

# Effect of the glucocorticoid receptor antagonist Org 34850 on fast and delayed feedback of corticosterone release

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## Abstract

We investigated the effect of the glucocorticoid receptor (GR) antagonist Org 34850 on fast and delayed inhibition of corticosterone secretion in response to the synthetic glucocorticoid methylprednisolone (MPL). Male rats were implanted with a catheter in the right jugular vein, for blood sampling and MPL administration, and with an s.c. cannula for Org 34850 administration. All experiments were conducted at the diurnal hormonal peak in the late afternoon. Rats were connected to an automated sampling system and blood samples were collected every 5 or 10 min. Org 34850 (10 mg/kg, s.c.) or vehicle (5% mulgofen in saline) was

injected at 1630 h; 30 min later, rats received an injection of MPL (500 µg/rat, i.v.) or saline (0.1 ml/rat). We found that an acute administration of MPL rapidly decreased the basal corticosterone secretion and this effect was not prevented by acute pretreatment with Org 34850. However, blockade of GR with Org 34850 prevented delayed inhibition of MPL on corticosterone secretion measured between 4 and 12 h after MPL administration. Our data suggest an involvement of GR in modulating delayed, but not fast, inhibition induced by MPL on basal corticosterone secretion.

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## Introduction

There is considerable evidence for the involvement of dysfunction of the hypothalamic–pituitary–adrenal (HPA) axis in the pathophysiology of stress related disorders. Moreover, clinical evidence suggests that overactivity of the HPA axis in depression (Dinan 1994) and chronic fatigue syndrome (Demitrack *et al.* 1991) could be due to alterations in the mechanisms through which glucocorticoids, secreted by the adrenal cortex, exert inhibitory negative feedback effects on the HPA axis (Dallman *et al.* 1987).

Based on the time of onset of glucocorticoid effects on HPA axis activity, negative feedback has been divided into three distinct time domains (Keller-Wood & Dallman 1984): fast feedback, which occurs within seconds or minutes; intermediate/early delayed feedback, which occurs within 2 h; and slow/late delayed feedback, which occurs within a range of >2 h to days. The mechanism underlying delayed feedback has been extensively investigated. It is clear that delayed feedback is due to a genomic action of glucocorticoids, which is mediated through an interaction with two different receptors: mineralocorticoid receptors (MRs) and glucocorticoid receptors (GRs; Reul & De Kloet 1985, Reul *et al.* 1987). How glucocorticoids mediate fast feedback, however, remains unclear. Evidence suggests that rapid non-

genomic glucocorticoid actions could be mediated by a membrane receptor (Orchinik *et al.* 1991).

Fast feedback has been largely investigated using either *in vitro* models (Johnson *et al.* 1982, Widmaier & Dallman 1984, bou-Samra *et al.* 1986, Dayanithi & Antoni 1989, Nicholson & Gillham 1989) or *in vivo* either using rats under acute stress conditions (Sayers & Sayers 1947, Gray & Munson 1951, Keller-Wood & Dallman 1984) or following corticotropin-releasing factor (CRF)-induced adrenocorticotrophin (ACTH) secretion (Hinz & Hirschelmann 2000). Fast feedback has also been described in man, with investigation of ACTH responses to glucocorticoid feedback in healthy subjects (Krishnan *et al.* 1991, Posener *et al.* 1997, Boscaro *et al.* 1998) and in depressed patients (Young *et al.* 1991, Juruena *et al.* 2006). We have sought to investigate the role of GRs in mediating negative feedback in unstressed animals utilising a GR antagonist. We used the high-affinity GR antagonist Org 34850 (Bachmann *et al.* 2003, Peeters *et al.* 2004) to probe the role of GR on the fast and delayed inhibition of corticosterone secretion induced by the synthetic glucocorticoid methylprednisolone (MPL). In contrast with previous studies in rats, where either stress or CRH was used to induce ACTH and corticosterone negative feedback, in this study glucocorticoids negative feedback was investigated during the physiological corticosterone circadian peak. We have recently shown that

acute administration of Org 34850 at the dose of 10 mg/kg has no effect on corticosterone release in either basal or stress conditions (Spiga *et al.* 2007). In the same study, we also showed that the same dose of Org 34850 was able to antagonise the inhibitory effect of MPL on stress-induced corticosterone secretion. We have now been able to use our automated blood sampling (ABS) system, which allows us to collect blood samples every 5 or 10 min for a period of up to 15 h, to investigate whether Org 34850 can inhibit the effect of MPL on the fast, intermediate and delayed inhibition of endogenous corticosterone secretion. Furthermore, this high-frequency sampling procedure has also allowed us to investigate the effect of MPL and Org 34850 on the pulsatile pattern of corticosterone release.

