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The relation between noradrenaline content of rabbit heart muscle and the amount of k-strophanthin needed to produce arrhythmias

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Possible relations between the sensitivity of the heart to the arrhythmia-inducing property of k-strophanthin and myocardial storage of noradrenaline were investigated. Sensitivity to the glycoside was increased in rabbits pretreated with reserpine in doses which depleted noradrenaline, but not in rabbits pretreated with tyramine in doses which are known to reduce noradrenaline content. Sensitivity to the glycoside was restored to normal by an infusion of noradrenaline not earlier than 72 hr after reserpine treatment, but not by an infusion given at 24 hr. Infusion of noradrenaline did not itself reduce the sensitivity to k-strophanthin in normal rabbits. The glycoside acutely reduced the myocardial store of adrenaline and noradrenaline in normal rabbits. The possibility that cardiac sensitivity to k-strophanthin is regulated by *bound* but not by *unbound* noradrenaline is discussed.

ACCORDING to Cairoli, Reilly, Ito & Roberts (1961) the effect of ouabain on myocardial behaviour is brought about through the liberation of noradrenaline. Tanz (1964) has expressed the view that "the presence of a significant concentration of catecholamines is necessary in order that the myocardium . . . be able to respond to exogenously administered cardiac glycosides. If this be true . . . then one might liken the normal role of cardiac catecholamines to a *permissive* action."

The possibility of a relation between heart sensitivity to k-strophanthin and myocardial concentration of noradrenaline has been investigated on the following premises: if the action of k-strophanthin on the heart in some way depends on the local stores of catecholamines it might be that (1) the glycoside does alter these stores, (2) their previous depletion modifies the cardiac response to k-strophanthin, (3) this response can be restored to normal by effecting a restoration of myocardial concentrations of noradrenaline.

Experimental

METHODS

Preliminary experiments were made to establish the reproducibility of a "sensitivity test" to k-strophanthin in the rabbit. The animal, maintained under light anaesthesia (20 mg/kg i.v. of sodium pentobarbitone and thereafter 0·025–0·050 mg/kg/min in a saline drip of 0·125–0·250 ml/min through the marginal vein of the right ear), was infused through the marginal vein of the left ear with 13·5 μg/min of k-strophanthin (in 0·16–0·20 ml of saline) until the appearance of electrocardiographic abnormalities. These generally consisted of bursts of ventricular extrasystoles or disordered atrioventricular conduction. This was taken as the end point, sensitivity being expressed in μg/kg of k-strophanthin received by the animal at the end point. When the same rabbit underwent a second test a similar degree of sensitivity to k-strophanthin was observed.

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The experiments were made in 45 rabbits of either sex (2–4 kg). After a first test the animals were grouped according to size and degree of sensitivity and k-strophanthin sensitivity was re-assessed (i) immediately after an infusion of noradrenaline, (ii) at different times after reserpine treatment, (iii) as in (ii) but with an infusion of noradrenaline given immediately before the test, (iv) immediately after treatment with tyramine, (v) as in (iii) except that a treatment with tyramine was interposed between the infusion of noradrenaline and the test.

Whenever possible the different treatments were made successively in the same animals.

Reserpine (Serpasil, Ciba) was given, 0·5 mg/kg i.p., each day for three days.

Infusion of noradrenaline, 250 μg/kg (20 μg/ml of saline with sodium metabisulphite 0·2%), was effected slowly to avoid arrhythmias due to high blood concentrations of the infused amine. For this reason the duration of infusion was usually not less than 5-6 hr in normal rabbits and 7-8 hr in reserpinised animals.

Tyramine hydrochloride was given to normal rabbits as four separate intramuscular injections of 20 mg/kg at one-hrly intervals, and to reserpinised animals as two doses of 20 mg/kg i.m., the first immediately after completion of the infusion of noradrenaline, and the second dose 30 min later. This was followed immediately by the k-strophanthin test.

