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Characterization of mice deficient in Melanocortin 2 receptor on a B6/Balbc mix background

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ABSTRACT

We have previously reported that Melanocortin 2 receptor ($MC2R^{-/-}$) deficient mice on B6 N5 generations exhibited macroscopically detectable adrenal glands with markedly atrophied zona fasciculata (zF) and lack of detectable levels of corticosterone, and reduced serum concentrations of aldosterone and epinephrine. All $MC2R^{-/-}$ mice on B6/N8 background die within 2 days after birth, while about half of the $MC2R^{-/-}$ mice on B6/Balbc mix background survived to adulthood. Both male and female $MC2R^{-/-}$ mice were fertile, suggesting that normal development and function of reproductive organs. $MC2R^{-/-}$ mice delivered from $MC2R^{-/-}$ dams failed to survive due to lung failure, suggesting that fetal or maternal corticosterone is essential for lung maturation. $MC2R^{-/-}$ mice failed to activate the hypothalamic–pituitary–adrenal axis in response to both immune and non-immune stimuli. $MC2R^{-/-}$ mice maintained glomerular structure and achieved electrolyte homeostasis by the activation of the renin–angiotensin–aldosterone system under low aldosterone and undetectable levels of corticosterone.

1. Introduction

The body responds to stress by activation of the hypothalamic–pituitary–adrenal (HPA) axis and release of glucocorticoids (GCs) under the control of ACTH. ACTH secreted from the anterior pituitary is in turn regulated by hypothalamic corticotropin-releasing hormone (CRH) and Arginine vasopressin (AVP). This HPA axis is regulated by negative feedback exerted by serum cortisol levels on both the hypothalamus and the pituitary gland. ACTH is also the main regulator of adrenal cortical growth. Tissue specific post-translational cleavage of the prohormone, proopiomelanocortin (POMC) gives rise to bioactive peptides including melanotropic peptides, ACTH and several endorphins. We have previously generated mice with an inactivation mutation of the Melanocortin 2 receptor (MC2R) gene, and reported that MC2R on fifth generation of backcrossing to C57/BL6 (B6/N5) leads to neonatal lethality in about three-quarters of $MC2R^{-/-}$ pups, possibly due to hypoglycemia (Chida et al., 2007). Those surviving to adulthood exhibited macroscopically detectable adrenal glands with markedly atrophied zona fasciculata (zF), lack of detectable levels of GC, and reduced serum concentrations of aldosterone and epinephrine (Chida et al., 2007).

GCs are secreted into the systemic circulation from the adrenal cortex and initiate a broad range of actions throughout the organism that regulate the function of multiple organ systems including the central nervous, endocrine, and immune systems. The physiological effects of GCs are mediated by intracellular glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR) that function as ligand-dependent transcription factors (Mangelsdorf et al., 1995). Even though MR has a high affinity for GCs, the majority of the physiological effects of GCs are thought to be mediated via the GR, which is expressed more ubiquitously and is a stronger transcriptional activator. Physiologically, MR is thought to act primarily as a high affinity receptor for mineralocorticoids to control the sodium/potassium balance in the kidney and large intestine.

$MC2R^{-/-}$ mice are valuable for familial GC deficiency studies, and a unique animal model for investigation of the physiological functions of GC. The physiological role of GR and MR in vivo have been extensively studied in conditional KO mice (Tronche et al., 1999; Berger et al., 2006), due to the neonatal death of

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global GR$^{-/-}$ mice and MR$^{-/-}$ mice. Comparison of MC2R$^{-/-}$ mice, which are completely deficient for GC, with GR$^{-/-}$ or MR$^{-/-}$ mice may provide valuable information on the possible roles of GC function independent of GR and/or possible roles of GR function independent of GC. In this study, by altering genetic backgrounds, we obtained MC2R$^{-/-}$ mice with improved survivability that can be used for general analyses of stress response and reproduction. We established MC2R$^{-/-}$ mice on a B6/Balbc mix background from previously established B6 congenic MC2R$^{-/-}$ mice strains. About half of them survived neonatal death and grew to adulthood. We found that MC2R$^{-/-}$ pups from MC2R$^{-/-}$ dams die due to lung failure. MC2R$^{-/-}$ mice failed to increase plasma corticosterone levels in response to restraint and LPS stimulation and MC2R$^{-/-}$ mice have preserved renal glomerular morphology.

