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Structure:action Relationships Among Some Desacetoxy Analogues of Pancuronium and Vecuronium in the Anesthetized Cat

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The hypothesis that the neuromuscular blocking potency of pancuronium and vecuronium depends on the two acetylcholine moieties present at positions 3 and 17 was tested in cats by examining the neuromuscular profile of several desacetoxy analogues. Blockade of sciatic nerve-induced contraction of the tibialis and soleus muscles, as well as the effects on vagal-induced bradycardia and on sympathetically induced contractions of the nictitating membrane, were studied. The bis-desacetoxy analogue of pancuronium (ORG 7931) was one-fifth as potent as the parent compound as a neuromuscular blocking drug and as a vagolytic agent, but the neuromuscular block was faster in onset and shorter in duration than that produced by pancuronium. The desacetoxy analogues of vecuronium (ORG 8730 and ORG 8764) also were less potent neuromuscular blocking drugs, and, in addition, produced more vagal block than did vecuronium itself. The neuromuscular block produced by these desacetoxy analogues was of more rapid onset and shorter duration than that produced by vecuronium. The results thus showed that the greater neuromuscular blocking potency of pancuronium and vecuronium is lost after removal of one or both of the acetylcholine moieties. An analysis of the relationship between neuromuscular blocking dose and duration of action revealed that it was reciprocal, and it is suggested that a nondepolarizing equivalent of suxamethonium, when discovered, may necessarily be a drug of relatively low potency. (Key words: Neuromuscular blocking drugs: desacetoxy analogues; pancuronium; structure-activity relationships; vecuronium.)

IN THE DESIGN OF pancuronium by Buckett *et al.*,[1] it was logically assumed that the two acetylcholine moieties at either end of the molecule (fig. 1) would impart great affinity for the motor endplate acetylcholine receptors and, therefore, increased neuromuscular blocking potency. This concept was supported by the fact that pancuronium was the most potent neuromuscular blocking drug available at the time of its development. However, although pancuronium had been previously shown to be more potent than the corresponding 3,17-bis-hydroxy compound (dacuronium[2]), this is not a true test of the importance of the acetoxy groups. By analogy with choline and acetylcholine, hydroxyl groups may positively impede interaction with the cholinoceptors, possibly as a result of hydrogen bonding with the aqueous milieu. Thus, for example, choline is much less potent than the simple ethyltrimethyl ammonium ion at muscarinic cholinoceptors,[3] presumably because the hydroxyl group in choline interferes with its interaction with the receptors.

In 1979, Organon chemists succeeded in synthesizing ORG 7931 (fig. 1). Previously unpublished preliminary studies with this compound in cats showed that it is, in fact, less potent (about one-fifth as potent) than pancuronium in its neuromuscular blocking action. However, ORG 7931 also differed from pancuronium in the time course of the block, and this observation, together with structure:activity data obtained from studies with vecuronium and its analogues,[4] suggested that additional desacetoxy analogues of pancuronium and vecuronium might be worthy of study. The present paper describes experiments with some of these analogues.

Materials and Methods

Cats of either sex weighing between 1.8 and 4.0 kg were anesthetized with a mixture of α-chloralose (80 mg/kg) and sodium pentobarbital (5 mg/kg) injected ip. After tracheal intubation, the cats were artificially ventilated with 18 ml/kg of air at a rate of 26 breaths/min. Blood gas analysis gave the following mean values; P_{O_2}, 96 ± 3 mmHg, P_{CO_2}, 31 ± 2 mmHg, *p*H, 7.39 ± 0.04.

Drugs were administered iv through a polythene cannula inserted into a femoral vein. Arterial blood pressure was recorded by means of a polythene cannula inserted into a femoral artery and connected to a Statham P23AC pressure transducer. The pulse pressure was used to trigger a Grass 7P4F EKG tachograph that measured heart rate.

