Anesthesiology
55:12–18, 1981

A Comparison of the Neuromuscular Blocking and Vagolytic Effects of ORG NC45 and Pancuronium

S. Lee Son, M.B.,* B. E. Waud, M.D.,† D. R. Waud, M.D., D. Phil.‡

ORG NC45, a neuromuscular blocking agent not producing tachycardia, was examined first, to establish the kinetics of the antagonism it produces, and second, to test the hypothesis that the tachycardia seen with pancuronium and gallamine reflects an action on vagal postganglionic nerve endings. The action of ORG NC45 was studied on end-plate depolarization and neuromuscular transmission in the guinea pig lumbrical muscle. Also, the effect of ORG NC45 on the response of the cardiac pacemaker to carbachol and on the response of the pacemaker to pre- and postganglionic vagal stimulation was examined in isolated guinea pig atria. ORG NC45 was a potent neuromuscular blocking agent (twice as potent as pancuronium) in this species and showed typical competitive kinetics with a dissociation constant of 0.0103 μM. However, ORG NC45 affected the atrial system only at very high concentrations and did not affect release of transmitter from vagal nerve terminals. These results thus confirm the hypothesis that presence or absence of vagolytic action goes hand-in-hand with tachycardia or its absence clinically. (Key words: Heart: atria; pulse rate. Neuromuscular junction. Neuromuscular relaxants: ORG-NC45; pancuronium. Parasympathetic nervous system: atropine; vagus.)

THE NEUROMUSCULAR BLOCKING AGENT ORG NC45, a pancuronium analog with a tertiary nitrogen at C_2, is a short-acting competitive neuromuscular blocking agent which does not produce cardioacceleration.[1–6] Although the drug is undergoing clinical investigation, the chemical kinetics of the block of motor end-plate receptors by ORG NC45 have not been examined. Therefore, the action on the end-plate electrical response was measured quantitatively and the dissociation constant for the reaction of ORG NC45 with the receptor was estimated. The present experiments were also carried out to examine the effect of ORG NC45 on the isolated guinea pig vagus-atrium preparation. In previous studies with pancuronium and gallamine,[7–9] we have postulated that neuromuscular blocking agents that produce tachycardia clinically, do so by an effect on the vagal nerve terminals. ORG NC45 provides a unique opportunity to test this hypothesis since it is so closely related chemically and pharmacologically to pancuronium, yet apparently lacks the property of producing tachycardia. While one might find other agents for such a test, it is preferable to use one which is also of interest clinically in its own right.

ABBREVIATIONS

K_B(lumbrical) = Dissociation constant for the reaction between a competitive antagonist B and the acetylcholine receptor at the motor end-plate. When the antagonist is given at a concentration equal to K_B, half the receptors will be blocked.

K_B(atrium) = Dissociation constant for the reaction between a competitive antagonist and the muscarinic receptors of the pacemaker.

ED_{50}(lumbrical) = Concentration of competitive antagonist reducing indirect twitch response to one-half of the control value.

Y_{50} = Calculated fraction of end-plate receptors occluded at ED_{50}(lumbrical);
$= ED_{50}(\text{lumbrical})/[ED_{50}(\text{lumbrical}) + K_B(\text{lumbrical})]$.

VB_{50} = Concentration of antagonist producing a half-maximal shift of the frequency-response curve to preganglionic vagal stimulation.

FB_{50} = Concentration of antagonist producing a half-maximal shift of the train-length response curve to field (*i.e.*, postganglionic vagal) stimulation. (The explicit calculation of both the VB_{50} and FB_{50} is rather complicated.[8,9] In essence, both parameters go from zero when there is no shift from the control curve, through 0.5 when the curve is half way down and over to the scale of abscissae to 1 when the response to nerve stimulation is completely abolished.)

pA_2 = An index of potency of surmountable antagonists. It is the negative logarithm (to base 10) of the molar concentration of antagonist shifting the dose-response curve to the right by a factor of 2. Thus, a pA_2 of 6 means 10^{-6}M will shift the curve twofold. Since a concentration equal to K_B will also shift the dose-response curve twofold, a K_B of 10^{-6} corresponds to a pA_2 of 6.

* Assistant Professor of Anesthesia, Harvard Medical School, and Senior Associate in Anesthesia, Brigham and Women's Hospital, Boston, Massachusetts 02115.

† Professor of Anaesthesia and Pharmacology, Department of Anaesthesia, University of Massachusetts Medical School, Worcester, Massachusetts 01605.

