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Relative importance of the enzymic hydrolysis of suxamethonium in plasma and tissues: studies in cats

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Summary

1. The effects of intravenous infusions of suxamethonium on the twitch tension of the indirectly stimulated tibialis and gastrocnemius muscles of anaesthetized cats were recorded. From these data the infusion rate giving a 50% reduction in twitch tension after 15 min (IR50), was calculated.

2. Marked inhibition of cholinesterase activity in plasma with less inhibition of cholinesterase activity in tissues, obtained by repeatedly withdrawing blood samples, incubating them with DFP and reinjecting them, had only a small effect on the IR50 of suxamethonium.

3. Injection of *iso*-OMPA produced marked inhibition of cholinesterase activity in plasma and tissues, and lowered the IR50 to 10% of that in controls. The IR50 in cats treated with *iso*-OMPA could be restored to normal only by raising the suxamethonium hydrolysing capacity of plasma 10–50 times above normal by the intravenous injection of purified cholinesterase of human plasma.

4. Exchange blood transfusion between normal cats and cats treated with *iso*-OMPA failed to affect the IR50 of suxamethonium in either of the two.

5. It is concluded that in cats, unlike in man, the effectiveness of suxamethonium is not determined by the cholinesterase activity in plasma but by the cholinesterase activity in tissue. The role in this of cholinesterase at the neuromuscular junction and other sites is discussed.

Introduction

We previously reported (Hobbiger & Peck, 1970) that in cats the neuromuscular blocking activity of suxamethonium, assessed by the effects of intravenous infusions of the drug, was independent of the activity in plasma of cholinesterase (acylcholine acylhydrolase; E.C. 3.1.1.8) which varied widely between animals. Furthermore, we found that if the capacity of plasma to hydrolyse suxamethonium was raised 22-fold above normal the effectiveness of suxamethonium was reduced by a factor of 1·6 only. On the other hand, after the injection of *iso*-OMPA (tetramono*iso*-propyl pyrophosphortetramide) in doses which markedly inhibited cholinesterase activity and had little or no effect on the activity of acetylcholinesterase (acetylcholine acetylhydrolase ; E.C. 3.1.1.7), an inverse correlation was observed between the activity of cholinesterase in plasma and the effectiveness of suxamethonium. On the basis of these findings we concluded that in cats, unlike in man (Kalow & Gunn, 1957), hydrolysis by cholinesterase in tissues must be the major factor determining the

effectiveness and duration of action of suxamethonium. To obtain direct evidence for this we studied the effects of independent variations of the activity of cholinesterase in plasma and tissues on the neuromuscular blocking action of suxamethonium, given by intravenous infusions. This paper reports the results obtained in these studies.

Methods

All experiments were performed on cats of either sex, weighing 2–4 kg, under general anaesthesia with pentobarbitone sodium, 0·24 mmol (60 mg)/kg intraperitoneally. The trachea was cannulated and artificial respiration with a Palmer pump was given when required. Rectal temperature was recorded by an Ellab Type TE3 thermocouple and maintained at 37–38° C by external heaters. Blood pressure was monitored in the left carotid artery using a Statham P23 HA pressure transducer coupled to a Devices type M4 recorder with a wax paper writeout.

The neuromuscular blocking action of suxamethonium was assessed from changes in twitch tension of either the soleus or gastrocnemius muscle, separated from each other. For this the limb was fixed by drills through the lower condyles of the femur and the calcaneum, held in a Brown-Schuster myographic stand. The sciatic nerve was stimulated with supramaximal rectangular pulses (0·2 Hz; 0·2 ms duration; 2–3 V) and muscle tension was recorded isometrically with Grass FT 10 force displacement transducers which were coupled also to the Devices type M4 recorder.

