11β-Hydroxysteroid Dehydrogenase in Cultured Hippocampal Cells Reactivates Inert 11-Dehydrocorticosterone, Potentiating Neurotoxicity

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11β-Hydroxysteroid dehydrogenase (11β-HSD) catalyzes the conversion of the glucocorticoid corticosterone (cortisol in humans) to inert 11-dehydrocorticosterone (cortisone). 11β-HSD activity is present in the hippocampus, where it is induced by glucocorticoids and stress *in vivo*, prompting suggestions that the enzyme may attenuate the deleterious effects of chronic glucocorticoid excess on neuronal function and survival. Two isoforms exist: 11β-HSD1, a bidirectional NADPH-dependent enzyme, and 11β-HSD2, an NAD^+-dependent exclusive 11β-dehydrogenase (corticosterone-inactivating enzyme). In this study, 11β-HSD1 activity and mRNA synthesis were demonstrated in primary fetal hippocampal cell cultures. Unexpectedly, the reaction direction in intact hippocampal cells was 11β-reduction (reactivation of inert 11-dehydrocorticosterone), although homogenization revealed that the enzyme was capable of 11β-dehydrogenation when removed from its normal cellular context. Dexamethasone (10^{-7} M) increased 11β-HSD activity in homogenates of hippocampal cultures (102% increase). In intact hippocampal cells, dexamethasone induced 11βreductase, not dehydrogenase. To determine the functional relevance of hippocampal 11β-reductase, glucocorticoid potentiation of kainic acid neurotoxicity was examined. Pretreatment of hippocampal cells with corticosterone reduced survival on kainate exposure. Hippocampal cell 11β-HSD activity was potently inhibited by carbenoxolone. Carbenoxolone had no effect on cell survival after kainate alone and did not alter the effect of corticosterone. 11-Dehydrocorticosterone also potentiated kainate neurotoxicity; this effect was lost, however, if 11β-HSD was inhibited with carbenoxolone. Thus, hippocampal 11β-HSD seems to be a functional 11β-reductase in intact cells. Measures to attenuate hippocampal 11β-reductase may reduce neuronal vulnerability to glucocorticoid toxicity.

Key words: NADPH; dexamethasone; glucocorticoids; carbenoxolone; corticosterone; kainic acid

11β-Hydroxysteroid dehydrogenase (11β-HSD) catalyzes the conversion of physiological glucocorticoids (corticosterone, cortisol) to inert 11-keto derivatives (11-dehydrocorticosterone, cortisone) (Monder and White, 1993). *In vivo*, 11β-HSD ensures selective access of aldosterone over corticosterone to mineralocorticoid receptors (MRs) in the distal nephron (Edwards et al., 1988; Funder et al., 1988); MRs are otherwise nonselective and bind corticosterone with similar affinity to aldosterone *in vitro* (Arriza et al., 1987, 1988). When 11β-HSD is congenitally absent or inhibited by licorice (or its derivative carbenoxolone), glucocorticoids illicitly occupy renal MRs, causing sodium retention and hypertension (Stewart et al., 1987, 1988, 1990).

Glucocorticoids, which are released from the adrenal cortex in response to circadian or stress-induced activation of the hypothalamic–pituitary–adrenal axis, subserve many roles in homeostasis and the response to stress. The brain is a key target for glucocorticoid action, which is mediated via both MRs and lower affinity glucocorticoid receptors (McEwen et al., 1986a; de Kloet, 1991; Seckl and Olsson, 1995). The hippocampus expresses a higher density of MRs than does the kidney, but these sites are occupied by corticosterone *in vivo* (de Kloet et al., 1975; Reul and de Kloet, 1985; McEwen et al., 1986a; de Kloet, 1991), suggesting that 11β-HSD is absent. However, several recent studies have demonstrated 11β-HSD activity, immunoreactivity, and mRNA expression in hippocampal cells (neurons) (Moisan et al., 1990; Lakshmi et al., 1991; Sakai et al., 1992), raising the possibility of an aldosterone-selective subset of hippocampal MRs (Moisan et al., 1990). Indeed, some data suggest that a proportion of hippocampal aldosterone binding is not readily displaced by corticosterone (McEwen et al., 1986b) and that not all functions of aldosterone and corticosterone in the hippocampus are identical (de Kloet et al., 1983). Administration of 11β-HSD inhibitors alters functional activity in the hippocampus *in vivo* (Seckl et al., 1991), although the mechanisms underpinning this effect are obscure. Hippocampal 11β-HSD is induced by chronic glucocorticoid excess or stress (Low et al., 1994b). Because chronic glucocorticoid excess exerts well documented deleterious actions on hippocampal cell function and survival (Landfield et al., 1978; Sapolsky et al., 1985, 1986; Sapolsky, 1992), it has been postulated that such induction of 11β-HSD is protective (Monder, 1991; Low et al., 1994b; Seckl and Olsson, 1995). Nevertheless, the presence of 11β-HSD activity in the hippocampus contradicts a majority of data, which indicate nonselective MRs at this site.

