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Chronic potassium depletion increases adrenal progesterone production that is necessary for efficient renal retention of potassium

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Modern dietary habits are characterized by high-sodium and low-potassium intakes, each of which was correlated with a higher risk for hypertension. In this study, we examined whether long-term variations in the intake of sodium and potassium induce lasting changes in the plasma concentration of circulating steroids by developing a mathematical model of steroidogenesis in mice. One finding of this model was that mice increase their plasma progesterone levels specifically in response to potassium depletion. This prediction was confirmed by measurements in both male mice and men. Further investigation showed that progesterone regulates renal potassium handling both in males and females under potassium restriction, independent of its role in reproduction. The increase in progesterone production by male mice was time dependent and correlated with decreased urinary potassium content. The progesterone-dependent ability to efficiently retain potassium was because of an RU486 (a progesterone receptor antagonist)-sensitive stimulation of the colonic hydrogen, potassium–ATPase (known as the non-gastric or hydrogen, potassium–ATPase type 2) in the kidney. Thus, in males, a specific progesterone concentration profile induced by chronic potassium restriction regulates potassium balance.

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Human consumption of Na^+ has increased well above physiological needs, whereas that of K^+ has dramatically decreased over the last 10,000 years.[1] Our organism has somewhat adapted in response, but these dietary alterations are at the origin of several pathologies. The need to understand the consequences of modern dietary habits is all the more important, given that high levels of Na^+ consumption, but also a low daily intake of K^+, have been positively correlated with the probability for developing hypertension.[2,3]

Among the primary response elements of the organism are the adrenal glands, which adapt steroid production to changes in Na^+ and K^+ consumption. The mechanisms of adrenal steroidogenesis regulation under low-Na^+ and high-K^+ diets have been the subject of many investigations.[4,5] Adrenal steroidogenesis pathways, leading to the synthesis of mineralo- or glucocorticoids, differ among species (for review, see ref. 6). In rodents, the production of aldosterone in the zona glomerulosa (ZG) is dependent on the two rate-limiting enzymes CYP11A1 and CYP11B2, which are upregulated under chronic low-Na^+ or high-K^+ diets.[7] In these species, the zona fasciculata (ZF) produces corticosterone because of the lack of adrenal expression of CYP17 in rodents.[8,9] Corticosterone production has been found to be stimulated under low-Na^+ or high-K^+ diets, and related to changes in CYP11A1 expression levels.[7] The factors or signals involved in the regulation of steroidogenesis under low-Na^+ and/or high-K^+ diets have been identified as angiotensin II or plasma K^+ itself (for review, see ref. 10). Although these conditions probably reflect the eating habits of our ancestors (who had easy access to food rich in K^+ and poor in Na^+, see ref. 1), nowadays, the situation is drastically reversed. Investigating the mechanisms of adaptation to modern dietary habits is thus necessary. Moreover, until now, attention was particularly focused on aldosterone and corticosterone production, even though the intermediate metabolites of steroidogenesis also present in plasma are possibly subjected to variations and could have specific functions as well.

Our first objective was therefore to study the mechanisms of adrenal adaptation to Na^+ loading or K^+ depletion by examining possible variations in the levels of not only the end products of adrenal steroidogenesis but also of the intermediate metabolites. The second goal was to investigate the renal response to these modifications.

For this purpose, we developed a mathematical model of steroidogenesis in rodents that identified a specific steroid concentration profile induced by chronic K^+ restriction; subsequent experimental measurements established that progesterone acts as a hormonal regulator of K^+ balance in this state by stimulating renal expression of the 'colonic' hydrogen, potassium–ATPase (cHKA, also known as the non-gastric H, K–ATPase or the H, K–ATPase type 2) in the kidney.

RESULTS

Building and validation of a mathematical model for adrenal steroidogenesis in rodents

To predict the effects of variations in the daily intake of Na^+ and K^+ on the plasma concentration of circulating steroids, we developed a mathematical model of steroidogenesis in mice (SI Methods online) using parameters summarized in Supplementary Table S1 online and Supplementary Figure S1A online. One fundamental assumption of our model is that any change in mRNA expression for a given enzyme is directly proportional to an increase or decrease in its activity. We validated our model by comparing predicted and measured plasma concentrations of aldosterone and corticosterone (a) under basal conditions (Supplementary Figure S1B and S1C online) and (b) under low-Na^+ and high-K^+ diets, using previously published data[7] (Supplementary Figure S1D, S1E online and Supplementary Table S2 online). We also performed a sensitivity analysis to identify the critical parameters affecting steroid production (see Supplementary Table S3 online). The analysis showed that the production of aldosterone, corticosterone, and progesterone depends primarily upon the activity of CYP11A1 and the activity and progesterone affinity of CYP21.

