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The relationship between the level of cholinesterase in plasma and the action of suxamethonium in animals

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Summary

1. The neuromuscular blocking action of suxamethonium, given by intravenous injection, and the effect upon it of *iso*-OMPA (tetra*iso*propyl pyrophosphoramide) in doses which produced marked selective inhibition of cholinesterase in blood were studied in anaesthetized rats and cats, and in mice.

2. In cats experiments were also carried out in which suxamethonium was given by intravenous infusion until an effect which remained constant with time was achieved. From the degree of neuromuscular block (under equilibrium conditions) obtained with different infusion rates the infusion rate for 50% reduction in twitch tension of the indirectly stimulated soleus and gastrocnemius muscles (IR50) was calculated. The effect on it of raising the suxamethonium hydrolysing capacity of blood and of selectively reducing the level of cholinesterase in blood by various doses of *iso*-OMPA was then investigated.

3. At relevant stages of each experiment cholinesterase activity in blood was determined with butyrylcholine or benzoylcholine and where appropriate with suxamethonium as substrate.

4. The results obtained show that in rats and cats the effectiveness of suxamethonium is unrelated to the level of cholinesterase activity in blood and that raising the suxamethonium hydrolysing capacity in the blood up to 22-fold (in cats) only reduces the IR50 by a factor of 1·6.

5. The enhancement of the effectiveness of suxamethonium in the three species (2- to 3-fold in rats, 2- to 4-fold in mice and 7- to 8-fold in cats under the conditions used for comparison) which follows the administration of *iso*-OMPA is attributable to inhibition of cholinesterase in the tissues.

6. It is concluded that the results obtained clearly indicate that the species studied do not give information as regards suxamethonium and its metabolism which is applicable to man.

Introduction

Suxamethonium is a substrate for cholinesterase and in man its effect on neuromuscular transmission is inversely related to the level of cholinesterase (acylcholine acylhydrolase; EC 3.1.1.8) in plasma (Kalow & Gunn, 1957; Neitlich, 1966). In view of this, and since an injection of purified human cholinesterase (Borders, Steven, Nowhill & Martin, 1955; Altland & Goedde, 1967) given before neuro-

muscular block has become established shortens the period of apnoea produced by suxamethonium, Litwiller (1969) concluded that in man suxamethonium is hydrolysed primarily in plasma and that this determines its potency and duration of action. This conclusion is not necessarily valid since cholinesterase is also found in high concentrations in many tissues.

As far as laboratory animals are concerned it is well established that inhibitors of cholinesterase potentiate the action of suxamethonium (Brücke, 1956) but Keleman & Volle (1966) who studied the effect of anticholinesterase 2-diethoxyphosphinylthioethyldimethylamine acid oxalate (217 AO) on the action of suxamethonium in cats, found that there was no linear relationship between the effectiveness of suxamethonium and reduction of the level of cholinesterase in plasma and that considerable inhibition of the enzyme in plasma was required before potentiation of the action of suxamethonium occurred. Furthermore, Fraser (1954) noted that inhibition of cholinesterase in the plasma of cats by physostigmine was maximal in less than 10 min while potentiation of the action of suxamethonium was greatest one hour after the injection of the anticholinesterase.

In previous studies (Hobbiger & Peck, 1969) it was shown that in the usual laboratory animals the level of cholinesterase in plasma, as measured with butyrylcholine as substrate, is often lower than the level of cholinesterase in human plasma and that these cholinesterases hydrolyse low concentrations of suxamethonium relative to butyrylcholine, and thus to acetylcholine, considerably slower than does the typical variant of human cholinesterase. The action of suxamethonium, given by intravenous injection, and the effect on it of *iso*-OMPA (tetra*iso*propyl pyrophosphoramide) in doses which inhibited cholinesterase in blood selectively therefore were investigated in cats, rats and mice. Since analyses based on infusions are more informative as regards metabolism of drugs than are analyses based on injections, studies were also made in cats to which suxamethonium was given by infusion before and after administration of *iso*-OMPA or in which the enzyme activity of the blood towards suxamethonium was raised by purified cholinesterase of human plasma.

Methods

Experiments on rats

Wistar rats of either sex, weighing between 180 and 250 g, were used. All experiments were performed on animals anaesthetized with pentobarbitone sodium, 0·16 mmol (40 mg)/kg intraperitoneally. The trachea was cannulated and artificial respiration using a Palmer pump was given when natural respiration became insufficient. Polyethylene cannulae were inserted into the right external jugular vein and the right carotid artery for the injection of suxamethonium and the withdrawal of blood samples respectively. The rectal temperature was recorded by an Ellab Type TE3 thermocouple and kept close to 38° C by manually controlled external heating. One of the legs was immobilized using a Brown-Schuster myographic stand, with a drill through the knee joint and a clamp gripping the distal part of the tibia and fibula. The sciatic nerve was stimulated with supramaximal rectangular pulses (0·2 Hz ; 0·2 to 0·3 ms duration ; 2–3 V) and the isometric tension of the soleus-gastrocnemius muscle group was recorded with a Grass FT10 force displacement transducer coupled to a Devices Type M4 recorder with wax paper writeout.

Experiments on mice

Adult white mice of the Tuck strain and of either sex, weighing between 20 and 40 g, were used. Suxamethonium was injected into the tail vein in volumes of 0·2–0·4 ml. Neuromuscular block was assessed by the time an animal was unable to maintain itself on a rotating drum (surface movement: 51 cm/min) covered by fine sandpaper and attached to and driven by a horizontally placed kymograph. For artificial respiration air delivered by a Palmer pump was blown into the oropharynx through a fine polyethylene tube.

