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

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Summary

1. Suxamethonium was given by intravenous infusion to anaesthetized Rhesus monkeys and the infusion rate required to produce after 10 min a 50% reduction in twitch tension of the indirectly stimulated gastrocnemius or tibialis anterior muscle (IR50) was calculated from results obtained with paired infusions of two concentrations, repeated at intervals. The IR50 values in three monkeys were (388, 958 and 1,010 nmol/kg)/minute.

2. In one experiment the cholinesterase activity in plasma was increased to 125 and then to 182% of the initial value by infusions of purified human cholinesterase (usual enzyme). This increased the IR50 by 34 and then by 60%, respectively.

3. Inhibition by *iso*-OMPA of cholinesterase activity in plasma and tissues (four monkeys) by more than 90% lowered the mean IR50 value to (69 ± 11 (S.E.M.) nmol/kg)/minute. Restoration of cholinesterase activity in the plasma of these animals by infusion of purified human cholinesterase raised the IR50's to values within the normal range.

4. It is concluded that in the Rhesus monkey most of the infused suxamethonium is hydrolyzed by the cholinesterase in plasma before reaching the motor endplates. This agrees with observations in man with usual cholinesterase and contrasts with experiments in the cat where the hydrolysis of suxamethonium by cholinesterase in tissues was found to be more important than the hydrolysis by cholinesterase in plasma.

Introduction

In man Kalow (1962) has demonstrated the presence of two forms of cholinesterase (acetylcholine acetylhydrolase ; *E*.C. 3.1.1.8) in plasma, the usual form being present in 96% of the population and the atypical form in approximately 0·0005%, leaving 4% of subjects with plasma containing both forms. Furthermore, Kalow & Gunn (1957) showed that in persons with usual cholinesterase the duration of apnoea after intravenous administration of suxamethonium correlated inversely with cholinesterase activity in plasma, indicating that most of the drug is hydrolyzed mainly in plasma before reaching the motor endplate.

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By contrast, in the cat suxamethonium is mainly hydrolyzed in tissues (Hobbiger & Peck, 1970, 1971). This conclusion is based on the observation that the rate of intravenous infusion of suxamethonium required to produce 50% reduction in twitch tension of the indirectly stimulated gastrocnemius or soleus muscle in 15 or 20 min (IR50), was unrelated to the plasma cholinesterase activity and was only slightly increased by a 20 fold increase in suxamethonium hydrolyzing capacity produced by infusion of purified usual human cholinesterase. Inhibition of cholinesterase in plasma and tissues by *iso*-OMPA (tetramono*iso*propyl pyrophosphortetramide) greatly reduced the IR50, while restoration of plasma suxamethonium hydrolyzing capacity to normal failed to restore the IR50 values.

The reasons for the difference between man and cat lie in the enzyme concentration and in the enzymic turnover of suxamethonium. Thus human plasma containing usual cholinesterase hydrolyzes suxamethonium, in low concentration, 44 times faster than does cat plasma. The level of cholinesterase in plasma is higher in man and the rate of hydrolysis of low concentrations of suxamethonium relative to butyrylcholine is 11 times as fast with human cholinesterase as with cat cholinesterase (Hobbiger & Peck, 1969). In addition, the mean circulation time (blood volume/cardiac output) in man is approximately double that in the cat (Altman & Dittmer, 1964; Spector, 1956) and this allows more time for hydrolysis in the blood in man compared with that in the cat.

The relative rates of hydrolysis of low concentrations of suxamethonium and of butyrylcholine are similar for plasma from the Rhesus monkey (*Macaca mulatta*) and human plasma with usual cholinesterase (Hobbiger & Peck, 1969). Experiments on the Rhesus monkey, designed to investigate the relative importance of cholinesterase in plasma and tissues in the hydrolysis of suxamethonium, therefore, were thought likely to be of more predictive value for man, and are described in this paper.