Effect of high-Na^+ and low-K^+ diets on aldosterone, corticosterone, and intermediate metabolites levels

To investigate how modern eating habits affect adrenal steroid production, we subsequently placed male mice under conditions of K^+ depletion or Na^+ loading. These two physiological stresses are known to induce a significant decrease in aldosterone levels[11] but their effects on the overall steroid production remain unclear. As shown in Figure 1a, K^+ depletion (black bars) induced significant changes in the expression of steroidogenic genes: the mRNA levels of StAR, CYP11A, and 3βHSD1 increased by a factor of 2.1, 2.8, and 2.3, respectively, whereas those of CYP11B1 and CYP11B2 decreased to 0.05 and 0.07 times their control levels (white bars), respectively. During chronic Na^+ loading (hatched bars), only the expression of CYP11B1 and CYP11B2 was found to be significantly altered. The steps mediated by CYP11A and CYP21 being critical for steroid production,

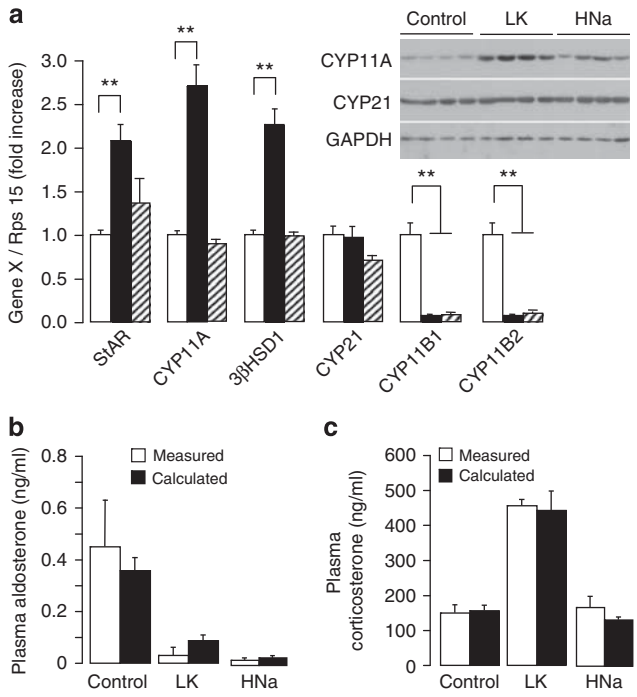

Figure 1 | Variations in steroid production and steroidogenic gene expression during potassium deficiency and sodium loading. (**a**) Expression of steroidogenic gene transcripts in adrenals from male mice fed a low-K^+ (black bars) or a high-Na^+ diet (hatched bars) for 8 days relative to that in male mice fed a control diet (white bars). Inset: adrenal expression of CYP21, CYP11A1 and GAPDH protein in male mice fed a control, low-K^+ or high-Na^+ diet for 8 days. Plasma aldosterone (**b**) and corticosterone (**c**) levels were both measured (white bars) and calculated (black bars) based upon the relative steroidogenic gene expression displayed in panel (**a**) for each individual ($n = 6$). Results are shown as means ± s.e.m. (significance is tested by non-paired Student *t*-test, $^{**}P < 0.01$). GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

we investigated the protein levels of these two enzymes. As shown in Figure 1a (inset), the expression of CYP21 is not affected by either diet, whereas that of CYP11A1 is increased under K^+ restriction.

The measured concentrations of plasma aldosterone (Figure 1b, white bars) in animals placed either under low-K^+ or high-Na^+ diets (0.05 ± 0.03 and 0.020 ± 0.005 ng/ml, respectively) are very similar to those calculated (black bars) using our model, and are much lower than the measured and calculated values in animals receiving the control diet. The calculated concentrations of plasma corticosterone (Figure 1c, black bars) are also in good agreement with measured values (white bars); both indicate that corticosterone levels increase specifically during K^+ restriction.