Recently, it has become apparent that there are at least two distinct 11β-HSD isoforms (Seckl, 1993). Target organs for aldosterone and the placenta express a high-affinity, NAD^+-dependent enzyme (11β-HSD2), which is an exclusive 11β-dehydrogenase (corticosterone inactivating enzyme) (Brown et al., 1993; Albiston et al., 1994). In contrast, the liver-derived isoform (11β-HSD1) is

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a lower-affinity, $NADP^+$/NADPH-dependent enzyme (Lakshmi and Monder, 1988; Agarwal et al., 1989). Expression of 11β-HSD1 cDNA in a range of cell lines encodes either a bidirectional enzyme (Agarwal et al., 1989) or a predominant 11β-reductase (Duperrex et al., 1993; Low et al., 1994a). 11β-Reductase activity, best observed in intact cells, activates 11-dehydrocorticosterone to alter target gene transcription and differentiated cell function (Duperrex et al., 1993; Low et al., 1994b). In homogenates of hippocampus, both dehydrogenation and reduction occur (Lakshmi et al., 1991), but the reaction direction in intact cells is unknown. We therefore have examined 11β-HSD activity and its function in primary cultures of fetal hippocampal cells.

MATERIALS AND METHODS

Cell culture media were obtained from Gibco (Paisley, UK); corticosterone (B), 11-dehydrocorticosterone (A), poly-D-lysine, insulin, apo-transferrin, putrescine, sodium selenite, and progesterone were obtained from Sigma (Poole, UK). Tissue culture plastics were from Costar UK Ltd (High Wycombe, Bucks, UK). [^{3}H]-1,2,6,7-Corticosterone ([^{3}H]B; ~ 72 Ci/mmol) was obtained from Amersham International (Aylesbury, Bucks, UK). [^{3}H]11-Dehydrocorticosterone ([^{3}H]A) was prepared by incubating [^{3}H]B with human placental extract, a concentrated source of 11β-dehydrogenase (11β-HSD2), as described previously (Low et al., 1994a). Purity was typically >99%, monitored on HPLC.

Primary hippocampal neuronal culture. The cell cultures were prepared according to a method derived from Mitchell et al. (1990). The medium (pH 7.3) contained DMEM with Glutamax-I (0.086%), 10% fetal bovine serum (FBS) or donor horse serum (DHS), 15 mM HEPES, 100 IU/ml penicillin, and 100 μg/ml streptomycin and glucose increased to 0.2%. Day 18 fetuses were removed by laparatomy, and the hippocampi were dissected into HBSS containing 15 mM HEPES, pH 7.4. The cells were incubated in trypsin-EDTA for 20 min, washed, and mechanically dissociated by trituration. Cells were seeded at a density of 0.8–1.0 × 10^6 cells/ml of medium and plated on 35 mm Petri dishes previously coated with 0.025 mg/ml poly-D-lysine. The cells were cultured in a water-saturated atmosphere at 37°C, 10% CO_2, for 3 d in the presence of serum, and then changed to defined (serum-free) medium (DMEM-F12 containing Glutamax-I, 10 μg/ml insulin, 100 μg/ml transferrin, 60 μM putrescine, 20 nM sodium selenite, 20 nM progesterone, 100 IU/ml penicillin, and 100 μg/ml streptomycin). The cells were maintained in this medium for 5 d with one-third of the medium changed every 3 d until experimentation. Under these conditions, the cultures contained 60–80% neurons with glia forming the remainder, as determined by immunostaining of specimen cultures with antisera to glial fibrillary axial protein and neuron-specific enolase.

11β-HSD activity. 11β-Reductase and 11β-dehydrogenase activity were determined in intact primary hippocampal cells by the addition of 25 nM [^{3}H]A or [^{3}H]B to the medium, as described previously (Low et al., 1994a). Aliquots of the culture medium were removed at intervals over 24 hr and put into ethyl acetate; the steroids were extracted, dried under N_2, and suspended in 100 ml of ethanol containing 2.5 mg/ml cold A and B. Steroids were separated by thin-layer chromatography (TLC) in chloroform/95% ethanol (92:8), and bands were visualized under ultraviolet light and scraped into scintillation vials containing 3 ml of liquid scintillant (Cocktail T, BDH, Lutterworth, UK). The radioactivity in each fraction was determined, and enzyme activity was expressed as the percentage conversion to reaction product (Low et al., 1994a). Blanks, ^{3}H-labeled steroids incubated in medium on dishes without cells and extracted as above, were subtracted.

