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Tissue- and temporal-specific regulation of 11β-hydroxysteroid dehydrogenase type 1 by glucocorticoids in vivo

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Abstract

11β-hydroxysteroid dehydrogenase type 1 (11β-HSD-1) catalyses the interconversion of active corticosterone and inert 11-dehydrocorticosterone. Short-term glucocorticoid excess upregulates 11β-HSD-1 in liver and hippocampus leading to suggestions that 11β-HSD-1 ameliorates the deleterious effects of glucocorticoid excess by its 11β-dehydrogenase activity. However the predominant activity of 11β-HSD-1 in vivo is 11β-reduction, thus generating active glucocorticoid. We have re-examined the time-course of glucocorticoid regulation of 11β-HSD-1 in the liver, hippocampus and kidney of adult male rats in vivo.

Sham operation markedly reduced 11β-HSD-1 mRNA expression in all tissues, and reduced 11β-HSD bioactivity in liver and hippocampus when compared to untouched controls. Adrenalectomy reduced 11β-HSD-1 expression in all tissues in the short-term (7 days), followed by subsequent recovery of enzyme activity by 21 days in liver and hippocampus. Dexamethasone replacement of adrenalectomised rats attenuated the initial decrease in hepatic 11β-HSD-1 activity, but by 21 days dexamethasone reduced activity compared to control levels.

Thus glucocorticoids regulate 11β-HSD-1 in a complex tissue- and temporal-specific manner. This pattern of regulation suggests glucocorticoids repress 11β-HSD-1 at least in the liver, a pattern of regulation more consistent with the evidence that 11β-HSD-1 is an 11β-reductase in vivo. Operational stress per se down-regulates 11β-HSD-1 which has implications for interpretation and design of in vivo studies of 11β-HSD-1.

1. Introduction

Glucocorticoids regulate many metabolic and homeostatic processes and comprise a key component of the response to stress. Recently it has become apparent that the tissue response to glucocorticoids is modulated not only by circulating levels of steroids and the cellular expression of their receptors, but also by the presence of isozymes of 11β-hydroxysteroid dehydrogenase (11β-HSD), which catalyse interconversion of physiologically active glucocorticoids (cortisol, corticosterone) and their inert 11-dehydro forms (cortisone, 11-dehydrocorticosterone) [1,2].

11β-HSD-1 is a NADP(H)-dependent enzyme, originally purified from rat liver [3]. 11β-HSD-1 is widely distributed, with highest expression in the liver. There is clear expression throughout the brain, notably in the hippocampus, as well as in the kidney [4,5]. Although 11β-HSD-1 shows bi-directional activity in tissue homogenates and purified microsomal fractions in vitro [3], this isoform is a predominant 11β-reductase (regenerating active glucocorticoids) in the liver in vivo [6,7]. Cultures of intact primary cells which continue to express endogenous 11β-HSD-1 (hepatocytes, hippocampal cells, lung cells), also show that the enzyme in its cellular context primarily catalyses 11β-reduction [8–10].

Previous studies conducted over 1–10 days have reported glucocorticoid induction of 11β-HSD activity and mRNA levels, both in the liver in vivo [11,12] and in cell culture in vitro [8,13,14], whilst adrenalectomy over this time frame attenuates hepatic 11β-HSD-1 [11]. Glucocorticoid regulation of 11β-HSD-1 also

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appears to be tissue-specific, since neither adrenalectomy, nor glucocorticoid administration affect 11β-HSD-1 mRNA levels in the rat kidney [15,16]. Glucocorticoid excess or inflammatory (arthritis) stress increase 11β-HSD-1 mRNA and enzyme bioactivity in the hippocampus [11,15]. However, the reasons for this are unclear, as chronic glucocorticoid excess is detrimental to neuronal function and survival [17,18], and 11β-reductase induction might be anticipated to potentiate neurotoxicity. Recent data from this group have shown an upregulation of 11β-HSD-1 21 days following adrenalectomy with respect to sham operation [19]. Some of these discrepancies may be explained by experimental design and/or differences in time-courses examined. Therefore we have investigated the time course of glucocorticoid control of 11β-HSD-1 in rat liver, kidney and hippocampus over a period of 21 days in more detail.