The model also served to predict the relative changes in the levels of pregnenolone, 11-deoxycorticosterone and progesterone induced by different diets. As reported in Table 1, an interesting prediction of the model is that in male mice fed a low-K^+ diet, the plasma concentration of progesterone increases by a factor 2.6 (Table 1). This change in progesterone levels concentration was found to be specific to chronic K^+ depletion, that is, no such large increase

Table 1 | Predicted plasma concentration of the intermediary metabolites of steroidogenesis in mice

	Low Na^+	High K^+	Low K^+	High Na^+
Pregnenolone	ND	ND	1.2	0.9
Progesterone	1.5	1.5	2.6	1.2
11-deoxycorticosterone	1.1	1.4	7.1	1.9

Relative levels of steroidogenic gene expression, as given in Supplementary Table 2 online and Figure 1a, were used to calculate the plasma levels of pregnenolone, progesterone and 11-deoxycorticosterone.

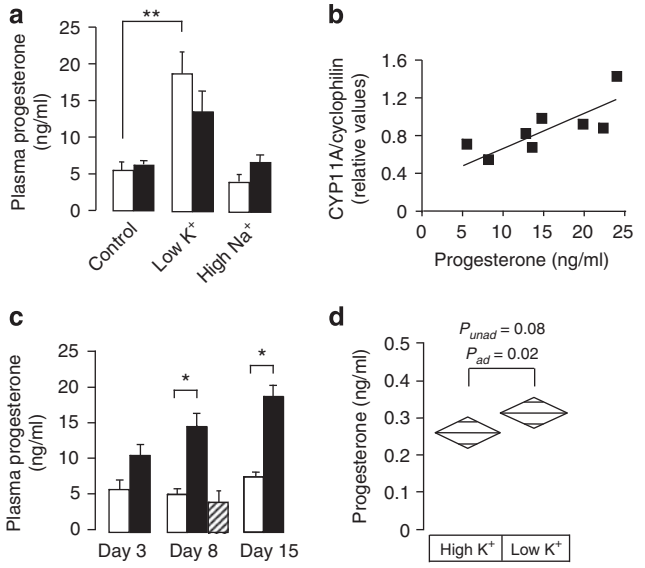

Figure 2 | Plasma progesterone concentration in males under different diets. (**a**) Plasma progesterone levels were measured (white bars) or calculated (black bars) after 8 days of different diets ($n = 6$). Results are shown as means ± s.e.m. (non-paired Student *t*-test $^{**}P < 0.01$). (**b**) Correlation between individual progesterone concentration (ng/ml) and level of CYP11A expression ($n = 8$). (**c**) Time-dependent progesterone production in response to K^+ restriction. Male mice were placed under normal (white bars) or low-K^+ diet (black bars) or low-K^+ diet with 1% of KCl in the drinking water (hatched bar). Results are shown as means ± s.e.m. (non-paired Student *t*-test, $^*P < 0.05$, day 3, $n = 40$ in each group; day 8, $n = 60$ in each group; day 15, $n = 55$ in each group; day 8 + KCl $n = 10$). (**d**) Plasma progesterone concentration measured in two groups of men selected according to their habitual daily K^+-intake. Results are shown as means with 95% confidence interval (diamonds).

was predicted under Na^+ loading (nor for the other two regimes).

Plasma progesterone level depends on dietary K^+ intake in male

To check this prediction, we measured experimentally the plasma concentration of progesterone in male mice placed under both diets for 8 days (Figure 2a, white bars). Other physiological parameters appear in Table 2. The progesterone level in the control group was 4.9 ± 1.1 ng/ml (similar to the predicted value, black bars) and did not vary significantly when mice were fed a high-Na^+ diet (Figure 2a). However, as predicted, K^+ depletion induced a significant increase in the plasma concentration of progesterone, to 18.8 ± 1.1 ng/ml. The correlation between the expression of the main rate-limiting enzyme, CYP11A, and the production of progesterone, shown in Figure 2b, can be described as a simple linear relationship ($r^2 = 0.73$). As shown in Figure 2c, the adrenal production of progesterone in male mice placed under K^+ restriction (black bars) was significantly increased after 8–15 days when compared to the control group (white bars). The stimulation of progesterone synthesis was reversible (not shown) and a direct consequence of dietary K^+ restriction since K^+ replenishment by addition of potassium to drinking water abolished the plasma progesterone increase (hatched bars). Similar results were obtained with ovariectomized female mice (Supplementary Figure S2 online), indicating that stimulation of adrenal production of progesterone is not gender-dependent.

