

SYMPATHOMIMETIC EFFECTS OF PANCURONIUM BROMIDE ON THE CARDIOVASCULAR SYSTEM OF THE PITHED RAT: A COMPARISON WITH THE EFFECTS OF DRUGS BLOCKING THE NEURONAL UPTAKE OF NORADRENALINE

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1 The effects of pancuronium bromide on the cardiovascular system of the pithed rat were examined. Pancuronium had two effects, a short-lasting cardiovascular stimulation following injection and a longer-lasting potentiation of responses to sympathetic nerve stimulation.

2 The initial effect of pancuronium was compared with that of tyramine. The cardioaccelerator but not the pressor responses to both pancuronium and tyramine were significantly reduced following sympathectomy with 6-hydroxydopamine (6-OHDA).

3 The action of pancuronium in potentiating sympathetic nerve responses was compared with that of known blockers of the neuronal uptake of noradrenaline (NA). Pancuronium (1 mg/kg) and cocaine (0.5 mg/kg) potentiated cardioaccelerator and pressor responses to sympathetic stimulation. These effects of pancuronium could be obtained following adrenalectomy and during neuromuscular blockade with gallamine. Pancuronium and uptake blockers potentiated the cardioaccelerator response to NA, reduced the response to tyramine, but did not affect the response to isoprenaline. Pancuronium and uptake blockers potentiated the pressor response to NA, but did not affect the response to tyramine or clonidine.

4 Following sympathectomy with 6-OHDA, pancuronium failed to potentiate cardioaccelerator and pressor responses to NA.

5 These results are discussed in relation to two main cardiovascular effects of pancuronium; an indirect sympathomimetic action and blockade of the neuronal uptake of NA.

Introduction

Pancuronium bromide is a steroid non-depolarizing neuromuscular blocking agent (Buckett, Marjoribanks, Marwick & Morton, 1968). In clinical use pancuronium can produce cardiovascular stimulation, part of which has been explained by a vagolytic action (Saxena & Bonta, 1970; Hughes & Chapple, 1976). However, two effects on the cardiac sympathetic innervation have been postulated from animal experiments; an indirect sympathomimetic action (Domenech, Garcia, Sasiain, Loyola & Oroz, 1976), and blockade of the neuronal uptake of noradrenaline (NA) (Ivankovitch, Miletich, Albrecht & Zahed, 1975).

We have now found two sympathomimetic effects of pancuronium bromide on the cardiovascular system of the pithed rat: (1) a short lived elevation of both heart rate and blood pressure following intra-

venous injection, (2) a longer-lasting potentiation of responses to both cardiac and vasopressor sympathetic nerve stimulation.

First the injection effect was investigated in control rats and in rats sympathectomized with 6-hydroxydopamine (6-OHDA) to determine whether an indirect sympathomimetic action was involved. Secondly the possibility that the potentiation of sympathetic nerve-mediated responses was due to blockade of the neuronal uptake of NA was investigated by comparison of the effects of pancuronium with those of known blockers of neuronal NA-uptake, cocaine (Trendelenburg, 1966) and desipramine (Titus & Spiegel, 1962).

A preliminary account of these results has been published (Docherty and McGrath, 1977a).

Methods

Male Wistar rats (250 to 300 g) were pithed by the method of Gillespie, MacLaren & Pollock (1970) and ventilated with 100% O₂ at a rate of 60 per min and stroke volume 1 ml/100 g (Clanachan & McGrath, 1976). Heart rate was recorded by use of the carotid arterial pressure signal to trigger a Devices instantaneous ratemeter. The right jugular vein was cannulated for drug injections.

Sympathetic nerve stimulation

Cardiac The pithing rod electrode was placed for optimal stimulation of the sympathetic outflow to the heart (10 mm electrode, C6-T1, 0.05 ms pulses, supra-maximal voltage). With this short pulse duration, no neuromuscular blocking agent was necessary since skeletal muscle twitching was acceptably small, allowing drug-free control responses. However, vasopressor responses were small and consisted mainly of a systolic response due to a positive inotropic cardiac effect, especially at low frequencies.

Vasopressor A second group of experiments involved stimulation of vasopressor nerves (10 mm electrode, T2-T6, 1 ms pulses, supramaximal voltage). In this case gallamine (10 mg/kg, i.v.) was given to reduce skeletal muscle twitching. Vasopressor responses were assessed by diastolic pressure changes since systolic pressure responses were influenced by the cardiac sympathetic response which was still present with these stimulation parameters.

In all experiments involving nerve stimulation, reproducible control responses were obtained before injection of the test drug, after which stimulation was repeated as soon as possible following recovery of heart rate and blood pressure to control levels. In addition, the order of stimulus trains of different frequency or pulse number was varied to rule out error due to a time-dependent decline in the effect of the test drug.

Sympathomimetic drugs

Interactions with pancuronium of three types of sympathomimetic drugs were studied: (1) noradrenaline (NA), directly acting and subject to neuronal uptake; (2) isoprenaline or clonidine, directly acting but not subject to neuronal uptake (Pluchino & Trendelenburg, 1968; Autret, Schmitt, Fenard & Petillot, 1971); and (3) tyramine, indirectly acting and subject to neuronal uptake before releasing NA (Trendelenburg, 1963). The doses were chosen to give approximately equivalent sub-maximal responses. Injection of each agonist was repeated until two consecutive responses were of similar magnitude. The test drug was then

administered and injection of agonists repeated once heart rate and blood pressure had returned to control levels. However, the effect of clonidine in control rats was compared with its effect in rats previously given pancuronium (1 mg/kg), since it was difficult to obtain consistent control responses to successive injections of clonidine.

