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THE INFLUENCE OF BRETYLIUM ON THE ACTION OF RESERPINE AND McNEIL–A–343*

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Abstract—Injection of bretylium into rats produced little or no change in content of catecholamines in heart; reserpine produced a marked decrease. The depletion of catecholamines caused by reserpine was reduced or abolished after pretreatment of the animals with bretylium. Bretylium depressed the increase in arterial blood pressure and cardiac phosphorylase *a* activity produced by administration of McNeil–A–343.

SYMPATHETIC blockade by bretylium was first demonstrated by Boura *et al.*[1] The inhibition of sympathetic activity and the hypotensive properties of this compound have since been the subject of a number of studies.[2–4] Bretylium does not interfere with the action of epinephrine or norepinephrine on effector cells; the drug has been shown, rather, to potentiate the action of circulating catecholamines.[5] apparently the blockade of sympathetic activity by bretylium is caused by an action on sympathetic nerve endings.[4]

In the present study the action of bretylium and reserpine on catecholamine content of rat heart was investigated. Since bretylium in some way interferes with the release of norepinephrine from tissue stores or nerve endings, the influence of this compound on the effects of ganglion stimulation was also studied.

METHODS

Catecholamine studies

Reserpine hydrochloride (100 μg base/kg) was injected intraperitoneally and bretylium *p*-toluene sulfonate (10 mg base/kg) subcutaneously into adult male albino rats. The animals were killed by decapitation at various times after drug administration. Two hearts were pooled for each sample, and chemical and, in some cases, biological determinations of catecholamines were made. The hearts were washed with water, placed in a solution of 5% trichloracetic acid and minced with a VirTis tissue homogenizer. Catecholamines were adsorbed on aluminium hydroxide and eluted at an acid pH according to the method of von Euler and Orwen.[6] After reaction with ferricyanide and addition of ascorbic acid and NaOH, the samples were analyzed in an

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TABLE 1. LONG-TERM EFFECTS OF BRETYLIUM AND RESERPINE ON HEART CATECHOLAMINES IN RAT

	Control	After start of experiment (hr)							
		12		24			48		
		B† + R‡	R	B + R	R	B	B + R	R	B
Mean* (μg/100 g)	71·4	53·4	22·4	69·7	12·7	74·0	41·9	24·8	62·4
s.e.m.	±3·07	±1·06	±0·41	±6·06	±0·82	±2·25	±2·09	±0·99	±1·89
N	10	4	5	7	10	10	7	8	8
P (compared with control)		< 0·01	< 0·001	> 0·5	< 0·001	> 0·5	< 0·001	< 0·001	< 0·05
P (R compared with B + R)			< 0·001		< 0·001			< 0·001	

* Fluorometric determination.
† B = bretylium, 10 mg base/kg injected s.c. 18 and 2 hr before experiment (injection of reserpine).
‡ R = reserpine hydrochloride, 100 μg base/kg i.p. at beginning of experiment.

Aminco-Bowman spectrophotofluorometer. Fluorescence was measured at 500 mμ after excitation at 436 and 400 mμ, and the amounts of epinephrine and norepinephrine were estimated. The recovery of norepinephrine added to the TCA extracts was 73·8 ± 2·01 per cent (N = 17). Final results were corrected for the loss of catecholamines during the isolation procedure. The concentration of epinephrine in the samples was found to be so small, compared with the norepinephrine concentration, that its contribution to the fluorescence could be neglected. Figures for norepinephrine were therefore not corrected for the presence of epinephrine. Biological estimations of catecholamines were made by the determination of blood pressure responses in cats treated with atropine.

Phosphorylase studies

The effect of bretylium on the sympathetic response to intravenous injection of McNeil-A-343 (100 μg/kg) was studied in open-chest rat preparations. Measurements were made of mean aterial blood pressure and heart phosphorylase according to methods previously described.[7] The animals were adrenalectomized 3 to 5 days before the experiments in order to limit the action of McNeil-A-343 to sympathetic ganglia. They were fed *ad lib* and allowed to drink 0·9% NaCl.

RESULTS

Excellent agreement was obtained between chemical and biological determinations of catecholamines. The mean norepinephrine content of heart in 10 determinations with normal rats was 65·6 ± 2·12 μg/100 g with the fluorometric method, and 65·2 ± 6·71 μg/100 g with the biological method.

The experiments reported in Table 1 demonstrate that administration of reserpine at a dose of 100 μg/kg intraperitoneally caused marked depletion of the norepinephrine content in heart.

In these experiments the effect of reserpine on the amount of norepinephrine in the heart was also determined in animals which had been injected subcutaneously with one dose of bretylium 18 hr and one dose 2 hr prior to the administration of reserpine. The striking observation was made that pretreatment with bretylium completely abolished the depletion by reserpine of heart catecholamines in the 24-hr experiments, and markedly reduced the effect of reserpine in the 12- and 48-hr experiments. The injection of bretylium alone had no significant effect on the norepinephrine content in heart after 24 hr, but caused a slight but statistically significant ($P < 0.05$) decrease after 48 hr.