Both vagus nerves were separated from the cervical sympathetic nerves and ligated. The right vagus nerve was stimulated by a train of impulses every 100 s (0.5 msec pulse duration at a frequency of 10 Hz for 10 s) applied to the cardiac side of the ligature. The stimula-

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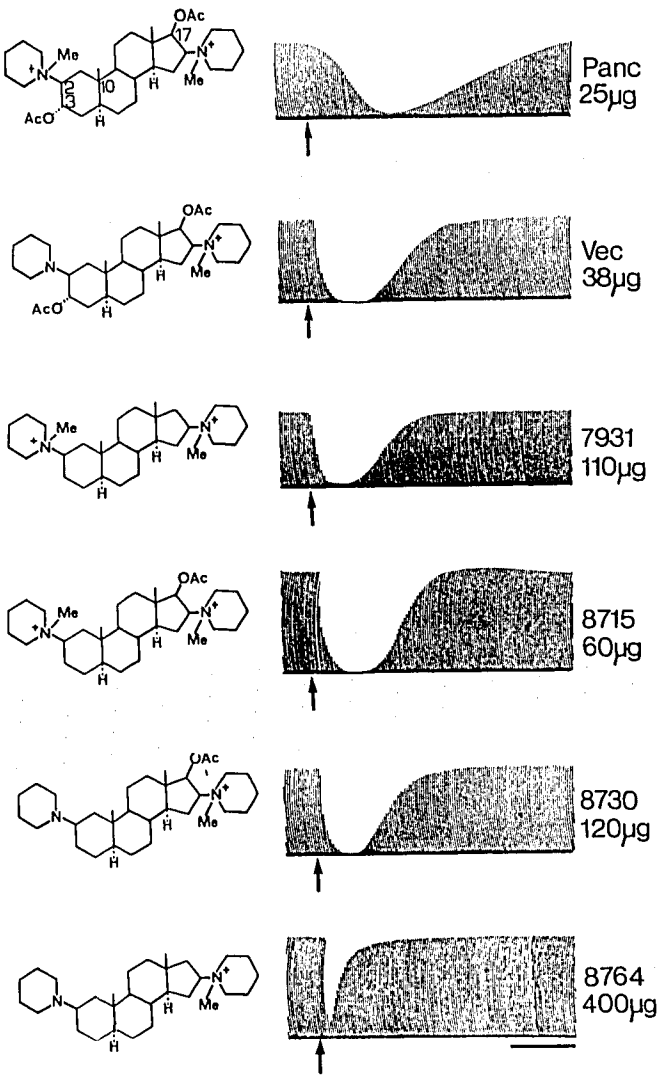

FIG. 1. Structures and typical blocks of the tibialis anterior muscle of six different cats produced by the compounds studied. Panc = pancuronium; vec = vecuronium; and the numbers are Organon code numbers. The doses are the doses/kg body weight. The time calibration corresponds to 5 min.

tion strength was adjusted to produce a constant decrease in heart rate of approximately 50%. In some experiments, acetyl-β-methylcholine (7–20 μg/kg) was injected in a dose that produced a bradycardia approximately equal to that produced by vagal stimulation.

The left cervical sympathetic nerve was ligated preganglionically and stimulated with a train of impulses every 100 s (0.5 msec pulse duration at a frequency of 10 Hz for 10 s). The stimulus strength was adjusted to produce a maximal contraction of the nictitating membrane. Contractions of the nictitating membrane were recorded by a Grass FTO3C force displacement transducer.

The sciatic nerve in the popliteal space was stimulated with rectangular pulses of 0.2 ms duration at a frequency of 0.1 Hz. The stimulation strength was adjusted to evoke maximal contraction of the tibialis anterior and soleus muscles, and then doubled. These two muscles were selected as examples of fast- and slow-contracting muscles, respectively.[5] Contractions of these muscles were recorded by Grass FT10C force displacement transducers. Body temperature and muscle temperature were maintained at 37–38° C by means of the heated operating table and heating lamps, and by bathing the muscles in warm mineral oil.

The drugs used were acetyl-β-methylcholine (Sigma), α-chloralose (British Drug Houses), sodium pentobarbital (Abbott), pancuronium bromide, vecuronium bromide, and ORG 7931, 8715, 8730, and 8764 (Organon). All of the compounds coded ORG (fig. 1) are either mono- or dibromides. They were synthesized and supplied to us by Drs. D. S. Savage and T. Sleigh. Analysis showed the following degrees of purity: ORG 7931, 92.2%, contained no steroidal impurities, but 1% diethyl ether and 6.8% water; ORG 8715, 95%, contained less than 2% steroidal impurity and less than 4% isopropanol; ORG 8730, 97%, contained no steroidal impurity, but 3% water; and ORG 8764, more than 98%, contained no steroidal impurity and less than 1% water. The syntheses of ORG 8715 and 8730 are described in European Patent 067,480. Stock solutions (2 mg/ml) of vecuronium bromide and of the compounds coded ORG were made in a citric acid buffer (citric acid 2.1 mg/ml in distilled water), and were diluted with 0.9% w/v NaCl solution immediately before injection. All other drugs were dissolved in NaCl solution.