We then investigated whether there is a similar correlation between K^+ intake and plasma progesterone levels in men. Plasma progesterone concentration was lower in the high-K^+ group than in the low-K^+ group (Supplementary Table S4 online and Figure 2d). The difference is significant ($P = 0.02$) when adjusted for the main characteristics of individuals. Performing step-by-step adjustment showed that the improvement in statistical significance was mainly due to habitual K^+ intake, which was itself correlated with plasma progesterone concentration ($P = 0.04$).

Relationship between urinary K^+ content and plasma progesterone

The specific increase in progesterone concentration following K^+ depletion suggests an adaptive response. To determine whether it plays a role in K^+ conservation, we measured simultaneously plasma progesterone concentration and the amount of K^+ excreted in urine (Figure 3, black dots) in male mice under K^+ restriction. Our results show that a high level of progesterone is correlated with more efficient potassium conservation ($r^2 = 0.538$). Adrenalectomized male mice, unable to produce progesterone in response to K^+ restriction (inset), could not reduce their urinary potassium excretion as efficiently as normal mice (Figure 3, white dots and inset) and therefore lost almost twice as much K^+ in their urine every day.

Progesterone stimulates cHKA expression in mouse cultured cells

To understand the mechanisms by which progesterone contributes to renal K^+ conservation, we incubated mouse collecting duct (mCCD) principal cell lines with progesterone (1 μg/ml) for 24 h and measured the expression of different genes known to be directly or indirectly involved in K^+ transport. As shown in Figure 4a, among the genes tested, progesterone induced a 5- and 3-fold increase in the expression of the cHKAα subunit (cHKα) and the Na,K-ATPase β1 subunit (NKA β1), respectively. We then examined the activity of cHKA in response to progesterone. Both ouabain-sensitive apical Rb^{86} uptake (Figure 4b) and

Table 2 | Physiological parameters of mice under a normal diet and following 8 days of K^+ depletion

	Plasma K^+ (mmol/l)	Plasma HCO_3^- (mmol/l)	Plasma pH	Δweight d8-d0 (g)	Food intake (g)
Control	4 ± 0.1	22.8 ± 0.9	7.18 ± 0.02	2.7 ± 0.4	3.6 ± 0.1
Low-K^+ diet (day 8)	2.5 ± 0.09**	23.4 ± 0.9	7.22 ± 0.01	−2.1 ± 0.3**	3.5 ± 0.1

Results are shown as mean ± s.e.m. (non-paired Student *t*-test, $^{**}P < 0.01$).

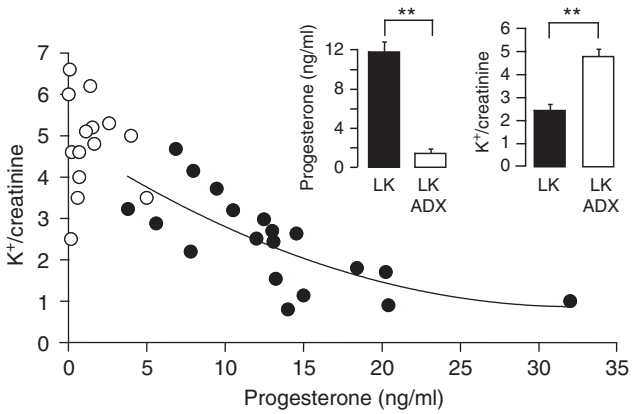

Figure 3 | Antikaliuretic action of progesterone during potassium restriction. Urinary potassium excretion versus plasma progesterone concentration in individual control (black dots) and adrenalectomized (white dots) mice after a 15-day low-K^+ (LK) diet. Inset: Mean plasma progesterone concentration and mean urinary potassium following a low-K^+ diet in control (black bars) or adrenalectomized (ADX, white bars) mice. Results are shown as means ± s.e.m. $^{**}P < 0.01$ (control mice, $n = 19$; ADX mice, $n = 15$).

K^+-dependent intracellular pH recovery after acidification (Figure 4c) were found to be stimulated by progesterone (100 ng/ml for 4–7 days). Interestingly, this stimulation was sensitive to RU486, a nuclear progesterone receptor antagonist (Figure 4c).