A second series of experiments was also carried out in which the short-term effects of reserpine were studied in control rats and in rats which had been previously injected with bretylium. The catecholamine content of heart was measured at various times up to 8 hr after the administration of reserpine, and both chemical and biological determinations were made. The results of these experiments are reported in Table 2.

It is seen that reserpine in the normal animals caused a slow depletion of the heart catecholamines. In the animals previously treated with bretylium, however, there was almost no effect of the drug. The depletion of heart catecholamines after reserpine and the marked inhibition of reserpine action by bretylium is seen clearly when the catecholamines were determined by the chemical method and also when bioassays

were used. We have no explanation for the fact that in the experiments with reserpine plus bretylium the bioassays gave consistently lower values than did the chemical method.

Experiments on heart phosphorylase were done in order to determine whether bretylium blocked the activation of this enzyme that occurs after the administration of McNeil-A-343.[7] The results of these experiments, in which open-chest rats were used, are reported in Table 3.

TABLE 2. SHORT-TERM EFFECTS OF RESERPINE AND OF RESERPINE + BRETYLIUM ON HEART CATECHOLAMINES OF RAT

Time after reserpine (hr)	Catecholamine content (μg/100 g)			
	Reserpine		Reserpine + bretylium	
	Chem. det.	Bioassay	Chem. det.	Bioassay
0	51·5 (4)	48·8 (2)	54·8 (4)	41·6 (2)
2	37·8 (2)	35·3 (2)	63·8 (4)	36·7 (2)
4	18·1 (4)	15·0 (2)	58·0 (4)	40·3 (2)
6	18·5 (4)	15·3 (2)	48·8 (4)	40·7 (2)
8	13·9 (4)	18·1 (2)	45·0 (4)	35·7 (2)

Number of determinations in parentheses; drug administration as indicated in Table 1.

TABLE 3. THE EFFECT OF BRETYLIUM ON THE ACTION OF MCNEIL-A-343 IN THE OPEN-CHEST ADRENALECTOMIZED RAT

Control rats				Bretylium-treated rats							
Blood pressure (mm Hg)			Phosphorylase *a*	Blood pressure (mm Hg)			Phosphorylase *a*	Blood pressure (mm Hg)			Phosphorylase *a*
Control	McNeil-A-343	Δ	(%)	Control	McNeil-A-343	Δ	(%)	Control	Saline	Δ	(%)
35	87	52	67·3	37	57	20	39·5	59	77	18	35·5
82	108	26	60·6	57	82	25	47·2	77	93	16	30·6
65	133	68	65·4	43	77	34	38·4				
90	132	42	67·4	58	96	38	34·3				

Bretylium, 10 mg/kg injected s.c. 18 and 2 hr before opening the chest. McNeil-A-343 was administered i.v., 100 μg/kg. At the height of the effect on blood pressure, the heart was removed from the animal and immediately frozen in dry ice–alcohol. The mean % phosphorylase *a* (± s.e.m.) in untreated, adrenalectomized rats given 1 ml saline/kg i.v. was 40·2 ± 1·60 (s.d. = 5·97, N = 14).

In the control animals the injection of McNeil-A-343 into the right external jugular vein produced a pronounced increase in blood pressure and in the activity of heart phosphorylase *a*, but in animals which had been pretreated with bretylium, the effects of McNeil-A-343 on blood pressure and on phosphorylase activity were markedly depressed.

DISCUSSION

Paasonen and Krayer[8] have shown that reserpine, acting peripherally, produces a decrease in catecholamine content in rabbit hearts. Experiments reported in this paper demonstrate that bretylium prevents this depletion of heart catecholamines

seen after reserpine administration. These results are in accord with the findings of Hertting *et al.*[9] that bretylium inhibited the spontaneous and reserpine-induced release of [^{3}H]-norepinephrine in the rat heart. Furthermore, it has been shown by Kuntzman *et al.*[10] that bretylium also inhibits the depletion of heart catecholamines caused by guanethidine.

Previous reports indicate that bretylium does not interfere with nerve conduction[11] nor does it block the effect of circulating catecholamines at receptor sites.[5] In addition the drug does not affect the level of heart catecholamines, as shown by the results of Cass and Spriggs[12] and confirmed in this paper.

Since sympathetic stimulation is ineffectual in reserpine-treated animals, the site of catecholamine depletion caused by this drug may be at or near the nerve endings. Results reported here indicate that bretylium also acts at the nerve terminals to prevent the release of norepinephrine produced by reserpine. This conclusion is supported by the results obtained in animals given McNeil-A-343 after prior administration of bretylium. The characteristic increase in blood pressure and cardiac phosphorylase seen with ganglion stimulation by McNeil-A-343 is markedly reduced by bretylium.

The inhibitory action of bretylium on the effects of McNeil-A-343 apparently is due to the blocking action of bretylium at the nerve terminals, which then prevents the release of endogenous norepinephrine and the subsequent stimulation of cardiac phosphorylase[7] otherwise seen with McNeil-A-343.

Added in proof—In an article published at the time of submission of this paper, Callingham and Cass[13] reported that bretylium decreased the effectiveness of reserpine in causing depletion of catecholamines in rat heart and spleen. Our work is in complete agreement with these findings.

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