

Invalidation of TASK1 potassium channels disrupts adrenal gland zonation and mineralocorticoid homeostasis

Dirk Heitzmann^{1,2}, Renaud Derand³,
Stefan Jungbauer¹, Sascha Bandulik¹,
Christina Sterner¹, Frank Schweda¹,
Abeer El Wakil³, Enzo Lalli³, Nicolas Guy³,
Raymond Mengual⁴, Markus Reichold¹,
Ines Tegtmeier¹, Saïd Bendahhou³,
Celso E Gomez-Sanchez⁵, M Isabel Aller⁶,
William Wisden⁷, Achim Weber⁸,
Florian Lesage³, Richard Warth^{1,9,*}
and Jacques Barhanin^{3,9}

¹Institute of Physiology, University of Regensburg, Regensburg, Germany, ²Clinic and Policlinic for Internal Medicine II, University of Regensburg, Regensburg, Germany, ³Institut de Pharmacologie Moléculaire et Cellulaire, CNRS and Université de Nice Sophia Antipolis, Valbonne Sophia Antipolis, France, ⁴Centre Hospitalo-Universitaire de Nice, Nice, France, ⁵Division of Endocrinology, GV Montgomery VA Medical Center, Jackson, MS, USA, ⁶Instituto de Neurociencias de Alicante, Consejo Superior de Investigaciones Científicas-Universidad Miguel Hernández, San Juan de Alicante, Spain, ⁷Institute of Medical Sciences, University of Aberdeen, Aberdeen, UK and ⁸Department of Pathology, University Hospital Zurich, Zurich, Switzerland

TASK1 (KCNK3) and TASK3 (KCNK9) are two-pore domain potassium channels highly expressed in adrenal glands. TASK1/TASK3 heterodimers are believed to contribute to the background conductance whose inhibition by angiotensin II stimulates aldosterone secretion. We used *task1*^{-/-} mice to analyze the role of this channel in adrenal gland function. *Task1*^{-/-} exhibited severe hyperaldosteronism independent of salt intake, hypokalemia, and arterial 'low-renin' hypertension. The hyperaldosteronism was fully remediable by glucocorticoids. The aldosterone phenotype was caused by an adrenocortical zonation defect. Aldosterone synthase was absent in the outer cortex normally corresponding to the zona glomerulosa, but abundant in the reticulo-fasciculata zone. The impaired mineralocorticoid homeostasis and zonation were independent of the sex in young mice, but were restricted to females in adults. Patch-clamp experiments on adrenal cells suggest that *task3* and other K⁺ channels compensate for the *task1* absence. Adrenal zonation appears as a dynamic process that even can take place in adulthood. The striking changes in the adrenocortical architecture in *task1*^{-/-} mice are the first demonstration of the causative role of a potassium channel in development/differentiation.

*Corresponding author. Institute of Physiology, University of Regensburg, Universitaetsstrasse 31, NWF III—VKL, Regensburg 93053, Germany. Tel.: +49 941 943 2894; Fax: +49 941 943 2896; E-mail: richard.warth@vkl.uni-regensburg.de

⁹These authors contributed equally to this work

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Introduction

The mineralocorticoid aldosterone is the major regulator of extracellular fluid and salt balance thereby controlling arterial blood pressure. The major target organ of aldosterone is the kidney, where it promotes Na⁺ retention and K⁺ secretion in the aldosterone-sensitive distal nephron. Dysregulation of aldosterone secretion leading to hyperaldosteronism is causal for about 3% of the cases of arterial hypertension (Nussberger, 2003). Moreover, aldosterone contributes to several aspects of cardiac fibrosis, cardiovascular dysfunction as well as progressive renal disease (Ibrahim and Hostetter, 2003; Remuzzi *et al*, 2005). The importance of aldosterone as clinical risk factor has been highlighted by recent clinical trials (Aldosterone Evaluation Study, (RALES); Eplerenone Heart failure and SURvival Study (EPHESUS)) demonstrating the beneficial effects of mineralocorticoid receptor antagonists in patients with heart failure (Chai and Danser, 2006).

The mechanisms regulating aldosterone secretion in glomerulosa cells of the adrenal cortex have been studied for already half a century. The most important physiological stimuli for aldosterone secretion are angiotensin II, high plasma K⁺, and, for a minor part, ACTH. Binding of angiotensin II to AT1 receptors stimulates phospholipase C, which in turn triggers InsP₃-dependent Ca²⁺ release from intracellular stores. The immediate phase of aldosterone secretion is followed by sustained secretion, which is dependent on membrane depolarization and activation of T- and L-type Ca²⁺ channels. High plasmatic K⁺ concentrations stimulate exclusively the sustained phase of aldosterone secretion via influx of Ca²⁺ through voltage-dependent Ca²⁺ channels (Lotshaw, 2001). In this respect, the glomerulosa cell appears to be a unique sensor of extracellular K⁺ (Spat and Hunyady, 2004): increases of the extracellular K⁺ concentration by approximately 1 mmol/l are sufficient to double aldosterone secretion; maximal secretion occurs at 8 mmol/l of extracellular K⁺ (Lotshaw, 2001). The basis for this unique sensitivity for plasma potassium concentration is a very high background K⁺ conductance, which makes the membrane voltage strictly follow the K⁺ equilibrium potential: at low extracellular K⁺, the membrane voltage is hyperpolarized; upon relatively small increases in extracellular K⁺, membrane depolarization between -80 and -70 mV suffices to

activate T-type Ca^{2+} channels in rat glomerulosa cells (Szabadkai *et al*, 1999; Lotshaw, 2001).

The relative contributions of molecularly identified K^+ channels to membrane depolarization and stimulation of aldosterone secretion have not been fully elucidated. Studies from genetically modified mice have pointed to a role of voltage-dependent KCNE1/KCNQ1 channels for the regulation of aldosterone secretion (Arrighi *et al*, 2001). Moreover, Ca^{2+} -activated MaxiK channels have been proposed as K^+ channels limiting aldosterone secretion (Sausbier *et al*, 2005). These K^+ channels are not constitutively open at rest and therefore cannot underlie the background conductance necessary to confer the exquisite extracellular K^+ sensitivity to glomerulosa cells. Electrophysiological recordings of glomerulosa cells have emphasized the importance for leak-type K^+ channels of the 2P domain (K2P) family (Czirjak *et al*, 2000). The human K2P channel family comprises 15 different members, which are characterized by their typical structure of four transmembrane domains and a tandem of two pore-forming loops (Lesage and Lazdunski, 2000). These channels exhibit little voltage dependence, remain open at negative membrane potentials, and can be modulated by a variety of physical and chemical stimuli such as external pH, membrane stretch, protein kinase A- or protein kinase C-dependent phosphorylation, and PiP_2 membrane depletion. Recently, *task1* (KCNK3) and *task3* (KCNK9), two K2P channels, were reported to be the dominant background channels in rat glomerulosa cells (Czirjak *et al*, 2000; Czirjak and Enyedi, 2002). In bovine glomerulosa cells, *trek1*, another K2P channel, is thought to be a major channel setting the membrane potential (Enyeart *et al*, 2004). The situation appears confusing mainly because the channels are poorly characterized beyond electrophysiological methods. However, a pivotal role of TASK1 is strongly suggested by the unusual high abundance of its messenger RNA in adrenal glands of several species including human and mouse (<http://symatlas.gnf.org/SymAtlas/>).

