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Differing potencies of muscle relaxants on rat and guinea-pig phrenic nerve diaphragm preparations

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The sensitivities of two in-vitro preparations to neuromuscular blocking agents have been compared. The guinea-pig phrenic nerve diaphragm preparation proved to be more sensitive to vecuronium, atracurium and pancuronium than the equivalent preparation from the rat. Only tubocurarine had a similar potency on the preparations from both species. This would suggest that the guinea-pig diaphragm would be the most appropriate bioassay preparation if only small quantities of drug were available. Small differences in the cholinesterase content of the preparations was not thought to be a likely reason for the differences between the two preparations.

The use of the rat phrenic nerve diaphragm preparation to assess neuromuscular blocking agents was described by Bulbring (1946). More recently the guinea-pig phrenic nerve diaphragm preparation has been used for this purpose (Healy & Palmer 1982). In the present study the sensitivity of the two preparations to a variety of neuromuscular blocking agents has been compared.

Methods

Rats and guinea-pigs were killed by a single blow on the head. Both phrenic nerve diaphragm preparations were dissected from Sprague-Dawley rats (200–250 g) or tricolour guinea-pigs (500–800 g) of either sex. The preparations were arranged for indirect stimulation by the application of rectangular pulses to the phrenic nerve (0·5 ms, 0·1 Hz and a voltage greater than that required to produce maximal stimulation).

The muscle was set up at a resting tension of 4 g and the whole preparation was immersed in Krebs-Henseleit solution at 37 °C and bubbled with 5% CO_2 in oxygen. Contractions of the muscles were recorded using an isometric transducer connected to a grass 79C Polygraph. After a loading dose and thorough washing, concentration-response curves were obtained using a randomized Latin Square sequence of drug administration. A 5 min contact time was used and at least a 20 min dose cycle. Separate preparations were used for each drug. Log EC50s were obtained on each preparation by calculating the best straight line by the method of least squares for % inhibition against log concentration for values between 16 and 84%. The neuromuscular blocking drugs were tubocurarine, suxamethonium chloride, atracurium besylate, vecuronium bromide and pancuronium bromide. Atracurium and vecuronium solutions were freshly prepared from powder and kept on ice. Atracurium solutions contained equimolar concentrations of ascorbic acid.

Some preparations were treated with 260 nM ecothiopate iodide which was prepared from the commercially available eye drops (Phospholine iodide). An equilibration time of 30 min was allowed with ecothiopate.

Measurement of cholinesterase inhibition. The cholinesterase activity of homogenates of rat phrenic nerve diaphragm preparations was assayed using the method described by Ellman et al (1961). Acetylthiocholine is hydrolysed by the cholinesterase in the tissue and the rate of hydrolysis is measured as the increase in yellow colour produced by the interaction of thiocholine with the dithiobisnitrobenzoate ion.

Results

In general, the guinea-pig was more sensitive to the neuromuscular blocking agents than the rat, the exception being tubocurarine (Table 1). The smaller EC50s for the drugs in the guinea-pig could not be attributed to the gross differences in the slope of the log dose-response curves as they were approximately parallel (Fig. 1). Only tubocurarine was approximately equipotent on the two preparations.

Assessment of cholinesterase activity demonstrated that the rate of hydrolysis of acetylthiocholine in the rat was slightly greater than in the guinea-pig. In contrast, the rate of hydrolysis of butyryl thiocholine was greater in the guinea-pig (Table 2). Since there were significant differences in cholinesterase activity between the two preparations it was decided to investigate whether irreversible inhibition of cholinesterase activity by ecothiopate affected the concentration-response relationships of neuromuscular blocking agents. On the rat preparation the inhibition of twitch tension produced by tubocurarine was antagonized as expected, but on the guinea-pig preparation inhibition of twitch tension was potentiated (Fig. 2). The effects of atracurium on the two preparations were unchanged by ecothiopate pretreatment.

