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A COMPARISON OF THE EFFECTS OF PANCURONIUM BROMIDE AND ITS MONOQUATERNARY ANALOGUE, ORG NC 45, ON AUTONOMIC AND SOMATIC NEUROTRANSMISSION IN THE RAT

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1 Potentiation by pancuronium of the effects of adrenergic nerve stimulation, previously shown in cardiovascular tissues, was also found in rat anococcygeus and vas deferens, *in vitro* or *in vivo*.

2 In the pithed rat, in the presence of pancuronium, pre-junctional α-adrenoceptor-mediated feedback inhibition of cardiac sympathetic transmission was uncovered at relatively low stimulation frequencies by phentolamine or yohimbine.

3 The effects of pancuronium and its mono-quaternary analogue Org NC 45 on autonomic and somatic neuromuscular transmission were compared, in the pithed rat. Org NC 45 was less potent than pancuronium at blocking somatic neuromuscular transmission by a factor of 2.1, at blocking cardiac, parasympathetic transmission by a factor of 538 and at potentiating sympathetic transmission by a factor of 33.

Introduction

The non-depolarizing neuromuscular blocker, pancuronium, produces tachycardia in some patients, suggesting an imbalance of cardiac autonomic control, which has been attributed, in part, to blockade of post-ganglionic, parasymapathetic transmission in the heart (Saxena & Bonta, 1970; Hughes & Chapple, 1976). Recent evidence suggests that potentiation of the cardiac sympathetic component might also be involved (Ivankovitch, Militech, Albrecht & Zahed, 1975; Domenech, Garcia, Sasiain, Loyola & Oroz, 1976; Docherty & McGrath, 1977; 1978). If so, this might provide a more satisfactory explanation than has, so far, been available for the clinical observations since vagal inhibition, alone, should not produce any considerable tachycardia without the presence of a sympathetic component and since tachycardia to pancuronium can still occur after atropine (Stoelting, 1972).

We have previously described two main actions of pancuronium on the cardiovascular system of the pithed rat, (1) an indirect sympathomimetic action leading to a cardioaccelerator effect and (2) blockade of the neuronal uptake of noradrenaline leading to potentiation of the cardiac and vascular effects of sympathetic nerve stimulation or of circulating noradrenaline (Docherty & McGrath, 1977; 1978).

We have now extended this study of these 'autonomic' effects of pancuronium: (1) to two non-cardiovascular preparations, rat vas deferens and anococcygeus, to demonstrate that such potentiation of adrenergic transmission is widespread in the rat; (2) to demonstrate that, like other drugs which block the neuronal uptake of noradrenaline, pancuronium will increase the effectiveness of the negative feedback control of noradrenaline release, which is exerted via pre-junctional α-adrenoceptors (see Docherty & McGrath, 1979); (3) to compare the effects of pancuronium and its mono-quaternary analogue Org NC 45 on (a) sympathetic, postganglionic adrenergic transmission, (b) parasympathetic, postganglionic cholinergic transmission and (c) somatic, neuromuscular, cholinergic transmission. In the anaesthetized cat, Org NC 45 retains much of the 'relaxant' activity of pancuronium but is relatively weaker in blocking post-junctional, cardiac muscarinic receptors (Durant, Marshall, Savage, Nelson, Sleigh & Carlyle, 1979). It was thus of interest to compare the additional 'sympathetic' effects in the pithed rat.

A preliminary communication of these results has been published (Docherty & McGrath, 1980).

Methods

Male Wistar rats (250 to 300 g) were used.

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In vitro *preparations*

Rats were killed by a blow on the head and exsanguination.

Isolated anococcygeus muscles were studied *in vitro* by the method of Gillespie (1972); the muscles were placed in 30 ml baths containing Krebs bicarbonate solution at 37°C and gassed with 95% O_2 and 5% CO_2. The muscles were threaded through silver wire electrodes and longitudinal isometric tension was recorded with Grass FT03 transducers. Tension was displayed on a Grass Model 7 polygraph or an S.E. Labs model 3006DL u.v. oscillograph.

Isolated vasa deferentia were each bisected transversely into two portions of equal length and set up as described above for anococcygeus: the contractile responses, to field stimulation of the intramural nerves, of such bisected portions of vas differ; (i) the epididymal portion displays a straightforward adrenergic response which is potentiated by blockade of the neuronal uptake of noradrenaline; (ii) the prostatic portion displays a response which is mainly 'non-adrenergic' and is not affected by any of the standard procedures which modify adrenergic transmission, including blockade of noradrenaline uptake (McGrath, 1978). To confirm that the effect of pancuronium on the epididymal portion was due to potentiation of adrenergic transmission, these experiments were repeated on tissues taken from rats pretreated with 6-hydroxydopamine (6-OHDA), which destroys the adrenergic terminals (Thoenen & Tranzer, 1968) and hence, selectively, removes the adrenergic response, leaving only a small, residual, 'non-adrenergic' component in the epididymal portion (Booth, Connell, Docherty & McGrath, 1978). Rats were sympathectomized by intraperitoneal injection of 6-OHDA (2 × 50 mg/kg on day 1; 2 × 100 mg/kg on day 4; rats killed on day 5 or 6). 6-OHDA was dissolved in de-oxygenated 0.9% w/v NaCl solution (saline) containing ascorbic acid (1 mg/kg).

