Br. J. Pharmac. Chemother. (1967), **29**, 302–308.

EFFECT OF RESERPINE AND PRONETHALOL ON THE THERAPEUTIC AND TOXIC ACTIONS OF DIGITALIS IN THE DOG HEART-LUNG PREPARATION

BY

G. FAWAZ

From the Department of Pharmacology, School of Medicine, American University of Beirut, Beirut, Lebanon

(*Received October* 12, 1966)

The possibility that release of endogenous catechol amines may play a role in the therapeutic and toxic actions of digitalis has been a subject of much experimentation and discussion in recent years. (For reviews of the literature see Levy & Richards, 1965; Morales-Aguilerá & Vaughan Williams, 1965; Benfey & Varma, 1966). Most arguments are based on results obtained after treatment with reserpine, known to deplete the heart of catechol amines, and after β-receptor blocking drugs such as dichloroisoprenaline and pronethalol although most authors realize that reserpine and pronethalol may act other than by depleting cardiac catechol amines or blocking their action. While the influence of reserpine and β-blockers on the inotropic effects of digitalis is still debatable, there seems to be agreement by most authors that these drugs can decrease, prevent or reverse the cardiotoxic actions of digitalis. Pronethalol has already found use in the clinic to treat digitalis intoxication (Taylor, Johnston & Jose, 1964).

The mammalian heart–lung preparation has been successfully used to study the quantitative aspects of digitalis uptake by the heart as well as its therapeutic and toxic effects (Weese, 1930; Farah & Maresh, 1948). The latter authors showed that the lethal dose of digitalis decreases as the infusion rate is diminished until a rate of administration is reached, the "optimal" rate, below which there is no further decrease in the lethal dose. However, regardless of the rate and within a wide range of infusion rates, the "therapeutic dose," the dose that just causes a fall in right atrial pressure in the failing heart, and the "irregularity dose," the dose that just produces premature ventricular systoles, bear a constant ratio to the "lethal dose," the dose that produces ventricular fibrillation or standstill.

In this paper, the failing dog heart–lung preparation is used to study the effect of reserpine and of pronethalol on the "therapeutic," "irregularity" and "lethal" doses of digitoxin and ouabain. Experiments on the effects of pronethalol on the "irregularity" and "lethal" doses of ouabain in intact dogs are also reported.

METHODS

Dog heart-lung preparations were made by the conventional Starling procedure using pentobarbitone anaesthesia (30–40 mg/kg pentobarbitone sodium in 10% alcohol). The blood donors

were anaesthetized with chloroform. The blood volume was about 900 ml. and the "peripheral resistance" was maintained at 6.8 mm Hg. The lungs were ventilated with air that was bubbled through water. The average "systemic" output (left ventricular minus coronary flow) before failure was about 750 ml./min. Failure was produced in each experiment by adding repeated doses of pentobarbitone until the initial "systemic" output was reduced to about one half. The total amount of pentobarbitone so needed varied from 50 to 150 mg or more. Electrocardiographic readings were taken every 5 min until the onset of irregularities when a continuous tracing was recorded. Otherwise, the procedure of Farah & Maresh (1948) was followed throughout. Reserpine was given as intraperitoneal injections of 0.5 mg/kg 48 hr and 24 hr before the experiment, and the heart–lung preparations were made from these animals using less anaesthetic. This reserpine treatment leaves no measurable amounts of noradrenaline in the heart (Fawaz & Simaan, 1963). Pronethalol (Alderline) was added to the venous reservoir in a dose of 20 mg. This was shown in preliminary experiments to block completely the positive chronotropic and inotropic effects of added noradrenaline (5 to 15 μg) for a period of at least 3 hr. Pentobarbitone was added 30 min after the pronethalol in order to produce failure.

The experiments on intact dogs were performed under pentobarbitone anaesthesia. Artificial ventilation with air (Starling pump) sufficient to depress spontaneous respiratory efforts was used in all experiments. The blood pressure was measured from a common carotid artery by a mercury manometer and both vagosympathetic trunks were cut. Electrocardiographic readings were taken as with the heart–lung preparations. Pronethalol was infused into a femoral vein at the rate of 0.25 mg/kg/min for the first 20 min and 0.1 mg/kg/min for the duration of the experiment. This treatment was found to block completely the positive chronotropic and depressor effects of isoprenaline (1 μg/kg) in every one of eight preliminary experiments (not reported in this paper). Ouabain (20 mg/l.) was infused at the rate of 1 ml./min 20 min after the start of the administration of pronethalol. Control experiments used dogs of the same sex and nearly the same weight as in the pronethalol experiments and were performed always on the same day.

