Effect of vasopressin 1b receptor blockade on the hypothalamic–pituitary–adrenal response of chronically stressed rats to a heterotypic stressor

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Abstract

Exposure to chronic restraint (CR) modifies the hypothalamic–pituitary–adrenal (HPA) axis response to subsequent acute stressors with adaptation of the response to a homotypic and sensitization of the response to a heterotypic stressor. Since vasopressin (AVP) activity has been reported to change during chronic stress, we investigated whether this was an important factor in HPA facilitation. We therefore tested whether vasopressin 1b receptor (AVPR1B) blockade altered the ACTH and corticosterone response to heterotypic stressors following CR stress. Adult male rats were exposed to CR, single restraint, or were left undisturbed in the home cage. Twenty-four hours after the last restraint, rats were injected with either a AVPR1B antagonist (Org, 30 mg/kg, s.c.) or vehicle (5% mulgofen in saline, 0·2/kg, s.c.) and then exposed to either restraint, lipopolysaccharide (LPS) or white noise. CR resulted in the adaptation of the ACTH and corticosterone response to restraint and this effect was not prevented by pretreatment with Org. Although we found no effect of CR on LPS-induced ACTH and corticosterone secretion, both repeated and single episodes of restraint induced the sensitization of the ACTH, but not corticosterone response to acute noise. Pretreatment with Org reduced the exaggerated ACTH response to noise after both single and repeated exposure to restraint.

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Introduction

Vasopressin (AVP) and corticotropin-releasing hormone (CRH) are the two main neuropeptides regulating the hypothalamic–pituitary–adrenal (HPA) axis. AVP, secreted from parvocellular neurons of the paraventricular nucleus (PVN) of the hypothalamus, stimulates, in synergy with CRH, the release of pituitary adrenocorticotropic hormone (ACTH) (Gillies *et al.* 1982, Antoni 1993, Aguilera *et al.* 1994). The effect of AVP on pituitary corticotrope cells is mediated through the activation of the vasopressin 1b receptor (AVPR1B; Aguilera *et al.* 1994).

AVP levels and secretion, as well as *AVP* mRNA expression within the parvocellular PVN, increase in response to repeated exposure to stressors such as restraint (de Goeij *et al.* 1991, Ma *et al.* 1997) immobilization (Bartanusz *et al.* 1993, Makino *et al.* 1995) and foot shock (Bartanusz *et al.* 1993, Sawchenko *et al.* 1993, Makino *et al.* 1995) or during the chronic stress of adjuvant arthritis (Chowdrey *et al.* 1995). Moreover, following chronic stress, an increased number of AVPR1B in the pituitary has been observed (Aguilera *et al.* 1994, Rabadan-Diehl *et al.* 1995). These data suggest that AVP plays an important role in regulating HPA axis activity under conditions of repeated or chronic stress.

In rodents, exposure to chronic stress alters the sensitivity of the HPA axis to further stressful stimuli. Rats exposed to repeated restraint show habituation of the pituitary–adrenal axis to subsequent acute restraint (Natelson *et al.* 1988, Chen & Herbert 1995, Ma *et al.* 1997, Ma & Lightman 1998, Stamp & Herbert 1999, Girotti *et al.* 2006). On the other hand, exposure to repeated restraint induces sensitization of the ACTH response to a novel stress (Hashimoto *et al.* 1988, Hauger *et al.* 1990). This has led to the current concept of habituation to the same (homotypic) stressor and sensitization to novel (heterotypic) stressors. There is evidence to suggest that AVP could be involved in both HPA axis habituation (Scaccianoce *et al.* 1991) and sensitization (Hashimoto *et al.* 1988).

In this study, we used a recently identified AVPR1B antagonist (Org), which is able to bind to the human recombinant AVPR1B with high affinity and >1000-fold selectivity over other members of the AVP receptor subfamily and a broad range (>60) of other receptors, ion channels, transporters, and enzymes (Presland *et al.* 2007, Craighead *et al.* 2008). We have recently shown that this AVPR1B

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antagonist can reduce ACTH and corticosterone responses to exogenous AVP in rats (Spiga *et al.* 2008). Furthermore, in the same report, we have shown that the same compound is able to reduce ACTH release induced by acute exposure to stress. Those data were consistent with the previous studies where the AVPR1B antagonist SSR149415 was found effective on stress-induced ACTH increase (Serradeil-Le *et al.* 2002, Ramos *et al.* 2006).