As in the case of nerve stimulation the vasopressor effects were assessed as the change in diastolic pressure.

Sympathectomized and adrenalectomized rats

Twelve rats were sympathectomized by intraperitoneal injection of 6-OHDA (2 × 50 mg/kg on day 1; 2 × 100 mg/kg on day 4; rats pithed on day 5 or 6) (Thoenen & Tranzer, 1968). 6-OHDA was dissolved in de-oxygenated 0.9% w/v Hale solution (saline) containing ascorbic acid (1 mg/kg). Experiments were carried out on these rats as previously described for control rats.

Other rats were adrenalectomized after pithing, via a mid-line incision in the abdomen.

Drugs used were clonidine hydrochloride (Boehringer Ingelheim), cocaine hydrochloride (Cockburns), corticosterone (Sigma), desipramine hydrochloride (Geigy), gallamine triethiodide (May & Baker), 6-hydroxydopamine hydrobromide (Aldrich), isoprenaline hydrochloride (Sigma), noradrenaline bitartrate (Koch-Light); pancuronium bromide (Organon) and tyramine hydrochloride (Sigma).

Drugs were dissolved in saline and injected intravenously in a volume of 1 ml/kg and washed in with 1 ml/kg saline. Control saline injections of 2 ml/kg were always given. Doses quoted are in terms of the salt, except for NA where doses are of the base.

Results

Cardiovascular stimulant effects of pancuronium bromide

Following injection, pancuronium bromide (0.1 to 10 mg/kg, i.v.) produced dose-related increases of heart rate and blood pressure which reached a maximum in 0.5 to 5 min, and declined to baseline within 2 to 10 min (Figure 1). The duration of responses was dose-related, being most prolonged with 10 mg/kg.

Continuous stimulation of the cardiac sympathetic nerves at 0.1 Hz raised the heart rate by 52.8 ± 4.5 beats/min ($n = 6$). Given during such periods pancuronium bromide produced a significantly greater tachycardia than in the absence of stimulation (Figure 1). This response was maintained for at least 5 min (at

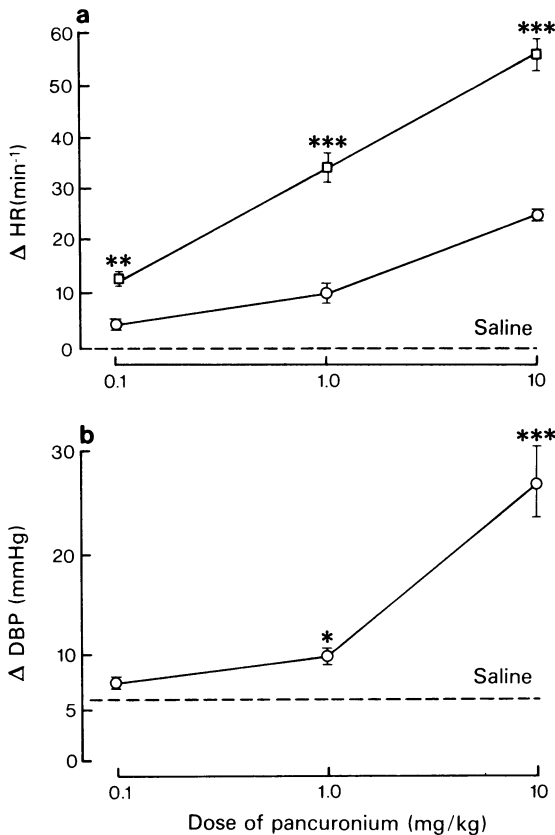


Figure 1 Cardiac and pressor responses to pancuronium bromide. (a) Cardiac responses. Effects in the absence of stimulation (○); effects during 0.1 Hz continuous stimulation at C6-T1 (□). Dashed line; mean effect of saline (2 ml/kg). All pancuronium effects were significantly larger than saline controls. ($P < 0.01$, Student's t test for paired data). Asterisks denote the significance of the difference between responses in the presence and absence of stimulation (Student's 2 sample t test). $n = 7-9$. (b) Pressor (diastolic) responses to pancuronium in the absence of stimulation (○). Dashed lines; mean effect of saline (2 ml/kg). Asterisks denote the significance of the difference between pancuronium responses and saline controls (Student's t test for paired data). $n = 12-19$. Vertical bars represent s.e. mean. (* $0.01 < P < 0.05$; ** $0.001 < P < 0.01$; *** $P < 0.001$)

which time sympathetic stimulation was terminated) and was clearly longer-lasting than the effect in the absence of stimulation.

These preliminary observations suggested that pancuronium had two distinct actions, the first short-lived and independent of nerve stimulation and the

second a longer-lasting potentiation of sympathetic nerve responses. They were therefore investigated separately.