11β-Reductase and 11β-dehydrogenase activity also were determined in homogenates of cultured hippocampal cells, broadly as described previously (Moisan et al., 1990; Low et al., 1994a). Cells were washed with PBS and homogenized in Buffer C [20 mM tricine, 1.07 mM $(MgCO_3)_4 \cdot Mg(OH)_2 \cdot 5H_2O$, 2.67 mM $MgSO_4$, 0.1 mM EDTA, 33.3 mM DTT, and 0.2 mg/ml coenzyme A] containing 0.1% Triton X-100. Protein was measured by Bradford's method (Bio-Rad protein assay kit, Bio-Rad, Hemel Hempstead, UK). Preliminary studies established conditions such that the amount of protein added was within the linear portion of the relationship between protein concentration and percentage substrate conversion. Thus, an aliquot of hippocampal cell homogenate (typically 64 μg protein/ml) was incubated at 37°C for 60 min with 10 nM [^{3}H]B (11β-dehydrogenase) or [^{3}H]A (11β-reductase), 400 μM $NADP^+$ or NADPH, and PBS to a total volume of 50 μl. Steroids were extracted and separated by TLC, and enzyme activity was calculated. Blanks were subtracted.

11β-HSD mRNA analysis. Expression of 11β-HSD1 and 11β-HSD2 mRNA was determined by PCR. In brief, total RNA was extracted from cell homogenates with guanidinium thiocyanate (Chomczynski and Sacchi, 1987). One microgram of RNA was subjected to reverse transcription in a total volume of 20 μl using the Promega Reverse transcription kit (Promega, Southampton, UK). A 2 μl aliquot of the reverse transcription reaction was used in the PCR reaction after denaturation at 96°C for 10 min. The PCR mix contained 1.5 mM $MgCl_2$, 40 μM dNTPs, 20 pmol of each primer, and 2.5 U of *Taq* DNA polymerase in a final volume of 50 μl. The primer pairs for 11β-HSD1 were 869P (5′-AAAGCTTGTCACA/TGGGGCCAGCAAA-3′), corresponding to nucleotides 178–207 of rat 11β-HSD1 cDNA, and 868P (5′-AGGATCCAG/AAGCAAACTTGCT-TGCA-3′), complementary to nucleotides 648–628 of rat 11β-HSD1 cDNA. The primer pairs for 11β-HSD2 were P2520 (5′-CAATGCTG-GCCTCAACATGGT-3′), corresponding to nucleotides 624–644 of the rat 11β-HSD2 cDNA, and P2521 (5′-GGTCCTGGGTTGTGTCAT-GAA-3′), complementary to nucleotides 1297–1277 of the rat 11β-HSD2 cDNA sequence (Zhou et al., 1995). Both sets of primers span intron sequences to distinguish DNA products (>4 kb for 11β-HSD1, ~0.93 kb for 11β-HSD2) from mRNA products (0.43 kb for 11β-HSD1, 0.63 kb for 11β-HSD2). The PCR conditions were 30 cycles of 96°C for 30 sec, 50°C for 45 sec, and 72°C for 90 sec, followed by extension at 72°C for 10 min.

Modulation of 11β-HSD activity in primary hippocampal cells. To determine whether 11β-HSD activity in intact hippocampal cells was inhibited by licorice derivatives, cultures were pretreated with carbenoxolone (10^{-6} M) for 24 hr, and enzyme activity in 11β-reductase and 11β-dehydrogenase directions was estimated, as above. Glucocorticoid effects on enzyme activity were determined by preincubation of cultured hippocampal cells with dexamethasone (10^{-7} M) for 72 hr before enzyme measurement in intact cells after 8 hr incubation with ^{3}H-labeled steroids (a period chosen to reflect submaximal 11β-reductase and detectable 11β-dehydrogenase activities).