Experiments on cats

Cats of either sex, weighing between 2 and 4 kg, were used. All experiments were performed under anaesthesia with pentobarbitone sodium, 0·24 mmol (60 mg)/kg intraperitoneally. In longer lasting experiments additional injections of up to 0·05 mmol/kg of pentobarbitone sometimes were required every 3–4 h for maintenance of the initial level of anaesthesia. The trachea was cannulated and artificial respiration with a Palmer pump was given when natural breathing became inadequate. A cannula was inserted into the right external jugular vein for the injection or infusion of suxamethonium. Blood samples, usually 3 ml, were collected from a cannula inserted into the right carotid artery. Blood pressure was measured in the left carotid artery using a Statham P23 HA pressure transducer coupled to a Devices type M4 recorder, with wax paper writeout (1 mmHg≡1·333 mbar). Measurements of rectal temperature and external heating were as described for rat experiments.

Infusions of suxamethonium were given using a Palmer continuous slow injection pump driving a 10 ml syringe and delivering 0·16 ml of fluid/minute. The total volume of fluid infused varied between experiments and at most amounted to 50–60 ml over a period of 10 h; this represents in a 3 kg cat an approximately 10% increase in extracellular fluid (not corrected for renal excretion, uptake by cells, and loss of blood due to removal of blood samples.

The neuromuscular blocking action of suxamethonium was assessed from changes in twitch tension of the indirectly stimulated soleus-gastrocnemius muscle group (when suxamethonium was given by injection) or of the two muscles, separated from each other (when suxamethonium was given by infusion). The limb was fixed by drills through the lower condyles of the femur and the calcaneum, held in a Brown-Schuster myograph stand. Stimulation of the sciatic nerve and recording of twitch tension were as described for experiments on rats.

For temporary occlusion of the ureters a single abdominal incision was made and loose threads were put around the ureters to serve as guides and reduce the time required for placing clamps. Bulldog clips covered with soft polyethylene were placed in position 15 min before testing the effects of occlusion on the response to suxamethonium.

Measurements of enzyme activities

(*a*) *Acetylcholinesterase.* Activity of this enzyme (acetylcholine acetylhydrolase; E.C. 3.1.1.7) was determined by the Warburg manometric technique (medium: 25 mM $NaHCO_3$, gas phase: 95% N_2:5% CO_2; 37°; pH 7·45) with 30 mM DL-acetyl-β-methylcholine chloride (mecholyl) as substrate.

(*b*) *Cholinesterase.* Activity of this enzyme, unless otherwise stated, was determined by the Warburg manometric technique with 10 mM butyrylcholine iodide as substrate. In studies of the correlation between effectiveness of suxamethonium and degree of inhibition of cholinesterase by *iso*-OMPA at individual stages of an experiment, the spectrophotometric method of Kalow & Lindsay (1955) with 50 μM benzoylcholine chloride as substrate was used because determinations by this method are much quicker than those by the Warburg method. In these experiments hydrolysis of benzoylcholine was determined with a Hilger Watts ultraviolet spectrophotometer from changes in absorption at 240 nm (medium: 67 mM phosphate buffer; 26° C).

Hydrolysis of suxamethonium was determined by the method described by Hobbiger & Peck (1969). Plasma was incubated at 37° C in 6·7 mM Sørensen's phosphate buffer, pH 7·4, with 25 μM suxamethonium chloride and hydrolysis was calculated from the difference between heights of contractions of the frog rectus muscle preparation, produced by aliquots removed from the incubate at intervals and by standard solutions.

Drugs, substrates and enzymes used

All blood samples were heparinized (10–20 i.u. heparin/ml blood) and plasma was separated by centrifugation at 3,000 rev/min for 15 min in a M.S.E. refrigerated centrifuge. Whole blood or plasma, diluted in the medium stated, were used for determinations of enzyme activities.

Purified human cholinesterase (a lyophilized preparation of plasma obtained from AB KABI, Sweden) was a powder which in solution hydrolysed 10 mM butyrylcholine at the rate of (62 μmol/mg powder)/h as compared with 395 μmol/ml by fresh human plasma. It had properties identical to those of the enzyme in crude plasma (Hobbiger & Peck, 1969): Dibucaine number: 81; fluoride number: 59; K_1 of suxamethonium for the hydrolysis of butyrylcholine: 0·16 mM; hydrolysis of 25 μM suxamethonium relative to that of 10 mM butyrylcholine: 1·2%. The purified human cholinesterase was dissolved in 25 mM $NaHCO_3$ and the tonicity of the solution was adjusted to that of 0·15 M (0·9%) NaCl by addition of solid NaCl.

The substrates used were: DL-acetyl-β-methylcholine chloride (Mecholyl; Koch-Light Laboratories, Ltd.); benzoylcholine chloride (Sigma London Chemical Co., Ltd.); butyrylcholine iodide (Sigma London Chemical Co., Ltd.); and suxamethonium chloride (Koch-Light Laboratories Ltd.). Rates of enzymic hydrolysis were always calculated from initial rates.

Solutions of *iso*-OMPA (tetra*iso*propyl pyrophosphoramide; Koch-Light Laboratories Ltd.), decamethonium (iodide; Koch-Light Laboratories Ltd.) and suxamethonium for injections or infusions were made in 0·15 M NaCl and prepared freshly each day.