2. Materials and methods

2.1. In-vivo studies

Male Han Wistar rats (200–250 g) were maintained under controlled lighting (lights on from 07.00 to 19.00 h) and temperature (22°C) with food and water available ad libitum. Groups of animals ($n = 5$/group) were bilaterally adrenalectomised (ADX) or sham-operated (Sham ADX) through dorsal incisions under halothane anaesthesia. Following operative procedures a sub-group of adrenalectomised animals were injected subcutaneously with 250 μg/kg dexamethasone (Sigma, Poole, UK) daily (ADX + DEX). The other subgroup of adrenalectomised animals and all Sham ADX animals were injected daily with vehicle (4% ethanol in 0.9% NaCl). Adrenalectomised rats were maintained on 0.9% saline. Rats were killed 2, 5, 7, 9, 15 or 21 days after surgery. Undisturbed animals served as controls. Trunk blood was collected into heparinised tubes, plasma separated and stored at −20°C. Corticosterone levels were measured by radioimmunoassay as previously described [20] in order to confirm the adequacy of adrenalectomy (data not shown). Liver, hippocampus and kidney were removed and dissected on ice for assay of 11β-HSD activity. An aliquot of each tissue was frozen on dry ice and stored at −80°C until extraction of RNA.

2.2. Quantitation of 11β-HSD activity

Tissues were homogenised in Krebs-Ringer bicarbonate buffer (118 mmol/l NaCl, 3.8 mmol/l KCl, 1.19 mmol/l KH_2PO_4, 2.54 mmol/l $CaCl_2$, 1.19 mmol/l $MgSO_4.7H_20$, 25 mmol/l $NaHCO_3$ and 0.2% glucose, pH 7.4) and assayed as described previously [11]. Briefly, following estimation of the total protein concentration of each homogenate colorimetrically (Bio-Rad protein assay kit, Hemel Hampstead, Herts, UK), homogenates (6.25 μg of liver protein, 250 μg of hippocampal protein, 25 μg of kidney protein; chosen to be in the linear portion of the relationship between protein concentration and enzyme activity) were incubated with 200 μmol/l NADP (Sigma, Poole, UK) and 12 nmol/l [^{3}H]-corticosterone (specific activity 84 Ci/mmol; Amersham International, Aylesbury, UK) in a total volume of 250 μl with Krebs-Ringer buffer supplemented with 0.2% bovine serum albumin for 10 min at 37°C. 11β-dehydrogenase activity was quantified in this assay as a measure of active enzyme since 11β-dehydrogenase activity was quantified in this assay as a measure of active enzyme since 11β-reductase is unstable in homogenates. Steroids were extracted with ethyl acetate and separated by HPLC with on-line β counter. 11β-HSD activity was expressed as conversion of corticosterone (B) to 11-dehydrocorticosterone (A), after correction for apparent conversion in reactions without homogenate (less than 3%).

2.3. Extraction and analysis of mRNA

Total RNA was extracted from tissues by the guanidinium thiocyanate method [21] and resuspended in diethylpyrocarbonate-treated water. RNA concentration and purity was assayed spectrophotometrically. Aliquots (20 μg) were separated on a 1.2% agarose gel containing 2% formaldehyde. RNA was blotted onto nitrocellulose membranes (Hybond-*N*, Amersham International, UK), prehybridized in 6 ml phosphate buffer (0.2 mol/l NaH_2PO_4, 0.6 mol/l Na_2HPO_4, 5 mmol/l EDTA), 3 ml 20% SDS and 100 μg denatured herring testis DNA (Sigma, Poole, UK) for 2 h at 55°C and hybridized at 55°C overnight in the same solution containing rat 11β-HSD-1 cDNA, labelled with ^{32}P-dCTP using a random primed DNA labelling kit (Boehringer Mannheim UK Ltd, Lewes, UK). Three 20 min washes were carried out at room temperature in 1 × SSC (0.3 mol/l NaCl, 0.03 mol/l sodium citrate), 0.1% SDS followed by a stringent wash at 55°C for 30 min in 0.3 × SSC, 0.1% SDS. Filters were exposed to autoradiographic film for 1–4 days. Filters were rehybridised with similarly labelled 7S cDNA probes to control for loading, as previously described [11]. Optical density was determined using a computer-driven image analysis system (Seescan plc, Cambs, UK). Values are expressed as a percentage of control levels.

