Brit. J. Pharmacol. (1955), **10**, 436.

ALPHA-COCAINE

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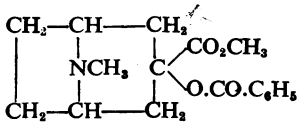
(RECEIVED JUNE 30, 1955)

Ever since Willstätter synthesized α-cocaine (I) in 1896, his statement that it did not possess local anaesthetic properties has been accepted, although his only evidence was that it did not have the numbing effect on the tongue characteristic of cocaine. α-Cocaine is a structural isomer of cocaine (II) and is closely related in structure to α-eucaine (III), one of the earliest synthetic local anaesthetics; consequently it is difficult to understand why Willstätter's statement was accepted



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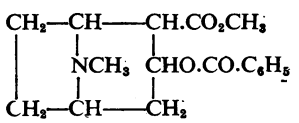

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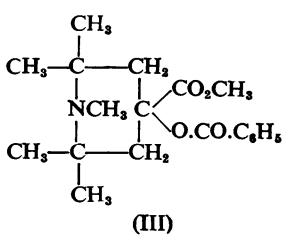

(III)

uncritically for so many years. Since tropinone, from which Willstätter synthesized α-cocaine, is now available commercially, we have repeated his synthesis and tested α-cocaine for local anaesthetic activity by other methods. α-Cocaine does, in fact, possess local anaesthetic properties, though inferior to those of cocaine. We have also investigated its ability to potentiate some of the pharmacological effects of adrenaline, as cocaine is well known to do; it was also compared with cocaine as an inhibitor of amine oxidase.

METHODS

Local anaesthetic activity was tested on the cornea of the rabbit by the method of Chance and Lobstein (1944) and also by the guinea-pig weal method and frog plexus anaesthesia method described by Bülbring and Wajda (1945). The effects of α-cocaine upon adrenaline responses were tested on the isolated uterus, the isolated auricles, and the isolated duodenum of the rabbit, on the perfused vessels of the rabbit ear, and on the blood pressure of the spinal cat. The effects of α-cocaine upon noradrenaline responses were tested on all but the first two of these preparations. α-Cocaine and cocaine were also compared as inhibitors of amine oxidase activity. This was measured by the manometric technique with the following solutions: 0.067M-phosphate buffer, *p*H 7.4; 0.01M-KCN; KOH/KCN (1 ml. N-KOH + 10 ml. N-KCN) (centre well).

The following preparations were made: cat liver was homogenized in 0.067M-phosphate buffer, *p*H 7.4, in a glass homogenizer and dialysed for 4 hours against running tap water. After dialysis one-fifth of its volume of 0.2M-phosphate buffer, *p*H 7.4, was added and the preparation stored frozen. Rabbit liver was prepared in the same way.

Rabbit uterus was finely chopped on a McIlwain chopper and suspended in 0.067M-phosphate buffer, *p*H 7.4.

Cat nictitating membrane was freed from fat, and the cartilage and Harderian gland were removed. The membrane was finely chopped and the whole mince was transferred to a manometer vessel. The oxygen uptake of R and L nictitating membranes of equal weight after dissection were compared in the presence of cocaine and α-cocaine respectively. No control observation without inhibitor was made owing to the difficulty of comparing nictitating membranes from different cats and to the difficulty of sampling pooled material.

RESULTS

Local Anaesthetic Activity. (a) Rabbit Cornea.—A comparison between the action of α-cocaine and that of cocaine was made by instilling solutions into the conjunctival sac of 13 rabbits. Cocaine was tested on one eye and α-cocaine was tested on the opposite eye. Cocaine hydrochloride was