Supramaximal field stimuli (0.5 ms pulses) were applied in trains at frequencies described in the text (anococcygeus) or as single pulses separated by 5 min intervals (vas deferens). Test drugs were added after reproducible control responses to field stimulation had been obtained.

The Krebs bicarbonate solution was of the following composition and was continuously gassed with 95% O_2 and 5% CO_2: (mM) NaCl 119, KCl 4.7, $MgSO_4$ 1.0, KH_2PO_4 1.2, $NaHCO_3$ 25, $CaCl_2$ 2.5 and glucose 11.1.

Pithed rats

Rats were pithed by the method of Gillespie, MacLaren & Pollock, (1970) and respired with 100% O_2 (see Clanachan & McGrath, 1976). Heart rate was measured from the right carotid arterial pressure wave by means of a Devices instantaneous ratemeter. Where appropriate, longitudinal isometric tension of the anococcygeus or vas deferens (Gillespie & McGrath, 1973; 1974) was monitored (Grass FT03 transducer) and displayed together with blood pressure and heart rate on a u.v. oscillograph (S.E. Labs model 3006DL). The pithing rod electrode was placed for stimulation of the sympathetic outflows to various organs (Grass S88 stimulator, supramaximal voltage, 10 mm electrode): cardiac sympathetic stimulation (C6-T1, 0.05 ms pulses); vas deferens (L1-L3, 0.5 ms pulses); (Gillespie & McGrath, 1974; Docherty & McGrath, 1979).

To assess neuromuscular blockade, a forelimb was pinned to the table and the tendon of flexor carpi radialis was exposed by a skin incision and blunt dissection. A loop of thread was tied round the muscle end of the severed tendon and attached to an isometric tension transducer (Devices 4151) under a resting tension of 10 g. In this way, while stimulating the cardiac sympathetic nerves with a single pulse every 2 min (0.05 ms pulses, C6-T1, supramaximal voltage) it was possible to assess, simultaneously, the effects of drugs on cardiac sympathetic and limb neuromuscular transmission (see also Clanachan & Muir, 1972).

In another set of experiments, α-adrenoceptor antagonists were injected during the maintained response to repetitive cardioaccelerator nerve stimulation at 0.1 Hz and stimulation was continued until the heart rate reached a new steady level. This procedure was followed in drug-free controls and then, in separate rats, after administration of pancuronium (2 mg/kg) (see Docherty & McGrath, 1979).

When stimulating at positions other than C6-T1, i.e. when monitoring the vas deferens, gallamine (20 mg/kg) was given to prevent skeletal muscle twitching; this was considered to be acceptable since gallamine did not significantly modify cardiac sympathetic transmission (see results).

To assess effects on parasympathetic transmission, trains of supramaximal pulses (50 pulses, 1 ms, 5 Hz) were applied to the peripheral end of the divided right cervical vagus; this produced a fall in heart rate.

With the exception of Org NC 45, drugs were dissolved in saline (0.9% w/v), were injected intravenously in a volume of 1 ml/kg and were washed in with saline (1 ml/kg). Control saline injections of 2 ml/kg were always given. Org NC 45 was dissolved in acetate buffer at pH 4. Control injections of buffer were given in this case. Each rat received only one test compound and different rats were used to assess different effects (excepting the cardiac sympathetic neuromuscular experiments).

Drugs used were atropine sulphate (B.D.H.), gallamine triethiodide (Flaxedil, May & Baker), nor-

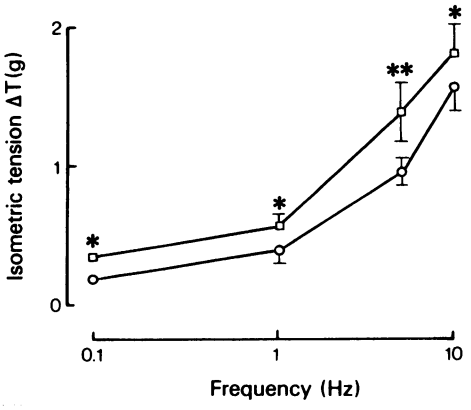

Figure 1 Effect of pancuronium (10 μM) on the isometric tension response of the anococcygeus *in vitro* to transmural stimulation for 10 s at frequencies of 0.09 to 10 Hz. Control responses (○), responses in presence of pancuronium (□). Vertical bars represent s.e. of mean. $n = 6$. Control responses and responses after pancuronium were compared by Student's t test for paired data: *$0.05 > P > 0.01$; **$0.01 > P > 0.001$).

