Antihypertensive Effect of Spironolactone (SC-9420) on Experimental Renal Hypertension

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INTRODUCTION

Over many years numerous reports have suggested that hypertrophy and hyperplasia in adrenal cortex, with an increase in sudanophilic lipids, occur more frequently in clinical renal and essential hypertension than in normotension^{1,2)}. The blood pressure of a majority of patients with malignant hypertension could be significantly lowered by removing the enlarged adrenals.³⁾

On the other hand, many investigators agreed that total adrenalectomy in dogs and rats with experimental renal hypertension resulted in a significant decrease of the hypertension.^{4,5,6,7)}

It has still not been clarified that the antihypertensive effect, caused by complete adrenalectomy, is due to a nonspecific effect of the cortical insufficiency or due in part to the disappearance of a pathogenic role of adrenal cortex in experimental renal hypertension.

Sturtevant^{8,9,10,11)} described that a synthetic steroid, SC-6584 reduced the blood pressure of rats with metacorticoid, metarenal and adrenal-regeneration types of hypertension. Spironolactone, SC-9420, 3 (3-keto-7 α -acetylthio-17 β -hydroxy-4-androsten-17 γ -yl)-propionic acid- γ -lactone, a new compound synthesized by Cella and Kagawa, blocks the effects of desoxycorticosterone acetate (DCA) on serum and urinary electrolytes in rats. It is reported that its oral potency as a DCA-blocker is about 5 times as much as that of SC-8109.

The purpose of the present study has been to know the effect of SC-9420 on blood pressure and electrolyte and water metabolism in experimental renal hypertensive rabbits.

EXPERIMENTAL MATERIALS AND METHODS

Nine male rabbits, averaging 3.0 kg. in weight, were placed individually in metabolic cages and divided randomly into 4 groups. Hypertension was produced

by the application of a figure-of-eight ligature to one kidney with removal of the contralateral kidney. Thereafter, the rabbits were provided with the standard diet, in which NaCl at the quantity of 10 mEq./kg. was supplemented, for about 50 days. Roughly two-third of the rabbits became hypertensive and the systolic blood pressure level rose to more than 100 mm Hg. The five hypertensive rabbits were divided into two groups. One group of 2 hypertensive rabbits was intramuscularly administered on the daily dose of 200 mg. of SC-9420, suspended in 5 ml. of sesame oil, for 6 days. The second group of 3 experimental renal hypertensive rabbits was given a similar dose of this compound orally for 6 days. The third group of two rabbits with normal arterial pressure received only the solvent (sesame oil) intramuscularly. The last group of two normotensive rabbits received 200 mg. of SC-9420 orally for the same period. All these groups were given 10 mEq. of sodium chloride, 25 mEq. of potassium and 60 ml. of water per kilogram of body weight, daily throughout the experimental period.

Urine was collected by means of an indwelling catheter and the volume and the sodium and potassium concentrations were measured on each day. Measurements of serum and urinary sodium and potassium were made by flame photometry. Body weight and blood pressure were recorded under the standard conditions at the beginning of each metabolic day. Blood pressure was indirectly estimated by the method of Watson.¹²⁾

SC-9420 was kindly provided by Dr. V. A. Drill, Director of Biological Research, G.D. Searle & Co.

RESULTS

The effects of SC-9420 are shown in Table I on renal hypertensive rabbits and in Table II on normotensive rabbits. The body weight showed little changes in all groups. The blood pressure was reduced to approximately normal levels in the hypertensive groups treated with spironolactone, but did not change in the normotensive groups. The reduced blood pressure began to rise again 3 or 4 days after the cessation of spironolactone therapy and returned to more than 100 mm Hg. on the 5th day (Fig. 1). In the hypertensive groups, there were increases in urinary sodium and urinary volume, mostly accompanied by a slight decrease in urinary potassium. A significant increase of serum potassium and a minor decrease of serum sodium were observed during spironolactone therapy, but no changes of serum electrolytes was observed in normotensive groups. There was no decrease of urinary potassium corresponding to this increase of serum potassium. It was observed that in all hypertensive groups the elevation of urinary volume and sodium excretion occurred on the second to fourth day of spironolactone administration and this effect continued for about 3 days after the withdrawal of spironolactone (Figs. 2 and 3). In the hypertensive rabbits the electrolyte effect of spironolactone administered orally was similar to the intramuscular injection.

TABLE I. Hypotensive Effect of Spironolactone on Renal Hypertensive Rabbits.

