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A Common Polymorphism in the Mineralocorticoid Receptor Modulates Stress Responsiveness

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Context: Mineralocorticoid receptors (MR) mediate the action of aldosterone on sodium resorption in kidney tubular cells, but in brain they respond to the glucocorticoid cortisol in stress regulation and cognitive processes.

Objective: The objective of the study was to investigate the role of the MR gene variant I180V in the neuroendocrine response to a psychosocial stressor and in electrolyte regulation.

Design: Associations between the MRI180V and outcome variables in a healthy cohort subjected to psychosocial challenge (Trier Social Stress Test) and in a mild hypertensive cohort exposed to acute salt loading (Weinberger's test) were investigated. *In vitro* transactivational assays were applied to compare the effects of cortisol and aldosterone on the MRI180V.

Results: Carriers of the MR180V allele showed higher saliva ($P < 0.01$), plasma cortisol ($P < 0.01$), and heart rate responses ($P < 0.05$) to the Trier Social Stress Test than noncarriers (MR180I). After 3 d of a normal salt diet and the Weinberger's test, no association was found with urinary sodium excretion, plasma aldosterone, and plasma renin activity or with changes in blood pressure, aldosterone, and renin responses. *In vitro* testing of the MR180V allele revealed a mild loss of function using cortisol as a ligand, compared with the MR180I allele. Significantly higher doses of cortisol were needed for half-maximal induction on the TAT-1 ($P < 0.002$), TAT-3 ($P < 0.03$), or mouse mammary tumor virus ($P < 0.02$) promoters, whereas maximal induction was not different. These differences were not observed using aldosterone as a ligand.

Conclusion: The findings reveal that cortisol and heart rate responses to a psychosocial stressor are enhanced in carriers of the MR180V variant. **(*J Clin Endocrinol Metab* 91: 5083–5089, 2006)**

GLUCOCORTICOIDS SECRETED by the adrenals control metabolic, immunological, cardiovascular, and behavioral homeostasis (1, 2). The increased cortisol levels observed during stress exert feedback on limbic brain structures to regulate and optimize cognitive aspects of the stress response. In brain the effects of glucocorticoids are mediated by two corticosteroid receptor types, the glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) (1). Whereas GR is ubiquitously expressed in the brain, MR resides in limbic regions, particularly in hippocampal neurons, in which it is coexpressed with the GR that binds cortisol with a 10-fold lower affinity (1, 3). MR and GR control coordinately the onset (MR) and termination (GR) of the stress response. Experiments in both human and animal models have shown that acute application of MR antagonists, such as spironolactone, not only increases cortisol levels under basal conditions but also in response to stress (4–10). In addition, central MR is also involved in the control of autonomic outflow as demonstrated in rats by modulation of stress-induced heart rate and blood pressure responses through central application of MR antagonists (11). Brain MR, which is already occupied at low basal levels of glucocorticoids, regulates the basal activity of the hypothalamus-pituitary-adrenal (HPA) axis and sets the threshold of activation of stress responsiveness. GR activation, at increasing levels of glucocorticoids during stress, leads to reduced activity of the HPA axis and facilitates the shutting down of the stress response.

To effectively cope with stress, the stress response needs to be activated rapidly in accordance with current specific needs but also must be efficiently terminated to limit potential harmful effects. Humans display a large degree of interindividual variability in HPA axis responsiveness after different stressors (12), which could have important implications for vulnerability to stress-related disorders, such as depression (13). Changes in corticosteroid signaling due to MR and/or GR variability could underlie this interindividual variability in stress responsiveness. Indeed, we recently showed that GR gene variants were associated with increased or decreased saliva and plasma cortisol responses

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Abbreviations: GLM, General linear model; GR, glucocorticoid receptor; HPA, hypothalamus-pituitary-adrenal; MMTV, mouse mammary tumor virus; MR, mineralocorticoid receptor; SNP, single nucleotide polymorphism; TAT, tyrosine amino transferase; TSST, Trier Social Stress Test.

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to acute psychosocial stress after the Trier Social Stress Test (TSST) (14). Therefore, we hypothesized that a change in MR function caused by a genetic polymorphism may also affect stress responsiveness.

