Chronic Corticosterone Administration Dose-Dependently Modulates Aβ(1-42)- and NMDA-Induced Neurodegeneration in Rat Magnocellular Nucleus Basalis

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Abstract

The impact of glucocorticoids on β-amyloid (Aβ(1-42)) and NMDA-induced neurodegeneration was investigated in vivo. Aβ(1-42) or NMDA was injected into the cholinergic magnocellular nucleus basalis in adrenalectomized (ADX) rats, ADX rats supplemented with 25%, 100%, 2×100% corticosterone pellets, or sham-ADX controls. Aβ(1-42)- or NMDA-induced damage of cholinergic nucleus basalis neurones was assessed by quantitative acetylcholinesterase histochemistry. Plasma concentrations of corticosterone and cholinergic fibre loss after Aβ(1-42) or NMDA injection showed a clear U-shaped dose–response relationship. ADX and subsequent loss of serum corticosterone potentiated both the Aβ(1-42) and NMDA-induced neurodegeneration. ADX + 25% corticosterone resulted in a 10–90 nM plasma corticosterone concentration, which significantly attenuated the Aβ(1-42) and NMDA neurotoxicity. ADX + 100% corticosterone (corticosterone concentrations of 110–270 nM) potently decreased both Aβ(1-42)- and NMDA-induced neurotoxic brain damage. In contrast, high corticosterone concentrations of 310–650 nM potentiated Aβ(1-42)- and NMDA-triggered neurodegeneration. In conclusion, chronic low or high corticosterone concentrations increase the vulnerability of cholinergic cells to neurotoxic insult, while slightly elevated corticosterone levels protect against neurotoxic injury. Enhanced neurotoxicity of NMDA in the presence of high concentrations of specific glucocorticoid receptor agonists suggests that the corticosterone effects are mediated by glucocorticoid receptors.

Both neuroprotective and neurodegenerative effects of glucocorticoids have been reported (4, 8). Lack of glucocorticoids as a result of adrenalectomy (ADX) induces degeneration of dentate gyrus neurones in the hippocampus (9, 10). On the other hand, kainic acid-induced hippocampal damage was shown to be decreased following ADX (11). Increased susceptibility of hippocampal neurones to metabolic or excitotoxic insults was observed after exposure to high concentrations of glucocorticoids (7, 12). Corticosterone can enhance cell death triggered by oxidative stress (4) and exacerbate degeneration of cholinergic nerve fibres after exposure to cholinotoxins (13). Stress-induced chronic elevations of corticosterone have been found to cause dendritic atrophy of rat hippocampal neurones (1–3). Glucocorticoids readily pass the blood–brain barrier and thus have direct access to neural tissue where they bind with high affinity to intracellular mineralocorticoid receptors and with low affinity to glucocorticoid receptors in both neurones and glia, and influence cellular homeostatic mechanisms (1–3). Glucocorticoids are of particular interest since it has become evident that these hormones can modulate neurodegenerative processes that occur in hypoxia and ischemia, stroke, neurotoxic trauma, seizures, ageing and Alzheimer’s disease (4–7).
CA3 cells (14), while chronic exposure to cortisol has been reported to damage CA2 and CA3 neurones in monkey hippocampus (15). Hypercortisolism after stroke (5, 16) and the chronically elevated basal glucocorticoid concentrations in Alzheimer’s disease (17) suggest a role of glucocorticoids in neurodegenerative mechanisms. Moreover, there is convincing evidence that exposure to high glucocorticoid concentrations has profound effects on the viability of nervous tissue during ageing (18). Most studies on the effect of glucocorticoids on nerve cell physiology and neuronal survival have been carried out on the hippocampus or hippocampal cell cultures, and much less is known about glucocorticoid-induced alteration of neuronal viability in other brain regions. In this respect the vulnerability of cholinergic neurones in the basal forebrain to neurodegenerative mechanisms and glucocorticoids is of particular interest, because of the reported loss of this cell group in Alzheimer’s disease (19, 20).

A major neuropathological hallmark of Alzheimer’s disease is the accumulation of amyloid-β peptides (Aβ). Several recent studies reported damage to cholinergic basal forebrain neurones and concomitant memory deficits after in vivo injections of Aβ in the magnocellular nucleus basalis (MBN) of the rat (21, 22). Moreover, evidence was provided that glutamate excitotoxicity, derangement of intracellular calcium concentration ([Ca²⁺]i) and oxidative stress are probably major steps in the neurodegenerative process triggered by Aβ (23–26). In accordance with this concept injections of the glutamate analogue NMDA into the MBN were characteristically followed by cholinergic cell damage and loss of cholinergic fibres in the neocortex that originate from the damaged MBN cells (22, 27).

