EFFECTS OF A NEW NEUROMUSCULAR BLOCKING AGENT (ORG 9426) IN ANAESTHETIZED CATS AND PIGS AND IN ISOLATED NERVE–MUSCLE PREPARATIONS


In the 1980s, two new neuromuscular blocking agents, vecuronium and atracurium, have been introduced into anaesthetic practice. Both compounds possess a duration of action intermediate between that of the short-acting suxamethonium and the longer-acting pancuronium. However, the drugs do not possess a sufficiently short onset time to allow rapid tracheal intubation unless large doses are used, with consequent prolonged paralysis of skeletal muscle.

Our recent efforts to develop new neuromuscular blockers for anaesthetic practice have concentrated on attempts to improve onset times. We have reported on two new non-depolarizing compounds with rapid onset and short duration, similar to those of suxamethonium [1]. A further objective has been to produce compounds with a non-depolarizing action, rapid onset but with duration and recovery times similar to those of vecuronium and atracurium. Additional criteria for the selection of compounds for detailed study were a potency greater than 10% of that of vecuronium, minimal autonomic and cardiovascular side effects, and chemical stability in aqueous solution.

From a series of monoquaternary amino-steroids, the compound Org 9426 (fig. 1) was selected as promising. In this communication we

SUMMARY

The effects of Org 9426 (the 2-morpholino, 3-hydroxy, 16N-allyl pyrrolidino analogue of vecuronium) were studied in anaesthetized cats and pigs and in isolated nerve–muscle preparations using tension and intracellular recording techniques. In isolated preparations, the effects of Org 9426 were antagonized by neostigmine. No contracture of the chick muscle preparation occurred. Org 9426 reduced the amplitude of endplate currents (EPC) in rat and snake muscle, but had no major effects on EPC decay characteristics, indicating a lack of endplate channel blocking action. In anaesthetized animals, no fasciculations were observed and the neuromuscular block was associated with tetanic and train-of-four fade and was antagonized by neostigmine. In anaesthetized cats and pigs, Org 9426 was approximately 20% as potent as vecuronium, its onset of action was twice as rapid as that of vecuronium in the cat and its duration of action was similar to that of vecuronium in both cats and pigs. It blocked the bradycardia produced by vagal stimulation only in doses greater than those necessary to produce neuromuscular block (ratios 7.2 in the cat and 4.4 in the pig—10–14% of the corresponding ratios for vecuronium). Ganglion block was seen only at doses several times those producing vagal block. In general the effects of Org 9426 on the cardiovascular system were slight, a small depressor effect occurring at high doses in the cat. The 17-hydroxy analogue, the potential metabolite of Org 9426, was approximately 20 times less potent than Org 9426 and is thus unlikely to contribute to the neuromuscular block produced by the parent compound.
describe its pharmacological profile in anaesthetized cats and pigs and in isolated nerve–skeletal muscle preparations. Anaesthetized cats were used primarily to predict neuromuscular blocking potency, onset time and autonomic effects. Anaesthetized pigs were used primarily to assess duration of action and cardiovascular effects. Isolated nerve–skeletal muscle preparations were used to assess mechanism of action. We have examined also some of the effects of 9943 (fig. 1), the 17-OH analogue and potential metabolite of Org 9426, in anaesthetized cats.

METHODS

Anaesthetized Animals

Anaesthetized cats

Cats of both sexes were anaesthetized with a mixture of α-chloralose 80 mg kg⁻¹ and pentobarbitone 5 mg kg⁻¹ injected i.p. Lungs were ventilated with air at a rate of 26 b.p.m. at a tidal volume of approximately 13 ml kg⁻¹, adjusted to maintain arterial pH at 7.37–7.47.

The right hind limb was immobilized and we recorded the contractile responses of the tibialis anterior and soleus muscles to single shock stimulation of the sciatic nerve. The sciatic nerve was stimulated at a rate of 0.1 Hz using rectangular pulses of 0.2 ms duration and of a strength greater than that required to produce a maximal twitch. This stimulation frequency was chosen to allow comparison of the results with test compounds with those for compounds reported previously in the literature. Contractions of the nictitating membrane were evoked in response to preganglionic stimulation of the cervical sympathetic nerve with trains (frequency 5 Hz, duration 10 s) of strength sufficient to produce maximal contractions of the nictitating membrane.