2. Materials and methods

2.1. Animals

Generation of MC2R deficient mice was described previously (Chida et al., 2007). All the mice were kept under specific pathogen-free conditions in an environmentally controlled clean room in the Laboratory Animal Research Center, Institute of Medical Science, University of Tokyo or in the Laboratory Animal Research Center, IMCJ. The experiments were conducted according to the institutional ethical guidelines for animal experiments and the safety guidelines for gene manipulation experiments. MC2R deficient mice were backcrossed into the C57BL/6J genetic background and intercrossed at N3, N4, N6, N7, and N8 generations to get MC2R$^{-/-}$ mice. MC2R$^{+/-}$ mice/B6 N8 mice were crossed with Balb/c mice and their pups were intercrossed and characterized in this study.

2.2. Blood analysis

12-Week-old male MC2R$^{+/-}$ or MC2R$^{-/-}$ mice were i.p. injected at 10:00 with either LPS from *Escherichia coli* serotype 0111:B4 (Sigma) (300 μg) or PBS. After 3 h, animals were sacrificed by decapitation and blood was collected. 12-Week-old male MC2R$^{+/+}$ or MC2R$^{-/-}$ mice were subjected to restraint for 30 min. Blood glucose level levels were measured by the glucose oxidase method (Terumo). Serum corticosterone levels were determined by RIA (Amersham, UK). Serum IL-6 levels were measured by ELISA (PharMingen, San Diego, CA) according to the manufacturer's instructions.

2.3. Histology

Fetal lung tissues (E18.5), adult heart and adult kidney tissues (6 months old) were fixed overnight in 4% paraformaldehyde in PBS, embedded in paraffin, and sections at a thickness of 6 μm were collected on slide glasses and stained with hematoxylin–eosin. Bright-field images were obtained by microscopy.

2.4. Quantitative real-time polymerase-chain-reaction (QRT-PCR) analysis

For determination of relative mRNA concentrations, total fetal lung and adult kidney RNA, isolated by sepazol were subjected to reverse transcription by Superscript III (Invitrogen). cDNA was analyzed by QRT-PCR with SybrGreen (Invitrogen) using the ABI7900HT Fast Real time PCR system (ABI). RPS3 mRNA was used for normalization. Primer sequences were designed using the Universal ProbeLibrary Assay Design Center (http://www.roche-applied-science.com/sis/rtpcr/upl/adc.jsp) (Roche Applied Science).

2.5. Statistical analysis

All values were calculated as means ± S.E.M. Comparisons of two groups were analyzed by the Student's *T*-test. In all analyses, a two-tailed probability of less than 5% (i.e. $p < 0.05$) was considered statistically significant.

3. Results and discussion

3.1. The effect of B6 background on the neonatal survival in MC2R$^{-/-}$ mice

We have previously reported that the majority of MC2R$^{-/-}$ mice under B6/N5 background died due to hypoglycemia before P2.5 (Chida et al., 2007). To determine whether the survival rate could be influenced by alleles present in the B6 genetic background, we analyzed the survival rate of MC2R$^{-/-}$ mice at each generation. The heterozygous mice at N3, N4, N6, N7, and N8 generations were intercrossed to generate mice homozygous for the MC2R mutation. As shown in Table 1, B6 genetic background significantly decreased the survival rate in MC2R$^{-/-}$ pups.

Table 1
The effect of B6 background on neonatal survival in MC2R$^{-/-}$ mice.

Background	+/+	+/−	−/−
N3	1	5	2 (25%)
N4	15	29	3 (6%)
N6	12	30	3 (7%)
N7	11	9	1 (5%)
N8	15	22	0 (0%)

The heterozygous mice at N3, N4, N6, N7, and N8 generations were intercrossed to generate homozygous for the MC2R mutation.