The approximate dose of each neuromuscular blocking drug necessary to produce 90–99% twitch depression was initially estimated from preliminary experiments. This dose was then injected into a test animal as a single bolus. If the block produced was outside the range of 90–99% twitch depression, the animal was left undisturbed for 2 h, by which time cumulative effects were undetectable, and a second adjusted bolus dose was then injected. Time course measurements were made on these early blocks corresponding to 90–99% twitch depression (table 1). The animals had received no other blocking drugs and only one previous dose of the same drug, thereby minimizing cumulative effects of the same drug and avoiding any cross-cumulative effects between drugs. If the second injection also produced a block outside the 90–99% range, the animal was used for a different purpose.

The doses to produce 50% twitch depression or 50% vagal inhibition (table 2) were determined from dose-response curves. In these experiments, a range of up to

TABLE 1. Effective Doses and Time Courses of Effects on the Tibialis Anterior Muscle

Compound	Dose to Produce 90–99% Twitch Depression (μg/kg)	Onset: Injection to Max. Block (Min)	Duration: Injection to 90% Recovery (Min)	Recovery Index: 25% to 75% Twitch Recovery (Min)
Pancuronium n = 9	21.5 ± 2.0	4.8 ± 0.3	15.0 ± 1.6	5.2 ± 0.45
Vecuronium n = 9	37.5 ± 1.9	4.0 ± 0.35	11.1 ± 1.0	3.2 ± 0.18
ORG 8715 n = 7	63.0 ± 6.5	3.2 ± 0.3	7.5 ± 0.61	1.9 ± 0.2
ORG 7931 n = 5	113.0 ± 8.3	2.2 ± 0.17	9.8 ± 0.65	3.2 ± 0.15
ORG 8730 n = 5	110.0 ± 11.5	2.4 ± 0.38	8.0 ± 1.2	2.4 ± 0.18
ORG 8764 n = 7	443.0 ± 71.0	1.2 ± 0.18	4.6 ± 0.96	1.4 ± 0.2

The numbers are the means ± SEM. Differences between means were compared by Student's t test and were considered significant when $P \leq 0.05$. All doses are significantly different from each other except for ORG 7931 and ORG 8730. Onset times for pancuronium, vecuronium, and ORG 8715 are not significantly different. All other drugs have significantly faster onset than vecuronium. ORG 8764 is significantly faster in onset than all other drugs. ORG 7931 and ORG 8730 are not significantly different from each other in their onset times. Vecuronium is significantly shorter in duration than pancuronium. ORG 8764 is significantly shorter in duration than all other drugs. The recovery index of vecuronium is significantly shorter than that of pancuronium. The recovery index of ORG 8764 is significantly shorter than that of all other drugs except ORG 8715.

five bolus doses was randomly injected, the largest being at least sufficient to produce 70% vagal inhibition. From 90 min to 2 h was allowed to elapse after recovery from any one dose before the next injection was given. Only one blocking drug was injected into any one animal in this series of experiments.

In a further series of experiments, the effects of two or more blocking drugs, one of which was always vecuronium, were compared in the same animals.

Results

The structures of the four desacetoxy compounds studied, and of pancuronium and vecuronium, are given in figure 1. All of the compounds blocked the twitch of the tibialis anterior and soleus muscles. There was little difference between the two muscles in the depth of twitch depression produced, but the effect on the soleus muscle was consistently longer lasting than that on the tibialis anterior (see, for example, the effect of ORG 7931 illustrated in figure 2). The time course of twitch depression was calculated for the tibialis anterior muscle in relation to the dose that produced 90–99% twitch depression. These data are given in table 1 along with the neuromuscular blocking doses. Experiments in which two or more drugs were compared in the same cat confirmed the time courses given in table 1. Figure 1 displays typical effects produced by each of the drugs. It should be noted that the twitch depression produced by ORG 8764, the least potent compound, was fastest in onset and shortest in duration.

None of the compounds, in doses large enough to produce complete muscle twitch depression, reduced contractions of the nictitating membrane evoked by preganglionic stimulation, indicating that they are devoid of ganglion blocking activity in neuromuscular blocking doses (see, for example, the absence of effect of ORG 7931 on the nictitating membrane in figure 2).

