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Simultaneous Comparison of Nicotinic Receptor Antagonists on Three Nicotinic Acetylcholine Receptors

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Abstract

The relative potencies of several nicotinic cholinoceptor antagonists in producing tetanic fade and reduction of striated muscle contraction were investigated in the isolated guinea-pig oesophagus as well as the guinea-pig and rat phrenic nerve-diaphragm preparations. Contractile smooth muscle responses to vagal stimulation, which involves ganglionic activation, were also measured simultaneously with striated muscle responses in the oesophagus.

The relative potency for inhibiting the response of oesophageal smooth muscle to vagal stimulation (20 Hz) was trimetaphan > mecamylamine > hexamethonium > tubocurarine > pancuronium. For oesophageal striated muscle, production of tetanic fade at 100 Hz and reduction in peak tetanic tension at 20 or 100 Hz showed a similar relative potency; pancuronium > tubocurarine > mecamylamine > trimetaphan > hexamethonium and similar results were obtained in the guinea-pig diaphragm for the antagonists investigated (pancuronium, tubocurarine and mecamylamine). In the rat phrenic nerve-diaphragm preparation, production of tetanic fade at 50 Hz and reduction in twitch or tetanic tension all showed the relative potency; tubocurarine > pancuronium > mecamylamine > trimetaphan > hexamethonium.

These findings indicate differences in the nicotinic cholinoceptor subtypes involved in vagal ganglionic responses and those in tetanic fade.

The guinea-pig oesophagus contains striated muscle along its length with some smooth muscle in the distal region (Irwin 1931; Ingelfinger 1958; Goyal & Paterson 1989) and acetylcholine can activate both types of muscle (Thomas & Trounce 1960). Vagal stimulation has been reported to produce a biphasic response; an initial contraction of the striated muscle followed by a contraction of smooth muscle due to preganglionic nerve stimulation (Beveridge & Taylor 1987). The presence of both ganglionic and striated muscle nicotinic receptors in the same preparation provided an opportunity to compare simultaneously the effectiveness of nicotinic-receptor antagonists on responses mediated by these receptors and additionally, their potency in producing fade of tetanic responses. There is considerable discussion in the literature regarding the subtype of the receptors involved in tetanic fade and whether prejunctional nicotinic receptors on motor neurons to striated muscle are mediators of the response (Bowman et al 1984, 1988; Wessler 1988; Wilson et al 1995). Evidence from in-vivo experiments indicates that tetanic fade is more evident with low doses of ganglion-blocking drugs such as hexamethonium than with pancuronium (Bowman & Webb 1976), but the prejunctional nicotinic receptor appears to have different characteristics from the ganglionic nicotinic receptor in that tetanic fade is not readily induced by another ganglion blocking drug, trimetaphan (Gibb & Marshall 1984; Bowman et al 1988).

In view of the current interest in subtypes of nicotinic acetylcholine receptors, the sensitivities of several nicotinic receptor antagonists on two putative types of neuronal nicotinic cholinoceptors, ganglionic and prejunctional, as well as the nicotinic receptor on the striated muscle in the oesophageal preparation were evaluated. Experiments were also conducted on the phrenic nerve-diaphragm preparation, for comparison, as many previous studies on tetanic fade have involved this tissue (Gibb & Marshall 1986; Wessler et al 1986, 1987; Tian et al 1994).

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Materials and Methods

Guinea-pig isolated oesophagus

The oesophagus with attached vagi was dissected from the thoracic inlet to the stomach of male guinea-pigs as described by Beveridge & Taylor (1987). The oesophagus was cut longitudinally and set up under 1·5 g tension in modified Krebs-Henseleit solution (30 mL) of the following composition (mM): NaCl 116, KCl 5·4, $MgSO_4.7H_2O$ 0·6, $NaH_2PO_4.2H_2O$ 1·2, $NaHCO_3$ 25, glucose 11·1, $CaCl_2$ 2·5, gassed with 95% O_2–5% CO_2. The bath temperature was maintained at 30°C to reduce spontaneous activity. The two vagi were threaded through bipolar electrodes positioned 1 cm from the oesophagus. Transmural stimulation could also be applied via two electrodes placed either side of the tissue. Contractions in response to vagal stimulation at 20 and 100 Hz for 1 s at 100 V and 0·5 ms pulse duration were recorded isometrically. Transmural stimulation at 20 Hz was also applied in some experiments with the same parameters as for vagal stimulation. The tissue was equilibrated with all antagonists for at least 30 min before the responses were evaluated. All responses were obtained at least in

duplicate to ensure equilibration with the antagonist had been achieved.

