Br. J. Pharmac. Chemother. (1966), **28**, 44–50.

SOME ASPECTS OF THE ANTI-ARRHYTHMIC ACTIVITY OF RESERPINE*

BY

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(*Received May* 10, 1966)

Although several studies have indicated that reserpine pretreatment antagonizes digitalis-induced ventricular arrhythmia (Roberts, Ito, Reilly & Cairoli, 1963; Erlij & Mendez, 1964; Boyajy & Nash, 1965), the nature of its antidigitalis action remains controversial. Boyajy & Nash (1965) attributed the antiarrhythmic properties of reserpine to "quinidine-like" myocardial depression, whereas Roberts *et al.* (1963), and Roberts, Ehrreich & Levitt (1965), suggested that the blockade was due to the action of reserpine in diminishing the influence of the adrenergic nervous system on the heart. If the antidigitalis action of reserpine is the result of "quinidine-like" myocardial depression, then reserpine should, like quinidine, have a broad spectrum of anti-arrhythmic activity. There is evidence which suggests that catecholamine-induced arrhythmias are not markedly affected by reserpine pretreatment (Fleming, 1962). However, since these experiments were not performed under experimental conditions comparable to those in which the antidigitalis effect of reserpine was observed, it is still uncertain whether reserpine, like quinidine, depresses catecholamine—as well as digitalis-induced arrhythmias. The experiments in the present study were designed to compare the effect of reserpine on digitalis- and on catecholamine-induced ventricular arrhythmia under similar experimental conditions. A preliminary report of this work has already been published (Levitt, Ciofalo and Roberts, 1966).

METHODS

Experiments were performed in cats anaesthetized with Dial-urethane (Ciba) (0.6 ml./kg), given intraperitoneally. Animals which were pretreated with reserpine 24 to 30 hr before the study, received Dial-urethane 0.3 ml./kg intraperitoneally. Changes in cardiac rhythm were recorded in Lead II of the electrocardiogram. In some animals, mean carotid arterial pressure was monitored on a mercury manometer; body temperature was measured rectally by means of a laboratory thermometer.

Induction of Arrhythmia. The "vagus-amine" test described by Roberts & Baer (1960) was used to study the ventricular arrhythmia induced by catecholamines and acetylstrophanthidin. This method is based on the fact that the structure in the heart possessing the highest inherent rhythmicity serves as the cardiac pacemaker. The inherent rhythmicity of the His-Purkinje system and the

* Supported by a grant from the U.S.P.H.S. No. HE-01165.

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‡ Work done during the tenure of a Post-doctoral Fellowship from the U.S.P.H.S. (2G-99).

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ventricle is normally masked by the higher rhythmicity of the S-A node, and becomes manifest only when either A-V block is present or the sinus node is slowed in one way or another to a point below the potential rate of the lower centres, giving rise to so-called ventricular "escape." In animals whose sinus rate is slowed by vagal stimulation to a "critical" point—namely, a rate just short of that at which the ventricle escapes (critical sinus rate)—relatively small increases in subatrial rhythmicity are needed for these centres to assume pacemaker dominance (Roberts, Standaert, Kim & Riker, 1956).

Following bilateral vagotomy, sinus activity was depressed by the application of 10 v, 5 msec stimuli to the distal end of the right vagus nerve for a period of 20 sec. To produce the necessary critical sinus rate, stimulus frequency was varied from 1 to 30 pulses/sec. The stimulus frequency varied from animal to animal, but, in each case, sinus activity was depressed to the same level, in that the rate was just above that which permitted ventricular escape. Therefore, the degree to which ventricular rhythmicity must be raised to usurp sinus dominance was approximately the same in all instances.

The smallest dose of adrenaline and isoprenaline which in combination with the critical sinus rate called for at least five ectopic beats was determined by a series of test doses. In the first trial, the dose of adrenaline was 1 or 2 μg/kg, whereas the dose of isoprenaline was 0.25 or 0.5 μg/kg. In the next trial, depending on the response to the previous dose, a dose two times as large or half as large as the previous dose of the amine was employed. This procedure was followed until the smallest dose of the amine causing arrhythmia was determined. In the test, the amine was injected 20 sec before the onset of vagal stimulation and at least 10 min were allowed to elapse between each injection of the catecholamine. Adrenaline and isoprenaline were administered by rapid intravenous injection.