Progesterone stimulates cHKA expression *in vivo* during K^+ restriction

The role of progesterone in mediating the effects of K^+ restriction on cHKA activity was further examined *in vivo*. Under the control diet, expression of the cHKα and plasma progesterone levels were both low (Figure 5a, white dots), but the increase in progesterone concentration that is induced by a chronic low K^+ diet was accompanied by a proportional increase in cHKα subunit expression (black dots, $r^2 = 0.799$). This stimulation of cHKα expression was markedly reduced by treatment with RU486 (gray dots) with a direct impact on the ability to efficiently retain K^+ (Figure 5b). To further confirm the role of progesterone, we pharmacologically inhibited steroidogenesis by injecting aminogluthetimide to mice on a low-K^+ diet. As shown in Figure 5c, this treatment reduced the stimulation of the cHKα subunit. Another approach consisted in investigating how adrenalectomized mice (ADX) respond to K^+ depletion. As shown in Figure 5d, after 8 days under a low-K^+ diet, renal expression of the cHKα subunit was not increased in ADX mice. It should also be noted that these mice handled K^+ restriction differently than normal mice, since 25% of them died when K^+ restriction was prolonged beyond 8 days.

Antikaliuretic action of progesterone is dependent on cHKA

To further establish the antikaliuretic effect of progesterone we first treated male mice with progesterone (3 daily injections raising plasma progesterone concentration from 6.8 ± 0.9 to 35 ± 3 ng/ml) and monitored biological and metabolic parameters (Supplementary Table S5 online). Plasma K^+, bicarbonate and pH were not modified by this treatment (not shown). As shown in Figure 6a, urinary potassium excretion (relative to creatinine excretion) gradually decreased after progesterone treatment, with a significant effect after the third injection when compared either to the control group at day 3 (unpaired Student *t*-test $P = 0.04$) or to the day −1 of the experiment (paired Student t-test, $p = 0.03$). This 20-25 % decrease in K^+ excretion cannot be linked to a putative effect on the mineralocorticoid receptor since sodium excretion was not affected (Supplementary Table S5 online). To confirm the involvement of cHKA in this antikaliuretic mechanism, we performed this experiment in C57Bl6 mice invalidated or not for the cHKα subunit.[12] Following treatment, progesterone concentration was 84 ± 9 and 65 ± 14 ng/ml in cHKα+/+ and −/−, respectively. However, the amount of excreted K^+ was significantly decreased (by 15–20%) in cHKα+/+ mice relative to the basal level, whereas K^+ excretion remained unchanged in the cHKα−/− group, as shown in Figure 6b. In these experiments, we did not observe other effects of progesterone on general metabolic parameters (Supplementary Table S5 online). These results indicate that the antikaliuretic action of progesterone is indeed mediated by the induction of cHKA.

DISCUSSION

Physiological adaptation to metabolic stresses requires that the organism be able to detect changes, to produce 'signals' that convey information to different target tissues and organs, and finally to react appropriately in response to a given perturbation. In this study, we established that the concentration profile of circulating adrenal steroids is drastically modified by Na^+ loading and K^+ restriction. To search for specific adaptive responses, we first developed a mathematical model of adrenal steroidogenesis, conceptually similar to previously published models of steroidogenesis in testes[13] and ovaries.[14] The model predicted that adrenal progesterone production in male mice increases specifically in response to dietary K^+ restriction. Subsequent experimental measurements revealed that progesterone acts as an