Genetic background has been shown to influence phenotype and survival of mutants in a variety of gene knockout animal models (Doetschman, 1999). Lethality for a variety of gene defects can be modified by allelic differences contributed by various inbred strain backgrounds. We have demonstrated that modifiers in the B6 genetic background reduced the survival of MC2R-null pups. Variation of disease onset and severity has been reported for familial glucocorticoid deficiency (FGD) patients with various mutations in MC2R (Clark et al., 2005). While many FGD patients show symptoms in the neonatal period and have undetectable circulating cortisol, others pass unrecognized until later childhood, and their cortisol deficiency might only be recognized after a short ACTH stimulation test. Penetrance variations in different populations suggest that genetic modifier loci or environmental factors should modulate the effect of MC2R mutation also in FGD patients.

3.2. The survival rate in MC2R$^{-/-}$ mice on B6/Balbc (BDF) background

To rescue neonatal death in MC2R$^{-/-}$ mice, we crossed MC2R$^{+/-}$ B6/N8 mice with Balb/c mice and their F1 offspring were intercrossed to generate mice homozygous for the MC2R mutation. To definitively analyze the mortality rate on B6/Balbc mix background, we collected all dead pups and analyzed their genotype and identified each pup at P14 by ear punch to analyze their genotype and follow their growth. On B6/Balbc mix background, 56% of MC2R$^{-/-}$ mice survive to adulthood. 22% died before P2.5, and 23% died between P14 and P28 (Fig. 1A). Mortality at weaning period is interesting because weaning is a crucial period where mice need to adapt to nutritional modifications. Hypoglycemia, due to an inability to adapt, may be the reason for death at weaning. To clarify this possibility, we measured blood glucose levels at P14. Levels were significantly decreased in MC2R$^{-/-}$ mice compared to MC2R$^{+/-}$ mice, while there was no significant difference in blood glucose level between dead and survived MC2R$^{-/-}$ mice (Fig. 1B). As we found aldosterone level was significantly decreased in adult MC2R$^{-/-}$ mice, it is possible that salt wasting may be the reason for death at this stage.

3.3. MC2R$^{-/-}$ mice have normal fertility

The ability of stress to interfere with reproductive functions and the association between the HPA axis and the hypothalamic–pituitary–gonadal (HPG) axis are generally recognized (Rivier and Rivest, 1991; Ferin, 1999; Kalantaridou et al., 2004). To analyze the potential effect of HPA disturbances in MC2R$^{-/-}$ mice on reproductive function, the fertility of male and female MC2R$^{-/-}$ mice was examined. Male MC2R$^{-/-}$ mice were capable of siring female mice with the average litter size. One male MC2R$^{-/-}$ mice sired 46 times

Table 2
MC2R$^{-/-}$ mice maintain normal litter size.

Mating pairs (female × male)	Litter size	*n*
−/− × +/−	6.8 ± 0.5	18
+/− × +/−	7.7 ± 0.5	22
+/− × −/−	7.8 ± 0.3	40

Mice carrying the MC2R mutation were mated, and their litter sizes were counted. Values are shown as the mean ± S.E.M.

in 6 months. Female MC2R$^{-/-}$ mice were also fertile and delivered their offspring with similar litter size compared to MC2R$^{+/-}$ mice (Table 2). However, we found a severely reduced survival rate among MC2R$^{+/-}$ pups delivered from MC2R$^{-/-}$ dams (D.C. unpublished observations). The reason for decreased survival observed in pups derived from MC2R$^{-/-}$ is currently under investigation. These observations indicate that the lack of MC2R does not affect fertility, however we do not necessarily deny a possible role of HPA axis in reproductive behavior (Heinrichs et al., 1997).

3.4. MC2R$^{-/-}$ mice delivered from MC2R$^{-/-}$ dams die due to lung failure

When female MC2R$^{-/-}$ mice were crossed with MC2R$^{+/-}$ males, almost all the surviving pups at P0.5 were heterozygous for the MC2R allele, suggesting that almost all the MC2R$^{-/-}$ pups were dead before P0.5. As GR$^{-/-}$ pups (Cole et al., 1995), CRHR1$^{-/-}$ pups (Smith et al., 1998), CRH$^{-/-}$ pups delivered from CRH$^{-/-}$ dams (Muglia et al., 1995) die at birth due to lung hypoplasia, we analyzed fetal lung histology at E18.5 and found that MC2R$^{-/-}$ pups from MC2R$^{-/-}$ dams had severe lung atelectasis (Fig. 2A). Consistently, the expression of surfactant apoprotein mRNAs (Sftpb, Sftpc and Sftpd) at E18.5 was reduced in MC2R$^{-/-}$ pups from MC2R$^{-/-}$ dams (Fig. 2B). As MC2R$^{-/-}$ pups from MC2R$^{+/-}$ dams and MC2R$^{+/-}$ pups from MC2R$^{-/-}$ dams are viable, either source of corticosterone, from the mother or pups, is sufficient for maturation of fetal lung, consistent with the observations for CRH$^{-/-}$ mice (Muglia et al., 1995).