Results

Neuromuscular block produced by single doses of suxamethonium before and after iso-*OMPA*

Experiments in rats

Single doses of 0·5–2 μmol/kg of suxamethonium, injected into the right external jugular vein, usually produced in the soleus-gastrocnemius muscle group only partial

neuromuscular block, while doses of 2–4 μmol/kg always produced complete neuromuscular block lasting for 8–13 minutes. If such doses were spaced at intervals of 20 min or more, the same dose produced a very similar effect on different occasions. After doses of suxamethonium up to 4 μmol/kg, which produced complete neuromuscular block, the rate of recovery of twitch tension from 10 to 50% of the initial tension, varied between 5 and 9 min in individual experiments.

Cholinesterase in blood was nearly completely inhibited by the intraperitoneal injection of a dose of *iso*-OMPA which reduced acetylcholinesterase activity in blood by only 25%. In seven control animals the rate of hydrolysis of 30 mM mecholyl was (17·7 ± 3·7 (S.E.M.) μmol/ml blood)/h and that of 10 mM butyrylcholine was (33·0 ± 6·1 (S.E.M.) μmol/ml blood)/h. Blood collected from four rats 1 h after the intraperitoneal injection of *iso*-OMPA, 30 μmol/kg, hydrolysed 30 mM mecholyl at a rate of (13·2 ± 0·6 (S.E.M.) μmol/ml blood)/h and 10 mM butyrylcholine at a rate of (0·6 ± 0·3 (S.E.M.) μmol/ml blood)/h. The relationship between dose of suxamethonium and its effect on neuromuscular block before and after *iso*-OMPA, in these four rats is shown in Fig. 1. *Iso*-OMPA increased the effectiveness of suxamethonium 1·9 to 3-fold as assessed by the dose which after *iso*-OMPA produced the same duration of complete neuromuscular block as was obtained with the highest dose given before *iso*-OMPA. Corresponding planimetric analyses gave similar values. No correlation was found between the level of cholinesterase in the blood of the four rats and the magnitude of response to a given dose of suxamethonium before *iso*-OMPA was given. The increased effectiveness of suxamethonium after *iso*-OMPA was mainly attributable to an increase in the depth of neuromuscular block with doses producing only partial paralysis before *iso*-OMPA and to an increase in the duration of complete paralysis with higher doses. The rate of recovery from complete neuromuscular block, measured by the time taken for twitch tension to recover from 10 to 50% of the initial value, was changed little by *iso*-OMPA. After doses of suxamethonium which produced after *iso*-OMPA a dura-

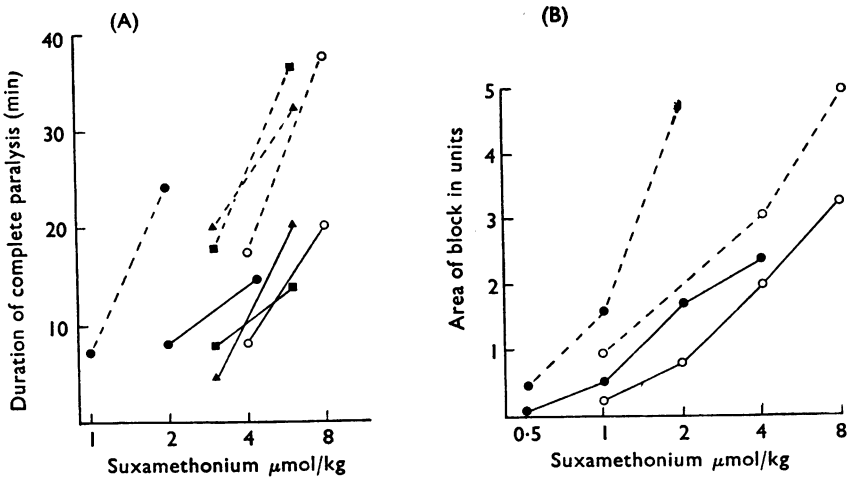

FIG. 1. Effect of iso-OMPA, 30 μmol/kg intraperitoneally, on neuromuscular block produced in the soleus-gastrocnemius muscle group by suxamethonium injected into the external jugular vein in anaesthetized rats. Neuromuscular block was assessed by the duration of complete block (A; four rats) and by planimetric measurement (in arbitrary units) of the area representing the twitch tension deficit (B; two rats). Continuous lines represent results before, and broken lines 30–150 min after the injection of *iso*-OMPA. For results in an individual rat the same symbol is used in A and B.

tion of block similar to that obtained with 2–4 μmol suxamethonium/kg before *iso*-OMPA, recovery times varied between 5 and 11 min as compared with 5–9 min in the absence of *iso*-OMPA. In a typical experiment 2 μmol suxamethonium/kg produced complete neuromuscular block lasting 8 min, and 5·2 min were required for recovery of twitch tension from 10 to 50%. Following *iso*-OMPA, 30 μmol/kg intraperitoneally, 1 μmol suxamethonium/kg, produced complete neuromuscular block lasting 7·2 min, and 4·8 min were required for recovery of twitch tension from 10 to 50%.

The increase in the effectiveness of suxamethonium produced by *iso*-OMPA was solely attributable to inhibition of cholinesterase since *iso*-OMPA, 30 μmol/kg intraperitoneally, had no effect on neuromuscular block produced by decamethonium.

