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Effects of K^+-Canrenoate on the Development of DOCA-salt Hypertension

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Abstract—The effects of K^+-canrenoate, a digoxin antagonist, on the role of digoxin-like factor in the development of DOCA-salt hypertension has been examined. DOCA-salt rats treated with 66 mg kg^{-1} day^{-1} of K^+-canrenoate (s.c.) presented a lower increase in blood pressure ($P<0.01$), less cardiac hypertrophy ($P<0.05$) and hypokalaemia ($P<0.05$) than non-treated DOCA-salt rats. K^+-canrenoate treatment did not lead to significant changes in urinary volume, Na^+ and K^+ urinary excretion or suppression of plasma renin activity in DOCA-salt rats. None of the parameters were significantly different between uninephrectomized-salt rats treated or non-treated with K^+-canrenoate. These data suggest a role for digoxin-like factor in DOCA-salt hypertension. However, the non-normalization of blood pressure observed in K^+-canrenoate DOCA-salt treated rats indicates that other factors contribute to the initiating mechanisms in this type of hypertension. Moreover, these data suggest that digoxin-like factor plays no role in the suppression of plasma renin activity induced by DOCA and salt treatment.

Haddy & Overbeck (1976) have suggested that the increase in blood pressure induced by Na^+ retention may be due to the rise in a sodium transport inhibitor known as "digoxin-like factor" (DLF), as its activity is similar to that of digitalis compounds and it also cross-reacts with digoxin antibodies (Gruber & Buckalew 1983). The action of DLF on the renal tubule results in natriuresis due to decreased sodium reabsorption thereby restoring it to normal levels. In addition, the effect of inhibition of the Na^+-K^+ pump on smooth muscle can produce: i) depolarization of the sarcolemmal membrane (Blaustein 1977), ii) an increase in intracellular Ca^{2+} by reducing Na^+-Ca^{2+} exchange (Blaustein 1977), and iii) an increased noradrenaline release at sympathetic nerve endings (Nakazato et al 1978). These events could result in an increase in peripheral resistance, the haemodynamic alteration responsible for sustained high blood pressure (Miller et al 1979).

K^+-canrenoate (KC), an active metabolite of spironolactone, besides competing for aldosterone receptors, may interfere with the mechanisms induced by endogenous DLF reported above. KC protects the heart from the toxic effects of cardiac glycosides (Yeh et al 1976), reverses the inhibition of renin release induced by digoxin (Finotti & Antonello 1982), and with canrenone, another active metabolite of spironolactone, is able to restimulate the Na^+-K^+ pump previously inhibited by ouabain (Finotti & Palatini 1981; Garay et al 1985). Moreover, it has been reported that canrenone is antihypertensive in a model where endogenous DLF is increased (De Mendonca et al 1985; Pamnani et al 1985).

In DOCA-salt hypertension we observed a marked natriuretic response to saline expansion, indicative of the presence of a natriuretic factor, and increased excretion of a urinary digoxin-like immunoreactive material, both related to rise in blood pressure (Vargas et al 1988). These alterations did not appear during Goldblatt hypertension (a renin dependent model). Thus, we decided to evaluate the role of DLF in the development of DOCA-salt hypertension, using KC as antagonist.

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Materials and Methods

Animals

Male Wistar rats, 100–125 g at the beginning of the experiment, were housed in individual metabolic cages for one week in a thermoregulated room, illuminated from 08.00 to 20.00 h. All the rats had free access to tap water and were fed with a commercial diet containing Na^+73, and K^+185 (m equiv kg^{-1}). Systolic blood pressure was measured twice a week. After a week of adaptation, the animals were divided into the following groups: Group I—uninephrectomized-salt rats. Left nephrectomy was carried out under ether anaesthesia and a 1% NaCl solution was given as drinking water (n = 10). Group II—uninephrectomized-salt rats, treated with K^+-canrenoate. These animals received the same treatment as group I and were injected subcutaneously with K^+-canrenoate (Searle Iberica S.A.) 66 mg kg^{-1} day^{-1} (n = 10). Group III—DOCA-salt rats; these were uninephrectomized, provided with 1% saline solution to drink and injected weekly with 12·5 mg deoxycorticosterone enantate i.m. (Cortiron Depot, Schering AG) (n = 10). Group IV DOCA-salt rats treated with K^+-canrenoate. The same treatment was used as in group II, plus 66 mg kg^{-1} day^{-1} of KC s.c. (n = 10). Groups I and II were injected with oleous Cortiron excipient and Groups I and III were injected with KCl 6·6 mg kg^{-1} day^{-1}.

