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THE ENZYMIC HYDROLYSIS OF COCAINE AND *ALPHA*-COCAINE

BY

H. BLASCHKO, JEAN M. HIMMS AND B. C. R. STRÖMBLAD
From the Department of Pharmacology, University of Oxford

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Some time ago Glick and Glaubach (1941) reported that cocaine is hydrolysed by rabbit serum; other mammalian species do not hydrolyse cocaine. In earlier work from this laboratory (Blaschko, Chou and Wajda, 1946) it was confirmed that cocaine is hydrolysed also in the blood of rabbits which do not contain the tropinesterase described by Glick and Glaubach.

α-Cocaine has recently become available (Foster, Ing, and Varagić, 1955), and we have taken the opportunity of examining α-cocaine as a substrate of mammalian esterases. A few experiments on the hydrolysis of benzamine (β-eucaine) are also reported.

METHODS

Human plasma was obtained by centrifugation of a sample of citrated blood stored for blood transfusions. Horse serum was a sample that came from the Lister Institute; ox and sheep serum was from blood obtained from the slaughterhouse. Most of the experiments with rabbit serum were carried out with the pooled sera of a number of tropinesterase-negative animals.

In the manometric experiments the total reaction volume in each manometer flask was 3.0 ml. The main compartment of the flask contained the enzyme sample and any inhibitors that were used; the volume was brought up to 2.7 ml. with Krebs bicarbonate-Ringer. The side bulb contained the substrate, in a volume of 0.3 ml. The temperature of the thermostat was 37.5° C., and the gas phase was a mixture of 95% N_2 and 5% CO_2. In most experiments a "substrate blank" was included, in order to correct for the slow, spontaneous hydrolysis of the substrates.

RESULTS

Hydrolysis of α-Cocaine in Different Species.—It was easy to demonstrate that α-cocaine was hydrolysed by rabbit serum. This hydrolysis, like the hydrolysis of cocaine, was seen with sera of both tropinesterase-positive and tropinesterase-negative animals. The rate of hydrolysis of α-cocaine exceeded that of cocaine, but differed a little in different experiments. We have not determined the saturation concentration of the rabbit esterase for α-cocaine, but there was no difference in the initial rates with a concentration of 10^{-3}M- and 10^{-2}M-α-cocaine; later the rates of hydrolysis fell off, and this fall was more marked with the lower concentration.

Horse serum hydrolysed α-cocaine, whereas cocaine was not attacked. The rate of hydrolysis with horse serum was of the same order of magnitude as with rabbit serum, and there was a similar slow falling off of the rate in an experiment with an initial α-cocaine concentration of 5×10^{-3}M.

Human plasma also hydrolysed α-cocaine, but it was without action on cocaine. Here, too, there was a slow decrease in the rate of hydrolysis with time.

With ox serum, there was only a very small increase in the rate of hydrolysis over that in the manometer flask which served as "substrate blank."

The Action of Eserine.—The hydrolysis of α-cocaine by horse serum and by human plasma was inhibited by eserine. This is seen in Fig. 1, which shows the rates of liberation of CO_2 with different eserine concentrations: 1a is from an experiment with horse serum; 1b from one with human plasma. It can be seen that with higher concentrations of eserine the inhibition of α-cocaine hydrolysis by human plasma was almost complete. Almost 20% of the activity of horse serum was left with 10^{-4}M-eserine; with 10^{-5}M- and 10^{-6}M-eserine the rates of hydrolysis were practically identical.