## Materials and Methods

### Subjects

All experiments were conducted on male adult 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 acclimatise to the housing facility for a minimum of 1 week prior to the start of experiments. Rats were maintained under standard environmental conditions ( $21 \pm 1$  °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 minimise the number of animals used and their suffering.

### Surgery and blood sampling

Animals were anaesthetised 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 heparinised (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 exteriorised through a scalp incision and then tunnelled through a protective spring that was anchored to the parietal bones using two stainless steel screws and a self-curing dental acrylic. Following recovery, the animals were housed in individual cages in the ABS room. The end of the protective spring was attached to a mechanical swivel that rotated through 360° in a horizontal plane and 180° through a vertical plane allowing the rats to

maximise freedom of movement. The cannulae were flushed daily with the heparinised saline to maintain patency.

### Drug treatments

Org 34850 (provided by Organon Laboratories Ltd, Newhouse, UK) was administered through the s.c. cannula in a 0.9% saline solution with 5% mulgofen (GAF Ltd, Manchester, UK), a detergent, which improves solubility, and the dose of 10 mg/kg dissolved in 1 ml vehicle.

Previous studies indicate that this is the threshold dose to pass the blood–brain barrier and bind the central GR (Bachmann *et al.* 2003).

In our study, we have chosen an administration route (via an s.c. cannula) that minimises any stress induced in the rat, by the injection. Due to limited solubility of Org 34850, the dose used in this study was the largest possible to administer via this route.

Vehicle controls were injected with 0.9% saline solution with 5% mulgofen (VEH). Vials containing 40 mg MPL (Solu-Medrone: MPL as the sodium succinate, Upjohn Pharmaceuticals, Crawley, UK) were reconstituted with the supplied diluent. The final dose (500 µg/rat) was administered in heparin–saline to achieve a total injection volume of 0.1 ml/rat and it was injected via the jugular cannula over a 30-s time interval; the control group received an injection of 0.1 ml heparin–saline (SAL). Both s.c. and i.v. injections were followed by the 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

Four experimental groups were used: VEH–SAL, Org 34850–SAL, VEH–MPL and Org 34850–MPL. For each group, eight animals underwent surgery and were then connected to the ABS. However, due to an excessive number of missing samples (> 10%), for part of the time period analysed, between one and three animals from each group were excluded from the study. The experiments were performed during the late light phase when basal levels of corticosterone are elevated. Blood sampling started at 1530. After 1 h basal sample collection, rats were injected with either Org 34850 or VEH. Thirty min later, rats from both VEH and Org 34850 were injected with either MPL or SAL.

The time domain of the two treatments was based on unpublished data showing that, in mouse, the half-life of Org 34850 (10 mg/kg, s.c.) is 4.5 h and it reaches maximum plasma concentrations within 30 min of injection (unpublished observations from the manufacturer).

The dynamics of the fast non-genomic response of corticosterone to MPL, and its modulation by Org 34850, were examined by rapid sampling over a period of 5.5 h starting at 1530, with samples collected every 5 min. Org 34850 or VEH were injected at 1630, 30 min before MPL or SAL administration. To investigate the delayed inhibition

induced by MPL and its modulation by Org 34850, sampling was carried out every 10 min until 0700 of the following day. 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 corticosterone RIA as described below.