MRs are promiscuous (3, 15) and can bind cortisol and aldosterone with nearly equal affinity. In kidney aldosterone is the main sole ligand of MR because the equally active glucocorticoids are metabolized by the intracellular enzyme 11β-steroid dehydrogenase type 2 in addition to other specificity-conferring mechanisms, such as ligand binding and cofactors (15, 16). In brain, MR is predominantly exposed to the naturally occurring glucocorticoid cortisol, which circulates at 100- to 1000-fold excess to aldosterone.

By studying naturally occurring MR gene variants, it is possible to gain further insight into the role of MR in the regulation of the stress response in humans. A putatively functional single nucleotide polymorphism (SNP) in the MR gene could, however, potentially affect both cortisol- and aldosterone-mediated MR effects in brain and kidney, respectively. Several MR gene polymorphisms are known (*e.g.* http://www.ensembl.org/) (17, 18), and, based on frequency (more then 5% carriers), location (*e.g.* MR protein domain involved in transcriptional regulation), and possible effect on MR function (*e.g.* amino acid change), we chose to test the MRI180V SNP for association with HPA axis and autonomic responses to the TSST. This SNP is located in exon 2 of the MR gene at position NR3C2 c.538A>G, changing an isoleucine to valine (codon 180, ATT to GTT, MRI180V; numbering corresponds to cDNA, in which +1 is the A of the first ATG, Human Genome Variation Society, www.hgvs.org/mutnomen/; on ensemble www.ensembl.org/index.html, the SNP is designated as rs5522 A/G).

The aim of the present study was to establish a role of this common SNP in the MR gene on the HPA axis and autonomic responsiveness in healthy volunteers subjected to an acute psychosocial stressor. Functional characteristics of the SNP were further examined *in vitro* with both cortisol and aldosterone as ligands. To discriminate between the involvement of MR in the regulation of either stress responsiveness and/or electrolyte balance, a group of mildly hypertensive patients submitted to the Weinberger's salt loading test were also investigated.

Subjects and Methods

Subjects

German cohort, subjected to the TSST. The present study sample consisted of 110 young males (mean age 18.7 yr), with mean body mass index of 21.7. Before the first experimental session, subjects were medically examined to rule out presence of diseases, and all subjects reported to be medication free. Because one objective of the investigation was to perform a heritability analysis of the HPA axis responses (not presented here), all subjects were twins (33 monozygotic and 22 dizygotic pairs; 10 monozygotic pairs, two dizygotic pairs, and three single dizygotic siblings carried at least one copy of the minor allele of the MRI180V; see below). The protocol was approved by the Ethics Committee of the Rheinland-Pfalz State Medical Association, and written informed consent was obtained from all participants.

Laboratory psychosocial stress protocol

Three challenge tests were performed between 1600 and 1700 h on 3 test days each separated by a 1-wk interval (for the entire protocol, see Ref. 14). Each day, 45 min before stressor onset (−45 min), a catheter was inserted in an antecubital vein, and subjects were instrumented with heart rate monitors. Subjects were asked to stand up at −10 min. At −2 min, a saliva and an EDTA blood sample were obtained. Subjects were then exposed to the TSST, which consists of a free speech and a mental arithmetic task of 15-min duration performed in front of an audience and a camera (19). Subsequently, subjects remained in an upright posture for 10 min, and saliva and EDTA blood samples were obtained at 1, 10, 20, 30, 45, 60, and 90 min after stress cessation.

Heart rate was measured at 5-sec intervals using a transmitter belt with a wrist receiver (Polar Sport Tester; Polar Electro, Büttelborn, Germany). From −40 min to +40 min relative to the stressor, HR measurements were divided into 23 data blocks: one block before and after stress seated (mean of a 15-min period; not included in statistical analyses), three blocks before and after stress upright (mean of 2 min), and 15 blocks during the TSST (mean of 1 min).

