Influence of Myocardial Catecholamines on the Cardiac Action of Ouabain

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Toxic and lethal doses of ouabain and concomitant changes in myocardial contractility were determined in reserpine-pretreated and control cats. Reserpine pretreatment did not diminish the degree of myocardial contractility induced by ouabain. The effect of reserpine pretreatment on the toxicity of ouabain depended upon the manner in which the glycoside was administered and the physiological state of the preparation. Depletion of myocardial catecholamines by reserpine led to decreased cardiac glycoside toxicity when the ouabain was administered in the form of intermittent infusions into intact animals. However, pretreatment with reserpine did not alter appreciably the toxicity of ouabain when the drug was continuously infused into open-chest animals. Ouabain *per se* tended to have little effect on myocardial catecholamine content.

NUMEROUS reports describing the influence of catecholamines upon the myocardial action of ouabain have been published in recent years.

Tanz (1) reported that augmentation of contractile force of cat papillary muscle caused by ouabain could be eliminated by dichloroisoproterenol (DCI). He also observed that no augmentation of contractile force was caused by ouabain in papillary muscles taken from reserpine-pretreated cats. On the other hand, Yelnosky and Erwin (2) found that ouabain produced as great an increase in myocardial contractile force and automaticity in the reserpine-pretreated dog as in the nontreated dog. Tanz (3) indicated that the positive inotropic action following ouabain in cat papillary muscle and in Langendorff preparations might be due to the release of endogenous myocardial catecholamines. Cession-Fossion (4) reported that ouabain lowers the norepinephrine content of the myocardium in the rat.

Morrow *et al.* (5) observed that in dogs the inotropic and arrhythmic dose of ouabain are independent of autonomic innervation and myocardial catecholamine stores, but that a major portion of the prolongation of atrioventricular functional refractory period (AVFRP) produced by ouabain is dependent upon autonomic innervation. Roberts *et al.* (6) conclude that catecholamine release is the mechanism responsible for arrhythmias caused by the combined action of small doses of acetylstrophanthidin (ac. stroph.) and vagal stimulation; large doses of ac. stroph. produced arrhythmias through another mechanism. They also observed that the increase in isometric tension developed in cat isolated papillary muscles after exposure to ouabain was not affected by reserpine pretreatment. Tanz (7), employing cat isolated papillary muscle preparations, reported that ouabain-induced augmentation could be reduced by pretreatment with either DIC or reserpine. Combined treatment with reserpine and DCI reduced still further ouabain-induced augmentation. The ability of DCI-treated or reserpinized muscles to respond to calcium was unaltered.

In view of these divergent results, experiments were carried out to determine the effect of catecholamine depletion on the toxicity, lethality, and myocardial contractile response to ouabain.

EXPERIMENTAL

A total of 78 experiments were performed using cats selected on the basis of the findings of Tanabe and Suzuki (8). Each cat was anesthetized with an intraperitoneal injection of 30 mg./Kg. of pentobarbital. Animals were positioned on their right sides with all limbs at approximately right angles to the long axis of the body. Infusion into the animal was achieved through the femoral vein using a polyethylene catheter (Clay Adams PE 90) and a constant infusion pump (Harvard Apparatus Co., model 600-0). Electrocardiograms and recordings of contractile force were taken employing a Sanborn Twin Viso recorder (model 60-13008) and Walton-Brodie arches (9). Standard limb lead II was used.

Ouabain solution (10 mcg./ml.) was prepared with physiological saline. Reserpine[1] was administered intraperitoneally as an aqueous solution. The dose employed was 3 mg./Kg. given 24 hr. prior to the experiment.

Appearance of cardiac arrhythmias was taken to indicate ouabain toxicity. Cessation of the heart beat was taken to indicate lethality.

Intact Animals (Intermittent Infusion).—A total of 20 experiments was performed, 10 with non-

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[1] Marketed as Serpasil by Ciba Pharmaceutical Co., Summit, N. J.