Effects of 11β-HSD on hippocampal cell vulnerability to kainate toxicity. To determine the functional relevance of 11β-HSD activity in primary cultures of hippocampal cells, the effects of corticosterone and 11-dehydrocorticosterone in the presence or absence of carbenoxolone on cell survival in response to kainic acid stimulation were determined, as described previously (Sapolsky, 1986; Packan and Sapolsky, 1990). In brief, cells were cultured as described above, but in the presence of 2% FBS to improve cell survival in the presence of kainic acid. This did not alter the predominant 11β-reduction in intact hippocampal cells or affect enzyme activity levels: corticosteroid levels are $<10^{-10}$ M in FBS (S.C. Low and J. R. Seckl, unpublished observations.) Plates of cells were pre-exposed to 11-dehydrocorticosterone (10^{-5} M) or corticosterone (10^{-5} M) in the presence or absence of carbenoxolone (10^{-6} M) for 24 hr. Controls included cultures exposed to carbenoxolone alone and cultures to which nothing was added. All cells then received kainic acid (10^{-5} M). After 48 hr, cells were washed in PBS, scraped into 1.2 ml of potassium phosphate buffer, pH 7.5, containing 0.5% Triton X-100, and lactate dehydrogenase activity was determined, using a kit (Sigma) according to the instructions of the manufacturer, as a measure of cell survival (Sapolsky, 1986; Packan and Sapolsky, 1990). Control plates that were not exposed to steroid or carbenoxolone were taken to represent 100% survival, and blanks represented 0% survival.

Statistics. Three to eight plates of cells were used for each data point. Data were assessed by ANOVA followed by Newman–Keuls *post hoc* test or Student's *t* tests, where appropriate. Significance was set at $p < 0.05$. Values are mean ± SEM.

RESULTS

11β-HSD in cultured hippocampal cells

Primary fetal hippocampal cell cultures showed clear 11β-HSD activity in cell homogenates. $NADP^+$-dependent 11β-dehydrogenase and NADPH-dependent 11β-reductase activity was clearly detectable after 1 hr incubation (Fig. 1). NAD^+-dependent activity was only just above basal enzyme activity (with no added cosubstrate; Fig. 1). PCR analysis showed clear expression of 11β-HSD1 mRNA, but no detectable expression of 11β-HSD2 mRNA (Fig. 2). Northern analysis showed only one hybridizing

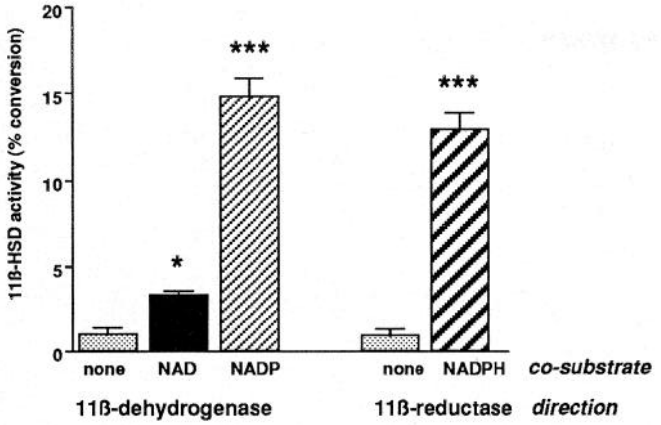

Figure 1. Cosubstrate dependence and reaction direction of 11β-HSD in homogenates of cultured primary hippocampal cells. Cosubstrates were added at 400 μM. Note the predominant $NADP^+$/NADPH-dependent reaction, typical of 11β-HSD1, and the obvious bidirectional activity in homogenates. $^*p < 0.05$ and $^{***}p < 0.0001$ compared with the appropriate control without exogenous cosubstrate.

species of 11β-HSD1 transcript, identical in size to the transcript in rat liver (data not shown), confirming previous studies of 11β-HSD1 transcripts in adult and fetal rat hippocampus *in vivo* (Moisan et al., 1990, 1992; Low et al., 1994b).

In contrast, predominant 11β-reduction was found (Fig. 3) in intact hippocampal cells, with clearly detectable conversion of inert 11-dehydrocorticosterone to corticosterone within 30 min of addition of steroid, and 78% conversion after 24 hr. This represented plateau activity because no further conversion occurred after 48 hr incubation with [^{3}H]A (data not shown). 11β-Dehydrogenase activity only became detectable after 8 hr incubation with [^{3}H]B (4 ± 1% conversion) and reached a mere 6.5 ± 1% conversion at 24 hr (Fig. 3). Pretreatment of cultures with the 11β-HSD inhibitor carbenoxolone (10^{-6} M) almost completely inhibited enzyme activity in intact hippocampal cells in

