A method for studying the pharmacodynamic profile of neuromuscular blocking agents on vocal cord movements in anaesthetized cats

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1 A new *in vivo* experimental method is described whereby the neuromuscular blocking effects of muscle relaxants can be investigated on the intrinsic laryngeal muscles of anaesthetized cats. The peripheral tibialis anterior muscle preparation is employed in the same animal to compare the blocking effect on both preparations.

2 The intrinsic laryngeal muscles react with different sensitivities to the neuromuscular blocking agents when compared to the tibialis anterior muscle.

3 The neuromuscular response in both muscle preparations is similar with steroidal agents but appeared to be different after suxamethonium or isoquinoline analogues.

4 It is concluded that this preparation may become a useful tool for studying new muscle relaxants developed to facilitate rapid intubation conditions.

Keywords: Larynx; muscle relaxants; skeletal muscle; vocal cords

Introduction

Following the introduction of (+)-tubocurarine in 1942 (Griffith & Johnson, 1942), several new neuromuscular blocking agents have been developed and are commonly used in clinical anaesthetic practice. Knowledge of the neuromuscular blocking effects of these agents in man is primarily based on measurements of twitch tension of the indirectly stimulated adductor pollicis muscle. However, it has been known for many years that different muscles in animals (Taylor *et al.*, 1964; Day *et al.*, 1982) and in man (Johansen *et al.*, 1964; Ali *et al.*, 1975; Derrington & Hindocha, 1988; Laycock *et al.*, 1988) differ in sensitivity towards the neuromuscular blocking effects of muscle relaxants. In addition, it has been reported that the magnitude and time-course of action of muscle relaxants differ significantly between the intrinsic laryngeal muscles and peripheral skeletal muscles (Gabriel, 1975; Baer, 1984). This may explain intubation difficulties and complications encountered during upper airway surgery (Baer & Pukander, 1982; Sia *et al.*, 1982). One of the objectives in the development of new neuromuscular blocking agents is that the 'ideal' relaxant should have a fast onset to facilitate rapid intubation (Savarese & Kitz, 1975). Therefore, we have developed a method in the cat, which allows the study of the neuromuscular blocking effects of muscle relaxants simultaneously on the (peripheral) tibialis anterior muscle and on vocal cord movement, the latter evoked by indirect stimulation of the intrinsic laryngeal muscles.

Methods

General

Adult cats (male and female, 2 to 4 kg body weight) were anaesthetized with pentobarbitone sodium (Nembutal) 40 mg kg⁻¹ intraperitoneally and, after cannulating the cephalic vein, an infusion with pentobarbitone sodium was started at a rate of 4.8 mg kg⁻¹ h⁻¹. Heart frequency was monitored with a BT-1200 Biotachometer and arterial blood pressure was measured by a Gould Statham P23Db pressure

transducer via a cannula (Angiocath 18G) inserted in a femoral artery. Another cannula was placed in the saphenous vein and was used for the administration of the muscle relaxant under study. The lungs were ventilated by means of a V5KG ventilator (Narco-Biosystems Inc.) using a tidal volume of room air of 15 ml kg⁻¹ at a frequency of 20 min⁻¹. End tidal *P*CO₂ was measured by a Capnograph Mark II and kept at 3.5–4.5 kPa. Rectal temperature was continuously measured and maintained at 37 ± 0.5 °C with a heated water mattress placed underneath the supine cat.

Peroneal nerve-tibialis anterior muscle preparation

The twitch tension of the tibialis anterior muscle, elicited by supramaximal square wave stimuli of 0.2 ms duration applied to the common peroneal nerve at a frequency of 0.1 Hz using a Grass S88 stimulator, was recorded with a Gould Statham UTC3 force displacement transducer and a physiopolygraph Varioscript 443 (Schwarzer) recorder.

Recurrent laryngeal nerve-vocal cord preparation

A median skin incision was made just caudally from the cricoideal cartilage towards the fossa jugularis. The sternomastoideal muscle was bisected along the media raphe to allow access to the underlying sternothyroideal muscles. These muscles were divided longitudinally along the junction of both muscle bellies and were pulled laterally, together with the underlying sternothyroideal muscles, exposing the trachea. A tracheotomy was performed approximately 3 to 4 cartilageal rings caudal of the cricoideus, and a Portex tracheal tube (4.5 or 5.0 mm internal diameter) was inserted and positioned with two ligatures. The right recurrent laryngeal nerve was carefully loosened from its surrounding tissues and attached to the stimulus electrodes, made of two U-shaped silver wires (diameter 0.6 mm), 8 mm apart. A sterile, inert non-conducting silicon oil was applied to the wound in order to prevent desiccation. Direct stimulation of the nuchal and laryngeal muscles was also prevented in this way. The nerve was stimulated supramaximally with square wave pulses of 0.2 ms duration and a frequency of 0.1 Hz using the apparatus described above.