Number of Rabbits	Treatment	Body Weight Kg.	Blood Pressure mm. Hg.	Serum		Urinary		
				Sodium mEq./L.	Potassium mEq./L.	Volume ml./d.	Sodium mEq./d.	Potassium mEq./d.
1	SC-9420 200 mg. per day i. m.	2.7	109			170	23	21
						150	23	21
						130	17	20
						180	20	20
		2.7	105	139	4.3	150	14	17
		2.7	95			175	22	27
						190	27	17
			68	136	4.5	210	26	17
						220	28	18
		2.9				210	19	21
			70	134.5	4.8	230	25	20
			62			140	25	22
						225	32	24
			62			210	27	20
		2.8				150	20	13
						170	23	18
			100	136	4.6			
2	SC-9420 200 mg. per day i. m.	3.2				140	19	12
			118			130	17	13
						130	20	15
						115	18	12
		3.25	116	143	4.2	110	15	14
			110			160	16	19
						140	20	14
			75	141.5	4.9	125	25	11
			65			140	27	15
		3.4	68			225	30	17
				139	5.0	260	32	16
		3.5	65			265	32	24
						235	34	18
						210	32	18
			70			170	18	16
		3.3		140	4.7	200	20	18
			110					
3	SC-9420 200 mg. per day i. m.	3.2	110			190	25	22
						200	30	21
			110			210	23	22
		3.0	114	143	3.3	200	34	23
			105			160	27	19
			85			190	25	22
		3.1	70	140	3.6	200	29	18
			67			230	35	23
						220	38	20
		3.2	58	141	3.5	220	36	25
						240	35	22
		3.2	68			230	33	20
						190	29	20
			88	142	3.6	170	40	30
		3.0						
			112					

4	SC-9420 200 mg. per day orally	3.1	108	148	4.6	240	29	21
		3.1	106			210	31	19
		3.1	100			250	27	23
			84	147	4.6	180	30	13
		3.1	72			250	25	28
			66			230	33	22
			66	143	4.9	250	35	21
			56			300	41	19
		3.2	54			320	38	20
			60	143	5.0	330	42	19
			70			280	44	20
		3.1	104			260	35	20
				146	4.5	230	40	21
						220	34	21
						240	39	23
5	SC-9420 200 mg. per day orally	2.9	100	146	4.4	250	30	23
		2.9	100			250	31	31
			105			210	26	26
		2.8	110	143	4.4	260	30	31
			98			230	26	26
		2.9	76			260	33	23
		2.9	78	141	4.6	250	30	26
		2.8	74			290	35	19
		2.8	68			300	39	23
		2.9	68	142	4.5	300	35	19
		2.9	78			320	34	21
						300	44	22
		2.9		145	4.3	280	35	20
			102			230	28	20
						210	26	20
		2.9				200	25	22

The antihypertensive effect of spironolactone in rabbits with experimental renal hypertension appeared immediately after start of administration, and the blood pressure stayed at normal levels (less than 100 mm Hg.) during the period of the administration. This changes of blood pressure were not coincident with those of the urinary sodium.

DISCUSSION

The possibility of a pathogenetic change of adrenal cortical function in experimental renal hypertension has been suggested by alterations in sodium and water metabolisms in renal hypertensive dogs and rats. In the rat with experimental renal hypertension, the adrenal cortex is enlarged and histological studies reveal hypertrophy of the zona glomerulosa¹³⁾ and hyaline droplet inclusions in the cells of the zona fasciculata.¹⁴⁾ Investigators agree that complete removal of the adrenal cortex in dogs and rats with acute and chronic experimental renal hypertension results in a significant decrease of the hypertension.^{4,5,6,7)} Laragh *et al.*¹⁵⁾ found that aldosterone plays an important role in the syndrome of malig-

TABLE II. Effect of Spironolactone or Sesame Oil Administration on Normotensive Rabbits.