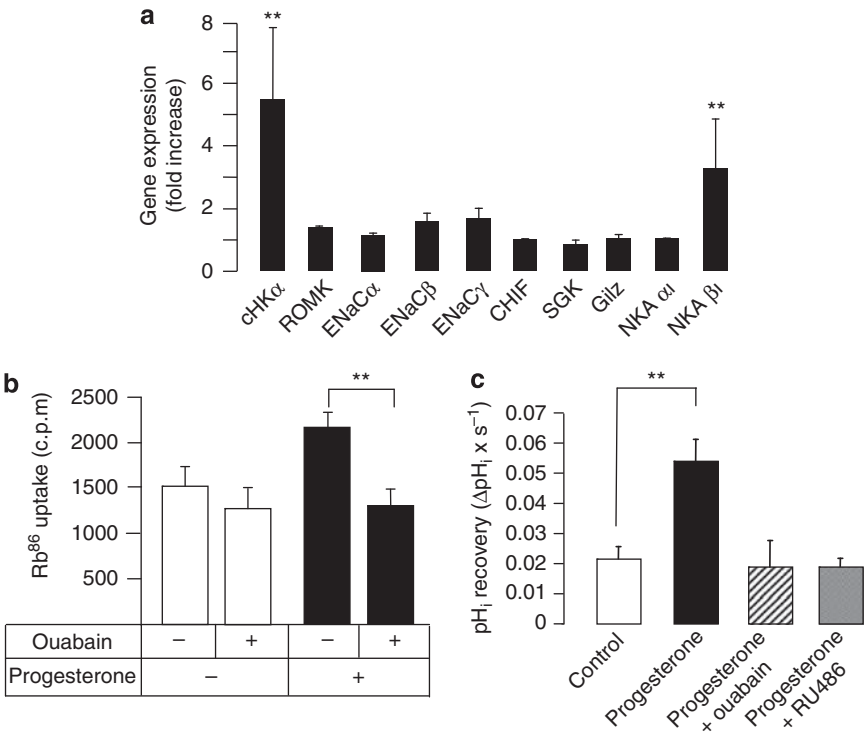

Figure 4 | Progesterone stimulates cHKA activity in mCCD cell line. (**a**) Expression of a set of genes involved in K^+ transport in mCCD cells, relative to cyclophilin expression, after incubation with 1 μg/ml of progesterone for 24 h. Results are expressed as fold-increase relative to vehicle-treated cells ($n = 2$ in triplicate). (**b**) Apical ouabain-sensitive rubidium uptake by mCCD incubated (black bars) or not (white bars) with progesterone (100 ng/ml) for 4 days. Results are shown as means ± s.e.m. (non-paired Student *t*-test, $*P < 0.05$, $**P < 0.01$). (**c**) K^+-dependent alkalinization of mCCD cells untreated (white bar, $n = 3$ in triplicate) or treated with progesterone (black bar, $n = 3$ in triplicate) or treated with progesterone and extemporaneously exposed to ouabain (hatched bar, $n = 3$ in triplicate) or concomitantly treated with progesterone and RU486 (gray bar, $n = 2$ in triplicate) (non-paired Student *t*-test, $**P < 0.01$). cHKA, colonic H, K-ATPase; mCCD, mouse collecting duct.

'antikaliuretic' hormone independently of gender and reproductive functions.

The present study is the first one to highlight a correlation between progesterone levels and daily K^+ intake in men. The finding that men with a low K^+ intake have a statistically higher level of progesterone may have important consequences, for instance in the development of gastric adenocarcinoma.[15]

Up to now, progesterone was thought to act on the kidney by exerting a putative anti-mineralocorticoid effect. In the particular physiological situation we investigated here (i.e., K^+ restriction), such an antagonistic mechanism is unlikely to be involved since aldosterone concentration decreases by 90% during chronic potassium depletion. On the other hand, progesterone has also been described as a partial and less efficient agonist for mineralo- or glucocorticoid receptors.[16,17] Such an effect is similarly unlikely here, since it would enhance K^+ secretion, whereas we observed K^+ retention in the present study. Moreover, expression of the mineralocorticoid- and glucocorticoid-induced genes (GilZ, ENaCα) was not modified in the kidney of mice fed a chronic low-K^+ diet (Supplementary Figure S3A and S3B online) and cells incubated with progesterone or dexamethasone displayed a completely different response (Supplementary Figure S3C online).

The presence of nuclear progesterone receptors in the distal nephron,[18] combined with the antagonist effect of RU486 on cHKα expression and its effects on urinary K^+ excretion, strongly suggest the involvement of this nuclear receptor in the renal action of progesterone.

A strong feature of dietary K^+ restriction is the renal switch from a high rate of K^+ secretion to efficient K^+ conservation mediated in part by stimulation of cHKA in the distal nephron.[19] Our results strongly suggest that this stimulation is under the control of progesterone, and that it acts to conserve K^+ more efficiently. We also showed that modification of adrenal steroidogenesis and progesterone production is a late effect, that is, adrenals start overproducing progesterone when plasma K^+ is already low (between the third and eighth day of a low-K^+ diet). Thus, this effect cannot be part of the hypokalemia-independent process described by McDonough's group.[20,21]

Two potential steroid response elements in the promoter of the cHKα gene have been identified[22] and correlated with the aldosterone-induced stimulation of cHKA in the colon under a low-Na^+ diet.[23] Based on our present observations, we speculate that the presence of these steroid response elements may partly explain how cHKA expression can be stimulated by progesterone and its nuclear receptor in low-K^+ conditions.