Effects on the cardiovascular system in the absence of nerve stimulation

Pancuronium (1 and 10 mg/kg) and tyramine (10 μg/kg) caused tachycardia in control rats, the increases in heart rate being (mean ± s.e. mean) 10.1 ± 2.4 , $n = 8$; 25.8 ± 3.3 , $n = 9$; and 43.3 ± 4.4 , $n = 6$ beats/min respectively. After sympathectomy with 6-OHDA the respective values were 4.6 ± 1.5 , $n = 9$; 7.1 ± 2.8 , $n = 7$; and 7.2 ± 1.6 , $n = 6$, the latter two being significantly smaller than the control counterparts ($P < 0.001$). This cardioacceleration to both pancuronium and tyramine was blocked by the β-adrenoceptor antagonist, propranolol. However, the pressor responses to both drugs were not significantly altered by sympathectomy.

Effects on the responses to stimulation of sympathetic nerves

(a) *Cardiac* Stimulation at C6-T1 produced increases in heart rate related both to frequency (Figure 2a and b) and pulse number (Figure 2c and d). With 20 pulses in the train, the frequency-response curve reached a plateau between 0.5 and 1 Hz.

Cardiac sympathetic nerve responses were reduced after sympathectomy. The curve for pulse number/response was shifted to the right (Figure 2c).

In control rats, pancuronium, 1 mg/kg, significantly potentiated responses to 20 pulses at 0.01 and 0.1 Hz but not at 0.5 or 1 Hz (Figure 2a) as did cocaine, 0.5 mg/kg (Figure 2b). However, even at 1 Hz, these two drugs potentiated responses to shorter pulse trains (Figure 2c and d). When the doses were increased, pancuronium (10 mg/kg) and cocaine (4 mg/kg) failed to produce any further potentiation of responses.

With a 10 or 20 pulse train at a frequency of 1 Hz, the response heights after pancuronium (1 mg/kg) were not significantly increased. The response durations, assessed as the time from peak response to 50% of peak response, were significantly increased ($P < 0.001$); cocaine (0.5 mg/kg) had similar effects (Figure 3).

After gallamine (10 mg/kg) the tachycardia to a single pulse (C6-T1 0.05 ms pulse) was $21.3 \pm 2.5 \text{ min}^{-1}$ ($n = 6$) compared with a control value of $21.9 \pm 2.1 \text{ min}^{-1}$ ($n = 6$) and a value in comparable rats after pancuronium (1 mg/kg) of $37.6 \pm 2.3 \text{ min}^{-1}$ ($n = 6$). When given after gallamine, pancuronium could still potentiate responses to sympathetic nerve stimulation in a manner related to both pulse number and frequency. For instance, the response to a single

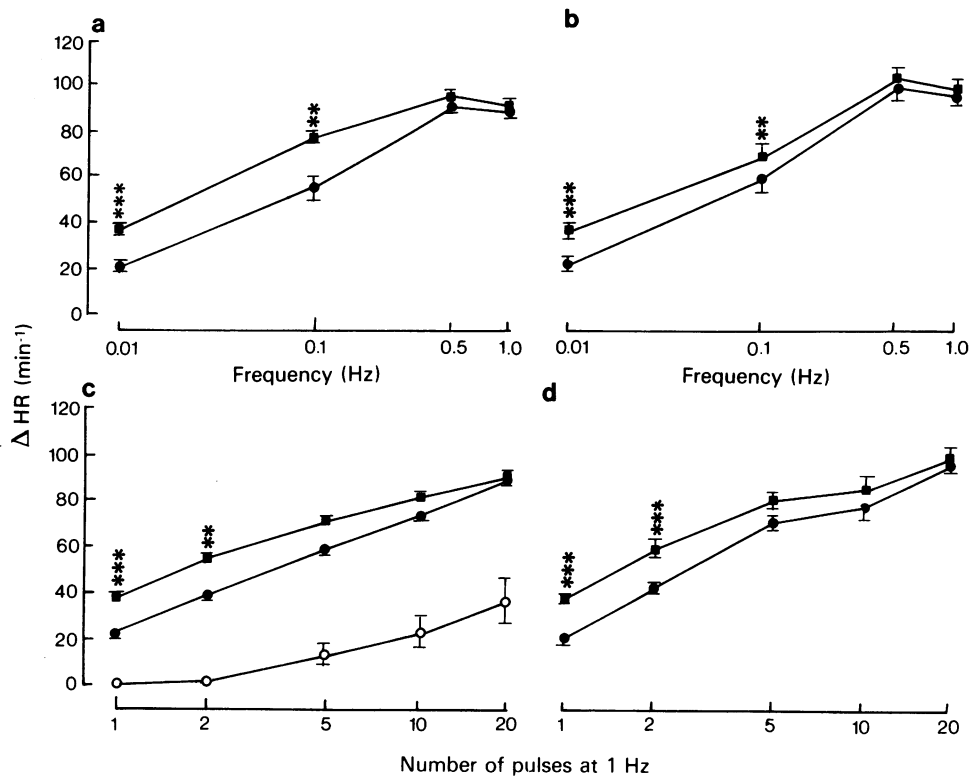


Figure 2 Comparison of the effects on cardioaccelerator responses of pancuronium (1 mg/kg) (a and c), and of cocaine (0.5 mg/kg) (b and d). (a and b) Responses to 20 stimulus pulses at frequencies of 0.01–1 Hz. Control (●); after pancuronium (a) or cocaine (b) (■). (c and d) Responses to trains of 1–20 pulses at 1 Hz. Control (●); after pancuronium (c) or cocaine (d) (■). Control responses in rats sympathectomized with 6-hydroxydopamine (○). Vertical bars represent s.e. mean. In each case $n = 6$. Asterisks indicate responses significantly different from controls (Student's t test for paired data: * $0.01 < P < 0.05$; ** $0.001 < P < 0.01$; *** $P < 0.001$).

pulse in the presence of gallamine (10 mg/kg) increased significantly ($P < 0.02$) to $35.8 \pm 4.2 \text{ min}^{-1}$ after the additional administration of pancuronium (1 mg/kg).

