RESEARCH PAPERS

THE INFLUENCE OF ANTICHOLINESTERASES ON THE NEUROMUSCULAR BLOCK PRODUCED BY SUXAMETHONIUM

BY R. KOHN* and D. BOVET

From the Istituto Superiore di Sanità, Rome

Received December 8, 1955

INTRODUCTION

THE short acting muscle-relaxing property of suxamethonium was demonstrated by Bovet and others[1] and confirmed by Phillips[2] and Walker[3]. Contrary to the findings with curare, anticholinesterase drugs increased the toxicity of suxamethonium in laboratory animals and prolonged the duration of its action[4–6].

Marotta and Carminati,[7] investigating the action of a number of anticholinesterases when injected before suxamethonium, found that they increased the duration of the neuromuscular block, and related this to the level of serum pseudocholinesterase. No such action was observed when the true cholinesterase was blocked. Similar results have been reported by Fraser[8], who showed that the inhibition of true cholinesterase by 284 C 51 (1 : 5-bis(4-allyldimethylammonium phenyl)-*N*-pentan-3-one dibromide) *in vivo* did not modify the neuromuscular blocking action of suxamethonium, whereas when pseudocholinesterase was also inhibited by the administration of eserine, a distinct prolongation of the effect was observed.

The extensive use of suxamethonium in endoscopy, electro-shock therapy and surgery has resulted in instances of prolonged apnœa which have been widely quoted in the literature[9–13].

To account for these occurrences it was suggested that the duration of action of suxamethonium was normally dependent upon the pseudocholinesterase level[14,15]. Bourne[16] observed prolonged apnœa in 15 out of a series of 1000 patients, in 10 of whom pseudocholinesterase values were determined. It was found that 8 patients possessed significantly low, and the other 2 comparatively low levels of this enzyme. On the other hand, however, prolonged apnœa has also been reported to occur in patients possessing normal pseudocholinesterase levels[17,18].

Owing to the conflicting theories advanced by various workers to account for these abnormal clinical findings and their relationship with pseudocholinesterase levels the present investigation was undertaken to examine further the effects of the anticholinesterases eserine and neostigmine on the neuromuscular blocking action of suxamethonium in the rabbit and dog.

* During the tenure of the Charter Travelling Fellowship awarded by the Pharmaceutical Society of Great Britain.

METHODS

Dogs were anæsthetised with chloralose (120 mg./kg. intravenously) and rabbits with urethane (1·5 g./kg. subcutaneously). The contractions of the gastrocnemius muscle, following electrical stimulation every 15 seconds of the cut peripheral end of the sciatic nerve, were recorded isometrically. The blood pressure from the carotid artery was registered by a mercury manometer and the abdominal respiration was recorded by a Marey tambour. Tracheotomy was performed and artificial respiration supplied when required.

Suxamethonium dichloride, neostigmine methylsulphate and eserine salicylate were used. All drugs were administered according to body weight by the intravenous route. The doses injected are expressed in terms of these salts.

The response to a dose of suxamethonium, selected to cause a reproducible partial neuromuscular block, was compared before and after administration of eserine or neostigmine (0·05 to 0·1 mg./kg.). In some experiments the dose of suxamethonium given after the anticholinesterases was varied to produce the same reduction in the height of contraction as that obtained before the administration of the anticholinesterases.

The degree of paralysis was expressed as the per cent. diminution of the initial height of contraction (complete paralysis = 100 per cent.). Duration of paralysis was estimated from the "half-return" of the contraction height to its initial level. In this way the effects of the anticholinesterases on both degree and duration of suxamethonium paralysis could be determined.

RESULTS

The effect of eserine in the rabbit

It was generally observed that doses of from 0·125 mg./kg. to 0·25 mg./kg. suxamethonium produced a partial paralysis which was fairly reproducible. After the injection of 0·1 mg./kg. eserine which had no effect on the muscular contractions it was found that similar doses of suxamethonium gave rise to a more intense and longer sustained muscular paralysis.

