Mineralocorticoid receptor expression and increased survival following neuronal injury

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Abstract
Glucocorticoids, acting via the mineralocorticoid receptor, are required for granule neuronal survival in the rat dentate gyrus. Whether this mineralocorticoid receptor-mediated neuroprotective effect has more general applicability is unknown. Here we report increased mineralocorticoid receptor expression in rat hippocampal and cortical neurons exposed in vivo to low levels of staurosporine and in rat hippocampal pyramidal neurons exposed in vitro to hypothermic transient global ischaemia. In both the cell culture system and the in vivo system increased mineralocorticoid receptor expression is associated with increased neuronal survival, and this increase is reversed by mineralocorticoid receptor antagonism. Modulation of mineralocorticoid receptor gene expression may therefore be an important target for reduction of brain injury in conditions caused by cerebral ischaemia including brain damage following cardiac arrest and stroke.

Introduction
Survivors of cardiac arrest may have profound cognitive and memory difficulties (Pusswald et al., 2000), and Petitot et al. (1987) found features of delayed hippocampal damage in patients dying after cardiac arrest. In animal models of cardiac arrest, transient global ischaemia causes delayed hippocampal damage in the form of CA1 pyramidal cell apoptosis (Sugawara et al., 1999) and this damage is strikingly reduced by intra-ischaemic hypothermia (Minamisawa et al., 1990). The mechanism(s) underlying this protective effect is not clear but may include reduced post-ischemic free radical production (Kil et al., 1996), prevention of protein kinase C translocation to cell membranes (Cardell et al., 1991) and reduced DNA fragmentation (Ferrand-Drake & Wieloch, 1999). Many animal models of cerebral ischaemia manifest biochemical features of apoptosis, and in such models pharmacological or genetic inhibition of apoptosis pathways results in reduced neuronal cell death and improved neurological performance (Choi, 1996).

Staurosporine causes apoptosis in a range of mammalian cell types including neurons in primary culture (Lobner & Choi, 1996). However, treatment with staurosporine in animal models of stroke paradoxically reduces neuronal cell death. This unexpected finding has been reported following focal (Chopp et al., 1999) and transient global (Hara et al., 1989) ischaemia. Moreover, staurosporine improves cognitive outcome following transient global ischaemia (Ohno et al., 1991). The mechanism of this neuroprotective effect is not known.

Glucocorticoids may promote cell death or enhance cell survival. High levels of glucocorticoids (cortisol, corticosterone) potenti ate neuronal injury in response to ischaemia (Sapolsky & Pulsinelli, 1985) and to excitotoxins (Supko & Johnston, 1994). The neurotoxic effects of elevated glucocorticoid levels are mediated through a lower affinity glucocorticoid receptor (GR) (Cho & Little, 1999). In contrast, complete removal of glucocorticoids by adrenalectomy results in profound apoptosis of granule cells in the dentate gyrus both perinatally and in adult life (Stolbvert et al., 1989). This effect is prevented by low-dose corticosterone replacement (Conrad & Roy, 1995) acting through a higher affinity mineralocorticoid receptor (MR), normally expressed highly only in hippocampus and septum, though GR may also play a neuroprotective role in early development (Woolley et al., 1991). MR signalling has previously been shown to have survival-promoting effects following kainic acid-induced neurotoxicity in rat hippocampus (McCullers & Herman, 2001), and to promote hippocampal expression of Bcl2 and Bcl-XL (Almeida et al., 2000).

In primary cultures of rat hippocampal cells, we observed increased expression of MR in apoptotic neurons. Given the known neuroprotective effects of MR signalling in hippocampus, this increase might represent an endogenous neuroprotective stress response which might form the basis for treatments in conditions such as cardiac arrest. Our hypotheses were that (i) neuronal injury results in increased MR expression; (ii) if so, this is associated with increased neuronal survival in these systems; and (iii) increased neuronal survival after transient global ischaemia in combination with hypothermia can be blocked by MR antagonism.