Vasopressor Stimulation at T2–T6 produced increases in diastolic blood pressure related both to frequency (Figure 4a and b) and pulse number (Figure 4c and d). In contrast to the cardiac responses, with 20 pulses in the train the frequency-response curve was still rising at 1 Hz.

Both pancuronium (1 mg/kg) and cocaine (0.5 mg/kg) significantly potentiated the responses to 20 pulses at all frequencies from 0.01 Hz to 1 Hz (Figure 4a and b); and to all train lengths from 1 to 20 pulses at 1 Hz (Figure 4c and d). When the doses were increased neither pancuronium (10 mg/kg) nor cocaine (4 mg/kg) produced any further potentiation of responses except for the response to a single stimulus pulse.

Effect of adrenalectomy Following acute adrenalectomy, pancuronium (1 mg/kg) and cocaine (0.5 mg/kg) still potentiated both cardiac and pressor responses to stimulation at low frequencies (0.1 Hz) and with short trains of pulses at 1 Hz (5 pulses). The immediate short-lived cardioacceleration and pressor responses to pancuronium were also present. At these frequencies and pulse numbers in normal rats, even at T2 to T6 which is near the optimal outflow to the adrenals, adrenal responses were minimal or undetectable.

Effects on responses to single stimuli

Since on a percentage basis the response to a single stimulus pulse was the most affected by pancuronium, the single pulse was used to assess potentiation using a range of doses. The absolute size of the post-drug responses is shown in relation to the corresponding controls since the effects of potentiating agents on

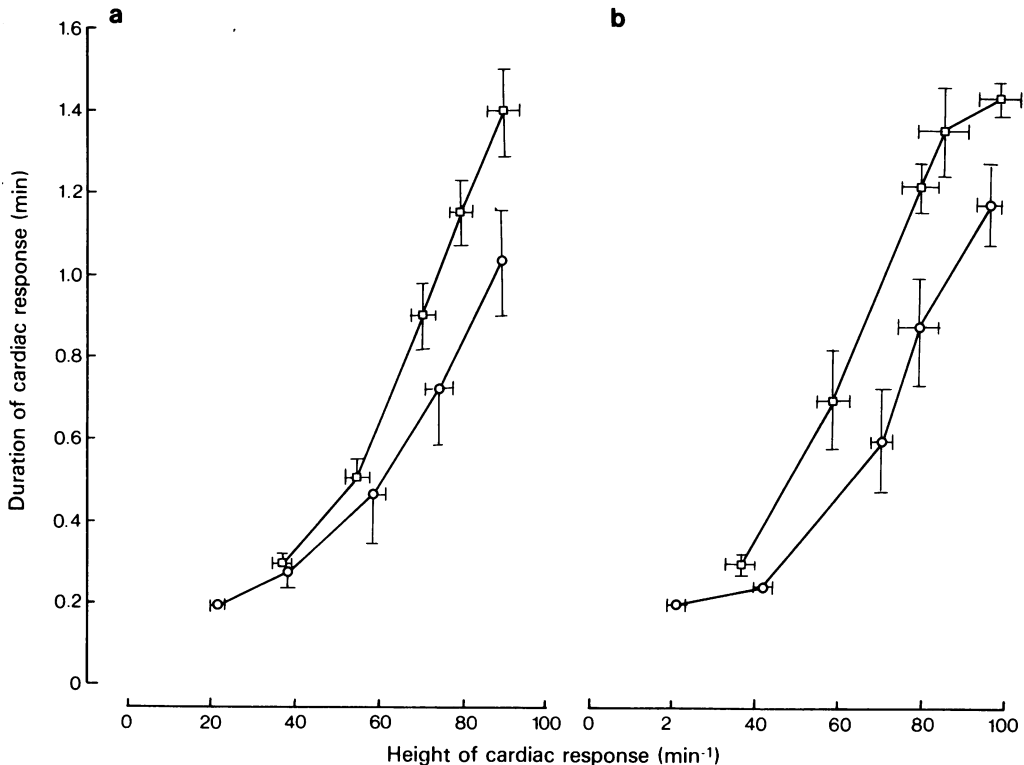


Figure 3 Effects of (a) pancuronium (1 mg/kg), (b) cocaine (0.5 mg/kg) on height and duration of the cardioaccelerator responses to spinal stimulation at 1 Hz, with trains of, from left to right in each case, 1, 2, 5, 10 and 20 pulses. Duration of response was assessed as time taken to fall from peak response to 50% of peak response. Control (○); in the presence of pancuronium (a) or cocaine (b) (□). Vertical bars represent s.e. mean. In each case $n = 6$.

the cardioaccelerator response in the pithed rat are critically dependent on the size of the control response (Docherty & McGrath, 1977b). Pancuronium (0.1 to 20 mg/kg) potentiated, in a dose-related manner, the cardioacceleration to single pulse stimulation (Figure 5a). Potentiation was maximal at 1 to 10 mg/kg. Cocaine (0.5 mg/kg) or desipramine (0.1 mg/kg) had effects similar in magnitude to the higher doses of pancuronium. Neither corticosterone (0.1 to 5 mg/kg) nor gallamine (10 mg/kg) potentiated the response to a single pulse.