In this study, we have investigated the specific contribution of *task1* K^+ channels to the control of aldosterone secretion using the *task1* knockout (*task1*^{-/-}) mouse as a model. We provide evidence that *task1*^{-/-} mice have a strongly impaired mineralocorticoid homeostasis resulting in salt retention, arterial hypertension associated with low plasma renin activity. Surprisingly, this phenotype was restricted to the female gender. This pathology was caused by abnormal zonation of the adrenal cortex in female mice. In young male mice, the same zonation defect was observed, which however regressed after puberty. Age- and gender-dependent *task3* expression might rescue the adrenal gland abnormalities.

Results

Plasma aldosterone, renin, and K^+ levels

On normal salt diet, adult *task1*^{-/-} mice had a higher plasma aldosterone concentration than wild-type and heterozygous mice (*task1*^{+/+} 394 ± 73 ng/l, *n* = 52; *task1*^{+/-} 235 ± 44 ng/l, *n* = 22; *task1*^{-/-} 2361 ± 452 ng/l, *n* = 55). This hyperaldosteronism was restricted to the female gender as males displayed normal aldosterone values (Figure 1). To assess the response upon physiological regulators of aldosterone secretion, the salt intake was modified by varying the salt

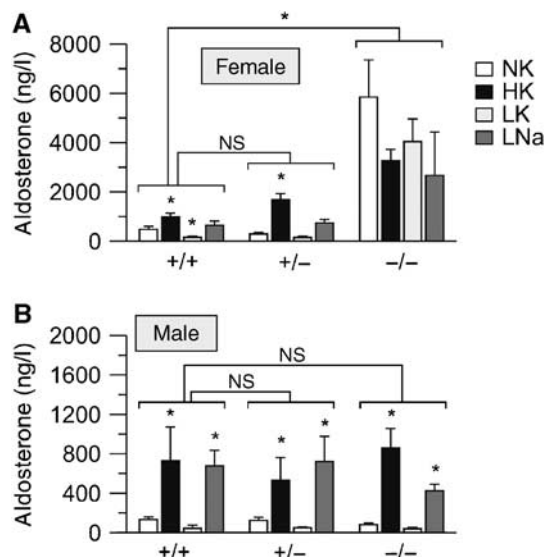


Figure 1 Aldosterone levels in *task1*^{-/-} mice. Plasma aldosterone levels of adult female (A) and male (B) mice on various salt diets are shown. In female *task1*^{+/+} (+/+) and *task1*^{+/-} (+/-) mice, aldosterone concentrations are not different from each other under the three diet conditions (*P* = 0.679), whereas they are considerably higher (*P* < 0.0001) in *task1*^{-/-} mice (-/-). In males, aldosterone levels varied with the diet independently from the genotype (*P* > 0.1). The numbers of female mice per genotype were 11–27 (+/+), 13–15 (+/-), and 17–32 (-/-). Corresponding numbers for males were 10–27 (+/+), 3–9 (+/-), and 9–23 (-/-). NK: normal K^+ (0.75%) diet; HK: high- K^+ (3%) diet; LK: low- K^+ (0.05%) diet; LNa: low- Na^+ (<0.005%) diet. *Indicates statistically different from normal K^+ diet (NK) or between the groups as indicated. NS: not significantly different.

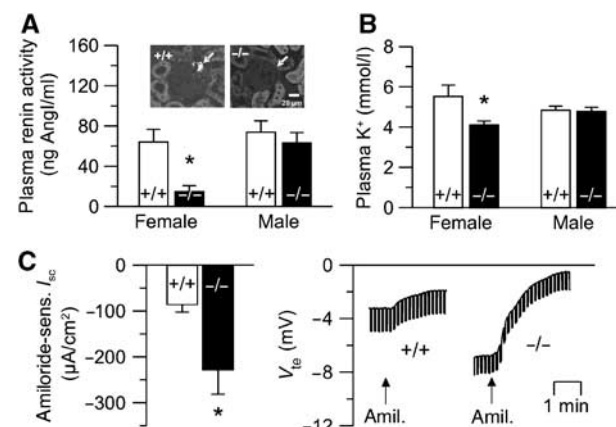


Figure 2 Primary hyperaldosteronism-associated symptoms under normal diet condition. (A) Female *task1*^{-/-} mice showed a strongly decreased plasma renin activity, which was paralleled by reduced renin-specific immunostaining in female *task1*^{-/-} kidney (right picture) compared with wild type (left picture) (*n* = 20). In males, renin activities were similar for both genotypes (*n* = 15). (B) Hypokalemia in female *task1*^{-/-} mice (*n* = 14); normokalemia in male mice (*n* = 15). (C) Ussing chamber experiments of distal colonic mucosa. As a measure of ENaC-dependent Na^+ reabsorption, amiloride-sensitive equivalent short circuit current (I_{sc}) was increased in female *task1*^{-/-} mice (left hand side; *n* = 14). An original experiment showing the effect of luminal amiloride (10 μM) on transepithelial voltage (V_{te}) is depicted on the right hand side. *Indicates statistically different from *task1*^{+/+} mice.

content of the diet. The response to high and low K^+ intake as well as low Na^+ was similar in heterozygous and wild-type mice of either sex. By contrast, female *task1*^{-/-} mice exhibited a total loss of the physiological control of aldoster-

one secretion; plasma aldosterone concentration was neither stimulated by high K^+ and low Na^+ diet nor was it decreased by low K^+ diet (Figure 1A). In female *task1*^{-/-} mice, invariable high plasma concentrations of aldosterone were paralleled by reduced plasma renin activity (Figure 2A), which is

