Curcumin reverses impaired cognition and neuronal plasticity induced by chronic stress

Ying Xu, Dan Lin, Shan Li, Gaowen Li, Subramaniam G. Shyamala, Philip A. Barish, Matthew M. Vernon, Jianchun Pan, William O. Ogle

J. Crayton Pruitt Family Department of Biomedical Engineering and Evelyn F. & William L. McKnight Brain Institute, University of Florida, Gainesville, FL, 32611, USA
Institute of Experimental Neurobiology, Wenzhou Medical College, Wenzhou, 325021, China

Abstract

Chronic stress occurs in everyday life and induces impaired spatial cognition, neuroendocrine and plasticity abnormalities. A potential therapeutic for these stress related disturbances is curcumin, derived from the curry spice turmeric. Previously we demonstrated that curcumin reversed the chronic stress-induced behavioral deficits in escape from an aversive stimulus, however the mechanism behind its beneficial effects on stress-induced learning defects and associated pathologies are unknown. This study investigated the effects of curcumin on restraint stress-induced spatial learning and memory dysfunction in a water maze task and on measures related neuroendocrine and plasticity changes. The results showed that memory deficits were reversed with curcumin in a dose dependent manner, as were stress-induced increases in serum corticosterone levels. These effects were similar to positive antidepressant imipramine. Additionally, curcumin prevented adverse changes in the dendritic morphology of CA3 pyramidal neurons in the hippocampus, as assessed by the changes in branch points and dendritic length. In primary hippocampal neurons it was shown that curcumin or imipramine protected hippocampal neurons against corticosterone-induced toxicity. Furthermore, the portion of calcium/calmodulin kinase II (CaMKII) that is activated (phosphorylated CaMKII, pCaMKII), and the glutamate receptor sub-type (NMDA2B) expressions were increased in the presence of corticosterone. These effects were also blocked by curcumin or imipramine treatment. Thus, curcumin may be an effective therapeutic for learning and memory disturbances as was seen within these stress models, and its neuroprotective effect was mediated in part by normalizing the corticosterone response, resulting in down-regulating of the pCaMKII and glutamate receptor levels.

Article Info

Article history:
Received 26 February 2009
Received in revised form 9 June 2009
Accepted 10 June 2009

Keywords:
Curcumin
Chronic stress
Learning and memory
Neuronal plasticity
CaMKII
Glutamate receptors

1. Introduction

The major factors involved in the age-related cognitive decline remain to be specified, but there is significant evidence indicating that chronic stress and neuronal vulnerability are interrelated events contributing to age-related pathologies. Clinical studies suggest the glucocorticoids (GCs) contribute to the progression and pathogenesis of Alzheimer's disease (AD) (Csernansky et al., 2006). The 'glucocorticoid cascade hypothesis' (Sapolsky et al., 1986) suggests that GCs initiate a cascade of events in the hippocampus and amygdala that leads to mood (Depression) and cognitive disorders (AD). GCs also modulate neurogenesis, changes in dendritic morphology and the number of synaptic connections (Sousa et al., 2008; Vyas et al., 2002). There is also a defined linkage between longevity and resistance to stress suggesting that aging and stress may affect common cellular mechanisms (Tatar et al., 2003). These observations suggest the need to develop therapeutics to alleviate the chronic stress response in aged individuals.