The Action of Nu 683.—This substance, the dimethylcarbamate of (2-hydroxy-5-phenylbenzyl)-trimethylammonium bromide, was first used by Hawkins and Gunter (1946) as a specific inhibitor of pseudo-cholinesterase. We have found that Nu 683 is also an inhibitor of the enzymic hydrolysis of α-cocaine by horse serum and by human plasma. This is illustrated by experiments with

5×10^{-3}M-α-cocaine as substrate, in which different concentrations of Nu 683 were used. The percentage inhibitions in the first 12 min. were :

Concentration of Nu 683	With horse serum	With human plasma
10^{-4}M	85%	100%
10^{-6}M	81 ,,	92 ,,
10^{-8}M	44 ,,	34 ,,

In another similar experiment with horse serum, the percentage inhibitions were:

with 10^{-4}M-Nu 683 ..	79%
,, 10^{-5}M ,, ,, ..	78 ,,
,, 10^{-6}M ,, ,, ..	77 ,,
,, 10^{-7}M ,, ,, ..	76 ,,

These experiments show that, as with eserine, most of the enzymic activity was inhibited, but that with horse serum there remained an activity which was relatively resistant to Nu 683.

Nu 683 (10^{-6}M) did not inhibit the hydrolysis of α-cocaine by rabbit serum.

The hydrolysis of benzoylcholine, a typical substrate of pseudo-cholinesterase, was inhibited by Nu 683.

Mixed-substrate Experiments.—In an experiment with rabbit serum, in which both α-cocaine and cocaine were used as substrates, the rate of hydrolysis with the two compounds present simultaneously was intermediate between the rate with either cocaine or α-cocaine alone. In the first 15 min. the amounts of CO_2 liberated were:

With cocaine 2×10^{-3}M:	16 μl. CO_2
,, α-cocaine 2×10^{-3}M:	39 ,, ,,
,, both substrates:	27 ,, ,,

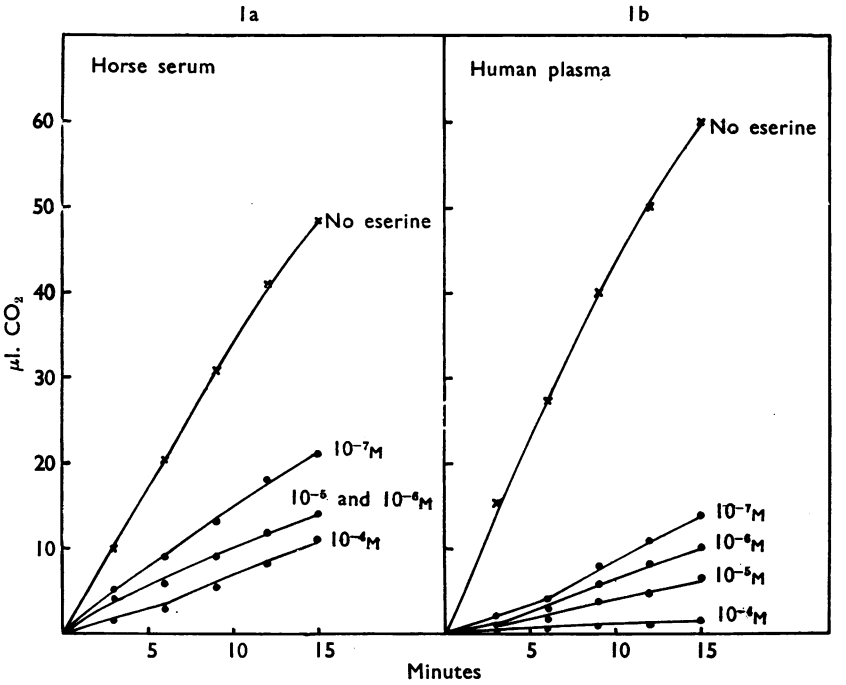

FIG. 1.—Inhibition of enzymic hydrolysis of α-cocaine by eserine. 1a: horse serum. 1b: human plasma. Abscissa, time in min. Ordinate, μl. CO_2 consumed. Initial concentration of α-cocaine: 0·005M. The eserine concentrations used are shown on the graphs.

In other words, the rate of the reaction in the mixed-substrate experiment was competitive, not additive.

A mixed-substrate experiment was also performed with horse serum; benzoylcholine and α-cocaine were the two substrates. The CO_2 liberated in the first 15 min. was:

With α-cocaine 5×10^{-3}M:	12 μl. CO_2
,, benzoylcholine 6×10^{-3}M:	51 ,, ,,
,, both substrates:	24 ,, ,,

In this experiment 0.2 ml. horse serum was used in each manometer flask.