Suxamethonium was given by infusion into the right external jugular vein, using a Palmer continuous slow injection pump driving a 10 ml syringe. The rate of infusion was either 0·13 or 0·16 ml fluid/minute. Each experiment consisted of a series of two consecutive infusions of suxamethonium, each lasting 15 min, with a 30 min interval between each pair of infusions. Unless theactivity of cholinesterase in tissue was markedly inhibited, 15 min were sufficient for a constant level of neuromuscular block to be approached at the end of the infusion. In a limited number of experiments the duration of each infusion was extended to 20 minutes. With each pair of consecutive infusions, suxamethonium was infused first at a rate giving between 20 and 60% reduction in twitch tension, and then at 1·4 or 1·5 times that rate. From the effects obtained with the two infusions the infusion rate required for 50% reduction in twitch tension at the end of the infusion (IR50) was determined graphically by plotting percentage reduction in twitch tension against log infusion rate of suxamethonium and taking the relationship between the two as linear.

Measurements of enzyme activities

Measurements of cholinesterase and phosphorylphosphatase activities were carried out by the Warburg manometric technique (medium: 25 mM $NaHCO_3$; gas phase: 95% N_2: 5% CO_2; 37° C; pH 7·45). For acetylcholinesterase the substrate was 30 mM DL-acetyl-β-methylcholine chloride (mecholyl; Koch-Light), while for cholinesterase the substrate was 10 mM butyrylcholine iodide (Koch-Light). Phosphorylphosphatase activity was determined with 5 mM di*iso*propyl phosphorofluoridate (DFP; Koch-Light) as substrate.

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 heights of contractions of the frog rectus abdominis muscle preparation, sensitized with the anticholinesterase tetraethyl pyrophosphate (TEPP; Albright & Wilson), produced by aliquots removed from the incubation mixture at intervals, and by standard solutions.

Enzyme preparations and drugs used

Acetylcholinesterase activity of blood was determined by using whole blood. Cholinesterase and phosphorylphosphatase activity in plasma was determined by using plasma separated from the red cells by centrifugation at 3,000 rev/min for 15 min in a M.S.E. refrigerated centrifuge. Blood samples, 3 ml, were collected from the right carotid artery in heparinized syringes (10 to 20 I.U. heparin/ml blood).

For determinations of the activities of the two cholinesterases in the small intestine a 5 cm long piece of bowel, located at 30–35 cm from the pylorus, was removed, weighed and ground by hand with acid-washed sand in a mortar containing also two parts of 25 mM $NaHCO_3$/one part of tissue. The homogenate, like whole blood and plasma, was further diluted with 25 mM $NaHCO_3$ before use for assays.

Purified human cholinesterase (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)/hour. The preparation was the same as that used in previous work (Hobbiger & Peck, 1970). For injection the dried powder 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.

Solutions of tetramono*iso*propyl pyrophosphortetramide (*iso*-OMPA; Koch-Light) and suxamethonium (Koch-Light) for injection or infusion, respectively, were made in 0·15 M NaCl and prepared freshly before use. DFP was made up as a 10 mM stock solution in dry propylene glycol from which dilutions were made with 0·15 M NaCl when required.

Results

Inhibition of cholinesterase in plasma without marked inhibition of cholinesterase in tissues

The anticholinesterase di*iso*propyl phosphorofluoridate (DFP), within a certain range of concentrations, markedly inhibits cholinesterase activity without affecting the activity of acetylcholinesterase (Koelle, Koelle & Friedenwald, 1950). In addition, DFP is hydrolysed by a phosphorylphosphatase in blood but the rate at which this occurs is species dependent and particularly high in rabbits (Mounter, 1963). If cat plasma is able to hydrolyse DFP sufficiently fast it might be possible by incubating blood samples with suitable amounts of DFP and then reinjecting them, to lower the cholinesterase activity in plasma to a much greater extent than that in tissues and at the same time to produce little or no inhibition of acetylcholinesterase activity. Experiments designed in this context gave the following results.