Here we report the impact of glucocorticoid manipulation on Aβ- and NMDA-induced neuronal damage in the MBN, which mimicks neuropathological aspects of Alzheimer’s disease and stroke, respectively. The Aβ- and NMDA-neurotoxicity was studied in rats that were pretreated by ADX or ADX and simultaneous implants of pellets with different cholesterol-corticosterone concentrations. The effect of pre-treatment with the glucocorticoid receptor agonists dexamethasone and RU28362 were also tested on NMDA-induced neuronal damage.

Materials and methods

Animals
Young adult male Wistar rats weighing 300–350 g were used for all experiments. The rats were group-housed at 24 °C and 55–65% humidity in a light:dark cycle (12 h:12 h) with lights on at 07.30 h. Rats had access to standard rat chow and tap water ad lib. All experimental procedures complied with the NIH Guide to the Care and Use of Laboratory Animals (1985) as well as the guidelines of the Animal Care Committee of the University of Groningen in accordance with the European Community Council Directive 86/609.

Aβ peptide synthesis
Aβ_{1-42} peptide was synthesized with amide at the C-terminal by a solid-phase technique involving Boc chemistry as previously described in detail (28). Briefly, peptide chains were elongated on 4-methylbenzhydrolamine (MBHA) resin (0.6–0.8 mmol/g) and the syntheses were carried out on an ABI 430 A (Biolytic Lab Performance, Inc. Fremont, CA, USA) automated peptide synthesizer. Couplings were performed with dicyclohexylcarbodiimide with the exception of asparagine, which was incorporated in OHB-ester form. The Boc group was removed by treatment with 50% trifluoroacetic acid in CH₂Cl₂. After completion of the synthesis, the peptides were cleaved from the resin with liquid hydrogen fluoride (HF). Free peptides were purified by reverse-phase high performance liquid chromatography (RP-HPLC) on an STS-Si-100S C18 column. The purity was checked by RP-HPLC on a W-Porex SC18S column. Ammonia acid analysis demonstrated the expected amino acid composition and electrospray mass spectrometry (ES-MS) gave the expected molecular ion.

Surgery
All surgery was performed under deep halothane (1.5% v/v; 2 L/min flow) anaesthesia. The adrenalecomies, i.e. incisions, i.v. cannulation, blood sampling and the perfusions were performed between 8 am and 3 pm. Adrenals were removed using a dorsal approach followed by s.c. implantation with corticosterone-cholesterol pellets (crystalline corticosterone and cholesterol, Sigma, St Louis, MO, USA) with an approximate weight of 150 mg. The pellets were divided into five groups: (1) sham-operated control rats (SHAM-ADX) implanted with pellets of 100% corticosterone (n = 24); (2) ADX rats (n = 12); (3) ADX rats with pellets of 75% corticosterone: 25 corticosterone (ADX + 25% CORT; n = 12); (4) ADX rats provided with one 100% corticosterone pellet (ADX + 100% CORT; n = 12); or (5) ADX rats with two 100% corticosterone pellets (ADX + 2 × 100% CORT; n = 12).

After 4 days, rats were anaesthetized with halothane (1.5% v/v; 2 L/min) and mounted in a stereotaxic frame. One μL of 0.2 mmol/μL Aβ or 60 mmol/μL NMDA (Sigma) was slowly injected into the right MBN at co-ordinates relative to Bregma A = −1.5 mm; L = 3.2 mm; and DV = 6.2; ramiun (0.5 μl at both rostems) with a Hamilton microsyring (38) and dissolved in ultrapure water, NMDA in 0.01 M phosphate buffered saline. Both drugs were prepared freshly and aliquots used within 3 h to maintain accuracy of MBN lesions. Half of the SHAM rats were injected with the vehicle alone.

Four SHAM-operated rats after i.c. injections were cannulated as described elsewhere (29) in order to measure their basal corticosterone concentration. Briefly, silicon cannula inserted into the jugular vein were attached to PE 50 tubing through a liquid swivel which allowed the collection of blood samples without disturbing the animals.