We have therefore used the AVPR1B antagonist Org to investigate whether AVPR1B plays a role in mediating the effect of repeated exposure to stress on the HPA axis response to both homotypic and heterotypic stressors. In the present study, the effects of chronic restraint (CR) on HPA axis responses to re-exposure to stress (homotypic and heterotypic) and to the administration of AVP were investigated. Moreover, the effect of the AVPR1B antagonist on these effects was investigated.

Materials and Methods

Subjects

All experiments were conducted on male Sprague–Dawley rats (Harlan-Olac, Bicester, UK) weighing 250–300 g at the time of surgery. Animals were group housed (four in each cage) and allowed to acclimatize to the housing facility for a minimum of 1 week prior to the start of experiments. Rats were maintained under standard environmental conditions (21 ± 1 °C) under a 14 h light: 10 h darkness schedule (lights on at 0515 h) and food and water were provided *ad libitum* throughout the experiment. All animal procedures were approved by the University of Bristol Ethical Review Group and were conducted in accordance with Home Office guidelines and the UK Animals (Scientific Procedures) Act, 1986. All possible efforts were made to minimize the number of animals used and their suffering.

Surgery and blood sampling

Animals were anesthetized with a combination of Hypnorm (0·32 mg/kg fentanyl citrate and 10 mg/kg fluanisone, i.m.; Janssen Pharmaceuticals, Oxford, UK) and diazepam (2·6 mg/kg i.p.; Phoenix Pharmaceuticals, Gloucester, UK). The right jugular vein was exposed and a silastic-tipped (i.d. 0·50 mm, o.d. 0·93 mm, Merck) polythene cannula (Portex, Hythe, UK) was inserted into the vessel until it lay close to the entrance of the right atrium. The cannula was pre-filled with pyrogen-free heparinized (10 IU/ml) isotonic saline. During the same surgery, an s.c. cannula, for drug administration, was inserted under the skin between the shoulder blades. The free ends of both cannulae were exteriorized through a scalp incision and then tunneled through a protective spring that was anchored to the parietal bones using two stainless steel screws and self-curing dental acrylic. Following recovery, animals were housed in individual cages in a sound-proof room. The end of the protective spring was attached to a mechanical swivel that rotated 360° in a horizontal plane and 180° through a vertical plane allowing the rats to maximize freedom of movement. The cannulae were flushed daily with the heparinized saline to maintain patency. Blood samples (0·2 ml) were collected by hand through cannula inserted in the jugular vein and stored in ice-cold eppendorf tubes containing 10 µl EDTA (0·5 M; pH 7·4) and 10 µl Trasylol (Aprotinin, 500 000 KIU/ml, Roche). Plasma was separated by centrifugation and then stored at −80 °C until processed for corticosterone and ACTH measurements.

Drug treatments

The AVPR1B antagonist (Org, provided by Schering-Plough Corporation, Newhouse, UK, Patent no. WO/2006/095014 A1) was administered through the s.c. cannula in a 0·9% saline solution with 5% mulgofen, a detergent that improves solubility (GAF Ltd, Manchester, UK), and administered at 2 ml/kg. Vehicle controls were injected with 0·9% saline solution with 5% mulgofen. AVP (Sigma) was administered via the jugular vein cannula at 100 ng (∼400 ng/kg) dissolved in 0·1 ml 0·9% saline. In all the experiments, the dose of Org used was 30 mg/kg. We have recently shown that this dose antagonized AVP-induced ACTH release. Both s.c. and i.v. injections were followed by an injection of 0·2 ml heparin–saline to flush out the cannula and ensure that the entire volume of drug had been received by the animal.