The learning and memory deficits associated with chronic stress may be alleviated using novel therapeutic strategies involving dietary and medicinal phyto-antioxidants. One such nutraceutical is turmeric which has been used throughout Asia as a food additive and a traditional herbal medicine. Turmeric's pharmacologically active substance is curcumin, the yellow pigment extracted from the rhizoma of Curcuma longa (Nafisi et al., 2009). Curcumin has extensive therapeutic properties, including anti-inflammatory and neuroprotective activities (Motterlini et al., 2000; Thiagarajan and Sharma, 2004). Previous studies in our laboratory have shown that curcumin inhibits the activity of monoamine oxidase (MAO) in different brain regions of mice (Xu et al., 2005a); MAO plays...
a central role in several neurodegenerative disorders, such as AD and depression (Xu et al., 2005b; Hirvonen et al., 2009). Moreover, our results show that the impaired escape from an aversive stimulus seen in rats exposed to chronic unpredictable stress was reversed if the animals were administered curcumin during the stressor period. Indeed stress-associated behavioral deficits were observed together with reduced synaptic proteins and neurotrophic factors expression. Therefore, the restorative effect of curcumin may be related to an increase in brain derived neurotrophic factor, phosphorylation of CAMP response element-binding protein, and maintaining normal levels of neurogenesis (Xu et al., 2006, 2007). Despite numerous studies on the beneficial effects of curcumin in various neurotoxicity models, its therapeutic potential in ameliorating memory and neuroendocrine abnormalities related to chronic stress is not fully understood. Therefore, the present study was designed to investigate the effects of curcumin on the restraint stress-induced spatial cognitive dysfunction and neuroendocrine changes using a water maze task. Moreover, the effects of curcumin on neuronal plasticity were assessed by the morphology of hippocampal neurons in rats exposed to chronic stress. To further examine the underlying mechanisms, the cytoprotective effects of curcumin against corticosterone induced toxicity, and the signal transduction pathway involved in the over expression of one of signaling molecules important for memory formation and neuroplasticity, phosphorylated calcium/calmodulin-dependent protein kinase (pCaMKII) and its downstream target excitatory N-methyl-D-aspartate receptor 2B (NMDA-R2B) were also assessed in primary hippocampal neurons.

2. Materials and methods

2.1. Animals and compound treatment paradigm

Male Sprague-Dawley (SD) rats, weighing between 230 and 250 g at the start of the experiment, were obtained from the Animal Center of Shanghai Branch, Chinese Academy of Sciences. Rats were housed four per cage under standard colony conditions, with a 12-h light/12-h dark cycle and given food and water ad libitum. Pregnant SD rats (18 days pregnant, 500 g) were obtained from the Animal Center of University of Florida and fetal pups were used for in vitro experiments. Experimental procedures were in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978) and the University of Florida Institutional Animal Care and Use of Laboratory Animals (IAUC) guidelines.

Curcumin, imipramine hydrochloride and corticosterone were purchased from Sigma Chemical Co. (St. Louis MO, USA). For oral administration (via gavage, p.o.), fresh curcumin was dissolved in peanut oil and diluted to the desired concentration on the day of the experiment. For intraperitoneal (i.p.) injection, imipramine was dissolved in distilled water. Curcumin (5, 10 and 20 mg/kg, p.o.) and imipramine (10 mg/kg, i.p.) were administered daily for 21 days.

For the in vitro assay, curcumin and corticosterone were dissolved in Dimethyl sulfoxide (DMSO) and ethanol, respectively, and the vehicle concentrations did not exceed 0.1% of the total volume in the experimental solutions. Imipramine was dissolved in Dulbecco’s modified Eagle’s medium (DMEM). Cells pretreated with curcumin (0.3, 0.62, 1.25 and 2.5 mM) or imipramine (5 mM) for 1 h, corticosterone (0.1 mM) was added and incubated for another 24 h before testing.

2.2. Restraint stress

Thirty-nine rats were randomly assigned to six groups (n = 6–8), which were non-stressed control, vehicle-treated, curcumin-treated (5, 10 and 20 mg/kg, p.o.) and imipramine-treated stressed rats. Restraint stress was performed using a rodent restrainer made of plexiglas that closely fit to the rats’ body (Rosenbrock et al., 2005). For chronic restraint stress, rats were put into the restrainers for 6 h everyday, between 9:00 AM and 3:00 PM, for 21 consecutive days. On day 22–24, the stressed rats were tested in the Morris water maze and then sacrificed to assess serum corticosterone levels and the morphology of hippocampal neurons.

2.3. Morris water maze (MWM)

The Morris water maze test was based on the method of Nicholas et al. (2006) with minor modification. The maze consisted of a black circular pool (1.7 m in diameter) and a transparent platform (35 cm diameter, 1.5 cm under the water level) filled with 25–27 °C water. A light source and patterns on the wall surrounding the pool served as extra maze cues. Rats (n = 6–8) were trained in six blocks consisting of three (60 s) trials separated by 20 min inter-block intervals during which the platform remained in the same location relative to the distal cues in the room. On each trial, rats were placed in the water at different start locations (E, S, W and N). One hour following the sixth block, the hidden platform was removed and rats were scored during a 60 s probe trial for latency to reach, and crossings over, the previous platform location (memory recall). Another probe trial was run 24 h after training to assess consolidation and retrieval of memory.