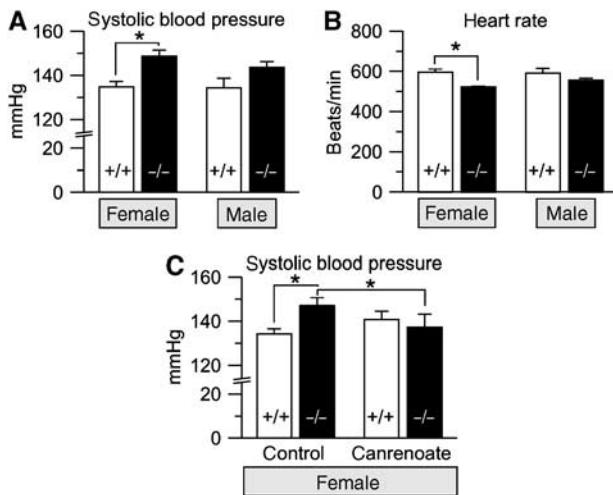


Figure 3 Hyperaldosteronism and hypertension in *task1*^{-/-} mice. (A) In tail cuff measurements, female *task1*^{-/-} mice displayed an increased systolic blood pressure compared with *task1*^{+/+} mice ($n = 20$). Male *task1*^{-/-} only tended to have a higher blood pressure ($P = 0.067$; $n = 20$). (B) Heart rate was lower in female *task1*^{-/-} compared with *task1*^{+/+} mice ($n = 20$). In male mice, the heart rate was not different between the genotypes ($P = 0.20$; $n = 20$). (C) Inhibition of the mineralocorticoid receptor by canrenoate normalized systolic blood pressure of female *task1*^{-/-} mice ($n = 12$, paired experiments). *Indicates statistically different.

indicative for primary hyperaldosteronism. Aldosterone stimulates K^+ secretion and Na^+ reabsorption in its target tissues. In female mice, plasma K^+ concentration was reduced (Figure 2B); plasma Na^+ concentration was not different between the genotypes. However, aldosterone-regulated Na^+ transport through the epithelial Na^+ channels (ENaC) was increased in the distal colon mucosa of female *task1*^{-/-} mice (Figure 2C).

Blood pressure and heart rate

Since enhanced salt retention in hyperaldosteronism can cause arterial hypertension, we assessed blood pressure by tail cuff measurements. Systolic blood pressure values of male *task1*^{-/-} were not significantly different from those of male wild-type mice. In female *task1*^{-/-} mice, however, systolic arterial blood pressure was 15 mmHg higher than in wild-type mice (Figure 3A). This increase in blood pressure was paralleled by a significant bradycardia, suggesting that the elevated blood pressure was not caused by a higher sympathetic nerve tone. In male mice, no difference in the heart rate was observed (Figure 3B).

To further evaluate the contribution of increased aldosterone concentration in female *task1*^{-/-} mice as cause for the arterial hypertension, the effect of the aldosterone receptor blocker canrenoate was tested in another set of experiments. Under control conditions, female *task1*^{-/-} mice displayed higher systolic blood pressure values than wild-type mice. Next, canrenoate was added to the drinking water (canrenoate 1.25 g/l) for 10 days. From days 6–10, arterial blood pressure was determined. Canrenoate led to a decrease of systolic blood pressure in *task1*^{-/-} but not in wild-type

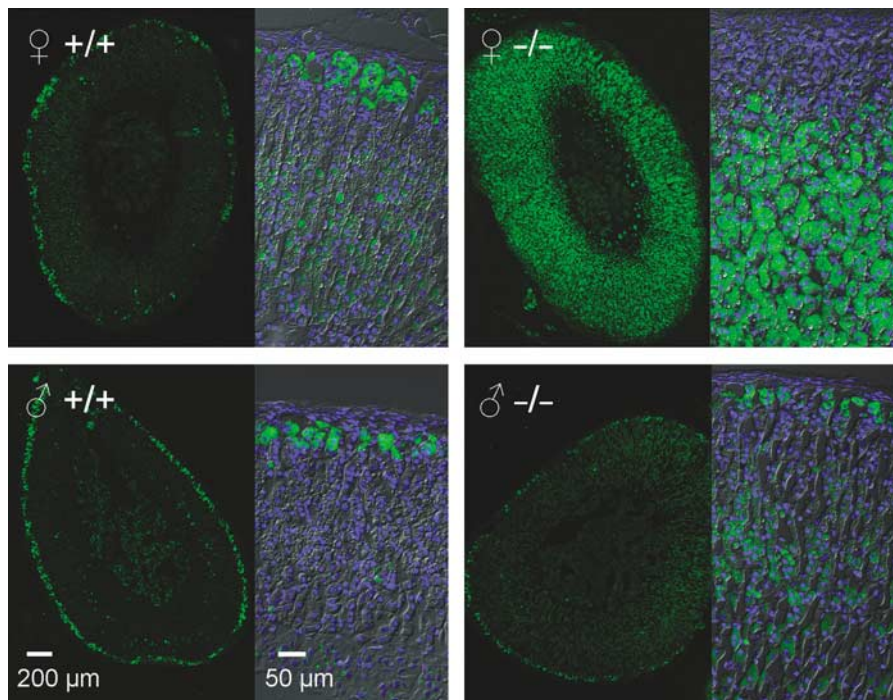


Figure 4 Mislocalization of aldosterone synthase in adrenal glands of *task1*^{-/-} mice is sex-dependent. In adult *task1*^{+/+} mice of either sex and in male *task1*^{-/-} mice, aldosterone synthase staining is restricted to the zona glomerulosa cells of the outer adrenal cortex. In female *task1*^{-/-} mice (right upper picture), however, the regular aldosterone synthase staining is disrupted and broadened to the inner parts of adrenal cortex. The right part of each panel shows higher magnification pictures of the adrenal cortex (aldosterone synthase in green, differential interference contrast in gray scale, nuclear staining with HOE33342 in blue).

mice, thereby diminishing the difference in blood pressure between the genotypes (Figure 3C). These data confirm that the arterial hypertension of female *task1*^{-/-} mice is caused by aldosterone-induced expansion of the extracellular volume.