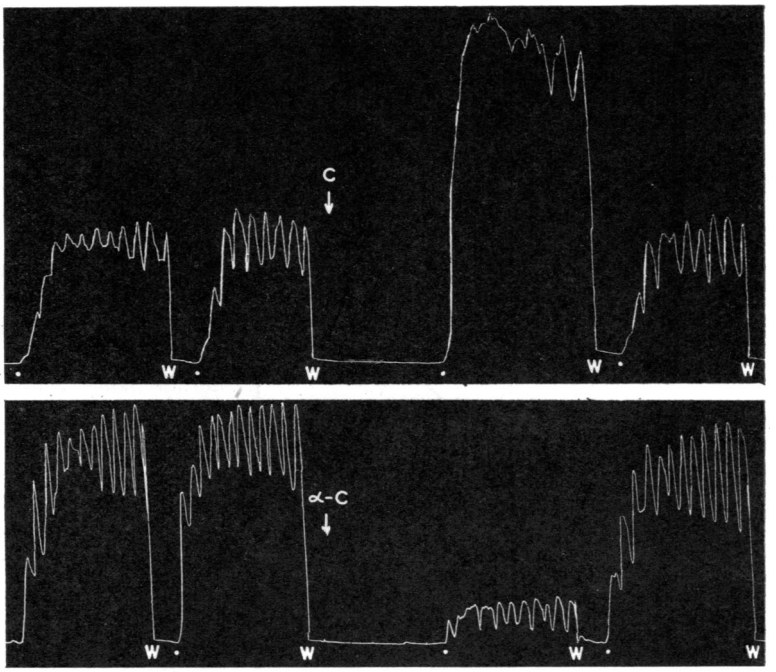

FIG. 1.—Contractions of isolated rabbit uterus in 10 ml. bath. Each white dot represents addition of 0·2 μg. adrenaline. W represents wash-out of bath. Arrow in upper tracing indicates addition of 0·2 mg. cocaine and that in the lower tracing addition of 0·2 mg. α-cocaine. Note that cocaine potentiated the effect of adrenaline, whereas the same dose of α-cocaine reduced the effect of adrenaline but did not abolish it entirely.

normally active in all the rabbits in solutions of strength 0.25–1.0% (w/v). In 3 rabbits α-cocaine was active in 1.0% solution, and in a fourth in 5% solution, but in 9 rabbits it was completely inactive, even in 5% solution.

(*b*) *Intradermal Injections in Guinea-pigs.*—In this method the injected area is tested at 5 min. intervals for 30 min., six pricks being made in each of the six tests. If there is no response to any prick the maximum score, representing full anaesthesia through the period, is 36. The results of the comparison between α-cocaine and cocaine are expressed in this way in Table I.

When the results of the two experiments were expressed graphically, it was found that 1 mg. α-cocaine was equivalent to 0.12 mg. cocaine in Expt. 1 and to 0.31 mg. cocaine in Expt. 2. The mean figure indicates that α-cocaine was approximately one-fifth as active as cocaine.

(*c*) *Plexus Anaesthesia in Frogs.*—In this method the response of the decerebrate frog to the application of dilute HCl to the skin of the hindlegs is determined and the time from the introduction of the anaesthetic solution into the abdominal cavity until the response fails is measured. The results are given in Table II, each figure for the time to failure of response being determined in 4 frogs.

TABLE I

COMPARISON OF α-COCAINE WITH COCAINE BY GUINEA-PIG WEAL TEST

Expt.	Substance	Concentration g./100 ml.	No. of Weals Tested	Degree of Anaesthesia Max. = 36
1	Cocaine	0·05	7	17
		0·1	8	24
		0·2	6	32
	α-Cocaine	0·1	2	3
		0·2	3	10
		0·35	8	13
		1·0	4	25
2	Cocaine	0·05	2	24
		0·1	3	35
	α-Cocaine	0·1	2	20
		0·3	3	30

When the results in Table II were expressed graphically, they showed that 0.6 mg. cocaine was equivalent to 1 mg. α-cocaine, so that α-cocaine was approximately three-fifths as active as cocaine.

TABLE II

COMPARISON OF α-COCAINE WITH COCAINE BY PLEXUS ANAESTHESIA TEST

Substance	Concentration g./100 ml.	Mean Time (min.) to Failure of Response (4 Frogs per Figure)
Cocaine ..	0·05	23
	0·1	18
	0·2	9
α-Cocaine ..	0·1	21
	0·2	14·5

Some observations were also made to see if the quaternized derivative, α-cocaine methiodide, had any local anaesthetic action. By the guinea-pig weal test α-cocaine methiodide was inactive up to 4 g./100 ml., and by the plexus anaesthesia test the activity was very slight and doubtful (up to 1 g./100 ml.).