Experimental protocol

The following parameters were measured weekly for three weeks: i) Systolic blood pressure of unanaesthetized rats was measured using the tail-cuff technique with a pneumatic sphygmomanometer unit (Technical Instruments, Inc.) connected to a two-channel recorder (Devices MX2). After the initial adaptation period, the rats were placed in plastic holders and set on a warm pad for each recording session.

Blood pressure (BP) was measured seven times, and the value used for data analysis was obtained from the mean of five recordings, ignoring highest and lowest measurments. ii) 24 h diuresis (UV), natriuresis (U_{Na}^+V) and kaliuresis (U_K^+V).

At the end of the third week of hypertension induction, the left carotid artery was catheterized and the cannula was exteriorized at the dorsum of the neck under equithesin anaesthesia. After a 48 h recovery period, mean arterial pressure (MAP) and heart rate (HR) were recorded directly (Bell and Howell type 4 transducer connected to a two-channel Devices MX2 recorder) and blood samples were subsequently taken to determine plasma renin activity (PRA) and serum electrolytes. PRA was determined in duplicate by angiotensin I radioimmunoassay, according to the method described by Haber et al (1969) after incubating the plasma for 1 h at 37°C, pH 6·0, using a commercial kit (CEA-IRE-SORIN, Gaf-sur-Ivette, France). Serum and urinary electrolytes were measured by flame photometry (Corning 435). The body, heart and left kidney were weighed and hypertrophy rates estimated as: body weight/heart or kidney weight.

Statistical methods

The three week time course of each of the four variables was analysed using a three factor nested model with three fixed effect factors (K-canrenoate, time, group) and a random effect factor (individual rats). The random effect factor was nested in K^+-canrenoate and groups were crossed. Subsequent analysis with Neumann-Keuls and t-Tukey schemes were carried out when the nested tests were significant. The comparison of each variable at its end point in the time course was carried out with one-way Anova analysis. When the overall ANOVA was significant, we then performed pairwise comparison using Bonferroni's method.

Results

Group III rats, when compared with their controls, Group I rats, showed: a) A progressive increase in systolic blood pressure ($P < 0{\cdot}001$ at the third week); b) An increase in diuresis ($P < 0{\cdot}001$) and natriuresis ($P < 0{\cdot}001$) from the first week, with no change in urinary K^+ excretion (Fig. 1).