Experimental procedures

Rats were either left undisturbed in their home cage as controls (HCC) or exposed to CR (exp. 1–4) or single restraint (SR, exp. 4). SR or CR (11 days) was performed in the room where the rats were housed and blood samples were collected. Restraint consisted of placing the rats for 1 h in cylindrical Perspex restrainers (diameter of 55 mm) provided with air holes to prevent overheating. The length of the restrainer was adjusted for the length of the rat in order to limit movement. On the sixth day of restraint, rats were implanted with both jugular and s.c. cannula as previously described. Experiments were performed 6 days after surgery, 24 h after the last restraint between 0900 and 1300 h, as following.

Rats from all groups were injected with Org (30 mg/kg, s.c.) or vehicle (5% mulgofen, 2 ml/kg, s.c.), 30 min prior to acute administration of AVP (exp. 1), exposure to acute restraint (exp. 2), administration of lipopolysaccharide (LPS; 100 µl; *Escherichia coli*; 055: B5; 250 µg/ml i.v.; Sigma, exp. 3), or exposure to white noise (96 dB, 10 min; exp. 4). We have recently shown that this compound, injected 30 min prior to AVP, is able to reduce both ACTH and corticosterone response to AVP (Spiga *et al.* 2008). Thus, this time point for Org administration in relation to both stress and AVP was used in the present study. Blood samples were collected by hand as described above. At the end of the sampling period, rats were overdosed with 0·5 ml Euthatal (200 mg/ml sodium pentobarbital; Merial, Harlow, UK). Blood samples were processed for ACTH and corticosterone measurements as described below.

Hormone measurements

ACTH and corticosterone levels were measured by IRMA and RIA respectively. For ACTH measurement, 80 µl plasma were diluted in 120 µl saline. Intra- and inter-assay coefficients of variation of the ACTH assay were 2·84 and 6·41% respectively. For corticosterone measurements, 5 µl of each plasma sample was diluted in 500 µl of a citrate buffer and processed in triplicate as previously described (Spiga *et al.* 2007). Intra- and inter-assay coefficients of variation of the corticosterone assay were 16·65 and 13·30% respectively.

Statistical analysis

All statistical analyses were performed using SPSS 11.5 for Windows (SPSS Inc, Chicago, IL, USA). Data are expressed as mean ± S.E.M.

The effect of restraint exposure (CR versus HCC or SR versus HCC) and Org treatment on the time course of ACTH and corticosterone secretion following acute challenge (AVP administration in exp. 1 or acute stress in exp. 2–4, time effect) was analyzed by repeated-measures ANOVA. Significant interactions between time and other factors (restraint and Org) were further analyzed by Fisher's least significant difference *post hoc* test. For clarity, results of HCC group from exp. 4 are represented in two different figures (see results). Statistical significance was set at $P \leq 0{\cdot}05$.

Results

Experiment 1. Effect of the AVPR1B antagonist Org on ACTH and corticosterone secretion induced by exogenous AVP

As expected, acute administration of AVP increased ACTH secretion (Fig. 1A; time effect: F (6,186) = 215·324; $P < 0{\cdot}0001$) and this effect was reduced by pretreatment with the AVPR1B antagonist Org in both HCC and CR rats (time × Org effect: F (6,186) = 35·054; $P < 0{\cdot}0001$). There was no significant effect of CR on the ACTH response to AVP or on the effect of Org.

AVP also increased corticosterone secretion (Fig. 1B; time effect: F (6,186) = 68·867; $P < 0{\cdot}0001$), and, as for ACTH, this effect was reduced by the AVPR1B antagonist Org (time × Org: F (6,186) = 8·633; $P < 0{\cdot}0001$). There was no significant effect of CR on the corticosterone response to AVP or on the effect of Org.