A pooled sample of plasma from two cats hydrolysed DFP (5 mM) at a rate of (4·8 μmol/ml plasma)/hour. With a pooled sample of plasma from two rabbits the

rate of hydrolysis was (118 μmol/ml plasma)/hour. This indicates that the rate of hydrolysis of DFP in plasma might be sufficient for effective hydrolysis of free inhibitor under the conditions stated.

In each of four cats 20 ml blood were withdrawn from the carotid artery into a heparinized syringe containing DFP, final concentration in an individual experiment 0·25, 1·2 or 12 μM. After inverting the syringe several times and waiting for 2 min the blood was reinjected into the cat via the external jugular vein. This was done 6 times over a period of 30 minutes. As shown in Table 1, incubation of blood with 1·2 μM DFP produced optimal results, that is marked inhibition of cholinesterase activity in plasma, much less inhibition of cholinesterase activity in the small intestine than in the plasma and little inhibition of acetylcholinesterase activity, limited to blood only. With 0·25 μM DFP inhibition was limited to the cholinesterase activity in plasma but amounted to only 64% and with 12 μM DFP cholinesterase activity of both plasma and the small intestine was markedly inhibited and this was associated with inhibition of acetylcholinesterase activity in blood.

In the two cats whose blood was incubated with 1·2 μM DFP the effect of incubation on the effectiveness of suxamethonium calculated from differences between three consecutive infusion rates of suxamethonium required before and after DFP for 50% reduction in twitch tension (IR50; see **Methods**), was as follows. In one experiment the IR50 of suxamethonium was reduced by 13%, whereas in the other experiment it was unaffected. This is illustrated by Fig. 1.

A control experiment showed that withdrawal and reinjection of blood itself had no measurable effect on the IR50 of suxamethonium.

Elevation by human cholinesterase of the suxamethonium hydrolysing capacity of the plasma in cats treated with iso-*OMPA*

Since the cholinesterase of human plasma hydrolyses suxamethonium, in low concentrations and relative to butyrylcholine, 11 times faster than does the cholinesterase of cat plasma (Hobbiger & Peck, 1969) it should be possible to restore selectively with an injection of purified cholinesterase of human plasma the suxamethonium hydrolysing capacity of the plasma of cats whose cholinesterase activity in both plasma and tissues has been lowered by a selective inhibitor of cholinesterase such as tetramono*iso*propyl pyrophosphortetramide (*iso*-OMPA). A study of the

TABLE 1. *Inhibition of the activity of cholinesterases, obtained by repeatedly incubating blood samples with DFP and then reinjecting them*

Number of experiment	μM DFP for incubation	% inhibition AChE in blood	ChE in plasma	AChE in small intestine	ChE
1	12	51	95	0	94
2	1·2	11	94	0	26
3	1·2	3	94	0	55
4	0·25	0	64	0	2

The table shows the percentage inhibition obtained in cats by withdrawing 6 times in 30 min 20 ml blood into a syringe containing DFP in the final concentration stated and reinjecting the blood 2 min later. Inhibition of acetylcholinesterase activity (AChE) in blood and of cholinesterase activity (ChE) in plasma was calculated from the activities before the first and 30 min after the last incubation. Inhibition of AChE and ChE activities in the small intestine were calculated relative to mean activities of four control cats. These means were: (25·9±0·7 (S.E.M.) μmol mecholyl hydrolysed/g tissue)/h and (168±8·6 (S.E.M.) μmol butyrylcholine hydrolysed/g tissue)/h with 30 mM mecholyl and 10 mM butyrylcholine, respectively, as substrates.

effect of an injection of purified cholinesterase of human plasma on the IR50 of suxamethonium in cats treated with *iso*-OMPA thus will give additional information on the role the cholinesterase in plasma plays in the hydrolysis of suxamethonium *in vivo*.