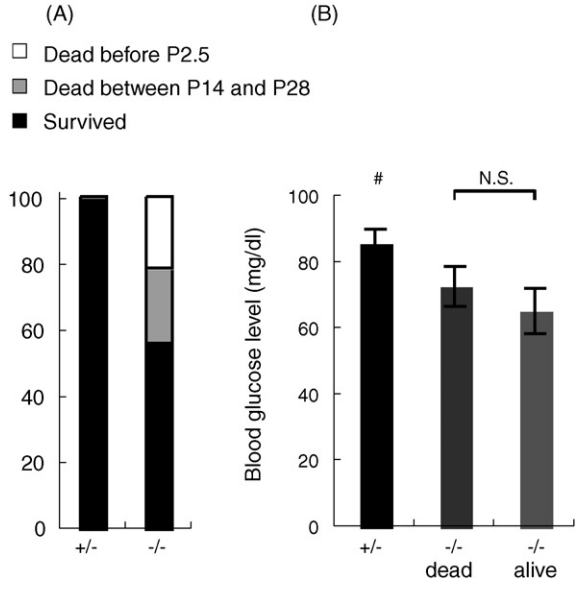

Fig. 1. Survival rate in MC2R$^{-/-}$ mice on B6/Balbc mix background. (A) On BDF background, 56% of MC2R$^{-/-}$ mice survived to adulthood. 22% of MC2R$^{-/-}$ mice died before P2.5, and 23% of MC2R$^{-/-}$ mice died between P14 and P28. The results were analyzed with (P0.5) MC2R$^{-/-}$ (n = 93) and MC2R$^{+/-}$ (n = 93) mice. (B) Blood glucose levels at postnatal day 14.5 at noon. MC2R$^{-/-}$ (n = 26) and MC2R$^{+/-}$ (n = 35) pups were analyzed. Data for MC2R$^{-/-}$ pups is shown and assessed separately based on whether they were alive (n = 19) or not (n = 7) at P28. Data are expressed as means ± S.E.M. Statistical significance was determined by ANOVA. $^{\#}p < 0.05$.

