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Possible protective action of antialdosterone compounds in myocardial necrosis in rats

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1. Experimental myocardial necrosis was produced in rats by injection of large doses of isoprenaline.

2. The effectiveness of four aldosterone antagonists in preventing this myocardial necrosis was studied.

3. The relative effectiveness of these compounds in preventing isoprenaline-induced necrosis was found to be Sc 5233>Sc 9420 (spironolactone)>Sc 11927 and Sc 8109.

4. The reported relative effectiveness of these compounds as aldosterone antagonists is Sc 11927>Sc 9420, Sc 8109>Sc 5233.

5. The significance of these findings is discussed.

Myocardial infarction may be associated with an imbalance between the available oxygen supply and the needs of the myocardium. Raab (1956) has claimed that such an imbalance can be produced by some catecholamines; Chappel, Rona, Balazas & Gaudry (1959) and Handforth (1962) have observed myocardial lesions in rats following injections of massive doses of isoprenaline.

It has been found that this infarct-like diffused necrosis produced by isoprenaline can be aggravated by pretreating the animals with steroids, particularly mineralocorticoids (Chappel, Rona & Gaudry, 1959). Aldosterone has been shown to aggravate the severity of the arrhythmias which occur in experimentally-produced myocardial infarction (Arora & Somani, 1962). Arora (1965) has reported that there is an increase in the urinary excretion of aldosterone both in experimentally induced myocardial infarction and in human myocardial infarction. Working with antialdosterone compounds, Selye (1960) found that spironolactone had a beneficial effect in preventing mineralocorticoid-treated, stress-induced myocardial necrosis.

The present work was undertaken to see the effect of other anti-aldosterone compounds, more or less potent than spironolactone in their antialdosterone activity, on isoproterenol-induced myocardial necrosis.

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Methods

Adult albino rats weighing between 200 and 250 g were used.

At the end of each experiment, all animals were killed ; the hearts were removed quickly, washed with water, gently dried on filter paper, weighed and fixed in 10% formol saline. Standard paraffin sections, 5 μ thick, were cut sagitally and stained with haematoxylin and eosin.

The experiments were divided into three groups:

1. *Treatment with isoprenaline.* A group of twenty animals was treated with isoprenaline sulphate ; 85 mg/kg per day was given subcutaneously for 2 days. All animals were killed on the third day.

2. *Treatment with anti-aldosterone compounds.* Four compounds were used:

Sc 5233: 3-(3-oxo-17β-hydroxy-4-androsten-17αyl) proprionic acid y-lactone.

Sc 8109: 19-nor-3(3-oxo-17β-hydroxy-4-androsten-17αyl) proprionic acid y-lactone.

Sc 9420 (spironolactone): 3-(3-oxo-7α-acetylthio-17β-hydroxy-4-androsten-17αyl) proprionic acid y-lactone.

Sc 11927: potassium 3-oxo-9α-fluoro-11β, 17β-dihydroxy-17α-pregn-4-ene-21-carboxylate.

These compounds were prepared for injection by dissolving 40 mg of each in 0.5 ml. of propylene glycol and diluting to 2.0 ml. with distilled water. A group of ten animals was used for each compound. The compounds were injected subcutaneously in daily doses of 20 mg/kg for 7 days. On days 6 and 7, isoprenaline sulphate, 85 mg/kg per day, was given subcutaneously in addition to the anti-aldosterone compounds. All animals were killed on day 8.

3. *Control groups.* A group of ten animals was given no treatment ; ten animals were injected daily with distilled water ; ten animals were injected daily with amounts of propylene glycol equivalent to the vehicle for the anti-aldosterone compounds.

Results

Based on the gross and microscopic appearances, the heart lesions were graded on a 5-point scale from grade 0 to grade 4. The criteria used are shown in Table 1.

The distribution of grades of lesions in each group of animals is shown in Table 2. The grades used to assess the lesions cannot form a normal distribution and so a non-parametric method was used to compare the effects of isoprenaline alone with the effects of isoprenaline given after treatment with the anti-aldosterone compounds. To do this, each set of results was arbitrarily arranged into two groups ; group A containing 0, 1 and 2 grades and group B containing 3 and 4 grades. The results of each treatment with an anti-aldosterone compound were then compared with the results of isoprenaline alone and the probability of difference calculated by Fisher's exact probability test. The values of P are shown at the foot of Table 2.

