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On the pharmacology of Org 6368 (2β, 16β - dipiperidino - 5α - androstan - 3α - ol acetate dimethobromide), a new steroidal neuromuscular blocking agent

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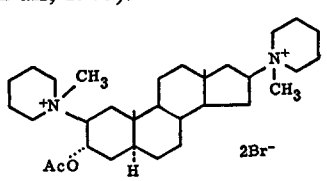
Org 6368 is a homologue of pancuronium bromide. Its interactions with other agents in the cat sciatic nerve-gastrocnemius muscle preparation revealed that paralysis was of the non-depolarizing type. This was confirmed in experiments using avian muscle. Org 6368 is a potent muscle relaxant being 2·4 times as potent as (+)-tubocurarine in the cat. Paralysis in the cat is rapid in onset and of appreciably shorter duration than that of pancuronium and (+)-tubocurarine. Repeated injections of the same dose of Org 6368 show no cumulative effect. Muscle relaxant doses generally cause a slight increase in both blood pressure and heart rate. Although its histamine-releasing capacity is greater than that of pancuronium it is less than that of (+)-tubocurarine. Org 6368 shares with pancuronium a very weak effect on both the muscarinic receptor and ganglionic transmission. Differences in the muscle relaxant profiles of Org 6368 and pancuronium are discussed.

Several of the undesirable side effects of (+)-tubocurarine as a neuromuscular blocking agent have been overcome by the introduction of the non-depolarizing bisquaternary steroidal compound, pancuronium bromide (Buckett, Marjoribanks & others, 1968). However, the need exists for a short acting non-depolarizing neuromuscular blocking agent with a rapid onset of action and which is free of side effects at therapeutic doses (Foldes, 1972). Preliminary pharmacological experiments revealed that Org 6368 (2β, 16β-dipiperidino-5α-androstan-3α-ol acetate dimethobromide) (I), a homologue of pancuronium bromide, may possess such a profile and the compound was submitted to a more detailed pharmacological investigation, the results of which are presented here. A preliminary account of some of the findings has been published in abstract form (Sugrue & Duff, 1973).



I

MATERIALS AND METHODS

In vivo *nerve-muscle preparations*

Unless stated otherwise, cats of either sex, 2–5 kg, were anaesthetized with pentobarbitone sodium (60 mg kg^{-1}, i.p.). The trachea was cannulated and artificial respiration applied throughout each experiment. The left sciatic nerve was exposed

in the popliteal space and the central end crushed. The peripheral end of the nerve was stimulated through a stimulus isolation unit by bipolar platinum electrodes. Rectangular pulses of 1 ms duration and at twice the strength required to evoke a maximal contraction were used. In preliminary experiments pulse widths ranging from 0·1 to 1 ms were used. No differences in drug effects were observed and a pulse width of 1 ms was subsequently used. The gastrocnemius-soleus muscle bundle of the left leg was partially dissected free from surrounding tissue and the Achilles tendon severed approximately 1 cm below its insertion into the calcaneus. A tension of 25 g was placed on the severed tendon and isometric tension changes were recorded on a Devices M4 recorder by means of a force displacement transducer. The frequency of stimulation was 0·05 Hz.

In other experiments cats were anaesthetized by injection of a mixture of α-chloralose (8 ml kg^{-1}, i.p. of a 1% solution) and pentobarbitone sodium (2·5 mg kg^{-1}, i.p.). The tibialis anterior and soleus muscles of the left leg were separated from neighbouring muscles and the tendon of insertion of each muscle cut. The left sciatic nerve was stimulated by supramaximal rectangular pulses of 0·1 ms duration and at a frequency of 0·1 Hz. With tetanic experiments, the soleus muscle of the left leg was indirectly stimulated by supramaximal rectangular pulses of 0·1 ms duration and at a frequency of 0·4 Hz. Tetanic stimuli of 10 s duration and at a frequency of 400 Hz were applied at 5 min intervals.

The hen sciatic nerve-gastrocnemius muscle preparation was set up in a similar manner to that described for the pentobarbitone sodium anaesthetized cat, except that anaesthesia was induced by the slow injection of pentobarbitone sodium (30 mg kg^{-1}) into the wing vein and that the frequency of stimulation was 0·1 Hz. The rat sciatic nerve-gastrocnemius muscle preparation was similar to that described for the pentobarbitone anaesthetized cat except that the tension on the severed tendon was 10 g.