It will be noted from Table I which summarizes five typical results that 0·125–0·25 mg./kg. suxamethonium caused 33 to 81 per cent. inhibition of the neuromuscular contraction, and the half-return required from 3 to 5½ minutes. After eserine the neuromuscular block produced by the relaxant was 57 to 100 per cent. and the time for the half-return was now 6 to 12 minutes.

In Figure I it will be observed that 0·25 mg./kg. suxamethonium produced 81 per cent. inhibition in the muscular contraction, and the time taken for half-return was 4 minutes. After 0·1 mg./kg. eserine the same dose of suxamethonium produced complete paralysis, and the time for the half-return was 10 minutes. Smaller doses of the compound were then injected in order to reproduce the initial reduction of contractions. When 0·075 mg./kg. suxamethonium was injected into the animal, the

TABLE I

THE EFFECT OF SUXAMETHONIUM BEFORE AND AFTER ESERINE IN THE RABBIT AND DOG

	Suxamethonium before eserine				Suxamethonium after eserine		Modification of neuromuscular blocking effect	
Dose of suxamethonium (mg./kg.)	Block per cent.	Duration of half return (minutes)	Dose of eserine (mg./kg.)	Effect of eserine on muscular contractions	Block per cent.	Duration of half return (minutes)	Intensity	Duration
Rabbits								
0·125	35	4	0·1	none	70	8	++	++
0·25	33	5½	0·1	,,	58	12	++	++
0·25	39	5½	0·1	,,	57	10	++	++
0·25	47	3	0·1	,,	74	6	++	++
0·25	81	4	0·1	,,	100	10	++	++
Dogs								
0·0375	80	5	0·1	,,	65	9	−−	++
0·05	47	3	0·1	,,	34	5½	−−	++
0·05	65	3½	0·1	,,	35	7½	−−	++
0·1	75	10	0·1	,,	63	15	−−	++

TABLE II

THE EFFECT OF SUXAMETHONIUM BEFORE AND AFTER NEOSTIGMINE IN THE RABBIT AND DOG

	Suxamethonium before neostigmine				Suxamethonium after neostigmine		Modification of neuromuscular blocking effect	
Dose of suxamethonium (mg./kg.)	Block per cent.	Duration of half return (minutes)	Dose of neostigmine (mg./kg.)	Effect of neostigmine on muscular contractions before suxamethonium	Block per cent.	Duration of half return (minutes)	Intensity	Duration
Rabbits								
1. 0·125	55	4	0·1	10 per cent. inhibition with slow return	61	6	+	+
2. 0·25	54	4	0·1	No inhibition	60	4½	(+)	(+)
3. 0·25	60	4	0·1	18 per cent. inhibition with partial return	62	6	no effect	+
4. 0·25	49	5	0·1	10 per cent. inhibition very slow return	73	8½	++	++
5. 0·25	55	8	0·1	34 per cent. inhibition without return	83	24	++	+++
Dogs								
1. 0·05	55	4	0·05	31 per cent. inhibition with complete return	48	9½	−	++
2. 0·075	41	4½	0·075	12 per cent. inhibition, complete return	31	6	−−	+
3.* 0·05	62	5	0·1	15 per cent. inhibition, incomplete return	30	11	−−	++
4. 0·05	42	4	0·1	48 per cent. inhibition with partial return	67	9	++	++
5. 0·075	64	4½	0·1	50 per cent. inhibition slow return	83	12	++	+++

* Atropine was given before neostigmine.

same type of paralysis curve as that given by 0·25 mg./kg. before the injection of eserine, was obtained. The percentage inhibition was 82 per cent. and half-return took 3¾ minutes. Thus the pattern of the block was duplicated when a fraction of the initial dose of suxamethonium was injected into the eserinised rabbit.

