The relative vagolytic potencies of six muscle relaxants in the rabbit

SHEILA E. DRANE*, MARTIN H. EVANS†, *A.R.C. Institute of Animal Physiology, Babraham, Cambridge CB2 4AT*

In addition to the production of muscular paralysis, most neuromuscular blocking agents also reduce the negative chronotropic response of the mammalian heart to vagal efferent impulses (vagolysis) and this action has been well documented for cat and dog heart (Mautner & Luisada 1941; Guyton & Reeder 1950; Jacob & Depierre 1950; Riker & Wescoe 1951; Abrahams & Hilton 1962; Bonta et al 1968: Saxena & Bonta 1970; Hughes & Chapple 1976).

Data were needed for cardiovascular research using the rabbit in which the hypothalamus was to be stimulated. One effect of such stimulation is a bradycardia mediated by vagal efferents (Evans 1976). The only quantitative data on the effects of muscle relaxants upon the negative chronotropic responses in rabbit heart appear to be in the in vitro study by Goat & Feldman (1972) on the effects of (+)-tubocurarine, gallamine and pancuronium on acetylcholine-induced bradycardia in the perfused heart. Some preliminary experiments by one of us had suggested that gallamine showed less vagolytic action in the rabbit than in the cat. We have therefore compared systematically the paralytic and vagolytic potencies of some muscle relaxants in the rabbit. The results, from four non-depolarizing and two depolarizing neuromuscular blocking agents, should allow a rational choice to be made when muscular paralysis is required during cardiovascular experiments on the rabbit, and also offers a degree of comparison with data on carnivores. Muscle relaxants used were: alcuronium chloride (Alloferin, Roche), decamethonium bromide (Sigma), gallamine triethiodide (Flaxedil, May & Baker), pancuronium bromide (Pavulon, Organon), suxamethonium bromide (Brevidil, May & Baker) and (+)-tubocurarine chloride (Burroughs Wellcome). All doses are given as weights of salts, dissolved in 0·9% NaCl saline such that the dose could be administered in 0·1–1·0 ml (occasionally up to 3·0 ml) injected rapidly intravenously. Only one substance was tested in any one animal.

New Zealand White rabbits were anaesthetized by urethane (1 g kg^{-1} i.v.) and α-chloralose (60 mg kg^{-1} i.v.); with additional doses to ensure full anaesthesia during muscular paralysis (confirmed by the stability of the arterial blood pressure and heart rate during periods of rest). Preparative surgery included tracheotomy and catheterization of the femoral vein and artery in one hindlimb. In the other hindlimb the lateral popliteal nerve was divided and the peripheral end placed on platinum wire stimulating electrodes. The tendon of the tibialis anterior muscle was freed from its insertion and firmly connected to an isometric strain gauge. The limb was fixed rigidly with steel pins in the bone. One vagus nerve was divided in the neck and the peripheral end laid on stimulating electrodes.

The lateral popliteal nerve was stimulated by a 1 s train of supramaximal shocks (1–5 V, 1 ms, 60 s^{-1}) repeated every 60 s. The resulting isometric muscle tension was recorded, as was blood pressure in the femoral artery from a Statham P23Db transducer, and the analogue from beat-to-beat heart rates (HR) derived digitally from the intervals between successive systolic pulses, on channels of a polygraph. Vagal stimulation commenced 30 s after the popliteal nerve stimulus, lasted 10 s, and consisted of supramaximal shocks (4–9 V, 1 ms) at a rate of 10 or 20 s^{-1}, chosen to cause slowing of the HR to about half the resting rate.