Italian cohort, testing salt sensitivity

Italian volunteers (33 females, 56 males, mean age 46.0 yr, mean body mass index 26.8), recruited by nine centers, gave written informed consent to participate in the study. All studies were performed in accordance with the Declaration of Helsinki guidelines. Patients had mild essential hypertension, *i.e.* mean systolic blood pressure 152 mm Hg and mean diastolic blood pressure 97.5 mm Hg, and were taken off antihypertensive medication 14 d before testing. After a normal sodium diet (150 mmol/d) for 3 d, patients were subjected to an acute salt loading (constant rate iv infusion of 2 liters of 0.9% NaCl carried out over 4 h) and salt depletion protocol (sodium restriction 50 mmol plus three doses of 37.5 mg furosemide) to evaluate the distribution of blood pressure sensitivity to salt (20). If the difference between the mean arterial pressures at the end of the salt loading and salt depletion period was greater than the median (10 mm Hg), the patient was classified as salt sensitive; otherwise he or she was considered salt resistant. Twenty-four-hour urinary sodium excretion, upright plasma aldosterone (after 2 h orthostatism), and plasma renin activity were measured after 3 d of normal sodium diet (150 mmol/d) just before the salt load. Postload plasma aldosterone and renin activity were measured 4 h after the beginning of the salt load.

Biochemical analysis

Saliva samples were kept at −20 C until analysis. EDTA blood samples were immediately stored on ice and were centrifuged within 30 min at 2000 × *g* and 4 C for 10 min. Plasma was then divided into aliquots and stored at −20 C until analysis. For biochemical analyses, see Wüst *et al.* (14).

Most urine electrolytes analyses were performed in the coordinating laboratory at the University of Naples. Urinary electrolytes were measured by an ion-selective electrode using a Beckman (Fullerton, CA) EA-2 electrolyte analyzer. Serum creatinine was measured by a colorimetric method using a COBAS-Mira spectrophotometer (Roche, Basel, Switzerland). The measurements of unstimulated plasma renin activity and plasma aldosterone concentration were performed in the Sassari participating laboratory by RIA, using commercially available kits (Technogenetics, Milan, Italy).

Determination of genotypes

In the German volunteers subjected to the TSST, we performed PCR amplification on the light cycler for genotyping. Briefly, lightcycler hybridization probes and additional primers were designed by TIB Molbiol (Berlin, Germany), and assays were performed according to the manufacturer's protocol.

The following primers were used: forward, TCCGTGAATGGTGACGT, and reverse, TCATGCTACTTAACGGACTTG.

The following probes were used: sensor, TAGGGCTTTTAACAACGGCG, and anchor, CAGACGGGCTTTTCTCATGACACATG.

The PCR was performed using standard conditions.

The Italian cohort was genotyped by direct sequencing using an ABI BigDye termination sequence kit (version 1.1; Applied Biosystems Inc., Foster City, CA) on an ABI 3700 DNA analyzer, and the reaction was performed according to the manufacturer's specifications.

Site-directed mutagenesis

The rs5522, an A to G change, leading to the I180V amino acid change, was created by site-directed mutagenesis using the Quick change site-directed mutagenesis kit (Strategene, La Jolla, CA) on the recombinant human MR-plasmid, containing part of exon 1 to the nontranslated region of exon 9 (pRSV-MR; courtesy of Dr. R. Evans, Gene Expression Laboratory and Howard Hughes Medical Institute, The Sulk Institute for Biological Studies, La Jolla, CA). The following primers were used to introduce the I180V: sense, 5′-3′, GGCGTCATGCGCGCCGTTGTTAAAAGCCCTAT; antisense, 5′-3′, ATAGGGCTTTTAACAACGGCGCGCATGACGCC.

After mutagenesis, the cDNA inserts were sequenced to assure absence of other mutations.

Transient transfections

For transfections, we used a similar protocol and the same batch of CV-1 cells as described by Meijer *et al.* (21). Cells were cultured in DMEM supplemented with 5% charcoal-stripped fetal calf serum (Gibco, Paisley, UK). Importantly, these cells did not show endogenous MR or GR activity (21). One day after transfection with MR-containing plasmids, the cells were treated with the indicated ligand. As control plasmid we used 1 ng pCMV-R (Promega, Madison, WI) coding for Renilla luciferase controlled by a cytomegalovirus promoter. After 24 h, the cells were harvested and firefly and Renilla luciferase activity were determined using the Promega dual label reporter assay and a luminometer (LUMAT LB 9507; Berthold, Vilvoorde, Belgium).

Western blotting

CV-1 cells were transfected with either of the MR variants (MR-ATT or MR-GTT; at codon 180) and cultured for another 24 h. The primary antibody MR1-18 1D5 [a generous gift by Dr. C. Gómez-Sánchez (Division of Endocrinology, University of Mississippi, Jackson, MS), raised against a common epitope of the human and rat MR (22)] was diluted 1:500 in 5% milk powder in Tris-buffered saline and Tween 20 and incubated for 1 h at room temperature. Tubulin was used as a reference to compare different isolates: we used a monoclonal antibody against γ-tubulin (T6557; Sigma, St. Louis, MO) at 1:1000 dilution.

