Br. J. Pharmac. Chemother. (1967), **30**, 143–154.

SOME FACTORS AFFECTING OUABAIN-INDUCED VENTRICULAR ARRHYTHMIA IN THE RESERPINE-TREATED CAT*

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(*Received December* 13, 1966)

A number of investigators have observed that reserpine, an agent which diminishes adrenergic nervous activity by catecholamine depletion, depressed ventricular arrhythmia induced by certain cardiac glycosides (Roberts, Ito, Reilly & Cairoli, 1963; Erlij & Mendez, 1964; Boyajy & Nash, 1965; Swamy, Hamlin & Wolf, 1965). This observation suggested to some investigators that catecholamines are necessary for the genesis of ventricular arrhythmia induced by digitalis materials (Roberts *et al.*, 1963; Swamy *et al.*, 1965). Although Roberts *et al.* (Roberts *et al.*, 1963; Roberts, Ehrreich & Levitt, 1965) have proposed that digitalis acts directly on the adrenergic nervous system, it is possible that physiological changes resulting from depletion of catecholamines—namely, fall in body temperature, heart rate or blood pressure—may account for the reserpine antagonism of the action of digitalis to induce ventricular arrhythmia.

It has been suggested that the slow heart rate following reduction in sympathetic influences may account for the reduction in the capacity of digitalis materials to induce ventricular rhythm disorders (Erlij & Mendez, 1964; Boyajy & Nash, 1966). In this regard, it has been demonstrated that the development of the positive inotropic action of digitalis is dependent on the number of cardiac muscle contractions (Sanyal & Saunders, 1958; Moran, 1963) and, therefore, it is possible that heart rate affects the development of ouabain-induced ventricular arrhythmia. The level of body temperature has also been shown to influence the reactivity of the ventricle to ouabain (Angelakos, Torres & Driscoll, 1958; Beyda, Jung & Bellet, 1961). It has been shown that reducing the body temperature of dogs to 24°–28° C doubles the dose of ouabain necessary to produce

* Supported by Grant HE 10916 from The National Heart Institute, United States Public Health Service. A preliminary report appeared in *Federation Proceedings* (1966), **25**, 382.

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death (Beyda *et al.*, 1961). Finally, some investigators (Moe, Malton, Rennick & Freyburger, 1948 ; Nickerson & Nomaguchi, 1949) have suggested that the level of arterial blood pressure is an important factor in the genesis of hydrocarbon-adrenaline arrhythmia ; it is possible that the level of blood pressure also influences the capacity of digitalis to induce ventricular arrhythmia.

This investigation was undertaken to determine whether changes in heart rate, body temperature and blood pressure affect the reactivity of the ventricle to digitalis, and whether they contribute to the anti-arrhythmic action of reserpine.

METHODS

Experiments were performed in cats anaesthetized by the intraperitoneal administration of 0.3 ml./kg Dial-urethane (Ciba) in reserpine-pretreated animals and 0.6 ml./kg Dial-urethane in all others. The electrocardiogram was recorded continuously on a two channel polygraph (usually Lead II). In some animals, mean carotid blood pressure was continuously monitored by means of a Statham pressure transducer, the output of which was recorded on a polygraph. Body temperature was measured continuously with a laboratory rectal thermometer.

In previous studies from this laboratory, attempts were made to control environmental temperature by keeping both laboratories and animal rooms at approximately 25° C, thereby minimizing the fall in temperature that may occur after reserpine and other procedures reducing adrenergic nervous activity. In the present study, experiments were performed at ambient temperatures in order to study the effects of changes in body temperature that may occur during the experimental procedure.

In all animals, the cervical vagus nerves were bilaterally crushed. Doses of ouabain were selected to produce ventricular arrhythmia and death in both control and reserpine-pretreated animals. It was determined that at least 125 μg/kg ouabain was needed for this purpose. In the present study, single doses of 125 or 250 μg/kg ouabain were employed. The times following the intravenous administration of ouabain to the development of ventricular extrasystole, then to sustained ventricular tachycardia (lasting at least 5 min), and finally to death due either to ventricular fibrillation or asystole were employed as the criteria for ouabain toxicity. This method was previously employed by Boyajy & Nash (1965) in their study of ouabain toxicity in dogs.