adrenaline bitartrate (Koch–Light), Org NC 45 (Organon; structure in Durant *et al.*, 1979), pancuronium bromide (Organon) and (+)-tubocurarine (Tubarine; Calmic). Doses of drugs given to pithed rats are expressed as grams of the salt with the exception of noradrenaline, which is given as grams of the base.

Where appropriate, comparison of responses before and after addition of a drug is made with a paired 't' test. ID_{50} was calculated as the dose of a drug producing 50% inhibition of a given response according to regression analysis of the linear part of the log dose-response relationship.

Results

Effects of pancuronium on adrenergic transmission

Potentiation of nerve-induced responses

Anococcygeus The effects of pancuronium were examined on the isometric tension response of the isolated anococcygeus muscle to field stimulation for 10 s at frequencies of 0.1 Hz (single pulses which induce individual contractions which do not summate) to 10 Hz. Pancuronium (10 μM) significantly potentiated the response at each frequency (Figure 1). Pancuronium (100 μM) produced significant further potentiation of responses at each frequency except 10 Hz. Cocaine (1 μM) potentiates responses in this frequency range; such potentiation should be regarded qualitatively since quantitative analysis of nerve-induced responses in anococcygeus *in vitro* is complicated by the simultaneous presence of an inhibitory nerve-mediated response (McGrath & Olverman, 1978).

The response of the isolated anococcygeus to noradrenaline (NA) (3 μM) was significantly potentiated from 0.98 ± 0.45 g in controls to 2.22 ± 0.64 g ($n = 4$, $P < 0.05$) in the presence of pancuronium (10 μM) and to 2.88 ± 0.70 g ($n = 4$, $P < 0.05$) in the presence of pancuronium (100 μM). Similarly, in each of 2 experiments, the contractile effect of NA (10 μg/kg, i.v.) on the isometric tension of the anococcygeus, monitored *in situ* in the pithed rat, was potentiated by pancuronium (1 mg/kg).

Vas deferens The effects of pancuronium were examined on the isometric tension response of the bisected vas deferens to single field stimuli *in vitro*. In the epididymal portion, whose response is predominantly adrenergic, pancuronium (10 or 100 μM) potentiated the response. In the prostatic portion, the response of which is 'non-adrenergic', the response was significantly increased only by the highest concentration tested, pancuronium (100 μM) (Table 1). Examination of the time course of the nerve-induced contraction revealed, however, that this latter increase was due to the emergence of an adrenergic response which is normally submerged within the declining phase of the contraction; this is similar to the effect found *in situ* (see Figure 4) and is identical to the effect of cocaine (McGrath, 1978).

In tissues taken from rats sympathectomized with 6-OHDA, pancuronium (1 to 100 μM) had no significant effect on the response of the prostatic portion ($n = 6$, $P > 0.05$) and increased the response of the epididymal portion only at 100 μM (table 1). This latter potentiation suggests a post-junctional site of action since, in addition, pancuronium, in this high concentration, increased spontaneous activity: this activity is found in vasa after 6-OHDA (see MacDonald & McGrath, 1980); post-junctional supersensitivity is thus present.

Taken together these results indicate that, *in vitro*, pancuronium (10 μM) potentiates the adrenergic but not the 'non-adrenergic' nerve-induced contraction of rat vas deferens; at higher concentrations post-junctional mechanisms may appear.

In the pithed rat, pancuronium similarly produces a selective potentiation of the adrenergic component (Figure 4).

Endogenous activation of pre-junctional α-adrenoceptors after pancuronium In the absence of pancuronium, during continuous stimulation of the cardio-accelerator fibres (C6-T1) at 0.1 Hz, neither saline nor yohimbine (100 μg/kg) increased the heart rate further (Figure 2a). After pancuronium (2 mg/kg), the response to stimulation at 0.1 Hz was increased. When

injected during stimulation, saline still had no effect but yohimbine (100 μg/kg) (Figure 2a) or phentolamine (20 μg/kg) (Figure 2b) increased heart rate. These effects of pancuronium are similar to those found previously with cocaine (Docherty & McGrath, 1979) and indicate that α-adrenoceptor-mediated feedback has been induced.

