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Succinylcholine Iodide (Celocurin)

A Synthetic Drug with a Curare-like Effect

L.-E. TAMMELIN

Research Institute of National Defence, Dept. 1, Sundbyberg and Institute of Organic Chemistry and Biochemistry, University of Stockholm, Sweden

The di-choline esters of the aliphatic dicarboxylic acids show a pronounced curariform action and Bovet [1,2] *et al.* and Fusco [3] in particular have studied an extremely large number of such compounds. These authors have tested clinically the di-(N,N-dimethyl-N-ethylammoniumethyl) ester of succinic acid. In Austria, co-operation between Die Österreichische Stickstoffwerke, Linz, and Ginzel *et al.*[4–6] and Brücke [7] in Vienna resulted in the close study of O,O-adipylcholine and this compound also was given clinical trials.

Löw and Tammelin [8] described a pure preparation of *O,O-succinylcholine iodide* and preliminary reports were given of its synthesis, pharmacology and biochemistry. Shortly afterwards Thesleff [9] and Holmberg and Thesleff [10] were able to claim that the succinylcholine, as prepared by Tammelin and Löw [11] was useful clinically. The preparation has since been subjected to close study in over two thousand cases, and no harmful side-reactions have been observed.

In 1951, simultaneously with but independently of the Swedish group, the Viennese research team published the results of clinical tests with succinylcholine chloride (Arnold [12]) and as far as can be judged from their limited material their results are largely in agreement with those obtained in Sweden.

Synthesis of Dicholine Esters of Dicarboxylic Acids

For the synthesis of compounds of this type three main methods have found favour. The acid chloride may be coupled with choline chloride directly, or with di-methylaminoethanol, followed by quarternisation with methyl iodide. A third method is to start from the diethyl ester of the carboxylic

acid, bring about the exchange reaction with dimethylaminoethanol, and quarternise with methyl iodide.

A brief description of the synthesis of succinylcholine has been given by Löw and Tammelin [8]. This substance is now produced on a large scale and sold for medical use under the name of CELOCURIN.

Phenylsuccinylcholine has been synthesized by the method below, which is better suited to laboratory conditions. As far as the author is aware, the substance has not been described before.

Phenylsuccinyl chloride: 1 Mole (194.2 g) of phenylsuccinic acid [13] is mixed with 2 moles (417 g) of PCl_3 in a flask fitted with a reflux condenser. One hundred ml of $POCl_3$ is added as solvent. When the reaction, initially vigorous, slows down, the mixture is heated at 60° C for 12 hours, then fractionally distilled giving phenylsuccinyl chloride b.p. 132—134° C/4 mm in 50 % yield.

Phenylsuccinylcholine Chloride. 1 Mole (231.2 g) of phenylsuccinyl chloride and 2 moles (278 g) of choline chloride are heated together in dioxan at 90° C in a flask equipped with a reflux condenser. After 18 hours a very viscous phase develops under the dioxan consisting largely of phenylsuccinylcholine chloride. This material was not purified.

Phenylsuccinylcholine Iodide. Phenylsuccinylcholine chloride, obtained as described above from 1 mole of phenylsuccinyl chloride is dissolved in a minimal quantity of water. 2.2 moles of potassium iodide as a saturated aqueous solution are then added, and the phenylsuccinylcholine iodide which precipitates is purified by dissolution in water and precipitation with ethanol. Yield: 10 % based on phenylsuccinic acid. Melting point 250° (decomp.). Found: C 39.0; H 5.6; N 4.5. Calc. for $C_{20}H_{34}O_4N_2I_2$ (620.2): C 38.7; H 5.5; N 4.5.

Pharmacodynamic Effect of Succinylcholine and Phenylsuccinylcholine.