Statistical analysis

All analyses on human cohorts were performed with SPSS statistical software, version 12.0 (SPSS Inc., Chicago, IL). For the *in vitro* results, the GraphPad Prism program, version 4 (GraphPad Software, Inc., San Diego, CA) was used.

Allele frequencies

Allele frequencies were calculated and analyzed for deviation from Hardy-Weinberg equilibrium using the χ^2 test.

TSST

First, all data were log transformed. To control for confounding influences of interdependence within twin pairs, we modeled this pairwise noninterdependence and applied a four-level mixed model (time within day within subjects within twin pairs), assuming a first-order autoregressive covariance structure of time within day. This model also controlled for the impact of two polymorphisms in the glucocorticoid receptor gene (*Bcl*I and N363S) that were previously assessed in this cohort (14).

General linear model (GLM) procedures do not allow controlling for influences of interdependence within pairs, but this more common statistical approach was also used to ensure the mixed model results. GLMs were computed to assess the repeated measures effect time, the between-subjects effect genotype, and the interaction time × genotype for TSST responses. To control for the influence of the above-mentioned GR gene variants, these two variables were also included as predictors in the GLMs. Due to the small number of carriers of the rarer allele of a third determined GR gene variant (ER22/23EK), the respective four subjects were excluded from the present analyses. Greenhouse-Geisser corrections were applied where appropriate, and only adjusted results are reported.

Salt-sensitivity testing

A χ^2 test was computed to assess whether the ratio of salt-sensitive to salt-resistant subjects differed significantly between the MR genotype groups (180I homozygotes *vs.* 180V carriers). Differences between MR genotype groups in blood pressure responses to the treatment and in parameters related to sodium handling (24 h urinary sodium, upright plasma aldosterone, plasma renin activity, and the comparison of aldosterone and renin levels before *vs.* after salt loading) were assessed with GLM procedures. To control for the influence of age, sex, and body mass index, these variables were also included in the models.

In vitro luciferase assays

Analysis of the dose-response curves was done in GraphPad, using a two-way ANOVA to test genotype-specific effects (indicated in text and legend).

Results

In the German cohort of healthy males, we found 27 subjects carrying at least one copy of the minor G allele of the MRI180V and 83 noncarriers, indicating an allele frequency of the minor allele of 12%.

Figure 1 shows mean endocrine and heart rate responses to the first, second, and third TSST exposure. Four-level mixed models revealed that carriers of the minor 180V allele showed significantly higher salivary and plasma cortisol responses (Fig. 1, A and B) than MR180I homozygotes across sessions as indicated by significant time × genotype interactions (salivary cortisol: $F_{7;\,1793.63} = 3.36$, $P < 0.01$; plasma cortisol: $F_{7;\,1822.29} = 5.31$, $P < 0.01$). No such difference was observed for ACTH responses (main effect genotype and time × genotype interaction: $F < 0.20$, n.s.; Fig. 1C). Remarkably, the MR180V allele was associated with not only adrenocortical activity after stress exposure but also with heart rate responses (Fig. 1D). Consistent with the elevated cortisol responses in MR180V carriers, we also observed significantly higher heart rate responses (time × genotype interaction: $F_{20;\,5738.28} = 1.75$, $P < 0.05$).

Although GLM procedures do not allow to control for influences of interdependence within twin pairs, this more common statistical approach was also used, and the results confirmed the mixed model findings (salivary cortisol, interaction time × genotype: $F_{3.21;\,301.4} = 2.86$, $P < 0.05$; plasma cortisol, interaction time × genotype: $F_{2.71;\,249.36} = 3.20$, $P < 0.05$; heart rate, main effect genotype: $F_{1;\,73} = 4.25$, $P < 0.05$).