#### Blood collection and corticosterone measurements

For collection of blood samples, animals were attached to the (ABS) system as previously described (Clark *et al.* 1986, Windle *et al.* 1997). Blood samples were partially diluted by the ABS so that each blood sample consisted of 37.7  $\mu$ l blood diluted in a ratio of 1:5 in heparinised saline. The corticosterone levels were measured by RIA. For the assay, 50  $\mu$ l each blood sample was further diluted into 50  $\mu$ l of a citrate buffer (pH 3.0). The samples were processed in duplicate and incubated overnight at 4 °C with 50  $\mu$ l  $^{125}$ I corticosterone tracer (Oxford Bio Innovation DSL Ltd, Oxford, UK) and 50  $\mu$ l rabbit anti-rat corticosterone primary antibody (kindly donated by G Makara, Hungary). On day 2, a charcoal/dextran solution was added to the samples, which were then centrifuged (15 min, 3120 g, 4 °C) and aspirated before being loaded onto a gamma counter. Intra- and inter-assay coefficients of variation of the corticosterone assay were 16.65 and 13.30% respectively. The detection limit of the corticosterone assay was 0.09 ng/ml.

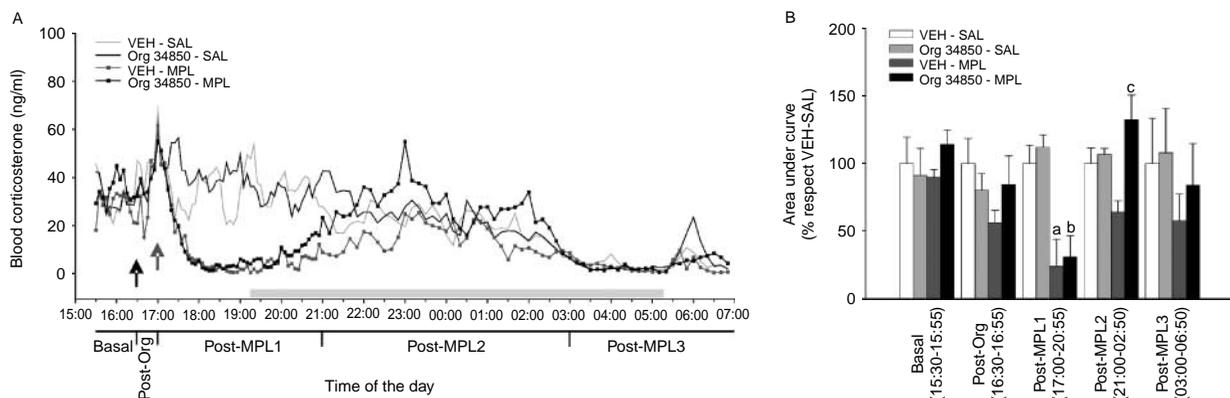
#### Statistical analysis

All statistical analyses were performed using SPSS 11.5 for Windows (SPSS Inc., Chicago, IL, USA). Data are expressed as mean (Fig. 1A), mean  $\pm$  S.E.M. (Figs 1B and 2) or individual blood corticosterone secretion profiles within each group

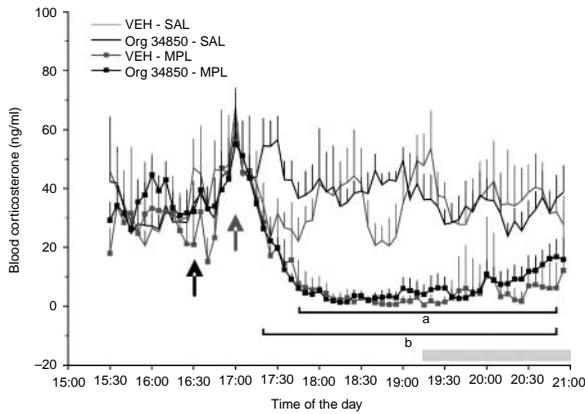
(VEH-SAL, Org 34850-SAL, VEH-MPL and Org 34850-MPL; Fig. 3). Based on the diurnal pattern of corticosterone secretion and the effect of MPL on corticosterone secretion, corticosterone profiles were divided into different time periods: basal (1530–1625 h); post-Org 34850 (1630–1655 h), post-MPL1 (1700–2055 h), post-MPL2 (2100–0250 h) and post-MPL3 (0300–0650 h). The data generated during each time period were analysed separately. To investigate the effect of MPL and Org 34850 administration on corticosterone secretion, the area under the curve (AUC; nanogram of corticosterone) for each experimental group was analysed. In the presence of a significant effect of either MPL or Org 34850 on AUC at any of the time frame analysed, the hormone profile for each animal was analysed for variation in pulse characteristics (mean daily corticosterone concentration and the number, height and frequency of corticosterone pulses) using the PULSAR algorithm (Merriam & Wachtler 1982). For the PULSAR algorithm, the following G-values were employed:  $G_1=5$ ,  $G_2=3$ ,  $G_3=2$ ,  $G_4=1.5$  and  $G_5=0.8$ , together with a peak splitting parameter of 5 (S.D. units). These values were obtained from visual inspection of the data, as recommended (Windle *et al.* 1998). AUC, single time points and PULSAR parameters were analysed using ANOVA; *post hoc* analysis examined the differences between groups using Fisher's least significant difference test. The level of significance was set at  $P \leq 0.05$ .