Similarly pressor responses to a single pulse were potentiated by pancuronium in a dose-related manner, the maximal effect being obtained with 10 mg/kg; cocaine (0.5 mg/kg) had similar effects (Figure 5b).

Effects on responses to sympathomimetic agents

Cardioaccelerator responses The effects of pancuronium, cocaine and desipramine were examined on the tachycardia to injection of NA, tyramine or iso-

prenaline (Table 1). The response to noradrenaline (100 ng/kg) was potentiated by pancuronium (1 and 10 mg/kg), by cocaine (0.5 mg/kg) and by desipramine (0.1 mg/kg). The response to tyramine (10 μ g/kg) was not significantly affected by pancuronium (1 mg/kg) or by cocaine (0.5 mg/kg), but was significantly reduced by pancuronium (10 mg/kg) and cocaine (4 mg/kg). The response to isoprenaline (10 ng/kg) was not significantly affected by pancuronium (1 and 10 mg/kg), nor by desipramine (0.1 mg/kg).

Following sympathectomy, pancuronium, in doses that potentiated the response to NA in control rats, failed to potentiate the response to NA (20 ng/kg). It was necessary to use this reduced dose of NA to produce a response comparable with that to 100 ng/kg in controls due to the increased sensitivity to NA produced by 6-OHDA (Table 1).

Pressor Responses Pancuronium (1 and 10 mg/kg), cocaine (0.5 mg/kg) or desipramine (0.1 mg/kg) significantly potentiated the pressor effects of NA, but did

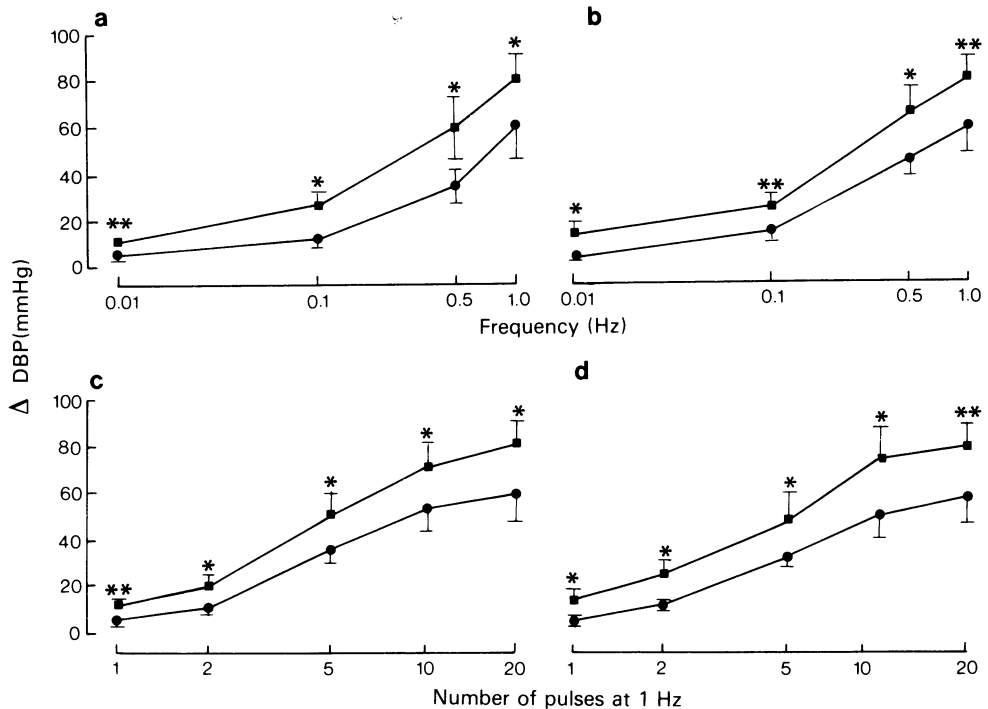


Figure 4 Comparison of the effects on vasopressor responses of pancuronium (1 mg/kg) (a and c) and of cocaine (0.5 mg/kg) (b and d). (a and b) Responses to 20 stimulus pulses at frequencies of 0.01–1 Hz. Control (●); after pancuronium (a) or cocaine (b) (■). (c and d) Responses to trains of 1–20 pulses at 1 Hz. Control (●); after pancuronium (c) or cocaine (d) (■). Vertical bars represent s.e. mean. In each case $n = 6$. Asterisks indicate responses significantly different from controls (Student's t test for paired data: * $0.01 < P < 0.05$; ** $0.001 < P < 0.01$).

not significantly affect the response to clonidine (10 $\mu\text{g}/\text{kg}$). However, pancuronium (1 and 10 mg/kg) and cocaine (0.5 and 4 mg/kg) had no significant effect on the response to tyramine (10 $\mu\text{g}/\text{kg}$) (Table 2) and pressor responses to higher doses of tyramine (100 or 1000 $\mu\text{g}/\text{kg}$) could not be significantly reduced by pancuronium (10 mg/kg) or cocaine (4 mg/kg).

