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THE PHARMACOLOGY OF PANCURONIUM BROMIDE (ORG.NA97), A NEW POTENT STEROIDAL NEUROMUSCULAR BLOCKING AGENT

BY

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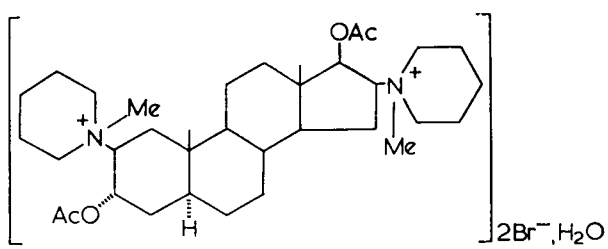
The incorporation of a biologically active group into a naturally occurring structure is an interesting approach to new pharmacological agents. For example, when an acetylcholine-like structure is incorporated in ring-A of 5α-androstane-17-one or 5α-pregnan-20-one, neuromuscular blocking agents of up to one-fifteenth the potency of tubocurarine are obtained (Lewis, Martin-Smith, Muir & Ross, 1967). Although of limited clinical value, such compounds present useful leads for further chemical exploration, particularly the synthesis of bisquaternary amino-steroidal salts. A series of 2β,16β-diamino-5α-androstane-3α,17β-diol dimethohalide derivatives was described by Buckett, Hewett & Savage (1967). One of them, 2β,16β-dipiperidino-5α-androstane-3α,17β-diol diacetate dimethobromide (code number Org.NA97; approved name pancuronium bromide), is a potent neuromuscular blocking agent and in the experiments described in this paper its actions were compared with those of tubocurarine chloride.



METHODS

Nerve-muscle preparations

Cats were anaesthetized with a mixture of chloralose (70 mg/kg) and sodium pentobarbitone (12 mg/kg) injected intraperitoneally; rabbits and dogs were anaesthetized with sodium pentobarbitone (45 mg/kg) injected intravenously, and hens with sodium barbitone (200 mg/kg) injected intravenously. In all species the trachea was intubated and artificial ventilation applied throughout each experiment. Twitches and tetani of a gastrocnemius muscle were elicited by stimulating the peripheral portion of the crushed sciatic nerve in the popliteal space with rectangular shocks of

0.5 msec duration and of about twice the strength necessary to evoke a maximal twitch. When the muscles were stimulated directly, stimuli of 1 msec duration were applied between a platinum wire inserted through the musculo-tendinous junction and an electrode attached to the pin through the femur. The strength of the shocks was such as to produce contractions equal in size to an indirectly elicited maximal twitch. Contractions were recorded by means of flat steel spring myographs writing on smoked paper. In cats, dogs and rabbits, blood pressure was recorded from a common carotid artery by means of a mercury manometer. Drugs were injected intravenously through a cannula in a jugular vein.

In two experiments on cats, maximal twitches of a tibialis anterior muscle were recorded and the muscle was prepared for the close-arterial injection of acetylcholine as described by Brown (1938).

In two further experiments on cats, gross muscle action potentials were recorded from the gastrocnemius muscle simultaneously with nerve action potentials recorded antidromically from the peripheral end of the severed S1 ventral root. After amplification by Tektronix (type 122) preamplifiers, the muscle and nerve action potentials were displayed simultaneously on a Tektronix (type 502) dual beam oscilloscope. The method used was identical with that described by Blaber & Bowman (1963).

Muscle temperature was recorded using a thermocouple probe (type RM4) connected to an electrothermometer (Ellab, Copenhagen, type TE3). In the experiment to show effects at different temperatures, the temperature of the muscle was varied by direct heating with a lamp or by surrounding the muscle with crushed ice, and by varying the operating table temperature.

Experiments were also made on isolated phrenic nerve-diaphragm preparations of rats (Bülbring, 1946). The preparation was suspended in a 100 ml. organ bath of Ringer solution containing (g/l.): NaCl, 8, KCl, 0.2, $CaCl_2$, 0.14, glucose, 2, $NaHCO_3$, 0.36, and bubbled with a mixture of 95% oxygen and 5% carbon dioxide. Twitches were elicited at a frequency of 4/min by stimulating the nerve with rectangular pulses of 1 msec duration and of a strength greater than that necessary to evoke a maximal twitch. The potency of pancuronium was compared with that of tubocurarine using a 2×2 assay design.

Rectus abdominis muscles of frogs were suspended in frog Ringer solution as described by Jindal & Deshpande (1960).

Conscious animals

Albino mice weighing 18–22 g were distributed into groups of ten and different concentrations of pancuronium or tubocurarine were infused into the tail veins of the mice in each group. The infusion was administered at a rate of about 0.125 ml./min through flexible nylon tubing (00 gauge) and the shaft of a size 16 hypodermic needle. The time at which each mouse failed to retain its grip on a vertical wire mesh screen (1 cm mesh) was noted and converted into infusion time/20 g body weight. Concentrations of drugs were adjusted to give a similar end point within 3 min of infusion. The duration of paralysis was assessed by noting the time interval until the animals could again retain their grip on the screen.

