

The Distribution of Procaine in Human Blood:

Relation to Potentiation of Succinylcholine

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Results of previous studies on human subjects have suggested that the interaction of procaine and succinylcholine might be explained by the distribution of the drugs among three compartments, each drug entering only two compartments and sharing one in common. Two of the compartments have been shown to be the neuromuscular junction (open to succinylcholine only) and plasma (open to both succinylcholine and procaine). The present study indicates that the red blood cells represent the remaining compartment (open to procaine only). When the capacity of the latter compartment is saturated, the excess procaine results in a dose-response curve measurable by respiratory depression in the rat. Succinylcholine is not hydrolyzed in this compartment. This parallels the findings in man and fits the three-compartment model previously hypothesized.

APNEA induced by succinylcholine can be prolonged if procaine is also injected.¹ Experiments in human subjects show that with a given dose of succinylcholine the delay is almost constant with doses of procaine ranging from 5.04 to 11.34 mg./kg., while larger doses (up to 38.26 mg./kg.) give rise to increasing periods of apnea; the resulting dose-response curve parallels that of succinylcholine alone.

This additive effect was tentatively explained by the following hypothesis²: these drugs might enter a three-compartment system in such a way that the first compartment was permeable to both drugs, the second to procaine only and the third to succinylcholine only. Limited competition between procaine and succinylcholine would then occur in the first compartment (horizontal area of the

curve, due to the entrance of procaine into the second compartment, doses 5.04 to 11.34 mg./kg.), maximum competition occurring only after this second compartment was saturated as evidenced by the steep slope of the dose-response curve (doses 11.34 to 38.26 mg./kg.). Two of these compartments have been identified. The first, obviously, is plasma, where both drugs compete for pseudocholinesterase in the intravascular space, and the third is the neuromuscular junction, where succinylcholine exerts its action and where procaine has no obvious effect.³ The second compartment has not been previously identified.

This paper is concerned with identification of the second compartment. Two facts suggest that it might be located in red blood cells. The first is that some patients subjected to large doses of intravenous procaine during general anesthesia with this drug develop cyanosis,⁴ this phenomenon being attributable to the combination of procaine with hemoglobin.⁵ The second is that procaine is also hydrolyzed by the true cholinesterase contained in red blood cells.⁶

Since two of the compartments are located in the vascular space it should be possible to demonstrate a double-curve phenomenon *in vitro*, using human blood and testing the paralyzing effect of the blood in an experimental animal. In other words, addition of a constant dose of succinylcholine and increasing doses of procaine to a constant volume of blood and injection of a sample into an experimental animal should reproduce the phenomenon observed in man. Moreover, the double curve should be absent if the same procedure were performed adding the drugs to plasma—that is, to a system lacking the supposed second compartment.

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Finally, to fulfill these theoretical conditions, and assuming that red blood cells form the second compartment, it should be found either that succinylcholine cannot enter the second compartment or that true cholinesterase does not hydrolyze succinylcholine. The latter statement seems to be correct, for hemolyzed red blood cells do not inactivate succinylcholine⁶ whereas they do inactivate procaine.⁵

Methods

EXPERIMENT 1

Human heparinized whole blood was incubated for five minutes at 37° C. in the presence of increasing doses of procaine (0.19, 0.29, 0.59, 0.88, 1.76, 2.64, 5.28, 7.92, 15.84, 23.75, and 47.50 $\mu\text{g./Gm.}$) dissolved in a constant volume, and in a dosage of 0.003125 ml./Gm. per rat, administered to rats concurrent with succinylcholine in a single dose of 0.7 $\mu\text{g./Gm.}$ per rat. The sample was injected intravenously into the rat immediately after preparation. Six rats were used for each dosage, for a total of 66 rats. The duration of respiratory depression was then measured.⁷ A constant minute ventilation was attained by means of a mechanical ventilator especially devised by us for use in rats so that differences in P_{CO_2} could be minimized, thus not hampering the return of spontaneous ventilation.

EXPERIMENT 2

This experiment was designed to investigate the action of true cholinesterase of red blood cells on procaine. Three ml. of human red blood cells obtained from citrated blood were washed three times in 0.9 per cent NaCl and resuspended in 3 ml. of an 0.9 per cent NaCl solution containing procaine at concentrations ranging from 3.99 up to 30.28 Gm./100 ml. The suspension of red blood cells was incubated for 5 minutes at 37° C. and later centrifuged for 20 minutes. A dose of 0.0025 ml./Gm. per rat of the supernatant was then injected intravenously into the rat immediately after a paralyzing dose (0.7 $\mu\text{g./Gm.}$ rat) of succinylcholine had been given by the same route. The duration of respiratory depression was determined. This experiment was repeated six times with 18 animals each.