Succinylcholine is remarkable for its relatively short-lived curare-like action. Like *d*-tubocurarine it paralyses the skeletal muscles; and, as with the former drug, the effect starts with the eyes and pharyngeal muscles, and ends with the diaphragm. The smallest dose that can paralyse the neck muscles in a rabbit (the headdrop dose) has been found to be between 0.20 and 0.25 mg/kg. Phenylsuccinylcholine, though it can produce the same effect in the rabbit, is considerably less powerful in this respect, the head-drop dose being 8 mg/kg. Typical of this substance, at a moderate dose level, is its power to produce a severe paralysis of the extremities, unaccompanied, however, by spontaneous cessation of respiration in rabbits.

Hydrolysis of Succinylcholine

Succinylcholine (SCh) hydrolyses in aqueous solutions. This property is of significance, where both the stability of the injection solution and the breakdown of the substance in the body are being considered. According to Whit-

taker [14] SCh on hydrolysis gives rise, in the first place, to a monoester of succinic acid (SmCh) and choline as follows.

$$\text{SCh} = \text{SmCh} + \text{Choline};\ \text{SmCh} = \text{Succinic acid} + \text{Choline}.$$

Thus the following equations may be derived for the hydrolysis of SCh:

$$\begin{aligned}(\text{SCh}) &= a-x\\ (\text{SmCh}) &= x-y\\ \text{succinic acid} &= \quad y\end{aligned}$$

$\frac{-\mathrm{d}(a-x)}{\mathrm{d}t} = k_1(a-x); \frac{\mathrm{d}y}{\mathrm{d}t} = k_2(x-y)$. Solution of the differential equations gives:

$$x = a(1-e^{-kt_1}) \text{ and } y = \frac{a}{k_1-k_2}(k_2e^{-k_1t}-k_1e^{-k_2t} + k_1-k_2)$$

If k_1 and k_2 are known, it is possible to follow the breakdown and formation of the three substances.

To investigate the tendency to hydrolyse, the present author has made use of, and closely adhered to, Hestrin's [14] method for the photometric determination of choline esters. This method is based on the formation of hydroxamic acid, which in its turn forms a coloured complex with the ferric ion. This complex exhibits maximum absorption at about 5 400 Å. A "Lumetron" photoelectric colourimeter with a filter for 5 300 Å and a 1 cm cell was used for the determinations.

The extinction (E) obtained is composite. That is:

$$E = E_1\ (\text{SCh}) + E_2\ (\text{SmCh}) \qquad (1)$$

Though this function is composite, it is of interest to express the extinction of SCh solutions as a function of time (t) under different conditions. The author has determined E as a function of (t) at a constant pH with different temperatures (a); with different pH (b); with different concentrations of SCh (c); and in a solution containing the cholinesterase from cobra venom (d).

Within the range of measurements a linear relationship between concentration and extinction is obtained, and E_2 is thereby found to be 230. E_2 from corresponding experiments with phenylsuccinylcholine is 205. These values were noted before any appreciable hydrolysis could take place.

a) 0.00266 M SCh-solutions in a buffer of the following composition were prepared: 45.2 ml of 0.1 M NaOH and 50 ml of 0.1 M KH_2PO_4 plus 4.47 g of KCl, the whole being diluted to 100 ml. Potassium chloride was added to make the experiment with cobra venom possible. The pH of the buffer following dilution was found to be 7.3. The results are presented in Fig. 1.

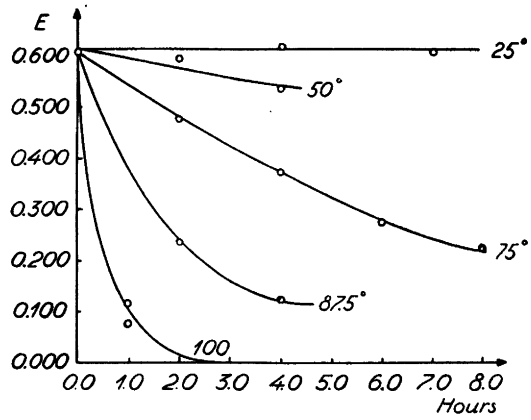

Fig. 1. Photometric determination of the rates of hydrolysis in a 0.00266 M succinylcholine solution at pH 7.3. The various curves represent the rates of hydrolysis at the temperatures given in the figure.