‡ Professor of Pharmacology, Department of Pharmacology, University of Massachusetts Medical School, Worcester, Massachusetts 01605.

Received from the Department of Anesthesia of the Brigham and Women's Hospital, and the Departments of Anesthesia and Pharmacology of the University of Massachusetts Medical School. Accepted for publication February 6, 1981. Supported by Grant NS 12255 from NINCDS.

Address reprint requests to Dr. S. Lee Son: Department of Anesthesia, Brigham and Women's Hospital, 75 Francis Street, Boston, Massachusetts 02115.

0003-3022/81/0700/0012 $00.85

Methods

Isolated organ preparations from guinea pigs were used. All experiments were performed in Krebs' solution of the composition (mM): Na^+, 138; K^+, 5.9; Ca^{++}, 2.5; Mg^{++}, 1.22; Cl^-, 123; $H_2PO_4^-$, 1.2; $SO_4^=$, 1.22; HCO_3^-, 25; plus glucose 2.08 g/l, and kept at 36–37° C. A mixture of 95 per cent oxygen and 5 per cent carbon dioxide was bubbled through the solution. Five types of experiments were carried out, two on lumbrical muscle preparations to observe the neuromuscular blocking action of ORG NC45, and three on isolated beating preparations of the right atrium to examine effects on the vagus and cardiac pacemaker.

Series 1

In the first series of experiments, the effect of ORG NC45 on carbachol-induced end-plate depolarization was measured in a lumbrical muscle preparation by the moving fluid electrode technique of Fatt[10] as previously described from this laboratory.[7] Carbachol dose-response curves in the presence of graded concentrations of ORG NC45 were used to derive an estimate of the drug receptor dissociation constant, K_B(lumbrical), for the reaction of ORG NC45 with end-plate receptors.

Series 2

In the second series of experiments, the effect of ORG NC45 on neuromuscular transmission was observed in a nerve-lumbrical muscle preparation.[11] The effect of graded concentrations of ORG NC45 was to depress the twitch response and the relationship was plotted as a twitch tension *vs.* ORG NC45 concentration response curve. The concentration of ORG NC45 needed to produce 50 per cent depression of the twitch response was expressed as ED_{50} (lumbrical). The receptor occupancy at which twitch response was one-half, Y_{50}, was also derived from the ED_{50} (lumbrical) and K_B (lumbrical) from the preceding experiments.[11] Parallel experiments were done with pancuronium for comparison.

Series 3

The effect of ORG NC45 in blocking muscarinic receptors at the cardiac pacemaker was determined in a manner analogous to the first set of experiments above.[7] The spontaneously beating right atrial preparation was slowed by the addition of carbachol to the bath. Cumulative dose-response curves were generated first in the absence and then in the presence of ORG NC45. The resulting shift of the dose-response curve was used to estimate the dissociation constant, K_B (atrium), for the ORG NC45-muscarinic receptor reaction.

Series 4

The ability of ORG NC45 to antagonize the bradycardia produced by stimulation of the vagus nerve was determined by a quantitative assay.[8] The experimental preparation consisted of the right atrium and attached right vagus nerve. Stimulation of the nerve trunk with pulses of 3 ms at 1, 2, 5, 10, 20, 30, and 50 Hz, produced graded degrees of slowing of the spontaneously beating atrium. A plot of slowing of heart rate against vagal stimulation frequency was obtained in the absence and in the presence of ORG NC45. The extent of the shift of the control curve by ORG NC45 was used as previously described,[8] to derive and ED_{50} or VB_{50} for the vagal blocking drug effect.

Series 5

The ability of ORG NC45 to block the response to activation of postganglionic vagal fibers was also determined. As in previous studies,[9] the spontaneously beating right atrium was suspended between two stimulating electrodes in Krebs' solution. A voltage applied to these electrodes drives current through both the muscle and nerve cells in the atrial wall, and was timed to stimulate the postganglionic vagal fibers during the refractory period of the atrial muscle fibers. Timing was accomplished by sensing atrial depolarization and using the ECG to synchronize the stimulator. The ECG was also used to derive the interbeat interval or its reciprocal, the heart rate. After several normal beats, a train of stimuli was applied during the refractory period of the next beat and the duration of the subsequent interval or its reciprocal, the heart rate measured. Stimulation of the postganglionic vagal fibers increased that interval and the degree of slowing of atrial rate increased with the number of pulses in the train of test stimuli. Graphs of slowing of heart rate against train length were derived in the absence and in the presence of graded concentrations of ORG NC45. From the shift between these curves, ED_{50} values for block of the effect of field stimulation, FB_{50} were obtained.[9]