Localization of aldosterone-producing cells in the adrenal cortex

The high aldosterone plasma concentrations of female *task1*^{-/-} mice pointed to an increased secretion by aldosterone-producing cells in the zona glomerulosa of the adrenal cortex. Interestingly, immunofluorescence experiments using an antibody directed against the aldosterone synthase (CYP11B2) disclosed a sex-dependent effect of the *task1* knockout on adrenocortical zonation. In wild-type mice of either sex, the aldosterone synthase-specific staining was localized exclusively in the glomerulosa cells of the outer layer of the adrenal cortex. In adrenal glands of female *task1*^{-/-} mice, however, the zona glomerulosa was virtually absent and aldosterone synthase-positive cells were observed in deeper zones of the adrenal cortex (Figure 4, right upper panels). In contrast, adult male *task1*^{-/-} mice did not show abnormal zonation patterns (Figure 4, right lower panels). High K⁺ diet is known to drastically increase aldosterone synthase expression in glomerulosa cells. Thus, we checked whether high K⁺ diet could unmask responsive glomerulosa-like cells in adrenal glands from female *task1*^{-/-} mice. Aldosterone synthase-specific staining was strongly increased after K⁺-rich diet in female *task1*^{+/+} mice. In female *task1*^{-/-} mice, no such increase could be observed (Figure 5A). Clearly, as observed for aldosterone itself, the aldosterone synthase expression is independent of the salt diet in female *task1*^{-/-} mice. Western blot analysis of aldosterone synthase (Figure 5B) and real-time PCR experiments (data not shown) further supported this conclusion.

The mislocalization of aldosterone synthase in the zona fasciculata raised the question whether aldosterone secretion could be under the control of ACTH instead of angiotensin II. Dexamethasone (6 mg/kg, s.c.) was administered to female mice for 3 days to decrease plasma ACTH concentrations. This treatment virtually suppressed aldosterone in female *task1*^{+/+} and *task1*^{-/-} mice (Figure 6). Therefore, hyperaldosteronism of female *task1*^{-/-} mice was fully glucocorticoid-remediable.

Corticosterone synthesis in adrenal glands

Next we investigated whether the replacement of the typical zona fasciculata by aldosterone-producing cells in female *task1*^{-/-} mice was paralleled by an impairment of corticosterone synthesis. To this end, expression of the corticosterone-producing enzyme 11beta-hydroxylase (CYP11B1) in adrenal glands was determined by semiquantitative real-time PCR. No difference in 11beta-hydroxylase expression was observed between *task1*^{-/-} and *task1*^{+/+}, but female mice showed a threefold higher expression level than male mice (data not shown), which is in agreement with previously published data (Bielohuby *et al*, 2007). Additionally, plasma corticosterone concentration was similar in both genotypes (female *task1*^{+/+} 391 ± 35 µg/l, n = 20; female *task1*^{-/-} 341 ± 26 µg/l, n = 41).

Dynamics of adrenal cortex zonation

The following series of experiments was aimed at elucidating possible mechanisms underlying the gender differences of

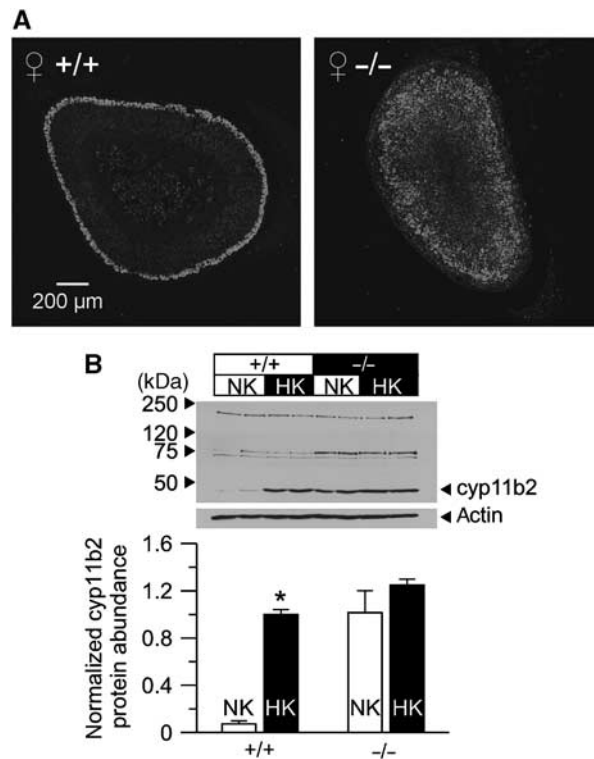


Figure 5 Effect of K⁺-rich diet on aldosterone synthase expression. (A) In female *task1*^{+/+} mice (left side), high-K⁺ diet strongly increased aldosterone synthase-specific staining in glomerulosa cells. In female *task1*^{-/-} mice (right side), high-K⁺ diet did not change localization and abundance of aldosterone synthase. (B) Western blot analysis of aldosterone synthase (cyp11b2) protein abundance in adrenal glands at normal K⁺ diet (NK) and high-K⁺ diet (HK). The upper trace shows a typical blot (the bands of higher molecular weight are considered non-specific), and the lower trace shows the quantification of aldosterone synthase protein expression (normalized to beta-actin; *task1*^{+/+}, n = 8; *task1*^{-/-}, n = 8). *Indicates statistically different from normal K⁺ diet (NK).

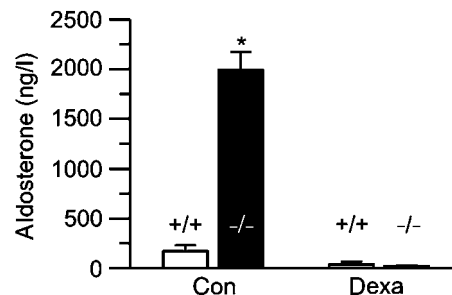


Figure 6 Glucocorticoid-remediable hyperaldosteronism in female *task1*^{-/-} mice. Plasma aldosterone concentration has been measured in *task1*^{+/+} and *task1*^{-/-} mice before (con) and after (dexa) administration of dexamethasone (n = 6). *Indicates statistically different from *task1*^{+/+} mice.

adrenal cortex zonation in *task1*^{-/-} mice. Obviously, mechanisms compensating for the absence of TASK1 were present in adult male *task1*^{-/-} but absent in female *task1*^{-/-} mice. Adrenal glands from mice of both sexes were compared before the age of puberty. Immunofluorescence of adrenal glands of mice at postnatal day 18 disclosed a normal zonation pattern of wild-type mice of either sex but abnormal zonation in male and female *task1*^{-/-} mice (Figure 7A).