Artificial ventilation was initiated about 1 h before administration of relaxant, the rate being made equal to that of spontaneous respiration and the amplitude adjusted to hyperventilation just sufficient to inhibit spontaneous attempts at respiration. This usually lowered the peak end-tidal CO_2 by about 0·5 percentage units (Beckman LB-1 gas analyser). During the latter part of this hour the muscle contraction tensions and HR responses usually became stable. As the animals were already being mechanically ventilated when paralysis commenced, the amplitudes of the post-paralytic responses could not be affected by changes in physiological state brought about by the artificial ventilation. If the response amplitudes were still drifting slightly at the end of the initial hour, this drift was analysed by linear regression and the regression line projected forward in time to establish corrected control amplitudes against which partially blocked responses were compared. In a few experiments the control period responses never stabilized sufficiently and the results were discarded.

Drug administration commenced with a sub-effective i.v. dose. Further doses were given at 2, 3, 5 or 7 min intervals (Table 1), the interval depending upon the duration of action of the drug. Each dose was calculated to raise the total to twice the previous level, except that a $1:\sqrt{2}:2$ geometrical progression was employed to span the steep portions of a dose-response curve. The total doses were calculated on the assumption that over the time of the experiment there was negligible elimination and effectively full accumulation of all doses. In most experiments on alcuronium, gallamine and suxamethonium the final accumulated dose was 512 times the first dose. In other experiments the ratio was usually 256:1 or, with (+)-tubocurarine and some of the pancuronium tests, 128:1.

The log dose: response curves were sigmoid, except that in addition there was distortion by augmentation of

* The work was performed while S.E.D. was on industrial training leave from the School of Studies in Pharmacology, University of Bradford, West Yorkshire BD7 1DP.

† Correspondence.

Table 1. The neuromuscular blocking potencies and vagolytic potencies of 6 compounds.

Compound	Drug cycle (min)	Doses giving 80% paralysis (mg kg^{-1}) means	range	Doses giving 20% vagolysis (mg kg^{-1}) means	range	Safety factors ratio means	Number of animals (n)
Suxamethonium	2	0·298 ± 0·030	0·23 — 0·40	—	>16·9	>42 to >96	5
Alcuronium	5	0·0165 ± 0·0012	0·014 — 0·019	0·410 ± 0·038	0·32 — 0·48	25·1 ± 2·4	4
Decamethonium	2	0·282 ± 0·036	0·19 — 0·38	5·33 ± 1·50*	2·80 — 8·00*	23·7 ± 9·6*	5
Pancuronium	2	0·0116 ± 0·0024	0·006 — 0·016	0·159 ± 0·021	0·11 — 0·21	15·3 ± 3·4	4
Gallamine	3	0·396 ± 0·036	0·29 — 0·47	1·840 ± 0·856	0·64 — 5·12	4·4 ± 1·9	5
(+)-Tubocurarine	7	0·398 ± 0·038	0·31 — 0·46	0·693 ± 0·180	0·48 — 1·23	1·7 ± 0·3	4

Safety factor is the ratio of the potencies, as defined in the text. In the case of suxamethonium the highest doses tested did not cause 20% vagolysis in any of the experiments tabulated, therefore the ratio must have been greater than the values given above. Means are given ± s.e.m.

* n = 3; in 2 other animals the highest doses (5·6 and 6·4 mg kg^{-1}) failed to cause 20% vagolysis and so the Safety Factors exceeded 16·7 and 22·6 respectively.

contraction tensions by sub-paralytic doses of decamethonium, gallamine and suxamethonium. The major portion of the curves could be straightened by plotting the percentage block on a probability scale against log dose. For these reasons it was not possible to find either the threshold or the 100% effective doses by extrapolation, so the dose which caused an 80% loss of muscle tension was found by interpolation. This value was then used for comparative purposes to represent a nearly paralytic dose. On this basis, pancuronium was the most potent muscle relaxant in the rabbit, followed by alcuronium, decamethonium, suxamethonium, gallamine and (+)-tubocurarine in that order. The numerical values are given in Table 1. All 6 muscle relaxants had dose-response curves with similar slopes, except for suxamethonium whose slope was less steep.