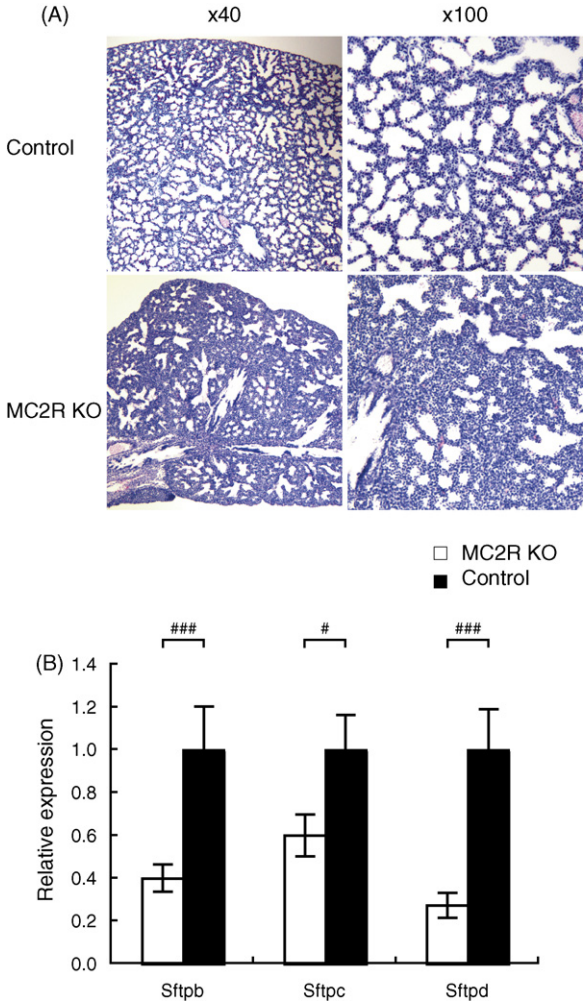

Fig. 2. Histological analysis of the fetal lung of MC2R$^{-/-}$ pups derived from MC2R$^{-/-}$ dams. (A) Hematoxylin–eosin staining of sections from the fetal lung of MC2R$^{+/-}$ or MC2R$^{-/-}$ pups from MC2R$^{-/-}$ dams. MC2R$^{-/-}$ female mice were mated with MC2R$^{+/-}$ heterozygous male and pups were obtained at E18.5 delivered by caesarean section. (B) Expression of surfactant proteins in fetal lung (E18.5) MC2R$^{-/-}$ (n = 8) and MC2R$^{+/-}$ (n = 6) mice from MC2R$^{-/-}$ dams were determined by QRT-PCR. Data are expressed as means ± S.E.M. Statistical significance was determined by T-test. $^{\#\#\#}p < 0.001$; $^{\#}p < 0.05$.

3.5. Serum corticosterone and IL-6 after acute restraint stress and LPS-induced immune challenge

The adult adrenal histology of MC2R$^{-/-}$ mice under B6/Balbc background was quite similar to B6/N5 background (data not shown). To determine whether MC2R is important for the activation of the HPA axis to stressors not associated with immune cell activation, MC2R$^{-/-}$ and MC2R$^{+/+}$ mice were restrained for 30 min followed by measurement of serum corticosterone (Fig. 3A). After restraint stress, serum corticosterone increased to 106.4 ± 16.7 ng/ml in MC2R$^{+/-}$ mice. In contrast, serum corticosterone in MC2R$^{-/-}$ mice was not detectable. To determine whether MC2R is important for activation of the HPA axis after immune system stimulation, MC2R$^{-/-}$ and MC2R$^{+/-}$ mice were injected with LPS (Fig. 3B). After LPS injection, serum corticosterone increased to 214.7 ± 7.5 ng/ml in MC2R$^{+/-}$ mice. In contrast, serum corticosterone in MC2R$^{-/-}$ mice was not detectable. As IL-6 has been

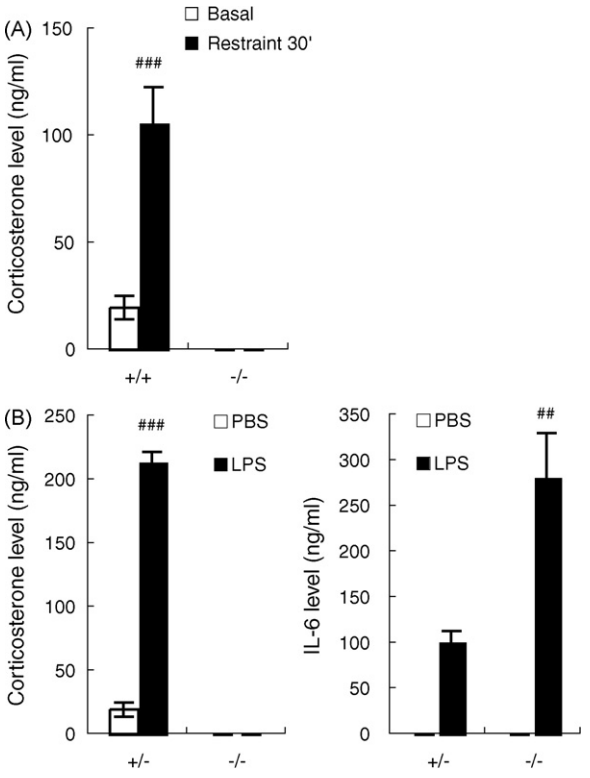

Fig. 3. Serum corticosterone and IL-6 levels after restraint or injection with LPS in MC2R$^{-/-}$ mice. (A) Basal serum corticosterone levels were measured in MC2R$^{-/-}$ ($n = 4$) and MC2R$^{+/+}$ mice ($n = 5$). Serum corticosterone levels were measured 30 min after restraint in MC2R$^{-/-}$ ($n = 4$) and MC2R$^{+/+}$ mice ($n = 5$). (B) Serum corticosterone and IL-6 levels were measured 3 h post-i.p. injection of LPS (μg/kg body weight) or PBS for MC2R$^{-/-}$ (PBS, $n = 5$; LPS, $n = 6$) and MC2R$^{+/-}$ mice (PBS, $n = 7$; LPS, $n = 8$). Statistical significance was determined by *T*-test. $^{\#\#}p < 0.01$; $^{\#\#\#}p < 0.001$.