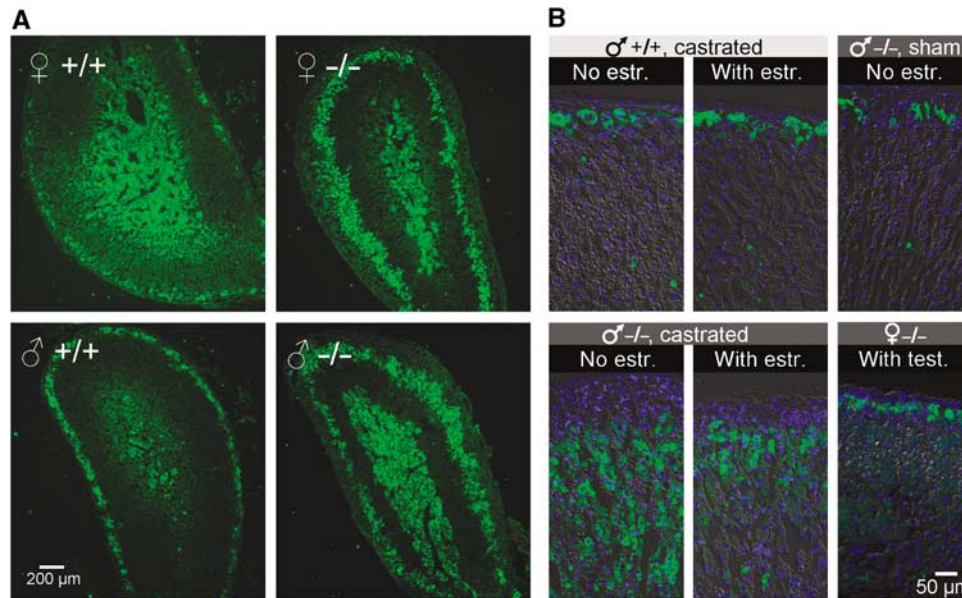


Figure 7 Age and sex dependence of adrenocortical zonation. (A) In 18-day-old *task1*^{+/+} mice of either sex, aldosterone synthase-specific staining is restricted to glomerulosa cells of outer adrenal cortex (left pictures). In *task1*^{-/-} mice of the same age, aberrant aldosterone synthase-specific staining is found in the mid-region of adrenal cortex (right pictures). The staining in the adrenal medulla is considered non-specific. (B) Effects of castration, estradiol (estr.), and testosterone (test.) treatment on aldosterone synthase localization. Male mice were castrated at the age of 5 weeks, followed by treatment with or without estradiol benzoate (4 μg/g/day, s.c., for 5 weeks). In male *task1*^{+/+}, castration (with or without estradiol treatment) did not affect adrenocortical zonation. Sham-operated male *task1*^{-/-} showed normal zonation, too (upper panel). In male *task1*^{-/-}, castration prevented normal zonation. Estradiol appeared to reduce aldosterone synthase expression without having clear effects on zonation patterns. In female *task1*^{-/-} mice, treatment with testosterone for 3 weeks induced normal zonation, highlighting the importance of androgens for adrenocortical rezonation in *task1*^{-/-} mice (lower panel).

To elucidate the possible role of sex hormones on adrenocortical zonation of *task1*^{-/-} mice in more detail, aldosterone synthase localization was determined in male mice after castration. Castration was performed at the age of 5 weeks (when male *task1*^{-/-} mice are not yet zoned) followed by an estrogen treatment. At the age of 10 weeks, castrated *task1*^{+/+} mice showed normal zonation irrespective of the treatment with or without estrogen (Figure 7B). Sham-operated *task1*^{-/-} mice also showed normal zonation. However, castration prevented normal zonation in *task1*^{-/-} mice. Treatment with estrogens did not enforce abnormal aldosterone synthase expression; if at all, high-dose estrogen treatment reduced aldosterone synthase expression without clearly restoring normal zonation pattern. These data suggested that the compensatory mechanisms allowing restoration of adrenocortical zonation in male *task1*^{-/-} mice were androgen-dependent. To further evaluate this hypothesis, 4-week-old female *task1*^{-/-} mice were treated with testosterone, and adrenocortical zonation was analyzed by aldosterone synthase-specific immunofluorescence. In fact, aldosterone synthase localization in female *task1*^{-/-} mice was redirected to the zona glomerulosa after testosterone treatment (Figure 7B).

Electrical properties of adrenocortical cells

To evaluate the contribution of *task1* channels for the whole-cell conductance, primary cultured adrenocortical cells of adult male *task1*^{-/-} and *task1*^{+/+} mice were examined by the patch-clamp technique. Cells from *task1*^{-/-} mice exhibited a more depolarized membrane voltage at resting conditions. In contrast to *task1*^{+/+} cells, *task1*^{-/-} cells displayed a non-significant change of whole-cell conductance upon acid extracellular pH. However, the membrane voltage, which is

more sensitive to small changes of K⁺ conductance, depolarized significantly at pH 6, suggesting the presence of other acid-sensitive K⁺ channels (e.g., homomeric *task3* channels) in *task1*^{-/-} cells (Figure 8A and B). Angiotensin II inhibited the whole-cell conductance and depolarized the membrane of *task1*^{+/+} and *task1*^{-/-} cells. Interestingly, the inhibition of the whole-cell conductance by angiotensin II was more pronounced than by acidic extracellular pH, indicating that angiotensin II—besides TASK-like channels—inhibits acid-insensitive channels, too. The *task1*-related K⁺ channel *task3* is also expressed in adrenal glands. Therefore, it is possible that *task3* inhibition underlies the acid-induced depolarization observed in *task1*^{-/-} cells. Actually, real-time PCR experiments disclosed a higher *task3* gene expression in adult male mice as compared with young mice. In females, *task3* expression did not appear to be age-dependent (Figure 8C).