Comparison of Org NC 45 with pancuronium

Cardiac sympathetic The effects of test drugs on the cardioaccelerator response to a single stimulus pulse and on the basal heart rate are shown in Figure 3. The 2 min separation of consecutive stimuli was sufficient for the return of the cardioaccelerator response to baseline, under control conditions. However, when the response was prolonged by drugs which block the re-uptake of transmitter NA part of the increase in baseline produced, e.g. by the higher doses of pancuronium or Org NC 45, may contain a contribution due to 'summation'. Increases following injection but before the next stimulus are, of course, exempt from this qualification and can be regarded as independent of nerve stimulation.

Pancuronium (0.1 mg/kg) produced a small but significant potentiation of the cardioaccelerator response to a single pulse; 0.3 mg/kg produced marked potentiation; 1 mg/kg produced maximal potentiation (i.e. as much as could be produced by the 'classical' uptake blockers desipramine or cocaine, see Docherty & McGrath, 1978). At these doses the elevation of heart rate, independent of nerve stimulation, was small. At pancuronium 10 mg/kg a marked elevation of the basal heart rate was produced with the consequence that the response to nerve stimulation, as measured by the change from the new baseline, was apparently smaller than with the lower dose of 1 mg/kg. This could be entirely explained by the elevation of basal heart rate. A major part of the elevation of basal heart rate was independent of nerve stimulation since it occurred after injection but before the next stimulus (Figure 3a).

Org NC 45 (1 mg/kg) produced a transient potentiation of the response to a single pulse, detectable at 1 min post-injection. At 10 mg/kg a potentiation of similar magnitude was found but this was still present 15 min after injection. Org NC 45 (0.1 mg/kg) produced a transient elevation of basal heart rate but no potentiation of the response to a single pulse. At both 1 mg/kg and 10 mg/kg a marked but short-lived elevation of heart rate occurred on injection; this did not overtly affect the response to nerve stimulation but made direct, quantitative comparison with pancuronium difficult (Figure 3b). We have not, therefore,

Table 1 Effects of pancuronium on the isometric tension response (ΔT) of the bisected vas deferens to single pulse transmural stimulation, *in vitro* in (a) control rats, (b) sympathectomized rats

(a) *Control rats*

	Concentration of pancuronium (μM)	Control response ΔT (g)	Response after pancuronium ΔT (g)	n	P<
E	1	1.31 ± 0.27	1.32 ± 0.27	5	NS
	10	1.28 ± 0.22	1.61 ± 0.26	6	0.01
	100	1.28 ± 0.22	1.91 ± 0.26	6	0.001
P	1	2.04 ± 0.25	2.03 ± 0.25	5	NS
	10	1.92 ± 0.23	1.94 ± 0.25	6	NS
	100	1.84 ± 0.27	2.12 ± 0.24	6	0.001

(b) *Sympathectomized rats*

	Concentration of pancuronium (μM)	Control response ΔT (g)	Response after pancuronium ΔT (g)	n	P<
E	1	0.23 ± 0.05	0.21 ± 0.04	6	NS
	10	0.23 ± 0.05	0.21 ± 0.05	6	NS
	100	0.23 ± 0.05	0.41 ± 0.09	6	0.05

Values are mean ± s.e. mean. Control responses and responses after pancuronium were compared by Student's *t* test for paired data (NS, not significant when $P > 0.05$). Epididymal (E) and prostatic (P) halves of vas.

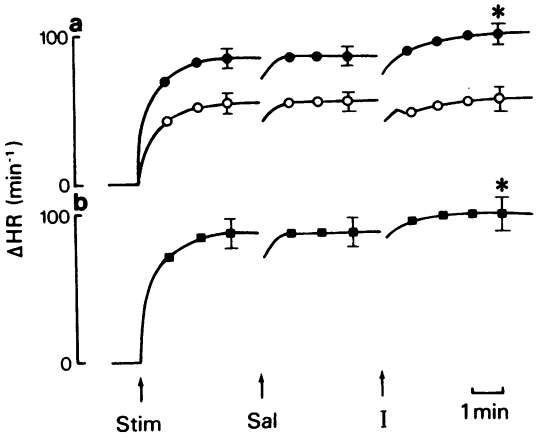

Figure 2 The effects of (a) yohimbine (100 μg/kg) or (b) phentolamine (20 μg/kg) when injected during continuous cardioaccelerator stimulation at 0.1 Hz (C6-T1, 0.05 ms pulses) in pithed rats. The rats had been given pancuronium (2 mg/kg) before the start of stimulation (filled symbols) or were drug-free (open symbols). Stimulation was started at Stim, saline was injected at Sal and yohimbine or phentolamine at I. The graphs were constructed from the mean heart rate taken every 10 s for the first minute after Stim, Sal or I and otherwise at 1 min intervals. ΔHR indicates the change of heart rate from the pre-stimulation level. For the sake of clarity, symbols with error bars (s.e. of mean) are shown only at certain points. $n = 5$. Responses before and after I were compared with a t test for paired data: $*0.05 > P > 0.01$.

attempted to produce an ED_{50} type of comparison but, instead, have taken the doses which produced similar prolonged potentiation (Org NC 45, 10 mg/kg; pancuronium, 0.3 mg/kg) to arrive at an approximate potency ratio of 33 (38 on a molar basis). Another justification for this course is that such large doses of Org NC 45 would have to be given in order to arrive at a full dose-response relationship that other, non-specific effects would arise. The threshold dose of Org NC 45 for cardiac effects was, however, approximately 1 mg/kg.