Methods

Materials
The Sigma Chemical Company (Dorset, UK) supplied all materials unless otherwise stated.
Cell cultures

Animals were maintained under controlled lighting (lights on from 07.00 to 19.00 h) and temperature (22 °C) with free access to food and water. Experiments were conducted in accordance with the provisions of the UK Animals (Scientific Procedures) Act 1984. Pregnant Wistar rats (Charles River Ltd, Margate, Kent, UK) at 18 ± 0.5 days of gestation were killed by cervical dislocation, and primary cortical and hippocampal neuronal cultures were prepared from fetal tissue. Following trypsinization and trituration, cells were collected by centrifugation, resuspended and plated onto poly-d-lysine-coated 12-well tissue culture plates (Biocoat™, Becton Dickinson Ltd) at a density of 3000 cells/mm² in 1.5 mL of Neurobasal™ medium supplemented with B-27 and 0.5 mM l-glutamine, and grown in a humidified incubator at 37 °C/5% CO₂. The medium was changed after 7 and 13 days in vitro (d.i.v.) and experiments were performed at 14 d.i.v. This protocol results in greater than 98% neuronal purity according to staining for MAP2 and GFAP (data not shown).

Immunohistochemistry

Cells at 14 d.i.v. were fixed with paraformaldehyde (4%, 4 °C) for 30 min. Fixed cells were washed with PBS, then incubated with PBS containing 1.5% serum and 0.1% Triton X100 (ST-PBS) for 1 h at room temperature. MR was detected using an affinity purified anti human MR goat polyclonal antibody (N-17; Santa Cruz Biotechnology, Santa Cruz, CA) at a dilution of 1 : 500 in ST-PBS (24 h at 4 °C) as previously described (Gesing et al., 2001), then incubated with biotinylated rabbit antibody IgG (1 : 200 dilution, 2 h at room temperature). Bound antibody was detected using the avidin–biotin–peroxidase system (Elite ABC goat kit; Vector Laboratories, Burlingame, CA) at a dilution of 1 : 500 in ST-PBS (24 h at 4 °C, 8 °C for 90 s followed by a final extension at 72 °C for 10 min). The resulting 635 bp product was then used as a template to generate internally deleted 467 bp MR cDNA fragment by PCR under the same conditions but using an internal linker 3’ primer (5’-CTTACACCAGGTCATCTTCAAGAAT-GCT-3’) as previously described (Gesing et al., 1989) (Oswel DNA Service, Southampton, UK). The PCR reaction comprised 30 cycles at 96 °C for 40 s, 45 °C for 45 s and 72 °C for 90 s followed by a final extension at 72 °C for 10 min. The resulting 467 bp fragment was amplified using primers 1 and 2 to generate a 485-bp MR competitor cDNA fragment which we subcloned into pGEM-T Easy (Promega). MR competitor RNA was synthesized by in vitro transcription (Promega) from Sal I linearized plasmid. The concentration of competitor RNA was determined by GeneQuant spectrophotometric analysis.

Competitive RT-PCR

A MR competitor fragment was generated by internal deletion of an MR cDNA fragment (Forster, 1994). The MR cDNA was obtained from rat kidney by RT-PCR using primers 1 (5’-AACGATCCAGAAGGCCCATCTTCAAGAAT-GCT-3’) and 2 (5’-TGCCATGTCGAGATGAAT-GCT-3’) (Patel et al., 1989) (Oswel DNA Service, Southampton, UK). The PCR reaction comprised 30 cycles at 96 °C for 40 s, 45 °C for 45 s and 72 °C for 90 s followed by a final extension at 72 °C for 10 min. The resulting 635 bp product was then used as a template to generate internally deleted 467 bp MR cDNA fragment by PCR under the same conditions but using an internal linker 3’ primer (5’-CTTACACCAGGTCATCTTCAAGAAT-GCT-3’) as previously described (Gesing et al., 1989) (Oswel DNA Service, Southampton, UK). The PCR reaction comprised 30 cycles at 96 °C for 40 s, 45 °C for 45 s and 72 °C for 90 s followed by a final extension at 72 °C for 10 min. The resulting 467 bp fragment was amplified using primers 1 and 2 to generate a 485-bp MR competitor cDNA fragment which we subcloned into pGEM-T Easy (Promega). MR competitor RNA was synthesized by in vitro transcription (Promega) from Sal I linearized plasmid. The concentration of competitor RNA was determined by GeneQuant spectrophotometric analysis.