Discussion

Hyperaldosteronism and hypertension

The disruption of the *task1* K⁺ channel gene allowed the identification of the physiological functions of this leak channel in determining mineralocorticoid homeostasis. Female *task1*^{-/-} mice exhibited hyperaldosteronism accompanied by marked arterial hypertension. The significantly lower heart rate of female *task1*^{-/-} mice compared with wild-type mice foreclosed the possibility that augmentation of the sympathetic nerve tone is causal for the arterial hypertension observed. Blockade of the mineralocorticoid receptor by canrenoate normalized high blood pressure in female *task1*^{-/-} mice without affecting it in wild-type mice. Thus, hyperaldosteronism-induced volume expansion seems to be the decisive factor for arterial hypertension related to

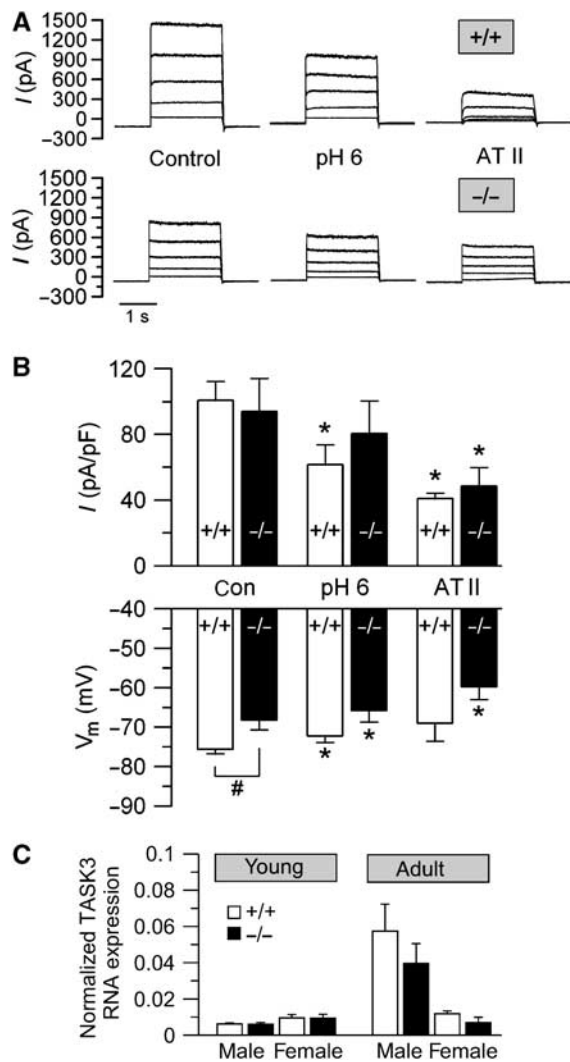


Figure 8 Adrenocortical TASK channel function and gene expression. Whole-cell current measurements in primary cultured mouse adrenocortical cells: (A) Typical traces of a *task1*^{+/+} (upper traces) and a *task1*^{-/-} cell (lower traces) under control conditions, at low extracellular pH, and after stimulation with angiotensin II (1 nM). Cells have been clamped from -95 to +30 mV (25 mV steps). (B) Summary of similar whole-cell measurements. For better comparison, Δ whole-cell currents (measured at a voltage step from -95 to +30 mV) have been normalized to cell capacitances (pA/pF; upper diagram). Membrane voltages have been measured in current clamp zero mode (lower diagram). *Indicates statistical difference from control; and # indicates statistical difference between *task1*^{+/+} and *task1*^{-/-}. (C) Age- and sex-dependent *task3* mRNA expression. Real-time PCR measurements revealed an increase in adrenal *task3* mRNA abundance in adult male mice compared with young and female mice (young mice (2–4 weeks of age), *n* = 48; adult mice (4–6 month of age) *n* = 14). *Task3* expression was not affected by the *task1* gene disruption.

task1 gene disruption. Under physiological conditions, the most important stimuli for aldosterone secretion are activation of the renin-angiotensin pathway and hyperkalemia. Female *task1*^{-/-} mice showed low plasma renin activity and low plasma K⁺ concentration, clearly indicating a defective regulation of mineralocorticoid secretion. Surprisingly, the disturbances in mineralocorticoid homeostasis were restricted to the female gender. Male *task1*^{-/-} mice were perfectly normal with regard to the plasma aldosterone levels and displayed normal regulation of aldosterone

secretion. Clearly, in male animals the *task1* channel is not an essential factor for adrenocortical function and its absence can be compensated.

Task1/task3 channel function

TASK1 belongs to a subgroup of 2P-domain K⁺ channels comprising TASK1, TASK3, and TASK5 (KCNK15). In heterologous expression systems, TASK5 was found to be inactive, whereas TASK1 and TASK3 were able to form functional homomeric channels (Karschin *et al*, 2001). Nevertheless, there is some evidence that TASK1 and TASK3 might form functional heterodimers *in vitro* and *in vivo* (Czirjak and Enyedi, 2001; Berg *et al*, 2004; Kang *et al*, 2004; Lotshaw, 2006). One might speculate that *task3* is the channel that compensates for *task1* gene disruption in adult mice. Accordingly, all previous studies on *task1*^{-/-} mice published so far have reported a moderate phenotype. The absence of severe neurological symptoms in *task1*^{-/-} mice has been interpreted as either compensation by *task3* channels in neurons in which the *task1* and *task3* coexpress, or that *task1* serves as a modulatory subunit for *task3* (Aller *et al*, 2005; Linden *et al*, 2006; Meuth *et al*, 2006). We observed a male-specific increase in TASK3 expression in adult mice that may account for the restoration of the normal adrenal gland function in male mice. In patch-clamp experiments, the membrane voltage of primary cultured adrenocortical cells of *task1*^{-/-} mice was more depolarized compared with cells of *task1*^{+/+} mice. In contrast to *task1*^{+/+} cells, acidic extracellular pH had no significant effect on the whole-cell conductance of *task1*^{-/-} cells in which acid-sensitive *task1* were absent. However, acidic pH still induced a significant depolarization in *task1*^{-/-} cells, likely because even small conductance changes (e.g., by inhibition of homomeric *task3* channels) can induce significant changes of the membrane voltage. In cells of both genotypes, the effect of angiotensin II was stronger than the effect of acidic pH, suggesting that angiotensin II probably leads to inhibition of ‘TASK-like’ and ‘non-TASK-like’ channels, which are insensitive to extracellular acidification. Creation of *task1/task3* double knockout mice will be necessary to definitively appreciate the contribution of *task3* to the membrane conductance and its eventual role for the late zonation process observed in male *task1*^{-/-} mice.

Task1 determines zonation of adrenal cortex

Classically, primary hyperaldosteronism is caused by adrenal gland hyperplasia or aldosterone-producing adenoma (Conn, 1955; Carroll *et al*, 1996; Ganguly, 1998), and one might expect such pathohistological findings in female *task1*^{-/-} mice. Examination of the morphology of paraffin-embedded adrenal glands of adult wild-type and *task1*^{-/-} mice allowed excluding any adenoma-like changes. Surprisingly, aldosterone synthase-specific immunofluorescence staining disclosed a dramatic change in functional zonation of adrenal glands: The localization of aldosterone-producing cells was shifted from the subcapsular region to the internal cortex, which is the typical site of corticosterone-producing cells. Nevertheless, corticosterone plasma concentrations and 11 β -hydroxylase gene expression were not modified in *task1*^{-/-} as compared with wild-type mice. These data indicate that despite the spectacular zonation defect, corticosterone production is not affected. To our knowledge no other

example showing such dramatic changes in the process of adrenal cortex zonation has been described so far.