Spironolactone (Sc 9420) given at 20 mg/kg did not produce a detectable difference between isoprenaline-alone as assessed by gross lesions but the microscopic lesions were significantly less severe. However, the group of animals given

spironolactone at 50 mg/kg showed significantly less severe lesions than isoprenaline alone as seen in both gross and microscopic lesions.

Compound Sc 5233 at 20 mg/kg gave significant protection against isoprenaline as seen in both gross and microscopic lesions. However, compound Sc 8109 at 20 mg/kg failed to show any protection against isoprenaline ; indeed, the combination of isoprenaline plus Sc 8109 produced significantly more severe lesions than those produced by isoprenaline alone. Similarly, compound Sc 11927 at 20 mg/kg failed to protect the hearts against isoprenaline but the combination of Sc 11927 with isoprenaline was not more toxic than isoprenaline alone.

Discussion

From these results, it appears that, for equivalent doses, compound Sc 5233 is more effective than spironolactone (Sc 9402) in protecting hearts against isoprenaline-induced myocardial necrosis. Compound Sc 8109, the 19-nor analogue of compound Sc 5233, is more active as an anti-aldosterone agent (Liddle, 1958) but failed to protect against isoprenaline-induced lesions. Finally, compound Sc 11927 which has been claimed to be twenty times more active than spironolactone in its anti-aldosterone activity (Ross, 1962), did not produce detectable effects in these experiments. Thus no correlation has been found between effectiveness in preventing isoprenaline-induced cardiac lesions and the relative activities of these compounds as aldosterone antagonists as reported by others. There is no obvious reason, however, why the reported relative activities of these compounds as aldosterone antagonists should not be applicable to these experiments.

TABLE 1. *Criteria used to grade the lesions*

Grade	Gross appearance	Microscopic appearance
I	Mottling of the apex and distal part of the left ventricle caused by intermingled pale and dark red streaks	Focal lesions of the subendocardial portion of the apex and/or the papillary muscle, composed of fibroblastic swelling or proliferation and accumulation of histiocytes
II	Well demarcated necrotic areas limited to the apex	Focal lesions extending over wider areas of the left ventricle, with right ventricular involvement (lesions included also oedema, mottled staining, fragmentation and segmentation of muscle fibres)
III	Large infarct-like necrosis involving at least one-third of the left ventricle and extending to the adjacent areas of interventricular septum and right ventricle	Confluent lesions of the apex and papillary muscles with focal lesions involving other areas of the ventricles and the atria. The lesions included vacuolar and fatty degeneration, granular disintegration and hyaline necrosis of the muscle fibres and marked capillary dilatation with haemorrhagic points
IV	Large infarct-like necrosis involving more than half of the left ventricle, interventricular septum and extending to the distal portion of the right ventricle	Confluent lesions throughout the heart, including infarct-like massive necrosis with occasionally acute aneurysm or mural thrombi. The latter lesions were usually apical but also occurred in the papillary muscle or right ventricle. The lesions were similar in character to those in grade III

TABLE 2. *Incidence of gross and microscopic heart lesions*

	Grade of lesions	Control+ solvent treated groups		Isoprenaline alone 85 mg/kg		Isoprenaline 85 mg/kg after: Sc 9420 20 mg/kg		Sc 9420 50 mg/kg		Sc 5233 20 mg/kg		Sc 8109 20 mg/kg		Sc 11927 20 mg/kg	
		g	m	g	m	g	m	g	m	g	m	g	m	g	m
	0	30	30					4		2	1				
	A 1					2	2	6	9	6	3				
No. of animals showing lesions	2			9	2	4	3		1	2	4			1	1
	3			8	13	3	5				2	9	7	6	6
	B 4			2	4	1							2	2	2
Total No. of animals		30		19		10		10		10		9		9	
P(Fisher's exact probability test)						0·25	0·005	0·005	0·001	0·005	0·001	0·01	0·04	0·07	0·76

g, Gradings based on gross lesions.
m, Gradings based on microscopic lesions.

The evidence that the combination of compound Sc 8109 with isoprenaline produced more severe lesions than isoprenaline given alone raises a question about the suitability of the doses which have been used. It is possible that different results might have been found if the compounds had been given in equiactive doses as judged by their relative activity as aldosterone antagonists. This question will have to be resolved in subsequent experiments.

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