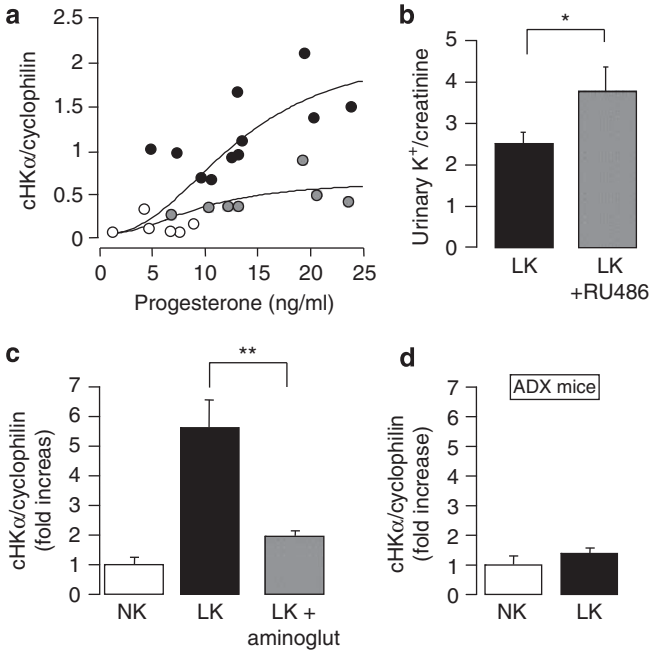

Figure 5 | Low-K^+ diet-induced expression of cHKα and urinary K^+ excretion are sensitive to RU486. (**a**) Correlation between plasma progesterone concentration and mRNA expression of cHKα in mice under normal diet (white dots) or low-K^+ diet (15 days, black dots) or low-K^+ (LK) diet + RU486 treatment (gray dots). Results are shown as means ± s.e.m. ($n = 7$–12 mice). (**b**) Urinary K^+ of mice under low-K^+ diet receiving RU486 (gray bar, $n = 7$) or not (black bar, $n = 12$). Results are shown as means ± s.e.m. (non-paired Student *t*-test, $^*P < 0.05$). (**c**) Expression of the cHKα subunit in mice fed a normal (NK) diet, (white bar, $n = 5$) or a low-K^+ diet with (gray bar, $n = 5$) or without (black bar, $n = 5$) aminogluthetimide. (**d**) Expression of the cHKα subunit under normal (white bar) or low-K^+ diets (black bar) in adrenalectomized male mice ($n = 7$).

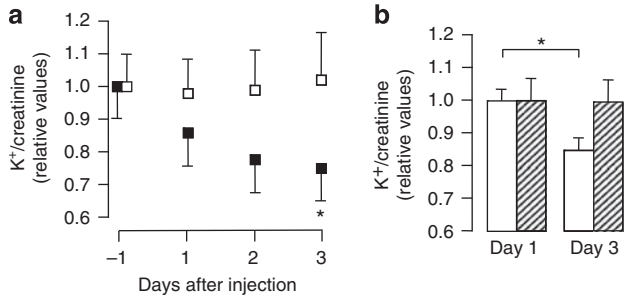

Figure 6 | Progesterone promotes urinary K^+ retention mediated by cHKA. (**a**) 24 h-urinary K^+-to-creatinine ratio relative to mean of the day before treatment (day 1) with progesterone (black squares) or vehicle (white squares). On day 1, K^+-to-creatinine ratio was 0.022 ± 0.002 and 0.021 ± 0.002 μmol/μmol, respectively. Results are shown as means ± s.e.m., $n = 18$; non-paired Student *t*-test at day 3, treated versus non-treated group, $^*P < 0.05$. (**b**) Same measurement in cHKα +/+ mice (white bars) or cHKα−/− mice (hatched bars). On day 1, K^+-to-creatinine ratio was 0.027 ± 0.001 μmol/μmol and 0.019 ± 0.001 μmol/μmol respectively. Results are shown as means ± s.e.m, $n = 6$ (Student *t*-test, $^*P < 0.05$).