Adrenal zonation is a dynamic process, which is dependent on age, sex, and species (Tanaka and Matsuzawa, 1995; Parker *et al*, 1997; Wotus *et al*, 1998). Apart from steroidogenic enzyme expression, a few markers have been identified that are expressed in different zones of the rodent adrenal cortex (Okamoto *et al*, 1997; Mitani *et al*, 1999). They include nuclear receptors of the NR4 family (Nurr1, NGFI-B) that have been associated with zone-specific transcriptional regulation of the aldosterone synthase and 3 β -hydroxysteroid dehydrogenase type II (HSD3B2) genes (Bassett *et al*, 2004a, b, c; Lu *et al*, 2004). However, the molecular mechanisms controlling adrenocortical zonation remain mostly unknown. The *task1* knockout mouse with its 'all or none' modified zonation appears to be a promising model to unveil these mechanisms.

Sex-dependent rescue of adrenal abnormalities

Gender difference usually suggests that sex hormones are involved in the respective physiological function. Therefore, adrenal gland morphology and zonation patterns were studied in animals before puberty. In fact, at 18 days of age, male *task1*^{-/-} mice exhibited the same severely compromised zonation of adrenal glands as females. At this developmental stage, wild-type mice of both sex showed already normal zonation patterns. Taken together, these observations indicate that *task1* is a K⁺ channel essential for the determination of adrenal gland zonation and aldosterone secretion in male and female mice before puberty. After puberty, unknown factors, possibly *task3* K⁺ channels, substitute for the absence of *task1* and promote compensatory mechanisms in male *task1*^{-/-} mice. To further evaluate the contribution of male sex hormones as factors triggering the restoration of the zonation process, we have investigated the effect of castration and treatment with estrogen. In male *task1*^{+/+} mice, castration did not affect adrenocortical zonation. However, castration abrogated the ability of male *task1*^{-/-} mice for developing normal zonation, suggesting a critical role of androgens for driving the compensatory mechanisms in male *task1*^{-/-} mice. The role of androgens for the compensation of the *task1* gene disruption was corroborated by the observation that female *task1*^{-/-} mice display normal aldosterone synthase distribution after testosterone treatment. The fact that regular zonation and biochemical properties of the adrenal cortex seem to be under the strict control of the expression of a K⁺ channel is certainly one of the most striking results from this study.

Clinical implications

Inactivation of *task1* leads to inappropriate high plasma aldosterone levels and, as a consequence, to fluid retention and arterial hypertension associated with low plasma renin activity. This hyperaldosteronism is fully glucocorticoid-suppressible. In humans, glucocorticoid-suppressible aldosteronism is due to a gene crossover recombination. This results in an 11 β -hydroxylase/aldosterone synthase chimeric gene that is expressed in the zona fasciculata and regulated by ACTH (Lifton *et al*, 1992; Pascoe *et al*, 1995). However, cases of glucocorticoid-suppressible aldosteronism that do not have a chimeric gene have been described in which no clear explanation for the abnormality was found (Gordon *et al*,

1995; Mulatero *et al*, 1998; Fardella *et al*, 2001). An interesting possibility is that abnormalities in the expression of TASK1, which is highly expressed in human adrenal gland (<http://www.ncbi.nlm.nih.gov/UniGene/ESTProfileViewer.cgi?uglist=Hs.645288>), might be responsible for a fasciculata expression of the aldosterone synthase in those patients with glucocorticoid-suppressible aldosteronism who do not have a chimeric gene.

In conclusion, physiological *task1* activity is required for normal development of adrenocortical zonation. After puberty, the dysregulation of aldosterone secretion and the adrenocortical zonation defect can be rescued in male *task1*^{-/-} mice implying androgen-driven upregulation of compensatory mechanisms, for example, increased expression of other K⁺ channels such as *task3*. At present, there are only very few cases of other K⁺ channels directly linked to a developmental disorder, for example, KCNJ2 (Kir2.1) whose mutations can cause Andersen Syndrome (OMIM 170390). However, in this syndrome the developmental changes are small and result in only discrete modifications of the adult organs. The *task1*^{-/-} mouse is the first example for a severe but reversible disorganization of the adrenal cortex and, thus, it represents a unique model to study adrenocortical zonation. In future studies, the availability of this mouse model might greatly facilitate the identification and characterization of cellular factors underlying differentiation and development of adrenal cortex.

Materials and methods

The *task1*^{-/-} knockout mouse

Task1^{-/-} knockout mice have been generated as described previously (Aller *et al*, 2005). The animals used in this study were backcrossed for seven generations into the C57Bl/6J genetic background. Mice were chronically maintained on a normal diet (chow, R03T-25; SAFE, 0.75% K⁺, 0.27% Na⁺). For the experiments, mice were fed high- (3%) or low- (0.05%) K⁺ diet (INRA, France), or low-Na⁺ diet (<0.005% Na⁺), for 2 weeks before the measurements. The animals had free access to food and water. The experimental protocols were approved by the local councils for animal care and were conducted according to the German and French law for animal care.

Blood analysis measurements

Blood was collected into heparin-treated capillary tubes from mice tails after small lateral incision. Samples were centrifuged and plasma was frozen and kept at -20°C. Aldosterone concentrations were determined in unextracted plasma using a solid-phase ¹²⁵I RIA kit (Immunotech, Marseille, France) with a very low cross-reactivity with corticosterone. Plasma Na⁺ and K⁺ concentrations were determined using an AVL 9180 Electrolyte analyzer (Roche, Switzerland). Corticosterone concentrations were measured using an ¹²⁵I RIA kit from MP Biomedicals, LLC (USA).

Blood pressure measurements

Blood pressure measurements were carried out by tail cuff manometry (TSE, Germany). Mice were conditioned to the measurement procedure during four consecutive days. Before measurement, the animals were allowed to stay up for at least 5 min in the measurement device, which was warmed to 37°C. Each day, blood pressure was determined as mean values of 10 cycles, with each cycle consisting of four individual measurements. For inhibition of the mineralocorticoid receptor, canrenoate (1.25 g/l plus sucrose 10%) was added to the drinking water for 10 days (blood pressure measurements were performed during the last 5 days). Before canrenoate treatment, a control period (5 days) was performed during which only sucrose (10%) had been added to the drinking water.

