INTERACTION OF COCAINE AND TYRAMINE ON THE ISOLATED MAMMALIAN HEART

By J. B. Farmer and B. Petch

From the Department of Pharmacology, Pfizer Limited, Sandwich, Kent

Received April 24, 1963

Cocaine added to the fluid perfusing the isolated guinea-pig heart antagonised the action of tyramine in reducing the noradrenaline content of the heart. The extent of the antagonism depended on the concentration of cocaine in the perfusion fluid and on the amount of tyramine administered to the heart. Cocaine exerted its antagonistic action in concentrations which had no effect on the release of noradrenaline from the isolated heart, as judged by the absence of change in the noradrenaline content of the heart, and by the absence of a decrease in heart rate. These results may account for the antagonism by cocaine of the sympathomimetic actions of tyramine.

Tainter and Chang (1927) observed that cocaine antagonised the pressor action of tyramine. Eakins and Lockett (1960) showed that the rise in catecholamine content of arterial blood produced by intravenous injections of tyramine was prevented after administration of cocaine. However the nature of the antagonism could not be ascertained from these experiments. The effect of cocaine on dose-effect curves for tyramine on the nictitating membrane, heart rate and blood pressure of the cat indicated that the antagonism was competitive (Trendelenburg, 1961.)

The sympathomimetic effect of tyramine on the guinea-pig and rat isolated heart is mediated by noradrenaline released from tissue stores (Davey, Farmer and Reinert, 1962, 1963; Davey and Farmer, 1963; Axelrod, Gordon, Hertting, Kopin and Potter, 1962). Since the mode of action of tyramine on the isolated heart has been established, this preparation was used for experiments with cocaine and its interaction with tyramine.

Methods

Guinea-pig hearts were perfused by the method of Langendorff, with Krebs' solution of the following composition (g./litre NaCl 6·9; KCl 0·35; $CaCl_2$ 0·28; $MgSO_4.7H_2O$ 0·28; $NaHCO_3$ 2·09; KH_2PO_4 0·16; glucose 1·0); the solution was at 36° and was gassed with oxygen 95 per cent and carbon dioxide 5 per cent. Tyramine hydrochloride (100 μg.) was injected into a cannula close to the heart every 5–10 min. In some experiments cocaine hydrochloride was added to the reservoir of Krebs' solution in the required concentration. The noradrenaline content of the whole heart was estimated by the method of Merrills (1962). The heart was homogenised in 0·3M perchloric acid, the noradrenaline was adsorbed on alumina from a neutralised aliquot of the perchloric acid extract and eluted by adjusting the pH. Noradrenaline was fluorimetrically estimated in the eluate. Thioglycollic acid was used as a

stabilising agent to make the method specific for the estimation of noradrenaline; tyramine, adrenaline, isoprenaline, 3/4-dihydroxyphenylalanine and cocaine produced no interference. The recovery of noradrenaline added was 90–95 per cent. Amounts of tyramine and cocaine are expressed as hydrochloride.

RESULTS

The Effect of Tyramine on the Isolated Guinea-pig Heart

The repeated administration of tyramine to the isolated perfused guinea-pig heart led to a gradual decrease in the positive inotropic response. The loss of a response to tyramine was accompanied by a decrease in the noradrenaline content of the heart and an increase in the noradrenaline content of the perfusate. These results on the loss of noradrenaline from the heart have been published previously (Davey and Farmer, 1963) but they are included in Fig. 1 to serve as a control for the later experiments.

The Effect of Cocaine on the Isolated Guinea-pig Heart

Perfusion of cocaine (1 to 5 μg./ml.) through the isolated hearts for 3 hr. was without effect on the noradrenaline content of the myocardium (see Table I), or on the force of myocardial contraction. Larger concentrations of cocaine (10 μg./ml.) slowed the rate and decreased the force of contractions. In some experiments, when the heart was perfused with 10 μg./ml. of cocaine, the heart stopped and the perfusion was discontinued. The time of perfusion was noted and the noradrenaline content of the heart was determined. After perfusion of the isolated heart with 10 μg./ml. of cocaine there was an increase in the noradrenaline concentration. The increase was proportional to the time of perfusion with cocaine (see Table I).