2.4. Measurement of serum corticosterone

Following behavioral testing, rats (n = 6) were sacrificed by decapitation and serum samples were collected to measure corticosterone concentrations on day 23. RIA of corticosterone was performed using [3H]-labeled corticosterone, antiserum and a standard solution in a kit obtained from the China Institute of Atomic Energy (Beijing, China). RIA was performed according to the manufacturer’s instructions. Inter- and intra-assay coefficients of variance were 6.5% and 4.5%, respectively, with a detection limit of 25 ng/ml.

2.5. Rapid Golgi staining

Whole brains were quickly removed and processed according to the directions of a rapid Golgi staining kit (FD NeuroTechnologies, Ellicott city, MD) on day 23–24. Sections (60 µm) were cut through the entire hippocampus (1.72 mm to 6.72 mm from the bregma, Paxinos and Watson, 2005) on a freezing microtome. The staining procedure followed previously established methods that successfully stain hippocampal pyramidal cells (Titus et al., 2007; McLaughlin et al., 2007). Sections were dehydrated in absolute alcohol, cleared in xylene, and coverslipped. Slides were coded during processing and decoded on the completion of analysis. For Golgi analysis, cells were chosen based on the following criteria: the cell body and dendrites were fully impregnated, the cell was relatively isolated from surrounding neurons, and the cell was located in the CA3 region of the hippocampus.

For morphological quantification of hippocampal neurons, 5 pyramidal neurons from each animal (6 rats/group; n = 180 neurons) were analyzed from area CA3 of the dorsal hippocampus (Yas et al., 2002). A camera lucida drawing tube attached to an Olympus microscope BX51 (Olympus, Tokyo, Japan) was used to trace selected neurons (400–500 µm) for subsequent computerized image analysis. Using the center of the soma as reference point, branch points and dendritic length were measured as a function of radial distance from the soma by adding up all the soma to the soma. The total length, the number of branch points, and the total surface area of the soma were calculated using analysis software (ImageJ, NIH).

2.6. Primary hippocampal culture

Pregnant (18 days) SD rats were anesthetized with 1 ml/kg of a mixture consisting of 100 mg/kg ketamine, 10 mg/kg acepromazine, and 100 mg/kg xylazine. The uterus was carefully separated from the abdominal viscera, the fetus was decapitated and its brain dissected. Hippocampi were chopped into fine pieces and cells harvested from a homogenized pool of 10 pup brains. Cells were plated at a density of 2 × 10^5 cells/ml on poly-L-lysine-coated culture plates (Nunc A/S, Roskilde, Denmark). Cultures were maintained in DMEM in a humidified incubator in an atmosphere of 10% CO2 at 37 °C. After 3 d, the DMEM solution was replaced with DMEM containing 1% cytosine arabinoside (ARC). Two days later, the solution was replaced with DMEM and the cells were cultured for an additional 7 d before use.

2.7. Neuronal viability assay

Neuronal cell death was determined by measuring the leakage of cytoplasmic lactate dehydrogenase (LDH) into the medium, according to a previously reported method (Tenenbaum et al., 2007). Briefly, hippocampal neurons were plated in 96-well plates for 12 days. Cells pretreated with curcumin (0.3, 0.62, 1.25 and 2.5 mM) or vehicle (DMSO) for 1 h, corticosterone (0.1 mM) was added and cells were incubated in a density of 2 × 10^3 cells/ml on poly-L-lysine-coated culture plates (Nunc A/S, Roskilde, Denmark). Cultures were maintained in DMEM in a humidified incubator in an atmosphere of 10% CO2 at 37 °C. After 3 d, the DMEM solution was replaced with DMEM containing 1% cytosine arabinoside (ARC). Two days later, the solution was replaced with DMEM and the cells were cultured for an additional 7 d before use.