Experiments in mice

A dose of 0·625 μmol of suxamethonium/kg, injected into the tail vein, produced neuromuscular block, that is, inability to stay on a rotating drum, in two out of five mice, lasting for 1 and 1·42 min, whereas all mice injected with 1·25 μmol of suxamethonium/kg died of respiratory failure. Suxamethonium, 1 μmol/kg, consistently produced neuromuscular block with a mean duration of $1{\cdot}75 \pm 0{\cdot}19$ (S.E.M.) min (sixteen mice). The same dose of suxamethonium given 3 h after the first dose produced neuromuscular block with a mean duration of $1{\cdot}47 \pm 0{\cdot}12$ (S.E.M.) min. In a second group of sixteen mice 1 μmol suxamethonium/kg produced neuromuscular block lasting for $1{\cdot}40 \pm 0{\cdot}11$ (S.E.M.) min. These mice were then injected intraperitoneally with 30 μmol *iso*-OMPA/kg, and 3 h later six of them were given 1 μmol suxamethonium/kg. All six mice died of respiratory failure. The remaining ten mice were given 0·5 μmol suxamethonium/kg and of them four died of respiratory failure. The mean duration of neuromuscular block in the six survivors was $4{\cdot}93 \pm 0{\cdot}78$ (S.E.M.) minutes.

No hydrolysis of butyrylcholine by blood collected 3 h after the intraperitoneal injection of 30 μmol/kg *iso*-OMPA was measurable, whereas a pooled sample of blood from sixteen control mice hydrolysed (105 μmol butyrylcholine/ml blood)/hour. Hydrolysis of mecholyl by blood of *iso*-OMPA treated mice was identical with that by blood of control mice.

Experiments in cats

In cats, 0·125–0·25 μmol of suxamethonium/kg, intravenously, produced partial neuromuscular block (as recorded from the combined soleus-gastrocnemius muscle group). Larger doses gave complete neuromuscular block. Recovery of twitch tension from 10 to 50% of the initial value took 3–4 min and comparable results were obtained when doses of suxamethonium were spaced at 20 min or more.

Selective inhibition of cholinesterase in blood was produced by *iso*-OMPA, 7·5 μmol/kg intravenously, as shown by the following results. Two cats were injected intravenously with 7·5 μmol *iso*-OMPA. Blood collected 3 h later hydrolysed 10 mM butyrylcholine at a rate of (1·8 and 2·3 μmol/ml blood)/h as compared with a hydrolysis of (54·4 and 53·1 μmol/ml blood)/h by blood samples collected before *iso*-OMPA was given. In the same cats the rate of hydrolysis of 30 mM mecholyl was reduced by the *iso*-OMPA treatment from 12·5 to 10·7 (μmol/ml blood)/h in one cat and remained unchanged in the second cat. A dose of 30 μmol *iso*-OMPA/

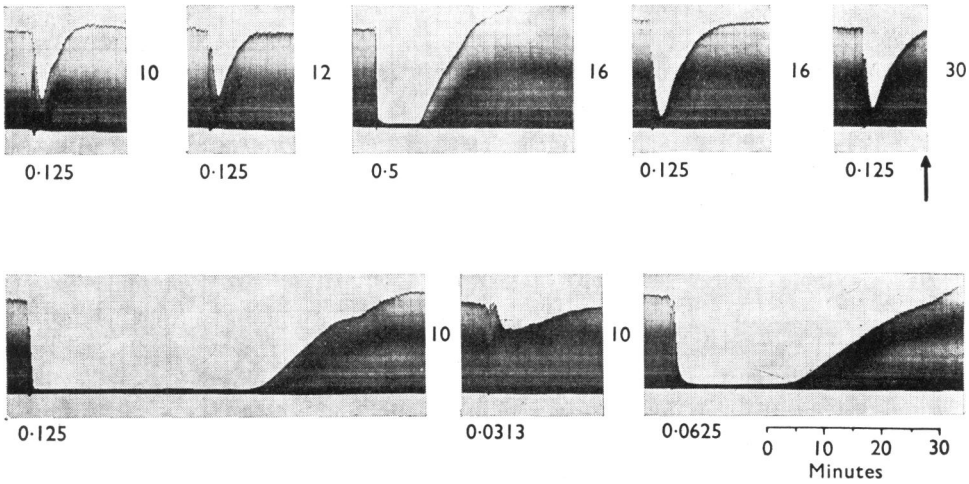

FIG. 2. Effect of *iso*-OMPA, 7·5 μmol/kg intravenously, on neuromuscular block produced by intravenous injections of suxamethonium in an anaesthetized cat (1·9 kg). The records show responses of the soleus-gastrocnemius muscle group to supramaximal stimulation of the sciatic nerve at 0·2 Hz. The figures between the records give the periods (in min) not illustrated and figures below the records are μmol/kg of suxamethonium injected. *Iso*-OMPA, given intravenously at the arrow, produced a 97% inhibition of cholinesterase in blood.