Other groups of rats were injected i.p. with 500 μg/kg of one of the glucocorticoid receptor agonists dexamethasone (Organon, n = 6) or RU28362 (Roussel-UCLAF, n = 6) 1 h before the NMDA injection. In this experiment a control NMDA-injected group (n = 6) received an i.p. injection with the vehicle alone.

After all Aβ or NMDA injections the rats survived for 11 days before being killed for histological processing.

Determination of plasma corticosterone concentrations
Before perfusion-fixation, blood samples were taken from the jugular vein cannula of SHAM rats and by cardiac puncture in the rest of the rats. The samples were collected in prechilled tubes containing a final concentration of 0.02% K-EDTA, centrifuged and the plasma stored at −20 °C until hormone measurement. Plasma concentrations of corticosterone were measured by an immunnoassay without extraction as described previously (30). Briefly, the antisera was raised in rabbit against corticosterone-carboxymethylxime-BSA. 125I-labelled corticosterone-carboxymethylxime-tyrosine-methyl ester was used as the tracer. The interference of plasma transcortin was eliminated by inactivating transcortin at low pH. The sensitivity of the assay was 0.1 pmol. Intra- and interassay variations were 6.4% and 23.8%, respectively.

Tissue preparation
The rats were killed by an overdose of sodium pentobarbital injected i.p. The brains were fixed by transcardial perfusion with 300 ml ice-cold fixative composed of 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4) at 27 ml/min, which was preceded by a short pre-rinse of heparinized saline. The brains were removed from the skull and postfixed for 3 h in the same PB, pH 7.4, for 6) or RU28362 (Roussel-UCLAF, n = 6) 1 h before the NMDA injection. In this experiment a control NMDA-injected group (n = 6) received an i.p. injection with the vehicle alone.

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Acetylcholinesterase histochemistry
Sections were stained for acetylcholinesterase (AChE) using a silver nitrate-intensification procedure (31). Briefly, the sections were incubated for 2 h at room temperature (RT) in a sodium-acetate-buffed solution (0.1 M, pH 6),
containing acetylthiocholine iodide (25 mg/50 ml medium), sodium citrate (0.1 M), copper sulphate (0.03 M) and potassium ferricyanide (5 mM). The sections were subsequently reacted in sodium sulphide (1% pH adjusted to 8.6) and silver nitrate (1%) for 2 min each, to reveal black staining for AChE-rich neurones and fibres.

**Quantifications**

Cholinergic neuronal damage after MBN lesioning was assessed by measuring the decrease in cholinergic cortical projection fibre that originate from the MBN neurones. AChE fibre density was quantified in layer V of the posterior somatosensory cortex according to a standard protocol (22) by using a Quantimet Q-600 HR computerized image analysis system (Leica, Rijswijk, the Netherlands). Surface area density of cortical AChE-positive fibres (the area covered by AChE-positive cholinergic fibres) (the total sampling area), given as percentages) was measured in three parietal cortical sections (at co-ordinates −1.3 mm, −1.5 mm and −1.7 mm (32)), representing the densest cholinergic innervation from the damaged MBN division (22). After background substraction and grey-scale threshold determination, the surface area of skeletonized AChE-positive fibres was computed in each parietal cortical section by using a 599-nm emission filter. The relative value of fibre reduction was calculated in pre-established quadrants as the percentage difference between the surface area density at lesioned and contralateral sides of the brain.

**Statistical analysis**

The relationship between AChE fibre loss and corticosterone concentration was determined by nonlinear regression analysis followed by residual analysis. Data of AChE fibre loss were expressed as means ± SEM. Analysis of variance followed by Student-Keuls-Newman comparison was used for group differences (P < 0.05).

**Results**

In the ADX rats the corticosterone concentration at the end of the experiments was in a range of 0–8 nM. Adrenalectomy and 25% corticosterone pellet implantation, and ADX with 100% corticosterone pellet implants resulted in concentrations of plasma corticosterone of 20–90 nM and 110–270 nM, respectively. In the ADX + 2 × 100% CORT rats plasma concentrations of corticosterone reached levels of 310–650 nM. In the cannulated SHAM-ADX rats according to our standard experimental surgery conditions, the basal corticosterone concentrations was in the same range as that of ADX + 25% CORT rats.