b) 0.00266 *M* solutions of succinylcholine were prepared in the buffer mixtures employed by Clark and Lub (Kolthoff [16]) with pH = 1, 4, 5, 6, and 8 respectively. The solutions were maintained thermostatically at a temperature of 75° C, since at this temperature the hydrolysis reaction proceeds at a convenient rate. At pH 4 or 5, no decrease in the extinction was observed, even after 6 hours. The rate of the breakdown reaction at the other pH values is shown in Fig. 2.

A sensitive test for a substance with curare-like action is the earlier described head-drop dose (H.D.) in rabbits. This test is very suitable for following the hydrolysis of SCh, since the H.D. of SCh is 0.22 mg/kg, and the H.D. for the monoester, according to Bovet *et al.*[2] 10 mg/kg. In three experiments with the same rabbit, it was shown that the dose in ml must be doubled if the solution has been at a pH of 7.3 for half an hour at 75° C. The half-life of SCh under the above-mentioned conditions is thus 30 minutes, and so $k_1 = 1.4$. The determination of this constant is admittedly not particularly accurate, but the error should not exceed 15 %. The value for k_1 indicates that the concentration of SCh may be neglected after 4 hours (see Fig. 3); and k_2 can be directly calculated from the extinction curve (Fig. 1) after that time. From the graph it is seen, that at pH 7.3, k_2 is 0.16

The vessels in which the reaction took place were fitted with reflux condensers which had been in use immediately beforehand. For this reason, the error due to "hold up" could be neglected. The vessels were placed in a water thermostat.

Results

a) and b) Results are given in Fig. 1 and Fig. 2.

c) Estimations of the concentrations of ester linkages in 0.00266 *M*, 0.0266 *M* and 0.0399 *M* solutions of SCh, in a buffer with pH 7.3, after 2, 4 and 6

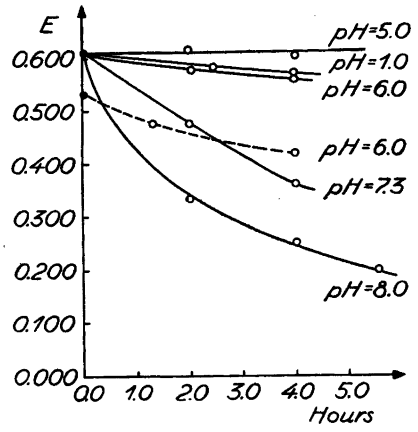

Fig. 2. Photometric determination of the rates of hydrolysis in solutions of succinylcholine (0.00266 M) and of phenylsuccinylcholine at 75° C. Continuous lines are for the hydrolysis of succinylcholine at the various pH values shown in the diagram. The dashed line is for the hydrolysis of phenylsuccinylcholine.

hours at 75° C show that the rate of breakdown is independent of the concentration.

d) The extinction of a 2.66×10^{-3} M solution of SCh in the buffer with pH 7.3 is reduced from 0.610 to 0.477 in 42 hours by the addition of cobra venom. A blank test without cobra venom reveals no such changes. The hydrolysis of SCh is therefore catalysed by the esterase in cobra venom.

By substitution of the value 0.0026 for a, and also the values for k_1 and k_2 in the kinetic equations, the curves in Fig. 3 are obtained.

Succinylcholine and Cholinesterases

An important clinical advantage possessed by SCh is its relatively rapid inactivation in the body, due mainly to its susceptibility to enzymatic breakdown and loss of curarising power. Glick [17] and Bovet-Nitti [18] found that SCh is broken down by bovine plasma at about one twentieth the speed with which acetylcholine is broken down by the same concentration of enzyme. The breakdown of SCh is prevented by cholinesterase inhibitors, such as prostigmine and eserine (shown by Bovet-Nitti [18]) and tetra-ethyl pyrophosphate (demonstrated by Löw and Tammelin [8]). The low rate of hydrolysis means that the results from all of the determinations described above must necessarily be approximate. For this reason it was considered to be impossible to identify with the aid of inhibition experiments the actual enzymes responsible. This held true, whether the Warburg or electrometric method (described by Tammelin and Löw [19] and Tammelin and Strindberg [20]) was used. The latter method was chosen for the investigations to be described here.