Experiment 2. Effect of the AVPR1B antagonist Org on ACTH and corticosterone secretion induced by acute restraint

Exposure to acute restraint induced a significant increase on ACTH secretion (Fig. 2A; time effect: F (8,256) = 56·552; $P < 0{\cdot}0001$). Moreover, there was a significant effect of CR on ACTH secretion induced by exposure to acute restraint (time × CR effect: F (8,256) = 38·883; $P < 0{\cdot}0001$), indicating that exposure to CR induced habituation of the ACTH response. In fact, all groups of CR rats, whether pretreated with vehicle or the AVPR1B antagonist Org, showed a significantly reduced ACTH response to acute re-exposure. There was however a significant effect of Org (time × Org effect: F (8,256) = 8·023; $P < 0{\cdot}0001$). Org reduced the ACTH response to restraint in HCC, compared with vehicle, whereas ACTH response in CR rats treated with Org was unchanged, when compared with HCC rats.

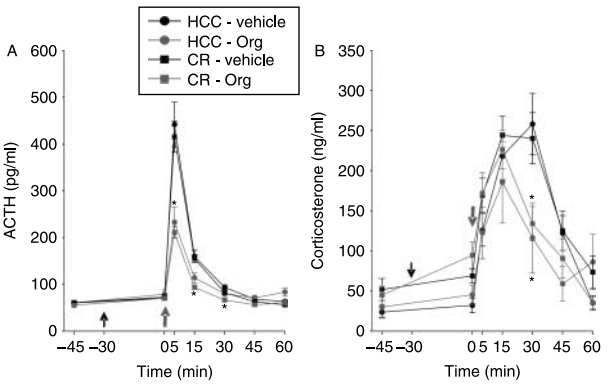

Figure 1 Effect of chronic restraint (CR) on (A) ACTH and (B) corticosterone responses to exogenous vasopressin (AVP) and modulation by a AVPR1B antagonist. Twenty-four hours after the last restraint, control rats (HCC) or CR rats (1 h/day for 11 days) received an acute injection of Org (30 mg/kg, s.c.) or vehicle (5% mulgofen in saline, 0·2 ml/kg, s.c.) 30 min prior to i.v. administration of AVP (100 ng/0·1 ml, i.v.). Black arrows indicate Org or vehicle injection, grey arrows indicate AVP injection. Data points represent mean (n = 6–10) ± S.E.M. *$P < 0{\cdot}05$: effect of Org in HCC and CR rats.

A similar habituation response was seen for corticosterone. There was a significant effect of acute restraint on corticosterone secretion (Fig. 2B; time effect: F (8,256) = 48·426;

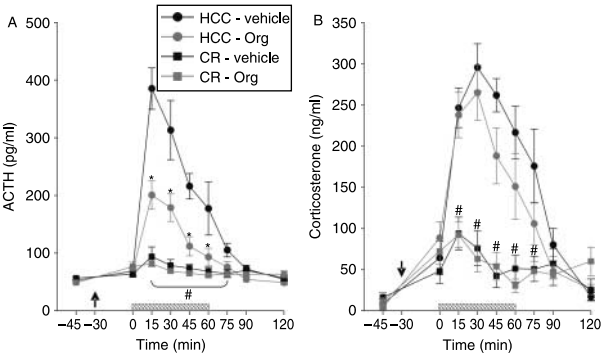

Figure 2 Effect of CR on (A) ACTH and (B) corticosterone responses to acute re-exposure to restraint and modulation by a AVPR1B antagonist. Twenty-four hours after the last restraint, home cage control rats (HCC) or CR rats (1 h/day for 11 days) received an acute injection of Org (30 mg/kg, s.c.) or vehicle (5% mulgofen in saline, 0·2 ml/kg, s.c.) 30 min prior to a challenge with a further restraint session (1 h). Black arrows indicate Org or vehicle injection, shaded bars indicate restraint stress. Data points represent mean (n = 7–11) ± S.E.M. #$P < 0{\cdot}05$: effect of CR in vehicle or Org treated rats. *$P < 0{\cdot}05$: effect of Org in HCC and CR rats.

$P<0{\cdot}0001$), and a significant effect of CR on the corticosterone secretory response to further acute restraint (time×CR effect: F (8,256)=27·375; $P<0{\cdot}0001$). CR rats pretreated with either vehicle or Org had a reduced corticosterone response to acute re-exposure to restraint stress. No effect of Org on corticosterone secretion induced by acute restraint was observed in HCC rats, nor did Org affect the corticosterone response in CR rats.