Methods

The experiments were performed on eight Rhesus monkeys (*Macaca mulatta*), seven females and one male, weighing 2·5 to 3·2 kg under general anaesthesia with pentobarbitone sodium, 0·1 mmol (26 mg)/kg intravenously. Cannulation of the trachea enabled artificial respiration by a pump to be used when required. Rectal temperature was measured using a Telethermometer YSI Model 42SC and maintained between 37° and 38° C by external heating. Blood pressure was monitored in the left carotid artery using a Statham pressure transducer, Model P23 AC, coupled to an inkwriting Grass Polygraph, Model 7.

The neuromuscular blocking action of suxamethonium was assessed from changes in twitch tension of either the tibialis anterior or gastrocnemius muscle. The left leg was immobilized by drills through the lower femoral condyles and the calcaneum. The sciatic nerve was stimulated with supramaximal rectangular pulses (0·2 Hz; 3–7 ms duration; 5–20 V) and muscle tension was recorded isometrically with a Grass force displacement transducer (FT 03) coupled to a Grass Polygraph recorder.

Suxamethonium chloride (Koch-Light Laboratories, England) was given by infusion into the left internal jugular vein with a continuous infusion pump which delivered a volume of 0·2 ml/minute. An individual experiment consisted of a

series of two consecutive infusions of suxamethonium, each of which lasted for 10 min, with a 30 min interval between individual pairs of infusions. Ten min was usually sufficient for a constant level of neuromuscular block to be approached, even if *iso*-OMPA had been given. With each pair of infusions suxamethonium was infused first at a rate giving between 20 and 60% reduction of twitch tension, and then at 1·5 to 2 times this rate. From the effects obtained with the two infusions the infusion rate required for 50% reduction in twitch tension (IR50) was determined graphically by plotting percentage reduction in twitch tension at the end of the 10 min infusion against log infusion rate of suxamethonium and taking the relationship between the two as linear. In any individual experiment comparable values were obtained in both muscles.

Measurement of enzyme activities

All determinations of enzyme activity were made by means of the Warburg manometric technique (medium: 25 mM $NaHCO_3$; gas phase: 95% N_2; 5% CO_2; 37° C; pH 7·45). For acetylcholinesterase (acetylcholine acetylhydrolase, E.C. 3.1.1.7) the substrate was 30 mM (±)-acetyl-β-methylcholine chloride (mecholyl; Sigma Chemical Co.) while for cholinesterase the substrate was 10 mM butyrylcholine iodide (Sigma Chemical Co.).

The cholinesterases of the Rhesus monkey and man (with usual cholinesterase) hydrolyze low concentrations of suxamethonium relative to butyrylcholine at very similar rates and thus measurements of the rate of hydrolysis of butyrylcholine in monkeys, that are given an injection of purified human cholinesterase can be taken as a measure of the suxamethonium hydrolyzing capacity before and after the injection of the human enzyme.

Enzyme preparations and drugs used

Blood samples of 3 ml were collected from the right carotid artery and heparinized (10–20 I.U. heparin/ml blood). The acetylcholinesterase activity of whole blood was determined using haemolysates made in 25 mM $NaHCO_3$. The cholinesterase activity of plasma was determined using plasma which had been separated in a refrigerated centrifuge at 3,000 rev/min for 15 minutes. For the determination of cholinesterase activity of the small intestine, a 5 cm length of jejunum was removed at the end of the experiment. The tissue was washed, weighed and then homogenized in 25 mM $NaHCO_3$ in a ground glass homogenizer. The cholinesterase activity of the homogenate was determined immediately after its preparation.

The purified cholinesterase of human plasma (lyophilized preparation of plasma containing usual cholinesterase obtained from AB KABI, Sweden) had the same properties as that used previously (Hobbiger & Peck, 1970 & 1971) and in 25 mM $NaHCO_3$ hydrolyzed 10 mM butyrylcholine at a rate of (51 μmol/mg powder)/hour. For injection, the preparation was dissolved in 25 mM $NaHCO_3$ containing the amount of NaCl which was required to bring the tonicity to that of a 0·15 M (isotonic) NaCl solution.

A stock solution of suxamethonium chloride (Koch-Light Laboratories, England) in 0·15 M NaCl was prepared freshly each day and final dilutions were made from it immediately before use.