Arterial pressure was recorded from the carotid artery using a Statham PC45 pressure transducer. The arterial pressure pulse triggered a cardiograph to display heart rate. Both vagus nerves were ligated and, at 100-s intervals, the right vagus nerve was stimulated with trains of 10 s duration at a frequency of 2–5 Hz and with pulses of 0.5 ms duration and strength greater than that required to produce a maximal reduction in heart rate. Contractile responses of muscles were recorded using Grass FTO3C and FT10C force displacement transducers. All responses were displayed on a Grass model 5 ink oscillograph.

Anaesthetized pigs

Domestic pigs (Landrace–Welsh cross) of both sexes (10.4–15.1 kg) were administered a tranquilizing dose (approximately 2 mg kg⁻¹) of azaperone approximately 20 min before induction of anaesthesia with 3–4% halothane in oxygen. Following induction, anaesthesia was maintained with α-chloralose 200 mg kg⁻¹ dissolved in polyethylene glycol (PEG 300) given slowly into a jugular vein. Approximately 1 h later, anaesthesia was supplemented with additional chloralose given by slow i.v. infusion (33 mg kg⁻¹ h⁻¹) which was continued throughout the investigation. The lungs were ventilated mechanically with room air via a tracheal cannula at a rate of 28 b.p.m. and tidal volume of 12–14 ml kg⁻¹. Arterial $P_{CO_2}$ was 4.3–5.1 kPa.

Arterial pressure was recorded via a polythene
catheter placed in the right carotid artery and connected to a Gould–Statham pressure transducer. Heart rate was monitored continuously using the arterial pulse pressure to trigger a Grass 7P4F cariotachometer or Devices instantaneous heart rate meter. Drugs were administered through a catheter in the contralateral jugular vein. Contraction of the tibialis anterior and soleus muscles were recorded by force displacement transducers (Grass FT10 or FT03). A resting tension of 30 g was applied to each muscle. Twitches were evoked every 10 s by stimulating the two branches of the sciatic nerve supplying the lower leg, immediately distal to the point where the nerve divided, using square wave pulses of 0.25 ms duration and at twice the voltage required to produce maximum contractions. In a small number of experiments trains-of-four and tetanic contractions were elicited by stimulating the motor nerve at 2 Hz for 1.9 s or at 50 Hz for 10 s, respectively.

**General experimental procedure**

Initial experiments were carried out by injecting doses of the neuromuscular blocking compounds at 1-h intervals until a range of neuromuscular blocks of 5–95% had been obtained on both the tibialis anterior and soleus muscles. Control experiments with vecuronium [2] indicated the reproducibility of the effects of neuromuscular blocking drugs injected at different times during the course of such experiments, which lasted for a maximum of 8 h. At the end of the experiments in cats, logarithmically increasing doses of the compounds were injected at 200-s intervals until block of the vagal and nictitating membrane responses was obtained. The time interval of 200 s was chosen as a compromise between the generally faster action of compounds of this type on the skeletal muscles than on the cardiac vagus neuroeffector junction, with the action on the nictitating membrane often being slower still. The experiments were designed to obtain the maximum amount of comparable information from each animal.

From the data, dose-inhibition graphs were constructed using a non-linear iterative curve-fitting routine (Levenberg–Marquadt method) and the doses producing 50% block of the responses to stimulation of the tibialis anterior and soleus muscles, the nictitating membrane and the vagus nerve were calculated. Time courses were measured from doses that produced neuromuscular block of 85–95%. Onset time was measured as time from injection to the first maximally depressed twitch. The recovery time was time from 75% block to 25% block, and the duration of action was time from injection to recovery to 90% of control twitch height in both anaesthetized cats and pigs. In some experiments, a dose equivalent to three times the ED₉₀ was injected towards the end of the investigation to evaluate the effects of clinically relevant higher doses. In one series of experiments, the effects of ED₉₀ and three times the ED₉₀ twitch-blocking doses of Org 9426 were tested on arterial pressure and heart rate in the absence of vagal or sympathetic stimulation. Results in human mixed fibre-type muscles would be expected to lie between those measured in the appropriate animal fast and slow contracting muscles.

Ease of antagonism of neuromuscular block produced by Org 9426 and vecuronium by the anticholinesterase neostigmine was assessed during steady state block maintained by continuous infusion. Bolus doses of the blocking drugs sufficient to produce approximately 90–95% twitch block on the cat soleus muscle were injected initially and this was maintained for at least 15 min by infusion. Incremental doses of neostigmine (10, 10, 20, 40 µg kg⁻¹) were then injected at 2–3 min intervals until a maximum level of recovery was seen.