Experiment 3. Effect of the AVPR1B antagonist Org on ACTH and corticosterone secretion induced by acute LPS

LPS induced a significant increase in ACTH secretion (Fig. 3A; time effect: F (9,216)=83·215; $P<0{\cdot}0001$), which was not altered by prior exposure to CR. The AVPR1B antagonist Org diminished the response to LPS compared with rats injected with vehicle (time×Org effect: F (9,216)=13·915; $P<0{\cdot}0001$) and this effect was significant in both HCC and CR groups between 30 and 120 min after LPS administration.

Corticosterone showed a similar pattern of response with a marked increase following LPS (Fig. 3B; time effect: F (6,216)=173·502; $P<0{\cdot}0001$), but no effect of CR on subsequent LPS-induced corticosterone secretion was observed. No effect of Org was observed in either HCC or CR rats.

Experiment 4. Effect of the AVPR1B antagonist Org on ACTH and corticosterone secretion induced by acute noise stress

Experiment 4.1. Chronic restraint Exposure to acute noise induced a significant increase in ACTH secretion (Fig. 4A; time effect: F (6,234)=159·054; $P<0{\cdot}0001$). There was also a significant effect of CR on the ACTH response to acute noise (time×CR effect: F (6,234)=7·881; $P=0{\cdot}0019$). In comparison with HCC rats, CR rats showed a higher ACTH response to noise, an effect that was significant within 5 min of the onset of noise ($P=0{\cdot}0026$). There was also a significant effect of Org (time×Org effect: F (6,234)=5·179; $P=0{\cdot}0132$). Despite the lack of effect of Org observed in HCC rats exposed to noise, CR rats pretreated with Org had a significantly reduced ACTH response to noise ($P<0{\cdot}05$) although no significant interaction between CR and Org (time×CR×Org effect) was found.

Acute noise also increased corticosterone secretion (Fig. 4B; time effect: F (6,234)=191·61; $P<0{\cdot}0001$), but in contrast to ACTH, there was no significant effect of CR on the subsequent corticosterone response to acute noise. Although repeated measure analysis revealed a significant effect of Org on corticosterone secretion induced by noise (time×Org effect: F (6,234)=4·340; $P<0{\cdot}003$) *post hoc* analysis showed no effect within the experimental groups at any of the time points analyzed.

Experiment 4.2. Single restraint Consistent with the results from exp. 4.1, acute noise induced a significant increase in ACTH secretion (Fig. 5A; time effect: F (6,180)=117·695; $P<0{\cdot}0001$). There was also a significant effect of single exposure to restraint on ACTH secretion induced by exposure to acute noise (time×SR effect: F (6,180)=3·595; $P=0{\cdot}031$). In comparison with HCC rats, SR rats showed a higher ACTH response to noise and an effect was significant within 15 min from the onset of the noise ($P=0{\cdot}0063$). There was also a significant effect of Org (time×Org effect: F (6,180)=5·7209; $P=0{\cdot}0045$). As described for exp. 4.1, although only SR rats pretreated with Org had a significantly reduced ACTH

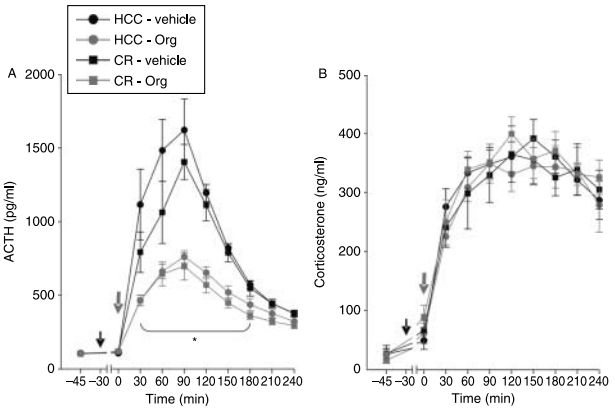

Figure 3 Effect of CR on (A) ACTH and (B) corticosterone responses to acute administration of lipopolysaccharide (LPS) and modulation by a AVPR1B antagonist. Twenty-four hours after the last restraint, home cage control rats (HCC) or CR rats (1 h/day for 11 days) received an acute injection of Org (30 mg/kg, s.c.) or vehicle (5% mulgofen in saline, 0·2 ml/kg, s.c.) 30 min prior to a challenge with acute LPS (250 µg, i.v.). Black arrow indicates Org or vehicle injection, grey arrows indicate LPS injection. Data points represent mean ($n=7$)±S.E.M. *$P<0{\cdot}05$: effect of Org in HCC and CR rats.