At the end of the third week, Group III rats in comparison

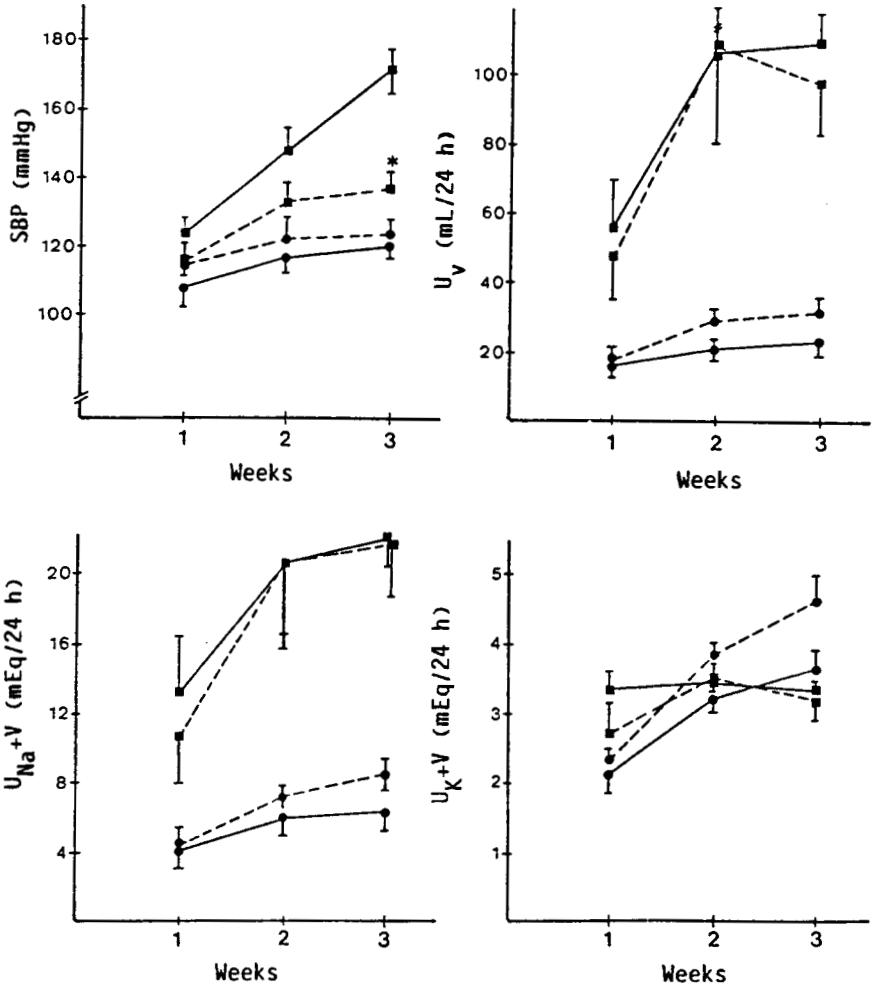

FIG. 1. Weekly changes in systolic blood pressure (SBP), measured by the tail-cuff method, urinary volume (U_V), urinary sodium ($U_{Na}+V$) and potassium excretion (U_K+V) in uninephrectomized-salt rats (Group I) (●——●), uninephrectomized-salt plus K^+-canrenoate rats (Group II) (● – – ●), DOCA-salt rats (Group III) (■——■) and DOCA-salt plus K^+-canrenoate rats (Group IV) (■ – – ■). Data are expressed as mean ± s.e.m. *Significant differences between DOCA-salt rats treated and non-treated with K^+-canrenoate. ($P < 0{\cdot}05$).

Table 1. Mean arterial pressure (MAP) and heart rate (HR) directly recorded, body weight/heart weight (Bw/Hw) and body weight/kidney weight (Bw/Kw) ratios as an index of hypertrophy, serum sodium, potassium and plasma renin activity (PRA) in unnephrectomized-salt and DOCA-salt rats treated and non treated with K^+-canrenoate at the end of the experiment.

Groups	MAP mmHg	HR Beats min^{-1}	Bw/Hw	Bw/Kw	Na^+ m equiv L^{-1}	K^+ m equiv L^{-1}	PRA ng mL^{-1} h^{-1}
I	94·6±4·3	450·4±14·0	317·1±8·5	174·1±11·2	142·5±0·4	4·80±0·18	6·56±1·7
II	98·3±6·0	430·0±10·4	318·8±12·1	178·8±7·6	143·8±0·9	4·96±0·16	4·08±1·17
III	173·0±7·4††	478·0±9·5	198·5±9·4†	101·5±3·3††	148·5±0·6†	3·45±0·15†	0·55±0·20†
IV	136·3±7·6**	375·0±15·6**	231·2±9·7*	116·0±5·03	147·8±0·9	4·28±0·29*	0·22±0·10

Values are means ± s.e.m. I = uninephrectomized. II = uninephrectomized-salt plus K^+-canrenoate. III = DOCA-salt. IV = DOCA-salt plus K^+-canrenoate. †$P<0·01$; ††$P<0·001$. III vs I. *$P<0·05$; **$P<0·01$; IV vs III.

with Group I rats presented: i) A high ($P<0·001$) mean arterial pressure (direct recording), with normal heart rate, ii) High serum Na^+ ($P<0·01$), low serum K^+ ($P<0·01$) and a marked inhibition of plasma renin activity ($P<0·01$), iii) cardiac ($P<0·01$) and renal ($P<0·001$) compensatory hypertrophy (Table 1).

Chronic administration of KC (66 mg kg^{-1} day^{-1}) to uninephrectomized-salt rats (Group II) did not modify any of the parameters studied throughout the experiments (Fig. 1), nor those determined at the end of the third week after 48 h of catheter implantation (Table 1). However, the same treatment in DOCA-salt rats (Group IV) produced a smaller increase in systolic blood pressure than that observed in non-treated (Group III) rats, significant differences being recorded in the third week ($P<0·05$) with no changes in 24 h urine volume or Na^+ and K^+ excretion (Fig. 1).

At the end of the experiment (Table 1), Group IV rats showed lower MAP values (direct recording) than Group III rats ($P<0·01$) as well as a marked reduction in HR in comparison with all the groups ($P<0·01$). Moreover, Group IV rats had lower serum K^+ ($P<0·05$) and less cardiac hypertrophy ($P<0·05$) than non-treated DOCA-salt rats (Group III).