The capacity of ouabain to induce ventricular rhythm disturbances was studied in 10 groups of animals as follows.

1. No procedures other than bilateral vagotomy were performed before ouabain administration.

2. Reserpine, 0.5 mg/kg, was administered intraperitoneally 24–30 hr before ouabain administration.

3. Reserpine, 5 mg/kg, was administered intraperitoneally 24–30 hr before ouabain administration.

4. Bilateral translumbar adrenalectomy was performed 1 hr before ouabain administration.

5. Reserpine, 0.5 mg/kg, was administered intraperitoneally 24–30 hr before the experiment and bilateral translumbar adrenalectomy was performed 1 hr before ouabain administration.

6. The animals in this group were treated in exactly the same way as in group 5 except that body temperature was maintained at approximately 37° C by means of an electric heating pad activated by a rectal thermometer through an appropriate relay circuit.

7. Reserpine, 5 mg/kg, was administered intraperitoneally 24–30 hr before the experiment and bilateral translumbar adrenalectomy was performed 1 hr before ouabain administration.

8. Hexamethonium, 2 mg/kg, was administered intravenously 10 min before ouabain administration.

9. Hexamethonium, 2 mg/kg, was administered intravenously to animals bilaterally adrenalectomized 1 hr previously. Ouabain was given 10 min after hexamethonium.

10. The right vagus nerve was stimulated to reduce sinus rate to 140–150 beats/min. For this purpose, mineral oil was introduced in the neck, and the temperature of the cervical tissues

maintained at 37° C by a heating lamp connected to an indwelling cervical thermometer via a relay circuit. Ten volt, 5 msec stimuli were applied to the distal end of the right vagus nerve. A stimulus frequency of 5–30 pulses/sec was used to depress heart rates to the desired level. The frequency of vagal stimulation, once established, was maintained throughout the entire experiment.

In all groups (1–10) ouabain, 125 μg/kg, was rapidly administered as a single intravenous dose. Ouabain, 250 μg/kg, was administered only to animals from groups 1, 2 and 5.

Animals were observed for development of ventricular rhythm disturbances up to 3 hr after the administration of ouabain. This time period was selected to reduce the influence of deterioration of the preparation on the responsiveness of the heart to ouabain.

The significance of differences between means was determined by Student's *t* test and differences in incidence of arrhythmia by the Chi square test for small populations. The standard error is given after each mean.

RESULTS

The effect of reserpine on the capacity of ouabain to induce ventricular rhythm disturbances

Reserpine depressed the capacity of ouabain, 125 μg/kg, to induce ventricular arrhythmia. In the control group, after ouabain was injected, ventricular extrasystoles occurred on the average in 2 min, ventricular tachycardia in 4.50 min and death in 11.3 min (Table 1). After 0.5 and 5 mg/kg reserpine, both the time to development of arrhythmia and the time to death were significantly prolonged ($P<0.05$). While the effect of the 5 mg/kg dose was not significantly different from that of the 0.5 mg/kg dose ($P>0.05$), in animals given 5 mg/kg reserpine, the time to death was generally longer and less variable. Thus, after 0.5 mg/kg reserpine, eight of 14 animals died after ouabain injection in a time interval within two standard deviations of the control mean (11.3 ± S.D. 6.1 min; 0–23.5 min). After 5 mg/kg reserpine, none of the animals died within this time interval. Furthermore, the standard error of the mean time to death induced by ouabain after 0.5 mg/kg reserpine was approximately 25% of the mean, while that of the mean time to death after 5 mg/kg reserpine was approximately 12%.

TABLE 1

THE EFFECT OF RESERPINE ON THE TOXICITY OF OUABAIN (125 μg/kg)

Ouabain, 125 μg/kg, was administered intravenously as a single dose to all animals. Reserpine (0·5 mg and 5 mg/kg) was administered intraperitoneally to separate groups of animals 24–30 hr before the experiment. Following the administration of ouabain, the time required for the development of the first ventricular extrasystole, of sustained ventricular tachycardia and of ventricular fibrillation was measured in all animals. Death was caused by ventricular fibrillation in all cases. The means and standard errors are given

Treatment	Cats (No.)	Time to first ventricular extrasystole (mean ± S.E.) (min)	Significance*	Time to ventricular tachycardia (mean ± S.E.) (min)	Significance*	Time to death (mean ± S.E.) (min)	Significance*
Control	24	2·00 ± 0·33		4·50 ± 0·48		11·3 ± 1·25	
Reserpine (0·5 mg/kg)	14	13·1 ± 3·7		23·3 ± 8·4		56·9 ± 14·4	
Reserpine (5 mg/kg)	10	14·4 ± 4·0		19·5 ± 4·8		76·4 ± 9·4	

* Means joined by the same vertical line are not significantly different. Those means not so joined differ significantly ($P<0·05$).