RNA (200 ng) from each sample was mixed with known concentrations of MR competitor RNA (12.5–100 pm) and the mixture subjected to reversed transcription for 1 h at 37 °C using the First Strand cDNA Synthesis Kit (Pharmacia) that employs random hexamer primers and Murine Moloney Leukaemia Virus reverse transcriptase. A 7.5-µL aliquot of each RT reaction product was PCR amplified using the primers 1 and 2 for 30 cycles of 40 s at 94 °C, 40 s at 54 °C, 40 s at 72 °C and a final extension of 10 min at 72 °C. Resulting amplification products were separated by electrophoresis on 1.5% agarose gels and visualized by ethidium bromide staining (Fuji film Image Analyser FLA2000R). Densitometric analysis was performed using the AIDA Image software package and MR concentrations determined as the concentration intercept of the logarithmic plot of density of sample RNA signal/competitor RNA signal vs. concentration of competitor.

Transient global ischaemia

All procedures followed the guidelines of the National Institute of Health and were approved by the Lund/Malmö ethical committee for experimental animals. Adult male Wistar rats (265–375 g; Møllegaard’s Breeding Centre, Copenhagen, Denmark) were housed in cages with free access to water and food until 24 h prior to surgery and after surgery until perfusion at the end of the experiment.
Rats were anaesthetized (3.5% halothane in oxygen/nitrous oxide) and ventilated and the halothane concentration reduced to 1.5%; muscle relaxation was achieved with Vecuronium bromide (Organon, Boxtel, the Netherlands; 2 mg/kg/h). We catheterized the superior cava vein and tail artery and vein to allow infusion or withdrawal of blood and for continuous measurement of arterial blood pressure and regular measurement of blood gases to maintain arterial PaO₂ at 100–120 mmHg, PaCO₂ at 35–45 mmHg and pH at 7.30–7.45. Body temperature was controlled by a rectal temperature probe and a heating pad. Head temperature was controlled with a heating lamp. We recorded bipolar EEG.

Body temperature was maintained at either 37 °C (normothermia) or 33 °C (hypothermia) by ice pads or a heating lamp. We induced ischaemia by reducing mean arterial blood pressure to 50 mmHg by withdrawal of blood and by clamping of both common carotid arteries; ischaemia was timed from the onset of an isoelectric EEG (Smith et al., 1984a). 10 min later we removed the arterial clamps and replaced the withdrawn blood along with 0.5 ml of 0.6 M sodium bicarbonate. In sham-operated control animals all procedures were performed except lowering of blood pressure and clamping of the carotids. We normalized body temperature using heating lamps and pads. Rats resumed spontaneously respiration after 20 min and were extubated and returned to their cage. Where appropriate, animals received s.c. injections of either spironolactone (100 mg/kg; n = 11) or vehicle (propylene glycol; n = 12) 1 h prior to ischaemia and 24 and 48 h after induction of hypothermic ischaemia.

Seven days later, rats were anaesthetised as before and their brain’s perfusion-fixed with 4% formalin. The paraffin-embedded brains were sectioned in 5 μm sections from Bregma –3.80 mm. Neuronal death was quantified as previously described (Smith et al., 1984b); one transverse section at the mid-hippocampal level was selected per animal and counted without knowledge of treatment allocation. Under these conditions, final neuronal necrosis is fully developed by 7 days while resorption of necrotic neurons is not yet extensive (Auer et al., 1984a, b; Smith et al., 1984a); this provides a reliable method of quantifying neuronal death similar to methods involving counting of live and dead cells (Drake et al., 1996). There are approximately 1000 pyramidal neurons visible at this level under control conditions, and 10 min normothermic transient global ischaemia leads to the death of 96% of these (Smith et al., 1984b).