An interval of at least ten days was left between consecutive tests to k-strophanthin sensitivity, and of at least 30 days between consecutive treatments with reserpine.

Electrocardiographic tracings were obtained using standard lead 2.

Adrenaline and noradrenaline contents of the ventricular myocardium, brain and whole adrenals were estimated by a procedure which incorporates modifications to the method of Lund (1949). This was preferred after a comparative evaluation with other methods (Angelucci, Ajello & Baldieri, 1963).

Tissues samples (half of the ventricles along a transverse section, half of the brain along a longitudinal section, one whole adrenal) were washed rapidly with cold saline, dried on filter paper, weighed and frozen at −40°. The frozen sample was homogenised in 0·4N perchloric acid, centrifuged at 30,000 *g* for 10–15 min at 5°, the supernatant, filtered through a Jena Glass G4 funnel if necessary, was transferred to a 50 ml beaker containing 12·5 mg of sodium metabisulphite and 200 mg of sodium edetate and made up to 25 ml with 0·4N perchloric acid. After mixing, prepared alumina (400 mg) was added and the contents were brought to and maintained for 5 min at pH 8·6 with continuous stirring and titrimetric control. Stirring was then stopped and the mixture left to settle (about 30 sec.) The supernatant was discarded by suction and the alumina, after rapid washing several times with cool distilled water with constant stirring using an L-shaped glass rod, was eluted twice with 2·25 ml of 0·2N acetic acid, with stirring for 10 min. The combined eluates were centrifuged at 30,000 *g* for 15 min at 5° and finally divided in two 2 ml portions; one sample was brought to pH 3 and the other to pH 6·5–7·0. Both samples were

oxidised with manganese dioxide, centrifuged, their supernatants each mixed with a solution of ascorbic acid 0·1% in 5N sodium hydroxide and the fluorescence read in a spectrophotofluorimeter at 400/540 mμ. From this reading the reading of the blank, obtained by shaking for 1 hr 250 times/min, was then subtracted. At the same time as the estimations in tissue extracts, recovery and standard estimations were made with 2 ml of solutions of adrenaline and noradrenaline (100 ng/ml) and mixtures of both amines. Concentrations of amines in the sample were calculated using the formula:

$$x = \frac{[fS\ pH\ 7 \cdot (fAd\ pH\ 3 : fAd\ pH\ 7)] - fS\ pH\ 3}{[fNor\ pH\ 7 \cdot (fAd\ pH\ 3 : fAd\ pH\ 7)] - fNor\ pH\ 3}$$

$$y = \frac{fS\ pH\ 3 - (x \cdot fNor\ pH\ 3)}{fAd\ pH\ 3}$$

where: x = noradrenaline concentration ng/ml in the sample; y = adrenaline concentration ng/ml in the sample; f = fluorescence reading; Ad = 2 ml standard solution of adrenaline (100 ng/ml); Nor = 2 ml standard solution of noradrenaline (100 ng/ml); S = sample 2 ml.

The method outlined affords a recovery of at least 90% and agrees in its limit of sensitivity of 0·5 ng/ml of final reaction mixture with the results obtained by Anton & Sayre (1962) using one of the most sensitive spectrophotofluorimetric methods.

To detect residual tissues amines in reserpinised animals a microprocedure was adopted. The whole heart or brain (respectively about 11 and 13 g in rabbits of average body weight of 3·5 kg) was extracted and the amount of alumina was reduced to 200 mg; elution was with 1·0 ml of 0·2 N acetic acid. 0·5 ml of the eluate was then oxidised at pH 7 and taken through the above procedure with proportionally reduced amounts of reagents. The final volume of the reaction mixture in the microcuvette was 0·82 ml. With this procedure it was possible to detect amounts of amines in excess of 1 ng/g of tissue, since the limit of sensitivity of the method is 0·5 ng/ml of final reaction mixture (the fluorescence reading of sample and its blank are respectively 18·5 and 10·0) No differential reading in this condition between sample and its blank indicates absence of appreciable quantities of amines. It is possible to recover from the brain homogenates of reserpinised animals 80% of an added 10 ng of noradrenaline [net fluorescence reading 24 (sample 54, blank 30)].