When assessed in the way shown in Figure 3, gallamine (10 mg/kg) produced a small maintained increase in heart rate but did not modify the nerve-induced responses, (+)-tubocurarine (10 mg/kg) did not affect resting heart rate but produced a short-lived (10 to 15 min) depression of nerve-induced responses and atropine (1 mg/kg) produced no detectable effect on either parameter.

Since it was difficult to determine the threshold for potentiation of adrenergic responses by Org NC 45 on the heart, due to the elevation of basal heart rate, we also compared the effects of pancuronium and Org NC 45 (each 1 mg/kg) on the response of the smooth muscle of the whole vas deferens to stimulation of its sympathetic nerves with a single pulse. The resulting isometric contraction of the vas is biphasic, the second phase being potentiated by drugs that block the re-uptake of transmitter noradrenaline (McGrath, 1978). Both pancuronium and Org NC 45 at 1 mg/kg could potentiate such a response but pancuronium had a greater effect (Figure 4). This confirms that the threshold dose for potentiation of sympathetic transmission by Org NC 45 is below 1 mg/kg.

Cardiac parasympathetic The dose-response curves for inhibition of the bradycardia to stimulation of the vagus indicated that pancuronium was more potent than Org NC 45 but less potent than atropine (Figure 5b). Calculation of the ID_{50}, i.e. the dose producing 50% inhibition of the response, gave values of: Org NC 45, 4.3 mg/kg; pancuronium, 0.008 mg/kg; i.e. a ratio of 538 (615 on a molar basis). The calculated ID_{50} for atropine was 0.003 mg/kg giving a pancuronium:atropine ratio of only 2.7 (2.5 on a molar basis).

Somatic neuromuscular transmission The dose-response curve for blockade of the foreleg 'twitch' to a single pulse indicated that pancuronium was more potent than, and had a steeper slope than, Org NC 45 or gallamine but was less potent than tubocurarine (Figure 5a). Calculation of the ID_{50} gave values of: Org NC 45, 0.38 mg/kg; pancuronium, 0.18 mg/kg; i.e. a ratio of 2.1 (2.4 on a molar basis). The calculated ID_{50} for tubocurarine was 0.11 mg/kg and for gallamine was 6.03 mg/kg giving molar ratios of pancuronium:tubocurarine, 1.7, pancuronium:gallamine, 0.036, Org NC 45:tubocurarine, 4.1.

The effects of Org NC 45 was faster in onset and shorter in duration than pancuronium or gallamine and were similar in onset but of shorter duration (for equivalent blockade) than those of tubocurarine (Figure 6). Comparison of doses producing equivalent, sub-maximal blockade, pancuronium (0.2 mg/kg), Org NC 45 (0.75 mg/kg), showed respective effects of: maximum inhibition, 64%, 70%; onset, injection to maximum, 5 to 6 min, 2 min; duration, injection to 90% recovery, 35 min, 7 to 8 min. Equivalent 'maximal' doses showed the same trend (Figure 6a and b).

Discussion

These results demonstrate that pancuronium potentiates the effects of adrenergic nerve stimulation in rat anococcygeus and vas deferens as well as in the heart and blood vessels. These effects could be shown *in vitro* or *in vivo* and, in the vas deferens, potentiation was selective for the adrenergic component of the nerve-induced contraction, the 'non-adrenergic' component being unaffected (see McGrath, 1978).