**Statistical analysis**

Time course data were analysed by one-way analysis of variance and groups discriminated by the Tukey–Kraymer HSD test, to allow for multiple comparisons [3]. Cardiovascular changes were compared with values obtained before administration of drug and tested for significant difference using Student’s paired t test.

**Isolated Nerve–Muscle Preparations**

**Tension recording**

Biventer cervicis nerve–muscle preparations from young chickens (3–10 days) and phrenic nerve–hemidiaphragm preparations from Sprague–Dawley rats (200–250 g) were dissected and mounted at 32 °C in Krebs solution of the following composition (mmol litre⁻¹): NaCl 118, KCl 5, CaCl₂ 2.5, NaHCO₃ 30, KH₂PO₄ 1, MgSO₄ 1, glucose 11 and of pH 7.4 when aspirated with 5% carbon dioxide in oxygen. The motor nerves were stimulated at a frequency of
ORG 9426—a new muscle relaxant

0.1 Hz with rectangular pulses of 0.2 ms duration and a voltage greater than that required to produce maximal twitches. Tension responses were recorded on a chart recorder by Grass FT03C force displacement transducers. Concentrations of blocking drugs sufficient to produce approximately 80% twitch block were allowed to equilibrate with the tissues for 30 min before their ease of antagonism by neostigmine \(5 \times 10^{-7} \text{ mol litre}^{-1}\) was assessed.

Intracellular recording

Microelectrode recordings were obtained from the phrenic nerve–hemidiaphragm preparation of the rat and from the costocutaneous nerve–muscle preparation of the North American garter snake \((Thamnophis sirtalis)\). The latter preparation is particularly suitable for voltage clamp recording from the neuromuscular junction because it possesses large diameter fibres which aid visualization and penetration of endplates. This, coupled with compact endplates, allows good control of membrane voltage over the entire endplate region when using the two-microelectrode voltage clamp technique.

In order to record evoked endplate currents (EPC) without accompanying muscle contraction, cut fibre preparations were used. Dissection and cutting of muscle fibres was performed in low K+ (2 mmol litre\(^{-1}\)) physiological solution perfused for approximately 30 min. Snake nerve–muscle preparations were mounted in a physiological salt solution of pH 7.1–7.2 containing (mmol litre\(^{-1}\)): NaCl 159; KCl 4.2; CaCl\(_2\) 1.5; MgCl\(_2\) 4.2; HEPES 1.0. Rat nerve–muscle preparations were mounted in Krebs solution of the same composition as that used for the tension experiments. The muscles were mounted in a Sylgard-coated Perspex dish and endplates were voltage clamped using glass capillary microelectrodes (resistance 2–10 M\(\Omega\)). Voltage recording electrodes were filled with potassium chloride 3 mol litre\(^{-1}\) and current passing electrodes were filled with potassium sulphate 0.6 mol litre\(^{-1}\) [4].

The nerves were stimulated through platinum electrodes at a frequency of 0.5 Hz with rectangular pulses of 0.05 ms duration and of strength sufficient to produce EPC. EPC were filtered by a 5-kHz low pass filter and recorded on magnetic tape (Racal 4DS bandwidth DC-5kHz). The currents were amplified and digitized by a Cambridge Electronic Design 502 laboratory interface connected to a DEC PDP 11/23 computer at a digitization rate of 25 kHz. Ten to 20 EPC were collected and averaged after alignment at the middle of their rising phase. EPC decayed as a single exponential function according to the following relationship:

\[ I(t) = I(0) \exp^{-\frac{t}{\tau}} \]

where \(I(t)\) is the current amplitude at time \(t\) after the peak, \(I(0)\) is the peak current amplitude and \(\tau\) is the decay time constant (calculated by computer from the reciprocal of the slope of the least squares regression line fitted to the semi-logarithmic plot of amplitude between 5% and 95% of the peak against time). EPC with a 10–90% rise time greater than 0.4 ms were discarded and only those with a voltage deviation of less than 1% of the driving force (holding potential–reversal potential) accepted. Experiments were carried out at room temperature (20–22 °C). Drug solutions were perfused through the tissue bath for 10 min by peristaltic pump, ensuring a complete change of solution.