Whereas in the brain MR is cortisol responsive and capable of modifying neuronal and HPA axis reactivity to stress (1), in the kidney MR mediates aldosterone effects on electrolyte balance. This prompted us to test the association of the MR180V allele with parameters of sodium handling in a cohort of mildly hypertensive subjects (n = 89) who underwent a Weinberger's test (20). Frequency of the minor allele was 9% in this group. First, after 3 d of a controlled normal salt diet (150 mmol/d), parameters related to sodium handling such as 24-h urinary sodium excretion, plasma aldosterone, and plasma renin activity did not differ between MR180I homozygotes and MR180V carriers (Table 1). Second, after the Weinberger's test, the ratio of salt-sensitive to salt-resistant subjects was not significantly different between MR180I homozygotes and MR180V carriers ($\chi^2_1 = 2.39$, $P = 0.12$). In addition, the comparison of plasma aldosterone and plasma renin levels before and after salt loading (4 h) re-

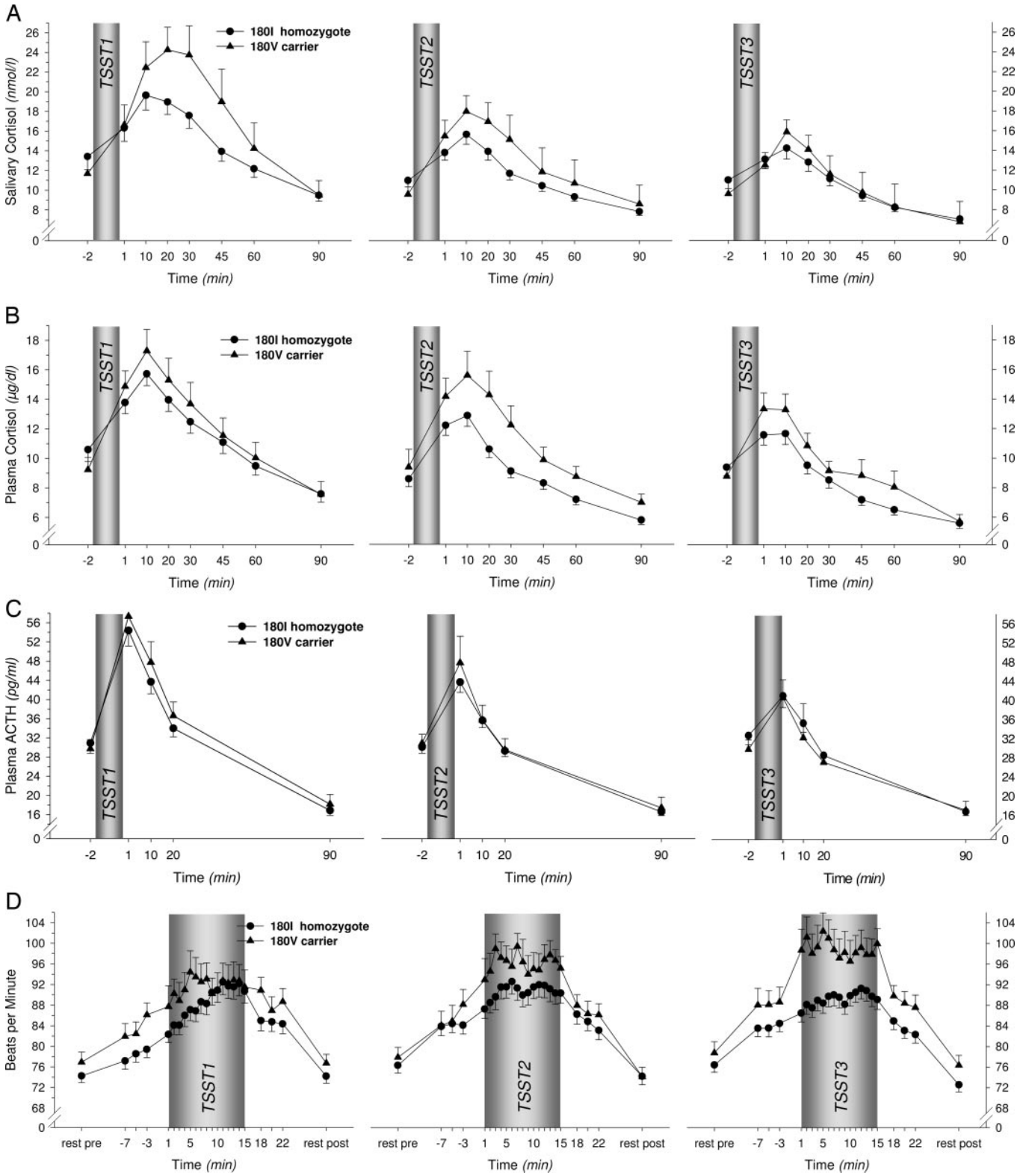

FIG. 1. Salivary cortisol (A), plasma cortisol (B), plasma ACTH (C), and heart rate responses (D) to the first, second, and third stress exposure in homozygous noncarriers (MR180I, n = 83) and carriers (MR180V, n = 27) of the minor G allele of the MRI180V (mean ± SEM). To graphically represent realistic mean cortisol and heart rate responses, figures were based on non-log-transformed data. For saliva cortisol, plasma cortisol, and heart rate, MR180V carriers had significant higher values ($P < 0.01$, $P < 0.01$, and $P < 0.05$, respectively), compared with the MR180I homozygotes. Plasma ACTH responses were not significantly different between the two genotypes.

TABLE 1. Blood pressure, cardiac frequency, and renal sodium handling as a function of the MR I180V genotype

Italian cohort (89 subjects)	Pretest cardiac parameters			3-d controlled salt intake (150 mmol/d)			Weinberger's test	
	Systolic (mm Hg)	Diastolic (mm Hg)	Heart (beats/min)	24-h urine Na excretion (mEq)	Aldosterone (ng/dl)	Renin (ng/ml·h)	Salt resistant (ΔBP $<$ 10 mm Hg)[a]	Salt sensitive (ΔBP $>$ 10 mm Hg)
MR180I (n = 76)	152 ± 1.8	98 ± 1.3	73 ± 1.1	173 ± 9	15 ± 1.2	2.1 ± 0.2	35	41