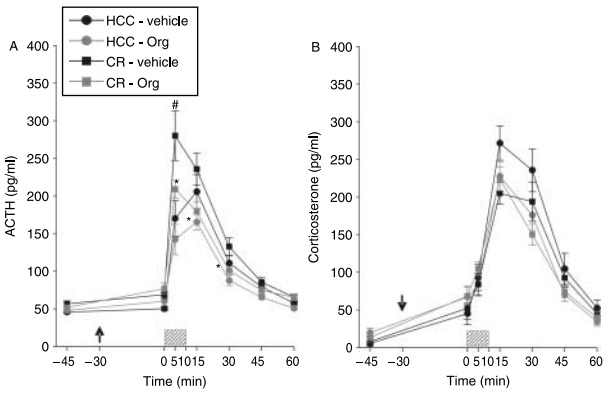

Figure 4 Effect of CR on (A) ACTH and (B) corticosterone responses to acute exposure to noise and modulation by a AVPR1B antagonist. Twenty-four hours after the last restraint, home cage control rats (HCC) or CR rats (1 h/day for 11 days) received an acute injection of Org (30 mg/kg, s.c.) or vehicle (5% mulgofen in saline, 0·2 ml/kg, s.c.) 30 min prior to exposure to noise (96 dB, 10 min). Black arrows indicate Org or vehicle injection, shaded bars indicate noise. Data points represent mean ($n=9$–12)±S.E.M. #$P<0{\cdot}05$: effect of CR in vehicle or Org treated rats. *$P<0{\cdot}05$: effect of Org in HCC and CR rats.

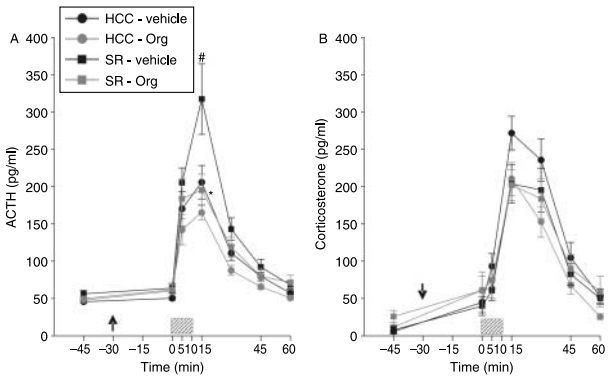

Figure 5 Effect of a single exposure to restraint on (A) ACTH and (B) corticosterone responses to acute exposure to noise and modulation by a AVPR1B antagonist. Twenty-four hours after restraint, home cage control rats (HCC) and rats exposed to restraint (SR) received an acute injection of Org (30 mg/kg, s.c.) or vehicle (5% mulgofen in saline, 0·2 ml/kg, s.c.) 30 min prior to exposure to noise (96 dB, 10 min). Black arrows indicate Org or vehicle injection, shaded bars indicate noise. Data points represent mean (n=7–8)±S.E.M. $^{\#}P<0·05$: effect of SR in vehicle or Org treated rats. $^{*}P<0·05$: effect of Org in HCC and SR rats.

response to noise (P=0·0031), no significant interaction between SR and Org (time×SR×Org effect) was found.

Acute noise also induced a significant increase on corticosterone secretion (Fig. 5B; time effect: F (6,180)=122·485; $P<0·0001$), but, as seen in exp. 4·1, there was no significant effect of single exposure to restraint on corticosterone secretion induced by acute noise. Although repeated measure analysis revealed a significant effect of Org on corticosterone secretion induced by noise (time×Org effect: F (6,180)=2·623; $P<0·042$), *post hoc* analysis showed no effect within the experimental groups at any of the time points analyzed.