The head of the cat was fixed and the jaws stretched by placing an oval metal ring (2.5 × 4 cm) behind the eyeteeth. A curved, pointed metal blade was introduced into the mouth,

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Figure 1 Schematic presentation of the laryngeal nerve-vocal cord preparation: (1) force transducer; (2) little hook (see text); (3) endotracheal tube; (4) stimulation electrodes; (5) muscle relaxation monitor; (6) recorders; (a) supraglottal area; (b) vocal cords; (c) trachea; (d) recurrent laryngeal nerve.

gently elevating the epiglottis ventrally and, hence, visualising the vocal cords. A force displacement transducer (Strain Measurement Devices Ltd., series 100; 0.5 N range) was modified by fixing a small, sharp, pointed hook at its end. This transducer was introduced through the mouth and the pharynx, and the hook was connected to the right vocal cord to quantify the abduction force evoked by right recurrent laryngeal nerve stimulation. A schematic presentation of the preparation is shown in Figure 1.

Experimental procedure

Both vocal cord and tibialis preparations were employed in the same animal to allow simultaneous measurement of the neuromuscular blocking effects of the administered muscle relaxants. In preliminary studies the doses of drugs producing approximately 75% depression of the single twitch height of the tibialis anterior muscle were ascertained (Table 1). After

intravenous injection of suxamethonium, (+)-tubocurarine, atracurium, vecuronium or pancuronium, the maximum block was measured, defined as the percentage of maximal depression of the single twitch height compared to the control twitches. Also, the onset time (the time from the end of injection to the attainment of maximum block) and the total duration (the time from the end of injection to the time when 90% recovery of the twitch height was achieved) were measured in both muscles after each administration of a muscle relaxant. Each muscle relaxant was given 3 times and all administered to a different cat.

Results

In these animals, heart rate was approximately 200 beats per minute. The mean arterial blood pressure was always greater than 120 mmHg throughout the experiments. In addition, the end-tidal PCO_2 and central temperature were stable. Both the peroneal nerve-tibialis anterior muscle and laryngeal nerve-vocal cord preparations appeared to be stable for at least 6 h in pilot studies. No signs of fatigue were observed in either muscle during that period.

The maximum blockade, the onset time and the total duration time for suxamethonium, (+)-tubocurarine, atracurium, pancuronium and vecuronium are presented in Table 1. Figure 2 shows results obtained with (+)-tubocurarine and pancuronium.

Following the administration of suxamethonium, (+)-tubocurarine or atracurium, the maximum blockade of the intrinsic laryngeal muscle was significantly less when compared to the tibialis anterior muscle. In addition, both the onset time and the total duration were shorter for the vocal cord paralysis. Because in one cat the maximal degree of muscle paralysis in the vocal cord preparation amounted to 10% after administration of suxamethonium, the duration could not be calculated and, therefore, only two duration times are listed in Table 1.

No differences were found between the preparations for the maximum blockade following injection of pancuronium and vecuronium. Both the onset time and duration were shorter in the vocal cord preparation for both drugs.

Discussion

Semiquantitative measurements of the neuromuscular blocking effects of the depolarizing muscle relaxant suxamethonium in man (Gabriel, 1975; Baer, 1984) has shown that the laryngeal muscles recover earlier than peripheral arm muscles. In addition, Gilly *et al.* (1986) and Streinzer *et al.* (1986) have attempted to quantify the laryngeal muscle relaxation in patients by electromyographic (EMG) recording of evoked responses elicited by surface electrodes stimulating the laryngeal nerve. They showed that, following a bolus dose of

Table 1 Neuromuscular blocking effects on the tibialis anterior and intrinsic laryngeal muscles in the cat, following approximately equi-effective doses of five routinely used muscle relaxants

Drug	Dose ($\mu\text{g kg}^{-1}$)	Max. block (mean) (%)					Onset (mean) (min)				Duration (mean) (min)			
Suxamethonium	30 T	66	66	74	(69)		1.3	1.5	1.9	(1.6)	3.5	3.7	4.2	(3.8)
	V	10	17	22	(16)		0.4	0.9	1.0	(0.8)	1.0	1.3		(1.2)
(+)-Tubocurarine	200 T	83	83	89	(85)		1.7	1.9	2.7	(2.1)	9.6	11.9	15.6	(12.4)
	V	23	23	25	(24)		1.5	1.7	2.4	(1.9)	5.2	5.5	7.6	(6.1)
Atracurium	75 T	74	80	91	(82)		2.4	2.7	3.8	(3.0)	8.9	8.9	9.0	(8.9)
	V	11	34	40	(29)		1.6	2.9	4.3	(2.9)	4.7	4.8	9.1	(6.2)
Vecuronium	20 T	69	74	91	(78)		3.7	4.0	4.7	(4.1)	9.7	10.4	13.7	(11.3)
	V	55	71	86	(71)		2.5	3.1	3.3	(3.0)	6.8	7.7	8.0	(7.5)
Pancuronium	15 T	43	65	85	(64)		6.8	4.8	4.1	(5.2)	15.4	12.9	26.9	(18.4)
	V	86	57	63	(69)		2.9	2.7	4.1	(3.2)	10.1	5.9	9.6	(8.5)