Rat and guinea-pig phrenic nerve diaphragm

Left and right hemidiaphragms were removed from male, hooded Wistar rats or guinea-pigs and placed in a 50-mL bath under 2 g tension at 37°C in a Krebs–Henseleit solution of the following composition (mM): NaCl 118, KCl 4·7, $MgSO_4.7H_2O$ 1·2, KH_2PO_4 1·2, $NaHCO_3$ 25, D-(+)-glucose 10 and $CaCl_2$ 2·5 and gassed with 95% O_2–5% CO_2. The preparation was stimulated at twice the minimal voltage required for maximal response (1·5–10 V) for a duration of 0·2 ms.

Reproducible control values were obtained for single twitches at a frequency of 0·1 Hz, for a train of 4 twitches (1·9 s train duration) at 2·0 Hz (rat only) and for both the response and fade during tetanic stimulation at 50 Hz for 1·9 s. A single dose of antagonist was administered to the preparation and the tissue was allowed to equilibrate for 20 min, different compounds being used on the two hemidiaphragms from any one animal. Stimulations were repeated at successive 10-min intervals for up to 1 h after addition of the drug or until constant responses were obtained. The tissue was then washed and allowed to recover for 10 min before a higher concentration of antagonist was administered. This process was repeated up to 7 times or until all responses were blocked.

Data evaluation

Oesophagus. Reduction in the peak contractile response of the smooth muscle to ganglionic stimulation induced by antagonists was expressed as a percentage of the control value.

The same procedure was used to calculate reduction in the response to tetanic stimulation at 20 and 100 Hz. Since tetanic fade was evident in control responses as well as in the presence of drugs, fade at 100 Hz was calculated using the formula:

$$\frac{\left(\frac{C_2}{C_1}-\frac{d_2}{d_1}\right)100}{C_2/C_1} \qquad (1)$$

where C_1 = initial peak height of control response, C_2 = response at the end of stimulation, d_1 = initial peak height response in the presence of drug, and d_2 = response at the end of stimulation in the presence of drug.

At least two concentrations of an antagonist were used which encompassed the EC50 values allowing estimation by interpolation.

Diaphragm. The decrease in the height of a single twitch in the presence of antagonists was expressed as a percentage of the control value. The fade observed in the presence of an antagonist over a train of four twitches or during tetanic stimulation was calculated as above, although control responses to either parameter showed no fade over the 1·9-s period of stimulation in the rat.

Dose-effect curves using 4 to 7 concentrations of each antagonist were plotted for twitch reduction, tetanus reduction, train of four fade and tetanic fade and the geometric mean concentration producing 50% effect (EC50) was obtained for the antagonists with each response. The curves were evaluated on a computer by fitting data points to a logistic function by the method of least squares (Parker & Waud 1971) and the resulting EC50 value was determined for each parameter.

Drugs

Drugs used were: hexamethonium bromide (Sigma, St Louis, MO), mecamylamine hydrochloride (Research Biochemicals Inc., Natick, MA), pancuronium bromide (Organon, Oss, The Netherlands), trimetaphan camsylate (Roche, Dee Why, Australia) and tubocurarine hydrochloride (Sigma, St Louis, MO).

Results

Oesophagus

The response to a 1-s stimulation of the vagus nerve gave rise to an initial contraction involving striated muscle and then a second contraction involving smooth muscle which reached a maximum after cessation of stimulation (Fig. 1). In addition, the initial contraction of striated muscle at 100 Hz was subjected to tetanic fade. Both contractile responses were inhibited and tetanic fade was increased by nicotine-receptor antagonists. The geometric mean EC50 values for the antagonists on the various responses are shown in Table 1.

