LOCAL ANÆSTHETICS AS INHIBITORS OF CITRATE SYNTHESIS

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FOLLOWING the observation[1,2] that the inhibitory action of cocaine on cellular respiration could be correlated with the ability of the drug to block the entry of active acetate into the Krebs' citric acid cycle, it became of interest to discover whether other local anæsthetics exhibited a similar action. In the present work we have extended our studies with cocaine to include other local anæsthetics.

EXPERIMENTAL AND RESULTS

Effects of local anæsthetics on citrate synthesis in yeast

Citric acid accumulates in a suspension of starved bakers' yeast when aerated at 30° C. with acetate as substrate in the presence of a sufficiently high concentration of magnesium[3]. The magnesium retards the further metabolism of citrate, with which it forms a complex[4], thereby disturbing the natural equilibrium catalysed by aconitase[5].

The effects of the local anæsthetics on the rate of citrate synthesis by the yeast in the presence of 0·2 M magnesium acetate were measured by the procedure as previously described[1,2]. Citrate was determined by the method of Natelson, Pincus and Lugovoy[6]. Measurements were made at 445 mμ using 1 cm. cuvettes in a Unicam Quartz Spectrophotometer. The drugs did not interfere with the method, provided that, during the oxidative bromination of citrate to pentabromoacetone, a sufficient excess of bromine was present to allow for that taken up by the anæsthetic. Results of some typical experiments are given in Table I.

TABLE I

EFFECTS OF LOCAL ANÆSTHETICS ON CITRATE SYNTHESIS IN BAKERS' YEAST

Experiment	Anæsthetic used	Citrate, μg./ml. yeast suspension/hour		Inhibition, per cent.
		Control	Anæsthetic (0·001 M)	
1	Cocaine Procaine	123	62 57	50 53
2	Lignocaine Amethocaine	130	60 13	54 82
3	Cinchocaine Lignocaine	157	4 85	97 46
4*	Procaine Lignocaine	120	62 115	48 < 5
5	Cinchocaine Amethocaine	130	10 26	92 80

*With some samples of yeast, lignocaine had no appreciable effect on citrate synthesis but typical inhibitions were obtained with the other local anæsthetics.

Effects of local anæsthetics on citrate synthesis in brain

The effects of local anæsthetics on the synthesis of citrate from pyruvate and oxaloacetate by rat-brain have been determined in the presence of barium chloride which inhibits the metabolism of citrate[7]. In a preliminary experiment, to which reference was made in a previous paper[2], cocaine was found to inhibit citrate synthesis in this system. Further investigation has revealed that the inhibitory action of cocaine is depressed by an increase in the concentration of oxaloacetate. In the presence of 0·01 M oxaloacetate, even cinchocaine (0·001 M) did not inhibit. In the present work, in order to compare the effects of other local anæsthetics with that of cocaine, conditions have been standardised by having the substrates and the drug present in equal concentration (0·001 M).

Mixtures (2·5 ml.), with and without local anæsthetics, containing rat-brain homogenate (≡ 100 mg. tissue) and barium chloride (to give 0·033 M final concentration) in Krebs' phosphate-saline, were shaken in the Warburg apparatus at 37° C. and *p*H 7·4. The substrate solutions (0·5 ml.) containing pyruvate and oxaloacetate, buffered to *p*H 7·4, were added from the side-arms after a preliminary incubation of 15 minutes. After 1 hour, 30 per cent. w/v trichloroacetic acid (1·5 ml.) was added to the contents of each flask and the citrate determined after heating to destroy citrate-simulating substances as recommended by Krebs and Eggleston[8]. The values obtained were corrected for the amount of citrate present at zero time.

The results of a typical experiment are given in Table II.

TABLE II

EFFECTS OF LOCAL ANÆSTHETICS ON CITRATE SYNTHESIS IN BRAIN

Each vessel contained rat-brain homogenate (100 mg. tissue), pyruvate (0·001 M), oxaloacetate (0·001 M) and barium chloride (0·033 M) in Krebs' phosphate-saline (3·0 ml.) at 37° C. and *p*H 7·4.

Anæsthetic (0·001 M)	Citrate, μg./100 mg. tissue/hour	Inhibition, per cent.
None	74	—
Cinchocaine	23	69
Amethocaine	34	54
Procaine	42	43
Lignocaine	46	38
Cocaine	48	35

Effect of local anæsthetics on sulphanilamide acetylation

Since the biosynthesis of citrate, either from acetate or pyruvate, involves the intermediate formation of active acetate, it is desirable to know whether the local anæsthetics interfere with this stage of the reaction. It has previously been shown that cocaine is not a general inhibitor of active acetate utilisation[2]. In the present work we have investigated the effects of other local anæsthetics on active acetate formation and utilisation, using the sulphanilamide acetylating system of liver as the test system.