#### Results

Corticosterone profiles were divided into different time periods shown in Fig. 1A: basal (1530–1625 h); post-Org 34850 (1630–1655 h), post-MPL1 (rapid non-genomic time domain, 1700–2055 h), post-MPL2 (delayed genomic domain, 2100–0250 h)



**Figure 1** Effect of Org 34850 on MPL-induced corticosterone secretion decrease. (A) Mean corticosterone secretion of rats injected with methylprednisolone (MPL, 500  $\mu$ g, i.v.) or saline (SAL, 0.1 ml, i.v.), 30 min prior to acute administration of Org 34850 (10 mg/kg, s.c.) or vehicle (VEH, 5% mulgofen in saline, 0.1 ml/kg, s.c.). Blood samples were collected using an automated blood sampling system (ABS) every 5 min (1530–2055 h) or 10 min (2100–0650 h;  $n$  = five to eight per group). Black arrow indicates Org 52186 or VEH injection; grey arrow indicates MPL or SAL injection and grey bar indicates darkness period (1915–0650 h). (B) Mean  $\pm$  S.E.M. of area under curve (AUC) value (as % of VEH-SAL) of blood corticosterone levels shown in A analysed during five distinct time frames: basal (1530–1625 h), post-Org 34850 (1630–1644 h), post-MPL1 (1700–2055 h), post-MPL2 (2100–0250 h) and post-MPL3 (0300–0650 h). <sup>a</sup> $P < 0.0001$  for VEH-MPL compared with VEH-SAL; <sup>b</sup> $P < 0.0001$  for Org 34850-MPL compared with Org 34850-SAL. <sup>c</sup> $P < 0.005$  for Org 34850-MPL compared with VEH-MPL.



**Figure 2** Magnification of Fig. 1A showing the effect of Org 34850 on MPL-induced rapid corticosterone secretion decrease (time frame shown: 1530–2055 h). Blood samples were collected using an automated blood sampling system (ABS) every 5 min. Black arrow indicates Org 52186 or VEH injection; grey arrow indicates MPL or SAL injection and grey bar indicates darkness period (1915–2100 h). <sup>a</sup> $P < 0.001$  for VEH–MPL compared with VEH–SAL; <sup>b</sup> $P < 0.001$  for Org 34850–MPL compared with Org 34850–SAL.