Tetramono*iso*propyl pyrophosphortetramide was obtained from two sources, Koch-Light Laboratories, England and K and K Laboratories, Inc., U.S.A.

Results

Suxamethonium was given by intravenous infusion to two Rhesus monkeys anaesthetized with 0·1 mmol pentobarbitone sodium/kg and their IR50, that is the rate of infusion of suxamethonium required to produce 50% reduction of twitch tension of the indirectly stimulated tibialis anterior or gastrocnemius muscle, was determined at 30 min intervals, as described under **Methods.**

In one monkey (female, 2·6 kg) the mean IR50 for the tibialis anterior muscle, based on 7 individual values obtained over a period of 6·5 h was (388 ± 29 (S.E.M.) nmol/kg)/minute. In the other monkey (male, 2·5 kg) the mean IR50 for the gastrocnemius muscle based on 9 individual values obtained over a period of 7·5 h was (958 ± 53 (S.E.M.) nmol/kg)/minute. No consistent changes related to time occurred in the IR50 values of either monkey. The enzyme activities of the first and second monkeys were: cholinesterase activity in plasma (332 and 386 μmol butyrylcholine/ml plasma)/h; cholinesterase activity in the intestine (194 and 183 μmol butyrylcholine/g tissue)/h; acetylcholinesterase activity in blood (49 and 46 μmol mecholyl/ml blood)/hour.

In a third Rhesus monkey (female, 2·8 kg) the mean IR50 for the gastrocnemius muscle, based on 3 individual measurements, was (1,010 ± 8 (S.E.M.) nmol suxamethonium/kg)/min and the activity of cholinesterase in plasma was (644 μmol butyrylcholine/ml plasma)/hour. Two infusions of 1 g purified cholinesterase of human plasma, separated by an interval of 3 h 15 min, were given and raised the cholinesterase activity of the plasma to (804 and 1,170 μmol butyrylcholine/ml plasma)/h respectively. The IR50's associated with this, each based also on 3 individual measurements, were (1,350 ± 16 (S.E.M.) and 1,610 ± 13 (S.E.M.) nmol suxamethonium/kg)/min. Increases of the cholinesterase activity of the plasma by 25% and 82%, therefore, raised the IR50 above its initial value by 34% and 60% respectively. The acetylcholinesterase activity of whole blood was (58 μmol mecholyl/ml blood)/h and cholinesterase activity in the jejunum was (221 μmol butyrylcholine/g tissue)/hour.

Experiments following reduction of cholinesterase activity by iso-*OMPA*

Five Rhesus monkeys were injected intravenously or intraperitoneally with the anticholinesterase *iso*-OMPA 1 or 3 days before measurement of the IR50 values in order to determine the effects on the IR50 of marked reductions of cholinesterase activity in plasma and tissues and of subsequent elevations of the cholinesterase activity of the plasma by the intravenous injection of purified cholinesterase of human plasma.

One monkey (female, 2·9 kg) was given 45 μmol *iso*-OMPA/kg intraperitoneally. Twenty-four h later the cholinesterase activity of the plasma was reduced from (538 μmol butyrylcholine/ml plasma)/h to (5 μmol butyrylcholine/ml plasma)/h and the acetylcholinesterase activity of whole blood was reduced from (96 μmol mecholyl/ml blood)/h to (27 μmol mecholyl/ml blood)/h. The mean IR50 value for the tibialis anterior muscle, based on nine individual measurements made over a period of 8·5 h, starting 24 h after administration of *iso*-OMPA, was (49 ± 7

(S.E.M.) nmol suxamethonium/kg)/minute. The individual IR50 values showed no consistent trend in any direction with time. Cholinesterase activity in the jejunum was (5 μmol butyrylcholine/g tissue)/hour.