Transmural stimulation of the oesophagus also gave the same response as vagal nerve stimulation (20 Hz), except that the second contraction was not affected significantly ($P > 0.05$) by trimetaphan or tubocurarine at any concentration investigated, suggesting that post-ganglionic stimulation was involved. Hexamethonium (300 μM) produced a 25·2 ± 4·9% reduction ($P < 0.05$) and mecamylamine (100 μM) a 13·3 ± 2·3% reduction ($P < 0.05$), while pancuronium (0·1, 0·3 or 30 μM) produced enhancement of the second contraction induced by transmural stimulation by 32·1 ± 4·8%, 39·7 ± 7·5% and 22·4 ± 0·3%, respectively ($P < 0.05$). At higher concentrations pancuronium caused inhibition of this response with an EC50 of 0·18 mM (0.02–1·56, 3).

Phrenic nerve diaphragm

The EC50 values in studies with the rat diaphragm for the reduction in the response to a single twitch, reduction in

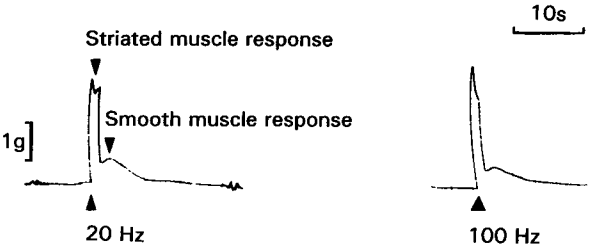

FIG. 1. Response of the guinea-pig oesophagus to a 1-s stimulation at 20 and 100 Hz. Note the tetanic fade evident in the striated muscle response. Vertical scale represents 1 g tension, time marker 10 s.

Table 1. Geometric mean EC50 values in μM (95% confidence limits)* for nicotine-receptor antagonists on various parameters in the guinea-pig oesophagus.

Drug	Tetanus reduction		Tetanic fade 100 Hz	Smooth muscle peak contraction reduction 20 Hz
	20 Hz	100 Hz		
Hexamethonium	764 (300–1943)	1164 (309–4385)	238(144–391)	51·5 (27·5–95·8)
Mecamylamine	130 (100–170)	140 (120–170)	29 (19–45)	25 (7.3–86)
Pancuronium	0·08 (0·03–0·23)	0·10 (0·04–0·22)	0·04 (0·02–0·07)	97 (28–340)
Trimetaphan	104(65–168)	149 (129–173)	40 (16–100)	5·3 (3·7–7·4)
Tubocurarine	0·57 (0·36–0·90)	0·66 (0·38–1·15)	0·20 (0·13–0·33)	89 (46–171)

* Values shown are the mean of 3 or 4 experiments except in the case of tetanic fade for hexamethonium where n = 6.

peak tetanic response at 50 Hz, the fade observed over a train of four twitches and to tetanic fade are shown in Table 2. The order of potency observed for the drugs on the response to each of the four stimuli was the same.

Experiments performed with the guinea-pig diaphragm using mecamylamine, pancuronium and tubocurarine showed a different order of potency to that observed in the rat diaphragm preparation, but the order was similar to the guinea-pig oesophagus for tetanic fade and the motor end plate responses (Table 3). Comparisons of the order of potency in the rat and guinea-pig tissues are shown in Table 4.

Discussion

The rank order of potency for the antagonists at the motor end-plate in rat diaphragm or guinea-pig oesophagus was similar, with the exception that pancuronium was more active than tubocurarine in the guinea-pig (Table 4). It is known that the rat shows a different relative potency for these two antagonists compared with other species (Buckett et al 1968; Derkx et al 1971; Buckett 1975) and this is supported by the results obtained on the guinea-pig diaphragm. The same relative potency for pancuronium and tubocurarine in the guinea-pig phrenic nerve-diaphragm preparation as that found in the present study has been reported previously (Birmingham & Hussain 1980, Bradshaw et al 1986). It is of interest that both these earlier studies found tubocurarine and pancuronium one-tenth as active in absolute terms at blocking single twitch responses, which appears to be due to the use of very short incubation times (3–5 min).