Drugs were injected into all species by means of a cannula in the external jugular vein. Cat blood pressure was recorded from the carotid artery through a cannula, the pulse pressure being relayed from a Bell and Howell pressure transducer to a Devices pre-amplifier linked to a Devices M4 pen recorder. The ecg was monitored using five subcutaneous needle electrodes and recorded. Heart rate was integrated from the ecg and recorded. In experiments investigating the negative chronotropic effect of vagal stimulation in the chloralose-pentobarbitone anaesthetized cat, the left vagus nerve was separated from the cervical nerve, ligated and supramaximally stimulated by 10 s trains of rectangular pulses (0·1 ms duration, 20 Hz frequency) applied at 5 min intervals.

In vitro *nerve-muscle preparations*

The rat and cat phrenic nerve-diaphragm preparations (Bulbring, 1946) were mounted in Tyrode solution at 29° and gassed with 5% carbon dioxide in oxygen. The composition of the Tyrode solution was (g $litre^{-1}$): NaCl, 8; KCl, 0·2; $CaCl_2$, 0·14; $MgCl_2$, 0·1; Na_2HPO_4,2 H_2O, 0·065; Na H_2PO_4, $2H_2O$, 0·36; glucose, 2. The phrenic nerve was stimulated supramaximally at a frequency of 0·2 Hz by rectangular pulses of 1 ms duration.

Ganglionic transmission

Blockade of ganglionic transmission was determined in the pentobarbitone sodium anaesthetized cat superior cervical sympathetic nerve-nictitating membrane prepara-

tion. A tension of 10 g was placed on the membrane and isometric tension changes recorded. The ipsilateral superior cervical sympathetic nerve was freed from the carotid artery and vagus, cut centrally and stimulated preganglionically with supramaximal rectangular pulses of 1 ms duration and at a frequency of 25 Hz. The nerve was stimulated for 15 s every 6 min. Drugs were injected via a cannulated jugular vein 2 min before the next period of stimulation.

Histamine release

Drug-induced histamine release was determined by the guinea-pig bronchoconstriction test of Konzett & Rossler (1940).

Antimuscarinic activity

Antimuscarinic activity was investigated by determining the ability of the drug to antagonize acetylcholine-induced contractions of the guinea-pig isolated ileum.

Drugs. Where applicable, doses refer to the salt.

RESULTS

The ability of Org 6368 to block indirectly induced contractions of the cat gastrocnemius muscle was compared with that of pancuronium, (+)-tubocurarine, suxamethonium and gallamine (Fig. 1). Except for (+)-tubocurarine ($P < 0.001$) log

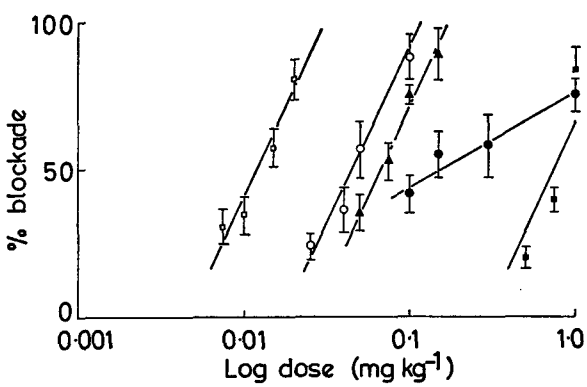


FIG 1. Dose/response curves for pancuronium (□), suxamethonium (○), Org 6368 (▲), (+)-tubocurarine (●) and gallamine (■) in the pentobarbitone sodium anaesthetized cat sciatic nerve-gastrocnemius muscle preparation. Each point is the mean of at least four values. Regression lines were constructed by the method of least squares.

dose/response curves paralled that of Org 6368. Graphically determined ED50 values are listed in Table 1. Thus Org 6368 is a potent neuromuscular blocking agent in the cat sciatic nerve-gastrocnemius muscle preparation being approximately 2·4 and 11·2 times as potent as (+)-tubocurarine and gallamine respectively. Org 6368 and pancuronium were compared for their ability to inhibit contractions of the *in vitro* indirectly stimulated cat diaphragm. Log dose/response curves were parallel and the ED50 values for Org 6368 and pancuronium were 0·71 and 0·18 mg ml^{-1} respectively. Thus the *in vitro* potency ratio of Org 6368 to pancuronium (0·24) was very similar to the corresponding *in vivo* value (0·18).