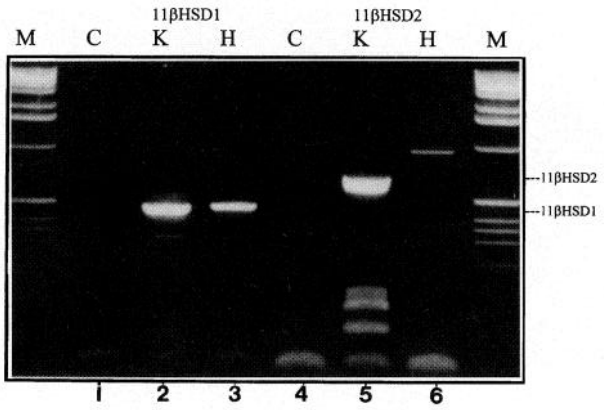

Figure 2. Presence of 11β-HSD1 mRNA in cultured primary hippocampal cells. Total RNA from 11-d-old cultures was subjected to reverse transcription followed by PCR using primers specific for 11β-HSD1 and 11β-HSD2. *Lane 1*, Negative control (*C*); *lane 2*, positive control kidney RNA (*K*); *lane 3*, hippocampal cell culture RNA (*H*); *lane 4*, negative control (*C*); *lane 5*, positive control kidney RNA (*K*); and *lane 6*, hippocampal cell culture RNA (*H*). *M* indicates marker lanes. Reactions in *lanes 1, 2,* and *3* contained 11β-HSD1-specific primers, and *lanes 4, 5,* and *6* contained 11β-HSD2-specific primers. Note the presence of a band in *lane 3* corresponding to hippocampal 11β-HSD1. The band in *lanes 5* and *6* migrating at ~0.9 kb corresponds to DNA-generated PCR product (see Results).

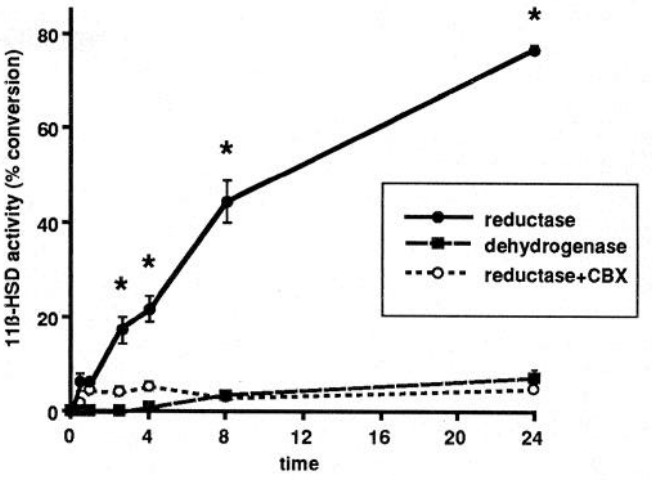

Figure 3. Reaction direction of 11β-HSD in intact primary hippocampal cells *in vitro*. [^{3}H]Corticosterone or [^{3}H]11-dehydrocorticosterone was added to the culture medium, and [^{3}H]corticosteroids in the medium were assayed at intervals. Note the predominant 11β-reduction in intact hippocampal cells. Carbenoxolone (CBX; 10^{-6} M) inhibits the reaction. $^*p < 0.01$ for 11β-reductase activity compared with either carbenoxolone-inhibited 11β reductase or 11β-dehydrogenase at the same time point.

both 11β-reductase (Fig. 3) and 11β-dehydrogenase (data not shown) directions.

Glucocorticoid modulation of hippocampal cell 11β-HSD activity

Treatment of primary hippocampal cell cultures with dexamethasone (10^{-7} M) for 72 hr increased 11β-HSD activity in cell homogenates (102% increase in 11β-dehydrogenase, 72% increase in 11β-reductase). In intact cells, this was exclusively an increase in 11β-reductase activity (by 43%), with no alteration in dehydrogenation detected (Fig. 4).

Effect of 11β-HSD on hippocampal cell survival in the presence of kainic acid

Preliminary experiments showed that both 10^{-7} and 10^{-5} M corticosterone potentiated kainic acid-mediated neurotoxicity, but this was considerably more apparent with the higher dose (data not shown). We therefore used the 10^{-5} M dose to study the effect of 11β-HSD in these cells. In the presence of corticosterone, kainic acid exerted significantly greater neurotoxicity than did kainic acid alone (Fig. 5), confirming previous studies (Sapolsky, 1986; Packan and Sapolsky, 1990). Carbenoxolone (10^{-6} M) did not alter the effect of corticosterone (10^{-5} M) on hippocampal cell loss in the presence of corticosterone, nor did 10^{-6} M carbenoxolone alone affect cell loss in the face of kainic acid stimulation (Fig. 5). 11-Dehydrocorticosterone (10^{-5} M) also potentiated kainic acid neurotoxicity and was at least as potent in this action as corticosterone (Fig. 5). However, inhibition of 11β-HSD with carbenoxolone (10^{-6} M) abolished the cytotoxic potentiating effects of 11-dehydrocorticosterone in hippocampal cultures.