It is tempting to speculate that males and females regulate their urinary K^+ excretion differently[24] precisely because of the ovarian production of progesterone. However there are many hormonal differences between the two sexes besides progesterone levels; further investigation is thus needed. During pregnancy, ion homeostasis is strongly modified because of multiple actions all along the nephron.[25,26] The amount of potassium retained during pregnancy reaches 300 mEq in women (around 15% of total tissue K^+ content, for review see ref. 27) but the mechanisms leading to this retention remain to be elucidated. Based on our results, we speculate that progesterone mediates, at least in part, pregnancy-induced K^+ retention via its antikaliuretic function.

MATERIALS AND METHODS

Ethical statement

All animal procedures were carried out in accordance with the French legislation for animal care and experimentation. The human study (SUVIMAX) was authorized by French authorities in 1994 (Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale, file number 706, and the Commission Nationale de l'Informatique et des Libertés, file number 94-042).

Mathematical model of steroidogenesis in rodents

The mathematical model of steroidogenesis is fully described in Supplementary Information online and is available upon request.

In vivo studies

Experiments were performed on CD1 mice (Charles River, L'Arbresle, France) weighing 24–26 g. Animals were fed the standard laboratory diet (Safe, France) (0.28% Na^+ and 0.6% K^+), a low-K^+ diet (0.28% Na^+ and 0% K^+) or a high-Na^+ diet (0.7% Na^+ and 0.6% K^+) *ad libitum* with free access to water. Progesterone (50 mg/kg/day) or RU486 (3 mg/kg/day) or aminogluthetimide (20 mg/kg/day) were injected sub-cutaneously (sc) every 24 h after dilution in an ethanol/sesame oil mixture or in water. Wild-type and knock-out mice for cHKα subunit[12] were also used for metabolic studies after 3 days of animal adaptation to encaging. Baseline 24-h urine volume, food and water intakes were measured and urinary creatinine, and electrolyte concentrations were determined on an automatic analyzer (Konelab 20i; Thermo, Cergy Pontoise, France). When indicated, urinary K^+ and creatinine were measured from urine spots by HPLC (Dionex).

mCCD cell culture

Mouse CCD cells ($mCCD_{cl1}$); a cell line established and described earlier were cultured as described in the original study.[28] In summary, cells were grown in supplemented DMEM/F-12 medium (Invitrogen) containing 50 nmol/l dexamethasone (Amersham Bioscience) for 5 days (growth medium), and then 3 nmol/l dexamethasone (filter medium) for a minimum of another 5 days. Rubidium fluxes and intracellular pH measurements are described in Supplementary Information online.

Human studies

The association between K^+ intake and plasma progesterone concentration was tested in 200 healthy men belonging to a previously described cohort (known as the SUVIMAX study)[29] (see Supplementary Table S4 online for parameters). To test whether plasma progesterone was associated with K^+ intake in men, two contrasted groups of 100 individuals were defined among the cohort

($n = 601$), those with the lowest K^+ intake (low-K^+ group) and those having the highest K^+ intake (high-K^+ group). Mean plasma progesterone concentration was compared between these two groups by a multivariate ANOVA test. All analyses were performed with the statistical discovery software JMP 8 (SAS, Cary, NC).

Other methods

More commonly used methods for measuring gene or protein expression as well as plasma progesterone concentration are described in Supplementary Information online.

DISCLOSURE

All the authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Table S1. Parameter values for mathematical model.
Table S2. Reported data on the expression of steroidogenic genes in rats under different physiological conditions.
Table S3. Sensitivity analysis of the adrenal steroidogenesis model.
Table S4. Plasma progesterone concentration and main characteristics of men according to dietary K^+ intake groups.
Table S5. Biological parameters of male mice treated or not with daily progesterone injection.
Figure S1. Validation of the mathematical model of adrenal steroidogenesis.
Figure S2. Time-dependent progesterone production in response to K^+ restriction.
Figure S3. Glucocorticoids are not involved in ion transporter regulation under a low-K^+ diet.
Supplementary Information. SI Mathematical Model of Steroidogenesis in Rodents.
Supplementary material is linked to the online version of the paper at http://www.nature.com/ki

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