cdot min^{-1}$ (range of infusion rates in four animals) maintained paralysis for 60 min at a steady state (95 ± 4% block). The rate of infusion required little adjustment after an appropriate rate was established. Recovery after discontinua-

Table 1. Neuromuscular Blocking Properties of GW280430A in the Rhesus Monkey: Comparison with Mivacurium

Dose, mg/kg	% Block	Time to 95% Twitch, min	Recovery Indices	
			25–75%	5–95%
GW280430A (n = 8)				
0.03	32.3 ± 8.1	3.1 ± 0.3	NA	NA
0.05	81.2 ± 4.1	5.0 ± 0.3	1.3 ± 0.2	NA
0.08	99.3 ± 0.3	6.3 ± 0.5*	1.4 ± 0.2*	3.8 ± 0.6*
0.20	100	8.5 ± 0.5*	1.5 ± 0.2*	4.1 ± 0.5*
0.40	100	10.0 ± 0.4	1.6 ± 0.2	4.4 ± 0.4
0.80	100	12.0 ± 0.7	1.6 ± 0.2	4.7 ± 0.6
1.60	100	13.7 ± 0.6	1.7 ± 0.2	4.9 ± 0.4
3.20	100	17.0 ± 0.9	2.1 ± 0.3	5.6 ± 0.5
Continuous infusions (n = 4)	95 ± 4	60 min	1.8 ± 0.1	4.6 ± 0.5
Mivacurium (n = 6)				
0.02	34.1 ± 11.3	10.2 ± 1.8	NA	NA
0.04	84.0 ± 6.7	14.8 ± 2.5	5.7 ± 0.8	NA
0.08	99.3 ± 0.7	18.0 ± 2.4	4.8 ± 0.6	12.0 ± 1.6
0.20	100	22.0 ± 2.6	5.5 ± 0.7	13.1 ± 1.5
0.40	100	NA	NA	NA
0.80	100	NA	NA	NA
1.60	100	NA	NA	NA

Experiments were performed in adult male rhesus monkeys under controlled ventilation with nitrous oxide–oxygen–halothane anesthesia. Block percentages, duration, and recovery indices refer to single twitch elicited at 0.15 Hz. All data are presented as mean ± SE.

* Highly significant differences ($P < 0.001$) *vs.* mivacurium.

NA = not applicable.

tion of the infusions occurred at a rate of 1.8 ± 0.1 min over the interval (25–75%) twitch height. This rate did not differ from rates noted after various bolus doses (fig. 3 and table 1). The same was true for the 5–95% interval. Figure 4 shows a typical recording from one of the four experiments where an infusion was given. The data indicate that the speed of recovery from GW280430A-induced blockade is little affected by administration by infusion for 60 min (see also figs. 2A and 3).

Mechanism of Action and Reversal. Neuromuscular blockade secondary to GW280430A showed nondepolarizing characteristics, such as fade of TOF and tetanus. The recovery slope was accelerated by edrophonium (0.5 mg/kg; fig. 5).

Neuromuscular Potency and Duration in the Cat. The ED_{95} for GW280430A was 0.11 ± 0.03 mg/kg. The total duration to 95% twitch recovery after doses of 0.2 mg/kg (approximately 1.8 × ED_{95}) was 5.4 ± 0.4