With the exception of vecuronium, all of the compounds, in neuromuscular blocking doses, reduced the bradycardia produced by vagal stimulation (figures 2

TABLE 2. The Neuromuscular Blocking and Vagolytic Potencies of Pancuronium, Vecuronium, and their Desacetoxy Analogues in the Chloralose-anaesthetized Cat

	Mean Doses (μg/kg) ± SEM to Produce 50% Twitch Depression		
	Tibialis	Vagus	Vagus/Tibialis
Pancuronium	21 ± 2	48 ± 3.8	2.3
Vecuronium	33 ± 2.1	3036 ± 196	92
ORG 7931	82 ± 5.3	197 ± 11.9	2.4
ORG 8715	59 ± 6.5	413 ± 38.8	7.0
ORG 8730	92 ± 6.0	190 ± 85	2.1
ORG 8764	358 ± 49	574 ± 115	1.6

The doses of pancuronium and vecuronium are the means of doses in 20 cats. The doses of ORG 7931, 8715, 8730, and 8764 are the means of doses in five or six cats. The vagus:tibialis ratio gives a guide to the selectivity of the compound for skeletal muscle compared to the heart. It should not be regarded as more than an approximation, since the dose-response curves for the two organs are not parallel, the doses differ with different frequencies of stimulation, and the number of animals (except for pancuronium and vecuronium) was small.

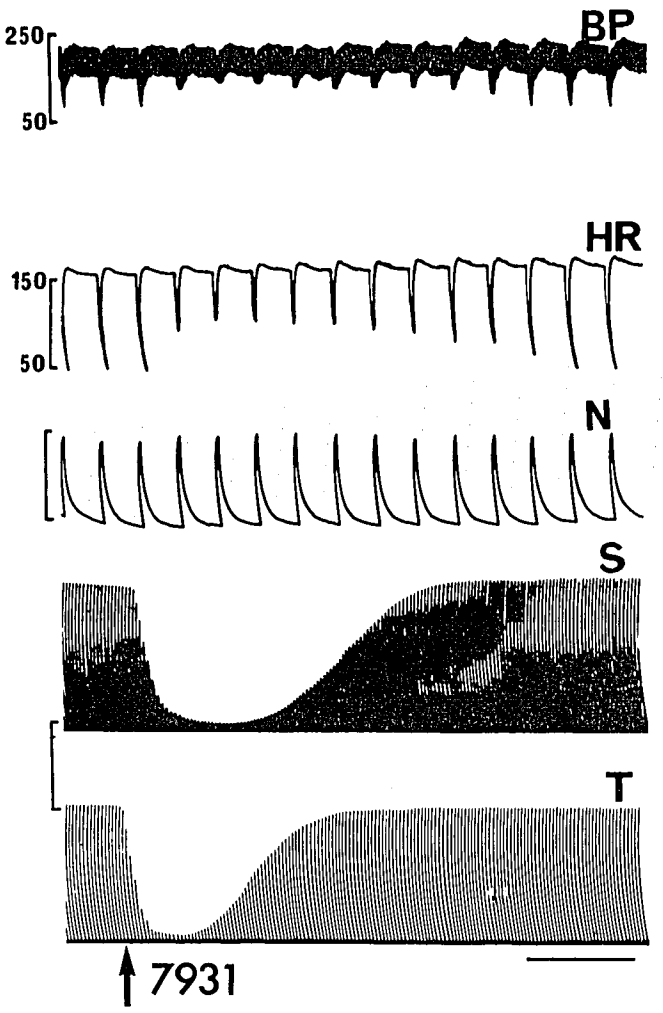

FIG. 2. Records from above downwards: Blood pressure (BP) calibrated in mmHg, heart rate (HR) calibrated in beats/min, nictitating membrane contractions (N), maximal twitches of the soleus (S) and the tibialis anterior (T) muscles evoked by stimulating the motor nerve at a frequency of 0.1 Hz. The right vagus was stimulated at 10 Hz for 10 s every 100 s to evoke a decrease in heart rate. The left cervical sympathetic nerve was similarly stimulated preganglionically to evoke contractions of the nictitating membrane. The tension calibrations are 5 g for the nictitating membrane, 0.5 kg for the tibialis anterior muscle, and 0.2 kg for the soleus. The time calibration corresponds to 5 min. At the arrow, 100 μg/kg of ORG 7931 was injected intravenously.