RESULTS

Anaesthetized Cats and Pigs

General activity

The new compound Org 9426 was compared with vecuronium in anaesthetized cats and pigs and with its potential metabolite Org 9943 in cats. None of the compounds produced muscle fasciculations or pre-block twitch augmentation.

Neuromuscular blocking potency and selectivity of action

In anaesthetized cats, the selectivity of the compounds for the neuromuscular junction was tested by assessing their potency in producing block at the neuromuscular junction, at the cardiac vagus neuroeffector junction and at a sympathetic ganglion. The effects of approximately equieffective 90% twitch blocking doses of Org 9426, Org 9943 and vecuronium are shown in figure 2. At neuromuscular blocking doses, Org 9426 produced only a small effect on the chronotropic responses to vagal stimulation and no effect on the responses of the nictitating membrane to preganglionic stimulation. Equieffective neuromuscular blocking doses of vecuronium produced no effect on either vagal or nictitating membrane responses, but the very large doses of Org 9943 required to produce neuromuscular block also
produced vagal block and block of the nictitating membrane (fig. 2).

In anaesthetized pigs, both Org 9426 and vecuronium were less potent neuromuscular blockers than in cats. This was particularly so with the soleus muscles (table I). In the pig, Org 9426 exhibited a small reduction in the chronotropic response to vagal stimulation at neuromuscular blocking doses.

Doses of the drugs producing 50% block of

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**Table I. Neuromuscular, vagal and ganglion blocking potencies of Org 9426 and its possible 17-hydroxy metabolite Org 9943, compared with vecuronium, in anaesthetised cats and pigs. ED$_{50}$ values are mean (SEM) [No. of experiments]. † Ratio (the margin of safety between the two effects at ED$_{50}$) =

<table>
<thead>
<tr>
<th></th>
<th>Tibialis</th>
<th>Soleus</th>
<th>Vagus</th>
<th>Nictitating membrane</th>
<th>Ratio †</th>
</tr>
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<tbody>
<tr>
<td><strong>Cat</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Org 9426</td>
<td>153 (13)</td>
<td>185 (18)</td>
<td>1329 (232)</td>
<td>4119 (561)</td>
<td>7.2</td>
</tr>
<tr>
<td>Org 9943</td>
<td>3188 (794)</td>
<td>3453 (299)</td>
<td>1985 (315)</td>
<td>5947 (948)</td>
<td>0.30</td>
</tr>
<tr>
<td>Vecuronium</td>
<td>31 (5)</td>
<td>26 (5)</td>
<td>1985 (315)</td>
<td>5947 (948)</td>
<td>76</td>
</tr>
<tr>
<td><strong>Pig</strong></td>
<td></td>
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</tr>
<tr>
<td>Org 9426</td>
<td>439 (11)</td>
<td>986 (30)</td>
<td>1935 (236)</td>
<td>&gt;2400</td>
<td>4.4</td>
</tr>
<tr>
<td>Vecuronium</td>
<td>78 (2)</td>
<td>214 (21)</td>
<td>2352 (372)</td>
<td>&gt;6400</td>
<td>30</td>
</tr>
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Fig. 2. Anaesthetized cat. Effects of Org 9426 400 µg kg$^{-1}$, Org 9943 3400 µg kg$^{-1}$ and vecuronium 55 µg kg$^{-1}$ on arterial pressure (AP), heart rate (HR), contractions (upward deflections) of the nictitating membrane (NIC pre) to pre-ganglionic nerve stimulation (2–5 Hz per 10 s every 100 s), and maximal contractions of the tibialis anterior and soleus muscles evoked by stimulation of motor nerves (0.1 Hz). The right vagus nerve was stimulated every 100 s for 10 s at 5–10 Hz to produce decreases in the heart rate (downward deflections of the HR trace). Induced decreases in heart rate were associated with concomitant decreases in arterial pressure.
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Table II. Time course of neuromuscular block in the anaesthetized cat following the injection of approximate ED$_{50}$ blocking doses of Org 9426, Org 9943 and vecuronium (mean (SEM) [No. of experiments])