MR180V (n = 13)	150 ± 3.4	100 ± 2.1	71 ± 3.1	149 ± 13	16 ± 2.5	1.8 ± 0.3	9	4

A cohort of mild Italian hypertensives was placed under controlled salt intake (3 d at 150 mmol/d) followed by a Weinberger's test of sodium sensitivity (see Ref. 20). Parameters of sodium handling (mean ± SE) include 24-h urinary sodium excretion, plasma aldosterone, and plasma renin. In the Weinberger's test, subjects are divided into resistant or sensitive according to the extent of change in blood pressure (ΔBP) following acute salt loading and salt depletion. No statistically significant genotype-dependent differences were observed.

[a] $\chi^2 = 2.39$, $P = 0.12$.

vealed no difference between MR180I homozygotes and MR180V carriers (plasma aldosterone response: main effect genotype and time × genotype interactions: $F < 1.6$, $P > 0.20$; plasma renin response: main effect genotype and time × genotype interactions: $F < 0.26$, $P > 0.60$).

To test ligand-dependent effects of the MR180V on transcription, *in vitro* luciferase reporter assays were conducted in CV-1 cells transfected with human MR with an ATT (major allele, coding for isoleucine, MR180I) or GTT (minor allele, coding for valine, MR180V) at codon 180, generated by site-directed mutagenesis. Protein expression did not differ between the two MR variants (relative expression MR180I *vs.* MR180V = 1.08 ± 0.3, three independent experiments, tubulin used for normalization), as analyzed on Western blot, making it unlikely that effects in the luciferase assays are caused by differences in protein expression (Fig. 2). MR regulation of luciferase transcription was tested with a tyrosine amino transferase (TAT)-1 (single hormone response element), TAT-3 (triple hormone response element), or mouse mammary tumor virus (MMTV) promoter (Fig. 3). Using cortisol, the MR180V allele was significantly less active on the TAT-1 (two-way ANOVA: $P = 0.0017$), TAT-3 ($P = 0.028$), or MMTV promoter ($P = 0.015$), compared with the MR180I. In particular, EC_{50} values, but not maximal responses (not shown), were higher for the MR180V allele. Importantly, none of these differences were observed on TAT-1 (two-way ANOVA: $P = 0.28$), TAT-3 ($P = 0.22$), or MMTV ($P = 0.35$) promoters when using aldosterone as a ligand.