2.4. Statistics

Data are the means ± S.E.M. of five replicates. Data

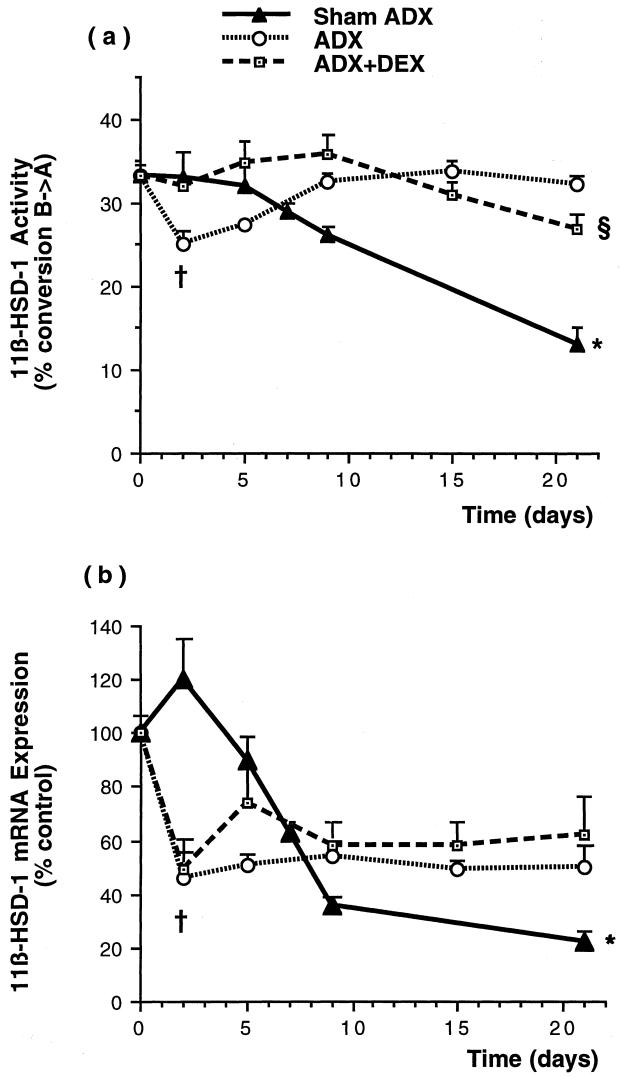

Fig. 1. Time course of the effect of adrenalectomy and dexamethasone replacement upon hepatic 11β-HSD-1. (a) 11β-HSD-1 activity and (b) 11β-HSD-1 mRNA levels in the livers of male rats following adrenalectomy (ADX), adrenalectomy with dexamethasone (ADX + DEX) or sham adrenalectomy (Sham ADX). Controls are untouched animals and are represented by the values on the y-axis at the 0 days time point. Data are expressed as (a) % conversion corticosterone (B) to 11-dehydrocorticosterone (A) ±S.E.M. or (b) percentage of mRNA levels (±S.E.M.) in untouched control animals; $n = 5$. $^{*}P < 0.001$ compared with control; $^{**}P < 0.01$ compared with control; $^{***}P < 0.05$ compared with control.