Rabbits (New Zealand white males) weighing 3.0–3.1 kg were distributed into groups of eight and the potency and duration of action of pancuronium compared with those of tubocurarine were assessed by the head-drop method of Haining, Johnston & Smith (1960).

Pancuronium was also injected intraperitoneally into day old chicks.

Ganglion blocking action

Ganglion blocking activity of pancuronium was assessed in two ways. (1) Its potency in depressing contraction of the isolated guinea-pig ileum evoked by nicotine was compared with that of tubocurarine and of hexamethonium (Feldberg, 1951). (2) Its potency in depressing contractions of the nictitating membrane evoked by preganglionic stimulation of the cervical sympathetic trunk of cats

under sodium pentobarbitone anaesthesia was compared with that of hexamethonium. The drugs were injected intravenously.

Atropine-like action

The potency of pancuronium in depressing contractions of the isolated guinea-pig ileum evoked by acetylcholine was compared with that of atropine. The superfusion method of Adam, Hardwick & Spencer (1954) was used.

Histamine release

Histamine release by pancuronium was investigated in two species. In cats, the delayed depressor response described by Collier & Macauley (1952) was studied and in guinea-pigs the broncoconstriction test of Konzett & Rossler (1940) was used.

Acute toxicity

LD50 values were determined in male albino Wistar rats (100–150 g) after intravenous injection, in male albino mice (18–22 g) after intravenous, intraperitoneal, subcutaneous and oral administration, and in rabbits (3.0–3.1 kg) after intravenous injection. Ten animals were used in each group and the method of calculation, based on 24 hr data, was that of Litchfield & Wilcoxon (1949).

Pancuronium was also injected intravenously in cats anaesthetized and artificially ventilated as described above. The doses were increased to determine the maximum tolerated dose.

Drugs

The drugs used were: (−) adrenaline acid tartrate (British Drug Houses Ltd.); edrophonium chloride (Roche Products Ltd.); hexamethonium bromide (May & Baker Ltd.); histamine acid phosphate (British Drug Houses Ltd.); neostigmine methylsulphate (Roche Products Ltd.); nicotine acid tartrate (British Drug Houses Ltd.); potassium chloride (Analar, British Drug Houses Ltd.); suxamethonium iodide (Koch-Light Laboratories Ltd.); tubocurarine chloride (Burroughs, Wellcome & Co.); and pancuronium bromide (Organon Laboratories Ltd.; NA97). Before use the drugs were dissolved in 0.9% w/v NaCl solution except for the isolated tissue preparations when the appropriate Ringer solution was used. The doses quoted in the text refer to the salts.

RESULTS

Neuromuscular blocking action

In doses of 0.02–0.05 mg/kg injected intravenously, pancuronium blocked the indirectly elicited maximal twitches of the gastrocnemius and tibialis anterior muscles of the cat. During the block of the indirectly elicited twitches, direct stimulation of the muscle produced normal contractions. In experiments in which nerve and muscle action potentials were evoked simultaneously, the same doses of pancuronium abolished the muscle action potentials but the nerve action potentials remained unaffected. These results locate the site of action of pancuronium at the neuromuscular junction.

Contractions of the tibialis anterior muscle of the cat evoked by close-arterial injection of acetylcholine 5 μg were completely blocked by the intravenous injection of pancuronium 0.01–0.02 mg/kg, even though the lower doses in this range only partially depressed the maximal twitches evoked by nerve stimulation. This result shows that the action of pancuronium is essentially a post-junctional anti-acetylcholine action resembling that of tubocurarine, and this conclusion is supported by results on the rectus abdominis of the frog in which pancuronium, in concentrations of 0.1–0.3 μg/ml. abolished contactures produced by acetylcholine (3 μg/ml.).

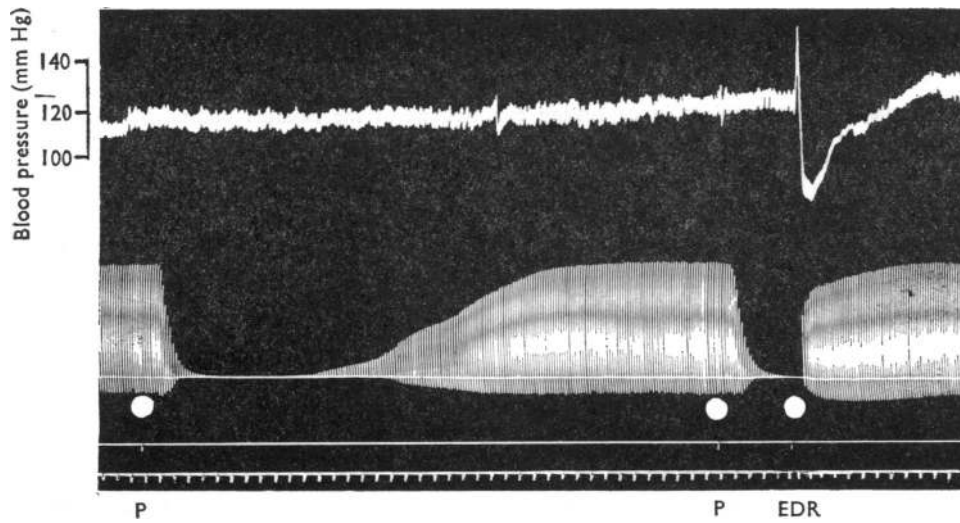


Fig. 1. Neuromuscular blocking action of pancuronium and its reversal by edrophonium. Cat, 3.3 kg. Chloralose-pentobarbitone anaesthesia. Lower trace. Sciatic-gastrocnemius preparation. Drugs intravenously: at P, pancuronium bromide 0.02 mg/kg; at EDR, edrophonium chloride 1 mg/kg. Upper trace: arterial blood pressure. Time scale: min.