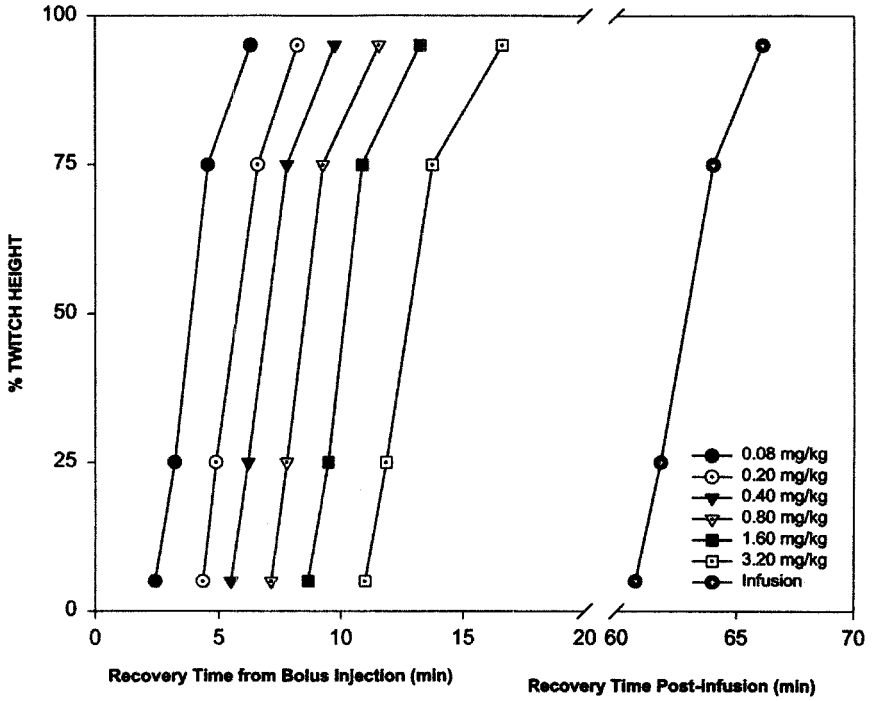

Fig. 3. Summarized recovery curves of the twitch response in the rhesus monkey after various bolus doses of GW280430A (n = 8 for each bolus dose) and 60-min infusions (n = 4). Twitch of the extensor digitorum was elicited at 0.15 Hz. The slopes of recovery from 25 to 75% do not differ significantly ($P < 0.05$) between 0.05 and 1.60 mg/kg. The speed of recovery of GW280430A is not related to dose within the range 0.05–1.60 mg/kg or to administration by infusion. SEs were omitted for clarity; they are presented in table 1.

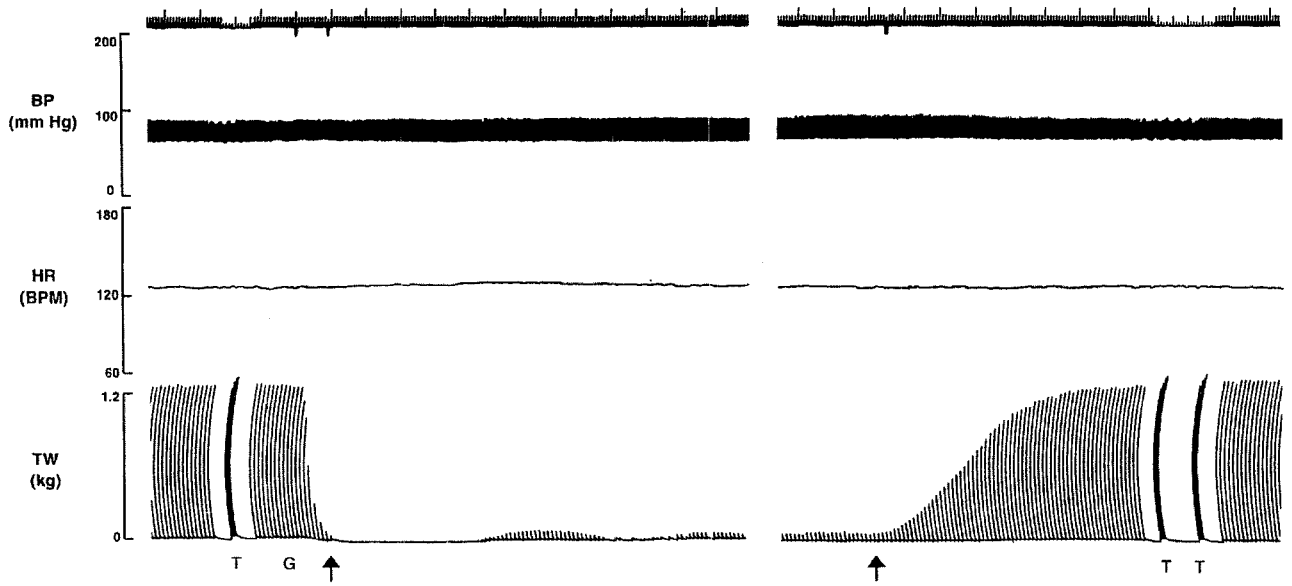

Fig. 4. Continuous infusion of GW280430A in an anesthetized rhesus monkey. Twitch (TW) of the extensor digitorum was elicited at 0.15 Hz. At *T*, train-of-four stimulation was interposed. At *G*, GW280430A, 0.08 mg/kg, was given as a rapid intravenous bolus, followed by infusion (*arrow*) at a rate between 20 and 38 $\mu g \cdot kg^{-1} \cdot min^{-1}$. Infusion was discontinued at the marker (*arrow*) on the *right*. Recovery times are as follows: 25–75%, 2.0 min; 5–95%, 5.0 min. A 50-min interval of record is not included (*space*). BPM = beats/min; BP = blood pressure; HR = heart rate.

min. The total duration of effect of mivacurium at 0.08 mg/kg (approximately 1.6 × ED_{95}) was 12.4 ± 0.7 min ($P < 0.05$ *vs.* GW280430A).