EXPERIMENT 3

Procaine in the same concentrations used in experiment 1 was added to whole blood, incubated and injected intravenously with succinylcholine given at a constant dose of 0.7 $\mu\text{g./Gm.}$ per rat. One hundred and ten animals were thus treated.

EXPERIMENT 4

This was performed in the same manner as experiment 1, but pooled plasma from five human donors were used instead of whole blood. Six rats were used for each dosage, for a total of 66.

EXPERIMENT 5

The cholinesterase activity of plasma and that of red blood cells were tested against a succinylcholine substrate. Heparinized blood from human donors was centrifuged, and after separation from plasma the red blood cells were washed three times in 0.9 per cent NaCl and frozen three times to produce hemolysis. A bioassay⁷ was then done, comparing the activity of plasma with that of hemolyzed red blood cells. Both plasma and red blood cells (0.0002 ml./Gm. rat) were separately incubated for five minutes at 37° C. in the presence of low and high doses of succinylcholine (0.7 and 1.4 $\mu\text{g./Gm.}$ rat) and immediately injected, in one dose per animal. Eight bioassays were performed with eight animals each.

EXPERIMENT 6

The effects of intact and hemolyzed red blood cells on a succinylcholine substrate were investigated. For this purpose bioassays were performed employing 0.9 per cent NaCl solution as the standard against two unknowns represented by intact and hemolyzed red blood cells obtained from human heparinized blood. Twelve animals were employed for each bioassay, four for each substance tested (intact red blood cells, hemolyzed red blood cells, and 0.9 per cent NaCl). The substances were incubated in the presence of high and low doses of succinylcholine (0.35 and 0.7 $\mu\text{g./Gm.}$ rat) immediately prior to injection.

Drugs used for all experiments were prepared as follows: procaine from 0.19 to 47.50 $\mu\text{g./Gm.}$ rat (ratio between doses, 1.5) was always diluted in the same volume of 0.9 per

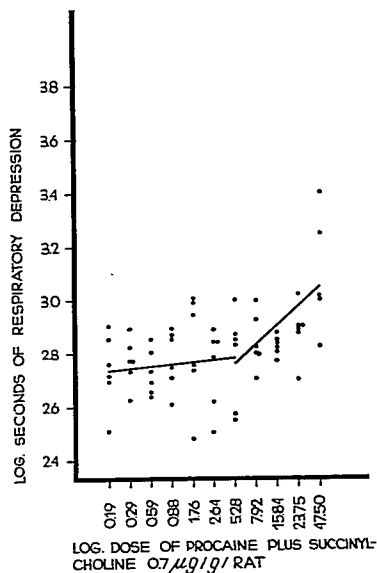


FIG. 1. Double-log correlation between succinylcholine (0.7 $\mu\text{g./Gm. per rat}$) and different doses of procaine with respiratory depression when these drugs were incubated in whole blood. Dose-response curve only appears after saturation of red blood cell compartment at 5.28 $\mu\text{g./Gm.}$ at dosage 7.

cent NaCl in redistilled water (0.003125 ml./Gm. rat). Experiment 2 employed larger doses of procaine to compensate for prolonged contact of the drug with whole blood. In experiment 6, doses of succinylcholine ranged from 0.35 to 0.7 $\mu\text{g./Gm. rat}$. The doses were randomized for injection, using a table of random numbers.

Experimental data from the experiments were submitted to double-log transformations in order to fulfill the requirements of linearity and stabilization of the variance.

Results

EXPERIMENT 1

Increasing doses of procaine plus a constant dose of succinylcholine 0.7 incubated in whole

blood prolonged the period of respiratory depression attributable to the known interaction between these drugs (fig. 1). The slope of the first portion of the curve is without significance, while that of the second is significant ($F = 13.35$; $P < 0.01$; $r = 0.57$). Both slopes differ from a mean slope, $P < 0.01$.