Neuromuscular block produced by pancuronium in anaesthetized cats exhibited the characteristics of block produced by non-depolarizing blocking drugs. Thus:

(1) The block was antagonized by edrophonium (1 mg/kg) (Fig. 1) and by neostigmine (0.1 mg/kg) (Fig. 2a).

(2) Suxamethonium in a dose of 0.1 to 0.2 mg/kg antagonized neuromuscular block produced by pancuronium (Fig. 2b).

(3) A small dose of tubocurarine (0.02 mg/kg), insufficient by itself to depress the twitches, augmented a partial block produced by pancuronium (Fig. 2c).

(4) Potassium chloride in a dose of 1 mg/kg antagonized partial block produced by pancuronium.

(5) Adrenaline in doses of 0.01 to 0.1 mg/kg antagonized partial block produced by pancuronium.

(6) During partial block of the maximal twitches produced by pancuronium, the tension of an indirectly elicited tetanus (50/sec for 8 sec) was poorly sustained and the post-tetanic twitches were temporarily increased in amplitude (Fig. 3).

(7) The block produced by pancuronium (0.012 mg/kg) in the cat was smaller in extent at low muscle temperatures. Muscle temperature was varied over the range 23°–43° C.

Absence of depolarizing action is expected of a bulky molecule such as pancuronium and was confirmed by results in avian and frog muscles in which there was no evidence of the contractual response characteristic of depolarizing drugs. In the anaesthetized hen, pancuronium in a dose of 0.05 mg/kg blocked the indirectly elicited maximal twitches. Fig. 4 compares the effects of pancuronium and tubocurarine on the gastrocnemius muscle