In four monkeys, all female and weighing 2·5–3·2 kg, cholinesterase activity in plasma and tissues was lowered markedly by *iso*-OMPA, given intravenously or intraperitoneally (Table 1). After the *iso*-OMPA treatment the IR50 values for suxamethonium were on average below 10% of those obtained in monkeys that had not been given *iso*-OMPA, as shown in Table 2. This table also gives information on the effect on the IR50 of infusions (over 10 min) of purified cholinesterase of human plasma which raised cholinesterase activity in the plasma to within the normal range. As can be seen the return of cholinesterase activity

TABLE 1. *Inhibition by* iso-*OMPA of the activity of cholinesterase (ChE) in plasma and jejunum and of acetylcholinesterase (AChE) in whole blood of Rhesus monkeys*

Monkey	Weight (kg)	Dose of *iso*-OMPA in μmol/kg	Interval to second (after *iso*-OMPA) enzyme measurement in h	ChE in plasma ((μmol butyrylcholine/ml)/h) Before *iso*-OMPA	ChE in plasma ((μmol butyrylcholine/ml)/h) After *iso*-OMPA	AChE in whole blood ((μmol mecholyl/ml)/h) Before *iso*-OMPA	AChE in whole blood ((μmol mecholyl/ml)/h) After *iso*-OMPA	ChE in the jejunum ((μmol butyrylcholine/g)/h)
1	2·5	24, i.v.	48	—	105	—	30	53
2	2·5	96, i.p.	72	375	13	68	15	11
3	3·2	72, i.p.	24	844	14	72	24	31
4	2·5	22, i.p.	24	964	0	—	39	14

The Table shows enzyme activities, expressed as (μmol substrate hydrolyzed/ml plasma, whole blood or gram tissue)/hour. The substrates for ChE and AChE were 10 mM butyrylcholine and 30 mM mecholyl, respectively. After collection of blood samples from the *iso*-OMPA treated monkeys for the assay of ChE and AChE activities in plasma and whole blood, respectively, IR50 values for suxamethonium and the effect on them of raising the ChE activity in plasma were determined (shown in Table 2). The animals were then killed and the jejunum was removed for assay of its ChE activity. Values not determined are indicated by —. *Iso*-OMPA was given in a single dose, except in the case of monkey 2 who received initially 48 μmol/kg and 24 h later was given the same dose again; in this monkey blood samples for the ' after *iso*-OMPA' assays were collected 72 h after the first *iso*-OMPA injection.

TABLE 2. *Effect on IR50 of suxamethonium of raising the cholinesterase (ChE) activity in plasma of* iso-*OMPA treated Rhesus monkeys*

Monkey	Dose in g of human ChE	ChE in plasma ((μmol butyrylcholine/ml)/h) Before Human ChE	ChE in plasma ((μmol butyrylcholine/ml)/h) After Human ChE	IR50 values ((nmol suxamethonium/kg)/min) Before Human ChE	IR50 values ((nmol suxamethonium/kg)/min) After Human ChE
1.	1	105	603	180±28	682±114*
2.	0·8	13	313	55± 3	320± 29†
3.	1	14	481	98±10	313± 26†
	1		824		507± 26†
4.	1	0	546	74±11	496± 32†
	1		903		725± 66*

This Table is a continuation of Table 1 and shows the effect of intravenous infusion over 10 min of purified cholinesterase of human plasma (human ChE) on the ChE activity in plasma of the *iso*-OMPA treated monkeys and on their IR50 for suxamethonium. Monkeys No. 3 and 4 received a second infusion of purified human cholinesterase after an interval of 2 h 45 min. The IR50 is the concentration of suxamethonium, given by infusion, required for 50% reduction in twitch tension of the indirectly stimulated gastrocnemius muscle (see **Methods**). All IR50 values are means of three consecutively obtained individual values. The significance of rises in the IR50 was assessed by a paired t-test; * and † represent P values of $<0{\cdot}05$ and $<0{\cdot}01$, respectively.

in plasma to normal was associated with a rise of the IR50 for suxamethonium to values seen in monkeys that had not been given *iso*-OMPA.