The smallest dose of acetylstrophanthidin required to produce arrhythmia during vagal stimulation was determined in the following manner. Acetylstrophanthidin was administered intravenously as a loading dose of 20 μg/kg. This initial dose was followed at 5-min intervals by doses of 10 μg/kg until arrhythmia appeared during the periods of critical sinus slowing. The vagus nerve was stimulated 4 min after each dose of acetylstrophanthidin. It is important to note that acetylstrophanthidin, in any of the doses used, did not induce arrhythmias in the absence of vagal stimulation.

Evaluation of anti-arrhythmic activity. The anti-arrhythmic action of reserpine and pronethalol is reflected in the increment in the dose of the catecholamine or acetylstrophanthidin necessary to produce ventricular escape. Some anti-arrhythmic agents have the capacity to lower background ventricular rhythmicity; therefore, to raise the rhythmicity of the ventricle to achieve pacemaker dominance larger doses of the arrhythmia-inducing agent might have been required than would have been needed if the prevailing rhythmicity of the ventricle had remained at a higher level. This was not a factor, since, following the administration of reserpine or pronethalol, the sinus rate was always reduced to the "critical" level.

Administration of reserpine and pronethalol. In one series of animals, reserpine was administered intravenously in a dose of 5 mg/kg. The reactivity of the ventricle to acetylstrophanthidin was tested in some animals 1 hr and in others 4 hr after the administration of reserpine (Fig. 1).

A separate group of animals was pretreated with reserpine 5 mg/kg intraperitoneally 24 to 30 hr before the experiment. To induce arrhythmia, acetylstrophanthidin was administered to some animals (Fig. 1) and adrenaline or isoprenaline (Table 1) was administered to others.

In still another group of cats, pretreated with reserpine (1 mg/kg) given intraperitoneally 24 to 30 hr before the study, reserpine (5 mg/kg) was administered intravenously and the animals challenged with adrenaline every half-hour for 4 hr (Table 2).

Pronethalol, 5 mg/kg, was administered intravenously at a rate of 2 mg/min to normal cats and animals pretreated with reserpine, 5 mg/kg, intraperitoneally 24 to 30 hours before the experiment. The reactivity of the ventricle to isoprenaline was tested 10 min after the administration of pronethalol.

The standard error of the mean is given and the significance of the difference between means was determined using the Student "t" test.

RESULTS

Effect of reserpine on catecholamine-induced arrhythmia. The smallest dose of the catecholamine necessary to produce arrhythmia in a setting of critical sinus rate was not affected by reserpine pretreatment. Reserpine (5 mg/kg i.p.) administered 24 to 30 hr before the experiment did not diminish the responsiveness of the ventricle to adrenaline or isoprenaline (Table 1). When reserpine was administered intravenously in

TABLE 1

INFLUENCE OF RESERPINE PRETREATMENT ON DOSE OF CATECHOLAMINE NECESSARY TO PRODUCE ARRHYTHMIA IN COMBINATION WITH VAGAL STIMULATION

The means and standard errors are given. The numbers in parentheses indicate the number of experiments. Reserpine (5 mg/kg) was administered intraperitoneally 24 to 30 hr before the experiment. The dose of isoprenaline and adrenaline was determined in a series of control animals and in a series of animals pretreated with reserpine. The frequency of vagal stimulation needed to produce " critical sinus rate " was used in every case. The dose of isoprenaline and adrenaline is expressed in terms of the free base

Pretreatment	Minimum dose of catecholamine needed to produce arrhythmia (μg/kg)	
	Adrenaline	
—	1·25±0·44 (5)	$P>0·05$
Reserpine	1·69±0·25 (8)	
	Isoprenaline	
—	0·32±0·095 (5)	$P>0·05$
Reserpine	0·15±0·040 (4)	

acute experiments without pretreatment the number of ectopic beats produced by a given dose of adrenaline after reserpine fluctuated widely and made quantification difficult, although in no instance did the number of ectopic beats fall below that produced before reserpine administration. When the experimental design was modified by pretreating the animals with reserpine, 1 mg/kg intraperitoneally, and approximately 24 hr later injecting reserpine, 5 mg/kg intravenously, these fluctuations were avoided. However, the reactivity to adrenaline was unaffected by all these methods of reserpine dosage. Furthermore, the response to adrenaline remained remarkably constant during the course of the experiment (Table 2).