In situ hybridization

In parallel experiments normothermic (37 °C) or hypothermic (33 °C) global cerebral ischaemia, and normothermic or hypothermic sham operations (n = 3 per group), were performed as described above except that spironolactone or vehicle was not given; sham-operated animals underwent all procedures except lowering of blood pressure and carotid clamping. 24 h after induction of ischaemia animals were decapitated and brains snap frozen. We thaw-mounted coronal brain sections (17.5 μm) onto chrome alum-treated slides. cRNA antisense probes were synthesized and labelled with [35S]-UTP by in vitro transcription as previously described (Bjartmar et al., 2000). We hybridized paraformaldehyde fixed and acetylated slides to MR (50 °C) or GR (55 °C) in a hybridization mixture of denatured riboprobe (3.3 MR or 3.5 GR × 10⁶ c.p.m.), 50% formamide, 0.6 M NaCl, 20 mM Tris-Cl pH 7.5, Denhardt’s solution, 1 mM EDTA, 0.2 mg/mL salmon sperm DNA, 10% dextran sulphate, 0.5 mg/mL yeast tRNA and 0.1 M DTT. Slides were washed with standard saline citrate (SSC), 0.01% SDS at 60 °C before incubation with RNase A, further washing and dehydration. After dipping in NTB2 photomulsion and exposure sections were counter-stained with Pyronin Y. We counted silver grains per neuron (counts per neuron) using the MCID M4 analysis system (Imaging Research Inc. Ontario, Canada).

Table 1. Effect of transient global ischaemia and hypothermia on expression of MR and GR in rat hippocampus

<table>
<thead>
<tr>
<th></th>
<th>Control (grain counts per neuron)</th>
<th>Ischaemia (grain counts per neuron)</th>
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<tbody>
<tr>
<td></td>
<td>Normothermia</td>
<td>Hypothermia</td>
</tr>
<tr>
<td><strong>MR</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CA1</td>
<td>53.2 ± 2.6</td>
<td>58.6 ± 4.1</td>
</tr>
<tr>
<td>CA2</td>
<td>75.4 ± 3.3</td>
<td>68.4 ± 6.2</td>
</tr>
<tr>
<td>CA3</td>
<td>47 ± 1.2</td>
<td>53.8 ± 3.2</td>
</tr>
<tr>
<td>CA4</td>
<td>44.4 ± 2.7</td>
<td>42.7 ± 4.5</td>
</tr>
<tr>
<td>DG</td>
<td>45.4 ± 0.7</td>
<td>49 ± 3.3</td>
</tr>
<tr>
<td><strong>GR</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CA1</td>
<td>40.2 ± 6.7</td>
<td>48.3 ± 8.6</td>
</tr>
<tr>
<td>CA2</td>
<td>18.6 ± 3.8</td>
<td>26 ± 9.1</td>
</tr>
<tr>
<td>CA3</td>
<td>20.6 ± 2.2</td>
<td>23.8 ± 1.7</td>
</tr>
<tr>
<td>CA4</td>
<td>16.1 ± 2.4</td>
<td>15.3 ± 2</td>
</tr>
<tr>
<td>DG</td>
<td>28.6 ± 4</td>
<td>37.3 ± 3.5</td>
</tr>
</tbody>
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*a = 3 per group (mean ± SEM). One way ANOVA with Tukey’s post hoc test: *P < 0.05 vs. normothermic control; **P < 0.05 v normothermic control and hypothermic ischaemia; ***P < 0.05 vs. normothermic control.