Results

Depolarization of the end-plate of the skeletal muscle preparation by carbachol was antagonized by ORG NC45. Figure 1 shows a representative example of experimental results. The dose-response curve shifts to the right in the characteristic "parallel" fashion clearly consistent with competitive kinetics. The dis-

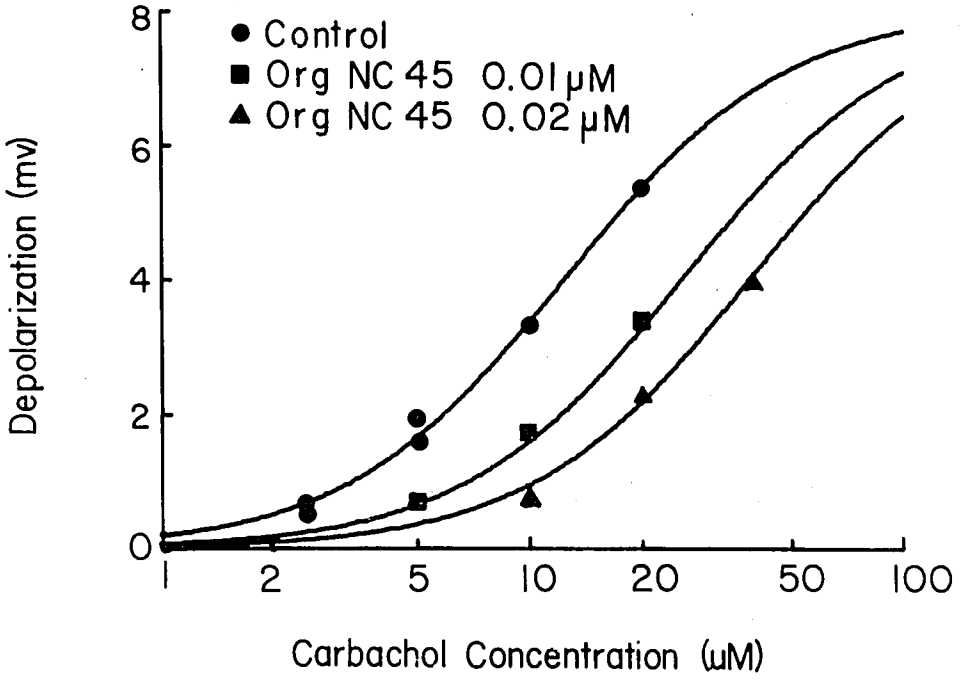

FIG. 1. Example of dose-response relationships obtained with ORG NC45 in guinea pig lumbrical preparation. Abscissae = concentrations of carbachol. Ordinates = depolarizations in mV (recorded with external moving fluid electrode). The circles (●) = controls; squares (■) = ORG NC45 0.01 μM; triangles (▲) = ORG NC45 0.02 μM.

sociation constant was estimated to be 0.0103 μM ± 0.0005 (SE). The effects of ORG NC45 and pancuronium on the twitch response to nerve stimulation are compared in figure 2. ORG NC45 was approximately twice as potent as pancuronium in this system. More importantly, the calculated receptor occupancy at 50 per cent twitch depression is 0.88 for ORG NC45, and 0.86 for pancuronium. Thus, both drugs seem to be acting by very similar mechanisms at the neuromuscular junction.

Figure 3 shows that the muscarinic receptors could be blocked by ORG NC45. The kinetics were competitive but the potency was low—the dissociation constant for the reaction was 5.79 μM ± 0.93 (SE).

Finally, figure 4 shows the relationship between concentration of ORG NC45 and the interference with the