DISCUSSION

These studies clearly demonstrate 11β-HSD activity in primary hippocampal cell cultures. A majority of the cultured cells were neurons, and it seems likely that the activity, taken together with previous immunocytochemical and *in situ* hybridization data showing a predominant neuronal localization of 11β-HSD in several brain regions *in vivo* (Moisan et al., 1990; Sakai et al., 1992), reflects 11β-HSD largely in neurons in the fetal hippocam-

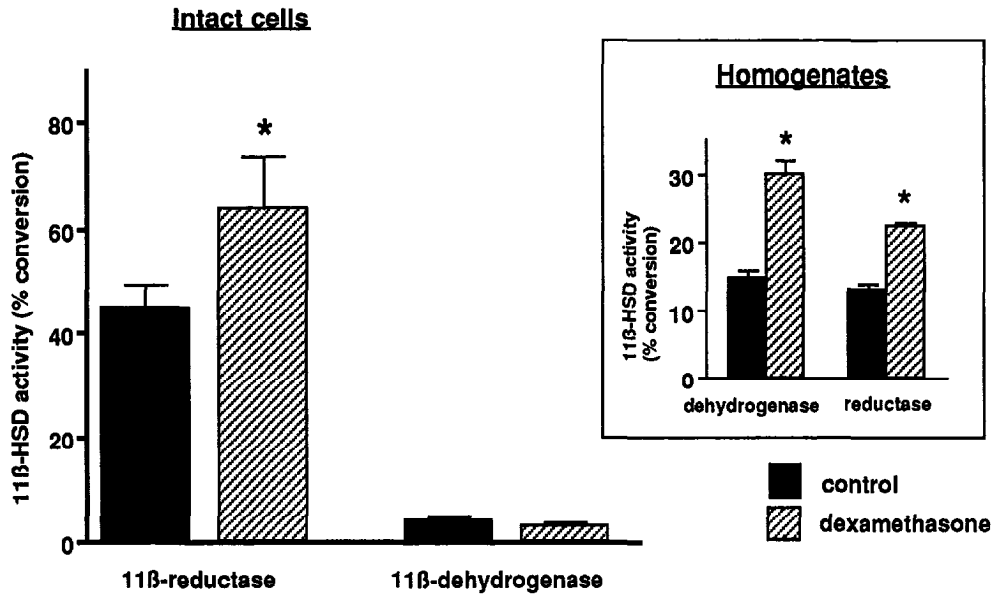

Figure 4. Effect of dexamethasone (10^{-7} M for 72 hr) on 11β-HSD activity in intact primary hippocampal cells and cell homogenates (*inset*). Note that dexamethasone induces hippocampal cell 11β-HSD activity *in vitro*, but the reaction is 11β-reduction in intact cells. $^{*}p < 0.05$ compared with control.

pal cultures. 11β-HSD activity was detectable in culture for more than 8 d, suggesting that it is stable under the conditions that were used. In intact hippocampal cells, 11β-reduction was clearly the predominant reaction direction. Dehydrogenation was barely detectable in intact hippocampal cells, and at the 8 hr time point at which 11β-dehydrogenation became detectable and 11β-reduction had not reached plateau values, the ratio of reduction to dehydrogenation was 11.5:1. Few previous studies have addressed the reaction direction of 11β-HSD in brain *in vivo*. Those reported have used peripheral injection of radiolabeled glucocorticoids and have examined the concentrations of 11-hydroxy to 11-keto products in target tissues (Burton and Tufnell, 1967). Uptake into the brain is low using such approaches, and in any event the data are complicated by peripheral conversion of steroids before entry into the brain, which largely reflects the balance of 11β-reduction in the liver and 11β-dehydrogenation in the kidney. No studies have examined 11β-reduction in brain *in vivo*, and indeed it is difficult to envisage satisfactory experimental protocols to overcome peripheral interconversion of corticoids without their direct infusion into the central nervous system.