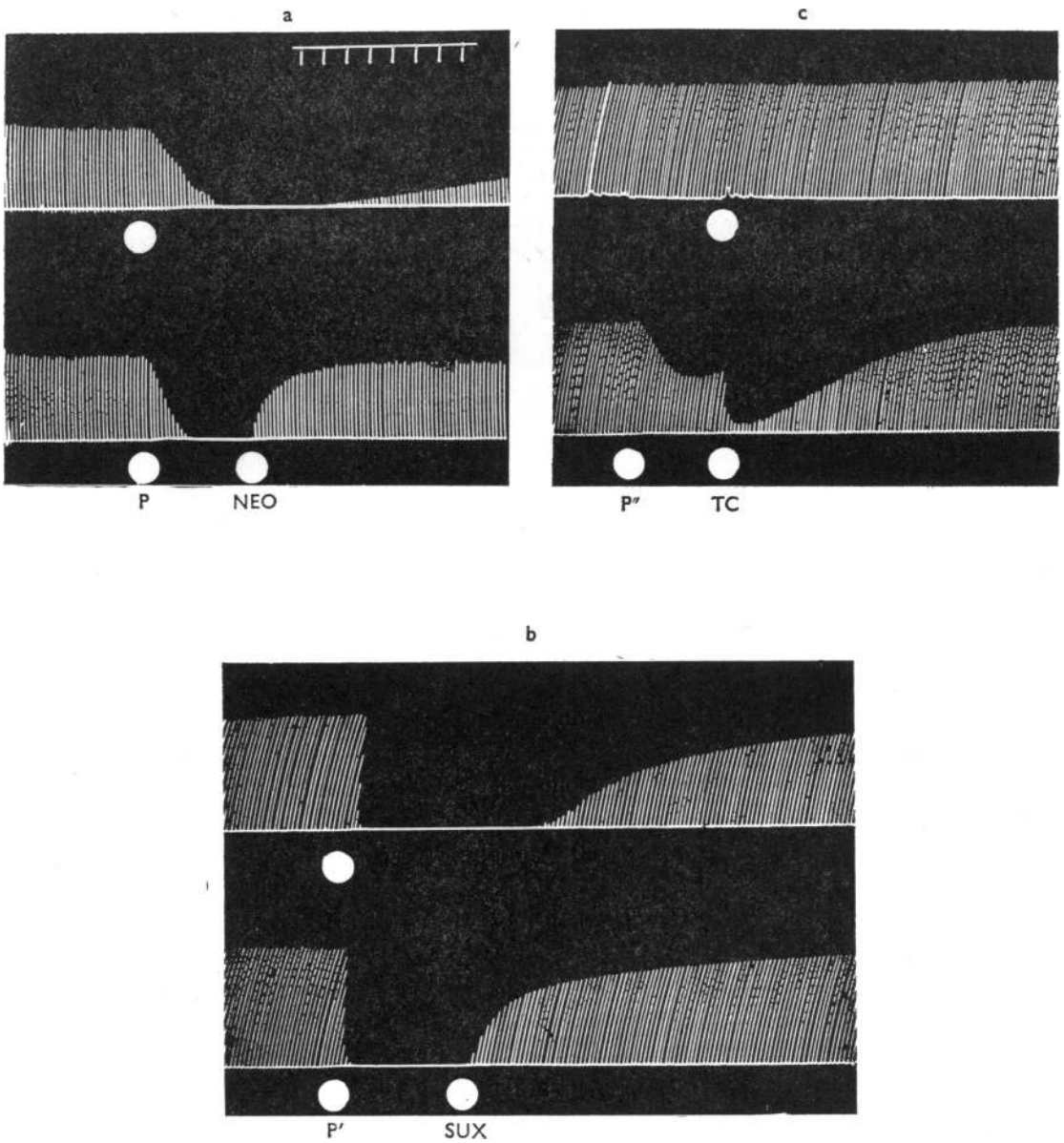


Fig. 2. Cat (2.2 kg) anaesthetized with chloralose-sodium pentobarbitone. Sciatic-gastrocnemius preparation. Drugs given intravenously. (a) Upper trace: at P, pancuronium bromide 0.013 mg/kg. Lower trace: at P, pancuronium bromide 0.013 mg/kg followed at NEO by neostigmine methylsulphate 0.1 mg/kg. (b) Upper trace: at P′, pancuronium bromide 0.02 mg/kg. Lower trace: at P′, pancuronium bromide 0.02 mg/kg, followed at SUX by suxamethonium iodide 0.125 mg/kg. (c) Upper trace: at TC, tubocurarine chloride 0.02 mg/kg. Lower trace: at P″, pancuronium bromide 0.015 mg/kg followed at TC by tubocurarine 0.02 mg/kg. Time scale: min.

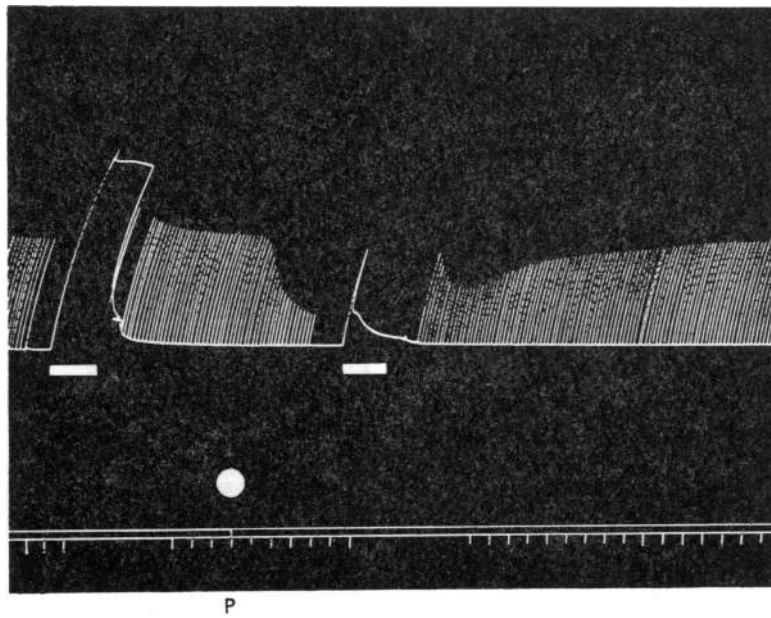


Fig. 3. Cat (2.4 kg) anaesthetized with chloralose-sodium pentobarbitone. Sciatic-gastrocnemius preparation. At P, pancuronium bromide 0.02 mg/kg intravenously. At bars, indirectly elicited tetanus (50/sec for 8 sec). Time scale: min, increased during tetanus.

of the hen and contrasts this effect with the contractual response to suxamethonium. In the conscious chick doses of pancuronium 1 mg/kg injected intraperitoneally induced flaccid paralysis and in the isolated rectus abdominis muscle of the frog, concentrations of 0.1–0.3 μg/ml. were without obvious effect by themselves but blocked responses to acetylcholine. All these results are characteristic of blocking drugs of the non-depolarizing type such as tubocurarine (see, for example, Bowman, 1964).

Relative potency

Table 1 gives the results obtained from experiments in which the potency and duration of action of pancuronium was compared with those of tubocurarine in different species. Figures 5 and 6 illustrate the results obtained in nineteen anaesthetized cats in which the potency and duration of action was compared with those of tubocurarine. Pancuronium was of the order of ten times more potent than tubocurarine (Fig. 5) on a weight basis, but the duration of action of equieffective doses (Fig. 6) was similar. Similar relative potencies were obtained from experiments on anaesthetized rabbits and hens, and in the rabbit head drop test (Table 1). In the anaesthetized dog, however, pancuronium was only about five times more potent than tubocurarine, although its duration of action was about the same (Table 1). In the mouse, pancuronium was only slightly more potent than tubocurarine and its duration of action was about three times longer (Table 1). In the isolated diaphragm of the rat, pancuronium was slightly less potent than tubocurarine and in the isolated rectus abdominis muscle of the frog it was about twice as potent