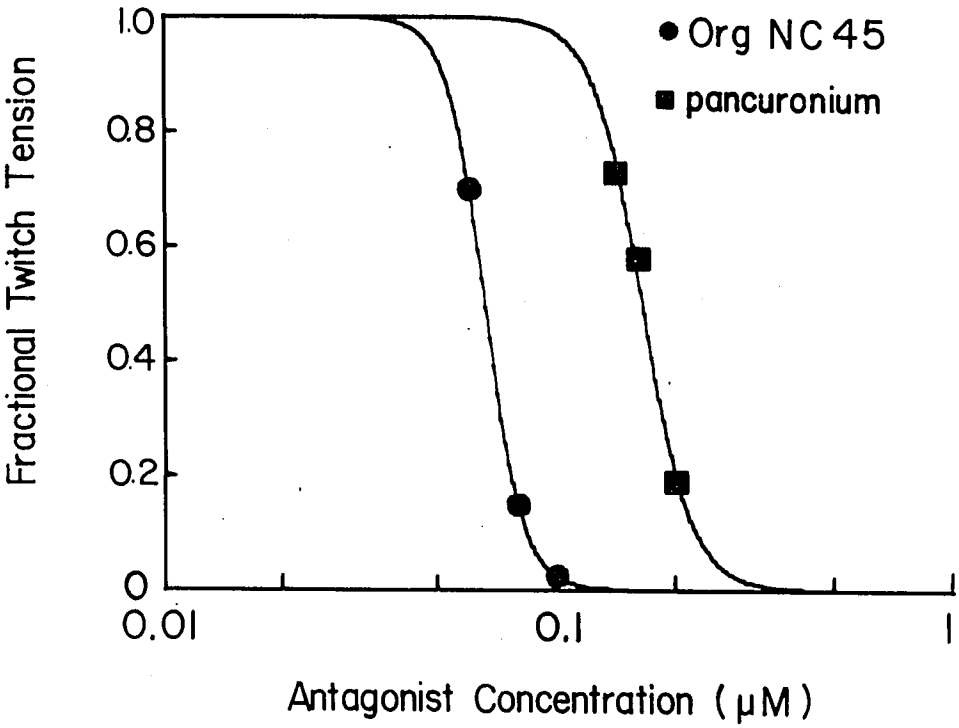

FIG. 2. Dose-response relationships of ORG NC45 and pancuronium on twitch response. Ordinates = twitch tensions as a fraction of control value. Abscissae = concentrations of agonist. The circles (●) = ORG NC45; squares (■) = pancuronium.

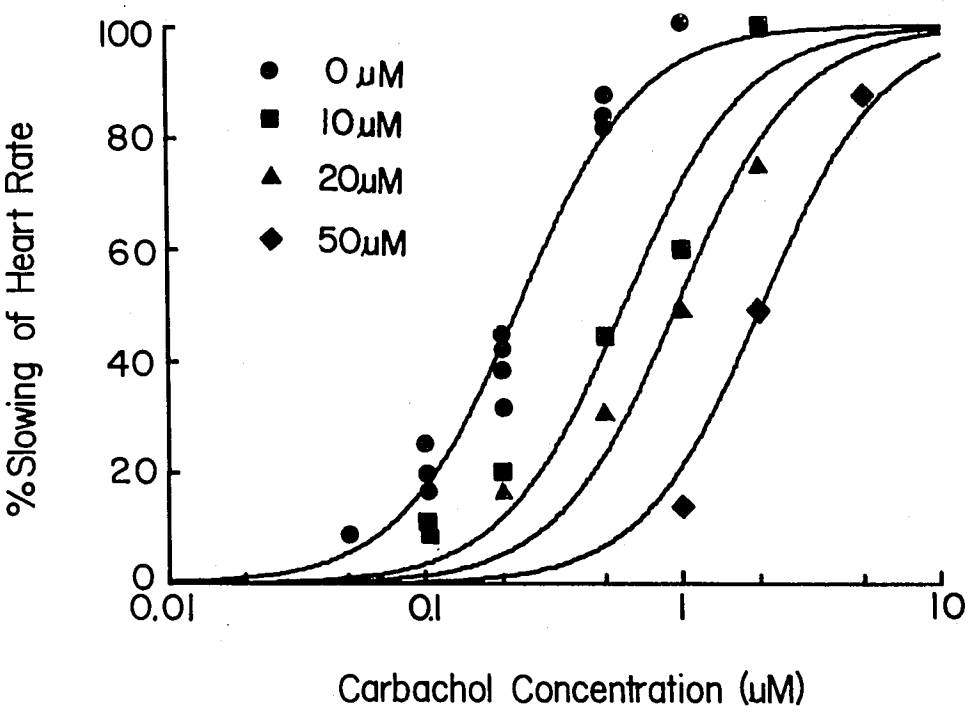

FIG. 3. Example of dose-response relationships obtained with ORG NC45 in guinea pig atrial preparation. Abscissae = concentrations of carbachol (μM). Ordinates = percentage of initial heart rate. The circles (●) = control; squares (■) = ORG NC45 10 μM; triangles (▲) = ORG NC45 20 μM; diamonds (◆) = ORG NC45 50 μM.