Following sympathectomy, pancuronium, in doses which potentiated the pressor response to NA in control rats, failed to potentiate the response to NA (20 ng/kg) (Table 2).

Discussion

These results demonstrate that pancuronium bromide can raise blood pressure and heart rate in the absence of all nervous tone and can additionally potentiate, in both amplitude and duration, responses to cardiac and vasopressor sympathetic nerve stimulation.

The first effect could be due to (a) a direct action on the pacemaker cells or smooth muscle; an indirect

action either by (b) displacement of noradrenaline from nerves or (c) potentiation of the effects of spontaneously released noradrenaline due to uptake blockade; or (d) a combination of these. In the case of cardiac responses β -adrenoceptors are involved since propranolol abolished the tachycardia to pancuronium.

We attempted to distinguish between direct and indirect effects by producing sympathectomy with 6-OHDA and comparing alterations in the responses to pancuronium with those to the known indirect sympathomimetic, tyramine. The cardiac responses to tyramine and to pancuronium 10 mg/kg were significantly reduced by sympathectomy whereas the response to pancuronium 1 mg/kg was not. This suggests that pancuronium may have two cardiac actions, a direct effect at relatively low doses and a further indirect sympathomimetic effect at high doses. The vasopressor responses to pancuronium (1 or 10 mg/kg) were not significantly affected by sympathectomy suggesting a direct action. However, since the responses to tyramine (10 $\mu\text{g}/\text{kg}$) were also unaffected,

