The effect of halothane on the action of alcuronium in the dog

R. S. Jones[1], R. Heckmann and W. Wuersch[2]

Veterinärchirurgische Klinik der Universität Zürich, Winterthurerstr. 260, CH-8057 Zürich (Switzerland), 22 December 1978

Summary. The effect of halothane on the action of alcuronium on neuromuscular transmission was studied in the intact dog. Electrical and mechanical evoked responses from stimulation of the ulnar nerve were recorded. The administration of 0.5% halothane prolonged the duration of action of alcuronium by about 50%.

It has been suggested that the duration of action of the neuromuscular blocking effect of the non-depolarizing muscle relaxants, in the human subject, is potentiated by halothane[3]. Halothane has been shown to potentiate the actions of pancuronium in the dog[4] and d-tubocurarine in the cat[5]. It has also been shown to potentiate the action of alcuronium in man[6]. In view of the paucity of information on the effects of halothane on alcuronium neuromuscular block in the dog it was decided to investigate the problem.

Materials and methods. The investigations were carried out in 4 Beagle dogs and the technique employed was that previously described[7]. After premedication with acepromazine and atropine anaesthesia was induced with thiopentone sodium and the trachea was intubated. A dose of 7 mg methadone was administered i.m. Anaesthesia was maintained with oxygen and nitrous oxide with controlled ventilation to produce a state of moderate hyperventilation. The experiments in the 1st group have been reported previously and the technique described[8]. In the 2nd, or halothane series 0.5% halothane was added to the delivered gases and this gave a range of 0.55–0.65% within the anaesthetic circuit. As soon as signs of electrical and mechanical muscular activity were observed atropine (0.6 mg) was administered followed by 0.5 mg of neostigmine. The observations were repeated once in each dog at intervals of not less than 1 week. The 1st group of experiments, without halothane, was completed before the 2nd group was commenced.

Results. The results are summarized in table 1. The duration of action of alcuronium from the time of administration to the return of spontaneous muscle activity, which was followed by the administration of atropine, and neostigmine, varied both between the experiments in the same dog and between dogs. This was seen in both groups of experiments either with or without halothane. In the 1st group the range varied from 51 to 109 min with a mean of 70 and a SD of ± 18 min. In the 2nd group, with halothane, the time to the return of spontaneous muscle activity varied between 85 and 175 min with a mean of 112 min and a SD of ± 35 min. The time to the return of full muscular activity, in the 1st group varied from 58 to 114 min with a mean duration of 75 min and a SD of ± 17 min. In the 2nd or halothane group the time to the return of full muscular activity varied from 91 to 181 min with a mean of 118 min and a SD of ± 37 min.

The statistical analysis using the Student t-test is summarized in table 2. A comparison was done between the mean times to the return of spontaneous muscular activity with and without halothane and of the times to restoration of neuromuscular transmission after atropine and neostigmine. There is a highly significant difference between the 2 groups, with and without halothane.

Discussion. The drugs used for the premedication and induction of anaesthesia in this series of experiments have not been shown to effect the action of muscle relaxant drugs.

The results demonstrate a definite effect by halothane on the duration of action of the neuromuscular blockade produced by alcuronium in the dog. This action is similar to that demonstrated in the human subject of potentiation of alcuronium by halothane[6]. It also shows the effect of halothane on alcuronium potentiation is similar to that described for pancuronium in the dog[4]. The neuromuscular depressant action of halothane has been shown to have several sites of action including the central nervous system[9], and the post synaptic membrane[10]. This investigation has demonstrated an approximate increase in the duration of action of pancuronium neuromuscular block of 50% by 0.5% halothane.

1 Present address: University Department of Anaesthesia, Royal Liverpool Hospital, Prescot Street, Liverpool L7 8XP (England).

2 We are indebted to Professor A. Mueller for facilities, help and encouragement. R.S. Jones was the holder of a Royal Society European Exchange Fellowship.

Table 1. A summary of the results. The 1st group is without halothane and the 2nd group with

Animal	Group 1 Time to return of muscular activity (min)	Time to return of full activity (min)	Group 2 Time to return of muscular activity (min)	Time to return of full activity (min)
D Experiment (1)	80	83	161	173
Experiment (2)	109	114	175	181
E Experiment (1)	58	63	92	97
Experiment (2)	51	58	85	92
F Experiment (1)	55	61	99	103
Experiment (2)	59	63	109	115
G Experiment (1)	71	77	86	91
Experiment (2)	76	81	90	96
Mean time and SD	70 ± 18	75 ± 17	112 ± 35	118 ± 37

Table 2. Statistical comparison of the results of the duration of action of alcuronium with and without halothane

Group 1 (no halothane) vs group 2 (halothane)			
Time to return of muscular activity. Significant difference of 2 means = 8.24	t = 5.12** df = 7	Time to return of full activity. Significant difference of 2 means = 9.11	t = 4.77** df = 7

3 S.A. Feldman, in: Major Problems in Anaesthesia, vol. 1, Saunders, London 1973.
4 C.L. Knight, P.K. Barnes and S.A. Feldman, Anaesthesia *33*, 139 (1978).
5 R. Hughes, Br. J. Anaesth. *42*, 826 (1970).
6 H. Grimmeisen, Anaesthetist *13*, 85 (1964).
7 R. Heckmann, R.S. Jones and W. Wuersch, Res. Vet. Sci. *23*, 1 (1977).
8 R.S. Jones, R. Heckmann and W. Wuersch, Res. Vet. Sci. *25*, 101 (1978).
9 A.J. Gissen, J.H. Karis and W.L. Nastuk, J. Am. med. Ass. *197*, 770 (1966).
10 R.L. Katz and G.J. Katz, Clin. Anaesth. *1*, 153 (1968).