TABLE I

NORADRENALINE CONTENT OF ISOLATED GUINEA-PIG HEARTS PERFUSED WITH COCAINE

Cocaine μg./ml.	Perfusion time hr.	Noradrenaline content μg./g.
0	3	1·21 ± 0·08 (5)
1	3	1·06 ● 0·2 (4)
2	3	1·20 ± 0·04 (4)
5	3	1·28 ± 0·27 (3)
10	1	1·92 ± 0·17 (3)
	2	2·49
	2¼	2·53 ± 0·12 (3) ± 0·19
	3	2·8

The Effect of Tyramine and Cocaine on the Noradrenaline Content of the Isolated Guinea-pig Heart

Cocaine antagonised the effect of tyramine in depleting noradrenaline. In experiments with a concentration of cocaine of 5 μg./ml. larger amounts of tyramine were required to diminish the noradrenaline content of the heart; the dose-response line for this effect of tyramine was shifted to the right but remained parallel to that obtained in the control experiment

(Fig. 1). When isolated hearts were given 3 mg. of tyramine (30 × 100 μg. doses) the depletion of noradrenaline produced by tyramine was progressively blocked by increasing concentrations of cocaine (1, 2 and 5 μg./ml.) (Fig. 2).

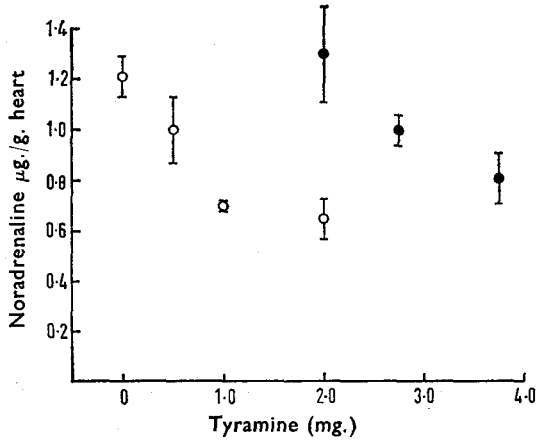

FIG. 1. The effect of tyramine on the noradrenaline content of the isolated perfused guinea-pig heart. Open circles hearts perfused with Kreb's solution, closed circles hearts perfused with Kreb's solution containing 5 μg./ml. cocaine.

The Effect of Cocaine on the Response of the Isolated Guinea-pig Heart to Tyramine and Noradrenaline

Guinea-pig hearts were perfused at the start of the experiments with Krebs' solution and control responses to tyramine and noradrenaline were obtained. The perfusion fluid was then altered to one which contained 5 μg./ml. of cocaine. After 10 min. had elapsed the response

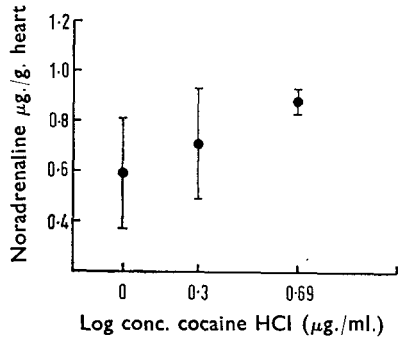

FIG. 2. The effect of increasing concentrations of cocaine on the depletion of heart noradrenaline produced by a standard dose of 3 mg. of tyramine (30 × 100 μg. doses).

to tyramine was almost abolished but the effects of noradrenaline were potentiated. Restoration of the perfusion fluid to Krebs' solution without added cocaine caused the partial return of the response to tyramine (Fig. 3).

Discussion

Macmillan (1959) suggested that cocaine prevented the sympathomimetic actions of tyramine on rabbits' isolated atria by blocking the release of noradrenaline from tissues stores. If these tissue stores of noradrenaline are those acted upon by impulses in postganglionic sympathetic nerves then it might be expected that cocaine would abolish the effects of nerve stimulation. But Trendelenburg (1959), found that cocaine has no effect on the output of noradrenaline from isolated spleens during stimulation of the postganglionic splenic sympathetic nerves. We have obtained similar results in experiments on the isolated cross perfused spleen of the cat. Noradrenaline output may even be increased during stimulation by intra-arterial injections of cocaine. Also, Hukovic and Muscholl (1962) found that cocaine increased the output of noradrenaline during stimulation of the sympathetic nerves to the isolated perfused rabbit heart.

