THE EFFECTS OF BRETYLIUM AND COCAINE ON NORADRENALINE DEPLETION

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Bretylium and cocaine possess the property of reducing the depletion of noradrenaline in rat hearts and spleens produced either by guanethidine or by reserpine. Their modes of action differ.

BOURA and Green suggested in 1959 that bretylium exerts its hypotensive action by preventing the release of noradrenaline from the nerve terminals when the postganglionic sympathetic nerves are stimulated. It has also been suggested (Fleckenstein and Bass, 1953; Macmillan, 1959) that cocaine prevents the release of noradrenaline from its stores. Since drugs such as reserpine or guanethidine release noradrenaline from sympathetically innervated tissues, it is of interest to discover whether bretylium or cocaine could reduce the effect of reserpine or guanethidine in depleting the rat heart and spleen of noradrenaline.

METHODS

Groups of 12 rats weighing 200–250 g. were used in most experiments. After treatment, they were killed by a blow on the head and their hearts and spleens removed as rapidly as possible. These tissues were weighed and stored at −10°. After extraction, their catecholamine content (adrenaline and noradrenaline) was estimated fluorimetrically as described by Cass and Spriggs (1961) and expressed in terms of noradrenaline.

Drugs were administered by subcutaneous injection. Reserpine, dissolved in the solvent of Pletscher, Shore and Brodie (1955), was given in doses of 0·1 mg./kg., except in the preliminary experiment where 3 daily doses of 1 mg./kg. were used. Guanethidine hemisulphate, dissolved in 0·01N HCl, was injected at a dose of 10 mg./kg. In the protection experiments, aqueous solutions of bretylium tosylate (30 mg./kg.) or cocaine hydrochloride (10 or 50 mg./kg.) were administered with the reserpine or guanethidine. All doses were calculated in terms of the base. Animals were killed either 6 or 18 hr. after the drugs.

In other experiments, a pressor effect indicative of stimulation of the sympathetic nervous system was induced in rats under urethane anaesthesia by the intravenous injection of 20 μg. of physostigmine (Varagić, 1955); cocaine (50 mg./kg.) was then injected subcutaneously and the response to physostigmine re-tested over the following 5 hr. at 30 min. intervals.

RESULTS

Preliminary experiments showed that whereas bretylium in 3 daily doses reduced the effect of guanethidine (also in 3 daily doses) in depleting the rat heart of noradrenaline, it failed to alter that of reserpine (3 daily doses of 1 mg./kg.). The dose of reserpine was therefore reduced to a single dose of 0·1 mg./kg. in the later experiments.

Action of Bretylium

Eighteen hr. after a single dose of bretylium the noradrenaline contents of rat heart (0·59 ± 0·05 μg./g.) or spleen (0·39 ± 0·03 μg./spleen) were not significantly altered. When given with reserpine or guanethidine,

TABLE I

EFFECT OF COCAINE (10 MG./KG.) ON THE DEPLETION OF THE NORADRENALINE CONTENT OF RAT HEART AND SPLEEN PRODUCED BY RESERPINE (0·1 MG./KG.) OR GUANETHIDINE (10 MG./KG.). ANIMALS KILLED 6 OR 18 HR. AFTER THE INJECTIONS

Time (hr.)	Drug	Noradrenaline (per cent of control)			
		Heart		Spleen	
		No cocaine	Cocaine	No cocaine	Cocaine
6	Saline	100	89	100	110
	Reserpine	4	10	8	12
	Guanethidine	19	22	19	32
18	Saline	100	92	100	126
	Reserpine	<9	<9	9	14
	Guanethidine	<9	<9	11	16

bretylium significantly reduced ($P < 0.001$) the release of noradrenaline produced by these agents. This is shown in Fig. 1. The reduction was greater in the heart than in the spleen, and in fact the noradrenaline content in the heart after bretylium and guanethidine did not differ significantly from the control value.

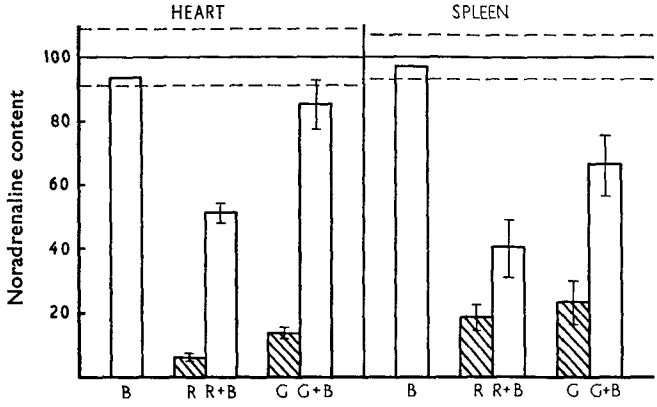

FIG. 1. Effect of bretylium (B, 30 mg./kg.) on the depletion of the noradrenaline content of rat heart and spleen produced by reserpine (R, 0·1 mg./kg.) or guanethidine (G, 10 mg./kg.). Animals killed 18 hr. after the injections; noradrenaline content expressed as percentages (± S.E.) of the control values.