Cardiovascular Effects in the Monkey

Changes in mean arterial pressure and heart rate were less than 10% after all doses of GW280430A up to and including 1.6 mg/kg (fig. 6A). At 3.2 mg/kg (approximately 53 × ED_{95}), a decrease in mean arterial pressure of 16.6 ± 5.6% and increase in heart rate of 12.4 ± 6.3% occurred. These cardiovascular changes were typically accompanied by facial erythema. The changes were short lasting (2–5 min). Although plasma histamine concentrations were not drawn, this combined response is nearly pathognomonic of histamine release and is termed as such in the Results and the Discussion. The dose ratio (ED Hist:ED_{95}) for this presumed side effect in the monkey is therefore approximately 53 *versus* the ED_{95} for neuromuscular blockade. Comparative data for mivacurium are shown in figure 6B and table 2. The comparative dose ratio for histamine release for mivacurium is approximately 13.

Autonomic and Cardiovascular Properties in the Cat

Data are presented in table 2. Neither GW280430A nor mivacurium inhibited either vagal or sympathetic autonomic responses at doses less than 25 × ED_{95}.

At a dosage of 1.0 mg/kg, GW280430A caused a brief (1–2 min) decrease in blood pressure (10–20%) and increase in heart rate (10–20%), accompanied by flushing of the skin of the shaved areas of the face and neck, suggestive of release of histamine. The dose ratio for presumed histamine release is therefore approximately 9 in the cat, compared with approximately 53 in the monkey (table 2).

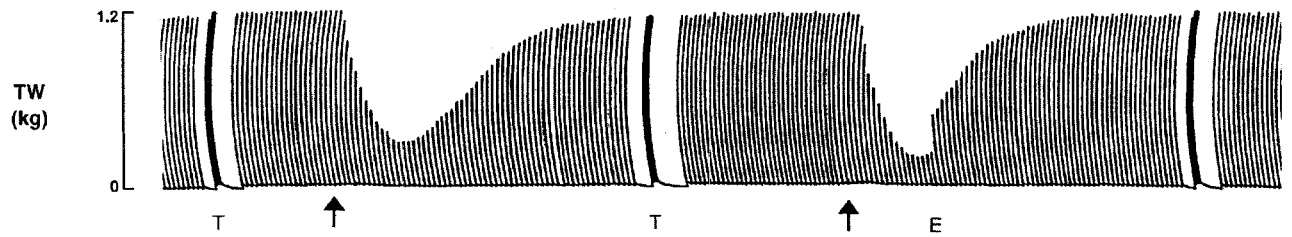

Fig. 5. Comparison of spontaneous recovery (*left*) and edrophonium-accelerated recovery (*right*) of GW280430A in the monkey. Continuous recording of the twitch (TW) of the extensor digitorum of the foot, elicited at 0.15 Hz. At *arrows*, GW280430A (0.05 mg/kg) was given intravenously. At *E*, edrophonium (0.5 mg/kg) was given intravenous together with atropine (0.1 mg/kg). Time scale (minutes) is at the *top*, and calibrations are at the *left*. At *T*, train-of-four stimulation was interposed.