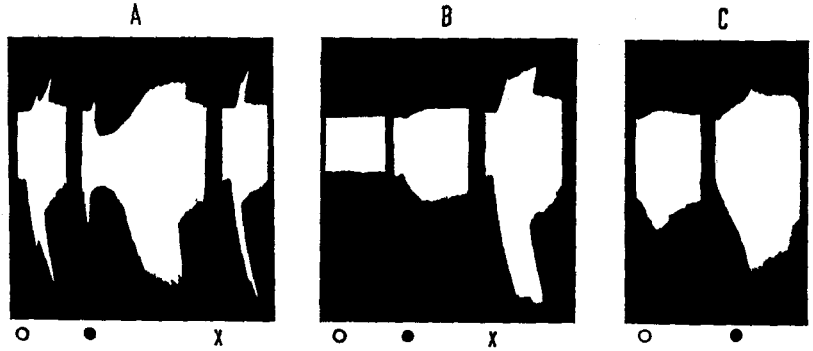

FIG. 3. The effect of cocaine on the positive inotropic response of the isolated guinea-pig heart to tyramine 10 μg. (○); 100 μg. (●) and noradrenaline 10 ng. (X). Between A and B, cocaine was added to the perfusion fluid at the concentration of 5 μg./ml. Between B and C, the perfusion fluid was altered to one without added cocaine.

The experiments of Trendelenburg (1961) indicated that there was a competitive antagonism between cocaine and tyramine. If the action of cocaine were to prevent the uptake of sympathomimetic amines into tissue stores then there would be no reason to suppose that the release of noradrenaline by nerve stimulation would be affected.

Our results show that the noradrenaline content of the heart muscle remained unchanged for up to 3 hr. in the presence of cocaine in concentrations of up to 5 μg./ml. With concentrations of 10 μg./ml. of cocaine the heart rate was slowed and there was an increase in the heart's content of noradrenaline. Our interpretation of these results is that the lower concentration of cocaine (less than 5 μg./ml.) did not interfere with the spontaneous release of noradrenaline but that higher concentrations (10 μg./ml.) caused a diminished release of noradrenaline. Macmillan (1959) observed a slowing of the rate of isolated rabbit atria with 7·5 to 10 μg./ml. of cocaine. Cocaine blocked the action of tyramine

and potentiated the action of noradrenaline on the guinea-pig heart in concentrations that did not affect the noradrenaline content of the heart. Therefore, it seems that cocaine can impair the uptake of tyramine and noradrenaline into tissue stores without affecting the spontaneous release of noradrenaline from the stores.

Davey and Farmer (1963) showed that tyramine depleted noradrenaline from the isolated guinea-pig heart; the degree of depletion was proportional to the amount of tyramine administered. Now it has been shown that cocaine antagonised the depleting effect of tyramine on the isolated heart. The line relating the dose of tyramine given to the amount of noradrenaline in the heart was shifted to the right by cocaine, but, it remained parallel to the original line. It was also shown that the depletion produced by a dose of tyramine was increasingly antagonised by increasing amounts of cocaine. These results are consistent with a competitive antagonism of tyramine by cocaine. Muscholl (1961) has shown that cocaine competitively antagonised the uptake of noradrenaline by the rat heart.

These results may explain the apparent discrepancy between the effect of cocaine on the release of noradrenaline from tissues by nerve stimulation on the one hand and by tyramine on the other.

Cocaine antagonises competitively the displacement of noradrenaline from tissue stores by tyramine and thus abolishes the sympathomimetic effect of tyramine. The enhanced release of noradrenaline from the spleen and heart during stimulation of the sympathetic nerves occurs because the re-entry of transmitter (noradrenaline) into tissue storage sites is prevented by cocaine.

References

Axelrod, J., Gordon, E., Hertting, G., Kopin, I. J. and Potter, L. T. (1962). *Brit. J. Pharmacol.*, **19**, 56–63.

Davey, M. J., Farmer, J. B. and Reinert, H. (1962). Communication to the Winter Meeting of the British Pharmacological Society, Jan., 1962.

Davey, M. J. and Farmer, J. B. (1963). *J. Pharm. Pharmacol.*, **15**, 178–182.

Davey, M. J., Farmer, J. B. and Reinert, H. (1963). *Brit. J. Pharmacol.*, **20**, 121–134.

Eakins, K. E. and Lockett, M. F. (1960). *Ibid.*, **12**, 513–517.

Hukovic, S. and Muscholl, E. (1962). *Arch. exp. Path. Pharmak.*, **244**, 81–96.

Macmillan, W. H. (1959). *Brit. J. Pharmacol.*, **14**, 385–391.

Merrills, R. J. (1962). *Nature Lond.*, **193**, 988.

Muscholl, E. (1961). *Brit. J. Pharmacol.*, **16**, 352–359.

Tainter, M. L. and Chang, D. K. (1927). *J. Pharmacol.*, **39**, 193–207.

Trendelenburg, U. (1959). *Ibid.*, **125**, 55–65.

Trendelenburg, U. (1961). *Ibid.*, **134**, 8–17.