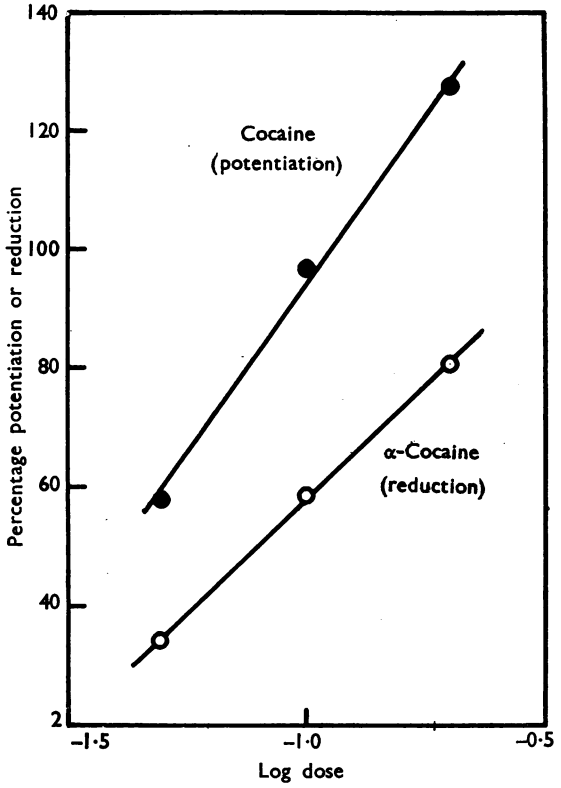

FIG. 2.—Graph of results obtained on the isolated rabbit uterus in a bath of 10 ml. ●——● shows percentage potentiation of effect of adrenaline in relation to log dose cocaine. ○——○ shows percentage reduction of effect of adrenaline in relation to log dose α-cocaine.

Isolated Rabbit Uterus.—Since cocaine is known to potentiate the action of adrenaline in stimulating the isolated rabbit uterus, observations were made to see if α-cocaine had the same action. It was found that α-cocaine had the opposite effect, depressing the action of adrenaline as shown in Fig. 1. Cocaine hydrochloride in concentrations from 5×10^{-3} to 5×10^{-1} mg./ml. potentiated the effects of adrenaline (5×10^{-6} to 4×10^{-5} mg./ml.) in five preparations, and of noradrenaline (6×10^{-5} to 2×10^{-4} mg./ml.). On the other hand, α-cocaine in concentrations from 5×10^{-3} to 8×10^{-1} mg./ml. reduced or abolished the effect of adrenaline in four preparations. In a fifth preparation showing much spontaneous activity, α-cocaine had no effect on the adrenaline response. In one experiment the potentiation produced by cocaine and the reduction of the adrenaline effect by α-cocaine were both in linear relation to the log dose as shown in Fig. 2.

Isolated Rabbit Duodenum.—In the isolated rabbit duodenum cocaine had little or no potentiating effect on the action of adrenaline, but the depression of the action of adrenaline by α-cocaine was still evident. This is illustrated in Fig. 3, and was the result obtained in four preparations. In two preparations cocaine potentiated the action of noradrenaline, whereas α-cocaine diminished it.

Isolated Rabbit Auricles.—When the auricles were suspended in Locke solution at 29° C., the presence of cocaine in a concentration of 2×10^{-3} mg./ml. potentiated the effect of adrenaline. α-Cocaine in the same or double the concentration occasionally potentiated the effect of adrenaline, but less so than cocaine; more frequently potentiation was absent or inhibition occurred. In seven observations potentiation occurred twice, reduction twice and no change thrice.