<table>
<thead>
<tr>
<th>Dose (µg kg$^{-1}$)</th>
<th>Tibialis</th>
<th>Soleus</th>
<th>Onset (min)</th>
<th>Tibialis</th>
<th>Soleus</th>
<th>Recovery 25–75% (min)</th>
<th>Tibialis</th>
<th>Soleus</th>
<th>Duration 90% (min)</th>
<th>Tibialis</th>
<th>Soleus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Org 9426</td>
<td>246 (27) [13]</td>
<td>311 (35) [12]</td>
<td>2.0 (0.1)</td>
<td>2.9 (0.3)</td>
<td>2.9 (0.3)</td>
<td>5.2 (0.5)</td>
<td>8.3 (0.7)</td>
<td>13.7 (1.2)</td>
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<td></td>
</tr>
<tr>
<td>Org 9943</td>
<td>4130 (691) [5]</td>
<td>4760 (375) [5]</td>
<td>2.4 (0.2)</td>
<td>3.0 (0.5)</td>
<td>3.0 (0.5)</td>
<td>4.5 (0.4)</td>
<td>9.3 (0.9)</td>
<td>13.6 (1.1)</td>
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</tr>
<tr>
<td>Vecuronium</td>
<td>39.7 (6.8) [7]</td>
<td>38.1 (6.7) [8]</td>
<td>4.3 (0.3)</td>
<td>5.0 (0.2)</td>
<td>3.0 (0.2)</td>
<td>5.2 (0.4)</td>
<td>10.1 (0.7)</td>
<td>17.4 (1.5)</td>
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</table>

Table III. Time course of neuromuscular block in the anaesthetized pig following the injection of approximate ED$_{50}$ and three times ED$_{50}$ doses of Org 9426 and vecuronium (mean (SEM))

<table>
<thead>
<tr>
<th>Dose (µg kg$^{-1}$)</th>
<th>Tibialis</th>
<th>Soleus</th>
<th>% Block</th>
<th>Tibialis</th>
<th>Soleus</th>
<th>Onset (min)</th>
<th>Tibialis</th>
<th>Soleus</th>
<th>Recovery 25–75% (min)</th>
<th>Tibialis</th>
<th>Soleus</th>
<th>Duration 90% (min)</th>
<th>Tibialis</th>
<th>Soleus</th>
</tr>
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<tbody>
<tr>
<td>ED$_{90}$</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Org 9426</td>
<td>696 (19)</td>
<td>1675 (60)</td>
<td>89 (2)</td>
<td>87 (1)</td>
<td>1.8 (0.1)</td>
<td>3.1 (0.2)</td>
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<tr>
<td>Vecuronium</td>
<td>153 (16)</td>
<td>410 (76)</td>
<td>90 (1)</td>
<td>86 (1)</td>
<td>1.8 (0.1)</td>
<td>3.2 (0.2)</td>
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<tr>
<td>3 × ED$_{90}$</td>
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</tr>
<tr>
<td>Org 9426</td>
<td>2237 (82)</td>
<td>—</td>
<td>100</td>
<td>—</td>
<td>0.8 (0.1)</td>
<td>—</td>
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</tr>
<tr>
<td>Vecuronium</td>
<td>422 (9)</td>
<td>—</td>
<td>100</td>
<td>—</td>
<td>0.8 (0.1)</td>
<td>—</td>
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<table>
<thead>
<tr>
<th></th>
<th>Tibialis</th>
<th>Soleus</th>
<th>Duration (min)</th>
<th>Tibialis</th>
<th>Soleus</th>
<th>Duration of complete block, tibialis (min)</th>
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<tbody>
<tr>
<td>ED$_{90}$</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Org 9426</td>
<td>3.3 (0.2)</td>
<td>5.7 (0.6)</td>
<td>9.9 (0.3)</td>
<td>16.8 (1.6)</td>
<td>—</td>
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<tr>
<td>Vecuronium</td>
<td>3.5 (0.4)</td>
<td>5.9 (1.6)</td>
<td>9.7 (0.8)</td>
<td>18.6 (4.8)</td>
<td>—</td>
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</tr>
<tr>
<td>3 × ED$_{90}$</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Org 9426</td>
<td>5.2 (0.2)</td>
<td>—</td>
<td>23.3 (1.2)</td>
<td>—</td>
<td>9.5 (0.6)</td>
<td></td>
</tr>
<tr>
<td>Vecuronium</td>
<td>5.1 (0.5)</td>
<td>—</td>
<td>22.0 (1.4)</td>
<td>—</td>
<td>7.9 (0.3)</td>
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</table>

each of the measured responses were calculated from dose-inhibition plots (table I). It may be seen that, depending on the muscle and animal, Org 9426 is three to six times less potent than vecuronium as a neuromuscular blocker. Org 9426 was slightly more potent than vecuronium in blocking the responses to either vagal or pre-ganglionic sympathetic stimulation. As a result, the margins between the neuromuscular blocking effects and the unwanted vagal and ganglion blocking actions were less than those for vecuronium; for example, the vagal: neuromuscular block ratio for Org 9426 was one-seventh (pig) to one-tenth (cat) that of vecuronium.