In contrast, in homogenates of hippocampal cells, 11β-dehydrogenase was readily detectable and indeed exceeded 11β-reductase activity. This confirms studies in homogenates of hippocampus *in vivo*, which show both dehydrogenation and reduction reactions (Lakshmi et al., 1991; Seckl et al., 1993). The basis for the discrepancy between predominant reduction in intact cells and dehydrogenation in homogenates of these cells is unclear but not unique to the hippocampus. Thus, transfection of COS7 cells with an expression plasmid encoding 11β-HSD1 produces exclusively 11β-reductase activity in intact cells, but potent dehydrogenation is revealed when the transfected cells are homogenized (Low et al., 1994a). Reaction direction has been suggested to be determined by the glycosylation status of the enzyme or the tissue cosubstrate condition (Agarwal et al., 1990; Monder and White, 1993). Variations in glycosylation cannot explain near-exclusive reduction in intact hippocampal cells when dehydrogenation predominates immediately after these cells are homogenized. Moreover, any variations in $NADP^+$/NADPH ratios are unlikely to be sufficient to account for the dramatic change in reaction direction observed, and even gross changes in these ratios in intact cells have little effect on reaction direction of 11β-HSD1, at least in the liver (Jamieson et al., 1995). Thus, it seems more likely that the subcellular context of the enzyme determines the reaction direction, with homogenization disrupting this. Whether or not the reductase component is unstable in homogenates, it is clear from these data that dehydrogenation is revealed by cellular disruption, but it is at most a minor reaction in intact hippocampal cells and, by implication, in the hippocampus *in vivo*. The lack of 11β-dehydrogenation in intact hippocampal cell cultures also concurs with the nonselectivity of hippocampal MRs *in vivo* (de Kloet et al., 1975, 1991; Reul and de Kloet, 1985; McEwen et al., 1986a). Whether 11β-HSD2 (an exclusive dehydrogenase) is present in the hippocampus is still debatable. Northern analysis shows no expression of 11β-HSD2 mRNA in whole (human) brain

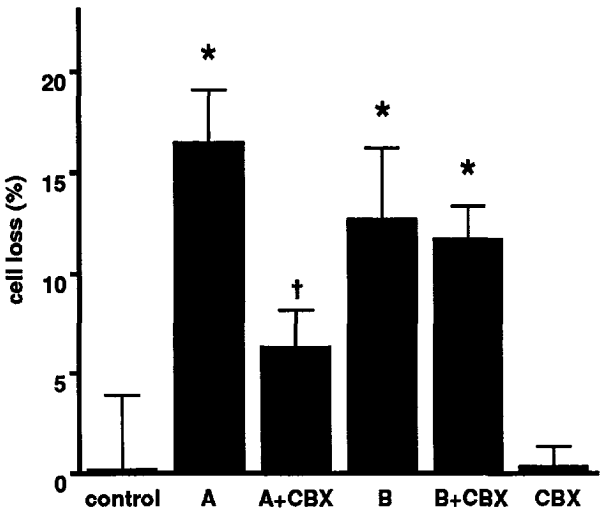

Figure 5. Effect of pretreatment with corticosterone (*B*; 10^{-5} M) and 11-dehydrocorticosterone (*A*; 10^{-5} M) with or without carbenoxolone (*CBX*; 10^{-6} M) on hippocampal cell loss after exposure to kainic acid (10^{-5} M). Carbenoxolone alone has no effect on cells and does not alter the neurotoxic potentiation of corticosterone (*B* vs *B+CBX*). However, 11-dehydrocorticosterone (*A*) toxicity is prevented by carbenoxolone (*A* vs *A+CBX*), indicating that hippocampal cell 11β-HSD acts as a functional 11β-reductase in this model. $^{*}p < 0.05$ compared with control; $^{\dagger}p < 0.05$ compared with *A* alone.

(Albiston et al., 1994), and *in situ* hybridization studies have failed to find 11β-HSD2 mRNA in the adult rat hippocampus (Roland et al., 1995). In contrast, a recent PCR-based study showed 11β-HSD2 transcripts in adult hippocampus (Zhou et al., 1995). Using a similar PCR approach, however, we could not detect 11β-HSD2 mRNA in primary hippocampal cell cultures and, importantly, found little NAD^+-dependent bioactivity. Thus, hippocampal expression of this MR-protective 11β-HSD isoform is probably negligible, at least in adult and late fetal life.

Previous studies have shown that chronic glucocorticoid (dexamethasone) excess or stress increase 11β-HSD1 mRNA expression and enzyme bioactivity (measured as dehydrogenation in homogenates) in the rat hippocampus (Low et al., 1994b). Because treatment of primary hippocampal cultures with dexamethasone increased 11β-HSD activity, it is probable that this effect of glucocorticoids is mediated directly on hippocampal neurons, and there is a putative glucocorticoid response element in the promoter of the rat 11β-HSD1 gene (Moisan et al., 1992). Moreover, preliminary data from transfection experiments with plasmids in which 11β-HSD1 promoter DNA is fused to a reporter gene suggest that a functional glucocorticoid response element lies within 3700 base pairs of the transcription start site (K. E. Chapman, M. Voice, R. Wallace, V. Lyons, and J. R. Seckl, unpublished observations). Although it has been proposed that induction of 11β-HSD in the hippocampus may protect vulnerable neurons from the deleterious consequences of chronic glucocorticoid excess (Monder, 1991; Low et al., 1994b), the activity induced by dexamethasone was exclusively 11β-reductase, a finding incompatible with this notion. Thus, hippocampal 11β-HSD may potentiate rather than attenuate the neurotoxic effects of chronic glucocorticoid overexposure.

