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ACTIONS OF TWO ALDOSTERONE ANTAGONISTS ON CAT PERFUSED KIDNEYS

BY

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The actions of a closed-ring [spironolactone, SC-9420, β-(7α-acetylthio-17β-hydroxy-3-oxoandrost-4-en-17α-yl)propionic acid lactone] and of an open-ring [SC-11480, potassium β-(17β-hydroxy-3-oxoandrosta-1,4-dien-17α-yl)propionate] spirolactone have been examined on the cat perfused kidney, in concentrations ranging from 5 to 20 mg per 150 ml. of blood. Both compounds had weak aldosterone-like effects and caused antidiuresis with retention of sodium, potassium and chloride. The urinary changes they induced summed with those of aldosterone, without competition. Neither spironolactone nor SC-11480 affected renal blood flow or glomerular filtration rate. Both compounds antagonized the reduction in renal vascular resistance caused by aldosterone in these preparations.

Kagawa, Cella & van Arman (1957) and Liddle (1957) introduced a group of steroidal lactones which antagonized the renal actions of exogenous mineralocorticoids in adrenalectomized animals. These compounds were believed to exert their effects by competitive inhibition of the actions of mineralocorticoids in or on the renal tubular cells, for three reasons: they were structurally related to aldosterone; they had themselves no demonstrable actions in adrenalectomized animals; but they inhibited the sodium retention due to a fixed dose of mineralocorticoid to an extent graded in respect of the log dose of the inhibiting compound used (Kagawa, 1960). We have confirmed these observations (Lockett & Roberts, 1963b).

The original explanation of the actions of spirolactones has, however, been made untenable for three members of this series of compounds, SC-11480 [potassium β-(17β-hydroxy-3-oxoandrosta-1,4-dien-17α-yl)propionate], SC-11927 and SC-9420 [spironolactone, β-(7α-acetylthio-17β-hydroxy-3-oxoandrost-4-en-17α-yl)propionic acid lactone] (Fig. 1) in one species. Each of these compounds fails to antagonize, but sums in renal effect with aldosterone in hypophysectomized rats (Lockett & Roberts, 1963b). This observation, and others made on the action of SC-11480 in cats (Lockett & Roberts, 1962), have shown that the diuretic naturetic effects of these compounds are mediated through the hypophysis.

Our present purpose is to show that SC-11480 and spironolactone (SC-9420) have aldosterone-like actions on the isolated perfused kidney of the cat, which are similar

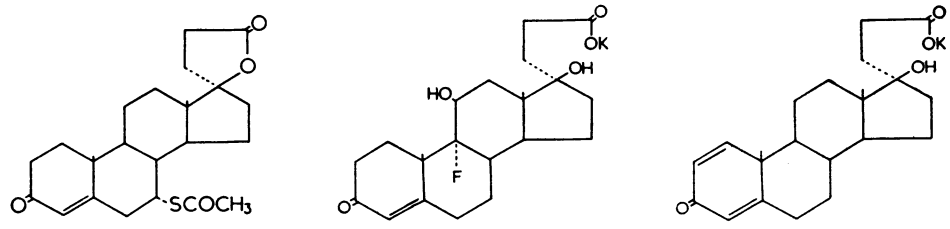

Fig. 1. Structural formulae of spironolactone (Aldactone, SC-9420), SC-11927 and SC-11480.