EXPERIMENT 2

The same double-slope response can be seen in figure 2, where increasing doses of procaine were incubated in red blood cells and injected intravenously in rats immediately after injection of a constant dose of succinylcholine but the effect was dependent on the dose of procaine: up to the third dose (22.46 $\mu\text{g./Gm. per rat}$) there was no increase in effect, while larger doses gave rise to significant changes

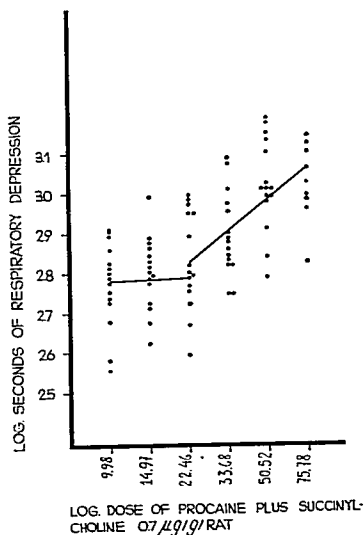


FIG. 2. Double-log correlation between succinylcholine (0.7 $\mu\text{g./Gm. per rat}$) and different doses of procaine with respiratory depression when these drugs were incubated in red blood cells. Observe dose-response curve is obtained only after 22.46 $\mu\text{g./Gm.}$ dosage 3, which represents red blood cell cholinesterase saturation. Only nine animals were employed for last dose.

in slope ($F = 26.13$; $P < 0.001$; $r = 0.58$). Analysis of variance shows that both slopes differ from a mean slope, $P < 0.05$. These results were found in five of the six experiments; the remaining experiment did not show this tendency and could easily be adjusted to a single slope.

In experiment 1 the dose at which the slope increased was $5.28 \mu\text{g./Gm.}$ per rat instead of $22.46 \mu\text{g./Gm.}$ per rat as in experiment 2, resulting from a more prolonged contact of procaine with red blood cells (five minutes of incubation plus 20 minutes of centrifugation). In the first experiment centrifugation was not done.

EXPERIMENTS 3 AND 4

There was no interaction *in vitro* between procaine and succinylcholine in whole blood.

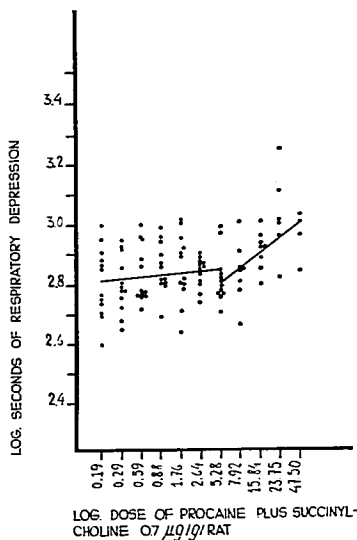


FIG. 3. Double-log correlation between succinylcholine ($0.7 \mu\text{g./Gm.}$ per rat) and different doses of procaine with respiratory depression, when only procaine was incubated in whole blood while succinylcholine was injected immediately after the injection of the incubate. Dose-response curve is seen after saturation of red blood cell compartment at $5.28 \mu\text{g./Gm.}$, dosage 7.

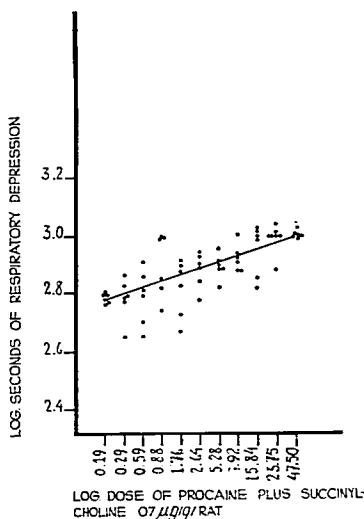


FIG. 4. Double-log correlation between succinylcholine ($0.7 \mu\text{g./Gm.}$ rat) plus different doses of procaine with respiratory depression, when these drugs were incubated in plasma. No double-response curve can be observed here.

This was demonstrated by incubating different doses of procaine in blood, injecting them intravenously with the same dose of succinylcholine. This procedure gave rise to a double slope (fig. 3) of the same type as that in figure 1. Up to $5.28 \mu\text{g./Gm.}$, the change in slope was not significant ($F = 0.87$; $P > 0.05$; $r = 0.10$); thereafter it was highly significant ($F = 20.54$; $P < 0.001$; $r = 0.60$). Analysis of variance between mean slopes for the seven first and last doses was highly significant, $F = 18.74$, $F_{0.001}$ for 1 and 120 degrees of freedom = 11.38.

With the same doses of procaine and succinylcholine incubated in plasma, a double slope was not found (fig. 4) (F slope for all doses = 34.89 ; $P < 0.001$; $r = 0.59$).