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**Results**

In primary cortical and hippocampal culture of rat fetal neurons we observed two components of MR immunopositivity; in addition to the expected nuclear staining in apparently healthy hippocampal cells, MR immunoreactivity was also seen in small, shrunken cells (cortical cultures shown in Fig. 1a). When these cultures were counterstained with Hoescht 33258 (which labels intact DNA) the strongest MR staining was associated with nuclear changes typical of apoptosis (Fig. 1b) implying increased MR abundance in cells undergoing apoptosis.

To determine whether increased MR is a more general feature of the neuronal response to injury we measured MR expression in cells exposed to a low level of injury. To do this we chose staurosporine, a fungal extract with diverse toxic effects but which, at low concentrations, has previously – paradoxically – been shown to have neuroprotective properties (Hara et al., 1990; Ohno et al., 1991; Chopp et al., 1999). Exposure of hippocampal neurons in primary culture to 10 nM staurosporine for 1 h resulted in a 136% increase in MR mRNA ($n = 3$, $P < 0.05$, Fig. 2a and b). This concentration of staurosporine does not cause cell death even after 24 h exposure. A similar effect was seen in primary cortical cell culture, where within 1 h of exposure to the same concentration of staurosporine there was a 71% increase in MR mRNA levels ($n = 4$, $P < 0.05$, Fig. 2c). The increased MR mRNA was translated, as western analysis showed that MR protein levels in cortical culture increased more than three fold after 4 h exposure to staurosporine ($n = 3$, $P < 0.05$, Fig. 2d and e).

To explore the mechanism of MR induction, we examined the effect of staurosporine on MR expression in primary cortical neuronal cultures following preincubation with the protein synthesis inhibitor cycloheximide. Under these conditions, the increase in MR mRNA with staurosporine was not altered ($n = 3$, Fig. 3a) suggesting new protein synthesis is not required for this effect. An alternative PKC inhibitor, H7, had no effect on MR expression at concentrations of 10 nM to 1 μM at either 1 or 2 h (data not shown), suggesting the effect of staurosporine on MR is not mediated via PKC inhibition. In contrast, preincubation with the MAP kinase inhibitor PD98059 (10 nM) completely abolished the increase in MR expression following staurosporine ($n = 3$, $P < 0.05$, Fig. 3b).

Next, we examined the effect of these changes on neuronal survival. In this primary cortical culture there is a spontaneous loss of neuronal viability between the 14 and 15 d.i.v. which reflects the spontaneous...
apoptosis demonstrated in Fig. 1. Using the MTS assay we quantify that loss of viability to be 7.0 ± 2.4% (n = 4; mean ± s.e.m.). We have tested the effect of the same low concentration of staurosporine on neuronal viability during this period in the presence of 130 nM corticosterone to ensure full occupancy of expressed MR. Staurosporine increased neuronal viability at 24 h (n = 6, P < 0.01, Fig. 4) equivalent to reduction in the anticipated decline in neuronal viability of around 45%.

To determine the role of increased MR expression in mediating this neuroprotective effect, we co-incubated neurons with spironolactone, a selective antagonist at MR which is without effect on GR signalling (Reul et al., 1990; Marienelli et al., 1998; Murphy et al., 1998). The neuroprotective effect of staurosporine was abolished by spironolactone (1 µM; Fig. 4). Spironolactone alone (at concentrations up to 100 µM) had no effect on neuronal survival.

To determine whether this in vitro effect has any relevance to neuroprotection in cerebral ischaemia in vivo, we examined the expression of MR in transient global ischaemia with and without hypothermia. In this paradigm, hypothermia at the time of the insult affords near total protection to hippocampal pyramidal neurons (Minamisawa et al., 1990). Normothermic transient global ischaemia had no effect on MR mRNA in any hippocampal subfield, but hypothermic transient global ischaemia resulted in increased hippocampal expression of MR selectively in the hippocampal CA3 and CA4 subregions at 24 h (P < 0.05; n = 3; Table 1). At 24 h there was no significant induction of MR in any cortical area, but the background level of expression was very low and a small increase cannot be excluded. Transient global ischaemia was associated with increased GR mRNA expression in every hippocampal region except CA2, and, except in the dentate gyrus, this increase was attenuated by hypothermia (Table 1).