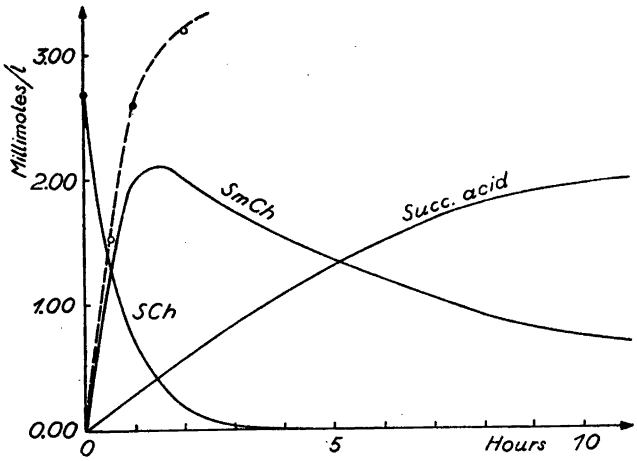

Fig. 3. Curves calculated from the rate constants determined for the hydrolysis of succinylcholine and succinylmonocholine. The continuous lines are for the breakdown of succinylcholine at pH 7.3 and 75° C. The dashed line gives the rate of liberation of equivalents of acid.

Apparatus.

The electrometric method makes use of the fact that acid formation due to the hydrolysis of the ester leads to a measurable change of pH in the buffer solution. Each of the six pairs of electrodes is connected successively to the pH-meter by means of an electrically driven switch of the drive shaft type. The pH is registered over a period of about 7 minutes and is represented as a short line for each of the six solutions. After about twenty minutes, three short lines are obtained from each solution, and when joined together give six lines, the slopes of which represent the rates of hydrolysis in the six solutions. The switch placed between the glass electrodes and the pH-meter must be well shielded, and the whole switch mechanism is therefore housed in an earthed copper chassis. Because of the high internal resistance between the glass and calomel electrodes the insulation between the incoming and outgoing leads, on the one hand, and the earth, on the other, has to be particularly effective. The desired result is achieved by ensuring that all leads entering the copper plate are at least 2 cm from the nearest metal by being sheathed in plexiglass. The rotating axle is made of glass and the cam wheel is of plexiglass and has a radius of 2 cm.

Cholinesterase-Inhibiting Effect of Succinylcholine and Phenylsuccinylcholine

By making use of Augustinsson's [21] graph (see Fig. 4), it is possible to determine the quantity of SCh which reduces the activity of the cholinesterases by half (I_{50}). Experiments have been made using plasma and erythrocyte haemolysate. Moreover, the effect of phenylsuccinylcholine as an inhibitor of the esterases in erythrocytes has been studied.

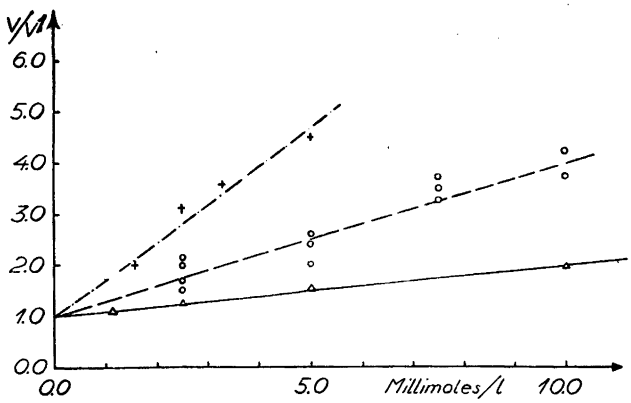

Fig. 4. Inhibition of cholinesterase as a function of the succinyl- and phenylsuccinylcholine concentration. v = reaction rate with uninhibited enzyme. v' = reaction rate with inhibited enzyme. The curve for the erythrocytes and phenylsuccinylcholine is represented by the continuous line; that for the erythrocytes and succinylcholine by the dashed line; and that for the plasma and succinylcholine by the dashed and dotted line.