The neuromuscular blocking potency of Org 9943 was approximately 20% that of Org 9426, but the vagal and ganglion blocking potencies were similar to those of Org 9426. In the case of the vagal block, the 50% vagal blocking dose was less than the 50% neuromuscular blocking dose, resulting in a vagal: neuromuscular block ratio of less than 1 (table I).

Time course of neuromuscular block

The time courses of the neuromuscular blocks produced by Org 9426, Org 9943 and vecuronium were compared in anaesthetized cats at approximately 90% twitch block (table II). Those of Org 9426 and Org 9943 were similar, with onset times significantly faster than that of vecuronium. However, the recovery times of the three compounds were not significantly different and the durations of action of Org 9426 and Org 9943 were only slightly shorter than that of vecuronium.

In the anaesthetized pig, which was used primarily to assess duration of action, doses of Org 9426 and vecuronium producing 90% block...
of the tibialis and soleus muscles were used, with three times the 90% blocking dose for the tibialis muscle. At the 90% blocking doses, the onset, recovery and duration times for Org 9426 in the pig were not significantly different from those of vecuronium (table III). At three times 90% blocking doses the recovery times of both Org 9426 and vecuronium increased by approximately 50%, while the duration was more than doubled (table III).

Effects on tetanus and train-of-four

Both Org 9426 and vecuronium produced a reduction of peak tetanic tension in anaesthetized pigs. This effect was accompanied by a rapid waning of tension (fade) during the period of tetanic stimulation. Post-tetanic augmentation of single twitch height occurred in the presence of both neuromuscular blockers (fig. 3) and both compounds produced train-of-four fade (fig. 4). In these experiments, the mean (SEM) T4:T1 ratios obtained at 25, 50, 75 and 90% recovery of T1 were 0.22 (0.03), 0.53 (0.02), 0.81 (0.02) and 0.97 (0.02) for Org 9426 and 0.21 (0.04), 0.46 (0.03), 0.78 (0.03) and 0.96 (0.01) for vecuronium (n = 4).

Ease of antagonism of neuromuscular block

The effects of both Org 9426 and vecuronium were antagonized well by neostigmine. Mean doses of neostigmine producing 50% antagonism, calculated from dose–recovery plots, were 14 (SEM 2) μg kg\(^{-1}\) (n = 4) in the case of Org 9426 and 19 (4) mg kg\(^{-1}\) (n = 3) after vecuronium.

Effects on heart rate and arterial pressure

In the cat, the effects of 90% twitch blocking doses of Org 9426 and three times this dose were examined on arterial pressure and heart rate in the absence of the intermittent vagal stimulation used in most of the experiments described. Org 9426 did not significantly affect heart rate at either of the two doses used (137 (11) to 141 (11) beat min\(^{-1}\) (n = 6) for ED\(_{90}\) dose; 144 (8) to 145 (9) beat min\(^{-1}\) (n = 6) for three times ED\(_{90}\) dose). The 90% blocking dose had no effect on mean arterial pressure (87 (16) to 89 (15) mm Hg), but the three times 90% blocking dose produced a slight but significant (P < 0.05) hypotension (from 85 (11) to 77 (11) mm Hg). At the very large doses required to produce neuromuscular block, Org 9943 produced a transient increase in arterial pressure (fig. 2).
TABLE IV. Concentrations of Org 9426 and vecuronium required to produce 80–85% block of the indirectly-elicited twitches of the isolated chick biventer cervicis and rat hemidiaphragm preparations, and the ease of antagonism of the neuromuscular block by neostigmine $5 \times 10^{-5}$ mol litre$^{-1}$. $n = \text{Number of experiments}$