To determine the functional implications of increased hippocampal MR in this paradigm, rats received spironolactone or vehicle prior to induction of ischaemia. Spironolactone had no acute or chronic effect on blood pressure or on any other physiological variable measured. In the hypothermic group few pyknotic dead cells were seen in any hippocampal region at 7 days (Fig. 5a). Animals which received spironolactone had many pyknotic dead cells in CA1 and the subiculum (Fig. 5b). Counting dead neurons from CA1 and the subiculum bilaterally demonstrated that MR inhibition resulted in a 8-fold increase in neuronal death, a significant reversal of the protective effect of hypothermia (n = 11–12; P < 0.05; Fig. 5c).

**Discussion**

We observed striking MR expression in a population of small, shrunken cells in primary culture of hippocampal and cortical neurons. Staining of intact DNA with Hoescht 33258 identified these cells as undergoing apoptosis, consistent with the findings of Ishitani et al. (1996), who reported spontaneous apoptosis in rat cortical culture developing after 15 d.i.v. Whilst MR expression is well documented in hippocampal cells in vitro and in vivo, where it mediates some degree of neuroprotection against apoptosis upon glucocorticoid depletion, in the cortex basal MR expression is much lower (Reul & de Kloet, 1985) and has not previously been associated with the promotion of neuronal survival.

Therefore we wondered whether induction of MR might be part of a more generalized neuronal response to activation of neuronal injury/death pathways which might promote survival. To examine this we used staurosporine, a classical inducer of apoptosis and inhibitor of protein kinase C, which at lower concentrations ‘paradoxically’ fails to cause neuronal death, but is neuroprotective (Hara et al., 1990; Ohno et al., 1991; Chopp et al., 1999). We found that staurosporine caused a rapid increase in MR mRNA and protein in cortical as well as hippocampal neurons in culture. The time course of MR induction was rapid and was not mimicked by the selective PKC inhibitor, H7, suggesting that it represents an alternative pathway activated by staurosporine. Staurosporine has also been reported to increase the activity of a p57 c-Jun NH2 kinase (Yao et al., 1997). Indeed, the effect of staurosporine did not require de novo protein synthesis and was blocked by the MAP kinase inhibitor PD98059, suggesting this pathway is involved in MR induction. To what extent these observations represent properties of staurosporine itself as distinct from a response to the cellular stress it induces is not clear. However, such cellular stresses frequently invoke activation of kinase cascades including the MAP kinases implicated here. Moreover, a more generalized ‘cell stress’ effect is supported both by our observation of increased MR immunopositivity in cells undergoing spontaneous apoptosis and by increased hippocampal MR expression seen following amygdala kindling (Clark et al., 1994).
We have clearly demonstrated that in the presence of corticosterone, the rise in neuronal MR mRNA and protein induced by staurosporine was associated with a promotion of neuronal survival in vitro equivalent to a 45% reduction in the spontaneous decline in neuronal viability normally observed over the same period. This survival-promoting effect was abolished by the selective MR inhibitor spironolactone, suggesting that the staurosporine-induced increase in neuronal MR is responsible, at least in large part, for this effect. So does this matter in vivo? Hypothemic neuroprotection following transient global ischaemia was associated with increased MR gene expression. While the neuroprotective effect of hypothermia has been attributed to effects on cerebral metabolism, other interventions which have more potent effects on cerebral metabolism are less effective neuroprotectants (Nemoto et al., 1996). This suggests that other mechanisms, such as the observed changes in MR expression reported here, may be involved. We have indeed demonstrated that MR mediates neuroprotection in vivo. This appears to be of biological importance, as a significant proportion of the neuroprotective effect of hypothermia was abolished by pretreatment with the MR antagonist spironolactone.

While these effects might have been mediated by possible hypertensive effects of the weak diuretic spironolactone, such effects were not anticipated at the doses used nor were they observed in our experiments. Spironolactone itself is not neurotoxic in vivo at similar doses (McCullers et al., 2002). Although the site of spironolactone action remains unclear, it is tempting to speculate that local MR antagonism in the CNS is important.