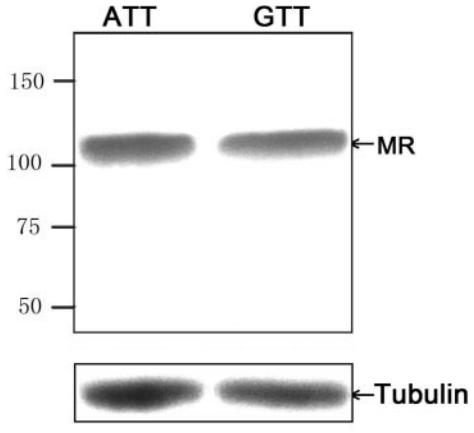

FIG. 2. Protein expression on Western blot of MR180I and MR180V. The ratio of MR180I (ATT, major allele) and MR180V (GTT, minor allele), as normalized to tubulin (*lower band*) did not differ by more than 8% (three independent experiments). The numbers on the Y-axis indicate the molecular weight.

Discussion

The current observations show that carriers of a functional MR gene variant, which *in vitro* shows a mild loss of transactivation effect of cortisol, have an enhanced endocrine and autonomic response to a psychological stressor. Based on data from studies in both animal and human models, it was proposed that MR exerts a tonic inhibitory control on HPA axis activity, both under basal conditions and in response to stress (1, 23). Although the genes for MR and GR are located on different chromosomes, all our analyses controlled for a possible impact of two GR SNPs, and we thus can rule out that the observed MR effects were significantly confounded by the previously reported association between GR SNPs and HPA axis responses to the TSST (14).

The MR gene polymorphism was also associated with an increase in autonomic output response during the TSST, as measured by the increased heart rate. Remarkably, no effect of GR polymorphisms on this autonomic response was observed (14). There is increasing evidence from animal studies that manipulation of central MR modulates autonomic outflow, as can be measured from altered cardiovascular parameters as well as altered adrenal responsiveness to ACTH (24, 25). However, it is not clear whether what we designate as increased autonomic output is indeed a result of increased sympathetic activity or decreased parasympathetic activity. Nevertheless, the increased heart rate during the TSST supports a role of MR in the onset of the stress response. The fast onset of these responses are in agreement with the fast effects described for corticosteroid actions mediated by MR in brain (26). Moreover, the use of unique brain-specific MR and GR knockout animal models (27) has established that MR also mediates fast membrane events in the brain. These findings demonstrate that central MR controlled functions in processing of stressful information may account for the altered heart rate and corticosteroid output observed in the carriers of the MR180V allele.

The observation of habituating cortisol and ACTH responses to repeated TSST exposure has been reported consistently for the TSST (28–30) and other stress protocols (31–33). Contrary to HPA axis responses across sessions, no response reduction was observed for heart rate. Comparable dissociations between the reactivity of the HPA axis and the sympathetic nervous system to repeated psychosocial stress have been previously reported (29, 34). Moreover, as is the case after antagonist treatment, the MR180V allele is not associated with increased stress-induced ACTH after the TSST. These data suggest that during the TSST sensitization of the adrenals to ACTH stimulation occurs resulting in enhanced cortisol output (7). Such an adrenal sensi-