In contrast, ganglionic blockade in the guinea-pig oesophagus showed a different rank order from neuromuscular junction blockade in both species. Comparison with data for the guinea-pig hypogastric ganglion (Birmingham & Hussain 1980) showed that, compared with tubocurarine, hexamethonium had the same relative potency in the vagal and hypogastric ganglia but both trimetaphan and pancuronium exhibited relative potencies with tubocurarine that were ca. 4-fold those in the guinea-pig oesophagus. Indirect measurement of ganglionic blockade, as in the present experiments, may be modified if the nicotinic-receptor antagonists have additional actions at sites such as the post-ganglionic nerve terminals or on the smooth muscle. This can be overcome to some extent by assessing the effects of the antagonists on post-ganglionic stimulation, which was achieved by evaluating their effects on responses to transmural stimulation. Thus, while trimetaphan did not significantly affect the response to transmural stimulation at any concentration investigated, both mecamylamine and hexamethonium in higher concentrations produced some inhibition of the smooth muscle response. This may be attributed to inhibition of responses to acetylcholine on muscarinic receptors as seen in gastrointestinal smooth muscle (Leung & Mitchelson 1982a). However, it is unlikely that this effect influenced the block of ganglionic stimulation as it only occurred with concentrations of the antagonists 4- 6-fold their ganglionic EC50 values. In the case of pancuronium, a facilitation of the response to ganglionic stimulation was evident at low concentrations. This may be due to an effect on prejunctional muscarinic autoreceptors on post-ganglionic cholinergic nerves as pancuronium possesses inhibitory effects on muscarinic receptors of the M_2 subtype (K_B ca. 0·1 mM) Leung & Mitchelson 1982b) and is about one-twentieth as active at muscarinic M_3 receptors (Leung & Mitchelson 1982b; Törőcsik et al 1989). This enhancement of the response to transmural stimulation by pancuronium suggests that the difference between the concentration

Table 2. Geometric mean EC50 values in μM (95% confidence limits)* for nicotine receptor antagonists on various parameters in the rat phrenic nerve diaphragm.

Drug	Twitch reduction	Train of four fade	Tetanus reduction	Tetanic fade	Twitch: tetanic fade	Twitch: train of four
Hexamethonium	3707 (2888–4758)	2711 (2379–3089)	2187 (1403–3407)	1431 (1135–1804)	2·59	1·37
Mecamylamine	81·4 (15·8–421)	102 (86·6–120)	181 (42·3–770)	74·6 (40·9–136)	1·09	0·80
Pancuronium	1·70 (1·28–2·26)	1·40 (1·18–1·66)	1·80 (0·57–5·58)	1·00 (0·91–1·10)	1·70	1·21
Trimetaphan	302 (261–349)	270 (237–307)	240 (135–427)	155 (103–234)	1·95	1·12
Tubocurarine	0·76 (0·63–0·93)	0·60 (0·47–0·77)	0·43 (0·34–0·54)	0·30 (0·25–0·38)	2·53	1·28

* Number of experiments = 4 for all drugs except for mecamylamine on train of four fade where n = 3.

Table 3. Geometric mean EC50 values, in μM, (95% confidence limits)* for nicotine receptor antagonists on various parameters in the guinea-pig phrenic nerve diaphragm.

Drug	Twitch reduction	Tetanus reduction	Tetanic fade	Twitch: tetanic fade
Mecamylamine	99·5 (73·8–134)	92·9 (71·3–121)	77·1 (70·7–84·1)	1·29
Pancuronium	0·12 (0·06–0·24)	0.10 (0·06–0·16)	0.11 (0·07–0·15)	1·09
Tubocurarine	0·66 (0·56–0·78)	0·56 (0·50–0·63)	0·48 (0·44–0·52)	1·38

* Number of experiments = 4 for all drugs.

required for inhibition of the motor end-plate nicotinic receptors and the ganglionic receptors is smaller than estimated.