<table>
<thead>
<tr>
<th>Chick biventer cervicis</th>
<th>Rat hemidiaphragm</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Drug concn (mol litre$^{-1}$)</strong></td>
<td><strong>Antagonism (%)</strong></td>
</tr>
<tr>
<td>Org 9426</td>
<td>$8.4 \times 10^{-7}$ ($n = 5$)</td>
</tr>
<tr>
<td></td>
<td>$1.6 \times 10^{-5}$ ($n = 4$)</td>
</tr>
</tbody>
</table>

$(n = 6)$. At three times the 90% twitch blocking dose, arterial pressure increased from 86 (7) to 93 (8) mm Hg and then decreased to 81 (7) mm Hg $(n = 6)$; the change in heart rate was from 120 (5) to 128 (7) beat min$^{-1}$. None of these changes was significant.

**Isolated Nerve-Muscle Preparations**

**Tension recording**

Both Org 9426 and vecuronium reduced the twitch response to single shock nerve stimulation in the chick biventer cervicis muscle preparation and in the rat phrenic nerve-hemidiaphragm preparation. In the chick preparation the neuromuscular block was not accompanied by an increase in the baseline tension of the preparation. For both Org 9426 and vecuronium, the concentration required to produce 80–90% twitch block under equilibrium conditions was much greater (60 times for vecuronium, 19 times for Org 9426) in the rat hemidiaphragm than in the chick biventer cervicis (table IV).

In both rat and chick preparations, neostigmine $5 \times 10^{-7}$ mol litre$^{-1}$ produced antagonism of the effects of both Org 9426 and vecuronium (table IV).

**Intracellular recording**

The effects of Org 9426 were studied at concentrations that produced an approximate 80% reduction of endplate current (EPC) amplitude in rat and snake muscle. As in the tension studies described above, there was a considerable species difference in the potency of Org 9426, which was 15 times more potent in the snake preparation than in the rat preparation.

In the snake preparation Org 9426 $(4 \times 10^{-7}$ mol litre$^{-1}$ reduced peak EPC amplitude by 84% at $-90$ mV. The effects of the drug were studied over a range of membrane potentials from $-30$ to $-130$ mV (fig. 5A, B). The percentage reduction in EPC amplitude was the same at all the holding potentials tested—that is, the reduction in amplitude was not voltage-dependent. At $-90$ mV, Org 9426 produced a slight but insignificant increase in the time constant of decay ($\tau$) of EPC, from 2.1 (0.3) to 2.3 (0.2) ms.

In the rat preparation Org 9426 $6 \times 10^{-6}$ mol litre$^{-1}$ reduced peak EPC amplitude by 83% at $-90$ mV. In this preparation, $\tau_{EPC}$ was reduced to
64% of control at \(-90\,\text{mV}\) but this decrease was not voltage-dependent. In the absence of Org 9426, control EPC were not well fitted by a single exponential function (fig. 5c), whereas in the presence of the drug the exponential fit was good (fig. 5d).

**DISCUSSION**

**Mechanism of action**

No evidence for a depolarizing mechanism of action of Org 9426 was found in the anaesthetized cat or pig. Thus the fasciculations and pre-block twitch augmentation associated with such a mechanism were absent, and tetanic and train-of-four fade were observed. In addition, no contracture was seen in the multiple-innervated fibres of the chick biventer cervicis muscle, which responds to depolarizing drugs with a sustained contracture [5]. We conclude that Org 9426 blocks neuromuscular transmission by a non-depolarizing mechanism of action.

Org 9426 produced no significant effect on \(\tau_{EPC}\) in snake muscle, indicating a lack of non-competitive endplate ion channel blocking activity that has been reported for other neuromuscular blockers including tubocurarine [6], pancuronium [7], gallamine [8] and other steroidal analogues [9, 10]. The measured reduction of \(\tau_{EPC}\) in rat muscle was similar to that reported for vecuronium and tubocurarine [10, 11] and the lack of voltage-dependence of this reduction makes a channel blocking mechanism unlikely. Such a non-voltage-dependent reduction of \(\tau_{EPC}\) has been ascribed to a reduction of repetitive binding of acetylcholine—that is, a postjunctional receptor blocking action of the drugs [11, 12]. The better exponential fit of the EPC in the presence of Org 9426 than in its absence is consistent with this receptor blocking mechanism.