TABLE I.—TOXIC AND LETHAL DOSE OF OUABAIN IN NONTREATED AND RESERPINE-PRETREATED CATS

Treatment	Intermittent Infusion		Continuous Infusion	
	Toxic Dose, mcg./Kg.	Lethal Dose, mcg./Kg.	Toxic Dose, mcg./Kg.	Lethal Dose, mcg./Kg.
Nontreated cats	76.1 (86.8–65.4)[a]	105.5 (116.2–94.8)	87.8 (100.7–74.9)	119.7 (132.6–106.8)
Reserpine-pretreated cats	105.0 (115.7–94.3)	139.6 (150.3–128.9)	87.0 (99.9–74.1)	122.0 (134.9–109.1)

[a] 95% confidence limits.

treated cats and 10 with reserpine-pretreated cats. Ouabain solution was infused for 1 min., followed by 1-min. infusions at 5-min. intervals. Rate of infusion was 1.23 ml./min.

Open-Chest Animals (Continuous Infusion).—Fifty-eight experiments were performed, 32 with nontreated animals and 26 following reserpine pretreatment. Of the 32 experiments carried out with nontreated cats, 20 received ouabain solution and 12 received isotonic saline for durations corresponding to those required to produce ouabain-induced toxicity and lethality.

Of the 26 reserpine-pretreated cats, 20 received ouabain solution until appearance of toxicity or lethality, whereas 6 cats received isotonic saline for equivalent periods.

Left lateral thoracotomy was performed, and respirations were maintained with a respirator (Harvard Apparatus Co., model 607). Ouabain was infused continuously at the rate of 0.49 ml./min. Electrocardiograms and recordings of contractile force were obtained as described above. At the toxic or lethal end point, a portion of right atrium was removed and assayed for catecholamine content (10).

RESULTS

Toxic and Lethal Doses.—Reserpine pretreatment significantly increased toxic and lethal doses of ouabain in intact animals receiving intermittent infusions. No significant change in dose levels was seen when ouabain was infused continuously into open-chest animals (Table I).

Inotropic Responses to Ouabain.—Ouabain produced significantly greater positive inotropic response in both nontreated and reserpine-pretreated cats than equal volumes of isotonic saline solution. A significant increase was also seen in the positive inotropic response to ouabain observed in reserpine-pretreated cats when compared with nontreated animals (Table II).

Atrial Norepinephrine Values.—A small statistically insignificant decrease in catecholamine values of the right atrium was seen in normal animals receiving ouabain (Table III).

DISCUSSION

When ouabain was administered in the form of intermittent infusions, significantly higher doses of the cardiac glycoside were required to induce arrhythmia in reserpine-pretreated cats than in untreated control cats. However, an absence of significant difference in dose levels was observed in animals receiving continuous infusions of ouabain.

Evidence has been reported relating the rhythmicity of the ventricles and the presence of endogenous catecholamines, *i.e.*, removal of the influence of catecholamines causes a fall in the intrinsic rhythmicity of the ventricles (11, 12). It would thus appear logical that a greater amount of ouabain would be required to raise the rhythmicity of the ventricles to a dominant rate in reserpine-pretreated cats than in untreated animals. The results obtained in our studies using intermittent infusions lend support to the above argument.

TABLE II.—CONTRACTILITY CHANGES IN NONTREATED AND RESERPINE-PRETREATED CATS

Treatment	Max. % Increase in Contractiity	
	Ouabain	Saline
Nontreated cats	53.7 ±7.0[a]	15.6 ±7.7
Reserpine-pretreated cats	71.9 ±8.6	17.4 ±12.2

[a] Standard error.

Roberts *et al.* (6) have also reported results which are in accord with the above hypothesis. Their observations on arrhythmia induced by combined action of ac. stroph. and vagal stimulation showed that reserpine pretreatment afforded significant protection against ac. stroph.-induced arrhythmia. They also report that doses of reserpine, which were sufficient to diminish the action of ac. stroph. to induce arrhythmia in combination with vagal stimulation, did not affect the arrhythmia induced by a large dose of ac. stroph. (150 mcg./Kg.). They thus characterized ventricular arrhythmias induced by ac. stroph. as being of two types: those induced by small doses of the drug and altered by reserpine pretreatment, and those induced by large doses of the glycoside and unaltered by reserpine pretreatment. They conclude that while catecholamine release is an important mechanism involved in the arrhythmia produced by small doses, another mechanism also plays a role in this action at higher dose levels.