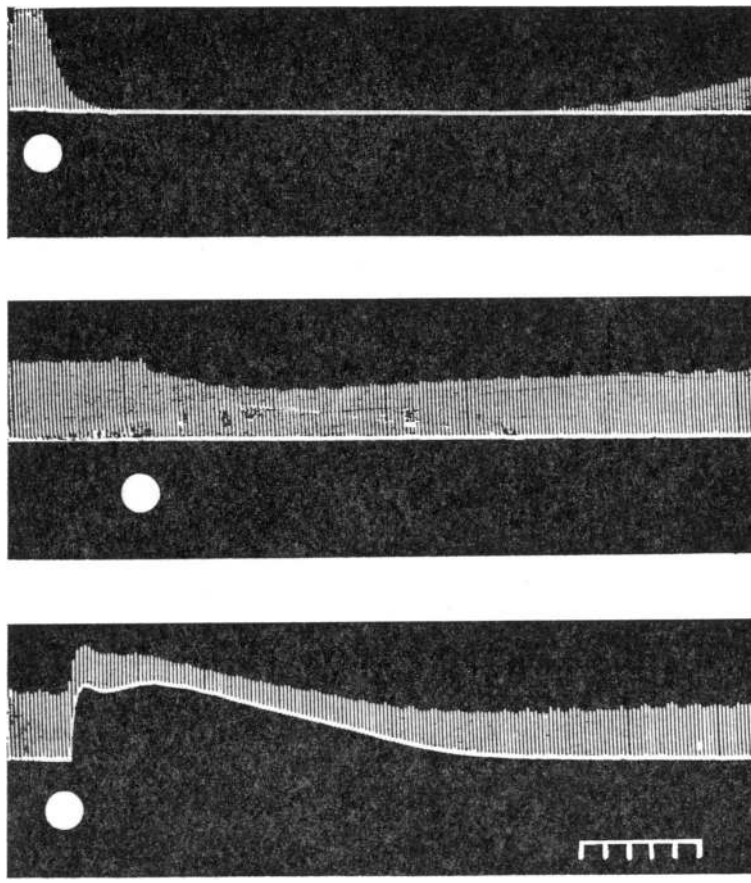


Fig. 4. Effect of neuromuscular blocking agents on avian muscle. Hen anaesthetized with barbitone. Sciatic gastrocnemius preparation. Drugs given intravenously. Upper panel: pancuronium bromide, 0.05 mg/kg. Central panel: tubocurarine chloride, 0.3 mg/kg. Lower panel: suxamethonium iodide, 0.05 mg/kg. Time scale: min.

(Table 1). Figure 7 illustrates the effective neuromuscular blocking doses of pancuronium and tubocurarine in the rabbit, the cat, the dog, the mouse and also in man (Baird & Reid, 1967).

Ganglion blocking action

The ganglion blocking potency of pancuronium compared with that of tubocurarine and hexamethonium is given in Table 2. Pancuronium is about four times less potent than hexamethonium and about half as potent as tubocurarine in blocking the response of the guinea-pig ileum to nicotine and is about eight times less potent than hexamethonium in blocking the contractions of the cat nictitating membrane evoked by preganglionic stimulation of the cervical sympathetic trunk. In none of the experiments on anaesthetized animals did pancuronium produce a fall in blood pressure confirming that its ganglion blocking action is weak.

TABLE 1

THE RELATIVE POTENCY AND DURATION OF ACTION OF PANCURONIUM IN DIFFERENT TESTS

The results represent relative potency and duration of action of pancuronium, where tubocurarine = 1·0. In anaesthetized animals the sciatic–gastrocnemius preparation was used. The relative potency and duration of action were determined graphically in comparison with tubocurarine, using dose-response lines from the same animal. Standard deviations are given except in the case of cats where a direct estimate was made from the lines of best fit from nineteen combined experiments and in hens when an approximation was obtained. In conscious animals the potency and duration of action compared to tubocurarine was determined in mice by direct graphical comparison of intravenous infusion times to loss of grip-strength and recovery, and in rabbits by the head-drop technique of Haining, Johnston & Smith (1960). Determinations of relative potency in isolated tissues were made using a 2×2 assay design in comparison with tubocurarine.

Species	No. of estimates	Relative potency (tubocurarine = 1·0)	Duration of action (tubocurarine = 1·0)
(a) Anaesthetized animals			
Rabbit	3	11·5 ±2·7	0·99±0·26
Cat	19	9·41	1·15
Dog	2	5·19±1·13	0·88±0·17
Hen	3	10 (approx.)	1 (approx.)
(b) Conscious animals			
Mouse	4	1·88±0·04	3·21±0·08
Rabbit	4	10·9 ±0·58	1·06±0·57
(c) Isolated tissues			
Rat diaphragm	6	0·60±0·004	—
Frog rectus abdominis	6	2·3 ±0·55	—

