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Note

Thin-layer chromatographic separation of ^{14}C-labelled succinyldicholine, succinylmonocholine and choline

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The application of the muscle relaxant succinyldicholine (suxamethonium) in anaesthesiology is widespread. In the human body, this compound is mainly hydrolyzed by serum cholinesterase (EC 3.1.1.8), and individuals with a genetically determined atypical form of this enzyme suffer from prolonged apnoea if they are given succinyldicholine during surgical operations[1–6]. The usual diagnostic test for this condition is based on the resistance of the variant (atypical) enzyme to inhibition with dibucaine[7]. While the "normal" enzyme is inhibited by more than 70%, heterozygotes and homozygotes with the "atypical" allele show inhibitions of 40–65% and less than 20%, respectively. Although most of the atypical homozygotes are predisposed to prolonged apnoea after treatment with succinyldicholine, not all of the apnoea cases encountered in clinical practice always show the variant nature of the serum enzyme. Nearly 30% of patients with prolonged apnoea episodes could not be classified as possessing known variants on the basis of dibucaine inhibition[8–10]. Since the substrate used in this test is usually benzoylcholine, no information is available on the ability of these sera to hydrolyze succinyldicholine and on the inhibition with dibucaine in the presence of this substrate.

We recently developed an assay of succinyldicholine hydrolysis[11] which employs a modified spectrophotometric method. Of 21 cases of prolonged apnoea which had normal enzyme activity for benzoylcholine and a normal inhibition with dibucaine, six sera had no detectable activity when tested with succinyldicholine as substrate[12]. Nine samples showed increased resistance to dibucaine inhibition in the presence of succinylcholine. In view of the possible implications of these results, it is necessary to develop a more sensitive assay in which even trace amounts of succinyldicholine hydrolysis could be detected with a relatively high accuracy. The use of labelled succinyldicholine allows the measurement[13] of its hydrolysis at concentrations of less than 10^{-6} *M*. However, the high-voltage paper electrophoresis used for the separation of the hydrolytic products is difficult for routine analysis, and the subsequent quantitation of the degradation products of succinyldicholine by means of a scintillation counter is very time consuming. Other methods[14,15] using filter-paper chromatography were not sufficiently sensitive for our purpose.

In this paper we report a simple method based on the thin-layer chromatographic (TLC) separation of ^{14}C-labelled succinyldicholine, succinylmonocholine and choline. The use of a suitable TLC scanner with an integrator allows a rapid quantitation of the degradation products and substrate.

EXPERIMENTAL

The ^{14}C-labelled succinyldicholine (succinyldi[methyl-^{14}C]choline iodide, specific activity 7.4 mCi/mmole) and choline ([methyl-^{14}C]choline chloride, specific activity 40 mCi/mmole) were purchased from Amersham Buchler, Braunschweig, G.F.R. Succinylmono[methyl-^{14}C]choline iodide (specific activity, 1 mCi/mmole) was supplied by Farbwerke Hoechst, Frankfurt/M., G.F.R. Thin-layer plates (20 × 20 cm) coated with a 0.01-cm layer of cellulose, and the various solvents used, were obtained from E. Merck, Darmstadt, G.F.R.

5 μl of an aqueous solution of each labelled compound (10^{-7} M) and a mixture of solutions of all three compounds were applied as small spots on the plate. Cold air was used to dry the spots. Several solvent systems were examined and the following systems gave a satisfactory separation of all of the compounds: (1) *n*-propanol–benzyl alcohol–water (5:2:2; development time, 6 h); (2) isopropanol–benzyl alcohol–water (5:2:2; development time, 5 h); (3) isopropanol–methanol–water (5:5:2; development time, 3–4 h) and (4) isopropanol–methanol–water (5:10:2; development time, 2–3 h). The solvent system 4 was the most suitable as it gave optimal separation with the shortest development time. Increasing further the concentration of methanol or water led to poor separation due to tailing. The chromatographs were run at room temperature in the ascending manner in a rectangular glass tank filled with 200 ml of the solvent mixture.