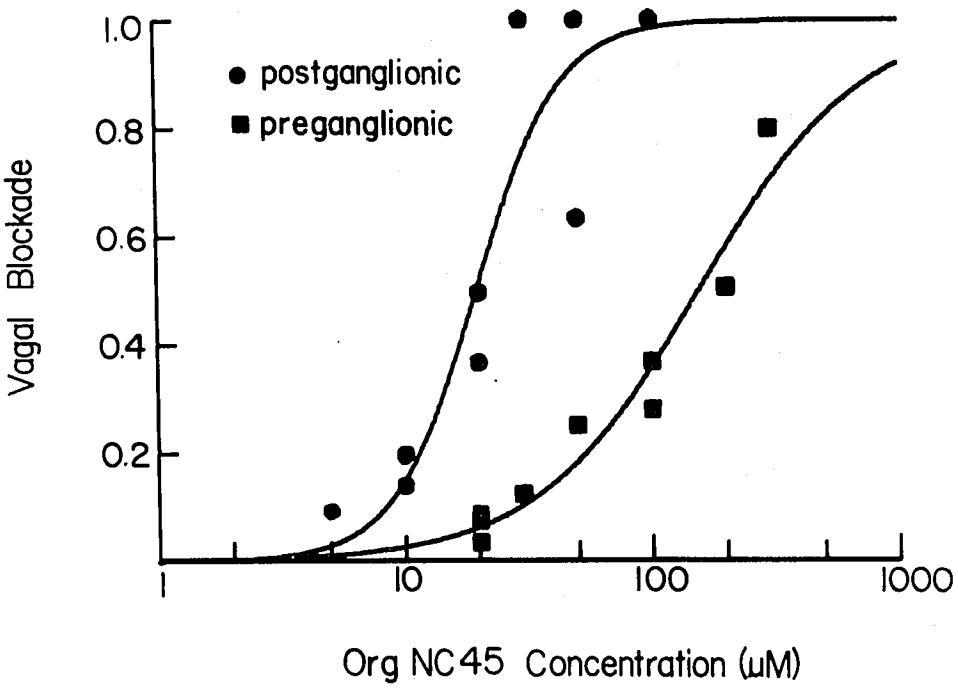

FIG. 4. Dose response relationships for ORG NC45 blockade of response to pre- and postganglionic stimulation. Abscissae = concentrations of ORG NC45 (μM). Ordinates = magnitude of block of response. The circles (●) = preganglionic stimulation; squares (■) = postganglionic stimulation. With both curves the magnitude of block was determined by comparing heart rate responses to graded nerve stimulation in the presence of a given concentration of ORG NC45 to those in the absence of the drug. With both pre- and postganglionic stimulation, the drug shifted the original frequency response curve down and to the right (cf figure 1 for Lee-Son and Waud).[8] An index was derived[8,9] which took the value 0 when there was no shift produced by the drug, and 1 when the drug shifted the curve all the way over and down to the scale of the abscissae. It is this index which is used as the ordinate in the present figure. Thus at low concentrations, ORG NC45 had no effect on either response to nerve stimulation, while by 100 μM the response to field stimulation was completely blocked while that to stimulation of the vagal trunk had been blocked about 40 per cent. Note that the relative location of the curves reflects the nature of the stimulation. The field stimuli were constrained to lie within the relatively brief refractory period of the atrial muscle. Therefore, field stimulation was not very vigorous. This explains why postganglionic stimuli appears paradoxically to be more sensitive to the drug than preganglionic stimuli. See also Discussion.

TABLE 1. Summary Estimates of Fractional Receptor Occlusion*

	ORG NC45	Pancuronium	Atropine
K_B (lumbrical)	0.0103 ± 0.0005 (n = 9)	0.025 ± 0.0014†	—
ED_{50} (lumbrical)	0.076 ± 0.0042 (n = 12)	0.151 ± 0.0068 (n = 9)	—
Y_{50}‡	0.88	0.86	—
K_B (atrium)	5.79 ± 0.93 (n = 4)	0.13‡	0.00063†
VB_{50}	156 ± 16 (n = 4)	0.532§	0.0116§
FB_{50}	19.7 ± 2.69 (n = 4)	0.292¶	0.004¶

* All values are μM ± SE except the measures of Y_{50} which have the dimensions of fraction of receptors blocked (n = number of experiments).