TABLE 2

THE EFFECT OF RESERPINE AND ADRENALECTOMY ON THE TOXICITY OF OUABAIN (250 μg/kg)

A single intravenous dose of ouabain, 250 μg/kg, was administered to all animals. Reserpine, 0·5 mg/kg, was administered intraperitoneally 24–30 hr before the experiment. In one group of cats pretreated with reserpine, bilateral translumbar adrenalectomy was performed 1 hr before the administration of ouabain. Following the administration of ouabain, the time to development of the first ventricular extrasystole, of sustained ventricular tachycardia and of ventricular fibrillation or asystole was measured in all animals. In three of five cats pretreated with reserpine and adrenalectomized, death was caused by ventricular asystole. All other animals died in ventricular fibrillation. The means and standard errors are given

Treatment	Cats (No.)	Time to first ventricular extrasystole (mean±S.E.) (min)	Significance*	Time to ventricular tachycardia (mean±S.E.) (min)	Significance*	Time to death (mean±S.E.) (min)	Significance*
Control	6	1·16±0·17†		2·00±0·26		4·00±0·68	
Reserpine (0·5 mg/kg)	11	1·19±0·44		2·82±0·47		5·09±0·72	
Reserpine (0·5 mg/kg) and adrenalectomy	5	6·20±2·50†		11·40±3·80		17·6 ±3·10	

* Means joined by the same vertical line are not significantly different. Those means not so joined differ significantly ($P<0·05$).

† P value of difference between these means is approximately equal to 0·05.

Although 0.5 mg/kg reserpine was effective in depressing arrhythmia induced by a single dose of 125 μg/kg ouabain, it was ineffective against arrhythmia induced by a single dose of 250 μg/kg ouabain (Table 2).

Influence of adrenalectomy on the toxicity of ouabain

Variable depletion of adrenal medullary catecholamines by small doses of reserpine may explain the greater variability in the capacity of ouabain to induce death following 0.5 mg/kg reserpine than following 5 mg/kg. Muscholl & Vogt (1958) have demonstrated in the cat that the level of adrenal medullary catecholamines is not readily affected by reserpine and that large doses of reserpine are needed to produce significant depletion. To explore the role of adrenal medullary catecholamines in ouabain-induced ventricular arrhythmia, ouabain was injected into cats that had not only been pretreated with reserpine but in which acute bilateral adrenalectomy (the entire gland was removed on both sides) had also been performed. The data are summarized in Table 3. Since in cats pretreated with 0.5 mg/kg reserpine and adrenalectomized, ventricular tachycardia and death did not usually occur during the 3 hr observation period, the time to development of ventricular rhythm disturbances could not be used as the means of comparison with other groups. The data are, therefore, expressed as incidence of arrhythmia or death. After 0.5 mg/kg reserpine, ventricular extrasystoles, ventricular tachycardia and death were observed in 100% of the animals. Even after 5 mg/kg reserpine, the incidence of ventricular extrasystoles, ventricular tachycardia and death was not altered. In animals pretreated with 0.5 mg/kg reserpine and also adrenalectomized, the incidence of death was significantly reduced from 100% to 17% (Chi square test, $P<0.05$). In animals previously given 5 mg/kg reserpine, adrenalectomy also resulted in significantly greater protection (Chi square test; $P<0.05$). After pretreatment with reserpine alone, nine of 10 animals died, while only two of six cats also adrenalectomized succumbed

(Table 3). The nine cats given reserpine alone all died in ventricular fibrillation, while the two also adrenalectomized died in asystole. These results suggest that even after 5 mg/kg reserpine maximum protection is not obtained if the adrenal gland is left *in situ*.