The statistical significance of the differences between results of the various treatments has been analysed with Student's *t*-test.

Results

SENSITIVITY TO K-STROPHANTHIN

Reproducibility of sensitivity. The quantity (μg/kg) of the infused glycoside in two successive tests in the same rabbit to elicit clearcut electrocardiographic abnormalities was remarkably constant (Table 1), even though in the different strains there were different degrees of sensitivity. These appeared to be correlated with the size of the adrenal

glands. Thus, the average sensitivity to k-strophanthin (in μg/kg) infused at a constant amount per min remained fairly constant for each group of four rabbits (adult animals, weight 2 kg), provided there were no large variations in body weight in successive tests. It was therefore possible to make comparisons among results obtained for different groups, provided they showed similar degrees of control sensitivity (first test), as well as among results obtained for the same group in different treatments, provided each group acted as its own control (first test).

TABLE 1. DEPENDENCE OF CARDIAC SENSITIVITY TO K-STROPHANTHIN IN RABBITS ON PREVIOUS TREATMENTS. Three successive tests were made on each group of four. Sensitivity was measured as μg/kg of the glycoside infused until appearance of arrhythmias on the ECG. Group A and B were infused with 13·5 and group C with 10·5 μg/min. Mean values ± s.e.

Group	First test			days of interval	Second test			days of interval	Third test		
	Weight kg	k-Strophanthin μg/kg	k-Strophanthin total μg		Weight kg	k-Strophanthin μg/kg	k-Strophanthin total μg		Weight kg	k-Strophanthin μg/kg	k-Strophanthin total μg
A	(no treatment before test) 2·63 ±0·23	246 ±9	649 ±57	14–143	(no treatment before test) 2·86[1] ±0·55	253[1] ±12	721[1] ±108	10–111	(after infusion of noradrenaline[5]) 3·42[2] ±0·56	214[2] ±15	728[1] ±80
B	(no treatment before test) 2·31 ±0·20	271 ±24	628 ±54	10	(24 hr after reserpine[4] treatment) 2·50[1] ±0·28	166[3] ±17	415[3] ±44	30	(24 hr after reserpine[4] treatment, with noradrenaline[5] infusion) 2·60[1] ±0·34	152[3] ±21	395[3] ±81
C	(no treatment before test) 2·15 ±0·00	398 ±43	853 ±87	10	(72 hr after reserpine[4] treatment) 2·41[1] ±0·40	263[3] ±23	633[3] ±102	30	(72 hr after reserpine[4] treatment, with noradrenaline[5] infusion) 2·76[3] ±0·24	385[1] ±52	1057[2] ±90

[1] Not different from the first test, $P > 0.05$.
[2] Different from the first test, $0.05 > P > 0.01$.
[3] Different from the first test, $P < 0.01$.
[4] 0·5 mg/kg each day for 3 days.
[5] 250 μg/kg in about 6–7 hr.

In most rabbits of equal body weight, for the same dose of k-strophanthin, abnormalities at the end point of the test consisted of ventricular extrasystoles, but in some animals disorders of conduction prevailed.

Sensitivity to k-strophanthin in rabbits pretreated with reserpine. Treatment with depleting doses of reserpine (0·5 mg/kg i.p. each day for three days) increased heart sensitivity to k-strophanthin. Data in Table 1 show that after reserpine treatment there was a significant reduction in the amount of glycoside necessary to reach the end point. After treatment with reserpine there was not only a reduction of the mean dose/kg needed to reach the end point, but also a significant reduction of the mean total dose administered, since the same rabbits were used in each of the successive tests even though there was an increase of mean body weight of the animals between one test and the next.