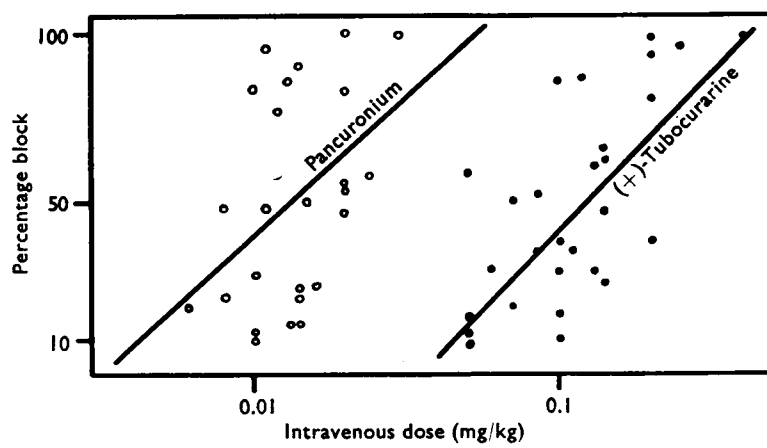


Fig. 5. Dose-response lines of best fit for neuromuscular block on the sciatic-gastrocnemius preparation of the cat anaesthetized with chloralose-pentobarbitone after pancuronium and tubocurarine. Abscissa (log scale): intravenous dose of drug in mg/kg body weight. Ordinate: percentage neuromuscular block. Results of nineteen experiments.

Atropine-like activity

Pancuronium was found to be almost devoid of atropine-like activity in the guinea-pig ileum. In comparison with atropine (potency = 1.0) the mean of three estimates of relative potency of pancuronium was 2.6×10^{-8}.

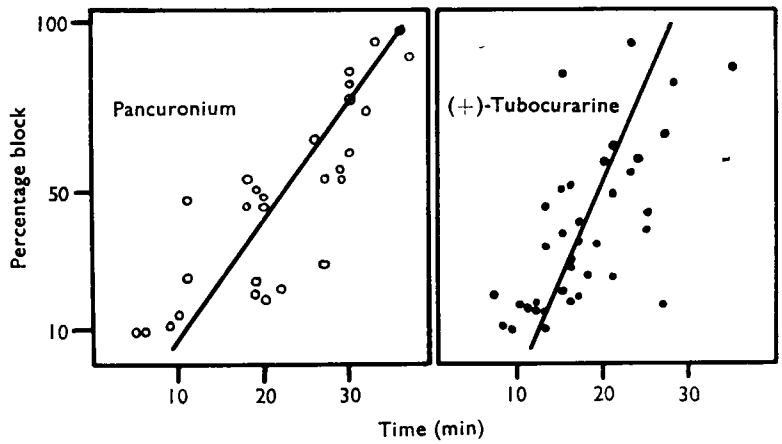


Fig. 6. Relationship between neuromuscular block and duration of action for pancuronium and tubocurarine on the cat sciatic-gastrocnemius preparation. Abscissa: duration in minutes. Ordinate: percentage neuromuscular block. Combined results of nineteen experiments computed to give lines of best fit.

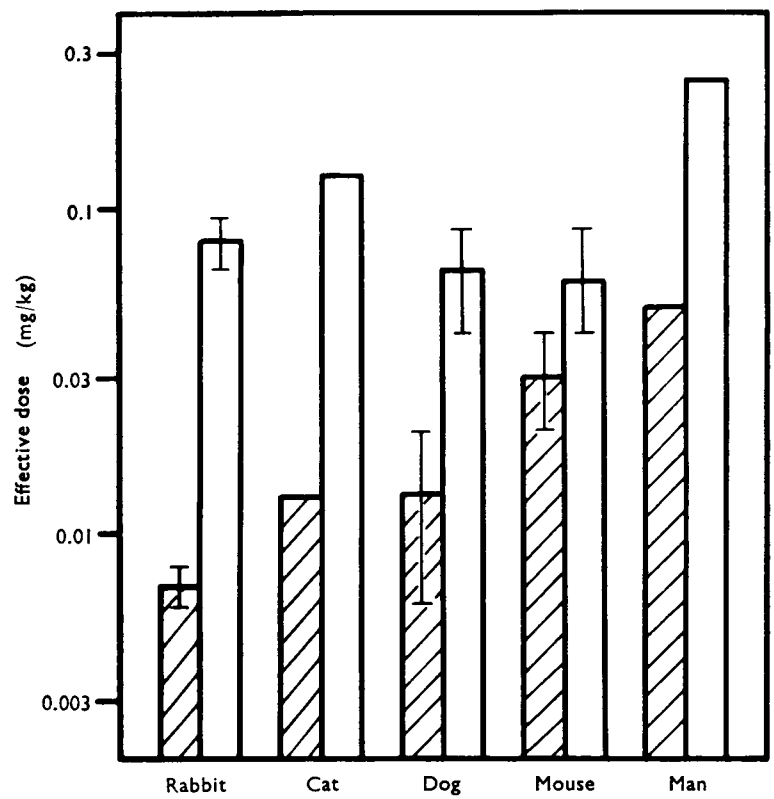


Fig. 7. The effective neuromuscular blocking doses of pancuronium bromide (▨) and tubocurarine (□) in various species (±S.D.). Rabbit, cat and dog sciatic-gastrocnemius preparation; mouse grip-strength. Values in man from Baird & Reid (1967) for block in ulnar nerve-hypothenar muscles.