TABLE 3

THE INFLUENCE OF RESERPINE AND ADRENALECTOMY ON THE TOXICITY OF OUABAIN (125 μg/kg)

A single intravenous dose of ouabain, 125 μg/kg, was administered to all animals. Reserpine, 0·5 or 5 mg/kg, was administered intraperitoneally to some animals 24–30 hr before the experiment. Bilateral translumbar adrenalectomy was performed approximately 1 hr before the administration of ouabain. The incidence of ventricular extrasystoles, ventricular tachycardia and ventricular fibrillation or asystole is expressed in terms of %. Death was caused by ventricular fibrillation except in the two cats pretreated with 5 mg/kg reserpine and adrenalectomized who died in asystole

Treatment	Animals (No.)	Incidence of ventricular extrasystole (%)	Incidence of ventricular tachycardia (%)	Incidence of death (%)
Control	24	100	100	100
Adrenalectomy	6	100	100	100
Reserpine (0·5 mg/kg)	14	100	100	100
Reserpine (5 mg/kg)	10	100	100	90
Reserpine (0·5 mg/kg) and adrenalectomy	6	67	50	17*
Reserpine (5 mg/kg) and adrenalectomy	6	67	67	33*

* Significantly different from the control and the group pretreated with the same dose of reserpine (Chi square test: $P<0·05$).

The added protection afforded by adrenalectomy in animals pretreated with reserpine was also evident when the large dose of ouabain (250 μg/kg) was used. After this dose of ouabain, ventricular rhythm disturbances occurred within the 3 hr observation period, and it was possible to determine the time to development of ventricular rhythm disturbances in all cases. The data are summarized in Table 2. In these animals, reserpine pretreatment did not influence the time to the development of arrhythmia and the time to death. However, adrenalectomy performed after reserpine pretreatment resulted in significant prolongation of the time to the development of ventricular rhythm disturbances ($P<0.05$). Although 250 μg/kg ouabain proved lethal to 100% of the animals regardless of treatment, in those animals adrenalectomized following the administration of reserpine, the mode of death was affected. While the controls as well as those animals treated with reserpine died in ventricular fibrillation, three of five animals also adrenalectomized died in asystole. Erlij & Mendez (1964) observed similar changes in the mode of death caused by digitalis following " exclusion " of the adrenergic nervous system.

The effect of adrenalectomy in animals pretreated with reserpine could be attributed to an increase in extracellular potassium consequent to the sudden removal of the influence of mineralocorticoids. This seems unlikely since adrenalectomy alone did not provide any protection against ventricular rhythm disturbances induced by ouabain. For example, in the six adrenalectomized animals, time to death was 13.7 ± 2.2 min compared with 11.3 ± 1.25 min in 24 control animals ($P>0.05$). All the adrenalectomized animals died in ventricular fibrillation (Table 3).

Influence of heart rate on the capacity of ouabain to induce arrhythmia

Since reserpine produced a marked decrease in heart rate in all doses (Table 5), it is possible that its protective action against rhythm disorders induced by ouabain is related

to this effect. To test this possibility, other procedures—namely, hexamethonium and continuous vagal stimulation—were employed to reduce the heart rate. The data are summarized in Table 4. After hexamethonium, although the heart rate was reduced to 163 beats/min, the time to ventricular extrasystoles, ventricular tachycardia and death was not affected. Furthermore, the capacity of ouabain to induce arrhythmia against a background of slow heart rates, on the average 143 beats/min, produced by continuous vagal stimulation was not significantly different from that in the control or the hexamethonium-treated group. However, in those animals pretreated with reserpine whose heart rates were of the same order of magnitude as those produced by hexamethonium or continuous vagal stimulation, there was a striking reduction in the toxicity of ouabain (Table 4).