T = peroneal nerve-tibialis anterior muscle preparation. V = recurrent laryngeal nerve-vocal cord preparation.

Figure 2 Experimental recordings of contractions of cat laryngeal nerve-vocal cord preparation (a) and peroneal nerve-tibialis anterior muscle preparation (b) in response to 0.1 Hz stimulation following intravenous administration of (+)-tubocurarine (200 µg kg⁻¹) or pancuronium (15 µg kg⁻¹).

0.06 mg kg⁻¹ vecuronium, the vocal cords gave varying degrees of blockade, ranging from 61–92%. In contrast, the skeletal adductor pollicis muscle was nearly completely (97–100%) blocked. These findings support clinical observations (Sia *et al.*, 1982).

Movement of the vocal cords involves a complex combination of action of the intrinsic laryngeal muscles, controlled by, in part, the superior but predominantly, by the inferior laryngeal nerves. The evoked movement of the homolateral vocal cord, after stimulation of the inferior or recurrent laryngeal nerve, is the resultant of the summation of contractions of the various activated muscles. Inhibition of vocal cord movement, necessary during intubation, can only be successful if all muscles involved are paralysed. Simple extrapolation of neuromuscular blocking effects of muscle relaxants at the adductor pollicis muscle to the vocal cords, as is done empirically in clinical practice, should be done with great caution. Contraction of the adductor pollicis muscle is simple and differs markedly from the abduction of the vocal cords. In addition, there are different microanatomical and histological structures and types of innervation (Rossi & Cortesina, 1965; Johnson *et al.*, 1973), which result in different neurophysiological manifestations (Buchthal & Rosenfalck, 1955; Buchthal, 1959) between muscles. Furthermore, differences in muscle temperature and blood supply, presumably responsible for different pharmacokinetic and pharmacodynamic effects at the site of action, i.e. the neuromuscular junction, must be taken into account.

Due to the small number of experiments in the various groups, the data of this study have not been statistically analysed. These should only be viewed as an illustration of the technical feasibility of the execution of these experiments.

In our experiments we have shown that, using single twitch measurements, intrinsic laryngeal muscles and the tibialis anterior muscle of the cat respond differentially to the neuromuscular blocking effects of muscle relaxants *in vivo*. Suxame-

thonium causes a more pronounced blockade of peripheral tibialis anterior muscle. Baer (1984) and Gabriel (1975) had comparable findings in man. In clinical practice, however, suxamethonium is primarily used for the rapid facilitation of intubation and should block pharyngeal and intrinsic laryngeal neuromuscular transmission totally. The isoquinoline derivatives, (+)-tubocurarine and atracurium, produce more pronounced blockade of peripheral skeletal muscle. The steroidal compounds, pancuronium and vecuronium, produced a more comparable degree of blockade in both types of muscle, more or less comparable with those found by Gilly *et al.* (1986) and Streinzer *et al.* (1986) for man. Both the onset time and duration were shorter at the vocal cords for both compounds.

Kharkevich & Fisenko (1981) investigated the sensitivity of acetylcholine receptors of six different skeletal muscles to muscle relaxants in cats, and showed that the sequence of relaxation of different muscles was variable with drugs of different chemical structures, but was similar after administration of analogous compounds of the same chemical group. The results of their studies are in agreement with those obtained in our *in vivo* preparations, although they did not investigate the laryngeal muscles. An explanation for this phenomenon has not yet been presented. Apparently, one can assume the existence of differences in types, organisation, or sensitivities of acetylcholine receptors in various groups of muscles. Changes in temperature among muscles or differences in local blood flow may contribute to these pharmacodynamic alterations, but they cannot be fully responsible.

This laryngeal nerve-vocal cord preparation, in combination with the tibialis anterior (skeletal) muscle, may become a useful tool for investigation of new compounds with neuromuscular blocking activities, which should have a high affinity for acetylcholine receptors in the intrinsic laryngeal muscles, in order to develop new neuromuscular blocking agents for rapid facilitation of optimal intubation conditions.

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