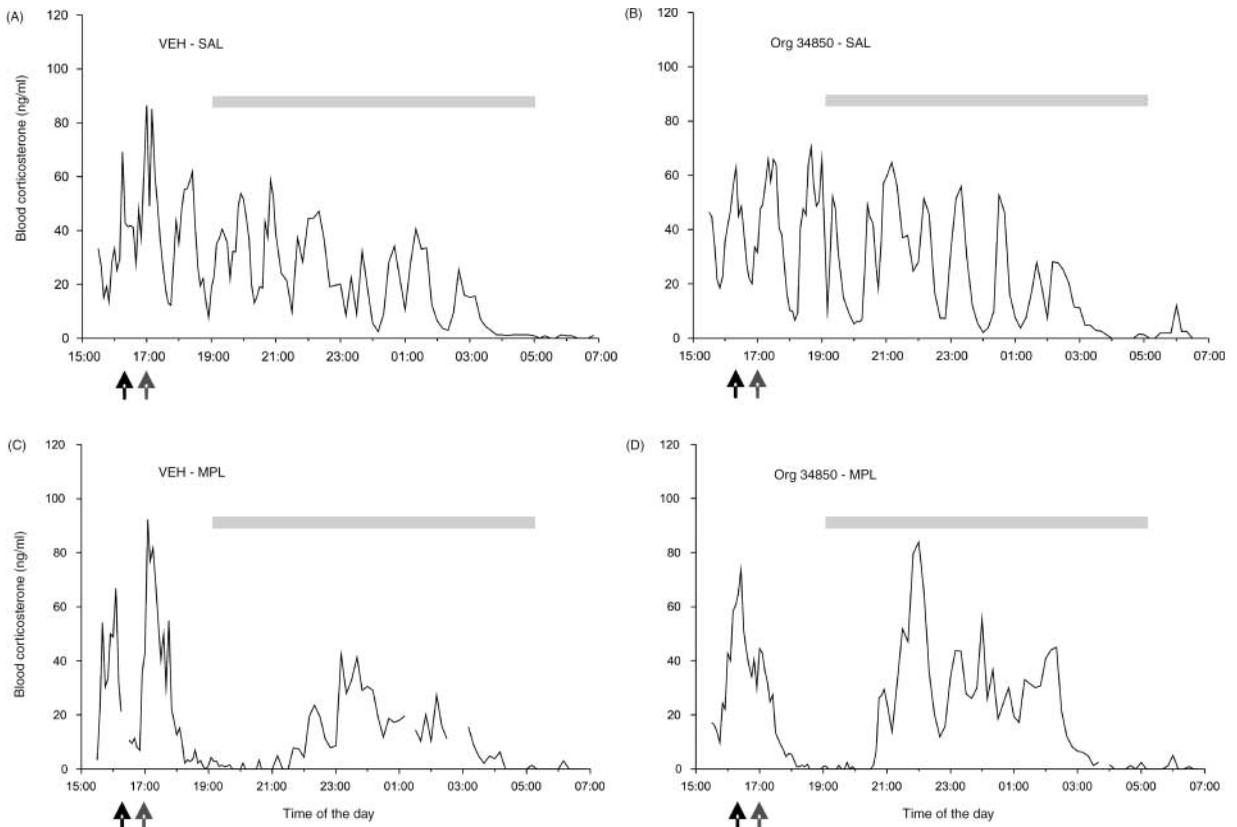
and post-MPL 3 (diurnal through, 0300–0650 h). The choice of the time frames for the analysis of corticosterone secretion with the four experimental groups is based on the experimental design of the study (basal and post-*Org 34850*), on the pattern and mechanism of effect of MPL (post-MPL1 and post-MPL2) and on the diurnal hormone variations (post-MPL3). There was no significant difference in basal corticosterone secretion (basal, 1530–1625 h) among the four experimental groups (Fig. 1B).

#### Effect of *Org 34850* on corticosterone secretion

As already shown in a recent study (Spiga *et al.* 2007), there was no significant effect of *Org 34850*, alone, on corticosterone secretion or on any of the parameters analysed to investigate corticosterone pulsatility pattern, at any of the time periods analysed (Fig. 1B).

#### Effect of *Org 34850* on rapid non-genomic corticosterone response to MPL

The rapid non-genomic effect of MPL administration on corticosterone secretion was analysed over a 4-h period



**Figure 3** Examples of individual 15.5-h corticosterone profiles of rats injected with either saline (SAL, 0.1 ml, i.v., A and B) or methylprednisolone (MPL, 500 µg, i.v., C and D) 30 min prior to acute administration of vehicle (VEH, 5% mulgofen in saline, 0.1 ml/kg, s.c., A and C) or *Org 34850* (10 mg/kg, s.c., B and D). Blood samples were collected using an automated blood sampling system (ABS) every 5 min (1530–2055 h) or 10 min (2100–0650 h). Black arrows indicate *Org 34850* or VEH injection; grey arrows indicate MPL or SAL injection and grey bars indicate darkness period (1915–0515 h).