TABLE 2

EFFECT OF RESERPINE ON ADRENALINE-INDUCED ARRHYTHMIA IN ANIMALS PRETREATED WITH RESERPINE

The means and standard errors are given. All animals were pretreated with reserpine (1 mg/kg, intraperitoneally) 24 to 30 hr before the experiment. The dose of adrenaline necessary to produce ventricular ectopic beats in combination with critical sinus rate was determined. The average dose of adrenaline was 1·70±0·16 μg/kg (8). Reserpine (5 mg/kg) was injected intravenously immediately after this determination. The animals were challenged at the time intervals indicated in the table with the same dose of adrenaline that was used before the reserpine injection. Each animal served as its own control

Time after reserpine injection (hr)	Ectopic beats (No.)	Animals (No.)
0	10·0±1·3	8
0·5	13·0±2·5	8
1	11·5±2·5	8
2	12·5±3·2	8
3	12·0±2·9	8
4	11·0±3·4	7

Effect of pronethalol on catecholamine-induced arrhythmia in reserpine-pretreated animals. To determine whether it is possible to block catecholamine arrhythmias in reserpine-pretreated cats, a β-adrenergic blocking drug, pronethalol, was administered. Although reserpine *per se* failed to depress the reactivity of the ventricle to catecholamines, pronethalol administered to animals pretreated with reserpine 24 to 30 hr before the experiment was effective. The average dose of isoprenaline necessary to produce arrhythmia in four reserpine-pretreated animals was increased by pronethalol, 5 mg/kg, from 0.15 ± 0.04 μg/kg to 2.5 ± 0.82 μg/kg ($P<0.05$). In five animals not treated with reserpine, pronethalol also raised the dose of isoprenaline necessary to produce arrhythmia from an average of 0.32 ± 0.095 μg/kg to an average of 2.7 ± 0.37 μg/kg ($P<0.05$). These observations agree with those of Black, Duncan & Shanks (1965), who reported that pronethalol blocked adrenaline-induced arrhythmia in animals pretreated with syrosingopine.

Effect of reserpine on acetylstrophanthidin-induced arrhythmia. Reserpine (5 mg/kg) administered intraperitoneally 24 to 30 hr before the experiment increased the dose of acetylstrophanthidin necessary to produce arrhythmia in combination with critical sinus rate. The data obtained in a series of 15 cats revealed that reserpine caused an increase in the acetylstrophanthidin threshold dose from an average of 28.8 μg/kg to an average of 64.0 μg/kg (Fig. 1).

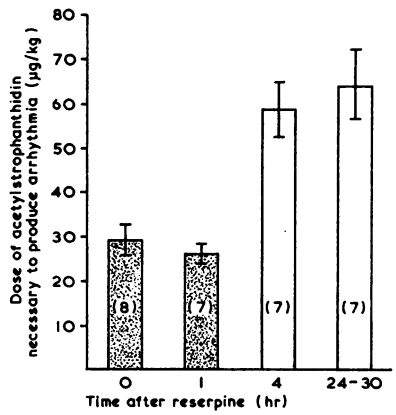

Fig. 1. Reserpine (5 mg/kg) was administered intravenously in the experiments performed 1 and 4 hr after its injection, while it was given intraperitoneally in the experiments conducted 24 to 30 hr after it was administered. The dose of acetylstrophanthidin necessary to produce arrhythmia was determined in the setting of critical sinus rate in the control and reserpine-treated animals. The vertical lines represent the standard error of the mean, and the numbers in parentheses the number of experiments.