Electrocardiographic tracings showed a pattern of abnormalities in reserpinised animals similar to that of the controls, though appearing at different dose levels of k-strophanthin.

RELATION BETWEEN NORADRENALINE AND K-STROPHANTHIN

Sensitivity to k-strophanthin after an infusion of noradrenaline in normal rabbits. Infusion of noradrenaline in normal rabbits before the k-strophanthin test increased heart sensitivity to the glycoside. A statistically significant difference ($0{\cdot}01 < P < 0{\cdot}05$) was found when comparing end point doses in μg/kg (Table 1).

Sensitivity to k-strophanthin after an infusion of noradrenaline in rabbits pretreated with reserpine. The infusion of noradrenaline in rabbits 72 hr after reserpine restored normal heart sensitivity to k-strophanthin. Data in Table 1 show that there was no significant difference between the dose of glycoside needed to reach the end point in controls and the dose in rabbits pretreated with reserpine when the test was preceeded by an infusion of noradrenaline.

The infusion of noradrenaline before the k-strophanthin test did not restore normal sensitivity when made within 24 hr after reserpine (Table 1).

Sensitivity to k-strophanthin after tyramine in normal and reserpinised rabbits. Tyramine is known to decrease catecholamine content of the myocardium so it might be expected to give results similar to those of reserpine. Treatment with tyramine in doses which effect a partial depletion did not modify sensitivity in normal rabbits nor in those infused with noradrenaline 72 hr after pretreatment with reserpine. The figures were: normal rabbits $249 \pm 10{\cdot}9$ and $291 \pm 31{\cdot}6$ μg/kg respectively without and with tyramine before the test to k-strophanthin; reserpinised rabbits infused with noradrenaline $281 \pm 24{\cdot}0$ and $263 \pm 19{\cdot}0$ μg/kg respectively without and with tyramine before the test to k-strophanthin.

THE RELATION BETWEEN CONCENTRATIONS OF NORADRENALINE AND HEART SENSITIVITY TO K-STROPHANTHIN

Table 2 shows that infusion of k-strophanthin until the appearance of electrocardiographic abnormalities reduced the content of noradrenaline in the myocardium. The degree of this reduction was relatively independent of the extent of the electrocardiographic abnormalities since in rabbits with similar degrees of reduction of noradrenaline, some showed only ventricular extrasystoles, some ventricular fibrillation and others died a few hours after the infusion. In rabbits so killed a substantial reduction of the noradrenaline content in the brain was found, while in rabbits which showed only clear cut but rapidly reversible electrocardiographic abnormalities the reduction in the brain was much less. In both groups a statistically significant difference from controls was found for the content of adrenaline in the adrenals.

The decrease of the noradrenaline content in myocardium and brain associated with k-strophanthin administration was not affected, as evidenced by data in Table 2, when the animals were previously infused with noradrenaline.

As data in Table 3 show, treatment with reserpine wholly depletes the stores of adrenaline and noradrenaline in myocardium, brain and whole adrenals. Repletion of normal stores did not occur in 24 hr from the last injection of reserpine; at 72 hr the process was beginning. An infusion of

TABLE 2. TISSUES CONCENTRATIONS OF ADRENALINE AND NORADRENALINE IN RABBITS INFUSED WITH K-STROPHANTHIN (13·5 μG/MIN) OR NORADRENALINE (250 μG/KG IN ABOUT 7–8 HR) OR WITH BOTH. Number of animals in parentheses.