RESULTS

Reserpine in the heart–lung preparation

It can be seen from Table 1 that previous treatment with reserpine in doses sufficient to deplete the noradrenaline stores has no effect on the "therapeutic," "irregularity" or "lethal" doses of digitoxin and ouabain in the isolated dog heart.

Pronethalol in the heart–lung preparation

The addition of 20 mg pronethalol to the venous reservoir of the heart–lung preparation resulted in an increase in heart-rate in each of the 13 experiments ranging from 16 to 64 beats/min (average 36). This dose of pronethalol, however, produced no failure as there was no change in right atrial pressure or "systemic" output. Pronethalol did not affect the lethal doses of digitoxin or ouabain. However, as can be seen from Table 1, in five of the digitoxin experiments there was no terminal ventricular fibrillation, but a ventricular standstill preceded by atrioventricular block and idioventricular rhythm. The "irregularity dose" of digitoxin after pronethalol ($71.5 \pm 2.7\%$, mean and standard error) is significantly greater than that in the controls ($54.5 \pm 1.6\%$), $P<0.001$.

Pronethalol in the intact animal

It can be seen from Table 2 that a continuous infusion of pronethalol in a dose just sufficient to block the chronotropic and depressor actions of isoprenaline did not affect the lethal dose of ouabain in dogs under pentobarbitone anaesthesia. It did, however,

TABLE 1

EFFECT OF RESERPINE AND PRONETHALOL ON DIGITALIS-INDUCED ARRHYTHMIAS IN THE DOG HEART–LUNG PREPARATION

The experiments with reserpine were performed on heart–lungs prepared from dogs pretreated with reserpine (0·5 mg/kg) 48 and 24 hr before experiment. In experiments with pronethalol, the latter (20 mg) was added to the reservoir at least 30 min before the infusion of digitalis. Rate of infusion of digitoxin or ouabain is in μ-mole/kg heart/min. Lethal dose is amount of digitalis in μ-mole/kg heart required to produce ventricular fibrillation or standstill. "Therapeutic" and "Irregularity" doses expressed as percentage of lethal dose at the given infusion rate denote beginning of decrease in right atrial pressure and start of ventricular premature systoles respectively. VF = Ventricular fibrillation, B = atrioventricular block, IVR = idioventricular rhythm, VS = ventricular standstill (no ventricular fibrillation)

Rate of infusion (μ-mole/kg/min)	Lethal dose (μ-mole)	Therapeutic dose (%)	Irregularity dose (%)	Terminal arrhythmias	Rate of infusion (μ-mole/kg/min)	Lethal dose (μ-mole)	Therapeutic dose (%)	Irregularity dose (%)	Terminal arrhythmias	Rate of infusion (μ-mole/kg/min)	Lethal dose (μ-mole)	Therapeutic dose (%)	Irregularity dose (%)	Terminal arrhythmias
	Digitoxin Controls					*Reserpine–Digitoxin*					*Pronethalol–Digitoxin*			
0·18	20·0	21	58	VF	0·16	21·8	21	61	VF	0·15	19·2	19	61	B, IVR, VS
0·15	19·8	19	56	VF	0·11	14·5	23	57	VF	0·14	14·8	22	66	VF
0·11	17·5	14	56	VF	0·10	14·1	23	60	VF	0·14	20·8	33	66	B, IVR, VS
0·099	16·8	18	47	VF	0·095	13·1	22	63	VF	0·13	16·2	25	84	VF
0·097	11·7	25	54	VF	0·09	17·0	19	47	VF	0·12	13·7	18	80	B, IVR, VS
0·073	9·2	24	56	VF						0·11	13·9	27	73	B, IVR, VS
										0·098	7·35	32	68	B, IVR, VS
										0·075	8·9	30	74	VF
	Ouabain Controls					*Reserpine–Ouabain*					*Pronethalol–Ouabain*			
0·16	6·7	23	57	VF	0·18	8·0	22	62	VF	0·15	6·6	33	62	VF
0·14	5·4	25	50	VF	0·12	5·4	22	62	VF	0·13	5·7	23	50	VF
0·12	6·4	19	57	VF	0·11	5·2	18	60	VF	0·12	5·7	21	68	VF
0·11	4·9	22	72	VF	0·105	4·3	25	72	VF	0·11	4·8	23	75	VF
0·10	4·2	25	50	VF	0·10	4·2	20	63	VF	0·10	4·9	21	52	VF