(post-MPL1, 1700–2050 h). An analysis of AUC using ANOVA revealed that there was a significant effect of i.v. injection of MPL on corticosterone secretion (AUC,  $F(3,28)=26\,159$ ,  $P<0.0001$ ; Fig. 1B) in both rats treated with VEH ( $P=0.0001$ ) and Org 34850 ( $P<0.0001$ ), when compared with rats from the same group but treated with SAL. No difference between rats treated with VEH or Org 34850, followed by MPL, was found ( $P=0.602$ ). In both VEH- and Org 34850-treated animals, the onset of effect of MPL was evident within 25 min of MPL injection and its inhibitory effect persisted for almost 4 h (Fig. 2). The decrease in corticosterone secretion induced by MPL in both VEH and Org 34850 groups was characterised by changes in the corticosterone pulsatility pattern (Table 1). An analysis of corticosterone profile using the PULSAR algorithm revealed that both VEH and Org 34850 animals treated with MPL showed a decreased mean corticosterone concentration ( $P<0.0001$  for both VEH- and Org 34850-treated groups), reduced number and frequency of the pulses ( $P<0.0005$  and  $P<0.005$  respectively for VEH- and Org 34850-treated groups).

#### Effect of Org 34850 on delayed genomic corticosterone response to MPL

Examples of individual 15.5-h corticosterone profiles of rats injected with either SAL or MPL, 30 min prior to acute administration of VEH or Org 34850 are shown in Fig. 3.

The onset of the delayed effect of MPL on corticosterone secretion was determinate based on the results of ANOVA analysis during the rapid non-genomic time domain showing a recovery, from MPL effect, for corticosterone secretion after 4 h of MPL administration (2100 h). During the post-MPL2 time period (2100–0250 h), a single injection of Org 34850 had no effect on corticosterone basal secretion compared with rats injected with VEH followed by SAL (Fig. 3A and B). In rats treated with VEH, although failing to reach statistical significance, there was a trend for MPL to reduce corticosterone secretion during the post-MPL2 time frame, compared with VEH animals treated with SAL ( $F(3,27)=3.63$ ,  $P=0.121$ ; Fig. 3A–C). During the same time frame, there was a significant increase in corticosterone

secretion in Org 34850–MPL compared with VEH–MPL ( $P=0.003$ ; Figs 1B and 3D). We used PULSAR to analyse the effects of Org 34850 and MPL on corticosterone secretion pattern (Table 2). For all the parameters analysed, no significant difference between VEH–SAL- and VEH–MPL-treated animals was observed. However, Org 34850–MPL-treated animals showed a significantly increased mean blood corticosterone compared with VEH–MPL ( $F(3,27)=3.100$ ,  $P=0.006$ ). There was also no significant difference in the mean number, mean height or frequency of corticosterone pulses between Org 34850–MPL- and VEH–MPL-treated groups. No differences in corticosterone secretion among the four experimental groups were found during the post-MPL3 time frame (Fig. 1B).

## Discussion

We have recently shown that administration of 500 µg MPL given i.v. partially inhibits the stress-induced increase of corticosterone secretion. This effect was evident within 75 min of MPL administration and 60 min of onset of the stressor (Spiga *et al.* 2007). This time course is within the time domain of both the rapid non-genomic mechanism of glucocorticoids and the more classic genomic mechanism, mediated by translocation of cytoplasmic GRs into the nucleus. Interestingly, the effect of MPL on stress-induced corticosterone secretion was completely blocked by acute pretreatment with Org 34850 (at the same dose used in the present study). This suggests that the effect of MPL on stress-induced corticosterone release, which may be by a non-genomic mechanism, is mediated by the activation of GR.

In the present study, we show that the same acute dose of MPL (500 µg), injected at the time of expected diurnal peak of corticosterone secretion in the evening (1700 h), induces a rapid decrease in corticosterone secretion. Our results are consistent with a previous study where higher doses of MPL (5 and 500 mg/kg) were shown to rapidly reduce corticosterone secretion (Boudinot & Jusko 1986). In our study, the onset of effect of MPL on corticosterone secretion was evident within 25 min of MPL administration, suggesting the

**Table 1** Mean  $\pm$  s.e.m. of PULSAR parameter measurements of vehicle–saline (VEH–SAL), Org 34850–SAL, VEH–methylprednisolone (MPL) and Org 34850–MPL rats during the post-MPL1 phase (1700–2055 h). Rats were injected with either vehicle (VEH, 5% mulfogen in 0.9% saline, 0.1 ml, s.c.) or Org 34850 (10 mg/kg, s.c.) 30 min prior to injection with saline (SAL, 0.1 ml, i.v.) or methylprednisolone (MPL, 500 µg/0.1 ml, i.v.). During the post-MPL1 phase (1700–2050 h), rats injected with MPL, pretreated with either VEH or Org 34850 showed reduced corticosterone concentration, reduced number of pulses and reduced pulse frequency, compared with rats treated with SAL