TABLE 4

THE INFLUENCE OF HEART RATE ON THE TOXICITY OF OUABAIN (125 μg/kg)

A single intravenous dose of ouabain, 125 μg/kg, was administered to all animals. The time to the development of the first ventricular extrasystole, of sustained ventricular tachycardia and of ventricular fibrillation was measured. In all animals death was caused by ventricular fibrillation. Reserpine (5 mg/kg) was administered to some animals 24–30 hr before the experiment. Hexamethonium (2 mg/kg) was administered 10 min before the administration of ouabain. In one group of animals, the right vagus nerve was stimulated to reduce heart rate to approximately that observed in reserpine pretreated animals. The means and standard errors are given

Treatment	Cats (No.)	Heart rate (mean±S.E.) (beats/min)	Time to first ventricular extrasystole (mean±S.E.) (min)	Time to ventricular tachycardia (mean±S.E.) (min)	Time to death (mean±S.E.) (min)
Control	24	229±6·7	2·00±0·33*	4·50±0·48*	11·3±1·25*
Reserpine (5 mg/kg)	8	155±7·5*	14·37±4·98	21·50±6·53	79·1±9·73
Hexamethonium (2 mg/kg)	6	163±4·3*	2·33±0·66*	6·33±0·56*	15·6±2·53*
Continuous vagal stimulation	6	143±5·1†	1·20±0·76*	5·16±1·11*	9·7±2·32*

* Indicates that the mean is not significantly different from any other mean marked with * in the same column ($P>0·05$).

† Significantly differs from all means in the column except that for reserpine ($P<0·05$).

In animals adrenalectomized following 5 mg/kg reserpine, the heart rate was lower than in those receiving 5 mg/kg reserpine alone ($P<0.05$) (Table 5); in the adrenalectomized group, there was also a greater degree of protection against rhythm disturbances induced by ouabain. On the other hand, although the heart rate in animals pretreated with 0.5 mg/kg reserpine and also adrenalectomized did not differ from the rate in animals given 0.5 mg/kg reserpine alone ($P>0.05$) (Table 5), adrenalectomy still caused additional protection against rhythm disorders induced by ouabain. This protection was of the same order of magnitude as that observed in adrenalectomized animals after 5 mg/kg reserpine (Table 3). It appears, therefore, that slowing of heart rate *per se* does not seem to influence ouabain toxicity, nor does it appear to account for the reserpine effect in depressing ventricular arrhythmia induced by ouabain.

The influence of blood pressure on the capacity of ouabain to induce arrhythmia

One half mg and 5 mg/kg reserpine significantly lowered the blood pressure from 162 mm Hg to 135 mm Hg and 99.4 mm Hg respectively ($P<0.05$). Five mg/kg reserpine

caused a lower blood pressure than 0.5 mg/kg ($P=0.05$). Blood pressures comparable to those observed after 5 mg/kg reserpine were noted in animals given hexamethonium after bilateral adrenalectomy ($P>0.05$). Since the latter were not protected against ouabain-induced ventricular arrhythmia it would appear that the reduction in blood pressure following reserpine does not account for the effect of reserpine on arrhythmia induced by ouabain. However, the blood pressure in adrenalectomized animals pretreated with reserpine (0.5 mg or 5 mg/kg) was significantly lower than that observed in any other group ($P<0.05$), and the possibility exists that the lowering of blood pressure following adrenalectomy accounts for its additional protection against arrhythmia induced by ouabain.

TABLE 5

THE EFFECT OF PHARMACOLOGICAL AND SURGICAL PROCEDURES REDUCING ADRENERGIC NERVOUS ACTIVITY ON HEART RATE, BLOOD PRESSURE AND BODY TEMPERATURE

The means and standard errors are given. The numbers in parentheses indicate the number of animals in each group. Reserpine (0·5 mg/kg or 5 mg/kg) was administered 24–30 hr before the measurements. Hexamethonium was administered 10 min before the measurements. Adrenalectomy was performed approximately 1 hr before the measurements. See text for statistical comparisons

Treatment	Average heart rate (beats/min)	Average mean arterial blood pressure (mm Hg)	Average body temperature (°C)
Control	229± 6·7 (24)	162 ± 5·9 (24)	37·4±0·23 (21)
Reserpine (0·5 mg/kg)	154± 8·1 (14)	135 ± 9·4 (11)	35·1±0·33 (14)
Reserpine (5 mg/kg)	149± 7·5 (10)	99·4±11·8 (7)	34·3±0·81 (10)
Hexamethonium (2 mg/kg)	163± 4·3 (6)	127 ±16·2 (6)	37·3±0·42 (6)
Adrenalectomy	218±13·4 (10)	120 ±11·4 (10)	35·4±0·64 (10)
Adrenalectomy and reserpine (0·5 mg/kg)	126±12·7 (8)	58·7± 8·2 (7)	34·0±0·76 (7)
Adrenalectomy and reserpine (5 mg/kg)	101± 7·9 (6)	45·0±12·5 (6)	32·1±0·63 (6)
Adrenalectomy and hexamethonium (2 mg/kg)	177±12·0 (6)	97·4±13·8 (6)	35·7±0·71 (6)