Discussion

In Rhesus monkeys repeated infusions of suxamethonium over periods of 7·5 h or more gave similar responses, a finding somewhat unexpected in view of the tachyphylaxis reported by Zaimis (1953) after intravenous 'bolus' injections of suxamethonium. The mean IR50 value of suxamethonium for three normal Rhesus monkeys was (785 ± 199 (S.E.M.) nmol suxamethonium/kg)/min: the cholinesterase activity in plasma of these monkeys was (454 ± 96 (S.E.M.) μmol butyrylcholine/ml plasma)/h, and the cholinesterase activity of jejunum was (199 ± 11 (S.E.M.) μmol butyrylcholine/g)/hour.

Increases in cholinesterase activity in the plasma of one monkey, by means of purified human cholinesterase (usual enzyme), were followed by proportional increases in the IR50 as illustrated in Figure 1.

Reduction of the cholinesterase activity in plasma and jejunum to 2 and 8%, respectively, of the activity in normal monkeys by administration of *iso*-OMPA, lowered the IR50 of four monkeys to 9%. Restoration of the cholinesterase activity of plasma without appreciably affecting tissue cholinesterase activity in

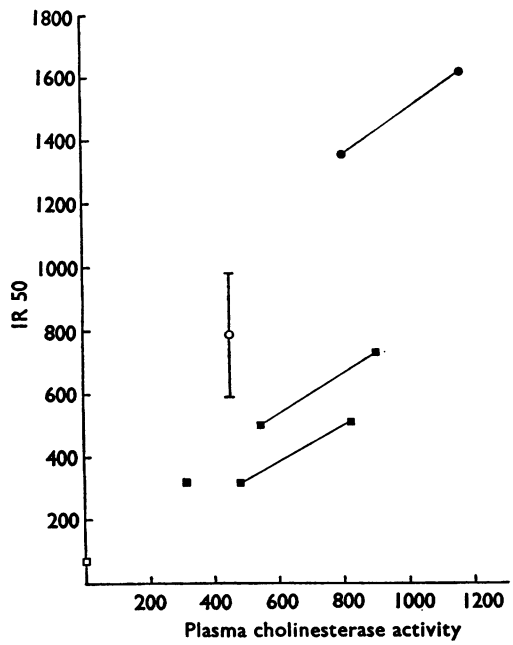

FIG. 1. Relationship between cholinesterase activity in plasma and IR50 for suxamethonium in seven Rhesus monkeys. The IR50 represents the amount of suxamethonium in (nmol/kg)/min, given for 10 min by intravenous infusion, required to produce 50% reduction in the response of the indirectly stimulated gastrocnemius or tibialis anterior muscle (see **Methods**). Cholinesterase activity in plasma was determined with 10 mM butyrylcholine as substrate and is expressed as (μmol substrate hydrolyzed/ml plasma)/hour. ○, Mean ± S.E.M. for three normal monkeys; ●, values in a normal monkey after two infusions of purified cholinesterase of human plasma; □, mean for four monkeys treated with *iso*-OMPA. The S.E.M. for this mean was 11; ■, values for three monkeys treated with *iso*-OMPA and then receiving an infusion of purified cholinesterase of human plasma. One of these monkeys received one infusion and the other two received two infusions.

three of these animals (monkeys 2, 3 and 4 of Tables 1 and 2) by infusions of purified human plasma cholinesterase (usual enzyme) raised IR50 values to within the range for normal animals as shown in Figure 1. As pointed out previously the concentration of cholinesterase in plasma and the turnover of suxamethonium by this enzyme are similar in Rhesus monkeys and man, and differ markedly from those in cats. Studies on the electrophoretic patterns of cholinesterase in plasma of different species by Goedde, Hofmann, Fuss & Omoto (1966) have also shown a close similarity between primate species and a marked difference between them and the enzyme in the cat. The experiments described in this paper show that a high turnover of suxamethonium in plasma is required for a correlation between enzyme activity in plasma and IR50 of suxamethonium. Studies in Rhesus monkeys, unlike those in the cat, give results which have a predictive value for man.

Inhibition of cholinesterase activity following *iso*-OMPA was accompanied by some inhibition of acetylcholinesterase activity in blood. It is unlikely that this produces any fundamental change in the effect of suxamethonium at the motor endplate, since in rats and cats such inhibition does not affect the neuromuscular blockade produced by decamethonium (Hobbiger & Peck, 1970).

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