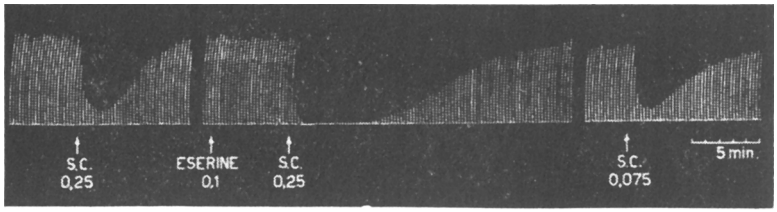

FIG. 1. Rabbit, 2·2 kg. under urethane anæsthesia; doses in mg/kg. Neuromuscular blocking action of suxamethonium before and after eserine. Contractions of the gastrocnemius muscle after electrical stimulation of the peripheral portion of the cut sciatic nerve.

The effect of eserine in the dog

In the dog, which was more susceptible to suxamethonium than the rabbit, doses of from 0·0375 mg./kg. to 0·1 mg./kg. gave the required partial muscular paralysis, i.e. generally one quarter of the dose needed in the rabbit produced the equivalent partial neuromuscular block in the dog.

The results of some representative experiments are shown in Table I.

The suxamethonium paralysis was from 47 to 80 per cent. and the time taken for the half-return was from 3 to 10 minutes. When eserine had been administered, suxamethonium produced from 34 to 65 per cent. neuromuscular block and the half-return was from 5½ to 15 minutes.

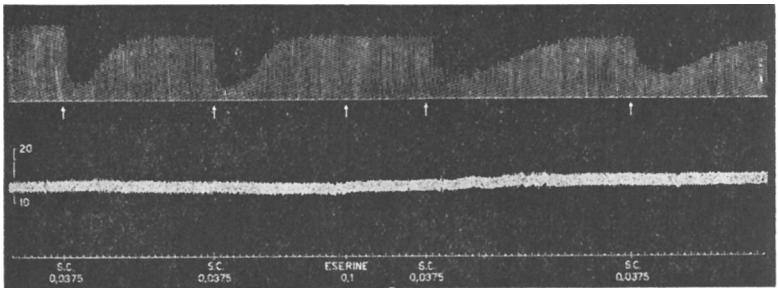

FIG. 2. Dog, 10 kg, under chloralose anæsthesia; doses in mg/kg. Neuromuscular blocking action of suxamethonium before and after eserine. Blood pressure from the carotid artery and contractions of the gastrocnemius muscle after electrical stimulation of the peripheral portion of the cut sciatic nerve.

Figure 2 shows that 0·0375 mg./kg. of the relaxant produced 77 per cent. and 81 per cent. inhibition in the muscular contractions, and the duration of the half-return was 4 and 5 minutes respectively. After eserine 0·0375 mg./kg. suxamethonium produced 67 and 61 per cent. inhibition and the time taken for the half-return was 9 and 8½ minutes.

It was clear that eserine in the dog did not potentiate the extent of the paralysing action of suxamethonium, as was observed in the rabbit; in fact some antagonism in the neuromuscular blocking action of the relaxant was noted. The duration of the paralysis was, however, distinctly increased.

The effect of neostigmine in the rabbit

A dose of 0·1 mg./kg. of neostigmine had little effect on blood pressure although it produced a distinct diminution in muscular contractions. Only in one experiment could no inhibition be observed, whereas in other cases the diminution in contraction varied from 10 to 34 per cent. The return to the original level was usually very slow and at times only partial. Suxamethonium before neostigmine gave degrees of inhibition varying from 49 to 60 per cent. and the time for the half return was from 4 to 8 minutes. The effect of 0·1 mg./kg. neostigmine on the response to the relaxant varied greatly from one experiment to another. In some experiments where neostigmine exerted only a slight action, a small increase in the intensity and duration of the suxamethonium paralysis was noted (Experiments 1, 2, 3, Table II). In others, (Experiments 4, 5, Table II), where the effect of neostigmine alone was especially prolonged, a substantial augmentation in the intensity and duration of the suxamethonium block could be observed.