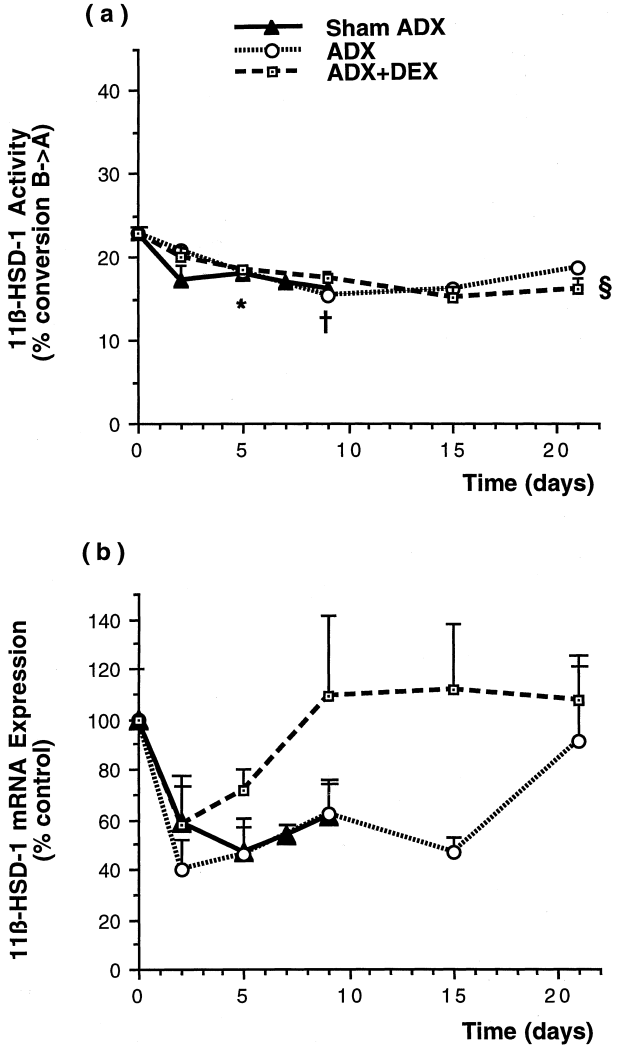

Fig. 2. Time course of the effect of adrenalectomy and dexamethasone replacement upon hippocampal 11β-HSD-1. (a) 11β-HSD-1 activity and (b) 11β-HSD-1 mRNA levels in the hippocampi of male rats following adrenalectomy (ADX), adrenalectomy with dexamethasone (ADX + DEX) or sham adrenalectomy (Sham ADX). Controls are untouched animals and are represented by the values on the y-axis at the 0 days time point. Data are expressed as (a) % conversion B to A (±S.E.M.) or (b) percentage of mRNA levels (±S.E.M.) in untouched control animals; $n = 5$. $^{*}P < 0.01$ compared with control; $^{**}P < 0.001$ compared with control; $^{***}P < 0.05$ compared with control.

were compared by ANOVA and Newman-Keuls post-hoc test. Significance was set at $P < 0.05$.

3. Results

3.1. Liver

Sham operation per se resulted in a progressive decrease in both 11β-HSD activity and 11β-HSD-1 mRNA expression in the liver over 21 days (Fig. 1) when compared to levels in untouched control animals. The decrease in 11β-HSD-1 mRNA levels preceded the decrease in activity. There was no recovery in 11β-HSD activity or 11β-HSD-1 mRNA levels over this time period. ADX produced a more rapid attenuation in 11β-HSD activity and 11β-HSD-1 mRNA than sham operation, such that both were minimal and significantly less than either sham or unoperated control levels at 2 days (Fig. 1). Subsequently, hepatic 11β-HSD activity rose following ADX, reaching control levels by 9 days, although 11β-HSD-1 mRNA levels remained below control levels over the time course