Pigeon-liver extracts were prepared from acetone-dried powder as described by Johnson and Quastel[9]. Mixtures (3·0 ml.), with and

without local anæsthetics, containing liver extract (1·0 ml. ≡ 60 mg. acetone-dried powder), sulphanilamide (100 μg.) and, in final concentrations, potassium phosphate buffer (0·02 M), sodium bicarbonate (0·028 M), magnesium chloride (0·005 M), sodium acetate (0·02 M), adenosine triphosphate (0·003 M), diphosphopyridine nucleotide (0·005 M) were gassed with a mixture of CO_2 (7 vol.) and N_2 (93 vol.) and shaken in the Warburg apparatus at 37° C. and *p*H 7·5. The sulphanilamide present at zero time and after 1·5 hours was determined by the method of Bratton and Marshall[10] and the acetylated sulphanilamide calculated by difference.

Results of typical experiments are given in Table III.

TABLE III

EFFECTS OF LOCAL ANÆSTHETICS ON ACETYLATION OF SULPHANILAMIDE IN PIGEON-LIVER EXTRACTS

Experiment	Anæsthetic used	Sulphanilamide acetylated, μg.	
		Control	Anæsthetic (0·002 M)
1	Cinchocaine Amethocaine	69	74 71
2	Lignocaine Procaine	73	73 15
3	Lignocaine Procaine	66	71 10
4	Cinchocaine Amethocaine	19	22 18

DISCUSSION

It has long been known that local anæsthetics depress cellular respiration and there have been several attempts to locate the site of the inhibitory action. Watts[11], who studied the oxidation of glucose, succinate and ascorbate by brain homogenates, came to the conclusion that the site of inhibition lay in the cytochrome system. Our experiments, in which we used lower concentrations of the drugs, suggest that there lies in the metabolic pathway of carbohydrate oxidation a site more sensitive than that of the general electron-transferring mechanism of the cytochromes.

The results of the present work show that typical, synthetic, local anæsthetics share with cocaine the property of inhibiting citrate synthesis, and that the more potent ones, like cinchocaine, have the stronger inhibitory action. These findings are of interest in providing further evidence that drugs which possess similar pharmacological activity behave similarly in their selective actions on the enzyme systems of living cells.

Whether the ability to depress cellular respiration is a property essential to local anæsthetic action is still an open question. Sherif[12], in experiments with cocaine, procaine and isolated nerve, obtained evidence that inhibition of oxidation and failure of conduction did not necessarily run parallel. Larrabee, Ramos and Bülbring[13] showed that local anæsthetics depress synaptic transmission without slowing the resting oxygen consumption. However, as McElroy[14] has pointed out, there may be two distinct systems, an "activity" and a "basal" system, and it is possible

that the local anæsthetics affect the former more strongly. It is known that the inhibitory action of narcotics, for example, is much greater on the respiration of stimulated nerve-tissue than it is on unstimulated tissue. Ghosh and Quastel[15] point out that the narcotics act on a specific phase of glucose or pyruvate oxidation which is potassium sensitive.

In our previous investigations[2] into the site of inhibitory action of cocaine, evidence was obtained that cocaine inhibited cellular respiration by blocking the entry of active acetate into the tricarboxylic acid cycle, and it was suggested that the condensing enzyme was primarily affected. The present evidence, that local anæsthetics behave like cocaine in inhibiting citrate synthesis while not interfering with sulphanilamide acetylation, supports this view and suggests that the inhibition may be competitive with regard to the substrate, oxaloacetate.

The inhibition of sulphanilamide acetylation by procaine calls for some comment. Of the drugs tested, procaine is the only one which, like sulphanilamide, has a free aromatic primary amino group. Further experiments (unpublished data) reveal that, in the absence of sulphanilamide, the procaine is itself acetylated and would therefore be expected to compete with sulphanilamide for available acetyl groups. The lack of inhibition of this system by cinchocaine, amethocaine and lignocaine implies that the local anæsthetics as a group behave like cocaine and do not exert their influence by interfering with the general utilisation of active acetate.

Johnson and Quastel[9] have suggested that narcotics, at low concentration, impede the oxidative synthesis of adenosine triphosphate. The possibility that local anæsthetics might act in this way deserves consideration. Preliminary investigations, however, using the enzyme systems employed by these workers, have yielded no evidence that adenosine triphosphate synthesis is directly affected.

Whether the local anæsthetics depress respiration by a direct, competitive inhibition of the condensing enzyme, or whether this action is secondary to some other effect and possibly associated with potassium concentration or membrane permeability remains a matter for further investigation.

Summary

Evidence is presented that typical, synthetic, local anæsthetics behave like cocaine in that they inhibit the synthesis of citrate, but do not affect the acetylation of sulphanilamide. The possibility that the condensing enzyme is competitively inhibited is discussed.

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