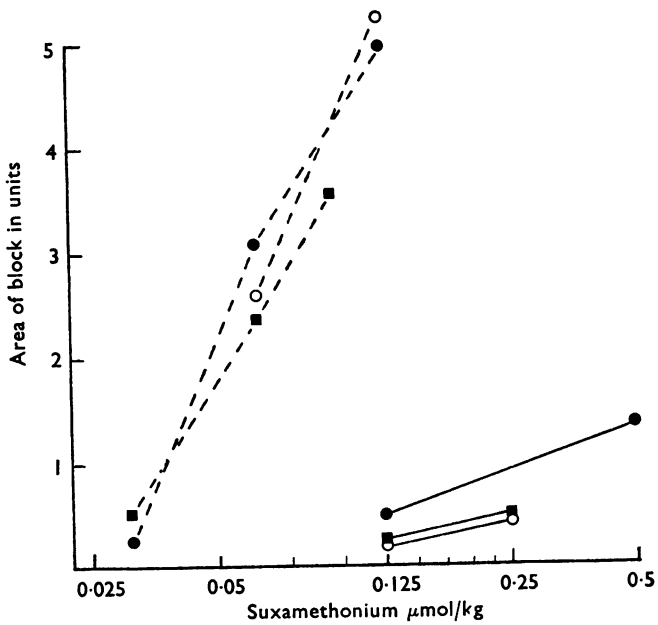

FIG. 3. Effect of *iso*-OMPA, 7·5 μmol/kg intravenously, on the neuromuscular block produced by intravenous injections of suxamethonium in three anaesthetized cats. Neuromuscular block was assessed by planimetric measurement (in arbitrary units) of the area representing the twitch tension deficit of the indirectly stimulated soleus-gastrocnemius muscle group. Cholinesterase activity in blood, determined with 10 mM butyrylcholine, was reduced by *iso*-OMPA to 3%, 3% and 7% in the three experiments as assessed by the difference between the enzymic activity of blood samples collected before the first and after the last injection of suxamethonium. Continuous lines: suxamethonium responses before *iso*-OMPA; interrupted lines: suxamethonium responses after *iso*-OMPA.

kg intravenously reduced cholinesterase activity activity in blood to 1%, but also markedly affected the hydrolysis of mecholyl which 4 h after the injection of *iso*-OMPA was 43% of the initial value (result in one cat).

The administration of *iso*-OMPA increased the effectiveness of suxamethonium in cats to a greater extent than it did in rats or mice. A typical result is illustrated in Fig. 2 and results obtained in three cats are summarized in Fig. 3. The increases in effectiveness of suxamethonium in the experiments summarized in Fig. 3 were 6·7, 8·0 and 7·1-fold according to the doses of suxamethonium which following *iso*-OMPA were equiactive to 0·25 μmol/kg of suxamethonium before inhibition of cholinesterase. Comparisons based on doses which after *iso*-OMPA produced the same effect as 0·5 μmol suxamethonium/kg given before inhibition of cholinesterase gave even higher values.

Iso-OMPA not only increased the depth of neuromuscular block or prolonged the duration of complete neuromuscular block produced by a given dose of suxamethonium, but also slowed considerably the rate of recovery of twitch tension. In a typical experiment, suxamethonium, 0.25 μmol, produced 88% reduction in twitch tension and recovery to 50% of the initial twitch tension took 3·6 min. Following *iso*-OMPA, 7·5 μmol/kg intravenously, suxamethonium, 0·06 μmol/kg, produced 83% reduction in twitch tension and recovery to 50% of the initial twitch tension took 22 minutes.

Control experiments showed that the administration of *iso*-OMPA, 7·5 μmol/kg intravenously, had no effect on the neuromuscular action of decamethonium.

Neuromuscular block produced by suxamethonium infusions in anaesthetized cats before and after iso-*OMPA*

The infusion into the external jugular vein of (0·0125–0·0375 μmol suxamethonium/kg)/min produced after an initial potentiation a gradual reduction of twitch tension which was of similar magnitude in soleus and gastrocnemius muscle.

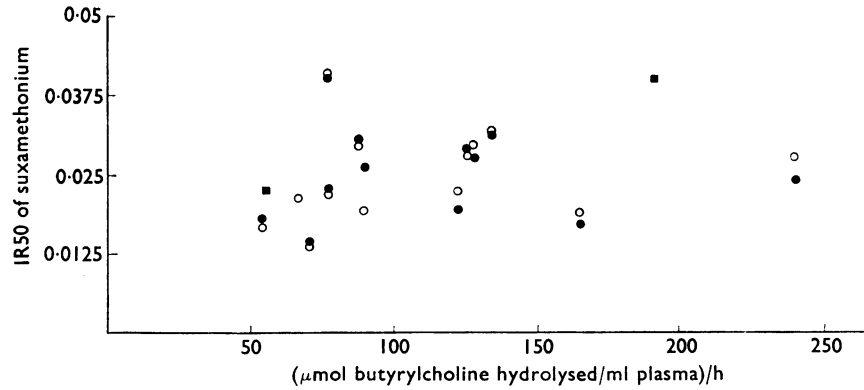

FIG. 4. Relationship between cholinesterase activity in plasma and IR50 for suxamethonium in anaesthetized cats. IR50 is the rate of infusion in (μmol suxamethonium/kg)/min into the right external jugular vein required for a 50% reduction in twitch tension under equilibrium conditions. Soleus (●) and gastrocnemius (○) muscle tension were recorded independently in thirteen cats; in two cats the tension of the combined muscle group (■) was recorded. Cholinesterase activity was measured with 10 mM butyrylcholine as substrate and the values plotted represent the mean of activity in plasma of blood collected before the first and after the last infusion of suxamethonium. Enzyme activity in the second plasma sample was usually slightly lower than that in the first because of haemodilution caused by the infusions.

After approximately 20–30 min a constant reduction in twitch tension was reached, that is, an equilibrium between rate of infusion of the drug and its rate of removal was obtained. Allowing 20–30 min for recovery between infusions only slight increases or decreases in sensitivity were seen over a period of hours. These changes were slowly progressive and in any given experiment in one direction only. Plotting percentage reduction in twitch tension at equilibrium, corrected for changes in sensitivity, against the logarithm of the infusion rate, the infusion rate for 50% reduction in twitch tension (IR50) was obtained. A comparison between the IR50 and the level of cholinesterase activity in plasma shows that the two parameters are unrelated (Fig. 4).