To examine this further, the effects of enzyme inhibition in both reductase and dehydrogenase directions was determined by using carbenoxolone. Potentiation of kainic acid neurotoxicity with 10^{-7} and 10^{-5} M corticosterone was found in primary hippocampal cell cultures, confirming previous studies (Sapolsky, 1986; Packan and Sapolsky, 1990). Although supraphysiological levels of steroids were used *in vitro*, physiological concentrations (10^{-7} M) are effective in potentiating kainic acid neurotoxicity in this system (Packan and Sapolsky, 1990; and these data), although the changes were small during the short time course used here. *In vivo*, corticosteroid effects take many days to become manifest (Sapolsky et al., 1985), and we have used higher concentrations to amplify effects in cell cultures. That the effect of corticosterone was unaltered by carbenoxolone in a dose that inhibited both reaction directions but had no direct effect on cell survival supports the relative lack of importance of 11β-dehydrogenation in these cells. 11-Dehydrocorticosterone, which has 10^4-fold lower affinity for receptors than does corticosterone (Ulmann et al., 1975; Armanini et al., 1983), was at least as effective as corticosterone in potentiating kainic acid toxicity. The functionality of 11β-reductase was clear, inasmuch as 11-dehydrocorticosterone was ineffective when 11β-HSD was inhibited by carbenoxolone. These data support the predominant 11β-reductase action of hippocampal 11β-HSD.

The reasons for an enzyme regenerating active glucocorticoids in the hippocampus are obscure. Hippocampal cells are exquisitely sensitive to glucocorticoid concentrations, with granular neuronal dysfunction and possibly death in the dentate gyrus after adrenalectomy (Sloviter et al., 1989; Gould et al., 1990) and pyramidal neuronal loss in the cornu ammonis with glucocorticoid excess (Landfield et al., 1978; Sapolsky et al., 1985, 1986; Sapolsky, 1985, 1992; Landfield and Eldridge, 1991). In the rat, corticosterone levels are very low during the day but show a pronounced diurnal increase in the evening. The biological importance of this rhythm is unclear, but the maintenance of many constitutive cellular functions may require more than the minimal levels of circulating corticosterone that pertain during the majority of the day. Thus, local 11β-reduction may increase corticosterone levels in specific tissues and, hence, 11-dehydrocorticosterone may form a circulating reservoir of inert corticosteroid for cell-specific activation (the apparent absence of 11β-HSD2 in the hippocampus suggests that 11-dehydrocorticosterone comes from the periphery rather than from local cellular production). Certainly in humans, cortisone (the equivalent of 11-dehydrocorticosterone) shows near-constant levels throughout the 24 hr period. Moreover, plasma concentrations of cortisone (which circulates largely unbound at ~100 nmol/l) approximate or even exceed "free" cortisol levels, providing plentiful substrate for 11β-reductase (Walker et al., 1992). Similarly, levels of 11-dehydrocorticosterone at ~50 nmol/l are found in rat plasma (R. Best and J. R. Seckl, unpublished observations), concentrations well in excess of "free" corticosterone levels during the diurnal nadir. Such cell-specific activation of an inert circulating form is not unique to glucocorticoids and may be analogous to the activation of thyroxine to tri-iodothyronine by 5′-monodeiodinase and testosterone to dihydrotestosterone by 5α-reductase in other tissues.

Why the activity of hippocampal 11β-reductase should increase with chronically elevated glucocorticoids also is unclear, because this seems to increase the neuron-jeopardizing effects of glucocorticoid excess. Perhaps the short- and medium-term metabolic and functional benefits of maximizing glucocorticoid exposure during stress outweigh any long-term detriments, particularly because potent negative feedback effects would be expected to rapidly attenuate glucocorticoid levels under most physiological circumstances. Further determination of the importance of hippocampal 11β-HSD activity will be assisted by the development of selective 11β-reductase inhibitors or transgenic animals lacking 11β-HSD1. Nevertheless, it is intriguing to speculate that measures to attenuate hippocampal 11β-reductase may reduce neuronal vulnerability to glucocorticoid toxicity in a target-specific manner, analogous to the effects of long-term maintenance of more generalized low glucocorticoid levels by adrenalectomy (Landfield et al., 1978) or increasing sensitivity to glucocorticoid negative feedback (Sapolsky et al., 1984; Meaney et al., 1988, 1993; Seckl and Olsson, 1995).

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