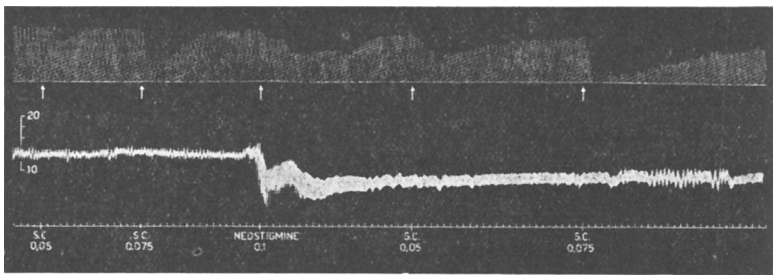

FIG. 3. Dog, 12 kg. under chloralose anæsthesia; doses in mg/kg. Neuromuscular blocking action of suxamethonium before and after neostigmine. Blood pressure from the carotid artery and contractions of the gastrocnemius muscle after electrical stimulation of the peripheral portion of the cut sciatic nerve.

The effect of neostigmine in the dog

Neostigmine when administered in a dose of 0·05 to 0·1 mg./kg. in the dog caused a fall in blood pressure which was concomitant with a reduction in the height of muscular contractions of up to 50 per cent. When atropine was injected (Experiment 3, Table II) the fall in blood pressure was prevented, and the muscular paralysis induced was slight. Suxamethonium before neostigmine produced degrees of inhibition in the muscular contractions from 41 to 64 per cent. and the time for the half-return was 4 to 5 minutes. An increase in the duration of the paralysis was noted when the relaxant was administered after neostigmine.

However, different results on the intensity of the neuromuscular block were obtained, which appeared to depend upon the previous effect of neostigmine alone. Figure 3 shows the direct effect of neostigmine on muscular contractions and on blood pressure. The inhibition in the contractions after neostigmine is 50 per cent. and the return very slow. Subsequent administration of suxamethonium leads to a distinct increase in the extent and duration of the neuromuscular block. In instances where the neostigmine paralysis was slight, or a complete return to the original height of contraction was rapidly obtained (Experiments 1, 2, 3, Table II), there was an antagonistic action towards suxamethonium and consequently the extent of the paralysis was diminished.

DISCUSSION

The experiments described indicate that, depending upon the animal species used, the neuromuscular block produced by suxamethonium may be modified in two different ways by the previous administration of anticholinesterases. The first may be illustrated by the action of eserine in the rabbit. In this species, eserine increases both the degree and duration of the block. As no significant alteration in the pattern of the curve was noted, this type of increased susceptibility may conveniently be termed a "dosage effect".

In the dog, however, eserine prolongs the duration of block while in effect decreasing its intensity. This may be described as a "time effect". Neostigmine produced different modifications of the suxamethonium response in the two species. The final result appeared to be mainly influenced by the direct previous action of neostigmine on the muscular contractions. In contrast to eserine, neostigmine when administered even in minute doses was found to provoke marked hypotension and diminution in muscular contractions.

The effect of neostigmine upon neuromuscular contraction has been referred to in the literature[19]. A direct effect of neostigmine on skeletal muscle has been reported by Riker and Wescoe[20]. Zaimis[21] has also drawn attention to the direct action of neostigmine on the neuromuscular junction. It may be of interest to mention that Jacobsohn and Kahlson[22] have demonstrated a difference in the anticurare activity between eserine and neostigmine.