In the experiments in which adrenergic nervous activity was reduced by reserpine or hexamethonium, ouabain caused a rise in blood pressure similar to that observed in animals not receiving reserpine or hexamethonium. This confirms our previous results obtained in anaesthetized dogs with surgically induced heart block (Roberts *et al.*, 1963). In these experiments, the increase in blood pressure produced by acetylstrophanthidin was not affected by ganglionic or adrenergic neuronal blockade.

Influence of body temperature on the capacity of ouabain to induce arrhythmia

Withrington & Zaimis (1961) have reported that reserpine causes a fall in body temperature. In the present study, the body temperature of anaesthetized animals pretreated with 0.5 mg or 5 mg/kg reserpine was lower than the temperatures observed in the anaesthetized control group (Table 5). In the control group, the average body temperature was 37.4° C while in animals pretreated with 0.5 mg/kg reserpine the body temperature was 35.1° C and in those treated with 5 mg/kg, it was 34.3° C ($P<0.05$). Thus a fall in body temperature, although not very large, was associated with the protective effect of reserpine. The body temperatures after adrenalectomy as well as after adrenalectomy and hexamethonium were also lower than the control temperatures

($P<0.05$). However, they were not significantly different from those observed in cats pretreated with either dose of reserpine ($P>0.05$). Since adrenalectomy alone as well as adrenalectomy combined with the administration of hexamethonium did not afford protection against rhythm disturbances induced by ouabain, the fall in body temperature after reserpine does not seem to account for the protection observed after the drug. This is supported by the finding that in six cats pretreated with 0.5 mg/kg reserpine and adrenalectomized, although body temperature was maintained at control levels (37.4 ± 0.23° C) by the use of a heating pad, the degree of protection was comparable to that observed when body temperature was permitted to fall. Thus four of six cats in the "heated group" and five of six cats in the group whose temperature was permitted to fall survived the 3 hr observation period. It appears that under the conditions of this study, body temperature does not influence the capacity of ouabain to induce ventricular rhythm disorders in the cat, nor does it account for the protective effect of reserpine against arrhythmia induced by ouabain.

The influence of sex on ouabain toxicity

Another factor which might have influenced ouabain toxicity is the difference in susceptibility of male and female animals to the action of ouabain to induce arrhythmia (Rodensky & Wasserman, 1964). The results of the present study were grouped so that comparisons could be made between male and female cats. The data are summarized in Table 6. It should be noted that the selection of animals for all experiments throughout the study was completely random and they were grouped according to sex for this comparison only. It is clear from the data that there is no significant difference in ouabain toxicity in male and female cats untreated or pretreated with reserpine.

TABLE 6

A COMPARISON OF OUABAIN TOXICITY IN MALE AND FEMALE CATS

A single intravenous dose of ouabain, 125 μg/kg, was administered to all animals. Reserpine (5 mg/kg) was administered 24–30 hr before the experiment. The time to the development of the first ventricular extrasystole of sustained ventricular tachycardia and of ventricular fibrillation was measured in all animals. Death was caused by ventricular fibrillation. The means and standard errors are given in all cases. One male cat pretreated with 5 mg/kg reserpine did not die and therefore was not included in the calculations of the mean time to death. The difference in the mean times to development of the rhythm disturbances between male and female cats is not significant ($P>0.05$)

Treatment	Cats (No.)	Sex	Time to first ventricular extrasystole (mean ± S.E.) (min)	Time to ventricular tachycardia (mean ± S.E.) (min)	Time to death (mean ± S.E.) (min)
Control	8	M	1·63 ± 0·42	4·25 ± 0·73	15·4 ± 5·5
	16	F	3·12 ± 0·87	5·39 ± 0·83	15·1 ± 3·4
Reserpine	5	M	10·3 ± 2·45	18·5 ± 5·44	83·3 ± 8·8
(5 mg/kg)	5	F	18·9 ± 7·54	27·4 ± 4·64	62·2 ± 6·9