TABLE 2

THE GANGLIONIC BLOCKING POTENCY OF PANCURONIUM *IN VITRO* AND *IN VIVO*

The results indicate relative ganglionic blocking potency where either hexamethonium or tubocurarine = 1·0, obtained using direct graphical comparison. *In vitro*, the nicotine-stimulated isolated guinea-pig ileum method of Feldberg (1951) was used, and *in vivo* contractions of the pentobarbitone-anaesthetized cats nictitating membrane in response to preganglionic stimulation of the cervical sympathetic trunk was utilized, drugs being injected intravenously.

Preparation	No. of estimates	Ganglionic blocking potency (± standard deviation)	
		Hexamethonium = 1·0	Tubocurarine = 1·0
Nicotine stimulated guinea-pig ileum	6	0·251±0·013	0·563±0·016
Nictitating membrane of anaesthetized cat	5	0·120±0·012	—

Histamine release

Bronchoconstriction produced by histamine and tubocurarine in anaesthetized guinea-pigs is illustrated in Fig. 8. Pancuronium, even at the very high dose level of 10 mg/kg, had no such effect, which indicates the lack of histamine-releasing action of the drug. This was confirmed by the stable blood pressure following injection of pancuronium in the cat treated with hexamethonium.

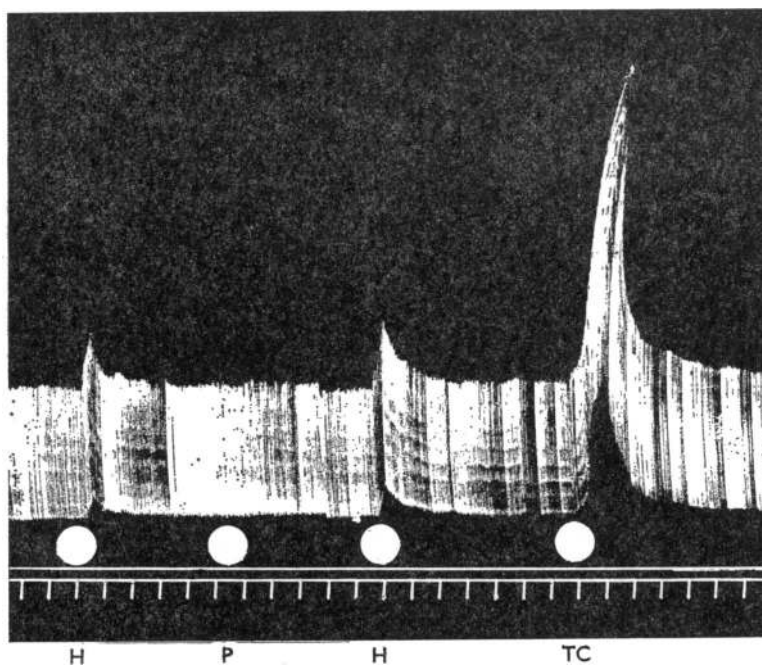


Fig. 8. Resistance to inflation of guinea-pig lungs *in vivo*. At H, histamine 2.0 μg; at P, pancuronium bromide 10 mg/kg; and at TC, tubocurarine chloride 10 mg/kg. Guinea-pig body weight, 300 g. Time scale: min.

Acute toxicity

The LD50 values for pancuronium in various species are given in Table 3. In all cases death was caused by respiratory failure arising from the neuromuscular blocking action of the drug. The results in mice show that toxicity is greatest following intravenous injection and least after oral administration. The high levels necessary by the oral route indicate that absorption is poor, as might be expected from this type of molecule. The rabbit is more sensitive to pancuronium than the mouse which in turn is much more sensitive than the rat. In anaesthetized and artificially ventilated cats, pancuronium up to 500 mg/kg produced no acute toxicity for up to 2 hr after injection.

TABLE 3

ACUTE TOXICITY OF PANCURONIUM IN VARIOUS SPECIES

95% limits of error are shown in parentheses.

Species	Route	LD 50 (mg/kg)
Mouse	Intravenous	0·047 (0·045–0·050)
	Intraperitoneal	0·152 (0·144–0·160)
	Subcutaneous	0·167 (0·158–0·175)
	Oral	21·9 (19·0 –25·2)
Rat	Intravenous	0·153 (0·136–0·172)
Rabbit	Intravenous	0·016 (0·015–0·018)

DISCUSSION

The results show that pancuronium is a potent neuromuscular blocking agent with a duration of action comparable with that of tubocurarine. The evidence suggests that its mechanism of action resembles that of tubocurarine. In contrast to tubocurarine, its ganglion-blocking potency and histamine releasing properties are weak and hence the side effects of hypotension and bronchospasm associated with tubocurarine are absent with pancuronium. These findings have been substantiated clinically (Baird & Reid, 1967). The data on species sensitivity indicate that dosage in the mouse on a mg/kg basis approximates most closely to the human dose (Fig. 7).