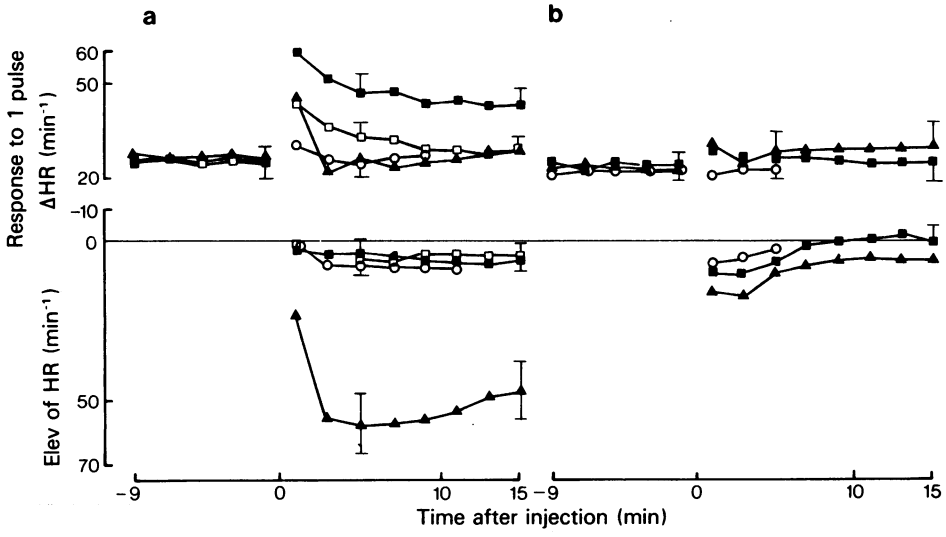

Figure 3 The effects of (a) pancuronium, (b) Org NC 45 on the cardioaccelerator response in the pithed rat to a single stimulus pulse (0.05 ms, C6-T1), given every 2 min. Five control responses are shown; drugs were injected at time zero, after which stimulation was repeated every 2 min starting 1 min after injection. Cardioaccelerator responses to stimulation (ΔHR) are shown in the upper part of the graph. Since the response to stimulation may be influenced by a change in basal heart rate caused by the test drug, the effects of the test drug on resting heart rate are shown in the lower part of the graph; the lower sets of symbols indicate the elevation in heart rate compared with the basal heart rate (taken as zero) at the point at which the drug was administered. The graphs are placed so that the zero line in the lower graph is also the base line for the upper graph. Thus, after injection of a drug, the vertical distance between similar symbols on the upper and lower graphs represents the deviation from the initial resting heart rate of the peak of each nerve-induced response, e.g. at pancuronium (10 mg/kg) 'responses' to each pulse appear to be relatively small because the baseline is elevated. Symbols indicate drug doses: 0.1 (○); 0.3 (□); 1 (■); 10 mg/kg (▲). For the sake of clarity, symbols with error bars (s.e. mean) are shown only at certain points. $n = 4$–6.

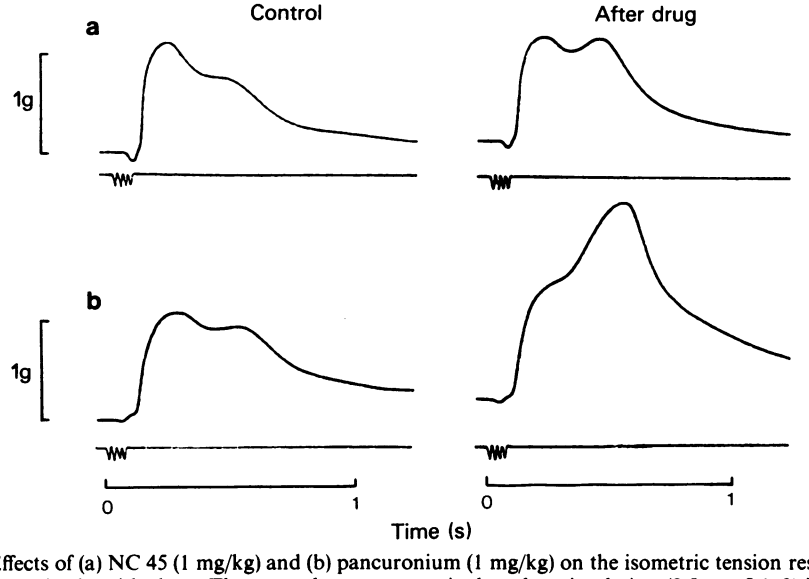

Figure 4 Effects of (a) NC 45 (1 mg/kg) and (b) pancuronium (1 mg/kg) on the isometric tension response of the *in situ* vas deferens in the pithed rat. The control response to single pulse stimulation (0.5 mg, L1–3) is shown on the left, and the response after injection of the test drug is shown on the right. Stimulus was given at time zero, as shown by the event marker below each trace. (a) and (b) are traces from different rats.