DISCUSSION

It has been suggested that reduction in heart rate may account for the reduced capacity of digitalis to induce arrhythmia after "exclusion" of adrenergic influences (Erlij & Mendez, 1964; Boyajy & Nash, 1966). The results of the present study show that reserpine antagonism of the action of ouabain to induce arrhythmia is not related to the fall in heart rate that followed the administration of reserpine. In animals, in which

heart rate was reduced to the same level as in the reserpine pretreated group by physiological means (continuous vagal stimulation) or by pharmacological means (ganglionic blockade with hexamethonium) ouabain toxicity was not affected. Furthermore, the added protection afforded by adrenalectomy in cats pretreated with reserpine was not associated with a further decrease in heart rate. Thus reduction in heart rate does not appear to influence the capacity of ouabain to induce ventricular arrhythmia. This conclusion differs from that of Boyajy & Nash (1966), who reported that there was a correlation between heart rate and the capacity of the digitalis materials to induce rhythm disturbances. It should be pointed out, however, that although all the surgical and pharmacological procedures they employed to exclude adrenergic nervous activity reduced the heart rate, they did not in every case result in protection against ouabain-induced ventricular tachycardia. Cervical transection (C_6), pithing of the spinal cord and cervical transection or chlorisondamine administration did not influence the capacity of ouabain to induce ventricular tachycardia. There did appear to be a correlation between the capacity of ouabain to induce ventricular fibrillation and the level of heart rate. Nevertheless, it should be emphasized that in their study, the heart rate was reduced in all cases by diminishing adrenergic nervous influences. It is possible that the reduction in heart rate and in the capacity of ouabain to induce fibrillation are two separate manifestations of the same action—namely, diminution in adrenergic nervous activity. In the present study, using, as a cholinergic mechanism, vagal stimulation, to slow heart rate, there appeared to be no direct relationship between the level of heart rate and the capacity of ouabain to induce ventricular rhthym disturbances. It is possible that if Boyajy & Nash (1966) had not only slowed the heart by excluding adrenergic influences but also by stimulating the vagus, they would have observed that the level of heart rate does not necessarily affect the capacity of ouabain to induce arrhythmia.

The finding that hexamethonium does not influence ouabain toxicity confirms the observations of Roberts *et al.* (1963) that ganglionic blockade by this agent does not affect the capacity of acetylstrophanthidin to induce ventricular tachycardia in dogs with surgically induced heart block. Our finding also agrees with the observation of Boyajy & Nash (1966) that ganglionic blockade by chlorisondamine does not influence the capacity of ouabain to induce ventricular tachycardia in the dog. While in the present study, the results indicate that in the cat hexamethonium does not provide protection against ouabain-induced ventricular fibrillation, Boyajy & Nash (1966) did demonstrate protection by chlorisondamine in the dog.

The findings of the present study confirm the observation of Withrington & Zaimis (1961) that reserpine reduces body temperature. Nevertheless, this effect of reserpine does not seem to be involved in the action of the drug which diminishes the capacity of ouabain to induce arrhythmia. After adrenalectomy, or adrenalectomy and the administration of hexamethonium, animals were not protected against rhythm disturbances produced by ouabain although their body temperature was lowered to the same level as that produced by reserpine. Furthermore, preventing the fall in body temperature that occurs in animals pretreated with reserpine and adrenalectomized did not modify the protection afforded by these procedures against rhythm disturbances induced by ouabain. These results are contrary to those of previous investigators (Beyda *et al.*, 1961), that reducing body temperature of dogs to 24–28° C diminished the reactivity of the ventricle to

digitalis. It is possible that the failure to observe an effect of hypothermia on ventricular arrhythmia induced by ouabain in the present study is due to the fact that temperatures did not fall below 30° C in any of the animals tested.