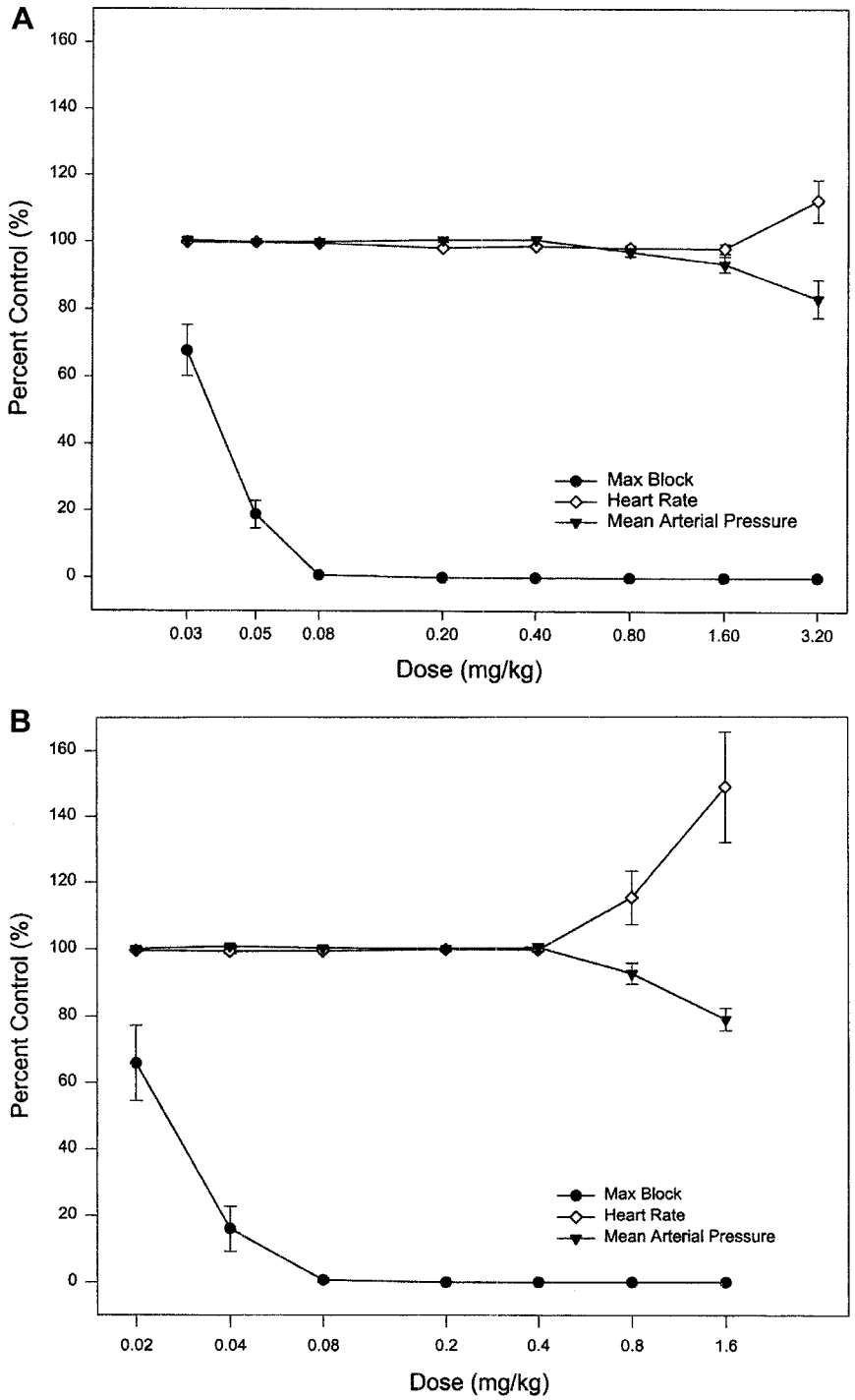

Fig. 6. (*A*) Dose–response curves for neuromuscular blockade, and heart rate and blood pressure changes after bolus doses of GW280430A in rhesus monkeys (n = 8). (*B*) Comparative data are given for mivacurium in the same animals (n = 6). Twitch of the extensor digitorum was elicited at 0.15 Hz. Control twitch = 100%. There is a fourfold increase in the dose ratio (ED Hist: ED_{95}) when GW280430A is compared with mivacurium, indicating a fourfold greater safety margin for the side effect of histamine release (see text).

Discussion

Desirable Characteristics

To modify the pharmacologic profile of mivacurium to produce a nondepolarizing relaxant that might replace succinylcholine, we believed that the following characteristics required adjustment: (1) acceleration of the onset and shortening the duration of action, (2) reduction of the side effects, (3) development of a nonbiological route of rapid inactivation to pharmacologically inert reaction products to eliminate clinical problems of prolonged duration of action in patients with reduced cholinesterase activity and to decrease the variability of duration of action, and (4) retention of a nondepolarizing blocking property. GW280430A seems to improve upon mivacurium in each category.