Plasma renin activity

Blood samples were taken from the tail vein. After an incubation of the mouse plasma (1.5 h, 37°C) with plasma of bilaterally nephrectomized male rats as renin substrate, angiotensin I (ng/ml/h) was generated. Afterwards angiotensin I (ng/ml/h) was measured by ¹²⁵I RIA (Byk & DiaSorin Diagnostics, Germany) to determine plasma renin activity.

Ussing chamber

Female wild-type and *task1*^{-/-} mice were killed by cervical dislocation and the distal colon was removed. After separation from the muscle layer, colonic mucosa was transferred into a mini Ussing chamber. Experimental solutions were warmed to 37°C by water jackets. The control solution contained (in mmol/l) HEPES 5, NaCl 140, Na-acetate 5, KH₂PO₄ 0.4, K₂HPO₄ 1.6, D-glucose 5, MgCl₂ 1, CaCl₂ 1.3, pH 7.4. Indomethacin (10 μmol/l) was added to prevent generation of endogenous prostaglandins. Data were collected and analyzed by a PowerLab setup (AD Instruments, Australia).

Immunofluorescence

Mice were anesthetized with isoflurane. After incision of the vena cava inferior, mice were perfused with 3% paraformaldehyde-containing PBS through a polyethylene catheter inserted into the abdominal aorta. After removing and freezing adrenal glands and kidneys, cryosectioning (10 μm) was performed on Polysine slides (Kindler, Freiburg, Germany). For unmasking epitopes, the sections were incubated in 0.1% SDS for 5 min. After washing with PBS and blocking with 5% bovine serum albumin (10 min), a polyclonal aldosterone synthase antibody raised in rabbit (dilution 1:50; Wotus *et al*, 1998) or a polyclonal renin antibody raised in chicken (dilution 1:300; Kurtz *et al*, 2007) was applied. Cy3 goat anti-rabbit (dilution 1:500; Dianova, Hamburg, Germany) or TRITC donkey anti-chicken (dilution 1:500; Dianova, Hamburg, Germany) was applied as secondary antibodies. The sections were examined with a filter wheel-based imaging system (Universal Imaging Corporation, Downingtown, PA, USA) mounted on an inverted microscope (Axiovert 200M; Zeiss, Jena, Germany; excitation 525–550 nm, emission 575–635 nm) and with confocal microscope (LSM 510 Meta; Zeiss, Jena, Germany; excitation 543 nm, emission 560–615 nm).

Hormonal treatment and castration

Five-week-old male mice were anesthetized with isoflurane and castrated via scrotal incision. For estrogen treatment, sexed mice received daily injections of estradiol benzoate (4 μg/g/day, s.c.). At the age of 10 weeks, mice were killed and used for immunofluorescence. To test the effect of androgens in females, female *task1*^{-/-} mice (4 weeks old) were injected twice a week with 1 mg testosterone (Androtardyl, Schering) dissolved in 50 μl sesame oil for 3 weeks (Wunderlich *et al*, 2005).

Real-time PCR

Adrenal gland total RNA was isolated using RNeasy mini kit (Qiagen, Hilden, Germany) and reverse transcribed into cDNA using M-MLV reverse transcriptase (Promega, Mannheim, Germany). TASK3-specific (sense primer: CAC TGT CAT CAC AAC TAT CCG; antisense primer: CAG CGT AGA ACA TAC AGA AGG) real-time PCR was performed using SYBR green (Qiagen, Hilden,

Germany) and beta-actin (sense primer: CCA CCG ATC CAC ACA GAG TAC TT; antisense primer: GAC AGG ATG CAG AAG GAG ATT ACT G) as reference gene in a LightCycler machine (Roche, Basel, Switzerland).

Western blot analysis

Adrenal glands from mice were rapidly frozen and stored at -80°C until used. Tissue was pulverized with mortar and pestle in liquid nitrogen and homogenized in RIPA (0.1% SDS, 1% Igepal, 0.5% sodiumdeoxycholate, with protease inhibitor cocktail in PBS, pH 7.4) using a 27-gauge needle. After incubation for 15 min on ice, cellular debris were removed by centrifugation at 10 000 g at 4°C for 10 min. Protein concentration of supernatant was determined by Bradford based protein assay (Bio-Rad). Equal amounts of protein were mixed with reducing Laemmli sample buffer (Bio-Rad) and separated by electrophoresis through 8% SDS-PAGE, transferred to a nitrocellulose membrane, rinsed with PBS, and blocked with 5% nonfat milk and 0.1% Tween in PBS for 1 h. Membranes were incubated overnight with the aldosterone synthase antibody (dilution 1:500; raised in rabbit; Wotus *et al*, 1998) in Tween-PBS with 0.1% BSA and 0.02% NaN₃, washed, and incubated with HRP-conjugated secondary antibody (dilution 1:7500) for 1 h and again washed. Images were developed with Western Blotting Luminol Reagent (Santa Cruz) and visualized on Medical X-ray film (Fotochemische Werke GmbH, Berlin).

Primary cell culture and patch clamp

During isoflurane anesthesia adult male *task1*^{-/-} and *task1*^{+/+} mice were perfused with a collagenase-containing Ringer-type solution (collagenase (type CLS II) 1 mg/ml; Biochrom, Berlin, Germany). Adrenal cortex was harvested, cut into small pieces, and digested for another 15 min at 37°C. Single cells and cell clusters were seeded on culture dishes (Falcon, Germany) and used for patch-clamp experiments 4–24 h after seeding. Whole-cell recordings were performed using an EPC-10 amplifier (Heka, Germany). The patch pipette solution contained (in mM) 95 K-gluconate, 30 KCl, 4.8 Na₂HPO₄, 1.2 NaH₂PO₄, 5 glucose, 2.38 MgCl₂, 0.726 CaCl₂, 1 EGTA, 3 ATP, pH 7.2. The extracellular Ringer-type solution contained (in mM) 145 NaCl, 0.4 KH₂PO₄, 1.6 K₂HPO₄, 5 glucose, 1 MgCl₂, 1.3 CaCl₂, 5 HEPES, pH 7.4. Differences in whole-cell currents induced by a voltage step from -95 to +30 mV were normalized to cell capacitance (pA/pF) as a measure of cell surface area. Leak subtraction was not performed. All experiments were performed at 37°C.

Statistics

Data are shown as mean values ± s.e.m. from 'n' observations. Paired as well as unpaired Student's *t*-test were used as appropriate. A *P*-value of <0.05 was accepted to indicate statistical significance.

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