For the assay of serum cholinesterase activity with succinyldicholine as substrate (unpublished results), 10 μl of serum were incubated with 10^{-6} M ^{14}C-labelled succinyldicholine in 0.066 M phosphate buffer (pH 7.4; final volume, 100 μl) for 15 min at 37°. The reaction was stopped by adding 20 μl of 20% sulphosalicylic acid. After centrifugation, 5 μl of the supernatant was applied to cellulose thin-layer plates and chromatographed with the solvent system 4. The labelled spots were scanned with an automatic radio thin-layer scanner (Dünnschicht-Scanner II, LB 2722) with a built-in integrator (Dual Ratemeter Integrator, LB 242 K) supplied by Laboratorium Prof. Dr. Berthold, Wildbad, G.F.R.

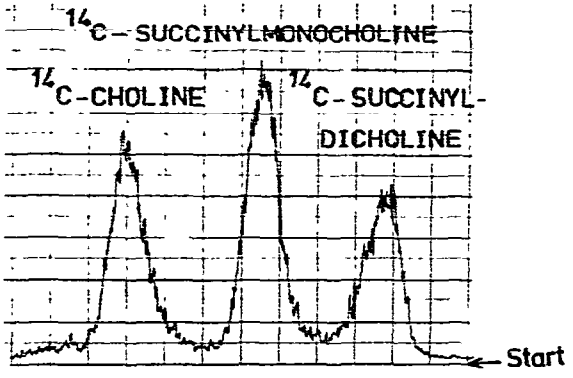

Fig. 1. Scan of thin-layer chromatogram of ^{14}C-labelled succinyldicholine, succinylmonocholine and choline. Solvent system, isopropanol–methanol–water (5:10:2). Running time, 2.5 h, at room temperature. Scanning time, 1 h in a Berthold LB 2722 scanner.

RESULTS AND DISCUSSION

The separation of labelled succinyldicholine, succinylmonocholine and choline is shown in Fig. 1, and the corresponding R_F values were 0.14, 0.31 and 0.54, respectively. The spontaneous hydrolysis of succinyldicholine was at a minimum in the neutral solvents used here. The separation time is much shorter compared to filter-paper chromatographic methods[14,15]. The use of a suitable integrator enables simultaneous determination of the degree of hydrolysis of succinyldicholine in enzyme assays.

We are now using this method to study the serum cholinesterase activity with succinyldicholine as the substrate in sera of various apnoea patients and members of their families; the results will be published elsewhere.

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REFERENCES

1 W. Kalow, *Pharmacogenetics*, Saunders, Philadelphia, Pa., 1962, p. 69.
2 H. Harris and M. Whittaker, *Nature (London)*, 191 (1961) 496.
3 H. W. Goedde, A. Doenicke and K. Altland, *Pseudocholinesterasen*, Springer, Berlin, Heidelberg, New York, 1967, p. 1.
4 H. W. Goedde, K. Altland and W. Schloot, *Ann. N.Y. Acad. Sci.*, 151 (1968) 742.
5 H. W. Goedde, K. Altland and W. Schloot, *Int. Titisee Workshop on Pharmacogenetics, Humangenetik*, 9 (1970) 197.
6 H. W. Goedde and K. Altland, *Ann. N.Y. Acad. Sci.*, 179 (1971) 695.
7 W. Kalow and K. Genest, *Can. J. Biochem. Physiol.*, 35 (1957) 339.
8 W. Kalow, *Anaesthesist*, 15 (1966) 13.
9 J. C. Thompson and M. Whittaker, *Acta Genet., Basel*, 16 (1966) 209.
10 H. W. Goedde, H. Münsch and K. L. Scholler, *Anaesthesist*, (1976), in press.
11 D. P. Agarwal, S. Schwenkenbecher, L. M. Srivastava and H. W. Goedde, *Z. Klin. Chem. Klin. Biochem.*, 13 (1975) 133.
12 D. P. Agarwal, L. M. Srivastava and H. W. Goedde, *Hum. Genet.*, 32 (1976), 85
13 H. W. Goedde, K. R. Held and K. Altland, *Mol. Pharmacol.*, 4 (1968) 274.
14 V. P. Whittaker and S. Wijesundera, *Biochem. J.*, 51 (1952) 348.
15 R. O. Davis and W. Kalow, *Can. J. Biochem. Physiol.*, 41 (1963) 1991.