Experiments with Benzamine (4-Benzoyloxy-2:2:6-trimethyl-piperidine).—This compound was readily hydrolysed by both human plasma and horse serum; it was less rapidly hydrolysed by rabbit serum, and with ox and sheep serum there was no hydrolysis.

The hydrolysis of benzamine was inhibited by Nu 683; this is shown in Table I.

TABLE I

INHIBITION OF HYDROLYSIS OF BENZAMINE (β-EUCAINE) BY NU 683

The percentage inhibitions are based on the hydrolysis during the 3–12 min. interval after tipping.

Source of Enzyme	Concentration of Nu 683		
	10^{-4}M	10^{-6}M	10^{-8}M
Rabbit serum	53%	13%	16%
Human plasma	98 ,,	89 ,,	48 ,,
Horse serum	100 ,,	96 ,,	51 ,,

DISCUSSION

As a result of these experiments it seems likely that in man and horse α-cocaine is inactivated by enzymic hydrolysis, whereas cocaine is not hydrolysed in human and horse plasma. In the rabbit, no similar difference between cocaine and α-cocaine was seen; both compounds were hydrolysed, α-cocaine somewhat faster than cocaine.

It seems quite possible that the catalyst of the hydrolysis of α-cocaine in man and horse is pseudo-cholinesterase. The hydrolysis was not only inhibited by eserine, but also by the rather more specific inhibitor, Nu 683; moreover, an inhibition of the hydrolysis of α-cocaine is obtained with concentrations of Nu 683 similar to those which inhibit the hydrolysis of benzoylcholine (Blaschko, Bülbring and Chou, 1949). Also in favour of the identity of the α-cocaine esterase with pseudo-cholinesterase are: first, the slow rate of hydrolysis with ox serum, a preparation that has no pseudo-cholinesterase activity; and second, the competitive rates of hydrolysis of α-cocaine and benzoylcholine in the mixed-substrate experiments. There is the possibility that in horse serum a second enzyme is responsible for the small eserine- (and Nu 683-) resistant hydrolysis of α-cocaine. Little, if any, of this second enzyme was present in the sample of human plasma used in our experiments.

The catalyst of the hydrolysis of α-cocaine in rabbit serum must be different from pseudo-cholinesterase. Firstly, there was no inhibition of α-cocaine hydrolysis by Nu 683, whereas the slow hydrolysis of benzoylcholine was inhibited, and, secondly, the rate of hydrolysis of a mixture of cocaine and α-cocaine was intermediate between the rates with either substrate alone. This latter observation suggests the possibility that in the rabbit the two esters are hydrolysed by one and the same esterase.

It is interesting that the hydrolysis of the other ester tested, benzamine, has a distribution which is similar to the distribution of pseudo-cholinesterase: the compound was rapidly hydrolysed by human plasma and horse serum, two preparations known to be rich in this type of cholinesterase; it was slowly hydrolysed by rabbit serum which is poor in this enzyme, and ox and sheep sera did not act on benzamine; it is known that ruminants do not contain pseudo-cholinesterase.

Observations like those reported in this study are of interest, as the time course of action of these drugs will depend on whether or not they are inactivated by hydrolysis. It can be expected from our findings that the relative potencies will depend upon the species used for biological assays.

SUMMARY

1. The enzymic hydrolysis of α-cocaine in mammalian plasma has been studied.

2. Horse serum and human plasma hydrolysed α-cocaine rapidly; the hydrolysis in horse serum was partly, that in human serum completely, inhibited by inhibitors of pseudo-cholinesterase.

3. Both α-cocaine and cocaine were hydrolysed by rabbit serum; the evidence suggests that the same enzyme is catalysing these reactions and that it is distinct from pseudo-cholinesterase.

4. Benzamine (β-eucaine) was hydrolysed rapidly by human plasma and horse serum, less readily by rabbit serum; it was not hydrolysed by ox or sheep serum.

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