The localization of [^{3}H]-desipramine in central nerve terminals studied with electron microscope autoradiography and subcellular fractionation

Z. Yavin, A. Biegon, R. Hofstein and D. Samuel[1]

Department of Isotope Research, Weizmann Institute of Science, Rehovot (Israel), 17 November 1978

Summary. The cellular and subcellular distribution of [^{3}H]-desipramine (DMI) in rat brain was studied by electron microscope (EM) autoradiography and by subcellular fractionation. A considerable proportion of label was found to be bound to the membranes of presynaptic nerve terminals, as well as to sites inside those terminals.

We have recently shown that, following the intracerebral administration of 6-hydroxydopamine (6-OH-DA), a marked reduction in the binding of [^{3}H]-desipramine (DMI) was found in all the rat brain areas investigated[2]. Furthermore, using light microscope autoradiography, we have demonstrated a preferential binding of [^{3}H]-DMI in the dopamine-rich caudate nucleus[2]. Since 6-OH-DA is known to be a relatively selective toxic agent for dopaminergic neurons of the caudate-putamen[3], this nucleus was selected for a more detailed electron microscope (EM) autoradiography. Investigation of binding of psychotropic drugs to particulate fractions from brain has been already established as a means of examining their subcellular distribution[4,5]. We now report on the localization of [^{3}H]-DMI in the rat brain, both by EM autoradiography and subcellular fractionation, following i.p. injection of the radiolabelled drug.

Materials and methods. Male Wistar rats were injected i.p. with 200 µCi/kg of [^{3}H]-desipramine-hydrochloride (44 Ci/mmole, IAEC-Negev, Beer-Sheva, Israel), diluted with unlabeled DMI (20 mg/kg), in a final volume of 0.5 ml saline.

30 min after injecting [^{3}H]-DMI, 8 rats were sacrified by decapitation and the pooled cerebral hemispheres homogenized in a solution containing 320 mM sucrose, 1 mM EDTA and 1 mM potassium phosphate buffer at pH 7.4. The homogenate was fractionated according to the method of Morgan et al.[6], with slight modifications. Aliquots (0.2 ml) of each fraction were taken for counting and protein determination[7].

Then 30 min after injecting the labelled drug, 2 rats were sacrified by perfusion with 4% paraformaldehyde, 0.5% glutaraldehyde and 0.54% glucose in 0.1 M phosphate buffer (pH 7.4), under nembutal anasthesia (50 mg/kg). The partially fixed caudate nuclei were carefully dissected into small cubes which were kept in the same fixative overnight and then post-fixed for 2 h in 2% buffered OsO_4, dehydrated and embedded in epon-araldite. Sections of 400–600 nm thickness were prepared for autoradiography by applying Illford L4 emulsion. Following exposure of 6–8 weeks, the sections were developed and examined by a Phillips 300 EM, after staining with methanolic uranyl acetate and with lead citrate.

Results and discussion. The subcellular distribution of [^{3}H]-DMI, expressed as cpm/mg protein, is summarized in the table 1. There was no loss of radioactivity (100% recovery) during the 1st centrifugation, 40% of the total radioactivity was lost during the Ficoll gradient step and an additional

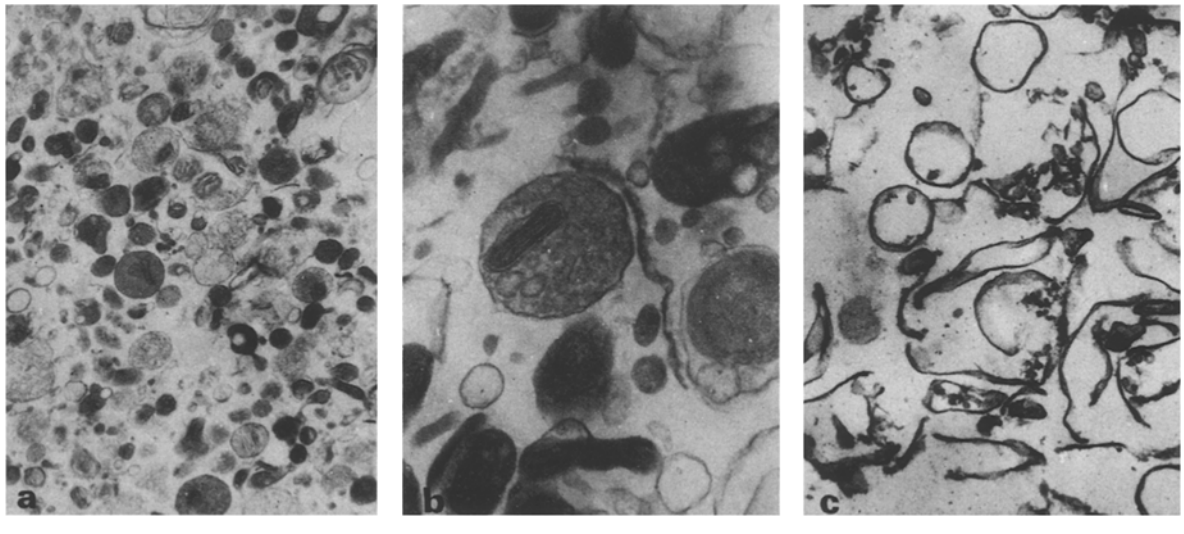

Fig. 1. EM micrographs of synaptosomal and SPM preparations: *a* Typical field of synaptosomes, prepared from pooled 12 and 16% discontinuous Ficoll gradients. × 15,000. *b* Synaptosomes as in figure 1a, at a higher magnification. × 35,000. *c* Typical field of the synaptosomal plasma membrane (SPM) fraction. × 30,000.