The rate of recovery of twitch tension after stopping the infusion, was measured in seventeen cats which had received suxamethonium at a rate which produced more than 75% reduction in twitch tension. The time for recovery of twitch tension from 25 to 75% of the initial tension was $2{\cdot}8 \pm 0{\cdot}2$ (S.E.M.) min. An increase in twitch tension above the initial pre-infusion level usually followed recovery and lasted several minutes.

Urinary excretion did not play an important part in the removal of suxamethonium under the experimental conditions, as shown by three experiments in which clamping of the ureters on the response to suxamethonium was investigated. In two animals no change in sensitivity to suxamethonium was observed. In the third animal clamping of the ureters increased the degree of neuromuscular block slightly, lowering the IR50 of suxamethonium by 8%.

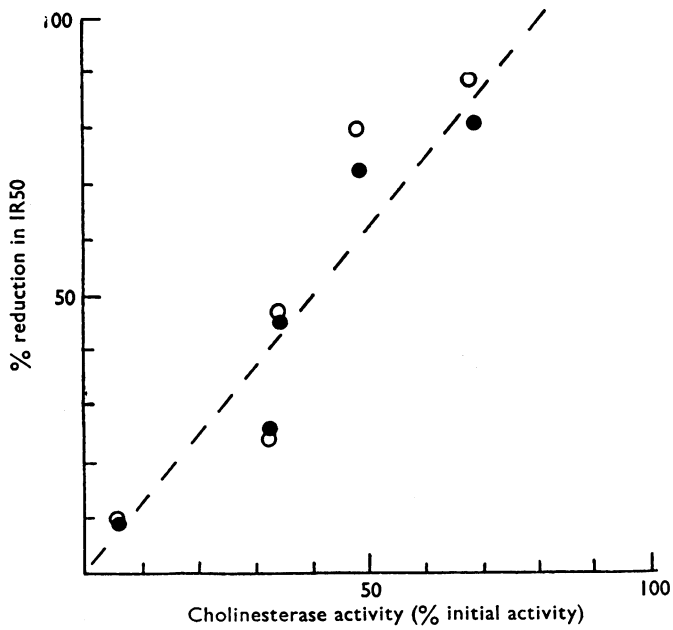

FIG. 5. Effect of iso-OMPA on the IR50 for suxamethonium in anaesthetized cats. Soleus muscle: ●; gastrocnemius muscle: O. The figure summarizes results in three cats, two of which were given a single dose of *iso*-OMPA whereas another received three doses. Cholinesterase activity was determined with 50 μM benzoylcholine as substrate and in each case represents the mean of the activity of plasma of samples of blood collected immediately before and after infusions of suxamethonium, the effect of which was used for assessing the IR50. An interval of 20 min was allowed between injection of *iso*-OMPA and the first infusion of suxamethonium following it.

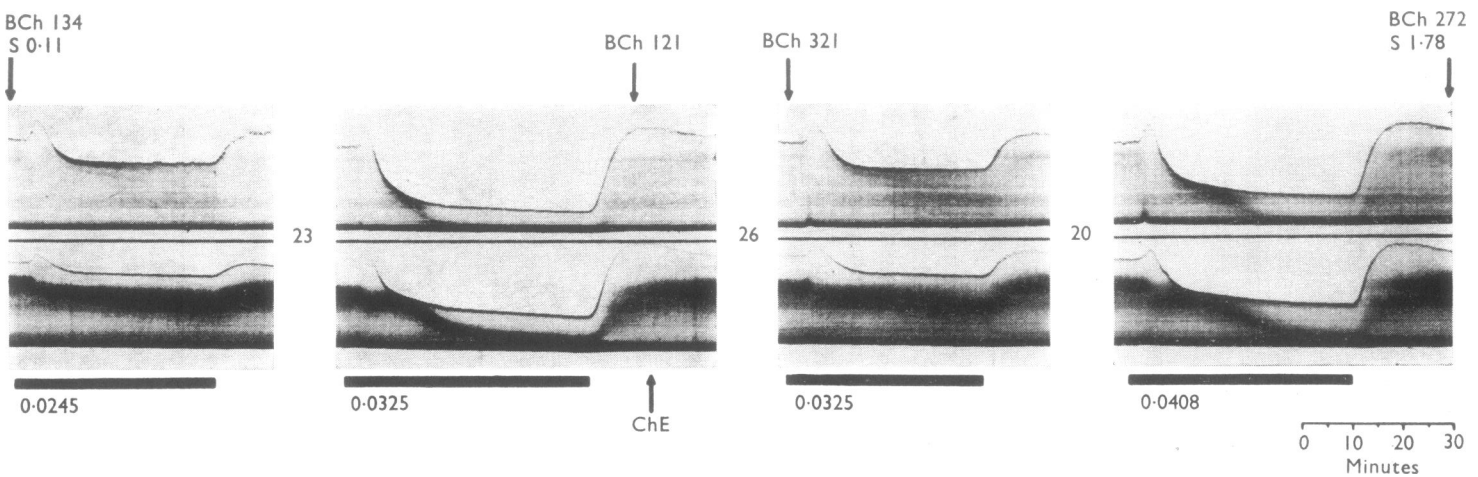

FIG. 6. Effect of raising the suxamethonium hydrolysing capacity of plasma by purified human cholinesterase on the neuromuscular block produced in an anaesthetized cat (2·4 kg) by infusions of suxamethonium indicated by the bars. The figures beneath the bars give infusion rates of suxamethonium in (μmol/kg)/min. Human cholinesterase (524 mg) was infused over 26 min from the arrow labelled ChE (record of experiment 4 summarized in Table 1). The upper and lower tracings show responses of the soleus and gastrocnemius muscles respectively. The figures above the top tracing give the rate of hydrolysis of 10 mM butyrylcholine (BCh) and 25 μM suxamethonium (S) in (μmol substrate hydrolysed/ml plasma)/h for blood samples collected at the arrows. The figures between the records give periods (in min) not illustrated.