Discussion

The plasticity of the HPA axis response to stress is of clear survival value to animals that need to adapt to changing physiological, pathological, and environmental conditions. The mechanism underlying this plasticity is however unclear. It is known that one aspect of HPA axis plasticity is its ability to show modification in response to the history of previous stressful experiences so that exposure to repeated stress leads to changes in the HPA axis response to subsequent challenges. The characteristics of the stressor, such as intensity (Natelson *et al.* 1988, Pitman *et al.* 1990), frequency (De Souza & Van Loon 1982, De Boer *et al.* 1990, Ma & Lightman 1998), and nature (i.e. psychological versus physical; for review see (Aguilera *et al.* 1994)) are important in the development of both habituation and sensitization of the HPA axis response to a further challenge with a stressor (see (Dallman 2007) for review). We have used the paradigm of repeated acute restraint, which has been shown to result in both habituation of the HPA axis in response to a homotypic stressor (restraint) and sensitization to heterotypic stressors.

Using this model, we have been able to confirm that the pituitary and adrenal responses to acute restraint were suppressed in rats exposed to restraint for the previous 11 days. Several studies have investigated the neuronal sites involved in HPA axis habituation. A decrease in *Fos* mRNA or Fos protein expression was found within the PVN (Girotti *et al.* 2006) and other brain areas related to the HPA axis, such as medial and central nuclei of the amygdala and lateral septum (Umemoto *et al.* 1994, Watanabe *et al.* 1994, Chen & Herbert 1995, Stamp & Herbert 1999). We have previously shown that in parallel with a progressive reduction of corticosterone response to acute restraint, exposure to repeated restraint also reduces CRF hnRNA expression within the PVN, but increases the *AVP* mRNA response to acute restraint (Ma *et al.* 1997, Ma & Lightman 1998). This increase in mRNA is also associated with increased levels of AVP in portal blood (Chowdrey *et al.* 1995). Taken together with the data from several other groups, these data suggest that AVP could be a good candidate as a modulator of the HPA axis response during repeated or chronic stress. We have therefore investigated the role of pituitary AVPR1B in mediating HPA axis habituation and sensitization using the selective AVPR1B antagonist Org. We have recently shown that acute administration of the AVPR1B antagonist Org in rat reduces the ACTH response to acute stress (Spiga *et al.* 2008) and those data are consistent with other reports where the AVPR1B antagonist, SSR149415 was described to be effective on reducing ACTH release induced by acute stress (Serradeil-Le *et al.* 2002, Ramos *et al.* 2006). Those studies showed that SSR149415 was effective at lower doses than the dose of Org used in our studies. However, the effect of SSR149415 on the activity of the HPA axis following repeated exposure to stress has not been investigated.

Pretreatment with Org does result in a reduction of the ACTH and corticosterone responses to exogenous AVP both in HCC and CR animals, confirming the effectiveness of this agent in antagonizing the pituitary corticotrope response to AVP. Org also reduces the ACTH – but not the corticosterone – response to acute restraint, confirming the modulatory role of endogenous AVP in this acute stress response (Spiga *et al.* 2008). The lack of effect of Org on corticosterone secretion induced by stress could be due to the fact that, even following Org treatment, ACTH levels are still high enough to induce a normal corticosterone response to stress. Moreover, a pituitary-independent modulation of adrenal corticosterone secretion cannot be excluded, as suggested by the evidence for modulation of adrenal glucocorticoid secretion by the sympathetic innovation of the adrenal gland (Engeland & Arnhold 2005).

AVPR1B blockade does not, however, alter the degree of habituation of the pituitary–adrenal response to repeated restraint, suggesting a different mechanism probably at a central level. This hypothesis is supported by the evidence that blockade of central mineralocorticoid receptors can prevent habituation of the corticosterone response to repeated restraint (Cole *et al.* 2000). Our data suggest that the effects of

AVP within the pituitary are not responsible for the development of habituation to restraint.