2.8. Immunoblot analysis

The hippocampal neurons were plated in six-well plates for 12 days. After curcumin or corticosterone treatment, cells were washed two times with cold PBS and lysed on ice with lysis buffer (20 mM Tris, pH 7.4, 140 mM NaCl, 1 mM EDTA, 1 mM sodium vanadate, 20 mM NaF, 2 mM sodium pyrophosphate, 1 mM phenylmethylsulfonyl fluoride, 10 µg/ml leupeptin, and 10 µg/ml aprotinin). Lysates were centrifuged at 1800 g for 10 min at 4 °C and the supernatants were collected. Supernatants containing 20 µg of protein/lane were separated by 10% SDS-PAGE gels. After electrophoresis, the separated proteins were electrophoretically transferred onto polyvinylidene difluoride membranes and were blocked in Tris-buffered saline with 0.1% Tween 20 (TBST) containing 5% non-fat dried milk for 1 h at room temperature.
Subsequently, the membranes were incubated with the appropriate primary antibodies overnight at 4°C (anti-αCaMKII: 1:1000, anti-αCaMKII (ptSer286); 1:1000, anti-Actin: 1:1000). Following three washes with TBS/T, the blots were incubated with the secondary horseradish peroxidase-conjugated antibody (1:100000) at room temperature for 1 h. The blots were washed again for three times by TBS/T buffer and the immunoreactive bands were detected by using the enhanced chemiluminescence method. Densitometer readings were used to quantitate the amount of protein in each treatment situation.

2.9. RNA extraction and real-time reverse transcriptase (RT)-PCR

After curcumin or corticosterone treatment, total cellular RNA was isolated using TriZOL reagent (TriZOL® Invitrogen) according to the manufacturer’s protocol and RNA (1 µg) was reverse transcribed using MJ MiniTA Gradient Thermal Cycler (Bio-Rad, Hercules CA, USA). The PCR reaction was performed using iCycler Real-Time PCR machine (Bio-Rad, Hercules CA, USA). SYBR Green (iQ SYBR Green supermix reagent, Bio-Rad) was added to each sample at a concentration of 50 nmol/L. For each primary culture tested, the reaction was performed with specific primers for β-actin: 5'-GTGAGAGCTCCTTTGCCAAC-3', 5'-TGAAGCAAGCACTGGTCATC-3'. PCR products were amplified in the iCycler real-time PCR machine followed by melt curve analysis and gel electrophoresis to verify specificity and purity of product. All the data were normalized to the housekeeping gene, β-actin.

2.10. Statistical analysis

All data were presented as mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) followed by a LSD test was used for statistical evaluation. Statistical significance was set at p < 0.05.

3. Results

3.1. Curcumin reverses impaired spatial memory under conditions of chronic stress

Restraint stress altered learning of the MWM in rats treated with vehicle as compared to control (non-stress) animals (Fig. 1). But, in the treated animals there was a significant decrease in their mean latency to the platform with increasing dosage of curcumin. In the initial training block there was no significant difference in latency times. There was a significant difference between groups in their latency to reach the platform during the subsequent 5 training blocks (blocks 2–6) [F(5,59) = 3.90, p < 0.001, ANOVA]. Post-hoc analyses show that stressed (stress + vehicle) rats took significantly longer to reach the platform from block 2 to block 6 compared to non-stressed rats (control) [p < 0.01]. This impairment was not present in the stressed rats administered curcumin (stress + curcumin, 10 and 20 mg/kg, p.o.) or imipramine (stress + imipramine, 10 mg/kg, i.p.); the latency time for the curcumin or imipramine-treated rats was significantly shorter than for the vehicle-treated stressed group starting from the second block (p′ < 0.01).

One hour after training, the platform was removed and rats were tested on a probe trial (Fig. 2). Stressed rats had longer latencies to locate the platform location than the non-stressed group (Fig. 2A) [F(5,59) = 4.59, p < 0.01]. This effect was reversed by curcumin (10 and 20 mg/kg) treatment [F(5,59) = 4.59, p < 0.01; p′ < 0.001]. Memory retention for the platform location was tested using a 24 h probe trial. Memory performance was worse in the stressed rats, shown by an increased latency time to find the platform (Fig. 2C). Curcumin (10 and 20 mg/kg) reduced the effects of chronic stress [F(5,59) = 3.20, p′ < 0.01]. Furthermore, as shown in Fig. 2B and D, chronic stress significantly decreased the number of platform crossing in both 1 h and 24 h testing trials as compared to non-stressed rats [F(5,59) = 5.67, p < 0.01; F(5,59) = 2.99, p < 0.05]. While curcumin had an overall enhancing effect on platform crossing at both of the higher doses, 10 and 20 mg/kg, [F(5,59) = 4.35, p′ < 0.01] on the 1h trials (Fig. 2B), and on the 24 hour trials (Fig. 2D) [F(5,59) = 3.13, p′ < 0.05]. These effects are similar to the classical antidepressant imipramine.