Table 1. *ED50 values (mg kg^{-1}) for neuromuscular blockade in the anaesthetized cat, hen and rat.* ED50 values were obtained from log dose/response regression lines constructed by the method of least squares. Each point was the mean of at least four values and a minimum of three doses was used for each dose/response curve.

	Species		
Drug	Cat	Hen	Rat
Org 6368	0·067	0·021	3·2
Pancuronium	0·012	0·014	0·068
(+)-Tubocurarine	0·158	0·23	0·046
Suxamethonium	0·046		0·46
Gallamine	0·750		5·6

Org 6368, like (+)-tubocurarine and pancuronium, did not elicit a contracture in the hen sciatic nerve-gastrocnemius muscle preparation. On the other hand suxamethonium did. Hence it would appear that Org 6368 is a non-depolarizing neuromuscular blocking agent. Reference to Table 1 reveals that Org 6368 and pancuronium were approximately equipotent in this preparation and were much more potent than (+)-tubocurarine. However, (+)-tubocurarine was more potent than Org 6368 and pancuronium in the rat sciatic nerve-gastrocnemius muscle preparation (Table 1). The resistance of the rat neuromuscular junction to Org 6368 was confirmed in the *in vitro* rat phrenic nerve-diaphragm preparation. The ED50 values for (+)-tubocurarine, pancuronium, suxamethonium and Org 6368 were 0·22, 0·54, 2·0 and 34·5 mg ml^{-1} respectively. The observation that the rat neuromuscular junction is more susceptible to (+)-tubocurarine than pancuronium is in agreement with the findings of others (Derkx, Bonta & Lagendijk, 1971).

Fig. 2A shows the effect of submaximal doses of Org 6368 and pancuronium on the cat sciatic nerve-gastrocnemius muscle preparation. To be noted is the more rapid onset and recovery of the Org 6368-induced block. Org 6368, pancuronium, (+)-tubocurarine, suxamethonium and gallamine were compared for onset and recovery. Onset of action was defined as the time required in min for maximum block to develop from time of injection whilst recovery was measured as the time needed from onset of recovery to 90% restoration of twitch height. The onset of action of Org 6368 rivalled that of suxamethonium and was appreciably faster than that of pancuronium,

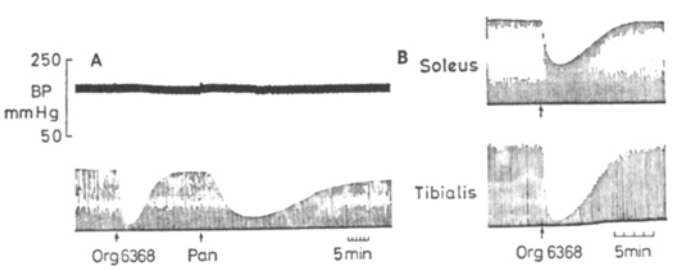


FIG. 2A. Effect of Org 6368 (0·1 mg kg^{-1}, i.v.) and pancuronium (0·015 mg kg^{-1},i.v.) on the pentobarbitone sodium anaesthetized cat sciatic nerve-gastrocnemius muscle preparation. Cat, 1·8 kg, time sale in min.
B. Effect of Org 6368 (0·15 mg kg^{-1}, i.v.) on the indirectly stimulated soleus and tibialis anterior muscles of the chloralose anaesthetized cat. Cat, 2·4 kg, time scale in min.

(+)-tubocurarine and gallamine. Recovery from an Org 6368-induced block was also rapid (Table 2).

The interactions of Org 6368 with other agents in the cat sciatic nerve-gastrocnemius muscle preparation strongly suggest that it is a non-depolarizing neuromuscular blocking agent. For example, a partial Org 6368-induced block was augmented by the intravenous injection of pancuronium, (+)-tubocurarine and gallamine at doses having negligible blocking effect when administered alone. Furthermore an Org 6368-induced block was antagonized by potassium chloride (10 mg kg^{-1}, i.v.), edrophonium (1 mg kg^{-1}, i.v.) and neostigmine (0·1 mg kg^{-1}, i.v.).