Discussion

Casanova et al (1986) reported a rise in plasma levels and urinary excretion of digoxin-like immunoreactive material (DLIM) in DOCA-salt hypertensive rats. High levels of plasma DLIM have also been observed in this model of hypertension (Kojima 1984) and low-renal mass hypertensive rats (Hout et al 1983; De Mendonca et al 1985; Pamnani et al 1985). These data suggest a role for DLF in low-renin hypertension models. In fact, data reported herein support this notion, since the treatment with K^+-canrenoate, a digoxin antagonist, markedly reduces arterial blood pressure in DOCA-salt rats (Group IV) (Fig. 1, Table 1). These data are in agreement with those published by Kojima et al (1982) who noted an important decrease in BP in DOCA-salt rats after acute administration of a digoxin-antibody. Treatment with canrenone, a metabolite of spironolactone, which is in equilibrium with canreonic acid following the administration of KC (Garret & Won 1971), also decreases BP in low-renal mass hypertension (De Mendonca et al 1985; Pamnani et al 1985). The mechanism by which spironolactone derivatives reduce BP in volume-dependent hypertension is not clear.

Several authors have suggested that interaction of KC with digitalis receptor sites may be more important for its antihypertensive action than the interaction with aldosterone receptors. Thus, both canrenone (Pamnani et al 1985) and K^+-canrenoate (Altman et al 1975) had no antihypertensive effect in rats with spontaneous hypertension, in which DLIM is not increased (Pamnani et al 1985) and canrenone reduces BP in low-renal mass hypertensive rats where DLIM is high (De Mendonca 1985; Pamnani et al 1985).

It is interesting that although KC produces a less marked increase in BP in DOCA-salt hypertension, it fails to maintain blood pressure within normal levels (Group IV vs Groups I and II, $P<0·001$, Table 1). Similar results have been obtained by other authors who attempted to block the pathogenic activity of DLF with canrenone (De Mendonca et al 1985; Pamnani et al 1985) or with digoxin-antibodies (Kojima et al 1982). Those results suggest two possibilities: i) Neither spironolactone derivatives nor digoxin-antibodies are able to adequately antagonize the pathogenic activity of the endogenous DLF, or ii) Other pathogenic factors contribute to produce high BP values.

Different authors have observed that the administration of canrenone to normotensive rats enhances BP (De Mendonca et al 1985; Pamnani et al 1985), an effect which has been attributed to a partial agonist action of the spironolactone derivatives on the digitalis receptor, when digitalis itself is not present in the medium (Finotti & Palatini 1981; Marchetti et al 1983; Garay et al 1985). However, no significant differences in BP between Group I and Group II rats (96·4±4·3 mmHg vs 98·3±6·0 mmHg, Table 1) were found. This discrepancy could be due to the fact that our control rats drank 1% saline solution and under these circumstances showed a rise in DLIM (Casanova et al 1986).

Group IV rats in comparison with the other groups showed a significant decrease in heart rate ($P<0·01$, Table 1). This effect could be the result of a direct effect of KC on the heart. However, this explanation is difficult to reconcile with the normal HR observed in Group II rats. Moreover, less cardiac hypertrophy was observed in Group IV rats than in Group III rats (Table 1), while no significant differences in body weight between these two groups were recorded. The reduced cardiac hypertrophy in Group IV rats may be due to a smaller rise in BP, since it also has been reported with other antihypertensive treatments in experimental (Motz & Straver 1984) and clinical hypertension (Tarazi & Fouad 1984).

KC did not significantly modify 24 h diuresis, natriuesis and kaliuresis in Group IV rats during the course of the experiment (Fig. 1). These data are in agreement with those reported in low renal mass hypertension (Pamnani et al

1985), where canrenone reduced BP without affecting Na^+ and water excretion. On the other hand, KC did not modify serum Na^+, but prevented the hypokalaemic effect induced by DOCA ($P < 0.05$, Table 1).

De Wardener & MacGregor (1982) suggested that the low plasma renin activity found in essential hypertension may be due, in part, to an effect of a circulating inhibitor of Na^+ transport on the juxtaglomerular cells. However, our results show that K^+-canrenoate treatment fails to modify the suppression of plasma renin activity in DOCA-salt hypertension rats, thus suggesting that DLF plays no role in the inhibitory effect on renin secretion produced by DOCA and salt treatment.

In conclusion, these results show that: a) Chronic treatment with KC did not affect any of the parameters studied in uninephrectomized-salt normotensive control rats. b) KC reduced the BP in DOCA-salt hypertensive rats with no modification in renal excretion of Na^+ and water. c) KC reduced cardiac hypertrophy and hypokalaemia produced by DOCA and salt treatment, and d) KC did not interfere with the inhibition of renin secretion in DOCA-salt hypertension.

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