TABLE 2

EFFECT OF PRONETHALOL ON OUABAIN-INDUCED ARRHYTHMIAS IN THE INTACT DOG

Pentobarbitone anaesthesia and artificial respiration were used. Both vagosympathetic trunks were cut. Pronethalol was infused at the rate of 0·25 mg/kg/min for 20 min then at 0·1 mg/kg/min for the duration of the experiment. Ouabain 1 : 50,000 was infused at the rate of 1 ml./min 20 min after the start of the pronethalol infusion. Each pronethalol experiment was performed on the same day as its corresponding number in the control experiments.
VF = Ventricular fibrillation, B = atrioventricular block, VS = ventricular standstill (no ventricular fibrillation)

Controls						Pronethalol treated					
Expt. No.	Dog weight (kg)	Time till VF or VS (min)	Time till ventricular premature systoles (min)	"Irregularity" dose in % of lethal dose	Terminal arrhythmias	Expt. No.	Dog weight (kg)	Time till VF or VS (min)	Time till ventricular premature systoles (min)	"Irregularity" dose in % of lethal dose	Terminal arrhythmias
1	6·5	40	25	62	VF	1	6·2	35	25	71	VF
2	7·5	70	45	60	VF	2	7·6	53	25	47	VF
3	7·9	46	15	33	VF	3	8·1	60	50	84	B, VS
4	11·0	49	35	71	VF	4	10·3	85	65	77	B, VS
5	10·6	55	30	55	VF	5	11·5	50	30	60	VF
6	11·8	50	25	50	VF	6	11·5	40	30	75	VF
7	14·2	44	30	68	VF	7	13·4	85	60	70	B, VS
8	14·6	90	30	33	VF	8	15·1	70	55	78	B, VS
9	16·0	50	25	50	VF	9	15·7	60	40	66	VF

significantly increase (from $53.6 \pm 4.6\%$ to $69.8 \pm 3.7\%$, means and standard errors) the dose required to produce premature ventricular systoles ($0.02 > P > 0.01$) and in four of nine dogs there was no ventricular fibrillation. During the 20 min after the administration of pronethalol there was a decrease in heart rate in all experiments ranging from 20 to 60 beats/min (average 30) and in four of nine dogs there was a measurable fall in mean blood pressure.

DISCUSSION

Catechol amines and the therapeutic action of digitalis

Our results lend no support to the belief that catechol amine release is involved in the therapeutic action of digitalis. Digitalis exhibited its full inotropic effect in failing heart–lung preparations prepared from reserpine-treated dogs or after pronethalol administration in a dose sufficient to block the action of injected catechol amines. It might, of course, be argued that digitalis liberates catechol amines from small stores not acted on by reserpine, and that liberated catechol amines act on receptors not reached by pronethalol. However, such arguments can neither be refuted nor substantiated at present.

Action of pronethalol on the isolated heart

Pronethalol had a substantial positive chronotropic action on the isolated dog heart. Our results do not indicate a positive inotropic action since pronethalol was added before the induction of failure by barbiturates. Even catechol amines produce only an increase in rate without a change in output or right atrial pressure if added to the nonfailing heart–lung preparation (unpublished data). Our results are in marked contrast to those of Schmier (unpublished), in which another β-blocking agent, propranolol, was found to exert negative chronotropic and inotropic actions on the dog heart–lung preparation. This shows that β-receptor blocking action *per se* is unrelated to any chronotropic or inotropic action exhibited by any particular β-blocking compound. Sekiya & Vaughan Williams (1963) and Benfy & Varma (1966) observed a slowing of heart rate in guinea-pigs after pronethalol. These findings are in harmony with those reported in this paper on intact dog but that action is probably indirect.

Catechol amines and digitalis-induced cardiotoxicity

The results of the experiments using isolated hearts prepared from reserpine-treated dogs indicate that catechol amine release is unrelated to digitalis-induced cardiotoxicity since there is no difference in the "irregularity" and "lethal" doses of ouabain and digitoxin between normal and reserpine-treated hearts. But here again one could also argue that reserpine may leave some cardiac catechol amine stores too small to be measured. Our results are not in harmony with those of Takagi, Zanuttini, Khalil & Bellet (1965) who studied the influence of reserpine on the toxic action of digoxin on intact dogs and of Roberts, Ito, Reilly & Cairoli (1963) who used cat papillary muscle and intact cats and dogs.