It has been proposed that the development of ventricular fibrillation following hydrocarbon-adrenaline administration is related, at least in part, to a rise in blood pressure (Moe *et al.*, 1948 ; Nickerson & Nomaguchi, 1949). It is possible that the level of blood pressure may also influence the capacity of the digitalis glycosides to produce ventricular rhythm disorders. Since reserpine caused a fall in blood pressure, the lower level of blood pressure might account for the protective action of reserpine against rhythm disorders induced by ouabain. However, in animals whose blood pressure was reduced to comparable levels following adrenalectomy and the administration of hexamethonium, reactivity to ouabain was not affected. Therefore it seems unlikely that reserpine-induced hypotension is responsible for the anti-digitalis effect of the drug. This finding agrees with the observation of Erlij & Mendez (1964) that hypotension induced by phenoxybenzamine did not alter the ventricular response to digitalis. However, adrenalectomy performed in animals pretreated with reserpine resulted in the lowest blood pressures and in the greatest degree of protection against arrhythmia induced by ouabain. Since comparable levels of blood pressure were not obtained in any of the other groups, the level of blood pressure cannot be excluded as a factor in the added protection afforded by adrenalectomy in animals pretreated with reserpine.

While the above considerations indicate that the body temperature, heart rate and blood pressure are probably not important factors in the induction of ventricular arrhythmia by digitalis or in the antidigitalis action of reserpine, the results of the present study do not exclude the possibility that the reserpine action is due to direct depression of ectopic foci. Direct depression has been suggested as the mode of action for reserpine by Boyajy & Nash (1965). However, reserpine pretreatment does not produce a quinidine-like effect on transmembrane action potential of rabbit atrium (Vaughan Williams, 1958). Furthermore, unlike quinidine, reserpine does not protect against arrhythmia induced by the two stage coronary ligation method of Harris (Maling, Cohn & Highman, 1959). In addition, Ciofalo, Levitt & Roberts (1966) have shown that while reserpine in large doses (5 mg/kg) depressed the capacity of acetylstrophanthidin to produce arrhythmia, the capacity of catecholamines to induce arrhythmia was not affected. Therefore it seems unlikely that reserpine protects against digitalis-induced ventricular arrhythmia by directly depressing myocardial reactivity to digitalis.

One of the most striking findings of this study is the observation that the action of reserpine to depress digitalis-induced ventricular arrhythmia was enhanced by adrenalectomy. It is known that, in the cat, depletion of catecholamines from the adrenal medulla by reserpine is not readily accomplished even when large doses are used (Muscholl & Vogt, 1958). The results of the present study show that in animals pretreated with 0.5 mg/kg reserpine, the degree of protection was greatly increased when they were also adrenalectomized. In fact, the degree of protection was even greater than that seen after 5 mg/kg reserpine. This suggests that 5 mg/kg reserpine does not produce complete depletion of the adrenal medulla in 24–30 hr. This is supported by the finding that adrenalectomy performed in animals pretreated with 5 mg/kg reserpine provided greater protection against arrhythmia induced by ouabain than that afforded by reserpine

alone. It may be argued that the added effect of adrenalectomy is due to an increase in the potassium content of the extracellular fluid in the absence of mineralocorticoids, but this does not seem likely since adrenalectomy alone is ineffective. The possibility exists that the adrenal medulla acts as a source of catecholamines in animals acutely treated with reserpine. Ouabain might either cause release of catecholamines from this storage site or the heart may take up circulating catecholamines spontaneously released from the incompletely depleted medulla. There is evidence which indicates that the heart depleted of catecholamines by reserpine is still capable of taking up catecholamines (Roberts *et al.*, 1963; Iversen, Glowinski & Axelrod, 1965). Such cardiac catecholamines may be available for digitalis action on the heart (Roberts *et al.*, 1963). In any event, adrenalectomy would result in reduced catecholamine availability and therefore in greater protection.

SUMMARY

1. Pretreatment of cats with 0.5 mg and 5 mg/kg reserpine prolonged the time to the development of ventricular rhythm disturbances induced by a single intravenous dose of 125 μg/kg ouabain; the larger dose of reserpine produced a more uniform effect. Reserpine did not appreciably affect the capacity of the large dose of ouabain (250 μg/kg) to induce rhythm disturbances.

2. Bilateral adrenalectomy did not influence the capacity of ouabain to induce arrhythmia. However, performed in animals pretreated with reserpine, adrenalectomy greatly enhanced the protective effect of reserpine against arrhythmia induced by both 125 and 250 μg/kg ouabain.

3. Changes in heart rate, body temperature and blood pressure did not modify the capacity of ouabain to induce ventricular arrhythmia nor did they account for the protective action of reserpine. However, in animals pretreated with reserpine the level of blood pressure may have been a factor in the added protection afforded by adrenalectomy against ouabain toxicity.

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