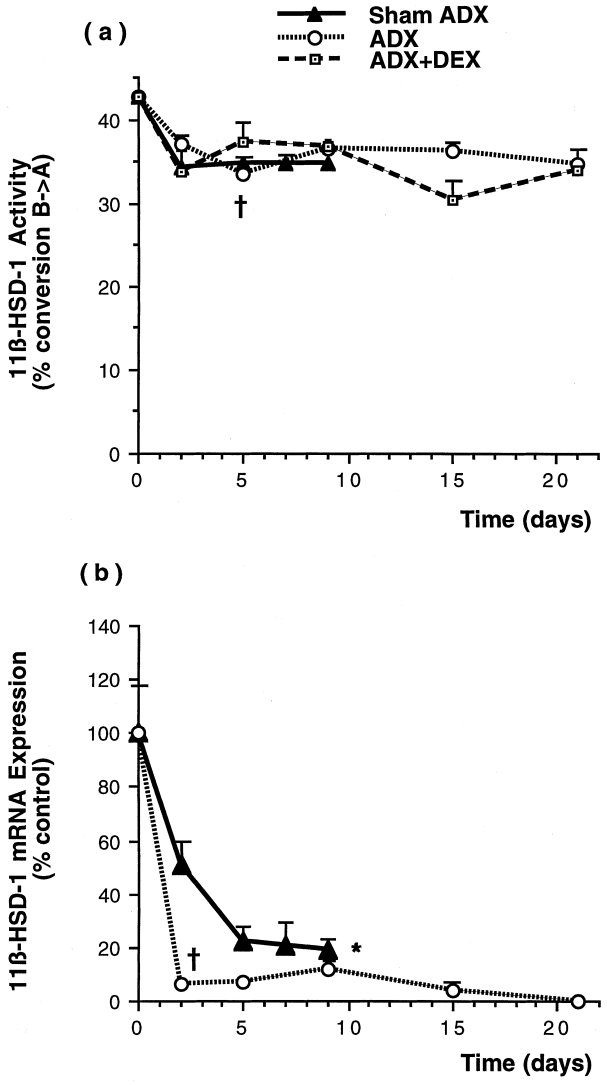

Fig. 3. Time course of the effect of adrenalectomy and dexamethasone replacement upon renal 11β-HSD-1. (a) 11β-HSD-1 activity and (b) 11β-HSD-1 mRNA levels in the kidneys of male rats following adrenalectomy (ADX), adrenalectomy with dexamethasone (ADX + DEX) or sham adrenalectomy (Sham ADX). Controls are untouched animals and are represented by the values on the y-axis at the 0 days time point. Data are expressed as (a) % conversion B to A (±S.E.M.) or (b) percentage of mRNA levels (±S.E.M.) in untouched control animals; $n = 5$. $^{*}P < 0.001$ compared with control; $^{**}P < 0.001$ compared with control.

examined. In ADX + DEX animals, hepatic 11β-HSD activity was maintained immediately after operation, but fell significantly after 21 days and was lower than following ADX alone (Fig. 1). At 21 days, sham operated animals had lower activity and mRNA expression than either the ADX or ADX + DEX animals.

3.2. Hippocampus

Hippocampal 11β-HSD activity was reduced within 2 days of sham operation and remained significantly lower than control levels until 9 days postoperatively (Fig. 2). ADX resulted in an initial fall in 11β-HSD activity in the hippocampus, reaching a nadir at 9 days, and although there was some recovery, activity was still significantly less than control levels at 21 days. ADX + DEX led to a decline in hippocampal 11β-HSD-1 activity reaching significance at 15 days. There was no difference in activity between the groups at any time point. Neither was there any difference in 11β-HSD-1 mRNA levels between or within treatments (Fig. 2), probably owing to the large standard errors, but there was a trend for 11β-HSD-1 in the hippocampus to fall following all manipulations and then to recover over the time course of the experiment.

3.3. Kidney

Overall 11β-HSD activity in the kidney was unaffected by sham operation (Fig. 3). There was, however, a small but significant decrease in renal 11β-HSD activity following ADX, with the lowest level at 5 days. Levels returned to control values by 9 days. Likewise, ADX + DEX reduced 11β-HSD activity at 2 days. At 21 days, both ADX and ADX + DEX reduced 11β-HSD activity compared with controls. In contrast, both sham operation and ADX resulted in a dramatic decrease in renal 11β-HSD-1 mRNA expression (Fig. 3) which did not recover over the time course examined.