EXPERIMENTS 5 AND 6

Succinylcholine was inactivated to a greater degree in plasma than in red blood cells. In this experiment (fig. 5) the potency ratio was

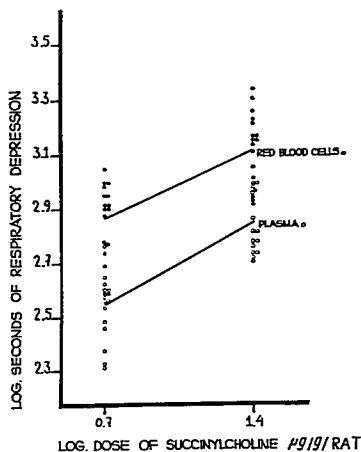


FIG. 5. Biological assay for establishing difference between activity of red blood cells versus plasma cholinesterase employing succinylcholine as substrate. This present figure represents a log-log transformation for the eight experiments. If plasma standard is taken as 100 per cent activity, then mean cholinesterase activity of red blood cells represent only 31 per cent of the standard. F for standard versus unknown = 69.92; $P < 0.001$, and index of precision $\lambda = 0.16$. Interaction between plasma and red blood cells is not present in the above figure, but was present in two of the eight bioassays.

3.2, but, actually it was demonstrated that red blood cells do not inactivate succinylcholine at all. In fact, when succinylcholine was incubated with either intact or hemolyzed red blood cells and the effect compared with succinylcholine incubated in 0.9 per cent NaCl (fig. 6), the red blood cells had no effect.

Discussion

The effect of procaine on the respiratory depression induced by succinylcholine in the rat was modified by prior incubation with human whole blood. When different doses of procaine were tested, a dose-response curve was obtained which could be separated after a double-log transformation into linear segments, the first a horizontal line and the second a rise paralleling the dose-response curve of suc-

cinylcholine. This phenomenon disappeared when procaine was incubated in plasma but appeared in the presence of washed red blood cells. Succinylcholine, on the other hand, was not inactivated by either intact or hemolyzed red blood cells.

Returning to the model suggested in a previous paper,² we may conclude that red blood cells fit the conditions attributed to the second compartment, that is, permeability to procaine but not to succinylcholine. In the present paper the red blood cell compartment could have been permeable to succinylcholine, but in either case the cholinesterase present in this compartment was active against procaine but not against succinylcholine. It follows that procaine permeates the first compartment (plasma) but is more specifically attracted by the second compartment (red blood cells) where it is inactivated by true cholinesterase, in turn inactive against succinylcholine. When the red blood cell compartment is saturated, any increase in dose of procaine competes with

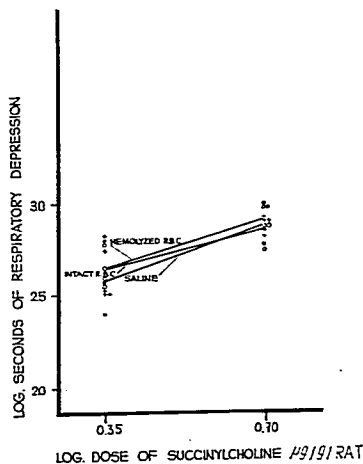


FIG. 6. Bioassay shows in a log-log correlation that cholinesterase activity of intact or hemolyzed red blood cells is not different from zero when tested against saline solution (zero standard). Mean index of precision $\lambda = 0.13$.

succinylcholine in the first compartment displacing succinylcholine, thus inducing a dose-response curve parallel to that of succinylcholine.

The experimental procedure in the present work can be used to assay the activity of red blood cell and plasma cholinesterase against normal standards. It also follows from the findings that patients with severe anemia should be given lower doses of procaine if this drug is used as a succinylcholine extender.

Summary

True cholinesterase of human red blood cells functions as an independent compartment for the hydrolysis of procaine when tested for interaction with succinylcholine *in vitro*. When the capacity of this compartment is saturated, the excess procaine induces a dose-response curve measurable by extended respiratory depression in the rat. Succinylcholine is not hydrolyzed in this compartment. This phenomenon fits the three-compartment model previously hypothesized to explain the interaction between succinylcholine and procaine in man.

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References

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8. Unpublished results.

Surgery

INTRAMUSCULAR INJECTION In Germany, intramuscular injections are made almost exclusively into the gluteal musculature. Accidental injection of almost any drug into or immediately beside the sciatic nerve may result in injection palsy. The safest site for injection is not the upper, outer quadrant of the buttock but rather the gluteus minimus muscle, well away from the sciatic and other large nerves. To define the surface anatomy of this safe area, place an index finger on the anterior superior iliac spine, the middle finger on the tubercle of the iliac crest and the palm on the greater trochanter. The injection can be made safely anywhere between the spread index and middle fingers. (Bay, E.: *Technique and Dangers of Intramuscular Injection*, *Germ. Med. Mth.* 13: 159 (April) 1968.)