Spinal Cat: Blood Pressure and Nictitating Membrane.—Experiments were carried out on four spinal cats. As shown in Fig. 4, the effects of adrenaline and of noradrenaline were usually not modified by the intramuscular injection of an amount of α-cocaine as great as 40 mg., but they were always potentiated by the injection of 8 mg. cocaine. The effects on the nictitating membrane were similar as shown in Fig. 4. In one preparation the injection of 80 mg. α-cocaine potentiated

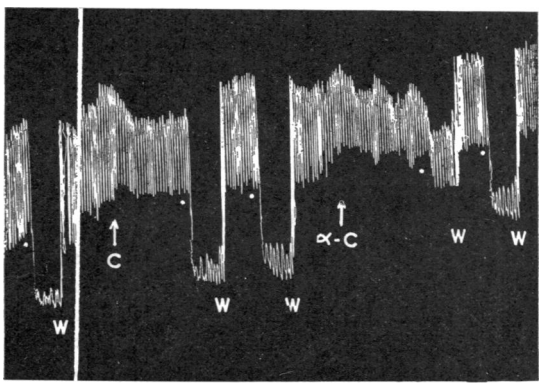

FIG. 3.—Rabbit duodenum in 10 ml. bath. Each white dot indicates the addition of 0·2 μg. adrenaline. W = washout of bath. At C, 0·3 mg. cocaine: at α-C, 0·3 mg. α-cocaine; only the latter depressed the adrenaline response.

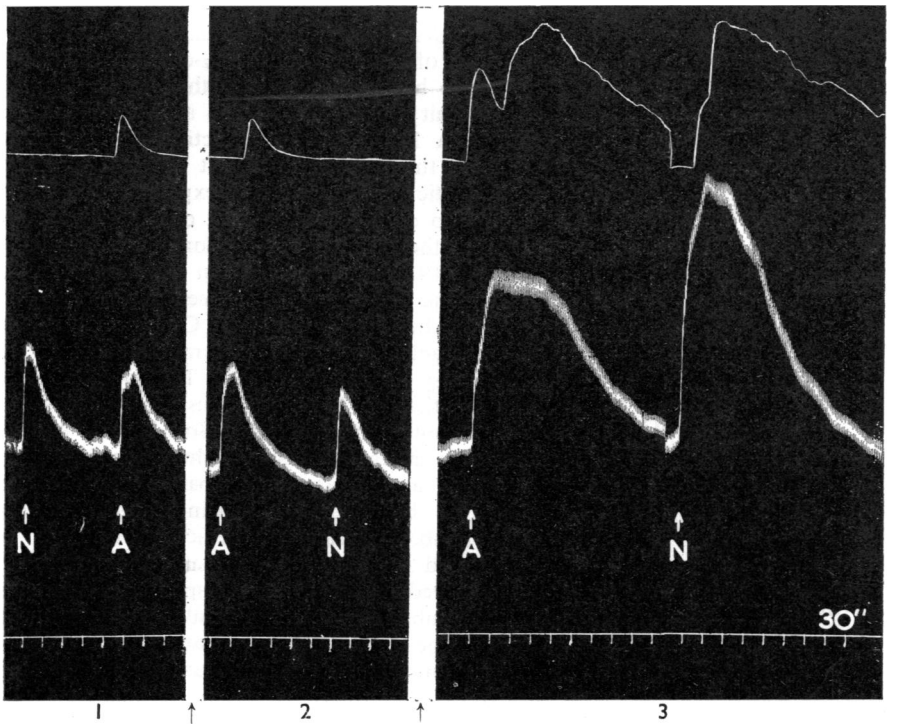

FIG. 4.—Spinal cat. Upper record: nictitating membrane; lower record: blood pressure. N=5 μg. noradrenaline; A=5 μg. adrenaline. Between 1 and 2, 40 mg. α-cocaine; between 2 and 3, 8 mg. cocaine intramuscularly. Note potentiation by cocaine but not by α-cocaine.