Number of Rabbits	Treatment	Body Weight Kg.	Blood Pressure mm. Hg	Serum		Urinary		
				Sodium mEq./L.	Potassium mEq./L.	Volume ml./d.	Sodium mEq./d.	Potassium mEq./d.
1	SC-9420 200 mg. per day orally	3.0	58	136	3.3	150	29	13
						210	29	20
						205	22	15
		3.0				150	23	13
						200	27	15
		3.1				150	24	16
		3.1	59	130	3.4	155	20	12
						150	21	14
		3.0				170	22	15
			59	137	3.0	140	15	12
						205	27	21
		3.1				200	32	16
			56	138	3.6	200	31	16
		3.1	58					
2	SC-9420 200 mg. per day orally	3.5	72			230	28	18
		3.5	68	147	4.6	210	29	18
			70			260	34	15
			70			200	30	17
		3.2	66			180	30	16
		3.4	66			180	30	16
		3.3	68	148	4.4	170	33	14
		3.2	62			200	27	14
			70			220	26	15
		3.1	60	149	4.5	220	31	18
			58			230	33	15
		3.1	56			200	31	12
			58	149	4.2	200	29	15
		3.2	60			180	31	16
			62			190	29	14
3	Sesame Oil 5cc i. m.	3.0	64	138	3.5	170	27	18
						140	23	21
		3.0	66	138	3.6	180	25	16
						190	29	18
						150	25	15
		2.9	64	137	3.5	160	26	20
						180	27	23
		3.0				170	23	16
			63	138	3.5	190	29	22
						140	25	18
		3.0		138	3.5	160	25	22
			65					

4	Sesame Oil 5cc i. m.	2.8	66	140	3.6	210	32	18
						220	32	21
		2.7	64			240	34	22
						220	31	19
						240	34	23
		2.8	62	143	3.4	190	28	19
						200	35	22
						220	31	18
		2.8	68	142	3.4	220	35	21
						200	36	20
						200	35	18
		2.8	66	141	3.5	220	39	22

Fig. 1. Effect of SC-9420 upon blood pressure in renal hypertensive and normotensive rabbits.

nant hypertension, by estimating aldosterone secretion.

Sturtevant¹¹⁾ showed that chronic administration of the hypotensive steroid, SC-6584, to rats actively treated with DCA-sodium chloride, tended to interfere with the pressor action of DCA, although these data were not definite. Hollander¹⁵⁾ reported that spironolactone had hypotensive effects in a half of hypertensive patients, but had not in normotensive subjects. From the present study, a large amount of spironolactone may be useful as antihypertensive agents to renal hypertension.

The present study showed its hypotensive effect for experimental renal hypertensive rabbits. At the same time, it was observed that urinary sodium and volume increased. Spironolactone has been reported to exhibit its charac-

Fig. 2. Effect of SC-9420 upon blood pressure, serum sodium and potassium and urinary volume, sodium and potassium in renal hypertensive rabbit.

teristic effects only in the presence of aldosterone-like steroid, and presumably the inhibition effect seems to occur in the kidney tubule where mineralocorticoid primarily works.¹⁷⁾ Thus, as aldosterone is present in a minute amount in normal animals, SC-9420 is relatively ineffective. So it is probable that in renal hypertension the level of aldosterone secretion is increased.

The effect of spironolactone on urinary electrolytes was the same as reported by Liddle.¹⁸⁾ There was an increase in urinary sodium, chloride and urinary volume, mostly accompanied by a slight decrease in urinary potassium. The elevation of serum potassium by spironolactone administration was not accompanied with proportionate reduction of urinary potassium. Presuming from this fact, it seems that intracellular potassium moved out to the extracellular space.

Blood pressure fell immediately after spironolactone administration and, thereafter, did not change significantly during the administration, but the increase of urinary sodium occurred later than the reduction of blood pressure. This fact, we feel, offers a strong evidence that the antihypertensive effect of spironolactone was not mediated by its action upon water and electrolyte metabolism. It is assumed that spironolactone itself has an action to decrease blood pressure. These findings indicate that the renal anti-aldosterone effects and the blood pressure effects of SC-9420 are separable and relatively independent actions. The mode of action which spironolactone induces a reduction in blood pressure is not clear. The antihypertensive and the natriuretic effects of spironolactone, after either oral or parenteral administration, were all almost the same.

SUMMARY

Spironolactone was administered to rabbits with experimental renal hypertension. The blood pressure of these rabbits was decreased during spironolactone therapy. A marked increase in sodium and water excretion was noted on the third day, but potassium excretion did not change at that time. Treatment with spironolactone caused a rise of serum potassium in hypertensive rabbits. It is probable that spironolactone moves intracellular potassium out to extracellular space.

Assuming from these results, water and electrolyte metabolism is not normal in the experimental renal hypertension, probably accompanied with excessive mineralocorticoid secretion. But the abnormal water and electrolyte metabolism, and hyperaldosteronism in experimental renal hypertension seem to play an independent different role in the development and maintenance of arterial hypertension.

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