The effect of reserpine (5 mg/kg) administered intravenously was determined 1 and 4 hr after its injection. The results are also summarized in Fig. 1. One hr after reserpine the reactivity of the ventricle to acetylstrophanthidin was not significantly affected. However, 4 hr after reserpine, the response to acetylstrophanthidin was markedly

diminished. In fact, the dose of acetylstrophanthidin necessary to produce arrhythmia in the setting of critical sinus rate was of the same order of magnitude as that needed 24 to 30 hr after the administration of reserpine. In a series of control cats prepared in exactly the same manner as the reserpine-pretreated animals, except that they did not receive reserpine, the dose of the acetylstrophanthidin required to induce arrhythmia shortly after the completion of surgery was 28.8 ± 3.25 μg/kg, while in cats tested 4 hr after surgery it was 23.3 ± 2.14 μg/kg. Thus the protection observed 4 hr after reserpine was not due to spontaneous changes in the experimental preparation.

Other effects of reserpine. One hr after the intravenous administration of reserpine (5 mg/kg), heart rate was not significantly different from that observed in the control group 1 hr after surgery (Table 3). After an interval of 4 hr, while the heart rate in both the control and reserpine-pretreated animals was markedly diminished, the heart rate in the reserpine-treated animals was significantly slower than that of the control group.

TABLE 3

EFFECT OF RESERPINE ON HEART RATE, BLOOD PRESSURE AND BODY TEMPERATURE

The means and standard errors are given. Reserpine, 5 mg/kg, was injected intravenously and measurements were made 1 and 4 hr after its administration. These data were obtained from some of the cats anaesthetized with Dial-urethane which were employed in the study of acetylstrophanthidin. The control measurements were made in cats which were prepared in exactly the same way as the reserpine-pretreated cats, except that they did not receive reserpine. The time 0 indicates the time that surgery was completed. The time interval indicated for the reserpine-treated group represents the period that had elapsed from the time of reserpine injection, whereas in the control series the time interval represents the period after the completion of surgery

Treatment	Cats (No.)	Time interval (hr)	Heart rate (beats/min)	Blood pressure (mm Hg)	Body temperature (°C)
Control	6	0	201·0±13·8	170·0±12·3	36·5±0·23
Control	6	1	187·5±13·4	150·3± 8·5	35·3±0·28
Reserpine	6	1	166·7± 8·6	96·0± 5·8*	35·6±0·54
Control	6	4	159·0±12·5	129·7±12·2	32·7±0·32
Reserpine	5	4	129·0± 5·3*	75·6±12·0*	33·8±0·46

* Significantly different from the control measured after the same time interval. ($P<0·05$)

After intervals of 1 and 4 hr, blood pressure in the reserpine-treated group was significantly lower than that of the control group ($P<0.05$). However, the blood pressure in both control and reserpine-treated groups after 4 hr did not differ significantly from that observed in these animals after 1 hr ($P>0.05$).

A significant fall in body temperature was noted after an interval of 4 hr in reserpine-treated as well as control animals. Most striking is the fact that the fall in body temperature following reserpine did not differ from that which occurred spontaneously in the controls. Roberts *et al.* (1963, 1965), showed that 24 hr after reserpine (5 mg/kg), heart rate and blood pressure were significantly lower than in control animals; body temperature, on the other hand, was not significantly different. These measurements were performed ½ to 1 hr after Dial-urethane was administered.

DISCUSSION

Most striking is the finding that reserpine pretreatment did not influence arrhythmia induced by catecholamines whereas it antagonized arrhythmia induced by acetylstrophanthidin. Even when reserpine was administered intravenously in animals pretreated with

the drug 24 to 30 hr before the experiment catecholamine-induced arrhythmia was not inhibited. Clearly, the anti-arrhythmic spectrum of reserpine is limited, compared with that of quinidine. Quinidine blocks arrhythmia induced by both catecholamine (Roberts & Baer, 1960) and acetylstrophanthidin (Lucchesi & Shivak, 1964 ; Levitt, Ehrreich & Roberts, 1964). Furthermore, quinidine suppresses arrhythmias produced by coronary ligation (Harris, Estandía, Ford & Tillotson, 1951), whereas reserpine pretreatment does not influence the development or nature of the arrhythmia (Maling, Cohn & Highman, 1959). Quinidine affects the rate of rise, overshoot and repolarization time of the transmembrane action potential (Vaughan Williams, 1958a), while reserpine does not (Vaughan Williams, 1958b). The evidence suggests, therefore, that reserpine is not a quinidine-like agent and that its antagonism of digitalis-induced arrhythmia represents a more specific action.