and 3 illustrate the effects of ORG 7931 and ORG 8764, respectively). That this effect was a consequence of an action on the cardiac muscarinic receptors (rather than on ganglionic nicotinic receptors or on other neural structures) was indicated by the fact that, in the same doses, the compounds also blocked the bradycardia produced by methacholine (data not shown). Vecuronium, as reported previously,[4] was without effect on cardiac responses to vagal stimulation unless very high multiples of neuromuscular blocking doses were used (>75 times the dose to produce 50% twitch depression). The neuromuscular blocking and vagolytic potencies of the compounds tested are summarized in table 2.

Removal of the two acetoxy groups from pancuronium (to give ORG 7931) decreased vagolytic potency and neuromuscular blocking potency to approximately the same degree, so that 7931 and pancuronium showed similar ratios of muscarinic/nicotinic receptor blocking activity. The bisquaternary compound lacking only the 3-acetoxy group (ORG 8715) possessed relatively weaker vagolytic effects than pancuronium and, therefore, appeared to be more selective than pancuronium for the nicotinic receptors at the neuromuscular junction. In contrast, removal of one or both acetoxy groups from vecuronium resulted in compounds (ORG 8730, ORG 8764) that possessed markedly higher affinity for the cardiac muscarinic cholinoceptors than did vecuronium. Indeed, in some experiments, ORG 8730 and ORG 8764 produced a greater block of the cardiac vagus than did pancuronium at a comparable neuromuscular blocking dose (fig. 3). Because of these pronounced effects on the vagus and their consequent propensity to cause tachycardia, these compounds have been discarded before the stage of clinical trials.

Two monoquaternary compounds, ORG 8730 and ORG 8764 (but not vecuronium), differed from the other compounds in that they consistently produced small rises in blood pressure and in heart rate (see effect of ORG 8764 in figure 3). This occurred despite both vagi having been cut centrally so that it was not a consequence of block of vagal tone. There was also a small rise in the background tone of the nictitating membrane, suggesting that all three effects—increase in heart rate, blood pressure, and nictitating membrane tone—may have been consequences of catecholamine release, although whether from sympathetic nerve endings or from the adrenal medullae was not determined.

Discussion

The results confirm the original proposal[1] that the acetylcholine moieties of pancuronium are important for interaction with the endplate cholinoceptors. The compound that lacked both the 3α- and the 17β-acetoxy groups (ORG 7931) was only about one-fifth as potent as pancuronium in its neuromuscular blocking action, and the compound that retained only the 17β-acetoxy group (ORG 8715) was about one-third as potent as pancuronium. The importance of the acetylcholine moiety at the ring D end of the molecule for interaction with the endplate cholinoceptors is further emphasized by consideration of ORG 8764, which lacks this moiety, and ORG 8730, the analogous compound that retains it. ORG 8764 was one-fourth as potent as ORG 8730 in blocking nerve-evoked muscle twitches.

As a generalization, it may be stated that duration of drug action, especially with long-acting drugs, is briefer the smaller the species of animal, presumably because of faster blood flow and metabolism. Hence, the absolute values describing time course of action in the cat would not be expected to be the same in humans. However, the relative time course in the cat (for example, whether a new drug is twice as fast in onset as vecuronium) may be taken as a guide to the likely time course in humans. The observation that the block produced by ORG 7931 was somewhat faster in onset than that produced by vecuronium suggested that the monoquaternary (tertiary nitrogen on carbon 2) analogue of ORG 7931 (*i.e.*, ORG 8764, which is stable in solution) might be a compound with clinical advantages. In other words, in addition to its stability, it could be expected that it might retain the time course of action of ORG 7931, yet be free from cardiovascular side effects, as is vecuronium. However, we found this not to be so. ORG 8764 had a faster onset and a briefer duration of action than ORG 7931, but it also produced unwanted cardiovascular effects. In fact, all four compounds that lacked the 3α-acetoxy group produced pronounced blocking effects on the cardiac muscarinic receptors. These results, when compared with those obtained with vecuronium, tentatively suggest that, in this series of compounds, it is not simply that a tertiary nitrogen on carbon 2 imparts freedom from cardiovascular effects, but that, in addition, the shielding presence of a 3α-acetoxy group (or presumably of some equivalent group) and the 10β-angular methyl group together are necessary in order to impede interaction with the cardiac muscarinic receptors.