TABLE 1. *Effect of purified human cholinesterase on the IR50 for suxamethonium in four anaesthetized cats*

Expt. number	Weight of cat (kg)	Before purified human cholinesterase: IR50 S	IR50 G	Hydrolysis in (μmol of substrate stated/ml plasma)/h: 10 mM BCh	25 μM S	After injection of purified human cholinesterase: Amount of enzyme injected (mg)	IR50 S	IR50 G	Hydrolysis in (μmol of substrate stated/ml plasma)/h: 10 mM BCh	25 μM S	Min after injection of enzyme when blood was collected for assay of enzyme activity
1	3·2	7·2	6·6	53	0·04	247	10·0	7·2	144 (169)	0·96	180
2	3·1	16·2	16·4	83	0·07	500	19·4	19·0	236 (274)	1·32	200
3	2·4	11·7	11·4	134	0·14	520	17·7	17·9	288 (368)	1·36	240
4	2·4	11·0	12·0	134	0·11	524	11·6 (14·6)	12·7 (16·2)	272 (321)	1·78	300

S, Result on soleus muscle ; G, results on gastrocnemius muscle ; BCh, butyrylcholine ; S, suxamethonium.
Values for enzymic hydrolysis before infusion of purified human cholinesterase are the activity of the plasma of samples of blood collected just before the first infusion of suxamethonium from which the IR50 value was calculated. Values for the enzymic hydrolysis after injection of the enzyme are based on the enzymic activity of blood samples collected immediately after the last infusion of suxamethonium. The table also shows in brackets values for enzymic activity of blood samples collected 20 min after the injection of enzyme and IR50 values based on effects of infusions of suxamethonium given immediately after that.

Since many of the experiments continued for several hours, the effect of varying the depth of anaesthesia on the response to suxamethonium was investigated. In a cat which had received the initial dose of pentobarbitone (0·24 μmol/kg) suxamethonium was infused at intervals in concentrations which allowed calculation of the IR50. Six hours after the initial dose and once more an hour later 0·06 μmol/kg of pentobarbitone were given intraperitoneally. These two doses of pentobarbitone had no effect on the IR50 of suxamethonium or on the blood pressure. A further dose of 0·06 μmol/kg of pentobarbitone, given 1 h after the previous dose, raised the IR50 of suxamethonium 1·4-fold and transiently lowered the blood pressure from 195 to 120 mmHg. An additional dose of 0·06 μmol/kg of pentobarbitone, again given 1 h after the previous dose, raised the IR50 of suxamethonium 1·9-fold relative to the IR50 before the first additional dose of pentobarbitone, and lowered the blood pressure from 175 to 90 mmHg. With the last two doses of pentobarbitone a level of anaesthesia was produced which was much deeper than that in any other experiment.

The injection of *iso*-OMPA increased the sensitivity to suxamethonium, and as Fig. 5 shows, an inverse relationship existed between the percentage of cholinesterase activity in plasma and the IR50 for suxamethonium when the cholinesterase level in plasma either was lowered stepwise by three intravenous injections of 0·15 μmol *iso*-OMPA/kg, given at two-hourly intervals, or by a single dose of 0·3 or 1·5 μmol *iso*-OMPA/kg, respectively. Under these conditions the length of time required for achieving or approaching a constant level of reduction in twitch tension was increased and the greater the inhibition of cholinesterase was, the longer it took for this. For example, when cholinesterase activity in plasma was reduced to 5% it took 60 min for an infusion to approach a near constant reduction in twitch tension as compared with 25 min required for an equilibrium before the injection of *iso*-OMPA.

Effect of raising the capacity to hydrolyse suxamethonium in the plasma, on neuromuscular block produced by suxamethonium in anaesthetized cats

In four cats the activity of the plasma in hydrolysing suxamethonium was raised 10- to 22-fold by intravenous infusion of purified human cholinesterase. This increased the IR50 values of soleus and gastrocnemius muscles only 1·6-fold at most (Fig. 6, Table 1).

The purified human cholinesterase had a half-life in blood ranging between 7·2 and 12·3 h in individual experiments as indicated by rates of hydrolysis of 10 mM butyrylcholine by plasma of blood samples taken before and at intervals after the injection of the enzyme. Calculations based on the same data and the activity of the infused enzyme show that the latter was distributed throughout a plasma volume of 40–45 ml/kg.

Discussion

The experiments described show that no correlation exists between the level of cholinesterase activity in the plasma of rats or cats and the doses of suxamethonium required to produce a given degree of neuromuscular block. Furthermore, a 22-fold elevation in the suxamethonium hydrolysing capacity of the blood of cats reduced the effectiveness of suxamethonium only 1·6-fold. These findings are contrary to

observations in man (Kalow & Gunn, 1957; Neitlich, 1966) and strongly indicate that in rats and cats hydrolysis of suxamethonium in blood plays very little or no part in determining the concentration of the ester reaching the motor end plate and its subsequent metabolic degradation. The reason for the difference between man and the laboratory animals used is at least two-fold. First, the circulation time and thus the period of contact of suxamethonium with cholinesterase in plasma is longer in man and second as shown in previous work (Hobbiger & Peck, 1969) the rates of hydrolysis of low concentrations of suxamethonium by cholinesterase of rats and cats expressed as a percentage of its turnover by human cholinesterase are only 24 and 9%, respectively.