The esterase was incubated for one hour in a solution consisting of 3 ml of Michel's buffer, plus 0.66 ml of erythrocyte haemolysate, diluted to thrice the volume of blood first used or plasma diluted to twice its volume plus 2.34 ml of a solution containing SCh. To this mixture was added 0.60 ml of an acetylcholine solution, prepared by dissolving 0.110 g of ACh iodide in 5 ml of water. The amount of acid formed was now determined at 25° C, with the help of the electrometric apparatus described above. As SCh and phenylsuccinylcholine gives rise to the formation of acid, a blank test was also done with SCh alone. The activities determined in this way were substracted from the results obtained in the inhibition experiments.

I_{50} is obtained from the diagram by finding the concentration which corresponds to $\frac{v}{v^1} = 2$. Thus the I_{50} value for SCh in plasma is 0.00135 and in erythrocytes 0.00335. I_{50} for phenylsuccinylcholine in erythrocytes is 0.01.

The enzymatic Breakdown of Succinylcholine

To ascertain if any particular organ plays a predominant role in the breakdown of SCh in the body, experiments were first of all performed on enzyme extracts made from various organs of the rabbit, as well as from the electric organ of the electric ray *Torpedo*.

The organs were extracted by grinding with kieselguhr in a mortar containing solution (A) to obtain a suspension as fine and homogeneous as possible. Solution (A) was prepared by adding 6 ml of Michel's buffer to 7.2 ml of water. One part by weight of the

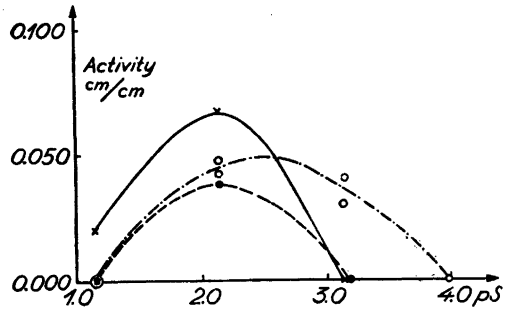

Fig. 5. Enzyme activity as a function of the negative logarithm of succinylcholine concentration during breakdown of succinylcholine. Dashed line for erythrocyte haemolysate. Dashed and dotted line for plasma. Continuous line for cobra venom.

organ was extracted with 5 parts by weight of solution A. The electrometric method was used in the investigation. The reaction vessel contained 3 ml of solution A, 3 ml of the enzyme extract, and 0.6 ml of a solution containing 0.219 g of SCh iodide per 5 ml of water. For purposes of comparison, the power of the enzyme extract to break down acetylcholine in an equimolar solution was determined, and 0.6 ml of a solution containing 0.110 g ACh iodide in 5 ml of water was used to assess this activity.

The results of these determinations are given in tabular form below.

Table 1.

Species Rabbit	Activities represented as	△ pH/min.
	SCh	ACh
Whole blood	0.0012	0.013
Liver	0.0012	0.015
Tibialis Anterior	0.0012	0.008
Heart	0.0030	0.008
Elect. Org. *(Torpedo)*	0.0030	0.019

The values given in the table for SCh are so low that the degree of accuracy of the method permits only a qualitative interpretation of results. It is clear, however, that SCh is broken down considerably more slowly than acetylcholine. Since the breakdown figures from the various organs differ only slightly, it would appear that the decomposition occurs at approximately the same rate in blood, liver and voluntary and smooth muscle.

The hydrolysis of SCh has also been studied by mixing it with the haemolysate from human erythrocytes, human plasma and cobra venom. In order to

ascertain if some optimal substrate concentration could be found, the quantity of the latter used was varied.