reported to increase during immune stress, we analyzed serum levels of IL-6. Serum IL-6 levels in MC2R$^{-/-}$ mice after LPS stimulation was significantly increased compared to MC2R$^{+/-}$ mice due to blunted negative feedback regulation by corticosterone, indicating that GCs are important regulators of inflammation.

3.6. Deficiency of MC2R maintained renal glomerular morphology and induced renal renin mRNA expression

We previously found that serum aldosterone levels were significantly decreased and the expression of angiotensin receptor 1b (AT1bR) was significantly induced in MC2R$^{-/-}$ mice (Chida et al., 2007). The observations are consistent with the recently described report that a small number of patients with homozygous nonsense mutations show biochemical evidence of mineralocorticoid deficiency (Lin et al., 2007). We found that the expression of renin mRNA was significantly increased in MC2R$^{-/-}$ mice (Fig. 4A), suggesting that the renin–angiotensin–aldosterone system (RAAS) was activated to maintain serum electrolytes and blood pressure under low aldosterone levels. As increased renin–angiotensin tone was suggested to play pathological roles in cardio-renal damage, we assessed cardiac morphology and renal glomerular structure by light microscopy in 6-month-old MC2R$^{-/-}$ mice and studied the possible effect of RAAS activation on cardiac hypertrophy and renal glomerular structure. We found that the histological parameters were not significantly different from WT mice (Fig. 4B and data not shown). These results suggest that low aldosterone level due to decreased ACTH signaling prevented cardio-renal damage under increased renin–angiotensin tones (Brown, 2003). HPA axis activation by stress may exacerbate cardiovascular disease by increasing aldosterone levels, therefore suppression of the HPA axis may be an important way to prevent the progression of the metabolic syndrome to cardiovascular disease.

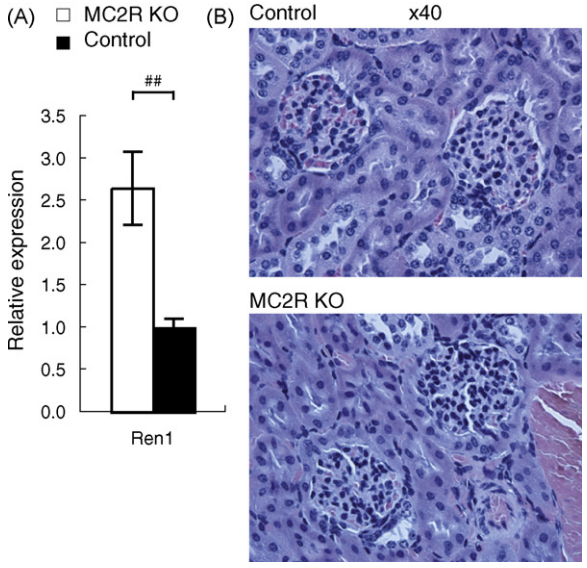

Fig. 4. Deficiency of MC2R maintained renal glomerular morphology and induced renal renin mRNA expression. (A) Expression of renal renin mRNA in adult kidney from 3-month-old MC2R$^{-/-}$ ($n = 5$) and MC2R$^{+/-}$ ($n = 4$) mice was determined by QRT-PCR. Data are expressed as means ± S.E.M. Statistical significance was determined by *T*-test. $^{\#\#}p < 0.01$. (B) Hematoxylin–eosin staining of sections from kidney of MC2R$^{-/-}$ mice or MC2R$^{+/-}$ mice.

In this study, we established an MC2R deficient mice line on a B6/Balbc mix background and characterized these mice for reproductive function, stress response and possible cardio-renal effects. MC2R$^{-/-}$ mice are a valuable animal model for studying familial GC deficiency, and a unique animal model to study the physiological function of GC and ACTH-MC2R signaling.