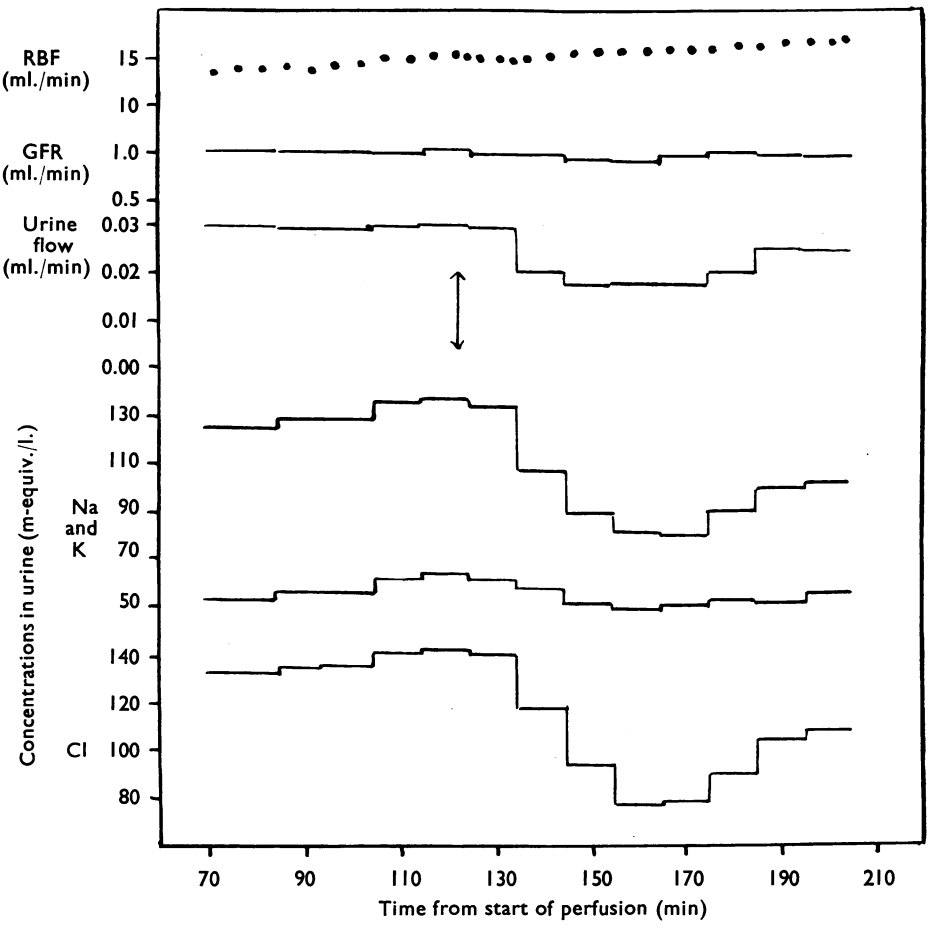

Fig. 2. Actions of SC-11480 (5 mg at arrow) on a kidney of 7.5 g, perfused at 128 mm Hg and 37° C with 150 ml. of blood. Ordinates, from above downward: renal blood flow (RBF, ml./min); creatinine clearance (GFR, ml./min); urine flow (ml./min); concentrations in urine (m-equiv./l.) of sodium (Na), potassium (K) and chloride (Cl). Abscissa: time in min from the start of the perfusion.

to their actions in hypophysectomized and in adrenalectomized and hypophysectomized animals, previously reported (Lockett & Roberts, 1963b).

METHODS

Eight heart-lung-kidney preparations were made in cats anaesthetized with chloralose, as described by Davey & Lockett (1960). Pulse pressure was adjusted by an air cushion in both series of experiments. The blood used to fill the perfusion circuit was obtained from male, female or neutered cats, anaesthetized with chloralose, in which the intracranial circulation was undisturbed. Heparin (200 units/100 ml. of blood) was used as anticoagulant.

Chemical procedures. Concentrations of sodium, potassium and creatinine in urine and plasma were estimated as by Davey & Lockett (1960).

Drugs. Spironolactone [SC-9420, Aldactone; β-(7α-acetylthio-17β-hydroxy-3-oxoandrost-4-en-17α-yl)propionic acid lactone] and SC-11480 [potassium β-(17β-hydroxy-3-oxoandrosta-1,4-dien-17α-yl)propionate] were kindly supplied by G. D. Searle & Co. These compounds were dissolved in propylene glycol, 20 mg/ml., warming, as necessary, in a water-bath not exceeding 60° C. The resulting solutions were added dropwise to the venous blood flowing back to the reservoir.

RESULTS

Cat kidneys, perfused at constant temperature and pressure with blood from normal animals, responded to concentrations of SC-11480 ranging from 5 to 20 mg/150 ml. of blood by reduction in the rates of urine flow and of excretion of sodium, potassium and chloride without change in renal blood flow or in creatinine clearance (Fig. 2). The concentrations of sodium, potassium and chloride in the urine typically decreased (Fig. 2), remaining almost unchanged only on one occasion. A latency of approximately 8 min preceded the urinary changes, which developed to a maximum in 30 min. The duration and the magnitude of the response varied with the dose.

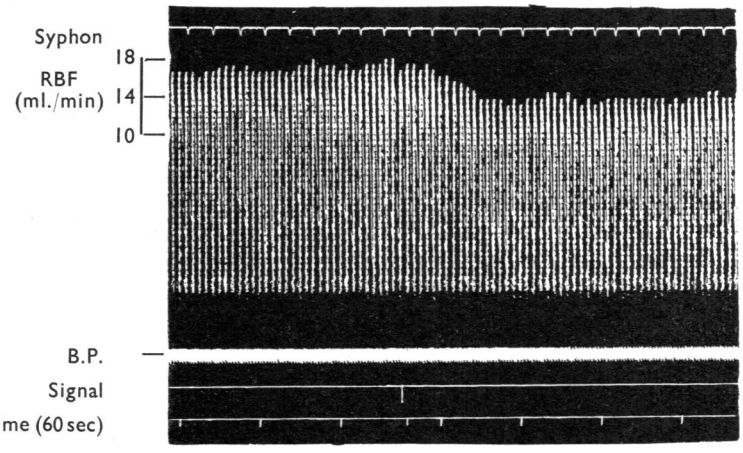

Fig. 3. Antagonism of the renal vasodilator action of aldosterone monacetate (5 μg/150 ml. of blood, given 12 min before records start), by SC-11480 (10 mg/150 ml.) at signal. Records, from above downward: syphon record indicating the returns of 8.5 ml. of extrarenal blood to reservoir; renal venous blood flow (RBF, ml./min); perfusion pressure (the line corresponds to 116 mm Hg); signal; time (60 sec). The kidney, 6.7 g, was perfused at 36° C.