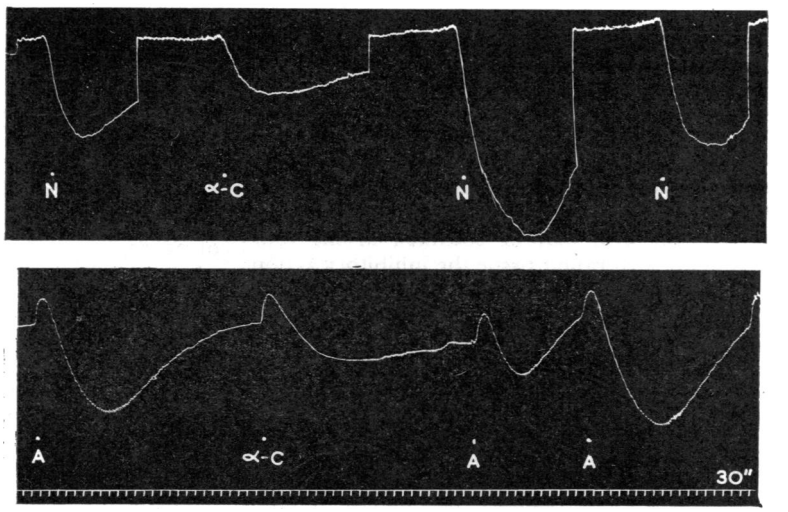

FIG. 5.—Record of outflow from perfused vessels of rabbit ear. Top: 0·001 μg. noradrenaline injected at N, and 0·2 mg. α-cocaine injected at α-C. Note potentiation of constrictor action of N. Bottom (taken from a different experiment): 0·001 μg. adrenaline injected at A, and 0·03 mg. α-cocaine at α-C. Note reduction of constrictor action of A.

the action of adrenaline and of noradrenaline, though much less so than a small dose (8 mg.) of cocaine.

Perfused Rabbit Ears.—Three experiments were carried out in which the isolated rabbit ear was perfused with Locke solution. In one of these the injection of 0.2 mg. α-cocaine potentiated the constrictor action of a subsequent dose of noradrenaline as shown in Fig. 5 (top). In a second experiment the injection of 0.03 mg. α-cocaine diminished the effect of a subsequent dose of adrenaline as shown in Fig. 5 (bottom). In the third experiment, α-cocaine again depressed the action of adrenaline whereas cocaine increased it.

Action of α-Cocaine on Amine Oxidase.—The results obtained for the inhibitory action of α-cocaine on the activity of amine oxidase are shown in Table III. There were seven experiments, three with cat liver and nictitating membrane, and four with rabbit liver and uterus. In

TABLE III

COMPARISON OF INHIBITORY ACTION OF COCAINE AND α-COCAINE ON AMINE OXIDASE

Expt.	Amine Oxidase Preparation	Tyramine Molarity	Cocaine Molarity	α-Cocaine Molarity	Inhibition %
1	Cat liver ..	0·005	0·01	—	40·0
	,, ..	0·005	—	0·01	38·0
2	,, ..	0·005	0·02	—	55·0
	,,	0·005	—	0·02	38·0
3	Cat nictitating membrane..	0·005	0·01	—	39·0
	,, ..	0·005	—	0·01	39·0
4	Rabbit liver	0·005	0·01	—	23 0
	,, ,,	0·005	—	0·01	32·0
5	,, ,,	0·005	0·02	—	44·5
	,, ,,	0·005	—	0·02	55·0
6	,, ,,	0·01	0·02	—	46·0
	,, ,,	0·01	—	0·02	54·0
7	Rabbit uterus	0·005	0·01	—	41·0
	,, ,,	0·005	—	0·01	49·0

The following solutions were used: enzyme, 1·0 ml.; tyramine, 0·05M, 0·2 and 0·4 ml.; cocaine and α-cocaine, 0·1M, 0·2 and 0.4 ml.; KCN, 0·01M, 0·2 ml.; KOH/KCN (centre well), 0·2 ml.; phosphate buffer, 0·067M, *p*H 7.4, to bring to final volume of 2·2 ml. The rate was measured as the extra oxygen uptake over that of the control during the period 0–20 min., except for Expt. 3.

the three experiments with cat tissue, the inhibitory action of α-cocaine on amine oxidase was practically identical with that of cocaine; in the four experiments with rabbit tissue the inhibitory action of α-cocaine was rather greater than that of cocaine.