† Lee Son and Waud.[7]

‡ Y_{50}, the fractional receptor occupancy associated with a 50 per cent reduction in the twitch response, is calculated as ED_{50}(lumbrical)/[ED50(lumbrical) + K_B(lumbrical)].[11] Mean values were used for the calculation.

§ Lee Son and Waud.[8]

¶ Lee Son and Waud.[9]

response to pre- and postganglionic vagal stimulation. Although the drug can block preganglionic stimulation, it does so only at very high concentrations. Similarly, the response to direct activation of the postganglionic vagal nerve fibers requires a high concentration—19.7 μM ± 2.69 (SE), approximately 250 times larger than that blocking the neuromuscular junction.

The summary estimates of K_B (lumbrical), ED_{50} (lumbrical), fractional receptor occlusion at the ED_{50} (lumbrical), K_B(atrium), VB_{50} and FB_{50} are collected in table 1. The corresponding values for pancuronium and atropine are also listed for comparison.

Discussion

The kinetic analysis of the interaction of ORG NC45 with carbachol shows typical competitive kinetics. Thus the preliminary conclusion that the drug is nondepolarizing[4] can be extended to a more specific model. Furthermore, the relation of twitch response to receptor occupancy is indistinguishable from that seen with other competitive blocking agents. Specifically, the receptor occupancy at 50 per cent depression of twitch response was 0.88 and 0.86 for ORG NC45 and pancuronium, respectively. This corresponds well with Y_{50} values of 0.86 and 0.89 for *d*-tubocurarine and fazadinium (AH8165), respectively.[11] These four antagonists all occupy the same fraction of the receptor pool for the same effect. Thus the anesthesiologist is fully justified in viewing the action of ORG NC45 at the neuromuscular junction as identical to that of tubocurarine or pancuronium and differing only in the concentration needed.

The fact that ORG NC45 behaves in such a typical manner and has a potency relative to pancuronium similar to that seen elsewhere[1–6] indicates the guinea pig does not behave idiosyncratically. This in turn means the guinea pig is a reasonable system in which to test our hypothesis about the mechanism of the tachycardia seen clinically with competitive neuromuscular blocking agents.

The dissociation constant of ORG NC45 at the atrial pacemaker, K_B(atrium), was found to be 5.79 μM. Marshall and Ojewole[3] report a pA_2 value of 4.6 for ORG NC45 at the guinea pig atrium. Our K_B of 5.79 μM corresponds to a pA_2 of 5.24. This difference may be due to differences in experimental conditions. Marshall and Ojewole used electrically driven left atrial preparations at 32°C and measured the negative inotropic action of pilocarpine and acetylcholine. Our experiments were done on spontaneously beating right atria at 37°C, with carbachol as the cholinergic agonist. (The latter conditions seem more closely relevant to the issue of tachycardia in patients.) It is interesting to note that the same group[5] subsequently have reported a value of 5.4 at 37°C.

The K_B(atrium) for ORG NC45 (5.79 μM) is much larger than that of pancuronium (0.13 μM) indicating that ORG NC45 is a much weaker (44 times) antagonist at the cholinergic receptors of the atrial pacemaker when compared with pancuronium. Similarly a VB_{50} of 156 μM shows that ORG NC45 is much less potent in blocking vagal stimulation of the atrial pacemaker when compared with pancuronium for which a VB_{50} of 0.532 μM was found.[8] These observations again indicate that our preparation, the guinea pig atrium, behaves similarly to the system of Durant *et al.*[2] who have already clearly demonstrated a lack of effect of ORG NC45 on the response to vagal stimulation.

The present experiments also indicate that ORG NC45 does not block at the vagal ganglion and thus agree with previous findings in sympathetic ganglia.[3,4] The somewhat involved argument may be outlined as follows. The cardinal feature of a ganglionic blocking agent is that it would block the response to preganglionic stimulation of vagus more than it would block a stimulus activating at a point distal to the preganglionic synapse. Unfortunately, a natural measure of interference with preganglionic stimulation is not available (ideally one might like to count the number of postsynaptic fibers that are responding, but this cannot be done in the heart where the ganglion cells are buried in the muscle and the postsynaptic fibers are, therefore, not accessible). We have to use