While several nicotine-receptor subtypes are known to exist on peripheral and central neurons (Lukas & Bencherif 1992; Sargent 1993) there are relatively few studies comparing the potency of several antagonists in functional studies on these sites (Lukas 1989; Mulle et al 1991). However, there appears to be sufficient diversity in those that have been studied to suggest that different subtypes of nicotine receptor could be present on vagal nerve endings in striated muscle compared with those in the vagal ganglia. Lukas (1989), for example, found different types of nicotine receptors in two neuronal cell lines. The nicotine receptor in the TE671 human medulloblastoma, resembled a neuromuscular junction-like nicotine receptor, while that in the PC12 rat phaeochromocytoma was a ganglionic-like nicotine receptor. Neither receptor had an antagonist-potency profile resembling that for any of the nicotine receptors in the oesophagus preparation.

Tetanic fade in both the guinea-pig tissues and the rat diaphragm preparation showed the same rank order of potency for the antagonists as for the motor end-plate (reduction in peak tetanic tension) in the respective species. Only minor differences were noted in the relative potencies of the antagonists for producing fade and block of twitch responses in the rat diaphragm compared with those reported by Gibb & Marshall (1986). Additional studies with other nicotinic-receptor antagonists including gallamine, pempidine and pentolinium have also not shown any differences in rank order for tetanic fade and inhibition of the twitch response in this preparation (Stevenson, unpublished).

While there is evidence of a pre-junctional facilitatory nicotinic autoreceptor on cholinergic motor nerve endings (Bowman et al 1984, 1988; Wessler et al 1987; Vizi & Somogyi 1989) and that the phenomenon of tetanic fade in the presence of nicotinic-receptor antagonists involves inhibition of this neuronal nicotinic receptor (Bowman et al 1984, 1988), the fade response failed to show any characteristics of a ganglionic neuronal nicotinic receptor as far as antagonist potency was concerned. The antagonists generally showed a greater potency for inducing fade compared with reduction in tetanic response. It has been pointed out that the nicotinic receptor associated with fade is likely to have different characteristics from ganglionic nicotinic receptors, as trimetaphan is not a potent activator of fade (Gibb & Marshall 1984; Bowman et al 1988), and this was found to be the case in the present experiments. It should also be noted that while inhibitory pre-junctional nicotinic autoreceptors may be present on motor nerve endings in the diaphragm (Wilson & Thomsen 1991, 1992), these only appear to be important with low-frequency stimulation (Tian et al 1994).

In conclusion our results support those of Tian et al (1994), who suggested that tetanic fade involves a nicotinic receptor related to the muscle type of nicotinic acetylcholine receptor rather than the neuronal type of nicotinic receptor found in ganglia.

Table 4. Relative potencies (equipotent molar ratios) for the various antagonists (values compared to tubocurarine = 1).

Tissue	Response	Rank order								
Guinea-pig oesophagus	Ganglion (20 Hz)	Trimet 0·06	>	Mec 0·28	>	Hex 0·56	>	Tubo 1	>	Panc 1·09
	Motor end-plate (100 Hz)	Panc 0·14	>	Tubo 1	>	Mec 212	≥	Trimet 227	>	Hex 1758
	Tetanic fade (100Hz)	Panc 0·20	>	Tubo 1	>	Mec 147	>	Trimet 202	>	Hex 1200
Guinea-pig diaphragm	Motor end-plate (50 Hz)	Panc 0·18	>	Tubo 1	>	Mec 166				
	Tetanic fade (50 Hz)	Panc 0·22	>	Tubo 1	>	Mec 161				
Rat diaphragm	Motor end-plate (50 Hz)	Tubo 1	>	Panc 4·2	>	Mec 418	>	Trimet 558	>	Hex 5093
	Tetanic fade (50 Hz)	Tubo 1	>	Panc 3·3	>	Mec 250	>	Trimet 517	>	Hex 4667

Trimet, trimetaphan; Mec, mecamylamine; Hex, hexamethonium; Tubo, tubocurarine; Panc, pancuronium.

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