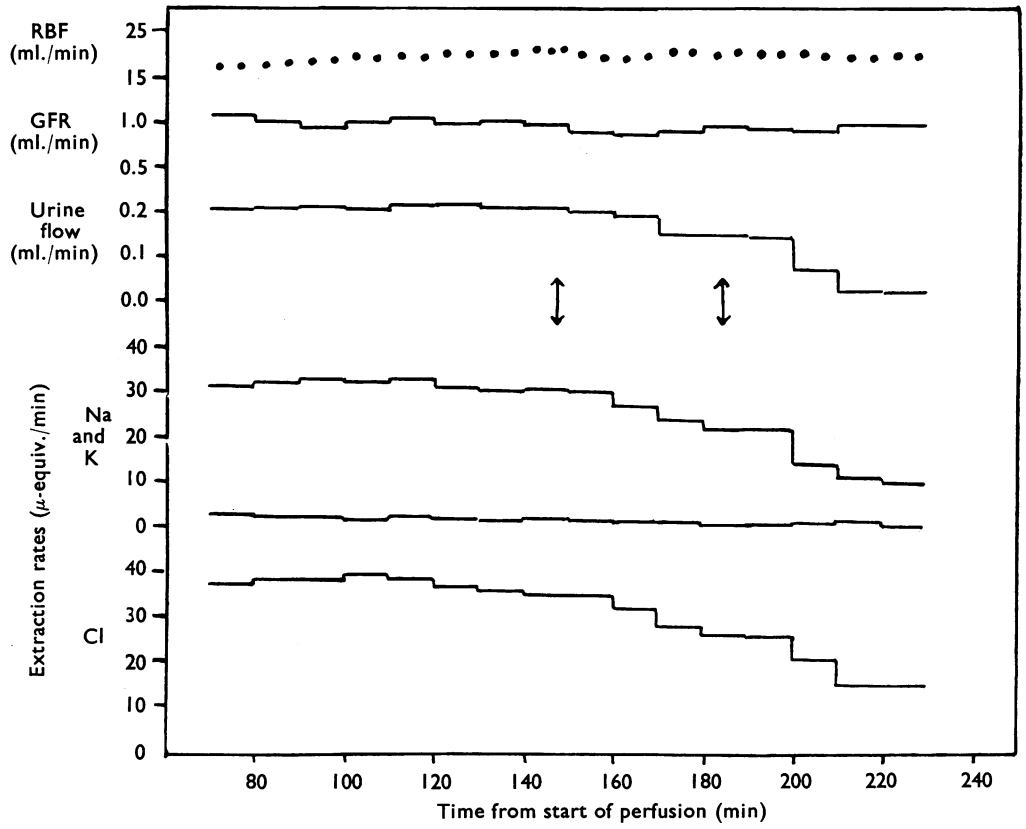

Fig. 4. Summation of the urinary effects of SC-11480 and aldosterone, shown by a kidney of 6.3 g perfused at 126 mm Hg and 36° C with 150 ml. of blood. 5 mg of SC-11480 and 5 μg of aldosterone monacetate were given at first and second arrows respectively. Ordinates, from above downward: renal blood flow, creatinine clearance and urine flows as in Fig. 2, then rates of excretion of sodium, potassium and chloride in μ-equiv./min. Abscissa: as for Fig. 2.

Hence the urinary changes induced by SC-11480 resemble very closely those caused by aldosterone when added to blood from intact donor animals as it perfuses a cat kidney (Davey & Lockett, 1960). The effects on the renal blood flow are, however, different. SC-11480 has no effect (Figs. 2 and 3) whereas aldosterone increases the blood flow (Davey & Lockett, 1960), except in the presence of SC-11480 (Fig. 4) or of spironolactone (Fig. 5).