heart rate as an indication of how many postsynaptic fibers have been blocked with the idea that the greater the ganglionic block, the less will be the cardiac slowing with preganglionic stimulation. Similarly, with postsynaptic activation we have to use reduction in heart rate response to carbachol as an index to block. To put a number on a drug's ability to block each type of stimulation, we have chosen arbitrary but clearly defined indices, the VB_{50} and K_B, respectively. To get the VB_{50} we noted that blocking drugs shifted the frequency response curve down and to the right, defined an index of such a shift, and chose the concentration which produced a half-maximal change in that index as the VB_{50}. The K_B (atrium) can be defined somewhat more directly as the concentration increasing the required dose of carbachol twofold. The problem arises when we wish to compare drugs without getting into a peaches-and-pears situation. The solution is to look at a drug which is known not to block at ganglia (*e.g.*, atropine). Atropine's relative effects then represent what one would expect from a drug without ganglionic activity. Thus, in the present experiments, atropine has a K_B(atrium) of 0.00063 μM and a VB_{50} of 0.0116 μM. The ratio is 18.4.[8] This ratio is what would be expected from a drug which does not block the cardiac vagal ganglia. Now, with the corresponding values for tubocurarine, the ratio becomes 1.5 (42.4 μM/28.2 μM),[8] indicating that, compared to atropine, tubocurarine blocks presynaptic stimuli preferentially. This result is in line with the well-known ganglionic blocking action of tubocurarine and confirms that our assay, though necessarily indirect and cumbersome, can detect a ganglionic block. We can now look at ORG NC45. It gave a ratio of 26.9 (156 μM/5.79 μM). Clearly, there is no sign whatsoever of a tendency to block presynaptic stimulation selectively, *i.e.*, ORG NC45 shows no blocking activity on cardiac vagal ganglia.

The next issue is to determine whether ORG NC45 has any action on vagal postganglionic nerve terminals such as that seen with pancuronium.[9] The natural measure of an action of a drug which blocks nerve terminals would be how much transmitter output was reduced. Unfortunately, it is not possible to measure acetylcholine output from vagal nerve endings directly and with precision. Therefore, we have resorted to an indirect measure based on the standard model for reaction of the transmitter A with the postsynaptic muscarinic receptor R:

$$A + R \rightleftharpoons AR \qquad (1)$$

Note that transmitter and receptor enter into this reaction symmetrically; one-half of A, for example, will produce the same effect on the concentration of AR as one-half of R. Thus, we can measure reduction in transmitter output in terms of an equivalent reduction in receptor capacity, a measure which is experimentally accessible. Since our measure of blockade of the response to stimulation of postganglionic axons, the FB_{50}, is once again an arbitrary index, it must be calibrated. As before, a reference drug can be used, in fact the same compound, atropine. From the K_B (atrium) for atropine the fraction of free receptors R/R_t (R represents the concentration of free receptors in equation 1 while R_t is the total concentration of receptors, one can only estimate fraction of receptors§) at the FB_{50} of atropine can be calculated[12] as follows:

$$\begin{aligned} R/R_t &= 1 - \text{fraction of receptors occupied} \\ &= 1 - FB_{50}/(FB_{50} + K_B) \\ &= 1 - 0.004/(0.004 + 0.00063) \\ &= 0.14 \end{aligned}$$

This small fraction reflects, incidentally, a large margin of safety for transmission at this synapse, *i.e.*, most (86 per cent) of the receptors have to be occluded to produce 50 per cent block of the response to nerve stimulation. The corresponding value found previously for tubocurarine was 0.16, illustrating a drug showing no sign of an action on the vagal nerve terminal, *i.e.*, the response to field stimulation was not blocked beyond what would be expected from block of receptors alone. In contrast, with pancuronium the value was 0.31 which indicates block of nerve stimulation when roughly twice as many receptors are available as was seen with atropine. In the framework of equation 1 this implies output of transmitter was reduced to approximately one-half by pancuronium.

With ORG NC45 the fraction of free receptors at the FB_{50} is

$$R/R_t = 1 - [19.7/(19.7 + 5.79)] = 0.23$$

corresponding to a reduction of transmitter output of only 40 per cent. Consideration of the variation associated with determination of the FB_{50} (fig. 4) suggests

§ The dissociation constant K_B is defined as $K_B = B.R/BR$ where B is concentration of free drug, R is concentration of free receptor, and BR is concentration of occupied receptors. Substituting the concentration of interest FB_{50} for B, and $R_t - BR$ for R, and rearranging yields $[FB_{50}/(FB_{50} + K_B)]$ for fraction of receptors occupied.