As the successive doses of drug accumulated, the negative chronotropic responses of the heart to vagal stimulation were affected. Slight augmentation was sometimes seen with near-paralytic doses of decamethonium and gallamine, and more rarely with alcuronium and pancuronium. All drugs reduced the HR responses after large doses. The percentage block was again plotted on a probability scale against log dose to achieve linearity, and therefore as with the muscle responses it was not possible to find the threshold dose. The dose: response curves for the drugs had similar slopes and these were much shallower than those of the muscle paralysing action. The dose that caused a 20% loss of the HR response was selected as representing a slightly vagolytic dose for comparative calculations, the numerical values found by graphical interpolation being given in Table 1.

Even the highest doses of suxamethonium used failed to cause a 20% vagolysis. This drug has a brief duration of action and so, although the final dose given equalled 50% of the total cumulative doses, we had to anticipate a significant failure for the earlier small doses to accumulate fully. Therefore it was given in large non-cumulative doses to two other rabbits (whose responses are not included in Table 1), with sufficient interval for full recovery between doses, and a 20% block of the HR response was obtained with doses 78 and 104 times the 80% paralytic dose.

The assumption that all doses accumulated fully introduces some error, but its magnitude is minimized by the use of progression times that are short compared with the duration of action, and by the geometrical progression of the doses which meant that the dose given in each bolus usually equalled the sum of all the preceding doses. The vagolytic doses we found can be compared with those of Goat & Feldman (1972) from their in vitro experiments on rabbit heart. Interpolation in their graphs indicates that the negative chronotropic responses to perfused acetylcholine suffered 20% reductions by pancuronium 0·2, (+)-tubocurarine 1·4 and gallamine 1·7 mg litre^{-1}. These figures agree reasonably with our in vivo figures, in mg kg^{-1}. The neuromuscular blocking doses in Table 1 also compare well with reported values. The determination of absolute potencies was not, however, our main object which was to establish the relative potencies of the vagolytic effects in the rabbit, compared with the muscle relaxant doses. Any error resulting from drug elimination could be expected to affect both the paralytic and the vagolytic figures to a similar extent.

If muscular paralysis is required during experiments on the cardiovascular system it is desirable to employ a paralysing agent with a high ratio: *dose that just fails to affect vagal bradycardia/dose that just achieves full paralysis.*

Because numerical values for this calculation could not be found accurately, for the reasons given, a working "Safety Factor" was defined for the purposes of the present study as the ratio: *dose that reduced vagal bradycardia by 20%/dose that reduced muscle contraction by 80%.*

The numerical values found for the 'Safety Factor' ratios of the 6 muscle relaxants are in Table 1. Suxamethonium appears to be the relaxant of choice for cardiovascular studies on the rabbit, judged by these criteria, having failed to cause a 20% block of the HR

response at any of the doses employed. 'Safety Factors' of about 78 and 104 were obtained in the two rabbits tested separately. The remaining 5 substances are tabulated in decreasing order of 'Safety Factor', the worst being (+)-tubocurarine with which some vagolytic action was seen even with doses that did not fully paralyse.

Comparison of the present results from the rabbit with data for the cat (Riker & Wescoe 1951; Bonta et al 1968; Hughes & Chapple 1976) suggest that the rabbit is relatively more resistant to the vagolytic effects of the non-depolarizing relaxants. The limited data for decamethonium (Abrahams & Hilton 1962) also suggests that the rabbit is more resistant than the cat to the vagolytic effects of this depolarizing relaxant.

It is a pleasure to thank Mr. M. R. Sutton for his conscientious technical assistance.