Additionally, the influence of chronic stress and chronic stress with drug treatment (curcumin or imipramine) on motor activity (swim speed) showed no significant difference in both the 1 h and 24 h testing (data not shown), suggesting that observed differences in performance were not the result of differences in activity level.

3.2. Curcumin reduces serum corticosterone levels during chronic stress

Rats exposed to the chronic restraint stress exhibited significantly elevated basal serum corticosterone levels (210 ng/ml) indicative of a moderate to high level of stress. As compared to the non-stressed control group (75 ng/ml) [F(5,50) = 8.85, p < 0.001] (Fig. 3). The stress-induced increases in serum corticosterone levels were significantly reduced, although not to baseline levels, in rats treated with curcumin (10 and 20 mg/kg, p.o.) [F(5,50) = 8.85, p < 0.01 and p < 0.001 vs. vehicle-treated, stressed rats] or imipramine [F(5,50) = 8.85, p < 0.001]. But, at the higher dosages (10 and 20 mg/kg) there was no significant difference in serum corticosteroid levels between the unstressed control group and the curcumin-treated chronic stress groups.

3.3. Curcumin prevents hippocampal dendritic remodeling under conditions of chronic stress

Under conditions of chronic restraint stress (6 h/21 d) the morphology of CA3 pyramidal neurons within the hippocampus appeared to be severely affected. This was seen by the qualitative differences in the number of dendritic branches between control (non-stress, Fig. 4A) and stress (stress + vehicle, Fig. 4B). With an increasing concentration of curcumin an increase in the number of dendritic branches was observed (compare Fig. 4B (stress + vehicle) with 4C (5 mg/kg curcumin), 4D (10 mg/kg), and 4E (20 mg/kg)). To get a quantitative measure of the effects of curcumin on dendritic remodeling in the hippocampus rapid Golgi staining was performed and a detailed segmental analysis was performed (see Materials and methods). To determine changes in morphology the number of branch points and dendritic length were determined as a function of radial distance from the cell soma (Fig. 5). The analysis showed that the number of dendritic apical branching points from chronically stressed rats decreased significantly as compared to neurons in the control non-stressed group (Fig. 5A). This can be seen comparing the segments from 150 µm [F(5,180) = 3.24, p < 0.01], 200 µm [F(5,180) = 14.92, p < 0.001], 250 µm [F(5,180) = 25.85, p < 0.001] to 300 µm [F(5,180) = 6.33, p < 0.001], all
statistical values were between control (non-stress) and stress + vehicle group. The apical dendritic length in the stress + vehicle group was also significantly different at 150 µm \[F(5,180) = 26.25, p < 0.001\], 200 µm \[F(5,180) = 23.50, p < 0.001\] and 250 µm \[F(5,180) = 13.69, p < 0.01\] as compared to the non-stressed control group (Fig. 5B). The atrophy of the basal dendrites, again comparing non-stress control with stress + vehicle group was as pronounced as that observed in the apical dendrites. The basal branch points were significantly decreased at 100 µm \[F(5,180) = 9.90, p < 0.001\], 150 µm \[F(5,180) = 7.10, p < 0.001\] and 200 µm \[F(5,180) = 7.75, p < 0.001\] in the chronically stressed rats (Fig. 5C). The basal dendritic lengths were decreased by 34%, 13% and 23% respectively at the segments measured at 100 µm \[F(5,180) = 93.61, p < 0.001\], 150 µm \[F(5,180) = 21.60, p < 0.01\], and 200 µm \[F(5,180) = 18.84, p < 0.01\], in the stressed versus non-stressed group (Fig. 5D).

The morphological changes within the CA3 neurons seen during chronic stress were apparent in rats treated with curcumin (5, 10 and 20 mg/kg) or imipramine (10 mg/kg). The number of apical dendritic branch points were greater at the dosages of 10 and 20 mg/kg curcumin as measured at the radial distance of 200 µm \[F(5,180) = 14.92, p's < 0.001\] vs. vehicle-treated, stressed group, 250 µm \[F(5,180) = 25.85, p < 0.01; p < 0.001\] and 300 µm \[F(5,180) = 6.32, p < 0.05; p < 0.001\] from the soma. There was also a significant different seen at the distance of 200 µm in the 5 mg/kg curcumin treatment group \(F(5,180) = 14.92, p < 0.01\); Fig. 5A). The length of the apical dendrites increased from 20% to 40% at segments 150, 200 and 250 µm, with the increasing dosage of curcumin (Fig. 5B). Similarly, imipramine (10 mg/kg) treatment also showed an increase in apical dendritic branch points \(p's < 0.001\) and dendritic length \(p's < 0.01\); Fig. 5A). In addition, curcumin