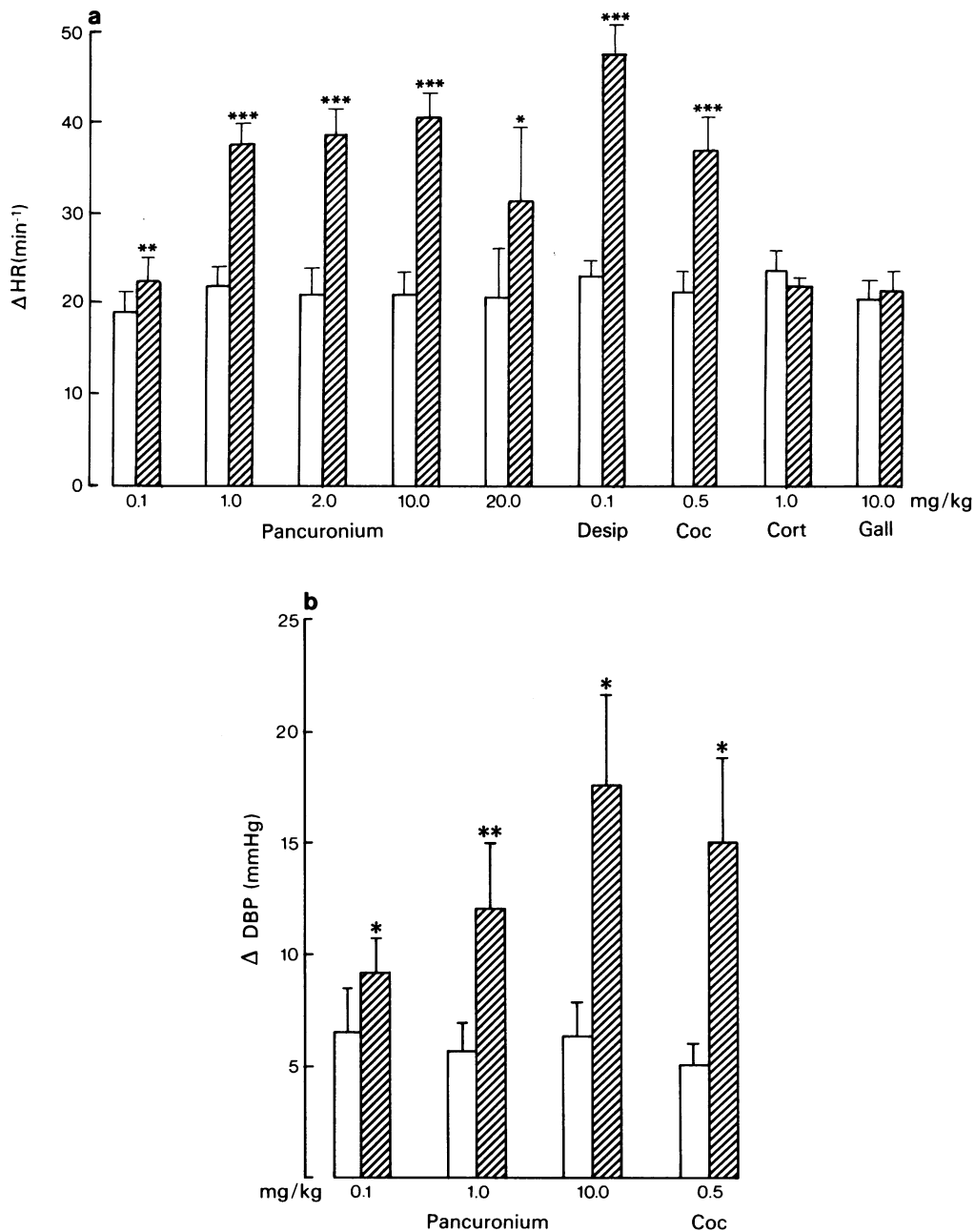


Figure 5 Effects of test drugs on cardioaccelerator or pressor responses to single stimulus pulses. Open columns: control; hatched columns: after test drug. (a) Cardioaccelerator. Test drugs from left to right; pancuronium (0.1, 1, 2, 10 and 20 mg/kg), $n = 5-13$; desipramine (Desip, 0.1 mg/kg), $n = 5$; cocaine (Coc, 0.5 mg/kg), $n = 8$; corticosterone (Cort, 1 mg/kg), $n = 3$; and gallamine (Gall, 10 mg/kg), $n = 6$. (b) Pressor. Test drugs from left to right; pancuronium (0.1, 1 and 10 mg/kg), $n = 5-8$; cocaine (Coc, 0.5 mg/kg), $n = 6$. Vertical bars represent s.e. mean. Asterisks indicate responses significantly different from controls (Student's t test for paired data: * $0.01 < P < 0.05$; ** $0.001 < P < 0.01$; *** $P < 0.001$).

Table 1 Effects of pancuronium and other test drugs on cardioaccelerator responses to injection of noradrenaline, tyramine or isoprenaline: effects of noradrenaline in sympathectomized rats were also examined

Agonist	Dose of test drug (mg/kg)	Control response	Response after test drug		n	P <
		ΔHR (mean \pm s.e. mean) (min ⁻¹)	ΔHR (mean \pm s.e. mean) (min ⁻¹)	ΔHR as % of control		
Noradrenaline (100 ng/kg)	Pancuronium (1)	26.8 \pm 4.3	44.8 \pm 3.7	177.8 \pm 21.8	4	0.001
	Pancuronium (10)	28.6 \pm 2.7	63.1 \pm 6.4	231.1 \pm 22.9	10	0.001
	Cocaine (0.5)	29.2 \pm 5.6	53.1 \pm 3.2	177.8 \pm 23.5	5	0.02
	Desipramine (0.1)	31.2 \pm 9.1	65.5 \pm 17.4	238.0 \pm 42.0	6	0.05
Tyramine (10 μ g/kg)	Pancuronium (1)	43.3 \pm 4.4	37.3 \pm 5.8	84.8 \pm 8.7	6	NS
	Pancuronium (10)	38.1 \pm 5.0	26.3 \pm 4.4	66.2 \pm 6.1	9	0.001
	Cocaine (0.5)	29.6 \pm 7.1	25.1 \pm 7.9	78.8 \pm 9.6	7	NS
	Cocaine (4.0)	29.6 \pm 11.1	16.6 \pm 9.3	45.7 \pm 11.6	5	0.01
Isoprenaline (10 ng/kg)	Pancuronium (1)	37.7 \pm 11.6	35.7 \pm 10.7	94.0 \pm 3.3	6	NS
	Pancuronium (10)	38.8 \pm 10.0	32.8 \pm 9.2	86.4 \pm 12.7	8	NS
	Desipramine (0.1)	40.0 \pm 19.0	35.0 \pm 17.4	77.9 \pm 20.5	5	NS
Sympathectomized rats-	Pancuronium (1)	47.3 \pm 8.3	42.3 \pm 7.7	89.9 \pm 4.5	7	NS
Noradrenaline (20 ng/kg)	Pancuronium (10)	41.0 \pm 6.9	31.8 \pm 7.5	73.2 \pm 6.2	8	0.01

Control responses and responses after test drug were compared by Student's *t* test for paired data (NS: not significant, when *P* > 0.05). Response after test drug was expressed as % of control for each rat.

the absence of an indirect effect cannot be definitely established.

Potential of sympathetic nerve responses by pancuronium showed clear characteristics of blockade of the neuronal uptake of noradrenaline. The relative susceptibilities to cocaine or pancuronium of responses to stimulation at different frequencies and train lengths were identical. Responses were always potentiated in height except where the control response approached maximal. Even in this latter case, responses, though unchanged in height, were pro-

longed which is to be expected if a major inactivation process were removed.

Another test for blockade of neuronal uptake of NA is the effect on responses to sympathomimetic amines whose actions may be modified by the uptake process. In the case of the cardiac response the results were straightforward. Pancuronium, cocaine and desipramine each blocked uptake since the response to isoprenaline was not affected, to noradrenaline was potentiated and to tyramine was inhibited. However, with the vasopressor response pancuronium and

Table 2 Effects of pancuronium and other test drugs on pressor responses to injection of noradrenaline, tyramine, or clonidine: effects of noradrenaline in sympathectomized rats were also examined