**Profile in anaesthetized animals**

In addition to the reversible non-depolarizing mechanism of action discussed above, we set several criteria pertaining to the profile of any new compound in anaesthetized animals. In terms of neuromuscular blocking potency, Org 9426 falls within the acceptable range, being much greater than 10% of the potency of vecuronium in both the species tested. The neuromuscular blocking potency of Org 9426 is similar to that of atracurium [13, 14]. The vagal:neuromuscular block ratio found for Org 9426 was much lower than that for vecuronium, the latter value confirming results from previous studies [14-16] which show that vecuronium has a high vagal ratio. Compounds with lower vagal ratios (in the region of 14-25), such as dimethyltubocurarine [13, 17] and atracurium [14, 18], have been shown in man to be free of cardiovascular side effects associated with vagal block [19-21]. Thus our use of vecuronium represents an especially stringent standard for neuromuscular/vagal block selectivity. From comparison of the vagal ratio of Org 9426 with those of other compounds tested in this way [13, 22, 23], it might be expected that the vagal ratio of Org 9426 would fall between those of drugs such as pancuronium and alcuronium, use of which in man has been associated with a mild degree of tachycardia, and drugs such as dimethyltubocurarine and atracurium. In anaesthetized animals, vagal tone is less than in humans and hence vagal block produces only small effects on heart rate. However, any sympathetically-mediated tachycardia, for example, noradrenaline re-uptake block, would be expected to be manifest in animal experiments. In the present experiments only a very small increase in heart rate was seen in the pig. Ganglion block required much higher doses of Org 9426 than those required to produce vagal block, and the ratio between ganglion and neuromuscular blocking activity was approximately 20. Thus it is unlikely that this action will be important in the overall cardiovascular profile of the drug. The small reduction in arterial pressure noted in the cat at three times the 90% twitch blocking dose was apparently not the result of ganglion block, as it was not accompanied by an effect on the nictitating membrane. Histamine release is not associated with this series of compounds [24]. A direct effect on the smooth muscles of the blood vessels may be more likely, but in any case the effect appeared to be too small to be important, and was not seen in the pig.

We also studied Org 9943, which is the 17-hydroxy derivative of Org 9426, and hence its potential metabolite. The 3-position of Org 9426 is not a potential point of immediate metabolism and it would be expected that only one hydrolysis product would be produced. The 3-hydroxy group of Org 9426 confers stability in aqueous solution at physiological pH and hence the compound has the potential to be formulated as a ready-to-use preparation. The results indicate that it is highly unlikely that the production of the...
metabolite would contribute to the neuromuscular block produced by Org 9426 or that there would be cardiovascular side effects or residual neuromuscular block attributable to the metabolite. In addition, from cat studies to date there is no evidence that Org 9943 is produced in measurable amounts after the administration of Org 9426 [R. D. Miller, 1988, personal communication].

The major objective of the work was to identify a compound with duration of action similar to that of vecuronium or atracurium, but with a substantially faster onset time. If the animal results are substantiated in man, the use of such a drug might allow tracheal intubation to be performed with this compound at lower multiples of the ED$_{90}$ dose than those necessary with such agents as vecuronium or atracurium, thus resulting in an overall shorter duration of action and obviating the need for use of the depolarizing drug, suxamethonium. We used the anaesthetized cat as a predictor of onset time and autonomic effects described above because the onset times of other pancuronium analogues that have been tested in both cats and humans, including pancuronium [16, 25], vecuronium [14, 16, 26], dacuronium [16, 25] and Org 6368 [27; W. L. M. Baird, personal communication], have shown a reasonable correlation between the two species. However, the cat has not proved a good predictor of the duration of action of all of the above compounds. In contrast, the pig has been a good predictor of duration of action and of cardiovascular effects [28], but is less useful than the cat in predicting onset times; for example, in the pig, vecuronium has a faster onset time than does pancuronium [28].

In the cat, we found that the onset time of Org 9426 was approximately 50% that of vecuronium and was similar to those obtained in a previous study with suxamethonium and some short duration aminosteroids [1]. However, recovery and duration values for Org 9426 were similar to those for vecuronium in both the cat and the pig. Thus the overall time course profile of Org 9426 is that of a drug of medium duration of action, similar to vecuronium or atracurium, but with a faster onset of action. We believe that if this rapid onset is also seen in humans, Org 9426 represents a potential advance in the field, as other recently introduced compounds including vecuronium and atracurium, and the newer compounds mivacurium [29] and doxacurium [30] require large doses to achieve satisfactory intubation conditions rapidly.

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