Selective inhibition of cholinesterase by *iso*-OMPA resulting in an inhibition of cholinesterase in blood by 95% or more, however, enhances the potency of suxamethonium. This can only mean that the inhibition of cholinesterase by *iso*-OMPA was not confined to cholinesterase in blood but the cholinesterase in tissues was also affected and that the latter—for example, at motor end plates—plays an important part in determining the action of suxamethonium. The presence of cholinesterase at motor end plates was demonstrated histochemically by Holmstedt (1957) and Eränko & Teräväinen (1967). In the case of rats the dose of *iso*-OMPA used increased the effectiveness of suxamethonium 2- to 3-fold but had no influence on the rate of recovery from neuromuscular block. On the other hand, *iso*-OMPA not only increased the effectiveness of suxamethonium, given by injection, in cats but also slowed the rate of recovery from neuromuscular block thus giving a 7- to 8-fold potentiation as assessed by doses which after *iso*-OMPA, were equiactive according to planimetric analysis to 0·25 μmol suxamethonium before *iso*-OMPA. It is possible that this difference between the two species is attributable to variations in the degree of inhibition of cholinesterase in the tissues of the two species. The results in mice indicate that the potentiation of suxamethonium obtained with *iso*-OMPA was 2- to 4-fold and since animals survived periods of weakness in excess of those which were compatible with life before *iso*-OMPA, it is likely that in this species, as in cats, *iso*-OMPA not only increased the effectiveness of suxamethonium but also slowed down the rate of recovery from neuromuscular block.

In some of the experiments on cats suxamethonium was given by infusion. This approach has the advantage over the injection technique of providing quantitative information on how much of the drug given is metabolized and excreted per unit time. It was found that under the experimental conditions excretion in urine played an insignificant role and that the degree of neuromuscular block obtained with a given rate of infusion was initially progressive but then became constant at a level which depended on the rate of infusion. This means that cholinesterase in tissue is either only partly saturated with suxamethonium in spite of its apparent high affinity for the enzyme (Hobbiger & Peck, 1969) or that the pool of cholinesterase participating in the hydrolysis of suxamethonium increases with increasing concentration of the ester.

The experiments of Kelemen & Volle (1966) showed that cholinesterase in plasma of cats can be markedly inhibited by 2-diethoxy-phosphinylthioethyldimethylamine acid oxalate (217 AO) before any potentiation of suxamethonium occurs. In our experiments an inverse relationship was shown to exist between the level of cholinesterase in the plasma and the effectiveness of suxamethonium given by infusion.

As shown by Burgen & Hobbiger (1951) the levels of inhibition of cholinesterases in blood and tissue are not identical and after low doses of an anticholinesterase, inhibition of the former can be much more pronounced than that of the latter. It is thus very likely that in the case of the experiments of Kelemen & Volle a discrepancy existed between the inhibition of cholinesterase in blood and tissue whereas in our experiments with *iso*-OMPA a more uniform level was obtained in both.

Zaimis (1953) observed that neuromuscular block produced in monkey, rabbit, hare and dog by decamethonium changed from a depolarization block to a competitive block (dual block) when the drug was given repeatedly. This was also observed with suxamethonium in monkey and dog. More detailed studies in cats by Jewell & Zaimis (1954) demonstrated that a dual block could be produced in the soleus muscle, a red muscle, but not the tibialis anterior, a white muscle. This was more pronounced with decamethonium than with suxamethonium and was least pronounced in the cat. In the present experiments soleus and gastrocnemius muscles were studied. The latter is a fast contracting white muscle (Buller, 1965). No marked changes in sensitivity to suxamethonium were observed with repeated injections or infusions of suxamethonium and soleus and gastrocnemius muscles responded similarly. Facilitation of twitch tension was seen during the early stage of infusion in most cats but varied in degree between animals. The anticholinesterase edrophonium (Hobbiger, 1952) given after the last infusion or injection increased the degree of neuromuscular block. Thus it is unlikely that a competitive type of neuromuscular block played a significant part in the experiments described.

As far as assessment of cholinesterase activity in the experiments described is concerned, there is no evidence for the existence of variants of cholinesterase in the plasma of the species used, unlike in man. Thus it does not matter which substrate is used for determining enzyme activity as long as the experimental aim is confined to establishing the relationship between enzyme concentration and effectiveness of suxamethonium in the same species. However, since the ratio at which cholinesterase of different species hydrolyses suxamethonium relative to other substrates varies greatly, the rates of hydrolysis of suxamethonium must be taken into consideration when comparisons are made between results obtained in different species. Our studies show that in the rat and cat, two species which hydrolyse suxamethonium relative to other substrates much slower than does the typical variant of human cholinesterase, inhibition of cholinesterase in the tissue modifies the effectiveness and in the latter species also the duration of action of suxamethonium. This undoubtedly indicates that cholinesterase in the tissue must also play some part in determining the effect of suxamethonium in man. On the other hand, cholinesterase in the plasma of rats and cats plays little part in determining the effectiveness of suxamethonium, whereas evidence obtained in man indicates the opposite. Commonly used laboratory animals such as the rat and the cat are therefore not suitable for providing information on the fate of suxamethonium which is directly applicable to man.

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