Cats injected intraperitoneally with 12 μmol *iso*-OMPA/kg had, 1–3 days later, 95 to 100% inhibition of cholinesterase activity in both plasma and small intestine without any inhibition of acetylcholinesterase activity. In such cats the infusion of suxamethonium ((2–5 nmol/kg)/min) produced a 50% reduction in twitch tension in 15 minutes. This represents on average an approximately 10-fold increase, relative to controls, in the effectiveness of suxamethonium. Since at high levels of inhibition of cholinesterase activity it took at least 60 min, as compared with 20–30 min in normal animals, to obtain a constant level of reduction in twitch tension the true value for the increase in sensitivity after the injection of *iso*-OMPA is slightly higher than the recorded value. As far as recovery of twitch tension after stopping the infusion of suxamethonium was concerned, the time taken for recovery from 25% to 75% of initial tension after infusions of suxamethonium which produced more than 80% but less than 100% reduction in twitch tension, was 5·4 ± 0·4 (S.E.M.) min in cats treated with *iso*-OMPA and 2·8 ± 0·2 (S.E.M.) min in control cats.

In cats which previously had been injected with 12 μmol *iso*-OMPA/kg, the intravenous injection of purified cholinesterase of human plasma reduced the effectiveness of suxamethonium. However, only when the suxamethonium hydrolysing capacity of the plasma had been raised more than 10-fold above that in normal cats was the effectiveness of suxamethonium in the *iso*-OMPA treated cats of an order comparable to that in control cats. Figures 2 and 3 illustrate results obtained in two typical experiments.

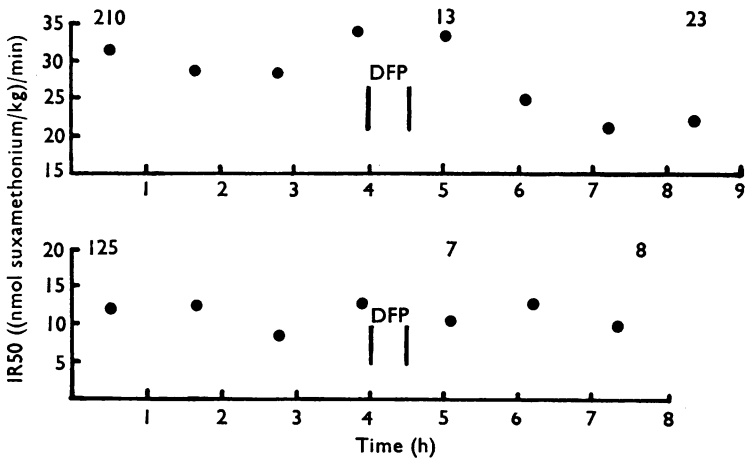

FIG. 1. Effectiveness of suxamethonium in two cats before and after inhibition of cholinesterase activity, predominantly in plasma. Samples of 20 ml of blood were withdrawn 6 times in 30 min, incubated for 2 min with 1·2 μM DFP and reinjected. Effectiveness of suxamethonium is represented by the IR50, that is infusion rate giving 50% reduction in twitch response after 15 min of infusion, for the soleus muscle. Abscissa: time in hours. Enzyme inhibition obtained in the two cats is shown in Table 1. The upper and lower record are from the cats, referred to in Table 1 as experiments 2 and 3, respectively. The figures represent cholinesterase activity in (μmol butyrylcholine hydrolysed/ml plasma)/h, with 10 mM butyrylcholine as substrate.