4. Discussion

Previous studies have reported glucocorticoid induction of 11β-HSD-1 activity and mRNA expression over 1–10 days in the liver and hippocampus in vivo and in various cell types in culture [8,11–15], with adrenalectomy reducing 11β-HSD-1 expression in vivo over the same time frame. However, the clear finding from the present work is that the effects of glucocorticoid manipulations in vivo are more complex and time-dependent. Moreover, sham operation/handling stress per se reduced expression of 11β-HSD-1.

Sham operation reduced 11β-HSD-1 mRNA expression and bioactivity in liver. mRNA levels also fell in kidney. The absence of activity changes in kidney throughout the study reflects the predominant contribution of the distinctly regulated 11β-HSD-2 isozyme to overall renal activity [15,16]. This fall in 11β-HSD-1 expression with sham operation remains to be explained. Operational stress could be responsible for the initial decrease, but the continued decline suggests the effect may be mediated by factors other than glucocorticoids. Nevertheless, other manipulations in vivo must be assessed against this changing control baseline.

In the liver, which expresses only the 11β-HSD-1 isozyme, the initial response to glucocorticoid manipulations confirmed previous findings [11,12] and concur with direct transcriptional induction of the 11β-HSD-1 gene promoter by glucocorticoids observed in vitro [22]. By 21 days hepatic 11β-HSD-1 activity was restored, with greater activity following adrenalectomy alone than with dexamethasone administration. This suggests that the secondary increase in 11β-HSD activity may be due to non-transcriptional control of 11β-HSD-1 e.g. reduced protein turnover. Adrenalectomy-associated induction of 11β-HSD-1 is more in accordance with its regeneration of active glucocorticoid in vivo. In chronically-adrenalectomised animals, the increase in 11β-reductase may be an attempt to compensate for the lack of intrahepatic glucocorticoid to sustain crucial glucocorticoid-regulated functions. 11-dehydrocorticosterone circulates at levels of around 50 nM in the rat (Andrew and Seckl, unpublished data), providing a reservoir of endogenous substrate for intrahepatocellular regeneration of corticosterone.

In the hippocampus, 11β-HSD-1 activity fell following adrenalectomy and then levels returned towards control, whilst dexamethasone administration to adrenalectomised animals resulted in a continued reduction of activity, indicating a similar pattern of glucocorticoid regulation to liver. The glucocorticoid regulation of 11β-HSD-1 in the hippocampus is of particular interest as the hippocampus has a crucial role in hypothalamic-pituitary-adrenal axis feedback [23,24]. The hippocampus is rich in corticosteroid receptors [25,26] and so 11β-HSD-1 has the potential to play a major role in modulating glucocorticoid action in this tissue [4,5]. Glucocorticoid excess and glucocorticoid deprivation both compromise hippocampal cell function and survival [27,28]. In vitro, hippocampal 11β-HSD-1 regenerates active glucocorticoid from substrate [9]. Thus it may be that the function of hippocampal 11β-HSD-1 is to ensure the provision of optimal levels of active glucocorticoid for the maintenance of essential neuronal functions when corticosterone levels are low (e.g. during the diurnal nadir), whilst negative-feedback provides rapid responses to avoid over-exposure to glucocorticoids under normal circumstances. Glucocorticoid-mediated regulation of hippocampal 11β-HSD-1 may help to ensure this protection of neurones. Corticosterone actions in the hippocampus are mediated via both glucocorticoid receptors and mineralocorticoid receptors [25,26], whereas dexamethasone is a specific agonist for the glucocorticoid receptor and as such dexamethasone administration is not entirely analagous to increases in endogenous glucocorticoids. Nevertheless, chronic (28-day) stress attenuates 11β-HSD-1 activity in tree-shrew liver and hippocampus [29], demonstrating that this pattern of glucocorticoid regulation of 11β-HSD-1 does operate under physiological conditions.

In summary, this study demonstrates that glucocorticoids regulate 11β-HSD-1 in a complex tissue- and temporal-specific manner. This pattern of glucocorticoid regulation is consistent with the notion the 11β-HSD-1 is a functional 11β-reductase regulating levels of active glucocorticoid at the tissue level.

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