Agonist	Dose of test drug (mg/kg)	Control response	Response after test drug		n	P <
		Δ DBP (mean \pm s.e. mean) (mmHg)	Δ DBP (mean \pm s.e. mean) (mmHg)	Δ DBP as % of control		
Noradrenaline (100 ng/kg)	Pancuronium (1)	22.5 \pm 3.2	30.8 \pm 5.3	136.8 \pm 10.9	6	0.02
	Pancuronium (10)	24.1 \pm 2.5	50.3 \pm 7.4	215.9 \pm 33.3	11	0.01
	Cocaine (0.5)	24.5 \pm 6.1	35.4 \pm 8.4	150.9 \pm 12.3	5	0.02
	Desipramine (0.1)	26.0 \pm 3.4	50.0 \pm 11.0	186.9 \pm 23.7	5	0.05
Tyramine (10 μ g/kg)	Pancuronium (1)	9.9 \pm 3.0	7.1 \pm 3.0	80.0 \pm 19.8	5	NS
	Pancuronium (10)	8.8 \pm 2.0	7.3 \pm 3.3	71.1 \pm 17.1	6	NS
	Cocaine (0.5)	9.7 \pm 1.7	8.3 \pm 1.9	82.4 \pm 6.6	7	NS
	Cocaine (4.0)	9.3 \pm 1.0	9.1 \pm 3.6	92.0 \pm 39.8	5	NS
Clonidine (10 μ g/kg)	Pancuronium (1)	54.0 \pm 7.6	47.4 \pm 4.0	87.8	10	NS
Sympathectomized rats	Pancuronium (1)	13.2 \pm 2.4	13.1 \pm 2.1	103.4 \pm 7.6	6	NS
Noradrenaline (20 ng/kg)	Pancuronium (10)	15.5 \pm 2.6	16.8 \pm 3.3	101.7 \pm 10.8	9	NS

Control responses and responses after test drug were compared by Student's *t* test for paired data (NS: not significant, when *P* > 0.05). Response after test drug was expressed as % of control for each rat, except in the case of clonidine where mean response after test drug is expressed as % of control mean response.

cocaine potentiated the response to NA, as might be expected with blockade of neuronal NA uptake, but did not block the response to tyramine. Nevertheless, doses of uptake blockers which potentiate the response to NA do not abolish the sympathomimetic effect of tyramine on several tissues including rat vas deferens (Barnett, Staub & Sychowicz, 1969) and rat iris (Lagercrantz, 1968). These latter findings may reflect the fact that such doses may not completely prevent the access of tyramine to the NA storage pools and will also potentiate the action of any NA which is released. The net effect will, therefore, depend on a balance between these two actions (see Trendelenburg, 1972). The pressor response to clonidine, an

agonist not subject to neuronal uptake (Autret *et al.*, 1971) was not affected by pancuronium (1 mg/kg), further confirming the prejunctional location of the potentiating effect of pancuronium.

Following 6-OHDA pretreatment, cardiac and pressor responses to NA were potentiated due to the absence of neuronal NA uptake (Iversen, 1967). In this latter case, pancuronium and cocaine both failed to potentiate the responses to NA, once more confirming that the potentiation in untreated rats was due to blockade of uptake.

The effectiveness of sympathectomy was indicated by the displacement to the right of the cardiac sympathetic nerve frequency-response curve. Residual re-

sponses at relatively high frequencies were found such as occur in tissues with a normally dense adrenergic innervation like anococcygeus even when adrenergic terminals can no longer be demonstrated histologically (McGrath, 1973).

This blockade of noradrenaline uptake by pancuronium is of obvious significance where pancuronium is employed as relaxant in experimental work where alterations in sympathetic transmission may be relevant. For example pancuronium has been employed to prevent muscle twitching in pithed rats (Gillespie *et al.*, 1970; Gillespie & McGrath, 1973; Clanachan & McGrath, 1976; Clanachan & Muir, 1978). Of these examples, the only ones in which blockade of NA uptake by pancuronium might have affected the result were investigations of the effects of anaesthetics on autonomic responses in the pithed rat (Clanachan & McGrath, 1976; Clanachan & Muir, 1978). Fortunately, the effects of the anaesthetic agent ketamine, which is the only one suggested to affect NA uptake (Nedergaard, 1973), were also investigated on pithed rabbits and cats in which gallamine was the relaxant and the results were similar to those in the rat (Clanachan, McGrath & Mackenzie, 1976). The present results indicate that gallamine does not share with pancuronium the additional effect of blockade of neuronal NA uptake. Similarly ketamine (1 to 50 mg/kg) failed to potentiate the cardioacceleration response to a single stimulus pulse in the absence of any neuromuscular blocker (authors' unpublished observations).

While our main interest was to clarify the effects

of pancuronium in animal experimentation these results may have clinical significance. We concentrated mainly on a dose of pancuronium of 1 mg/kg in order to obtain large enough effects to be analysed by pharmacological methods. While this is far in excess of the common clinical dose of 0.1 mg/kg (Coleman, Downing, Leary, Moyes and Styles, 1972), in the pithed rat prolonged blockade of skeletal muscle twitching requires at least 1 mg/kg (see Gillespie, MacLaren & Pollock, 1970; Clanachan & McGrath, 1976). It has also been demonstrated in the pithed rat that while pancuronium (0.08 mg/kg) produces approximately 90% block of the twitching of anterior tibialis muscle in response to 0.1 Hz stimulation of the spinal somatic motor outflow, there is virtual recovery after 5 min (Clanachan & Muir, 1972). Even at the lowest dose which we tested of pancuronium, 0.1 mg/kg, cardiac sympathetic responses were significantly potentiated. It remains a possibility, therefore, that at clinical levels of 0.1 mg/kg those patients who are most susceptible to blockade of neuronal uptake of NA might respond with cardiovascular stimulation. This does not contradict the hypothesis that part of the cardiovascular stimulation which occurs in some patients may be due to a vagolytic action (Hughes & Chapple, 1976) but may suggest an additional effect.

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