---

**Fig. 2.** Latency to reach the platform and the number of platform crossings during the 1 h (A, B) and 24 h (C, D) probe trials of the water maze after 21 days of treatment with curcumin or imipramine (mean ± SEM, n = 6–8). *p < 0.05 and **p < 0.01 vs. non-stressed control group. *p < 0.05, **p < 0.01 and ***p < 0.001 vs. the vehicle-treated stressed group.

**Fig. 3.** Effect of curcumin on serum corticosterone levels in restraint-stressed rats. Each column represents the mean ± SEM of 6 rats. ****p < 0.001 vs. non-stressed control group. ***p < 0.01 and **p < 0.001 vs. the vehicle-treated stressed group.
at 10 or 20 mg/kg increased the number of basal branch points at the radial distance of 100 μm \([F(5,180) = 9.90, p < 0.05; p < 0.01]\), 150 μm \([F(5,180) = 7.10, p < 0.001]\) and 200 μm \([F(5,180) = 7.77, p < 0.001]\) (Fig. 5C). The basal dendritic length also increased from 12% to 43% at segments 100, 150 and 200 μm when curcumin was administered at 10 or 20 mg/kg (Fig. 5D). These effects of curcumin were similar to those observed for imipramine. Overall, this data shows that with increasing dosages of curcumin there are more dendritic branching points and increased dendritic length under conditions of chronic stress.

### 3.4. Curcumin blocks corticosterone-induced toxicity in primary hippocampal neurons

Corticosterone activation of the glucocorticoid receptors at high concentrations is known to initiate a molecular cascade that causes significant neuronal cell death (Crochemore et al., 2005). The ability of curcumin to block the induction of cell death by corticosterone was tested in primary hippocampal cells. Cultured hippocampal neurons were treated with 0.1 mM corticosterone or vehicle and a LDH cytotoxicity assay was performed to quantitatively measure the cell death and cell lysis. Comparative analysis of the corticosterone-treated group (vehicle + corticosterone) versus the control (vehicle-treated group) showed a marked increase in OD (490 nm) values \([F(6,36) = 11.73, p < 0.001, \text{Fig. 6}]\), showing a loss in the total number of cultured neurons. Curcumin (0.62, 1.25 and 2.5 μM) or imipramine (5 μM) significantly decreased neuronal loss as measured by LDH release, indicating that it could protect cells from corticosterone-induced toxicity \([F(6,36) = 11.73, p's < 0.01 \text{ vs. corticosterone-treated group}]\). This data suggest the curcumin can effectively block corticosterone induced neuron death in primary hippocampal cells.

### 3.5. Corticosterone induced phosphorylation of CaMKII is blocked by curcumin in primary hippocampal neurons

Autophosphorylation of CaMKII at threonine 286 switches the kinase from Ca\(^{2+}\)-dependent to Ca\(^{2+}\)-independent activity (Miller and Kennedy, 1986). This activation is thought to promote memory formation and in particular to increase place spatial information (Cacucci et al., 2007). In contrast to these observations autophosphorylation of CaMKII also occurs during restraint stress (Suenaga et al., 2004) and after traumatic brain injury (Atkins et al., 2006) in hippocampal neurons. The amount of pCaMKII between the corticosterone-treated neurons and vehicle-treated controls was seen to be significantly different \([F(6,36) = 19.14, p < 0.001, \text{Fig. 7A}]\). The increase in the amount of phosphorylated CaMKII (pCaMKII) was significantly decreased at the three higher corticosterone concentrations (0.62, 1.25 and 2.5 μM) or imipramine (5 μM) respectively with 1h exposure \([F(6,36) = 19.14, p's < 0.01; p < 0.001 \text{ vs. vehicle-treated corticosterone group}]\). Total CaMKII protein levels in the hippocampal neurons of vehicle-treated group without corticosterone and corticosterone-treated groups did not significantly differ \([F(6,36) = 0.24, p > 0.05]\) (Fig. 7B).