Increased MR gene expression was limited to the CA3 and CA4 pyramidal subfields. In this model, delayed cell death, and therefore any neuroprotective effect of hypothermia, occurs almost exclusively in the subiculum and CA1 and CA2 subregions. This dissonance between one population of neurons manifesting increased MR expression and a second population in which the protective effects were observed was not apparent in our in vitro cell culture experiments. Of course, it may be that increased MR expression would have a protective effect in CA3 and CA4 given a more severe insult sufficient to cause damage to these areas. Alternatively, the effects, both in vivo and in vitro, might be explained by a neuroprotective mechanism requiring cell-cell communication. For instance MR signalling may induce the release, either by neurons in culture or by CA3 and CA4 cells projecting to the CA1 and CA2, of some survival factor. McCullers & Herman (1998) have shown that MR blockade leads to reduced basal Bcl2 expression in both CA1 and CA3 and to increased p53 expression in CA3 following kainic acid induced toxicity; increased Bcl2 expression in CA1 might be due to release of a survival factor by neurons originating in CA3 and CA4. Similarly, CA3 and CA4 pyramidal neurons provide a significant excitatory influx to the CA1/2 region; modulation of excitatory inputs to this region by increased MR in CA3 and CA4 might bring about the protective effects seen.

As well as effects on MR expression following transient global ischaemia, hypothermia was also associated with reduced hippocampal GR expression compared with normothermic ischaemic animals. The biological importance of this observation is not clear, but it provides further evidence of a role for modulation of steroid receptors in the response to ischaemia. There is some evidence for autoregulation of steroid receptor expression, and it may be that reduced GR expression is a consequence of increased MR expression. However, the protective effects of staurosporine in vitro and of hypothermia in vivo cannot be explained by alterations in GR signalling alone, because the promotion of survival associated with increased MR expression in both systems was blocked by a specific inhibitor of MR signalling, spironolactone, which is without effect on GR signalling (Marinelli et al., 1998; Murphy et al., 1998). Furthermore, in preliminary experiments the GR antagonist RU38486 was without effect on survival in our cell culture system (data not shown).

MR is almost completely occupied in animal models of stroke because of high levels of endogenous steroids seen as part of the stress response to injury combined with the high affinity of MR for the endogenous steroid corticosterone. This is also true of our cell culture system. Because of the relatively lower affinity of steroids for GR (Reul & de Kloet, 1985), further increases in circulating steroid concentration would have greater impact on potentially neurotoxic GR signalling than on MR signalling. In this context, increased MR expression may have evolved as a mechanism for increasing MR signalling without a concommitant increase in GR signalling.

The mechanism by which increased MR expression protects neurons is not clear. MR activation alters NMDA receptor and calcium-channel subunit gene transcription and hence receptor and channel composition, reducing NMDA receptor activity and neuronal calcium influx (Nair et al., 1998). Importantly, MR signalling also reduces expression of the proapoptotic protein p53 and increases expression of the apoptosis inhibitory protein Bcl2 (McCullers & Herman, 1998). More recently, exposure to levels of corticosterone sufficient to activate MR but not GR have been shown to increase expression of Bcl2 and Bcl Xl in rat hippocampus (Almeida et al., 2000). This could be of substantial importance in reducing delayed (mainly apoptotic) cell death after transient global ischaemia and focal ischaemia. The neuroprotective consequences of MR activation might therefore be due to a combination of direct inhibition of excitotoxic processes and promotion of an apoptosis-resisting internal milieu.

In summary, we have demonstrated, both in vitro and in vivo, that MR expression is increased following neuronal injury and that this has a positive effect on neuronal survival. This establishes the regulation of
MR expression as an important therapeutic target in conditions causing cerebral ischaemia such as cardiac arrest.

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Abbreviations

GR, glucocorticoid receptor; MR, mineralocorticoid receptor.

References