In this series of studies, where GW280430A was one of a large group of compounds to be evaluated, it was a major advantage to perform the studies in a colony of animals (a group of eight rhesus monkeys) that were studied repeatedly, at approximately 3- to 8-week inter-

Table 2. Autonomic and Cardiovascular Effects of GW280430A and Mivacurium* in the Cat and the Monkey

Animal	Measurement	GW 280430A	Mivacurium
Monkey	ED_{95} (NMB) (mg/kg ± SE)	0.06 ± 0.002	0.06 ± 0.009
Monkey	ED Hist† (mg/kg)	3.20	0.80
Monkey	Dose ratio $\left[\frac{\text{ED Hist}}{ED_{95}\text{NMB}}\right]$	53.3	13.3
Cat	ED_{95} (NMB) (mg/kg ± SE)	0.107 ± 0.03‡	0.047 ± 0.003
Cat	ED_{50} (vagus) (mg/kg ± SE)	2.80 ± 0.41	2.00 ± 0.55
Cat	Dose ratio $\left[\frac{ED_{50}\text{ vagus}}{ED_{95}\text{NMB}}\right]$	26.2	42.6
Cat	ED_{50} (sympathetic) (mg/kg ± SE)	3.22 ± 0.56	>5.12
Cat	Dose ratio $\left[\frac{ED_{50}\text{ sympathetic}}{ED_{95}\text{NMB}}\right]$	30.1	>109

Experiments determining autonomic properties were performed in adult male cats. Twitch of the tibialis anterior was elicited at 0.15 Hz. Bradycardia (vagal response) and nictitating membrane contraction (sympathetic response) were elicited at 20 Hz for 5 s every 3–5 min. Dose ratios for histamine release were generated from experiments performed in adult male rhesus monkeys where blood pressure and heart rate were recorded continuously. Twitch of the extensor digitorum of the foot was elicited at 0.15 Hz.

* Unpublished data. † ED for histaminoid phenomena: greater than 10% decrease in mean arterial pressure and greater than 10% increase in heart rate, and facial erythema. ‡ Significant difference, $P < 0.05$.

ED Hist = dose producing a cardiovascular/cutaneous response suggestive of histamine release; NMB = neuromuscular blockade.

vals. Surgical procedures were performed under sterile conditions, and anatomic structures (tendons, arteries) were preserved so that each animal eventually developed a history of responses to various compounds. As a result, highly reliable comparisons focusing on the above characteristics of new compounds were obtained in only a few days. Within 18 months, GW280430A was selected as an appropriate representative of the series (the lead compound). A direct comparison was then made with mivacurium to make a judgment regarding possible future clinical testing. This report is a description of that comparison.

Brief History

There have been many attempts to replace succinylcholine. Efforts from 1960 to the early 1970s and then from the 1980s to the early 1990s were reviewed.[10,11] During this time, the steroidal compounds have been studied extensively but have not yet achieved the time course of action of succinylcholine in humans because the high clearances required in humans (most likely around 50–100 ml · kg^{-1} · min^{-1}) have hitherto not been possible in this type of compound.[12–22] Rapacuronium is a current example. The duration in humans is much longer than predicted in animals.[23] Unfortunately, because of an unexpectedly severe side effect (bronchospasm), rapacuronium has been withdrawn.[7,8,24] The bronchospasm has been ascribed to M2 receptor blockade and is most likely not due to histamine release.[25,26]

Many bisquaternary diester-type compounds have been studied as potential substrates for plasma cholinesterase whereby the desired high clearance rate might be achieved. Most of these, although short-acting in some cases in humans (such as BW 785U), have also been failures because of unacceptable cardiovascular side effects.[4] The symmetric benzylisoquinolinium diester mivacurium has achieved modest clinical acceptance.[27,28]

Atracurium[29,30] and cisatracurium[9,31] also deserve mention as examples of symmetric benzylisoquinolinium diesters that undergo largely chemical degradation by the Hofmann elimination.[29–34] The chemical degradation of these compounds results in a rather consistent duration of effect and recovery slopes that are unaffected by dosage, by duration of administration, or by organ failure.[31]

Neuromuscular Blocking Properties of GW280430A

GW280430A and related compounds have recently been described in a brief medicinal chemical report[35] and in a more extensive article where some structure–activity relations are summarized by Boros *et al.*[36] The asymmetric mixed-onium compound GW280430A showed greater potency and less cardiovascular effect than related symmetric substances while retaining an ultrashort duration of action.[35,36]

The data herein show that GW280430A is a candidate for evaluation as a potential nondepolarizing alternative or replacement for succinylcholine in the clinic. It