The possibility of producing a nondepolarizing equivalent of suxamethonium has often been disputed, but these results show that it is possible to produce a nondepolarizing blocking drug with rapid onset and of brief duration of action, at least in the cat. ORG 8764 fulfilled these criteria, and has, therefore, provided a stimulus to continue the search. ORG 8764 itself is not suitable for further development, since its cardiovascular effects are pronounced. ORG 8764 lacked potency, and results with a large number of pancuronium and vecuronium analogues have shown that, in general, fast onset, coupled with brief duration, may be produced only with compounds of relatively low potency. Consideration of conditions in the biophase at a motor endplate may explain this. In general, in accordance with the law of mass action, a high concentration of molecules might be expected to be necessary to achieve rapid receptor block and, therefore, rapid paralysis. It is possible, of course, to deliver a high concentration of any drug, and, in experiments in cats (unpublished) and pigs,[6] we have confirmed that, with a sufficiently large

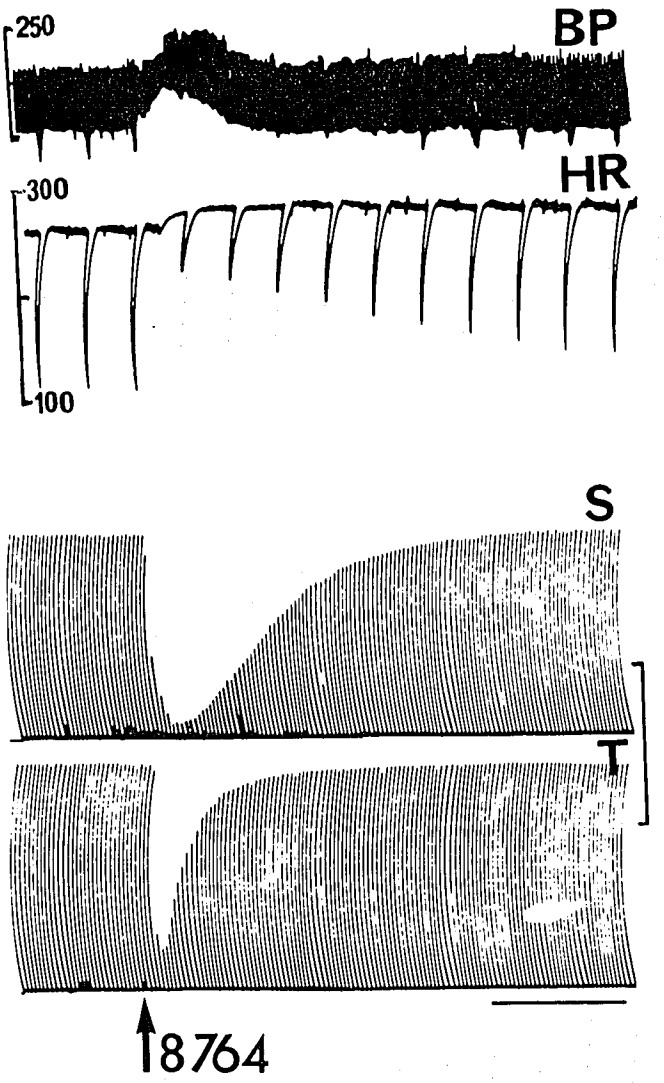

FIG. 3. Records from above downward are as in figure 2 except that nictitating membrane contractions were not recorded. At the arrow, 300 μg/kg of ORG 8764 was injected intravenously. The time calibration corresponds to 5 min.

dose, all neuromuscular blocking drugs tested, including pancuronium and vecuronium, produce block of very rapid onset. However, with highly potent drugs, which by definition have high affinity for the receptors, rapid onset can be achieved only at the expense of a very prolonged duration of action. For brief duration, low affinity for the receptors (and therefore low potency) is necessary. The only obvious exceptions to this would be drugs whose molecules, perhaps including those in the biophase, are very rapidly inactivated, or which, through some separate mechanism, have built-in self-antagonistic action. Theoretical considerations indicate that onset time must obviously reach a lower limit, no matter how large the dose, since it cannot be shorter than the circulation time. Likewise, potency