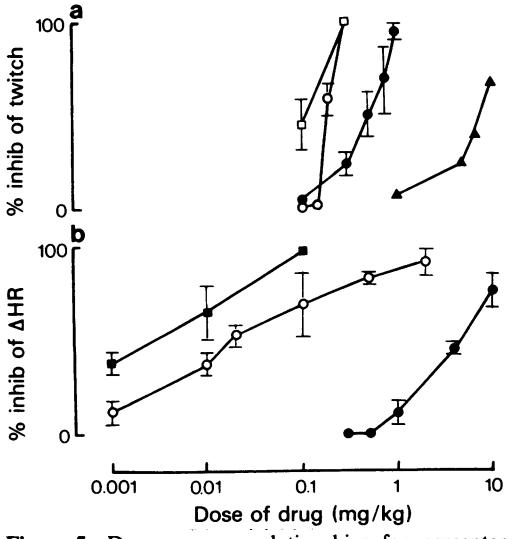

Figure 5 Dose-response relationships for percentage inhibition of (a) the twitch of flexor carpi radialis to stimulation of the spinal outflows at C6-T1 with a single pulse (0.05 ms) every 2 min, and (b) the fall in heart rate (ΔHR) to stimulation of the cervical vagus with trains of 50 pulses (1 ms) at 5 Hz delivered every 2 min, each in the pithed rat. Since the effects of these drugs are long-lived, a full dose-response relationship could not be obtained in each rat. Below is shown, for each compound, the number of rats at each data point (n = x) followed by the total number of rats to obtain that dose-response relationship. The lower numbers in each range indicate those for the extreme doses. (a) Tubocurarine (□, n = 3–4, 7 rats), pancuronium (○, n = 4–6, 17 rats), Org NC 45 (●, n = 3–6, 14 rats) and gallamine (▲, n = 2–4, 8 rats). (b) Atropine (■, n = 3, 3 rats), pancuronium (○, n = 3–6, 16 rats) and Org NC 45 (●, n = 2–4, 8 rats).

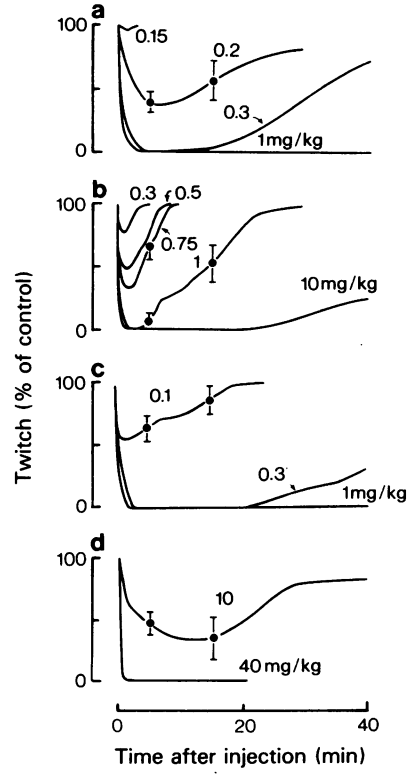

Figure 6 Time courses of neuromuscular blockade in flexor carpi radialis of the pithed rat produced by (a) pancuronium, (b) Org NC 45, (c) tubocurarine and (d) gallamine. (a) Pancuronium: 0.15 mg/kg, n = 2; 0.2 mg/kg, n = 4; 0.3 mg/kg, n = 4; 1 mg/kg, n = 5. (b) NC 45. 0.3 mg/kg, n = 2; 0.5 mg/kg, n = 2; 0.75 mg/kg, n = 4; 1 mg/kg, n = 6; 10 mg/kg, n = 6. (c) Tubocurarine: 0.1 mg/kg, n = 3; 0.3 mg/kg, n = 4; 1 mg/kg, n = 4. (d) Gallamine. 10 mg/kg, n = 4; 40 mg/kg, n = 2. For the sake of clarity, symbols with error bars (s.e. of mean) are shown only at certain points.

The antagonism by pancuronium at muscarinic receptors at the postganglionic parasympathetic junction has been found to display some cardio-selectivity when the dose-relationship was compared with that of atropine (Saxena & Bonta, 1970; Marshall & Ojewole, 1979). The potentiation of adrenergic transmission, however, was similar in the heart to that in either the vas or anococcygeus.

In the pithed rat, the threshold for potentiation of the response to cardioaccelerator nerve stimulation was slightly lower than the threshold for neuromuscular blockade, measured simultaneously. When pancuronium is used as a relaxant, therefore, a general increase in the effectiveness of the sympathetic nervous system might be expected in every tissue in which the re-uptake of noradrenaline is an important part of the inactivation process following transmitter release. Since a blockade of postganglionic parasympathetic transmission occurred with an even lower threshold, pancuronium might tip the autonomic balance towards the sympathetic side in a manner which could make reflex compensation difficult. Furthermore, the blockade of neuronal re-uptake of noradrenaline, by raising the concentration of noradrenaline outside the varicosity, not only potentiates the post-junctional effect but increases the degree of pre-junctional, α-adrenoceptor-mediated inhibition (Docherty & McGrath, 1979). Hence in the presence of pancuronium (2 mg/kg) the α-adrenoceptor antagonists, yohimbine and phentolamine, potentiated the tachycardia produced by low frequency cardioaccelerator stimulation by blocking the inhibitory, prejunctional effect of transmitter noradrenaline.