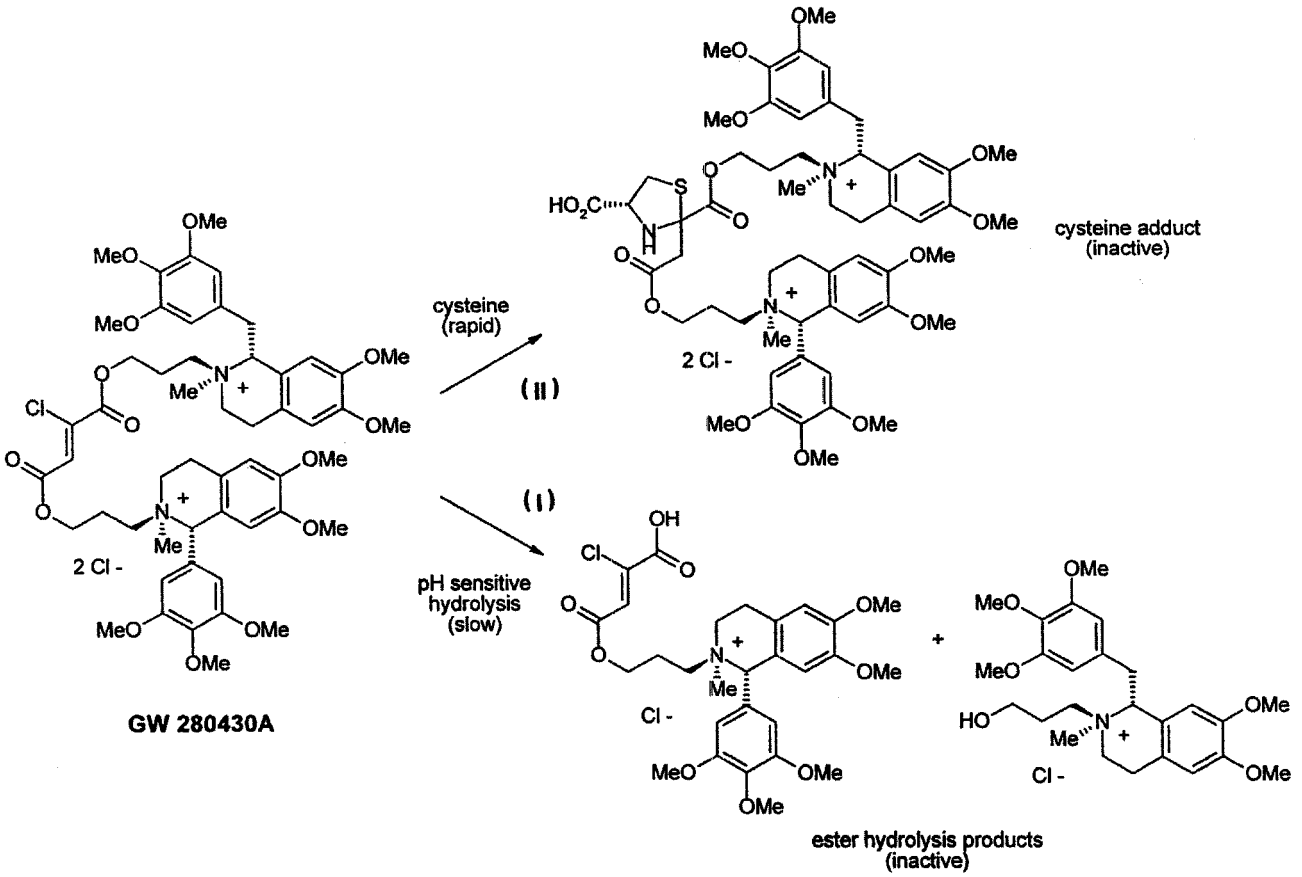

Fig. 7. Degradation and inactivation of GW280430A. This occurs by two novel processes: pH-sensitive chemical hydrolysis (reaction I, *lower pathway*) and adduction of cysteine, replacing chlorine and saturating the fumarate double bond to yield a new five-membered heterocyclic ring (reaction II, *upper pathway*). The products of both reactions are presumably inactive. Reaction II (cysteine adduction) is most likely the rate-limiting step with respect to the kinetics of GW280430A (see text).

shows desirable characteristics of faster onset and shorter duration *vis-à-vis* mivacurium and is readily administered by infusion to maintain stable levels of block. GW280430A is much shorter acting than succinylcholine and mivacurium in the dog, an early signal that its kinetics might not be governed primarily by cholinesterase activity (W. B. Wastila, Ph.D., Burroughs Wellcome Co., Research Triangle Park, North Carolina, unpublished data regarding the duration of action of mivacurium and succinylcholine in the dog, 1981–1982, and P. M. Heerdt, M.D., Ph.D., Weill Medical College of Cornell University, unpublished data regarding the duration of action of mivacurium and GW280430A in the dog, 1997–1998).