Action of Cocaine

Cocaine (10 mg./kg.) also did not significantly alter the noradrenaline contents of the heart and spleen when estimated 6 or 18 hr. later. When given with reserpine or guanethidine, cocaine had no effect on the release

of noradrenaline produced by these agents (see Table I). When the larger dose of cocaine (50 mg./kg.) was given and the animals were killed 6 hr. later, significant reductions ($P < 0.05$) in the release of noradrenaline were found (Fig. 2). Again, cocaine did not significantly alter the noradrenaline contents of the heart or the spleen. The larger dose of cocaine also failed to reduce the pressor action of physostigmine in the anaesthetised rat: in fact, it slightly potentiated the response.

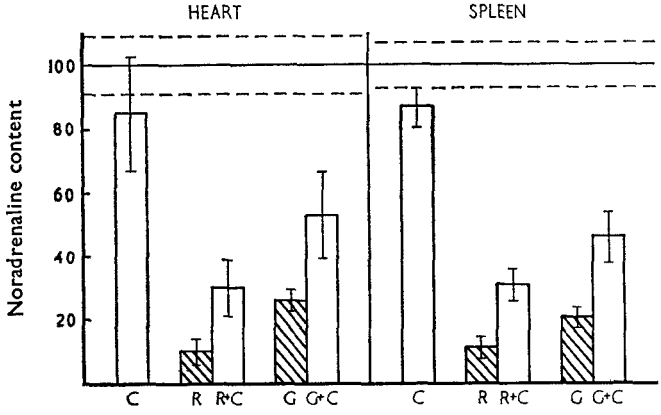

FIG. 2. Effect of cocaine (C, 50 mg./kg.) on the depletion of the noradrenaline content of rat heart and spleen produced by reserpine (R, 0·1 mg./kg.) or guanethidine (G, 10 mg./kg.). Animals killed 6 hr. after the injections; noradrenaline content expressed as percentages (± S.E.) of the control values.

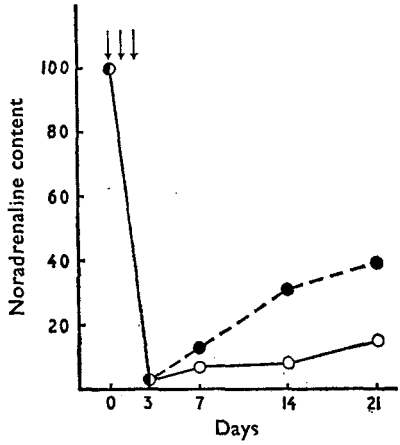

FIG. 3. Effect of daily doses of bretylium (30 mg./kg.) on the depletion and replacement of noradrenaline in rat heart produced by reserpine (3 daily doses of 1 mg./kg., at the arrows). Noradrenaline content expressed as percentages of the control values: reserpine and saline (○——○); reserpine and bretylium (●- - - -●).

Replacement of Noradrenaline

Daily doses of bretylium for 21 days produced no alteration in the noradrenaline content of rat heart, yet given with 3 daily doses of reserpine

(1 mg./kg.) and then by itself for the next 18 days, it produced an increased rate of recovery of noradrenaline. This is shown in Fig. 3.

Discussion

Bretylium has been shown to reduce the loss of noradrenaline in the heart and spleen of rats produced by reserpine or guanethidine. This suggests that bretylium acts by preventing the release of the noradrenaline from its stores or the access of the releasing agent to these stores. However, Varagić, Lešić, Vuco and Stamenović (1961) reported that bretylium prevented the release from sympathetic nerve endings of noradrenaline produced by the intravenous injection of physostigmine into the intact rat. Moreover, Hertting, Axelrod and Patrick (1962) using radioactive techniques found that bretylium blocked both the release and uptake of ^{3}H-noradrenaline in rat heart. These same authors showed also that guanethidine 4 hr. after administration, partially inhibited the reserpine-induced release of noradrenaline. This observation adds weight to the suggestion of Cass and Spriggs (1961) that guanethidine has a dual mode of action; a primary bretylium-like action and a secondary reserpine-like action.

In the present experiments, large doses of cocaine also antagonised the action of reserpine or guanethidine in depleting tissue noradrenaline. Whereas Macmillan (1959) suggested that cocaine blocked the release of noradrenaline from the stores, Trendelenberg (1959) found that cocaine did not alter the release of noradrenaline during stimulation of the splenic nerves and that it potentiated the pressor effects which followed this stimulation. Later, Trendelenberg (1961) showed that cocaine antagonised the pressor action of tyramine and suggested that this was due to competition with tyramine, thus preventing its access to the noradrenaline store. Hertting, Axelrod and Whitby (1961) have recently shown that cocaine prevents the uptake of infused ^{3}H-noradrenaline. Further, the larger dose of cocaine used in the present experiments did not reduce the pressor response to injected physostigmine. Thus it seems likely that the action of cocaine is to block the access of noradrenaline, or of its releasing agents to the stores of noradrenaline; the release of noradrenaline after nerve stimulation is unaffected.

Although it has been shown that both bretylium and cocaine possess the ability to reduce the degree of depletion of noradrenaline after treatment either with reserpine or with guanethidine, they appear to do so by different mechanisms. Bretylium probably acts by preventing the release of noradrenaline and cocaine by preventing the access of the releasing agents to the noradrenaline store.

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