	Corticosterone concentration (ng/ml)	Number of pulses	Pulse height (ng/ml)	Pulse frequency (pulse/h)
VEH–SAL	34.23 $\pm$ 4.9	3.8 $\pm$ 0.3	66.84 $\pm$ 7.3	0.97 $\pm$ 0.05
Org 34850–SAL	40.85 $\pm$ 3.6	3.5 $\pm$ 0.4	71.27 $\pm$ 5.9	0.89 $\pm$ 0.10
VEH–MPL	9.11 $\pm$ 1.4 <sup>†</sup>	1.5 $\pm$ 0.3*	58.15 $\pm$ 6.2	0.38 $\pm$ 0.07*
Org 34850–MPL	10.84 $\pm$ 1.6 <sup>§</sup>	1.7 $\pm$ 0.2 <sup>‡</sup>	61.86 $\pm$ 12.7	0.42 $\pm$ 0.05 <sup>‡</sup>

\* $P<0.0005$ , <sup>†</sup> $P<0.0001$  compared with VEH–SAL; <sup>‡</sup> $P<0.005$ , <sup>§</sup> $P<0.0001$  compared with Org 34850–SAL.

**Table 2** Mean  $\pm$  s.e.m. of PULSAR parameter measurements of VEH–SAL, Org 34850–SAL, VEH–MPL and Org 34850–MPL rats during the post-MPL2 phase (2100–0250 h). Rats were injected with either vehicle (VEH, 5% mulgofen in 0.9% saline, 0.1 ml, s.c.) or Org 34850 (10 mg/kg, s.c.) 30 min prior to injection with saline (SAL, 0.1 ml, i.v.) or methylprednisolone (MPL, 500  $\mu$ g/0.1 ml, i.v.). During the peak phase (2100–0250 h), rats injected with MPL and pretreated with Org 34850 showed increased corticosterone concentration compared with rats pretreated with VEH. No difference in any other parameter analysed was found among the four experimental groups

	<b>Corticosterone concentration</b> (ng/ml)	<b>Number of pulses</b>	<b>Pulse height</b> (ng/ml)	<b>Pulse frequency</b> (pulse/h)
VEH–SAL	20.4 $\pm$ 2.3	2.8 $\pm$ 0.3	41.9 $\pm$ 3.0	0.47 $\pm$ 0.05
Org 34850–SAL	22.6 $\pm$ 1.2	2.6 $\pm$ 0.6	47.7 $\pm$ 2.6	0.43 $\pm$ 0.10
VEH–MPL	14.1 $\pm$ 1.1	1.8 $\pm$ 0.4	33.4 $\pm$ 5.9	0.31 $\pm$ 0.07
Org 34850–MPL	26.9 $\pm$ 4.9*	3.1 $\pm$ 0.5	59.2 $\pm$ 11.7	0.52 $\pm$ 0.08

\* $P < 0.05$  compared with VEH–MPL.

activation of a non-genomic mechanism to suppress HPA axis activity. However, acute pretreatment with the glucocorticoid antagonist Org 34850 was unable to antagonise the effect of MPL, suggesting a mechanism that does not involve a genomic mechanism following GR activation.

There is an electrophysiological evidence in guinea-pig ganglion neurons for rapid effects of cortisol on cell membranes, which can be reversed by the glucocorticoid antagonist RU 38486 (Hua & Chen 1989), and *in vitro* data suggesting involvement of GR activation in fast, intermediate and delayed feedback (Dayanithi & Antoni 1989). Our results, however, are more consistent with previous *in vivo* data which show that rapid negative feedback effects of exogenous glucocorticoids can be demonstrated on CRF-induced ACTH secretion in rats, and that these were not affected by the acute administration of the glucocorticoid antagonist RU 486 (Hinz & Hirschelmann 2000).