SHAM-ADX rats receiving a vehicle injection of ultrapure water or PBS into the MBN showed a limited damage to the MBN, with an average reduction of 3.5 ± 1.2% AChE positive fibres in the ipsilateral somatosensory cortex, similar to previous studies. In the SHAM-ADX control rats, injection of 60 nmol NMDA into the MBN resulted in a profound decrease of 42.1 ± 1.8% of cholinergic fibres, while injection of 0.2 nmol of Aβ caused a AChE fibre loss of 18.42 ± 1.2% (Figs 1A, 2A).

The polynomial regression analysis showed an asymmetrical U-shaped dose-response relationship between the corticosterone concentration and the induced cholinergic fibre loss in the somatosensory cortex after NMDA or Aβ injection in the basalis complex (Figs 1B, 2B). The complete absence or extremely low corticosterone values in the ADX rats was accompanied by a significantly enhanced neurotoxic effect of

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Effect of different plasma corticosterone concentrations on percentages of cholinergic fibre loss in the cortex after unilateral injections of NMDA in the cholinergic magnocellular basal nucleus (MBN). AChE-positive fibres in the neocortex at the lesioned side of the brain measured by image analysis were calculated as percentage of fibre loss compared to the contralateral control side of the brain. (A) Histogram of AChE-positive fibre loss in the different experimental groups expressed as mean values ± SEM. In all cases the NMDA-induced cell damage in the nucleus basalis was preceded by a sham-ADX, ADX or ADX and corticosterone pellet implantation (*P < 0.05; **P < 0.01). (B) Scatterplot of the corticosterone concentrations and the percentages of AChE fibre loss. The equation of the polynomial fitting curve is: y = 71.64 – 0.57x2 + 0.001x2 – 6x1 – 8.9x0 – 12.3x2. The inset displays the direct relation between corticosterone concentration and excitotoxic brain damage. The horizontal dashed line indicates the level of NMDA injury in control animals.

both of the NMDA and the Aβ injections into MBN, in which case cholinergic fibre loss was 67.1±1.7% and 23.5±1.3%, respectively (Figs 1A, 2A, 3A,B, 4A,B). This corresponds to an increase of the excitotoxic damage of 59% and 27% for the NMDA- and the Aβ-induced lesions, respectively.

The increased vulnerability to NMDA neurotoxicity after ADX was clearly prevented by corticosterone replacement in the concentration range of 20–90 nM, since the fibre loss in the ADX-25% CORT group did not significantly differ from the reduction in the SHAM-ADX group (40.9±2.33%, Fig. 1A). However, corticosterone concentrations in the ADX-25% CORT group had a more dramatic protective effect on Aβ toxicity and significantly decreased Aβ-induced AChE fibre loss compared to the Aβ-induced damage in the SHAM-ADX operated rats (8.4±1.65%, Figs 2A, 4A), which represents a neuroprotective effect of 54%. Interestingly, the effects of the ADX-25% CORT group in both the Aβ and NMDA lesion experiments revealed an apparent minor dichotomy. The three lowest corticosterone values (40 and 33 nM in the NMDA and Aβ experiments, respectively) corresponded with an initial reduction of injury, while the three highest corticosterone values (averages of 76 and 81 nM in the NMDA and Aβ experiments, respectively) were followed by a slightly higher degree of injury (arrows in insets in Figs 1n, 2n).

Administration of corticosterone by a 100% corticosterone pellet in the ADX animals, which yielded plasma concentrations of 110–270 nM of corticosterone, potently and significantly attenuated cholinergic neuronal damage after both NMDA and Aβ deposits in the MBN. In these experimental groups the cortical fibre reductions after NMDA or Aβ injections were 18.77±2.9% and 3.87±1.71%, which can be expressed as a neuroprotection of 55% against NMDA lesion, and 79% against Aβ infusion.

This neuroprotective pattern of midrange corticosterone concentrations was reversed by implants of 2×100% pellets. In these experimental conditions with plasma corticosterone concentrations of 310–650 nM the NMDA lesion of the MBN resulted in a loss of 69.5±4.93% cholinergic fibres, which is a significant enhancement of 65% of the NMDA damage in SHAM-ADX rats. The presence of very high corticosterone concentrations evoked a similar effect in the Aβ-induced MBN damage and yielded a loss of cholinergic cortical fibres of 17.11±3.1%. This was in striking contrast to the midrange corticosterone effects but was not different from the degree of injury after Aβ injection in the SHAM-ADX control rats (Fig. 2A).