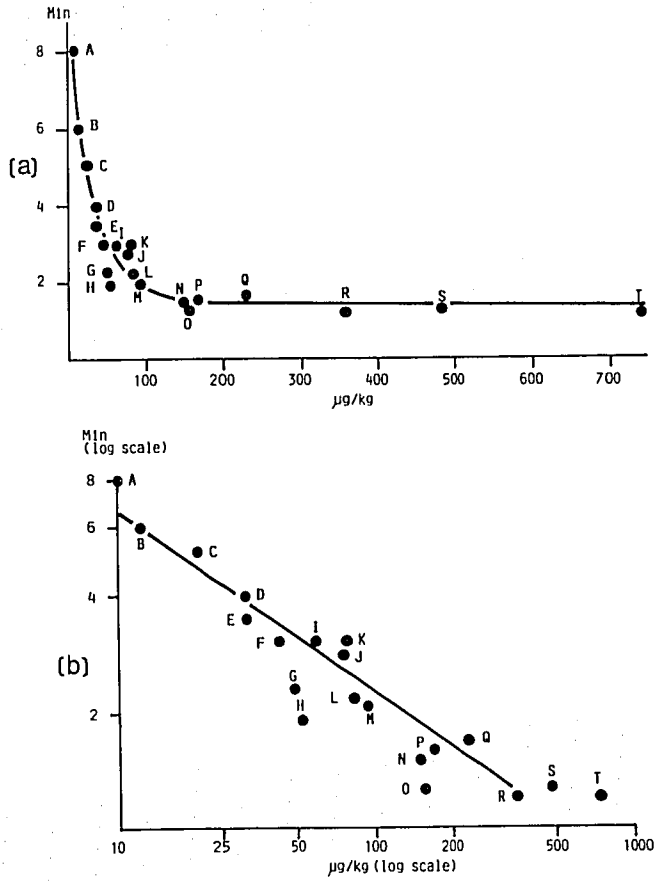

FIG. 4. *a.* The relationship between effective dose and onset time for 20 chemically related aminosteroidal neuromuscular blocking drugs. The effective dose (abscissa) is the mean dose to produce 50% twitch depression (0.1 Hz) of the tibialis anterior. The 50% blocking dose was used because it can be measured more accurately than the 90–99% blocking dose. The onset time (ordinate) is the mean time from injection to the maximum effect after a dose producing 90–99% twitch depression. The 50% and 90–99% blocking doses were determined by the same procedures as those described under *Materials and Methods. b.* The same data plotted as log dose against log onset time. A = pipecuronium; B = ORG 8788; C = pancuronium; D = vecuronium; E to M and O to T = the ORG compounds numbered 9274, 9360, 9273, 8715, 6502, 9216, 7931, 8730, 7617, 9275, 6368, 8764, 9382, and 7684, respectively. N is the compound RGH-4201.[7] The compounds that are the main subjects of this paper are C, D, I, L, M, and R. The syntheses and actions of the remaining Organon compounds are to be described in separate papers in the chemical literature.

must reach a limit, since, even if every molecule were to be effective, a fixed number of receptors must be blocked at each endplate to block transmission. Thus, if potencies, or the reciprocals (*i.e.*, doses to produce a particular effect), of a range of compounds are plotted against their onset times, it might be expected that the former would become asymptotic towards a figure related to the minimum number of receptors that have to be blocked, and the latter would be asymptotic towards the circulation time. We have, in fact, plotted such a curve for 20 Organon aminosteroid compounds related to pancuronium or vecuronium (fig. 4a). The results confirm the above theoretical consideration, the graph obtained having the form of a rectangular hyperbola. Such a curve may be converted to a straight line by plotting the logarithms of both variables (fig. 4b). In general, such a correlation can be tested only with a series of chemically related drugs, which would be expected to be governed by broadly similar metabolic and pharmacokinetic variables. An exception to the generalization might occur if an individual member of a series possessed some uniquely different characteristic, such as an exceptionally low volume of distribution that would lead to an increased biophase concentration relative to the dose. Consequently, the relationship can only be a generalization; some exceptions are likely to occur.

It seems, however, that the nondepolarizing equivalent of succinylcholine, when discovered, is likely to be a drug of relatively low potency compared to others in the same chemical group. The larger dose required carries with it the possible disadvantages of higher cost and greater likelihood of side effects, and it may be necessary to accept a compromise incorporating some tolerable disadvantages to gain the required time course of action.

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