Treatment	Myocardium ng/g		Brain ng/g		Adrenal gland ng/mg	
	Adrenaline	Noradrenaline	Adrenaline	Noradrenaline	Adrenaline	Noradrenaline
Controls (4)	128±40	1816±283	127±51	343±89	785±60	172±36
30 min after infusion of noradrenaline (3)	379	2088				
k-Strophanthin: lethal doses (6)	170[1]	1183				
	103[1]	967	45	76	572	
	248[2]	718	31	35		
	192[2]	932	27	36	612	
	216[3]	979	22	110	592	
		1130[3]	32	54	645	
	M185±54[4]	984±163[5]	31±8·5[5]	62±31·4[5]	605±31·1[5]	77±32[5]
k-Strophanthin: test doses (4)	96±54[4]	982±139[5]		264±35[4]	634±51[5]	
k-Strophanthin: test doses after infusion of of noradrenaline (2)	145	714		205		
	62	649		209		
	M103	681		207		

[1] Died with ventricular fibrillation during the test.
[2] Died after the test (more than 3 hr).
[3] Died after the test (within 3 hr).
[4] Not different from controls, $P > 0.05$.
[5] Different from controls, $P < 0.01$.
M = mean.

noradrenaline 24 hr after reserpine pretreatment was able to partially replenish stores of adrenaline and noradrenaline in the heart and in the adrenal glands (Table 3). But this infusion, although producing a significant response, was not enough to restore normal sensitivity to k-strophanthin. However if the infusion of noradrenaline was given 72 hr

TABLE 3. TISSUES CONCENTRATIONS OF ADRENALINE AND NORADRENALINE IN RABBITS TESTED FOR CARDIAC SENSITIVITY TO K-STROPHANTHIN AFTER TREATMENT WITH RESERPINE FOLLOWED OR NOT FOLLOWED BY INFUSION OF NORADRENALINE. Number of animals in parentheses.

Treatment	Myocardium ng/g		Brain ng/g		Adrenal gland ng/mg	
	Adrenaline	Noradrenaline	Adrenaline	Noradrenaline	Adrenaline	Noradrenaline
Controls (4)	128±40	1816±283	127±51	343±89	785±60	172±36
k-Strophanthin test[3] only (4)	96±54[4]	982±139[5]		264±33[4]	634±51[6]	77±32[5]
24 hr after reserpine[1] treatment. No test (2) ..	<1	<1	<1	<1	0·3	0·05
Infusion of noradrenaline[2] 24 hr after reserpine[1] treatment. No test (4)	112±54	107±37	5	3	139±	15±9
Infusion of noradrenaline[2] 24 hr after reserpine[1] treatment. Test[3] (2)	<1	<1	—	—	—	—
72 hr after reserpine[1] treatment. No test (2)	66	22	8	5	5	0·8
	101	34	8	6	9	1
Infusion of noradrenaline[2] 72 hr after reserpine[1] treatment. Test[3] (4)	97±47[4]	1076±247[5]	23±13[6]	54±15[6]	332±115[5]	82±42[6]

[1] 0·5 mg/kg i.p. each day for 3 days; last injection 72 or 24 hr before test or killing.
[2] 250 μg/kg in about 7–8 hr.
[3] k-Strophanthin 13 μg/min i.v.
[4] Not different from controls, $P > 0.05$.
[5] Different from controls, $P < 0.01$.
[6] Different from controls, $0.05 > P > 0.01$.

after treatment with reserpine, the myocardial stores were restored to normal as well as the sensitivity to k-strophanthin.

It was not possible to ascertain the concentration of noradrenaline in the myocardium after the infusion since the animals were then tested for sensitivity to k-strophanthin which, on its own, decreased the normal myocardial content of adrenaline and noradrenaline.

Discussion

Our experiments confirm already reported evidence that k-strophanthin decreases myocardial noradrenaline (Cession-Fossion, 1962). Depletion of catecholamine stores by reserpine resulted in an increase in heart sensitivity to the glycoside-induced arrhythmias. Repletion of myocardial stores of noradrenaline in reserpinised rabbits by an infusion of this amine restored normal sensitivity to the production of arrhythmias by k-strophanthin.

Since the increase above the normal of the noradrenaline content in the myocardium, which can be obtained by infusing the amine into normal rabbits, was not associated with a decrease of heart sensitivity to k-strophanthin, it could be argued that this sensitivity depends on the absolute value of the concentration of the amine.