Other facets of the neuromuscular blocking profile of Org 6368 include a preferential block of fast contracting muscles as indicated by a greater degree of block in the indirectly stimulated tibialis anterior than in the soleus of the cat (Fig. 2B). A similar

Table 2. *Onset and recovery times of neuromuscular blocks induced by various agents in the anaesthetized cat.*

Compound	Dose (mg kg^{-1})	n	% Block ± s.e.	Time to maximum block (min ± s.e.)	Time to 90% recovery (min ± s.e.)
Org 6368	0·15	10	83·3 ± 3·2	1·7 ± 0·1	5·3 ± 0·3
Suxamethonium	0·1	12	84·3 ± 7·5	1·4 ± 0·1	9·3 ± 2·1
Pancuronium	0·03	7	86·7 ± 8·5	4·8 ± 0·4	13·4 ± 2·8
(+)-Tubocurarine	0·3	7	66·3 ± 10·0	4·9 ± 0·7	14·1 ± 3·4
Gallamine	1·0	4	88·0 ± 6·0	3·0 ± 0·5	14·1 ± 2·1

selectivity has been reported for pancuronium (Bonta & Goorissen, 1968). With the frequency of tetanic stimulation employed in this study (400 Hz) post-tetanic twitches are markedly increased in amplitude for about 2 min. Webb & Bradshaw (1973) have shown that (+)-tubocurarine and gallamine, at doses having no effect on twitch height, depressed the post-tetanic augmentation of twitch tension of the cat soleus muscle. This finding was interpreted as indicating a prejunctional site of action. Org 6368, at doses having no effect on neuromuscular transmission, also depressed the post-tetanic augmentation of twitch tension of the cat soleus muscle. This observation suggests that it acts prejunctionally in addition to having a postjunctional locus of action. Finally, recovery by the indirectly stimulated cat gastrocnemius muscle was unaltered by repeated injections of the same dose (0·075 mg kg^{-1}). With (+)-tubocurarine repeated injections of the same dose (0·15 mg kg^{-1}) resulted in a marked lengthening of the recovery period (Fig. 3). Repeated injections of the same dose of pancuronium evoked a cumulative effect.

Neuromuscular blocking doses of Org 6368 generally caused a slight tachycardia and a slight transient rise in the blood pressure of both the pentobarbitone sodium and chloralose anaesthetized cat. Typical Org 6368-induced increases in blood pressure are shown in Fig. 3. The induced tachycardia would appear to be mediated at least in part, by an atropinic action on the cardiac vagus since the negative chronotropic effect following stimulation of the cardiac vagus of the chloralose anaesthetized cat was partially blocked by low doses of Org 6368. Such an effect has also been demonstrated for pancuronium (Saxena & Bonta, 1970). The administration of large doses of Org 6368 (doses up to 10 mg kg^{-1}) did not elicit marked adverse effects on either cat blood pressure and heart rate.

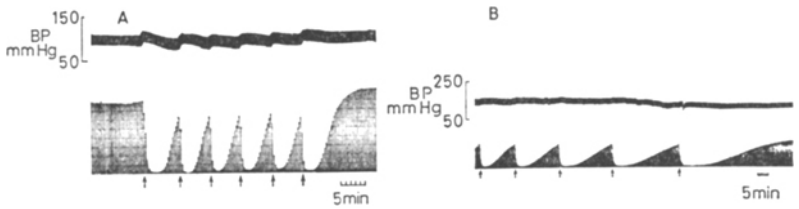


FIG. 3. Effect of repeated injections of the same dose of Org 6368 and (+)-tubocurarine on the pentobarbitone sodium anaesthetized cat sciatic nerve-gastrocnemius muscle preparation. (A) Cat, 2·6 kg. At arrows Org 6368 (0·075 mg kg^{-1}, i.v.), time scale in min. (B) Cat, 2·7 kg. At arrows (+)-tubocurarine (0·15 mg kg^{-1}, i.v.) time scale in min.