The reaction mixture in these experiments contained the following: 3 ml of Michel's buffer, 2.94 ml of the solution of SCh iodide, and 0.66 ml of undiluted plasma or erythrocyte haemolysate. In experiments with cobra venom the latter was dissolved in Michel's buffer (0.33 mg/ml). The composition of the mixture was 3 ml of Michel's buffer containing cobra venom, 2.94 ml of SCh solution and 0.66 ml of redistilled water.

In Fig. 5 the activity has been plotted against the negative logarithm of the substrate concentration. The curves obtained in this way link low, and, consequently only approximate values, but they do show that the optimal values are obtained at about the same pS as those found in the corresponding acetylcholine experiments (Tammelin and Strindberg [20]).

Discussion

The picture of SCh hydrolysis given by Fig. 3 shows that it is not at all certain that the values of the enzyme activities obtained by means of the Warburg or electrometric methods give a direct measure of the quantity of SCh broken down per unit time. The amount of carbon dioxide formed, and the change in the pH both depend on the number of equivalents of acid set free. For monoesters, such as acetylcholine, the number of acid equivalents parallels the amount of ester hydrolysed but with the diester the relationship is complicated by the formation of the dibasic acid. In this case the acid equivalents form more rapidly than the diester breaks down by spontaneous hydrolysis. See Fig. 3. By comparing the activities determined at the onset of hydrolysis for different enzyme preparations the error due to this may at times be neglected, and the figures obtained for the activities can always be accepted as a measure of the number of ester linkages hydrolysed.

As no particular organ has turned out to have a marked effect on the hydrolysis of SCh, it seems reasonable to suppose that the cholinesterases found in the blood and the neuromuscular end-plate, where the action of the drug is realised, are chiefly responsible for its inactivation. The rapid breakdown at the end-plates must therefore be of considerable significance. Experiments with enzyme extracts of the electric organ, which can be regarded as a huge end-plate, suggest that the cholinesterases in end-plates should strongly effect the rate of SCh hydrolysis.

The I_{50} determinations indicate that cholinesterase is not inhibited by SCh to an extent that can be of any importance for its physiological actions. However, the determinations indicate that cholinesterase has about the same affinity for SCh as for acetylcholine. Moreover, the enzymatic hydrolysis of SCh proceeds at a rate, which is 5—40 %

of the rate for acetylcholine. Both these observations prove that combination of cholinesterase with SCh must take place in the body. The complex once formed exists for a longer time than the corresponding acetylcholine complex. The physiological significance of such a complex is admittedly uncertain, but it is more than likely that the SCh-esterase complex formed in the blood must affect significantly the distribution of SCh in the body. It is furthermore, by no means inconcievable that the formation of such a complex may lead to inactivation of the curarising properties of SCh, if the affinity of the latter for the enzyme overshadows its tendency to accumulate at the end-plates, causing neuromuscular block and consequent paralysis.

SUMMARY

The synthesis of phenylsuccinylcholine has been described.

The hydrolysis of succinylcholine (Celocurin) and phenylsuccinylcholine have been followed using Hestrin's method for photometric determinations. The effect of succinylcholine concentration as well as pH and temperature on the stability of the ester linkages have been studied by this method. It has also been shown that the hydrolysis of succinylcholine is catalysed by cobra venom.

An electrometric apparatus for the determination of the esterase activity was modified to allow six readings in rapid succession.

The cholinesterase-inhibiting actions of succinylcholine and phenylsuccinylcholine have been determined and evaluated as I_{50}.

The hydrolysis of succinylcholine by enzyme preparations from different organs has been studied.

The enzymatic breakdown of succinylcholine has been studied as a function of the substrate concentration.

The relationship between the amount of succinylcholine hydrolysed and the number of equivalents of acid set free in this reaction, and the role played by the cholinesterases in changing the clinical picture after the injection of succinylcholine is discussed.

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