The i.p. injection of dexamethasone and RU 28362 1 h before the intraparenchymal injection of NMDA in the MBN significantly potentiated the NMDA-induced cholinergic cell damage and concomitant cortical cholinergic fibre loss compared to the values after NMDA and vehicle injection

![Figure 2](https://example.com/figure2.png)

**Fig. 2.** Effect of different plasma corticosterone concentrations on percentages of cholinergic fibre loss in the cortex after unilateral injection of Aβ in the cholinergic magnocellular basal nucleus (MBN). AChE-positive fibres in the neocortex at the lesioned side of the brain were expressed as percentage fibre loss compared to the contralateral control side of the brain. (a) Histogram of AChE-positive fibre loss in the different experimental groups expressed as mean values±SEM. Aβ-induced cell damage in the nucleus basalis of rats pretreated by sham-ADX, ADX or ADX and corticosterone supplement. (***P<0.005; **P<0.01). (b) Scatterplot of the corticosterone concentrations and the percentage of AChE fibre loss. The equation of the polynomial fitting curve is: \( y = 25.4 - 39x + 0.002x^2 - 6x^3 - 9.1x^4 - 11.8x^5 \). The inset displays the direct relation between corticosterone concentration and neuronal damage after Aβ injection. The horizontal dashed line indicates the level of Aβ-induced neuronal injury in control animals.
(39.7 ± 2.0%). The dexamethasone and RU28362 pretreatments resulted in an AChE fibre loss of 61.3 ± 2.9% and 64.3 ± 2.1% (Fig. 5), which is an enhancement of excitotoxic injury of 45% and 52%, respectively.

Discussion
The present data provide in-vivo experimental evidence that corticosterone exerts a powerful effect on Aβ- or NMDA-induced injury to cholinergic neurones of the nucleus basalis complex and their cortical projection fibres. The nature of this effect was dependent on the plasma corticosterone concentration and could vary from enhancement of neurotoxic mechanisms to neuroprotection. Graphically expressed, the dose–response relationship between the plasma corticosterone concentration and neurotoxicity exhibits a ‘U’-shaped curve. It appeared that a very low corticosterone range as in ADX animals potentiated both the Aβ- or the NMDA-induced cholinotoxicity in the MBN, while this potentiation was absent at a basal corticosterone range of 20–90 nM. Moderately elevated corticosterone concentrations of 110–270 nM significantly and strongly reduced excitotoxic injury,
whereas this neuroprotective effect was reversed to an increase of neurotoxic damage evoked by Aβ or NMDA at high corticosterone concentrations in the ‘stress’ level range of 310–650 nM. The finding that such increased damage after NMDA injections was mimicked by application of the glucocorticoid receptor agonists dexamethasone or RU28362 indicates that the corticosterone effects are mediated by glucocorticoid receptors. These data corroborate the findings of Cintra et al. (33) who reported the presence of glucocorticoid receptors in nucleus basalis neurones.

The mechanisms underlying the observed effects of corticosterone on the induced neurotoxicity likely involve a number of physiological and cellular components. This is not only suggested by the impressive impact of the hormone treatment but also by the U-shape of the dose–response relation, which is not uncommon for actions of glucocorticoids on neuronal tissue (34, 35). In general, corticosterone profoundly affects the excitability of nerve cells and ionic conductances (34, 36), regulates neuronal energy supply and metabolism (37, 38), and influences [Ca^{2+}] homeostasis (39–41) all of which...
are essential for neuronal functioning. In the present investigation regarding the impact of corticosterone on experimental neurodegenerative processes, three conditions could be distinguished associated with very low, intermediate and very high corticosterone concentrations that each characteristically influenced neurotoxic processes.

Absence or very low corticosterone levels and neurodegeneration

The present data clearly indicate that absence of corticosterone or very low corticosterone concentrations aggravate experimental neuronal damage after the NMDA or Aβ injections. There is ample evidence for increased sensitivity of neurones in the absence of corticosterone. Previous reports have shown that removal of the adrenal glands results in degenerative changes in the dentate gyrus of the hippocampus (9, 10, 42). The mechanism of neurodegeneration under very low concentrations of corticosterone is unclear but a critical role of [Ca^{2+}] may be implicated. The most prominent effect of ADX appears to be that the voltage-gated Ca^{2+} currents display a very large amplitude (40), with a concurrent small Ca^{2+}-dependent K^+ conductance (18). Furthermore this effect may be chronic since absence of corticosterone increases the relative numbers of L-type Ca^{2+} channels (43). Since NMDA injection increases the Ca^{2+} influx via NMDA receptor channels and secondarily via voltage-gated Ca^{2+} channels (44, 45) this process can synergistically act on the ADX-induced rise of [Ca^{2+}], thereby increasing the probability of neuronal cell death.