In relation to known drugs, pancuronium is one of the most potent neuromuscular blocking agents yet reported. A naturally occurring steroidal alkaloid, malouétine (3β,20β-bis dimethylamino-5α-pregnane bismethochloride) and its steroisomers (Khuong Huu-Lainé & Pinto-Scognamiglio, 1964) possess similar potency to tubocurarine but produce hypotension when injected. Recently, Alauddin, Caddy, Lewis, Martin-Smith & Sugrue (1965) and Bamford, Biggs, Davis & Parnell (1967) synthesized and tested stereoisomers of dialkylamino- and cyclic amino-steroids substituted at positions 3 and 17; they all possessed lower potency than tubocurarine in cat or monkey. In pancuronium, the acetyl groups present at positions 3 and 17 adjacent to the 2,16 amino functions could afford some steric compression in rings A and D of the steroid molecule, so that ring-A is held in an intermediate skew position between the possible rigid configurations (boat or chair), which might be optimal for association with a receptor site, and thus contribute to the high potency obtained.

SUMMARY

1. The neuromuscular blocking activity of a new amino-steroid, pancuronium (2β,16β-dipiperidino-5α-androstane-3α,17β-diol diacetate dimethobromide) is described.

2. Pancuronium possesses up to ten times the potency of tubocurarine according to the species used for testing, while possessing similar duration of action. Its potency in the mouse most closely approximates to that in man.

3. Pancuronium has a non-depolarizing mode of action, is free from histamine releasing properties and ganglion blocking potency, and does not produce hypotension.

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REFERENCES

ADAM, H. M., HARDWICK, D. C. & SPENCER, K. E. V. (1954). Assay of histamine on the isolated guinea-pig intestine by the method of superfusion. *Br. J. Pharmac. Chemother.*, **9**, 360–366.

ALAUDDIN, M., CADDY, B., LEWIS, J. J., MARTIN-SMITH, M. & SUGRUE, M. F. (1965). Non-depolarizing neuromuscular blockade by 3α,17α-bis (quaternary ammonium) 5α-androstanes. *J. Pharm. Pharmac.*, **17**, 55–59.

BAIRD, W. L. M. & REID, A. M. (1967). The neuromuscular blocking properties of a new steroid compound, pancuroniun bromide. *Br. J. Anaesth.*, **39**, 775–780.

BAMFORD, D. G., BIGGS, D. F., DAVIS, M. & PARNELL, E. W. (1967). Neuromuscular blocking properties of stereoisomeric androstane-3,17-bisquaternary ammonium salts. *Br. J. Pharmac. Chemother.*, **30**, 194–202.

BLABER, L. C. & BOWMAN, W. C. (1963). Studies on the repetitive discharges evoked in motor nerve and skeletal muscle after injection of anticholinesterase drugs. *Br. J. Pharmac. Chemother.*, **20**, 326–344.

BOWMAN, W. C. (1964). Neuromuscular blocking agents. In *Evaluation of drug activities: pharmacometrics*, vol. 1, ed. Laurence, D. R. & Bacharach, A. L., pp. 325–351. London and New York: Academic Press.

BROWN, G. L. (1938). The preparation of the tibialis anterior (cat) for close arterial injection. *J. Physiol., Lond.*, **92**, 22–23 *P.*

BUCKETT, W. R., HEWETT, C. L. & SAVAGE, D. S. (1967). Potent steroidal neuromuscular blocking agents. *Chim. Ther.*, **2**, 186.

BÜLBRING, E. (1946). Observations on the isolated phrenic nerve-diaphragm of the rat. *Br. J. Pharmac. Chemother.*, **1**, 38–61.

COLLIER, H. O. J. & MACAULEY, B. (1952). The pharmacological properties of "Laudolissin", a long-acting curarizing agent. *Br. J. Pharmac. Chemother.*, 7, 398–408.

FELDBERG, W. (1951). Effect of ganglion-blocking substances on the small intestine. *J. Physiol., Lond.* **113**, 483–505.

HAINING, C. G., JOHNSTON, R. G. & SMITH, J. M. (1960). The neuromuscular blocking properties of a series of bisquaternary tropeïnes. *Br. J. Pharmac. Chemother.*, **15**, 71–81.

JINDAL, M. N. & DESHPANDE, V. R. (1960). Neuromuscular blockade by streptomycin and dihydrostreptomycin. *Br. J. Pharmac. Chemother.*, **15**, 506–509.

KHUONG HUU-LAINÉ, F. & PINTO-SCOGNAMIGLIO, W. (1964). Activite curarisants du dichlorure de 3β,20α-bistrimethylammonium 5α-pregnane (Malouétine) et de ses stereoisomères. *Archs int. Pharmacodyn. Thér.*, **147**, 209–219.

KONZETT, H. & RÖSSLER, R. (1940). Versuchsanordnung zu Untersuchungen an der Bronchialmuskulatur. *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.*, **195**, 71–74.

LEWIS, J. J., MARTIN-SMITH, M., MUIR, T. C. & ROSS, H. H. (1967). Steroidal monoquaternary ammonium salts with non-depolarizing neuromuscular blocking activity. *J. Pharm. Pharmac.*, **19**, 502–508.

LITCHFIELD, J. T. & WILCOXON, F. (1949). A simplified method of evaluating dose-effect experiments. *J. Pharmac. exp. Ther.*, **95**, 99–113.