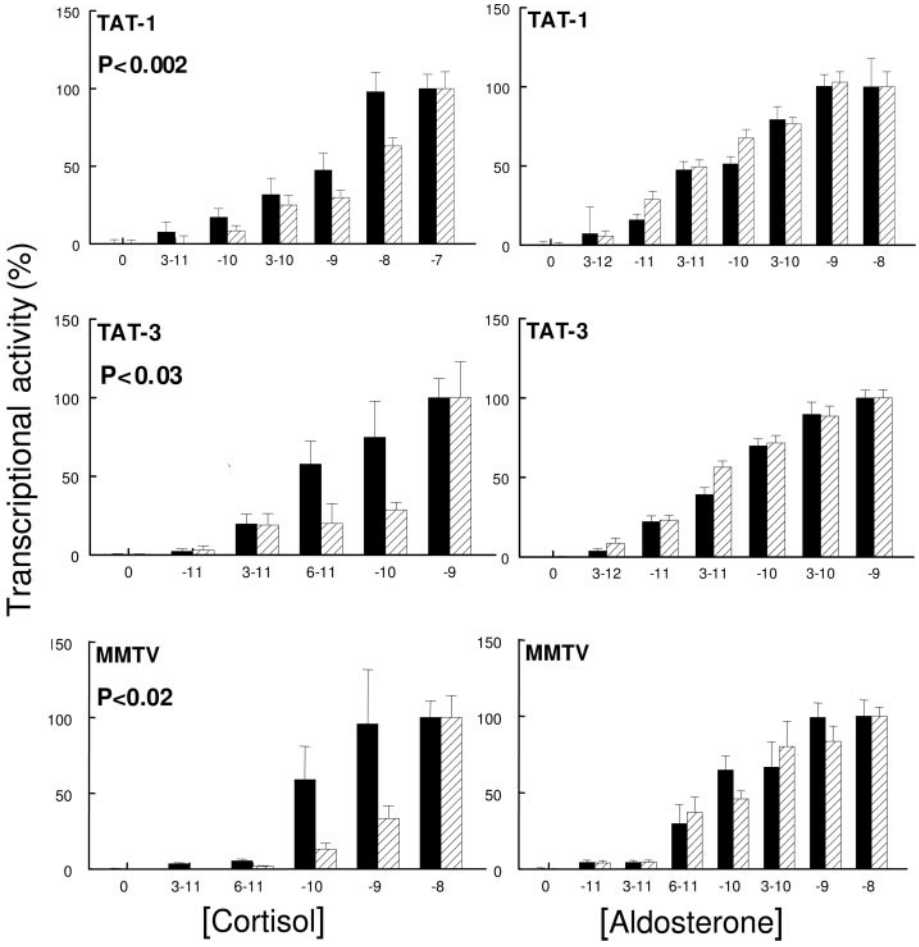

FIG. 3. Cortisol and aldosterone driven TAT-1, TAT-3, and MMTV activity in CV-1 cells. Concentrations of steroids are indicated in log units. Luciferase activity (transcriptional activity) has been normalized to both the zero and the highest steroid concentration. On TAT-1 (*upper panel*, $P < 0.002$), TAT-3 (*middle panel*, $P < 0.03$), and MMTV (*lower panel*, $P < 0.02$) promoters, a mild loss of function of the MR180V (*hatched bar*, minor allele), compared with the MR180I (*black bars*, major allele), was found using cortisol as ligand. No such differences were observed using aldosterone.

tization may be achieved via, for example, splanchnic nerve input (25, 35).

Because MR mutations result in either a salt-losing phenotype or hypertension (36), we wondered whether milder modifications of MR activity might result in a modification of salt sensitivity, blood pressure, or renal sodium handling under normal salt intake. Analysis of an Italian cohort that had been submitted to a Weinberger's salt sensitivity test (20) suggests that the MRI180V polymorphism may not influence volume regulation in response to acute changes of salt intake or aldosterone and plasma renin activity after sodium load. Under normal controlled salt intake, the MRI180V genotype also did not influence urinary sodium excretion, which depends, in the absence of major changes in plasma aldosterone, proximal tubular sodium, and fluid reabsorption and blood pressure, in part on MR activity. However, additional testing in a larger cohort is needed to establish firmly the seeming lack of effect of the MRI180V genotype on electrolyte regulation.

Interestingly, the *in vitro* data reveal a mild loss of function of the MR180V when cortisol is used as a ligand; no changes were observed with aldosterone. These *in vitro* data are in line with the observed *in vivo* effects of the MR180V variant on stress responsiveness but not electrolyte balance. In fact, differences between aldosterone and cortisol binding kinetics to MR have been reported. For example, the off-rate of aldosterone from MR is approximately five times lower, compared with cortisol (37). Possibly as a result of this, aldosterone is more potent in transactivation than cortisol (37, 38), as we also showed here. Furthermore, differences between aldosterone and cortisol on intramolecular interaction in MR and ligand-specific recruitment of cofactors influencing gene transcription have been described (16, 39, 40). The amino acid change in the MR180V is located in the N terminus of the molecule and may affect the intramolecular interactions or binding of specific coactivators, rather than ligand binding. However, the specific mechanism of the MRI180V remains to be elucidated. Together these data suggest that MR possesses an additional selection level for cortisol *vs.* aldosterone effects (15, 16). The presently described MR180V seems to provide us with a tool to differentiate cortisol from aldosterone-induced effects further.

In conclusion, the common MR180V allele permits enhanced responses in cortisol secretion and heart rate to a psychosocial stressor, whereas this gene polymorphism seemingly does not modulate the life-sustaining functions of aldosterone on sodium balance. This stress effect exerted by the polymorphism refers to the function of the cortisol-responsive MR in the limbic brain. Therefore, the MR180V variant may modify vulnerability for the precipitation of stress-related disorders such as depression.

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