TABLE 4. CARDIAC SENSITIVITY TO K-STROPHANTHIN AND MYOCARDIAL CONCENTRATIONS OF ADRENALINE AND NORADRENALINE IN RABBITS. SENSITIVITY WAS MEASURED AS μG/KG OF THE GLYCOSIDE GIVEN UNTIL APPEARANCE OF ARRHYTHMIAS ON THE ECG RECORD. Number of animals in parentheses.

Treatment	k-Strophanthin[1] test μg/kg	Myocardium ng/g	
		Adrenaline	Noradrenaline
Group 1: Controls (4)	—	128±40	1816±283
Group 2: test only (4)	305±36	96±54[4]	982±139[5]
Group 3: 72 hr after reserpine[2] treatment (2)	—	83	28
Group 4: First test (4)	271±24	—	—
Second test at 24 hr from reserpine[2] treatment	166±17[5]	—	—
Third test, preceded by infusion of noradrenaline,[3] at 24 hr from reserpine[2] treatment	152±21[5]	<1	<1
Group 5: First test (4)	398±43		
Second test at 72 hr from reserpine[2] treatment	263±23[5]		
Third test, preceded by infusion of noradrenaline[3] at 72 hr from reserpine[2] treatment	385±52[4]	97±46[4]	1076±247[5]

[1] 10·5 μg/min for groups 2 and 5; 13·5 μg/min for group 4.
[2] 0·5 mg/kg i.p. each day for 3 days; last injection 72 or 24 hr before test or killing.
[3] 250 μg/kg in about 7–8 hr.
[4] Not different from controls or first test, $P > 0.05$.
[5] Different from controls of first test, $P < 0.01$.

In Table 4 the degree of heart sensitivity to k-strophanthin is compared with the concentrations of noradrenaline in the myocardium in different experimental situations: it appears that for sensitivity to remain at normal values, a minimal local concentration of noradrenaline is needed which is larger than that found 72 hr after reserpine treatment, when increased sensitivity still persists. This minimal concentration can be built up by an appropriate suitably timed infusion of noradrenaline.

After reserpine depletion the immediate repletion of the myocardial store of *unbound* noradrenaline is still possible (Kirpekar & Furchgott, 1964). Repletion of the store of *bound* noradrenaline—by far the larger part of the amine store in the heart—remains inhibited for some days at least (Bhagat & Shideman, 1964). Since an infusion of noradrenaline can restore the concentration of the amine to normal only if given at a critical time after reserpine treatment (72 hr), the substantial increase in concentration of the *bound* form of noradrenaline would appear to be the phenomenon with which recovery of normal heart sensitivity to k-strophanthin is associated.

In favour of the hypothesis that *bound* noradrenaline is involved in the regulation of heart sensitivity to k-strophanthin is the fact that we found tyramine, in doses which partially reduce the *unbound* concentration of noradrenaline in the myocardium, did not interfere with heart sensitivity to the glycoside both in normal rabbits and in rabbits infused with noradrenaline 72 hr after reserpine treatment. Doses of tyramine which maximally deplete the myocardial amines, still leave enough noradrenaline to maintain an optimal functional transmission (Chidsey, Harrison & Braunwald, 1962); moreover the infusion of noradrenaline in the dog 24 hr after reserpine restored cardiac responsiveness to tyramine but not to sympathetic stimulation (Gaffney, Chidsey & Braunwald, 1963). It is this part of total myocardial catecholamine, not depleted by tyramine and not replaceable by an infusion of noradrenaline in the first hours after reserpine, which may also be associated with the sensitivity of the heart to the arrhythmia-inducing property of k-strophanthin.

During the preparation of this paper we learned of the increased toxicity of k-strophanthin to guinea-pig heart-lung preparations 24 hr after reserpine (Carpi & Oliviero, 1965).

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