MPL is a synthetic glucocorticoid that binds to both GR and MR (Grossmann *et al.* 2004). It is therefore possible that MPL-induced rapid corticosterone secretion inhibition could be mediated by the activation of MR. Indeed, we have recently found that the MR antagonist canrenoate can activate the HPA axis (Atkinson *et al.*) and can also antagonise the rapid feedback induced by MPL (Atkinson *et al.* unpublished). These data also fit with another report where electrophysiological evidence for rapid MR-mediated responses are described (Karst *et al.* 2005). This is further supported by evidence that, in both rats and man, MRs are also activated throughout the 24 h period both at the circadian peak of corticosterone secretion, and at the circadian trough (Bradbury *et al.* 1994, Spencer *et al.* 1998, Young *et al.* 2003). There is also recent data using a novel prednisolone suppression test in man that suggest an involvement of MR in dysfunctional feedback mechanism in depressed patients (Jurruena *et al.* 2006).

Due to the unstable nature of the ACTH peptide and the relatively large volumes of blood required for the ACTH measurement, the blood collected in this study by the sampling system could not be used to ascertain ACTH levels throughout the entire sampling period. Therefore, we have no evidence that MPL will reduce ACTH secretion within the same rapid domain as corticosterone. In light of this, an

involvement of the adrenal glands as site of effect of MPL could not be excluded.

The activity of the HPA axis is characterised by an endogenous diurnal rhythm (Jasper & Engeland 1991). Cortisol in man, and corticosterone in rodents, is released in a pulsatile manner throughout the 24-h period and it is the variation in the amplitude and frequency of these pulses that determines the diurnal rhythm (Windle *et al.* 1998). An acute administration of Org 34850 to rats did not alter the secretory characteristics of corticosterone secretion in keeping with our previous findings. Indeed, an acute administration of Org 34850 has no effect on corticosterone diurnal rhythm, whereas sub-chronic (5 days) treatment with the same drug induces an increase in the corticosterone secretion over the 24-h cycle, with an increased number of pulses, pulse height and frequency.

Here we found that, in the rapid feedback time domain, the reduction in corticosterone secretion by acute administration of MPL was also characterised by a reduction in pulsatility that lasted for 4 h. Indeed, following MPL administration, rats treated with vehicle or with Org 34850 showed a reduction in the number and frequency of corticosterone pulses.

In the delayed feedback domain we found that Org 34850 significantly increases plasma levels of corticosterone in rats that were subsequently injected with MPL. Moreover, analysis of corticosterone profiles using PULSAR algorithm revealed significantly greater levels of corticosterone in rats injected with MPL and pretreated with Org 34850 compared with rats injected with MPL but pretreated with vehicle only. No effect on pulse number, height or frequency was found between the two groups with the delayed feedback time domain.

Unlike the effects of MPL in the rapid feedback time domain, which is unaffected by the presence of the GR antagonist, the effects in the delayed time domain do appear to be mediated through GR.

It is unclear whether the rapid non-genomic and the delayed genomic effect of glucocorticoids are mediated by central structures involved in HPA axis modulation, such as the prefrontal cortex, hippocampus, amygdala and paraventricular nucleus or by the pituitary gland or a combination of these. There is also some evidence that MPL is only poorly able to penetrate the blood–brain barrier as a result of the

P-glycoprotein pump (Koszdin *et al.* 2000). Interestingly, we also have data that the synthetic glucocorticoid agonist dexamethasone, which is an excellent substrate for the P-glycoprotein pump, is also able to induce a rapid decrease in corticosterone secretion in rats following i.v. injection (unpublished observations; Andrews). These data would support the idea that the rapid effect of MPL is at a pituitary rather than at a central level. It should be noted that there is evidence for the presence of MR as well as GR within the anterior pituitary (De Kloet *et al.* 1975, De Nicola *et al.* 1981, Reul *et al.* 1990). The physiological role of this pituitary MR remains unknown, although MR in the hippocampus and in the anterior pituitary appears to be differentially regulated by glucocorticoids (Hugin-Flores *et al.* 2004). Additional studies with site-specific administration of GR antagonists would be necessary to investigate this further.

In summary, our data suggest that the effect of MPL on fast/intermediate feedback is via a non-genomic mechanism, which is not mediated through GR, whereas delayed feedback can be antagonised by the selective glucocorticoid antagonist Org 34850.

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## Disclosure

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