Indeed, two chemical routes of inactivation–degradation, both unrelated to cholinesterase activity, can account for the brief duration of GW280430A.[36] These are shown in figure 7. First, chemical (pH-sensitive) alkaline hydrolysis occurs in plasma or buffer at the ester linkage adjacent to chlorine (reaction I). The more important pathway, however, is most likely a rapid reaction of GW280430A with free cysteine in plasma. This is a type of Michael addition where cysteine replaces chlorine (reaction II) to form a new heterocyclic ring near the benzyl quaternary head. The new molecular geometry induced by this ring formation seems to yield an apparently inactive addition product (fig. 7). The latter might undergo further degradation; further investigation of this possibility is needed. Cysteine adduct formation is a novel chemical mechanism of inactivation of neuromuscular blocking drugs by an addition reaction, first described in GW280430A.[36]

Structure–Activity Characteristics and Nomenclature

The asymmetries in the molecule of GW280430A contribute to an improved profile *versus* mivacurium and to a novel route of degradation. Therefore, this type of compound deserves to be named differently from "ancestral" symmetric benzylisoquinolines, to which it is related only in the single R-benzyl substituted quaternary. The asymmetries (fig. 1) in the structure of GW280430A are (1) the different quaternary heads, (2) the different stereochemistry of the two heads (R in the benzyl group and S in the phenyl group), (3) the positioning of chlorine on only one side of the double bond,

and (4) the *trans*relationship of the two sides of the molecule across the double bond (fumaric acid). The synthesis of GW280430A is stereospecific, producing a single isomer[36] (R. Mook, Ph.D., E. Boros, Ph.D., V. Samano, Ph.D., GlaxoSmithKline, Research Triangle Park, North Carolina, data on file, 1996–1998).

Autonomic Effects

In the cat, GW280430A only weakly inhibits vagal and sympathetic responses: The dose ratios of more than 25 $\times$ ED_{95} for neuromuscular blockade in both cases (table 2) suggest that heart rate increases due to vagal block and decreases in blood pressure due to sympathetic ganglionic block are not likely in the clinical dose range for GW280430A. Furthermore, because this dose ratio is approximately 10 times higher for GW280430A than for rapacuronium,[23] M2 receptor–induced bronchospasm is also unlikely in humans.

Cardiovascular Responses

In the monkey, the point of bifurcation where a decrease in blood pressure and an increase in heart rate suggestive of histamine release occurred was at 3.2 mg/kg for GW280430A and at 0.8 mg/kg for mivacurium (compare figs. 6A and B). Despite the similarities in neuromuscular potency, dosing of mivacurium was halted after the final dose of 1.6 mg/kg because the cardiovascular effects were moderate (fig. 6B), well beyond the 10% change defined in the protocol as ED Hist. Furthermore, care was taken to ensure the animals' welfare because these were experiments in primates, where the animals were to be recovered for future study. GW280430A therefore has approximately four times less tendency than mivacurium to produce this side effect, *i.e.*, apparent histamine release, in the monkey because the neuromuscular potencies of the two blockers are identical.

The above has been noted together with facial flushing during clinical studies of various symmetric benzylisoquinolines, including mivacurium. The dose ratio of ED Hist:ED_{95} for mivacurium in humans is 2.5. Our comparisons performed in the monkey suggest that GW280430A should show a higher dose ratio for this side effect in patients, with respect to mivacurium.[37]

Conclusions

The neuromuscular blocking effect of GW280430A is significantly shorter than that of mivacurium. The side effect profile of GW280430A is much reduced in these experiments *vis-à-vis* mivacurium, suggesting potentially more stable cardiovascular responses, compared with mivacurium, in human subjects. The degradation of GW280430A *via* two novel pathways seems advantageous *versus* cholinesterase-catalyzed hydrolysis, as in the case of mivacurium.[27] These factors indicate that GW280430A is a promising candidate for clinical evaluation.

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