The potentiation of the neuromuscular blocking action of suxamethonium by anticholinesterases may be related to its hydrolysis in the organism. Thus Glick[23] and Bovet-Nitti[4] demonstrated the rapid hydrolysis of the compound by pseudocholinesterase, and to a less significant extent by the true cholinesterase of erythrocytes. Eserine appears to interfere with the destruction of suxamethonium owing to the reduction in the circulating cholinesterase level. It has also been shown that the ali-esterase present in the liver and kidney hydrolyses suxamethonium[24]. In the "dosage effect" observed in the rabbit the interference with the hydrolysis of suxamethonium by anticholinesterases may account for the results obtained. In the interpretation of the time effect, however, additional factors must be considered. Thus the

diminution in the intensity of the paralysis noted in the dog may be related to the general antagonism which is known to exist between anticholinesterases and curare-like drugs. In the case of decamethonium whose mechanism of action is very similar to that of suxamethonium, "decurarising" effects after the administration of anticholinesterases were reported by Bovet *et al.*[25] and by Brand[26]. The antagonistic effect of anticholinesterases towards curare was generally interpreted as an inhibition of the hydrolysis of acetylcholine liberated at the motor end-plate thus facilitating neuromuscular transmission. The particular pattern of the time effect of cholinesterase inhibition may be explained as a combination of the simple potentiation related to the slowing in suxamethonium hydrolysis in the body with the so-called "decurarising" action of eserine related to the endogenous acetylcholine metabolism.

It may be assumed that the effect of anticholinesterases on the paralysis produced by suxamethonium is a consequence of a complex series of interactions. Thus both eserine and neostigmine may interfere with: (*a*) the enzymatic hydrolysis of the compound in the circulation and in the liver, (*b*) its enzymatic hydrolysis by the enzyme concentrated at the level of the motor end-plate, and (*c*) the metabolism of acetylcholine which acts as mediator in neuromuscular transmission. The relative importance of these various factors may be responsible for the different tracings obtained in these experiments.

The various factors enumerated above should be considered in advancing an explanation for suxamethonium apnœa observed clinically. Even if the suggestion be accepted that the prolonged apnœa encountered after its administration is caused by reduction of cholinesterase, which is supported to some extent by experimental evidence, it is clear that this cannot account for all the cases reported in the literature.

In the experiments which have been recorded, the duration of suxamethonium paralysis was increased up to 3 fold after anticholinesterases, even when repeated doses of neostigmine or eserine had been administered and muscarinic effects were observed.

Durrans[27] considered that thiopentone used to induce anæsthesia was responsible for prolonged apnœa, and Barron[28] and Beecher and Todd[29] advanced the view that depression of the respiratory centre, which could be relieved by the administration of nikethamide, was involved in the phenomenon.

Davis *et al.*[30] have shown that prolongation in the duration of suxamethonium paralysis in animals may be increased by ventilation with 20 per cent. carbon dioxide. Among the factors involved in suxamethonium apnœa Dripps[31] suggested central depression, alterations in the tissue concentrations of carbon dioxide and oxygen, potassium deficiency, and changes in the flow of blood to the muscle.

It has been shown by E.E.G. tracings both in man and in experimental animals that curare-like drugs have no action on the cortical electrical activity when administered, as usual, intravenously. Smith *et al.*[32] demonstrated that in man the normal blocking of α waves after external stimuli was still elicited, when $2\frac{1}{2}$ times the dose of tubocurarine required

to produce respiratory paralysis was given. Confirmation of these findings has been reported in the rabbit by Bovet and Longo[33], who showed that the blocking action was still present after the administration of 100 times the LD50 of suxamethonium. This illustrates the lack of action of natural and synthetic blocking agents on the central neuronal synapses which mediate conduction of stimuli from the periphery to the cortex. In the general review of Toman and Davis[34] it is stated that "if curare exerts a central action it is excitory and not depressant." Although the cholinesterase levels of patients are likely to influence the duration of prolonged apnœa as observed clinically, this would not appear to be the only factor involved.

SUMMARY

1. The effects of the anticholinesterases eserine and neostigmine in modifying the neuromuscular block induced by suxamethonium have been investigated in the rabbit and dog. Eserine in the rabbit potentiated the action of suxamethonium both in the duration and intensity of the neuromuscular paralysis. This has been termed a "dosage effect".