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References

Berger, S., Wolfer, D.P., Selbach, O., Alter, H., Erdmann, G., Reichardt, H.M., Chepkova, A.N., Welzl, H., Haas, H.L., Lipp, H.P., Schutz, G., 2006. Loss of the limbic mineralocorticoid receptor impairs behavioral plasticity. Proc. Natl. Acad. Sci. U.S.A. 103, 195–200.

Brown, N.J., 2003. Eplerenone: cardiovascular protection. Circulation 107, 2512–2518.

Chida, D., Nakagawa, S., Nagai, S., Sagara, H., Katsumata, H., Imaki, T., Suzuki, H., Mitani, F., Ogishima, T., Shimizu, C., Kotaki, H., Kakuta, S., Sudo, K., Koike, T., Kubo, M., Iwakura, Y., 2007. Melanocortin 2 receptor is required for adrenal gland development, steroidogenesis, and neonatal gluconeogenesis. Proc. Natl. Acad. Sci. U.S.A. 104, 18205–18210.

Clark, A.J., Metherell, L.A., Cheetham, M.E., Huebner, A., 2005. Inherited ACTH insensitivity illuminates the mechanisms of ACTH action. Trends Endocrinol. Metab. 16, 451–457.

Cole, T.J., Blendy, J.A., Monaghan, A.P., Krieglstein, K., Schmid, W., Aguzzi, A., Fantuzzi, G., Hummler, E., Unsicker, K., Schutz, G., 1995. Targeted disruption of the glucocorticoid receptor gene blocks adrenergic chromaffin cell development and severely retards lung maturation. Genes Dev. 9, 1608–1621.

Doetschman, T., 1999. Interpretation of phenotype in genetically engineered mice. Lab. Anim. Sci. 49, 137–143.

Ferin, M., 1999. Stress and the reproductive cycle. J. Clin. Endocrinol. Metab. 84, 1768–1774.
Heinrichs, S.C., Min, H., Tamraz, S., CarmouchÈ, M., Boehme, S.A., Vale, W.W., 1997. Anti-sexual and anxiogenic behavioral consequences of corticotropin-releasing factor overexpression are centrally mediated. Psychoneuroendocrinology 22, 215–224.
Kalantaridou, S.N., Makrigiannakis, A., Zoumakis, E., Chrousos, G.P., 2004. Stress and the female reproductive system. J. Reprod. Immunol. 62, 61–68.
Lin, L., Hindmarsh, P.C., Metherell, L.A., Alzyoud, M., Al-Ali, M., Brain, C.E., Clark, A.J., Dattani, M.T., Achermann, J.C., 2007. Severe loss-of-function mutations in the adrenocorticotropin receptor (ACTHR, MC2R) can be found in patients diagnosed with salt-losing adrenal hypoplasia. Clin. Endocrinol. (Oxf.) 66, 205–210.
Mangelsdorf, D.J., Thummel, C., Beato, M., Herrlich, P., Schtz, G.N., Umesono, K., Blumberg, B., Kastner, P., Mark, M., Chambon, P., Evans, R.M., 1995. The nuclear receptor superfamily: the second decade. Cell 83, 835–839.
Muglia, L., Jacobson, L., Dikkes, P., Majzoub, J.A., 1995. Corticotropin-releasing hormone deficiency reveals major fetal but not adult glucocorticoid need. Nature 373, 427–432.
Rivier, C., Rivest, S., 1991. Effect of stress on the activity of the hypothalamic–pituitary–gonadal axis: peripheral and central mechanisms. Biol. Reprod. 45, 523–532.
Smith, G.W., Aubry, J.M., Dellu, F., Contarino, A., Bilezikjian, L.M., Gold, L.H., Chen, R., Marchuk, Y., Hauser, C., Bentley, C.A., Sawchenko, P.E., Koob, G.F., Vale, W., Lee, K.F., 1998. Corticotropin releasing factor receptor 1-deficient mice display decreased anxiety, impaired stress response, and aberrant neuroendocrine development. Neuron 20, 1093–1102.
Tronche, F., Kellendonk, C., Kretz, O., Gass, P., Anlag, K., Orban, P.C., Bock, R., Klein, R., Schutz, G., 1999. Disruption of the glucocorticoid receptor gene in the nervous system results in reduced anxiety. Nat. Genet. 23, 99–103.