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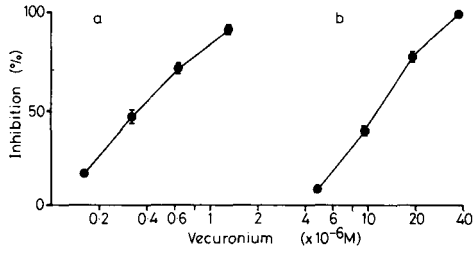

FIG. 1. Log concentration-response relationships for vecuronium bromide on the twitch tension produced by the guinea-pig (a) and rat (b) phrenic nerve diaphragm preparations. Results are means ± s.e.m., n= 8.

Table 1. A comparison of the sensitivity of the rat and guinea-pig diaphragms to a variety of neuromuscular blocking agents. Results are expressed as the geometric mean molar EC50 ± s.e.m.

	EC50 × 10^{-6} M ± s.e.m.		Potency ratio	
	Rat	Guinea-pig	Rat	Guinea-pig
Tubocurarine	1·5 (+0·20–0·18) n = 9	1·88 (+0·14–0·13) n = 9	1	1
Suxamethonium	15·1 (+0·33–0·32) n = 6	10·1 (+2·3–1·8) n = 9	10·06	5·37
Atracurium	15·5 (+0·80–0·76) n = 9	0·75 (+0·10–0·08) n = 9	10·33	0·40
Vecuronium	11·0 (+0·73–0·69) n = 8	0·37 (+0·07–0·04) n = 9	7·33	0·20
Pancuronium	4·965 (+0·47–0·44) n = 9	0·29 (+0·05–0·043) n = 7	3·31	0·15

Discussion

The guinea-pig diaphragm is more sensitive than the rat diaphragm to most neuromuscular blocking agents and thus it would be the most suitable tissue to use when small amounts of the agents are available, for example, bioassay of body fluids or testing of a novel compound. It is of interest that Bowman (1964) reported that the rat was particularly sensitive to tubocurarine but not to other neuromuscular blocking agents. Clearly the guinea-pig preparation is more sensitive to more compounds than that of the rat. Although we demonstrated some differences in the cholinesterase activity in the two preparations, they were not large enough to explain twenty-fold differences in sensitivity between the preparations. Unpublished experiments have shown that neostigmine, an effective anticholinesterase, antagonizes tubocurarine on both preparations. Thus the differential effect of ecothiopate on tubocurarine-induced neuromuscular blockade seen in this study was unhelpful. It is possible that the differences observed between the two preparations are due to differences in the receptor binding sites.

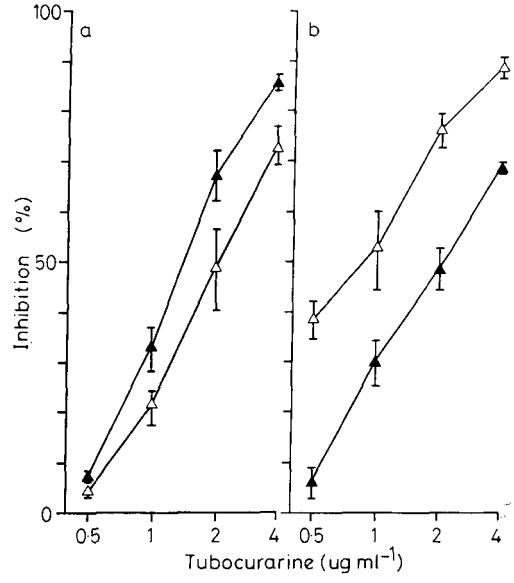

FIG. 2. Effect of pretreatment with 260 nM ecothiopate iodide on the inhibition of twitch tension produced by tubocurarine on the rat (a) and guinea-pig (b) phrenic nerve diaphragm preparations. Results are means ± s.e.m. of not less than 6 experiments: (△) control, (▲) ecothiopate pretreatment.

Table 2. The cholinesterase activity of rat and guinea-pig diaphragm muscle. Results are expressed as means ± s.e.m., $^*P < 0·05$.

	(Mol litre^{-1} min^{-1} (g tissue)$^{-1}$)	
	Rat	Guinea-pig
Rate of hydrolysis of acetylthiocholine	1·43 × 10^{-6} ± 0·06 (n = 12)	1·20 × 10^{-6} ± 0·08* (n = 11)
Rate of hydrolysis of butyrylthiocholine	0·06 + 10^{-6} ± 0·01 (n = 6)	0·24 ± 10^{-6} ± 0·03* (n = 10)

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