The combination of pancuronium and prejunctional α-adrenoceptor antagonists, e.g. phentol-

amine, yohimbine, may therefore produce tachycardia and should be avoided, e.g. in the removal of phaeochromocytoma.

In these circumstances, it would be more logical to avoid pancuronium, rather than to opt for a 'selective' α_1-adrenoceptor antagonist, e.g. prazosin, since part of the pressor response, in both man and rats, to α-adrenoceptor agonists is mediated by post-junctional receptors which are resistant to prazosin and have a profile of agonist specificity similar to the α_2-adrenoceptors which are found pre-junctionally (Moulds & Jauernig, 1977; Drew & Whiting, 1979; Docherty, MacDonald & McGrath, 1979).

Further interactions with pancuronium might also be anticipated with other drugs which affect the neuronal or extraneuronal uptake of noradrenaline, e.g. trycyclic antidepressants, corticosterone, and particularly with anaesthetics such as ketamine which potentiate the effects of circulating catecholamines (Clanachan & McGrath, 1976; McGrath & Mackenzie, 1977).

Org NC 45 was 2.1 times less potent as a muscle relaxant than pancuronium but was less potent in its effects on sympathetic and parasympathetic transmission by 33 and 538 times, respectively. In addition, the onset of, and recovery from, relaxation were more rapid for Org NC 45. This suggests that, in the rat, Org NC 45 displays two qualities that could be of advantage in its use as a relaxant: (1) less side-effects from interference with autonomic transmission; (2) more flexibility in control of relaxation. It should, however, be noted that the drug is not devoid of autonomic side-effects despite being relatively better than pancuronium, viz: (a) With each drug tested the dose-response curves for blockade of parasympathetic transmission were considerably shallower than for blockade of somatic transmission. Consequently the ID_{50} values should be used with caution when comparing effects at these two sites. This is particularly striking for pancuronium which, at doses at and below 0.1 mg/kg can actually produce a considerable blockade of the cardiac parasympathetic without a relaxant effect. With Org NC 45 at 1 mg/kg, which is the lowest dose which produced more than 90% inhibition of somatic transmission, significant inhibition of parasympathetic transmission was found and, as the effect on the vas deferens indicated, sympathetic transmission was also potentiated. (b) On injection, Org NC 45 at 1 mg/kg and above produced an increase in heart rate that was independent of autonomic transmission (see Docherty & McGrath, 1978).

It is clear that the doses of the steroid-relaxants which are required to produce 'relaxation' in the rat are larger than those in other species, e.g. ID_{50}: present results, rat, 0.180 mg/kg; Durant *et al.* (1979) anaesthetized cat, soleus muscle, 0.018 mg/kg (both for pancuronium). The ratio of relaxant effects between pancuronium and Org NC 45, however, remained constant in the same two studies, i.e. ID_{50}, NC 45/pancuronium: rat, 2.1; cat, 1.9. Another factor may be the choice of muscle. Work in progress indicates that the rat soleus is more sensitive to pancuronium than the flexor carpi radialis used in the present study (N.A. Flavahan & J.C. McGrath, unpublished observations) and Clanachan & Muir (1972) could demonstrate a considerable relaxant effect of pancuronium (0.08 mg/kg) in both soleus and tibialis of the pithed rat.

It is also possible that the effects on autonomic transmission found in this study bear little relationship to those in other species. It is difficult to compare this study with that in anaesthetized cats since the difference in experimental protocol is so great and since the extent of basal autonomic tone may differ greatly. For example the similarity of the ID_{50}s for parasympathetic blockade with Org NC 45, i.e. rat, 4.3 mg/kg; cat, 2.1. mg/kg contrasts with the difference for pancuronium, i.e. rat, 0.008 mg/kg; cat, 0.062 mg/kg (present study, Durant *et al.*, 1979). The apparently high dose in the cat with pancuronium might, however, be necessary due to a physiological antagonism from potentiation of the cardiac sympathetic nerves or even simply because sympathetic tone was present. The slope of the dose-response curve for blockade of parasympathetic transmission is, however, so oblique that the difference in the calculated ID_{50}s is of no great significance, the more important point being the low thresholds for parasympathetic blockade. In addition, preliminary experiments with pithed rabbits indicate that while rabbits are more sensitive to the relaxant effects of the steroids than are rats, the autonomic effects (sympathetic and parasympathetic) appear at identical doses in the two species (J.R. Docherty, G.N.C. Kenny & J.C. McGrath, unpublished observations). The 'autonomic' effects of pancuronium thus show more consistency between species than the relaxant effects.

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