Exchange blood transfusions between iso-*OMPA treated cats and normal cats*

The cholinesterase activity in the plasma of cats which had been injected on the previous day with *iso*-OMPA was raised also by exchange transfusion of blood between cats treated with *iso*-OMPA and one or two normal cats. For this a cannula was inserted into the right carotid artery of the cat treated with *iso*-OMPA and the normal cat and connected via a two-way connector and short tube to the external jugular vein of the other cat. Twenty millilitres of blood were withdrawn simultaneously from the carotid artery of both cats into a 20 ml syringe containing

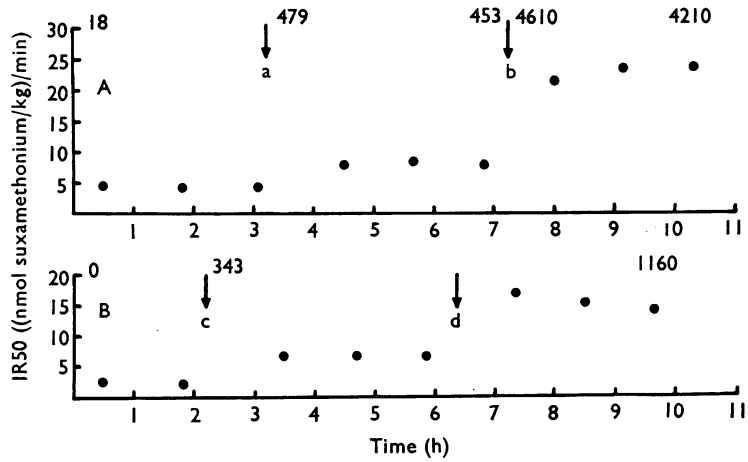

FIG. 2. Change of effectiveness of suxamethonium by purified cholinesterase of human plasma in cats pretreated with *iso*-OMPA.
A: cat, 3·6 kg, injected with *iso*-OMPA 12 μmol/kg intraperitoneally 1 day previously. B: cat, 3·9 kg, injected with *iso*-OMPA 12 μmol/kg intraperitoneally 3 days previously. The IR50 values were calculated from the responses of the gastrocnemius muscles to 15 min infusions of suxamethonium. Abscissa: time in hours. The figures represent rates of hydrolysis of 25 μM suxamethonium in (nmol suxamethonium hydrolysed/ml plasma)/h (value for normal cats: 101 ± 10 (S.E.M.)). Symbols a, b, c and d denote intravenous injections of 177, 1,210, 350 and 710 mg, respectively, of purified cholinesterase of human plasma.

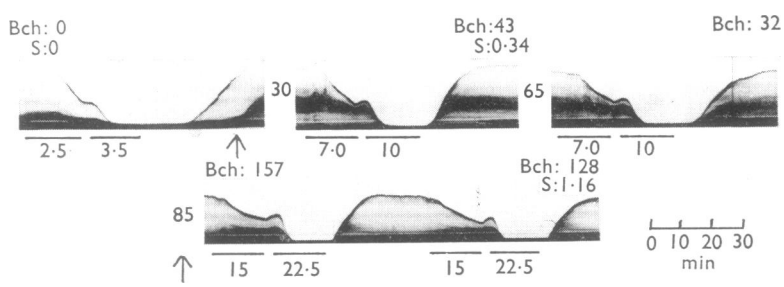

FIG. 3. Change of effectiveness of suxamethonium by purified cholinesterase of human plasma in a cat, 3·9 kg, treated with 12 μmol *iso*-OMPA/kg intraperitoneally 3 days previously. The figure shows part of the record (responses of the gastrocnemius muscle to indirect stimulation) on which the data illustrated in the lower record of Fig. 2 are based. Infusions of suxamethonium are marked by bars and the figures beneath them are rates of infusion of suxamethonium in (nmol/kg)/minute. Figures above the tracings are hydrolysis rates in (μmol/ml plasma)/h with 10 mM butyrylcholine (BCh) and 25 μM suxamethonium (S) as substrates. At the first and second arrows 350 and 710 mg, respectively, of purified cholinesterase of human plasma were given intravenously. Figures between the records are periods (in min) not illustrated.

1 ml of heparinized saline (10 I.U. heparin/ml). The blood in each syringe was then injected simultaneously into the external jugular vein of the respective recipient cats. This was repeated at least 9 times.