¶ At the FB_{50} for both drugs the effect is the same, therefore,

$$AR_{nmb} = AR_{atr}$$

(where nmb refers to the neuromuscular blocking agent being compared to atropine). From equation (1) this means

$$A_{nmb} \times R_{nmb} = A_{atr} \times R_{atr}$$

the value of 0.23 may not be significantly different from the reference value 0.14. An estimate for a standard error of the difference between values of 0.23 and 0.14, obtained[13] from standard errors of the FB_{50} and K_B(atrium), is 0.06. Thus the 0.14 plus two standard errors includes the value of 0.23 found with ORG NC45 (but, incidentally, not that for pancuronium). This means there is no experimental basis for an action of ORG NC45 on postganglionic vagal nerve terminals and our original hypothesis is sustained. ORG NC45, a drug which is extremely similar in structures to pancuronium, differs pharmacologically both in not producing tachycardia *in vivo*, and in not showing any sign of an effect on vagal postganglionic nerve terminals.

In summary, ORG NC45 behaves kinetically like a typical competitive neuromuscular blocking agent with a dissociation constant of 0.013 μM. ORG NC45 does not show any effect on the vagal nerve terminal. This lack of effect on the vagal control of the atrial pacemaker is consistent with the reports of lack of cardiac effect.[1–6] and confirms the hypothesis that an action on vagal nerve terminals underlies the tachycardia produced by pancuronium and gallamine.

(where A_{atr} and A_{nmb} are the control transmitter output and output in the presence of the neuromuscular blocking agent)

or

$$A_{nmb} \times R_{nmb}/R_t = A_{atr} \times R_{atr}/R_t$$

Therefore,

$$A_{nmb}/A_{atr} = (R_{atr}/R_t)/(R_{nmb}/R_t)$$

or, for pancuronium,

$$= 0.14/0.31 = 0.45$$

Thus, compared to control, the output is reduced 55 per cent.

The authors thank Organon Laboratories for kindly supplying the ORG NC45.

References

1. Booij LHDJ, Edwards RP, Sohn YJ, et al: Cardiovascular and neuromuscular effects of ORG NC45, pancuronium, *d*-tubocurarine and metocurine in dogs. Anesth Analg (Cleve) 59:26–30, 1980
2. Durant NN, Marshall IG, Savage DS, et al: The neuromuscular and autonomic blocking activities of pancuronium, ORG NC45, and other pancuronium analogues in the cat. J Pharm Pharmacol 31:831–836, 1979
3. Marshall RJ, Ojewole JAO: Comparison of the autonomic effects of some currently used neuromuscular blocking agents. J Pharmacol 66:77–78, 1979
4. Marshall IG, Agoston S, Booij LHDJ, et al: Pharmacology of ORG NC45 compared with other non-depolarizing neuromuscular blocking agents. Br J Anaesth 52:11S–19S, 1980
5. Marshall RJ, McGrath JC, Miller RD, et al: Comparison of the cardiovascular actions of ORG NC45 with those produced by other non-depolarizing neuromuscular blocking agents in experimental animals. Br J Anaesth 52:21S–31S, 1980
6. Crul JF, Booij LHDJ: First clinical experiences with ORG NC45. Br J Anaesth 52:49S–51S, 1980
7. Lee Son, Waud BE: Potencies of neuromuscular blocking agents at the receptors of atrial pacemaker and the motor end-plate of the guinea pig. ANESTHESIOLOGY 47:34–36, 1977
8. Lee Son S, Waud DR: A vagolytic action of neuromuscular blocking agents at the pacemaker of the isolated guinea pig atrium. ANESTHESIOLOGY 48:191–194, 1978
9. Lee Son S, Waud DR: Effects of non-depolarizing neuromuscular blocking agents on the cardiac vagus nerve in the guinea pig. Br J Anaesth 52:981–987, 1980
10. Fatt D: The electromotive action of acetylcholine at the motor end-plate. J Physiol (London) 111:408–422, 1950
11. Waud BE: Kinetic analysis of the AH8165-receptor interaction at the mammalian neuromuscular junction. ANESTHESIOLOGY. 46:94–96, 1977
12. Waud DR: Pharmacological receptors. Pharmacol Rev 20:49–88, 1968
13. Kendall MG, Stuart A: The Advanced Theory of Statistics. London, Griffin Publishers, 1958, pp 231–232