Blockade of ganglionic transmission was determined in the cat superior cervical sympathetic nerve-nictitating membrane preparation. The ED50 values for Org 6368, pancuronium, hexamethonium and (+)-tubocurarine were 6·6, 3·6, 0·81 and 0·42 mg kg^{-1} respectively.

The ED50 values for Org 6368 and (+)-tubocurarine in the guinea-pig bronchoconstriction test were 8·0 and 2·6 mg kg^{-1} respectively. Pancuronium at a dose of 20 mg kg^{-1} did not cause bronchoconstriction.

The effect of Org 6368 on the muscarinic receptor, as determined by ability to antagonize acetylcholine-induced contractions of the guinea-pig isolated ileum, was very weak. Org 6368 and pancuronium were equi-effective and both were 1000 times less active than atropine.

DISCUSSION

Org 6368 is structurally very similar to pancuronium, the sole difference being the replacement of the C-17 acetoxy substituent in pancuronium by hydrogen.

Org 6368, like pancuronium, has a very weak effect on both the muscarinic receptor and ganglionic transmission. However, its histamine-releasing capacity, as determined by the guinea-pig bronchoconstriction test, is greater than that of pancuronium although less than that of (+)-tubocurarine. The effects of Org 6368 on the cat cardiovascular system are very similar to those of pancuronium. Neuromuscular blocking doses usually evoke a slight increase in both blood pressure and heart rate. The latter may be due in part to an atropinic effect on the cardiac vagus although other possible mechanisms cannot be excluded. For example, increased plasma catecholamine concentrations have been reported following pancuronium administration (Nana, Cardan & Domokos, 1973).

The neuromuscular blocking profiles of Org 6368 and pancuronium show certain similarities. For example, both are non-depolarizing neuromuscular blocking agents as revealed by experiments using avian muscle and by interactions with other drugs in the cat sciatic nerve-gastrocnemius muscle preparation. In addition both act preferentially on fast contracting muscles, e.g. the cat tibialis anterior. However, marked quantitative differences also exist. Org 6368 is much less potent than pancuronium in blocking neuromuscular transmission in the rat. *In vivo* and *in vitro* studies reveal Org 6368 to be approximately one fifth as active as pancuronium in the cat. Grip strength studies in man have shown Org 6368 to be approximately 0·16 times as potent as pancuronium, an observation in close agreement with that reported for the cat in this study (Baird, 1974). Repeated injections of the same dose of Org

6368 show no cumulative effect in the cat sciatic nerve-gastrocnemius muscle preparation. On the other hand, a cumulative effect is observed with pancuronium. Perhaps the most striking difference between pancuronium and Org 6368 in the cat sciatic nerve-gastrocnemius muscle preparation is the more rapid onset and shorter duration of action of the latter. Several studies have shown that recovery from paralysis induced by non-depolarizing neuromuscular blocking agents in primates is longer than that observed in cats and tends to parallel that in man (Biggs, Davis & Wien, 1964; Bamford, Biggs & others, 1967; Busfield, Child & others, 1968; Hughes, 1972; Brittain & Tyers, 1973). Org 6368 was studied in the sciatic nerve-gastrocnemius muscle preparation of two baboons and recovery, whilst being shorter than that of pancuronium, was appreciably longer than that observed in cats (Sugrue, Duff, Baird & Miller, unpublished). Hughes (1972) has postulated that the brevity of action of BW.403C65 [(5,5-octa-methylenebis-7,8-dihydro-6,7,7-trimethyl-1,3-dioxolo (4,5-g) isoquinolinium methyl sulphate] in some species may be related to rapid accumulation by the liver as was demonstrated in the cat but not in the monkey. Furthermore, recovery from a BW.403C65-induced neuromuscular block was slower in monkey than in cat. Hence it is of interest to note that the cat liver has a much greater avidity for Org 6368 than for pancuronium (Agoston, personal communication) and this may contribute to the short duration of action of Org 6368 in the cat. However, the possibility cannot be excluded that other factors such as altered receptor affinity and/or differences in metabolism and hence plasma half lives, may contribute to the observed differences between Org 6368 and pancuronium.

In summary, the over-all pharmacological profile of Org 6368 is similar to that of pancuronium in some respects but markedly differs in others. Thus, the neuromuscular blocking profile of pancuronium is markedly altered by changing its C-17 substituent.

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