Three cats were injected intraperitoneally with *iso*-OMPA, 3, 6 and 12 μmol/kg respectively, one day before the exchange transfusion of blood. The rates of hydrolysis of 10 mM butyrylcholine by plasma obtained before exchange transfusion were (15, 0 and 0 μmol butyrylcholine/ml plasma)/h, respectively. After exchange transfusions of 190, 290 and 300 ml of blood, respectively, with normal animals, the values were (89, 68 and 12 μmol butyrylcholine/ml plasma)/h, respectively. In normal cats rates of hydrolysis ranged from 55 to 240 μmol butyrylcholine/ml.

The cholinesterase activities in the small intestine of the three cats treated with *iso*-OMPA (39, 21 and 7 μmol butyrylcholine/g tissue)/h, respectively, as compared with (168±8·6 (S.E.M.) μmol/g tissue)/h in normal cats.

The elevation of cholinesterase activity in plasma obtained by exchange transfusion in the three cats failed to raise the IR50 of suxamethonium to any appreciable extent. Conversely, a reduction of cholinesterase activity of up to 40% in the plasma of a normal cat by exchange transfusion with a cat which a day earlier had received *iso*-OMPA also failed to lower detectably the IR50 of suxamethonium.

Discussion

The experiments reported above show that in cats marked inhibition of cholinesterase activity in plasma by DFP under conditions which give much less inhibition of cholinesterase activity in tissue, as reflected by the level of enzyme activity in the small intestine, has only a small influence on the effectiveness of suxamethonium, given by intravenous infusion. On the other hand, marked inhibition of cholinesterase activity in both plasma and tissues by *iso*-OMPA increases the effectiveness of suxamethonium approximately 10-fold and a return of the effectiveness of suxamethonium to normal limits is only obtained if the suxamethonium hydrolysing capacity of the plasma of *iso*-OMPA treated cats is raised to a level which is 10–50 times the normal level. This was accomplished by the intravenous injection of purified cholinesterase of human plasma which hydrolyses suxamethonium faster than does the cholinesterase of the cat (Hobbiger & Peck, 1969). Replacement of the blood of cats treated with *iso*-OMPA by blood from normal cats and the reverse approach has not influence on the effectiveness of suxamethonium.

The results clearly show that the major factor determining the effectiveness and duration of suxamethonium in cats is not cholinesterase in plasma but cholinesterase in tissues. In the cat cholinesterase is present at the motor endplate (Holmstedt, 1957) and the rate of recovery from neuromuscular block produced by suxamethonium is the same in the presence and absence of blood flow through the muscle (Argent, Dinnick & Hobbiger, 1955). It seems reasonable to suggest that little suxamethonium is hydrolysed in the blood and that it is the cholinesterase at motor endplates which plays the essential role in determining the effectiveness and duration of action of suxamethonium which reaches the motor endplate. The cholinesterase at other sites in the tissues will serve an essential role in as far as it hydrolyses that fraction of the dose of suxamethonium which enters tissues other than muscle, preventing its return into the blood stream.

Evidence obtained in man indicates that a large fraction of suxamethonium is hydrolysed in the blood stream before it reaches the neuromuscular junction (Kalow & Gunn, 1957; Kalow, 1959; Kalow, 1962). The most likely explanation for the difference between man and cat is that the enzymic hydrolysis of suxamethonium, in low concentrations and calculated from the hydrolysis rate/ml plasma, in human plasma is 44 times greater than that in cat plasma (Hobbiger & Peck, 1969). This difference will be accentuated by the difference in circulation time between the two species. In man the circulation time, represented by blood volume divided by cardiac output, is approximately double that in cats. Calculations based on rates of hydrolysis of suxamethonium/ml plasma (Hobbiger & Peck, 1969) and circulation time indicate that other common laboratory animals should resemble the cat in so far as hydrolysis in blood is not an essential factor determining the effectiveness of suxamethonium.

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