It has been suggested that the antagonism of the digitalis arrhythmia by reserpine is due to diminished adrenergic nervous activity (Roberts *et al.*, 1963 ; Levitt *et al.*, 1964), though Boyajy & Nash (1965) pointed out that since reserpine is effective against digitalis arrhythmia just 4 hr after its administration, it is unlikely that this effect could be due to reduction in adrenergic nervous activity. In the present study also, reserpine was shown to be effective against digitalis arrhythmia 4 hr after injection, although it was not effective 1 hr after injection. This indicates that the effect of reserpine required time to develop. While generally a 24-hr period is allowed to elapse to obtain catecholamine depletion, it is possible to develop marked depletion even after 4 hr (Dahlström, Fuxe & Hillarp, 1965). Thus, only 4 hr after pretreatment, the antidigitalis effect of reserpine could still be due to a diminution in adrenergic nervous activity in the heart.

Some investigators have noted a fall in body temperature following pretreatment with reserpine (Withrington & Zaimis, 1961 ; Erlij & Mendez, 1964). Since hypothermia has been reported to depress the reactivity of the ventricle to digitalis (Beyda, Jung & Bellet, 1961), it is possible that the antidigitalis effect of reserpine is related to the fall in body temperature. The results of the present investigation do not support this view. First, there was no difference in the body temperature of reserpine-pretreated animals and the control group. Second, despite the marked fall in temperature in the control group 4 hr after surgery, the reactivity of the ventricle to acetylstrophanthidin was similar to that in the control group when body temperature was at normal levels. The difference between these results and those of Beyda *et al.* (1961) are probably related to differences in the degree of hypothermia. In this investigation, the lowest point to which body temperature fell was approximately 32° C whereas in the study of Beyda *et al.* (1961) temperatures were considerably lower (approximately 25° C).

Although reserpine lowers blood pressure, this action could not be correlated with its anti-arrhythmic effect. Thus, 1 hr after reserpine, a time interval at which ventricular reactivity to acetylstrophanthidin was not affected, the blood pressure had fallen to levels similar to those observed 4 hr after the administration of reserpine.

The action of reserpine which causes slowing of the heart seemed to parallel its anti-arrhythmic effect. This observation supports the suggestion of Erlij & Mendez (1964) that the slowing of the heart rate due to reduction of adrenergic influences may play a role in reserpine antagonism of digitalis arrhythmia. In this regard, Levitt, Ciofalo & Roberts (1966) have recently demonstrated that slowing the heart rate with

hexamethonium or vagal stimulation to levels similar to those observed following reserpine, did not affect ouabain-induced arrhythmia. It seems, therefore, that the slowing of heart rate and the antagonism of digitalis-induced arrhythmia may be two separate manifestations of the same action—namely, reduction in adrenergic nervous activity.

SUMMARY

1. Four hr after reserpine (5 mg/kg) was injected intravenously the reactivity of the ventricle to acetylstrophanthidin was depressed but that to catecholamines was not influenced. In animals pretreated with reserpine (5 mg/kg) intraperitoneally 24 to 30 hr before the experiment the ventricular response to acetylstrophanthidin was diminished while that to catecholamine was unaffected. In animals pretreated with reserpine (1 mg/kg) intraperitoneally 24 to 30 hr before the experiment, the intravenous injection of reserpine (5 mg/kg) also failed to influence ventricular reactivity to catecholamines.

2. The antagonism of digitalis-induced ventricular arrhythmia by reserpine was not related to changes in body temperature or blood pressure.

3. It was concluded that the anti-arrhythmic effects of reserpine are more limited than those of quinidine.

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