Exposure to repeated restraint also sensitizes the HPA axis response to a novel stressor, and we have investigated the effect of CR on pituitary–adrenal responses to the heterotypic stressors LPS and noise stress. Chronic stress had no effect on the ACTH and corticosterone response to LPS. This may relate to the potent immunological activation of the HPA axis by LPS and is consistent with previous observations (Fernandes *et al.* 2002). It is possible that the dose of LPS used in this study induced maximum ACTH and corticosterone release. Previous reports described the sensitization of both ACTH and corticosterone response to LPS 24 h after exposure to acute stress; however, a much lower dose of LPS was used in those studies (Johnson *et al.* 2002*a*,*b*, Dallman 2007). Therefore, lower doses of LPS may be needed to determine whether repeated restraint would induce sensitization of ACTH and corticosterone response to LPS.

Chronic stress did however result in sensitization of the pituitary response to the noise stressor with an increase in the ACTH response 5 min after the onset of the stress. It was very interesting to note that even a single episode of restraint was enough to sensitize the ACTH response to noise applied 24 h later, with an increased ACTH response 15 min after the onset of the stress. There was however no effect of CR or SR on the corticosterone response to acute noise. Similar discrepancies between pituitary and adrenal responses to stress have been previously described (Gagliano *et al.* 2008) and may be related to the modulation of adrenal response to ACTH via adrenal sympathetic innervation (Engeland & Arnhold 2005).

Org reduced the ACTH response to noise in CR rats, despite its lack of effect during CR. In fact, regardless of previous exposure to restraint, Org reduced the ACTH responses to noise stress. We also found a similar effect of Org on the corticosterone response to noise with a small but significant reduction in corticosterone which paralleled the reduced ACTH response.

With respect to the ACTH response to noise in rats exposed to acute restraint, there was a significant effect of Org on ACTH, which was independent of a history of previous episodes of acute restraint. The effect of noise was stronger in rats exposed to acute restraint compared with naïve rats. We also found a modest, but significant effect on the corticosterone response to noise.

The exaggerated ACTH response to noise in both CR and SR rats was reduced by treatment with Org, supporting an involvement of AVP on the HPA axis response to stress. These data suggest that the vasopressinergic system is involved in sensitization of the HPA response in this situation. The mechanism of this could involve both increased release of portal AVP (Chowdrey *et al.* 1995) and/or the described increase in AVPR1B density in the anterior pituitary (Aguilera *et al.* 1994, Rabadan-Diehl *et al.* 1995). Although chronic exposure to cold stress does seem to increase pituitary sensitivity via increased AVPR1B activity, this does not seem to be the explanation in our current studies, as there is change neither in sensitivity to exogenous AVP nor in their response to Org.

Our observation that Org reduced the exaggerated ACTH response to noise found in rats treated with vehicle is consistent with the work of Fred Tilders who showed that a single exposure to stress could result in increases in median eminence AVP lasting 11 days (Schmidt *et al.* 1996). The same authors also showed that subsequent activation of the HPA axis during this period resulted in an increased ACTH and corticosterone response (Schmidt *et al.* 1995).

The long-lasting effect of a single exposure to stress on ACTH has also been reported in others studies (Johnson *et al.* 2002*a*, Armario *et al.* 2004, O'Connor *et al.* 2004), and effects on basal *Crh* mRNA levels in the PVN and increased stress-induced *Fos* mRNA in the PVN have been described 24 h after exposure to an acute inescapable shock (O'Connor *et al.* 2004).

Our data suggest that pituitary AVPR1B and portal AVP could play an important role in the response to stress not only in naïve rats but also in the sensitized ACTH response to a heterotypic stressor in chronically restrained animals. Since stress-induced sensitization of pathways regulating the HPA axis has been implicated in the pathogenesis of psychiatric disease, in particular in depression (Pariante *et al.* 1995, Heim *et al.* 2008), the use of a AVPR1B antagonist could represent an interesting therapeutic avenue for the treatment of these mood disorders.

Declaration of interest

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