This mechanism may also apply to Aβ-induced neurodegeneration in the MBN in the absence of corticosterone. Aβ can potentiate glutamate toxicity (46, 47) by the generation of transmembrane Ca^{2+} fluxes, whereas a role for NMDA receptors was postulated in Aβ-induced Ca^{2+}-entry, as Aβ toxicity was significantly attenuated in the presence of NMDA receptor antagonists (24, 48, 49). Aβ peptides inhibit glutamate uptake by astrocytes (50) and trigger a rise in extracellular glutamate (28) which in the absence of corticosterone can increase Aβ-induced excitotoxicity via elevated [Ca^{2+}], and subsequent neuronal cell death.

Elevated concentrations of corticosterone (20–90 nM and 110–270 nM): possible neuroprotective mechanism

The detrimental effects of ADX on excitotoxic brain damage in the present experimental set-up were effectively compensated by a slight elevation of plasma corticosterone that resulted from 25% corticosterone pellet implantations. In these cases with corticosterone concentrations from 20 to 90 nM the experimental neuronal damage was either equal to or less than the injury level after NMDA or Aβ injections in SHAM-ADX rats, which demonstrates that low concentrations of corticosterone are essential for neuronal survival.

Glucocorticoids are pivotal for optimal neuronal function by their profound influence on ionic membrane conductances, as demonstrated in a number of electrophysiological studies notably of hippocampal cells (18, 34, 35). Low concentrations of corticosterone result in a small voltage-gated Ca^{2+} current and a steady ionic flow through amino acid receptors, mediated by corticosterone effects on mineralocorticoid receptors (36). In contrast, at elevated corticosterone concentrations in a physiological range which activates glucocorticoid receptors, the Ca^{2+} currents are relatively large and the amino acid receptor-mediated responses reduced (36, 39). This larger corticosterone-evoked Ca^{2+} current was demonstrated in hippocampal pyramidal cells to enlarge the afterhyperpolarization, which attenuates the sensitivity of these cells for excitatory amino acid stimulation. Since nucleus basalis neurones are endowed with glucocorticoid receptors, elevated corticosterone concentrations may thus render the MBN neurones less sensitive to excitotoxic input (18, 34, 40). Interestingly, the lower physiological range of corticosterone concentrations significantly attenuated the Aβ-induced neuronal injury. The reason for this is not clear but we anticipate that Aβ toxicity may be influenced not only by corticosterone but also by additional adrenal hormones. In this respect ADX and corticosterone suppletion leads to a sustained steady-state corticosterone concentration and will influence neuronal viability different from the characteristic circadian rhythmicity of basal corticosterone in adrenally intact conditions.

Exposure to high (310–650 nM) glucocorticoid levels: enhanced neurodegeneration

In contrast to the neuroprotective effects of midrange corticosterone concentrations, high levels of corticosterone in ADX rats reversed this process and enhanced the neurotoxicity of Aβ, and in particular of NMDA. A high concentration of glucocorticoids in itself can lead to neurodegeneration and...
cell shrinkage (51, 52). Prolonged elevation of corticosterone was shown to increase the vulnerability of nerve cells to neurodegenerative processes in ageing, cerebral ischemia and possibly other neurodegenerative diseases (6, 7, 15, 35).

The main current hypothesis to explain the high glucocorticoid-induced neuronal degeneration assigns a pivotal role to energy crisis (6) due to inhibition of glucose transporters over the endothelial and neuronal membrane (37, 53) and a rise of \([Ca^{++}]\) which can chronically stimulate degradative enzymes (11, 54) and oxygen radical formation (55). Indeed, very high glucocorticoid concentration induces \(Ca_{++}^{2+}\) influx via voltage gated \(Ca^{++}\) channels (18, 40) and indirectly via elevation of extracellular glutamate level (29, 56, 57). These \(Ca^{++}\) related events may contribute to enhancement of both \(Aβ\)- and NMDA-induced neuronal cell death in the MBN at high corticosterone concentration in our experimental conditions. This is important in view of evidence that excitotoxic mechanisms are attributed essential roles not only in stroke and cerebral ischemia, but also in Alzheimer’s disease (58).

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References
494 Glucocorticoids and neurodegeneration