2. Eserine, in the dog, diminished the intensity of the paralysis produced by suxamethonium. The duration of the muscular block was increased, however, and this type of action was termed a "time-effect".

3. Neostigmine in the rabbit increased the duration and intensity of the suxamethonium paralysis.

4. With neostigmine in the dog two effects were noted. Where neostigmine gave rise to a strong muscular action, an increase in the duration and intensity of the suxamethonium block was obtained. In the absence of a substantial muscular action by neostigmine an increase in the duration with a diminution in the intensity of the suxamethonium paralysis was observed.

5. The different types of tracings which were obtained experimentally have been discussed in relation to possible explanations of suxamethonium apnœa as observed clinically.

REFERENCES

1. Bovet, Bovet-Nitti, Guarino, Longo and Marotta, *Rend., Ist. Super. San.*, 1949, **12**, 106.
2. Phillips, *J. Amer. chem. Soc.*, 1949, **71**, 3264.
3. Walker, *J. chem. Soc.*, 1950, 193.
4. Bovet-Nitti, *Rend., Ist. Super. San.*, 1949, **12**, 138.
5. Castillo and De Beer, *J. Pharmacol.*, 1950, **99**, 458.
6. Löw and Tammelin, *Acta physiol. scand.*, 1951, **23**, 78.
7. Marotta and Carminati, *Arch. Int. Pharmacodyn.*, 1953, **98**, 255.
8. Fraser, *Brit. J. Pharmacol.*, 1954, **9**, 429.
9. Bourne, Collier and Somers, *Lancet*, 1952, **262**, 1225.
10. Cowan, *Anæsthesia*, 1954, **9**, 23.
11. Harrison, Seward and Skinner, *Anæsthesia*, 1954, **9**, 21, 22.
12. Richards and Youngman, *Brit. med. J.*, 1952, **1**, 1334.
13. Walker, *Lancet*, 1954, **266**, 103.
14. Foldes, *Lancet*, 1952, **263**, 245.
15. Hall, Lehmann and Silk, *Brit. med. J.*, 1953, **1**, 134.
16. Bourne, *Brit. J. Anæsth.*, 1953, **25**, 116.
17. Wallemacq, *Acta Anaesth. Belg.*, 1953, **4**, 53.
18. Wolfers, *Brit. med. J.*, 1952, 2, 778.

19. Briscoe, *Lancet*, 1936, **230**, 469.
20. Riker and Wescoe, *J. Pharmacol.*, 1946, **88**, 58.
21. Zaimis, *J. Physiol.*, 1951, **112**, 176.
22. Jacobsohn and Kahlson, *Arch. f. Physiol.*, 1938, **79**, 27.
23. Glick, *J. biol. Chem.*, 1941, **137**, 357.
24. Bovet-Nitti, 1955, Personal communication.
25. Bovet, Bovet-Nitti, Guarino, Longo and Fusco, *Arch. Int. Pharmacodyn.*, 1951, **88**, 1.
26. Brand, *Experientia*, 1952, **8**, 273.
27. Durrans, *Lancet*, 1952, **263**, 539.
28. Barron, *Brit. med. J.*, 1952, **2**, 833.
29. Beecher and Todd, *Ann. Surg.*, 1954, **140**, 2.
30. Davis, Ellis, Reese and Grosskreutz., *Anæsthesiol*, 1955, **16**, 333.
31. Dripps, *Ann. Surg.*, 1953, **137**, 145.
32. Smith, Brown, Toman and Goodman, *Anæsthesiol.*, 1947, **8**, 1.
33. Bovet and Longo, *Electroencephalography and Clin. Neurophys.*, 1953, **5**, 225.
34. Toman and Davis, *Pharmacol. Rev.*, 1949, **1**, 425.