Pronethalol, on the other hand, had an influence on the cardiotoxic action of digitalis both in isolated hearts treated with digitoxin and anaesthetized vagotomized dogs treated with ouabain. The most relevant finding is an increase in the "irregularity" dose, the

dose which is just sufficient to produce premature ventricular systoles. Furthermore, in nearly half of the experiments no ventricular fibrillation was observed as was the case in all control experiments, but rather a ventricular standstill preceded by atrioventricular block and idioventricular rhythm. It is not clear why pronethalol did not produce the same changes in the heart–lung preparations treated with ouabain. There was no demonstrable change in the lethal dose, however, and our values are comparable with those reported by Farah & Maresh (1948), although these authors used much wider ranges of infusion rates. It is difficult to make very accurate comparisons of lethal doses between normal and pronethalol-treated preparations since the lethal dose depends on the rate of the infusion per mass of heart tissue and that cannot be ascertained before the experiment is terminated (Farah & Maresh, 1948). The change in pattern of digitalis-induced cardiotoxicity after pronethalol is not necessarily due to β-receptor blockade. Our negative results with reserpine speak against the involvement of catechol amines. Furthermore, the changed electrocardiographic pattern—namely, block, idioventricular rhythm and standstill—is very reminiscent of the action of quinidine on isolated dog hearts and intact dogs. In a previous paper (Fawaz, 1955) it was shown that doses of quinidine ranging between 30 and 140 mg, and which were sufficient to depress the myocardium as judged by a decreased output and increased right atrial pressure, did not influence the lethal dose of ouabain in the dog heart–lung preparation but that in all cases the terminal arrhythmia was indeed ventricular fibrillation. Benfey & Varma (1966) working on the isolated guinea-pig atrium reported that pronethalol and propranolol possess an anti-fibrillatory property equal to that of quinidine and that it is not directly related to their β-blocking action.

One must be on one's guard against applying results obtained on isolated hearts and anaesthetized vagotomized dogs to human material. Yet, if any conclusion is to be drawn from our experiments it is that one should exercise caution in using pronethalol to prevent digitalis-induced arrhythmias. For if the lethal dose of digitalis is not altered by pronethalol regardless of whether the end result is ventricular fibrillation or cardiac arrest, an increase in the "irregularity" dose is anything but salutory. Ectopic beats are sometimes the first warning against digitalis intoxication and a physician would rather receive that warning when he is half-way, than three-quarters of the way, to the fatal arrhythmia.

In the experiments of Sekiya & Vaughan Williams (1963) on guinea-pigs, pronethalol (5 mg/kg) abolished the ventricular fibrillation, did not increase the "irregularity" dose (dose required to produce ectopic extrasystoles), and significantly increased the dose required to produce cardiac arrest. With 15 mg/kg pronethalol both the "irregularity" and the lethal doses were very significantly increased. Unfortunately, the "therapeutic" dose cannot be measured under these conditions yet the ratio of the "irregularity" to "lethal" dose (399 : 465) is uncomfortably close. It would be interesting to repeat Sekiya & Vaughan Williams' (1963) work on the failing guinea-pig heart–lung preparation so that the "therapeutic" dose can also be measured.

SUMMARY

1. In heart–lung preparations from reserpine-treated dogs and made to fail with barbiturate, ouabain and digitoxin counteracted the heart failure just as in hearts

prepared from normal dogs. Furthermore, the " therapeutic," " irregularity " and " lethal " doses of these last two drugs remained unchanged.

2. Pronethalol (20 mg) caused an increase in heart-rate of dog heart–lung preparations without a change in right atrial pressure or " systemic " output. Barbiturate failure after pronethalol could be completely counteracted by subsequent infusions of ouabain or digitoxin. Pronethalol did not alter the therapeutic and lethal doses of ouabain and digitoxin. It did, however, significantly increase the " irregularity dose " of digitoxin. Furthermore, in five of the eight heart–lung preparations treated with pronethalol and digitoxin there was no ventricular fibrillation but a standstill preceded by block and idioventricular rhythm.

3. In vagotomized dogs anaesthetized with pentobarbitone, a continuous infusion of pronethalol just sufficient to block the chronotropic and depressor actions of isoprenaline, did not influence the " lethal " dose of ouabain but significantly increased the " irregularity dose." In four of the nine experiments the terminal arrhythmia was block and ventricular standstill without fibrillation.

4. It is concluded that catechol amine release is not involved in the therapeutic action of digitalis and that the change in the pattern of digitalis cardiotoxicity after pronethalol is not necessarily related to its β-blocking action.

5. The clinical implications of the observation that pronethalol increases the " irregularity " but not the " lethal " dose of digitalis in dogs are discussed.

This study has been supported by a grant from the American Heart Association. I am indebted to Dr. R. Tabbara for his advice in the interpretation of the electrocardiograms, to Ciba (Basel) for the reserpine and to I.C.I. for the pronethalol.

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