DISCUSSION

α-Cocaine has been found to have a local anaesthetic action when injected into the skin of the guinea-pig, and also when allowed to act on the nerves entering and leaving the spinal cord of the frog in the lumbar region. The action in both tests was found to be weaker than that of cocaine. The results on the rabbit cornea were ambiguous; in nine of thirteen rabbits α-cocaine (5%) was found to have no local anaesthetic action, but in one rabbit a 5% solution was active and in three rabbits a 1% solution was active. These results indicate that α-cocaine is not a reliable surface anaesthetic and probably explain Willstätter's failure to observe anaesthesia of the tongue.

Since the original observation of Fröhlich and Loewi (1910) the potentiation of the action of adrenaline by cocaine has been repeatedly confirmed. In 1926 Tainter and Chang showed that cocaine acted differently on tyramine, reducing or abolishing its pressor effect. Tainter (1930) also showed that on the blood pressure of the spinal cat the potentiation of adrenaline became steadily less with doses of cocaine higher than 10–15 mg. per kg., and in the same paper recorded that psicaine, benzoylpseudotropeine and β-eucaine were unable to potentiate the action of adrenaline. He found further that these substances were unable to reduce the pressor response of tyramine. Hence substances closely similar in constitution to cocaine lacked its potentiating or depressant actions on sympathomimetic amines.

An effect of α-cocaine on the adrenaline response was found present in isolated organs; thus on the auricles of the rabbit potentiation, reduction and no alteration of the action of adrenaline were all observed. When potentiation occurred it was less than that produced by the same concentration of cocaine. On the isolated uterus of the rabbit α-cocaine diminished the action of adrenaline whereas cocaine increased it. This difference is not necessarily a difference in kind from the action of cocaine; Burn and Tainter (1931) observed that cocaine diminished the action of adrenaline in inhibiting the isolated uterus of the cat. On the small intestine of the rabbit α-cocaine depressed the action of adrenaline, cocaine having little or no effect. On the blood pressure of three spinal cats, α-cocaine did not modify the pressor action of either adrenaline or noradrenaline in amounts up to 40 mg., but in one cat the effects of adrenaline and noradrenaline were potentiated after the injection of 80 mg. In the vessels of the rabbit ear, small doses of α-cocaine (0.03 mg.) depressed the action of adrenaline in two experiments; in one experiment a large dose (0.2 mg.) potentiated the action of noradrenaline.

Since Philpot (1940) showed that cocaine exerted an inhibitory action on amine oxidase, tests were made with α-cocaine and cocaine in parallel. With tyramine as substrate, α-cocaine

was found to have the same inhibitory action as cocaine on amine oxidase from cat liver and nictitating membrane, and rather more inhibitory action than cocaine on amine oxidase from rabbit liver and rabbit uterus. The suggestion of Burn and Robinson (1952) that the potentiation of noradrenaline and of adrenaline by cocaine might be due to inhibition of amine oxidase seems therefore to be incorrect.

Summary

1. α-Cocaine was found to possess local anaesthetic activity when examined by intradermal injection into guinea-pigs or by application to the lumbar nerve plexus in frogs. By the former test it was one-fifth as active as cocaine, by the latter it was three-fifths as active. α-Cocaine was without appreciable action on the cornea of the rabbit eye.

2. In isolated preparations of the rabbit α-cocaine occasionally potentiated the action of adrenaline on the auricles, though it was less active than cocaine; more frequently reduction or no alteration of the adrenaline response was observed. It depressed the action of adrenaline on the uterus, intestine, and perfused-ear preparations.

3. In the spinal cat α-cocaine did not modify the actions of adrenaline or noradrenaline on the blood pressure and the nictitating membrane in doses five times larger than potentiating doses of cocaine.

4. α-Cocaine and cocaine had similar inhibitory effects on preparations of amine oxidase from the cat and the rabbit.

This work was done while one of us (V. V.) held a World Health Organization Fellowship. Our thanks are due to Mrs. F. J. Philpot for the observations on amine oxidase, and to Miss Roneen Hobbs for the estimations of local anaesthetic activity.

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