The antidiuresis accompanied by retention of sodium, potassium and chloride which was induced by SC-11480 summed with that of aldosterone in each of six preparations (Fig. 4). This was so whether the compound was added to the blood at intervals (from 5 to 80 min) before the aldosterone, with the aldosterone, or when the effects of the hormone had already developed. Antagonism of the effects of aldosterone and SC-11480 was demonstrable only in respect of renal blood flow. The rise in renal blood flow which characterizes the action of aldosterone on this

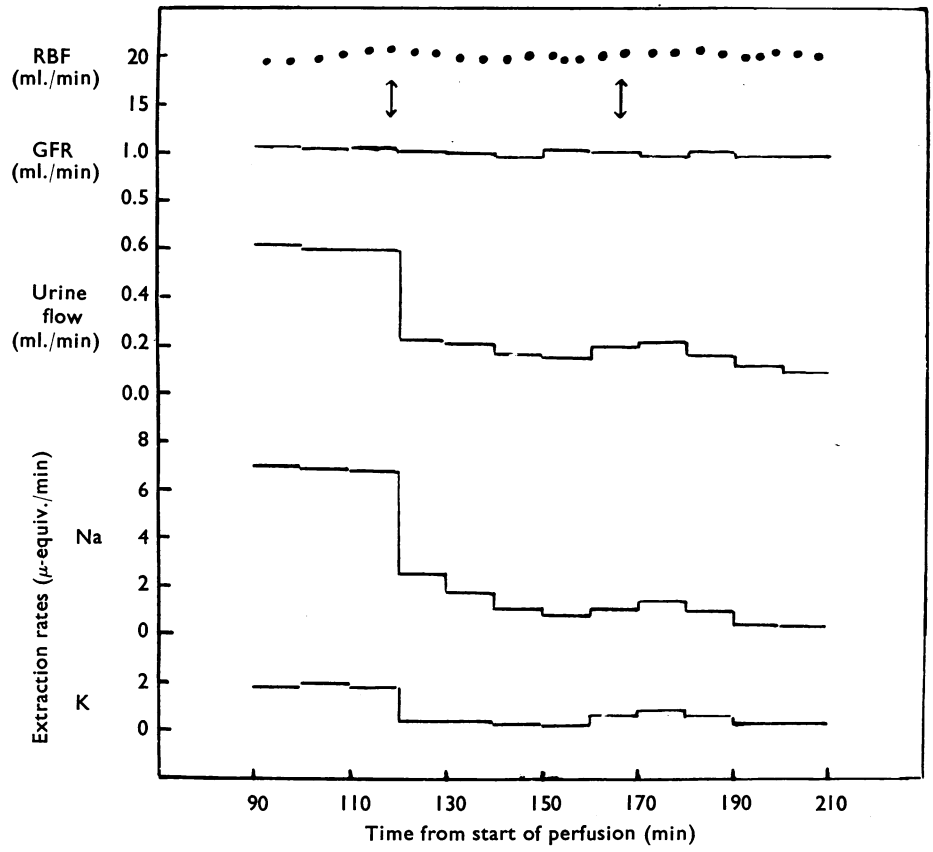

Fig. 5. Summation of the urinary effects of spironolactone and aldosterone shown by a kidney of 5.7 g, perfused at 120 mm Hg and 36.5° C with 150 ml. of blood. 5 mg of spironolactone and 5.0 μg of aldosterone monacetate were given at first and second arrows respectively. Ordinates (omitting that for chloride) and abscissa as for Fig. 4.

preparation (Davey & Lockett, 1960) was observed in each of three perfused kidneys which had received no prior treatment with a spirolactone. SC-11480 and spironolactone (5 or 10 mg/150 ml. of blood) caused rapid and complete reversal of the vascular actions of aldosterone in each experiment (Fig. 3). Aldosterone was, however, without vascular effects in each of five preparations which had been previously treated with a spirolactone (Figs. 4 and 5).

Spironolactone had actions on the cat perfused kidney that differed from those of SC-11480 in two points alone (cf. Figs. 3 and 4 with Fig. 5): spironolactone had the more powerful action and the shorter latency.

DISCUSSION

It is evident that SC-11480 and spironolactone have actions on the mammalian nephrons which resemble and sum with those of aldosterone ; they do not compete. This observation accords with the finding that the ability of these compounds to

antagonize the renal tubular actions of aldosterone in the whole animal (Kagawa, Cella & van Arman, 1957; Liddle, 1957; Lockett & Roberts, 1963b) is mediated by the hypophysis in rats (Lockett & Roberts, 1963b) and in cats (Lockett & Roberts, 1962), possibly by suppression of the secretion of growth hormone (Lockett & Roberts, 1963a). Moreover, since the immediate direct renal actions of SC-11480 and spironolactone resemble those induced by these compounds in hypophysectomized and in adrenalectomized and hypophysectomized animals (Lockett & Roberts, 1963b), these actions are more readily attributable to the unchanged compounds than to metabolites.

By contrast, both SC-11480 and spironolactone are effective antagonists of the decrease in renal vascular resistance which aldosterone induces in the isolated kidney (Davey & Lockett, 1960) but do not themselves influence renal blood flow.

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