June 29, 1979

REFERENCES

Abrahams, V. C., Hilton, S. M. (1962) Br. J. Pharmacol. Chemother. 18: 194–203

Bonta, I. L., Goorissen, E. M., Derkx, F. H. (1968) Eur. J. Pharmacol. 4: 83–90

Evans, M. H. (1976) J. Physiol. (London) 260: 205–222

Goat, V. A., Feldman, S. A. (1972) Anaesthesia 27: 143–148

Guyton, A. C., Reeder, R. C. (1950) J. Pharmacol. Exp. Ther. 98: 188–193

Hughes, R., Chapple, D. J. (1976) Br. J. Anaesth. 48: 59–68

Jacob, J., Depierre, F. (1950) Arch. Int. Pharmacodyn. Ther. 83: 1–14

Mautner, H., Luisada, A. (1941) J. Pharmacol. Exp. Ther. 72: 386–393

Riker, W. F., Wescoe, W. C. (1951) Ann. N.Y. Acad. Sci. 54: 373–392

Saxena, P. R., Bonta, I. L. (1970) Eur. J. Pharmacol. 11: 332–341

An improved technique for the partial purification of SRS-A

T. B. Lee*, J. Fuher, M. C. Holroyde, J. Mann, J. R. Bantick, *Department of Biochemical Pharmacology, Fisons Ltd., Pharmaceutical Division, R & D Laboratories, Bakewell Road, Loughborough, Leicestershire, U.K.*

Slow reacting substance of anaphylaxis (SRS-A) is released immunologically from several animal tissues and is believed to be a major mediator in the pathogenesis of human asthma. A simple technique for the purification of SRS-A would greatly help the study of this substance. Several multistep techniques have been reported, many based upon the use of Amberlite XAD-2, -7 and -8 resins as originally described by Orange et al (1973). However, only the latter authors quantify the separation of SRS-A from histamine at the Amberlite chromatography stage. Furthermore, workers in this field (Orange 1978; Bach et al 1979) report that Amberlite chromatography gives extremely variable recoveries of SRS-A.

For our investigations we required SRS-A of reasonable potency, free from histamine and physiological salts. After considering the methods available in the literature we elected to use Amberlite XAD-8 in a single step process. An anaphylactic perfusate containing SRS-A was produced from perfused isolated lungs of sensitized guinea-pigs by the method of Engineer et al (1978). The perfusate was collected between 2 and 20 min after antigen injection. In all experiments indomethacin (1 μg ml^{-1}) was present in the perfusion fluid to increase the yield of SRS-A and prevent the formation of prostaglandins (Engineer et al 1978). The perfusates were pooled and centrifuged at 1000 *g* for 20 min at 4°C to remove contaminating cells. All bioassays of SRS-A were performed on guinea-pig isolated ileum in the presence of atropine (10^{-6} M) and mepyramine (10^{-6} M). Histamine was assayed fluorimetrically. Recoveries of SRS-A were determined by comparison of the activity of the purified sample with an aliquot of the original perfusate which had been stored at −20°C until the time of assay on the following day. Previous experiments using freeze-dried SRS-A as a standard had shown that storage of crude perfusate for this length of time did not lead to loss of activity.

After some preliminary investigative work an experiment was performed using conditions as described by Orange et al (1973). In this experiment guinea-pig lung perfusate (50 ml) was chromatographed on a glass column (2·5 cm diameter) containing pre-washed Amberlite XAD-8 (65 g). The SRS-A recovery in the ethanolic fraction was 63%, but this fraction also contained 76% of the original histamine. This was contrary to the findings of Orange et al (1973) who reported a complete separation of SRS-A and histamine.

Because it has been suggested that SRS-A may bind more strongly to Amberlite at a pH below 7 (Jakschik, personal communication), we investigated the effect of pH on the separation of SRS-A and histamine. Table 1 summarizes some of the results obtained using 10 ml perfusate and 10 g Amberlite XAD-8 in columns 1 cm diameter, in which the Amberlite had been equilibrated with phosphate buffers of different pH. The anaphylactic perfusate was adjusted with buffer to the same pH before addition to the column. The column was eluted with buffer (40 ml) and then 80% ethanol. In these experiments the elution of SRS-A and histamine was carried out at the same pH.

* Correspondence.