While the experimental methods used in our studies differ from those of Roberts *et al.* (6), the results are comparable to those obtained in the experiments. Reserpine pretreatment afforded protection against ouabain-induced arrhythmias in cats receiving intermittent infusions, a result similar to that obtained in their experiments with ac. stroph. and vagal stimulation. No such protective effect was seen in our experiments or in those of Roberts and co-workers where continuous infusions and large doses of the drug, respectively, were used. It should also be mentioned that a similar absence of difference in the arrhythmia sensitivity of reserpine-treated and nontreated animals was observed by Morrow *et al.* (5) when ouabain was administered in the form of a continuous infusion.

TABLE III.—ATRIAL NOREPINEPHRINE VALUES IN NONTREATED CATS INFUSED WITH OUABAIN AND SALINE[a]

Ouabain		Saline	
Toxic Level, mcg./Gm.	Lethal Level, mcg./Gm.	Toxic[b] Level, mcg./Gm.	Lethal[c] Level, mcg./Gm.
1.76	1.46	2.36	1.77
(2.38–1.14)[d]	(1.99–0.91)	(3.13–1.59)	(2.46–1.08)

[a] Saline infused for periods corresponding to those necessary to obtain ouabain-induced toxicity and lethality. [b] Duration of infusion = 18 min./Kg. [c] Duration of infusion = 25 min./Kg. [d] 95% confidence limits.

Erlij and Mendez (13) reported a modification of digitalis intoxication by excluding adrenergic influences on the heart. Their experiments showed that reserpinization, sympathectomy, or administration of pronethalol altered the cause of death from digitalis-like compounds. Normally, cardiac glycosides caused death by inducing ventricular fibrillation. However, dogs and cats undergoing the above treatments usually died of ventricular arrest. The type of toxicity induced by continuous intravenous infusion of digitoxin is reported by these authors to be more likely to be affected by alteration of adrenergic tone than toxicity induced by ouabain. It is interesting to note in this context that in our experiments only 20% of the reserpine-pretreated cats receiving continuous infusion of ouabain died of ventricular arrest, whereas in animals receiving intermittent infusions of ouabain, 60% died of ventricular arrest.

The authors feel justified in suggesting that the manner of administering ouabain determines the nature of its toxicity in reserpinized hearts. The relationship between catecholamines and ouabain-induced toxicity cannot be stated with precision. It is possible that digitalis intoxication may consist of two components: one in which the removal of adrenergic influences causes a change in the nature and intensity of intoxication, and the other, an apparently direct component of digitalis glycoside toxicity whose intensity remains unaltered by removal of adrenergic influences. It is our belief that these components are clearly manifested in the above studies involving the use of continuous and intermittent infusions.

Ventricular contractility was not diminished by reserpine pretreatment. Contractility was studied by using a strain gauge arch to measure the peak rate of development of tension rather than peak systolic tension. Discussion of the principles involved and advantages inherent in this procedure are reviewed by Blinks and Koch-Weser (14) and Mommaerts and Langer (15). The results obtained are in agreement with those of Roberts *et al.* (6) and Morrow *et al.* (5).

The authors feel that the increase in contractile response to ouabain observed in reserpinized hearts when compared with nontreated hearts should not be construed as an indication of increased sensitivity of the former. The resting tension of reserpinized hearts has been reported by Withrington and Zaimis (16) to be one half that of the nontreated hearts. It is necessary therefore to recognize this factor when evaluating any changes in contractility observed in the catecholamine depleted heart.

SUMMARY

Experiments were carried out to study the effect of reserpine pretreatment upon inotropic and arrhythmic responses of the heart to ouabain. Ventricular contractility remained unaffected by reserpine pretreatment. The nature of ouabain intoxication was altered; it was more evident in reserpine-pretreated animals receiving intermittent infusions of ouabain than in animals receiving continuous infusions of the drug. A small, statistically insignificant depletion of catecholamines was seen as a result of ouabain infusion.

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