### 3.6. Curcumin prevents corticosterone induced increase in NMDA-R\(_{2B}\) mRNA expression in primary hippocampal neurons

Corticosteroids are known to increase the mRNA levels of the NMDA-R\(_{2B}\) within the hippocampus (Nair et al., 1998). Due to the dynamic interactions between the NMDA-R\(_{2B}\) and CaMKII (Colbran, 2004), we decided to determine if curcumin had an effect on NMDA-R\(_{2B}\) mRNA levels. NMDA-R\(_{2B}\) mRNA levels were measured in primary hippocampal neurons in the presence and absence of 0.1 mM corticosterone. The mRNA levels were also measured in the presence of increasing concentrations of curcumin or imipramine (Fig. 8). NMDA-R\(_{2B}\) mRNA levels were increased almost 3-fold in hippocampal neurons following exposure to 0.1 mM corticosterone for 24 h \([F(6,36) = 2.36, p < 0.01 \text{ vs. vehicle-treated group with corticosterone; Fig. 8}]\). This increase in mRNA levels was prevented by treating the cells with curcumin for 1 h prior to corticosterone exposure at curcumin concentrations of 0.62, 1.25 and 2.5 μM \([F(6,36) = 2.36, p's < 0.05; p < 0.01 \text{ vs. vehicle-treated corticosterone group}]\), respectively. This result shows that curcumin can prevent corticosterone induced increase in NMDA-R\(_{2B}\) mRNA expression in primary hippocampal neurons. The NMDA-R\(_{2B}\) mRNA levels were also decreased after imipramine treatment in the presence of corticosterone \([F(6,36) = 2.36, p < 0.05]\).

### 4. Discussion

The present study demonstrates that, in an animal model, treatment with curcumin can reverse impaired cognition induced
by chronic restraint stress. In addition to the maintenance of cognitive function, a normalizing of blood serum corticosterone levels, and a reversal of neuronal dendritic retraction within pyramidal cells of the hippocampus were also seen in animals that were treated with curcumin. Curcumin was also seen to exert a neuroprotective effect against chronic stress-induced neuronal death in primary hippocampal neurons. In addition, curcumin was demonstrated to block stress-induced phosphorylation of CaMKII along with the concurrent up-regulation of the (NMDA-R2a) glutamate receptor.

Stress is an unavoidable life experience that can disturb cognitive processes and neuroplasticity. In the present study rats were subjected to chronic restraint stress, which may emulate the psychosocial stress that humans encounter in daily life, for 21 consecutive days and then treated with vehicle or curcumin. In the MWM, the untreated stressed rats had significantly longer than normal escape latencies to reach the platform and showed a deficit in spatial learning. In the chronically stressed group that received curcumin (10 and 20 mg/kg) the animals learned faster and had shorter escape latencies than the vehicle-treated stress group. Moreover, the stressed rats without curcumin treatment performed poorly in subsequent testing compared to non-stressed rats on probe trials 1 h after acquisition and 24 h later, indicating impaired memory recall and retrieval, respectively. Curcumin (10 and 20 mg/kg) prevented these behavioral abnormalities and restored both memory measures (spatial memory and learning) in chronically stressed rats. The similar effect was seen by chronic treatment with the positive drug imipramine. Our preliminary data showed that 21 days after chronic stress the plasma concentration of curcumin was 55–120 ng/ml with 10 and 20 mg/kg curcumin administration. Curcumin is highly lipophilic and should have no trouble entering the central nervous system. It exhibits activity against neurologic diseases, such as AD and depression with an optimum doses of 2.5–25 mg/kg body weight in mice (Lim et al., 2001; Xu et al., 2005a). Therefore, the present results support and confirm our hypothesis that curcumin could ameliorate spatial learning and memory dysfunction induced by chronic stress.
A limbic-HPA axis feedback dysfunction has been reported both in chronically stressed rats and AD patients (Landfield et al., 2007). A central feature of the limbic-HPA stress response is the synthesis and the secretion of glucocorticoids (cortisol in primates and corticosterone in rats) from the adrenal cortex. Consequently we measured the blood serum corticosterone levels in our stressed animal in order to quantifying the degree of stress the animals were experiencing. It was seen that restraint stress significantly increased corticosterone levels in their blood to moderate or high levels as compared to the non-stressed group. In the animals treated with curcumin blood serum levels of corticosterone were reduced to normal or non-stress levels. These results are consistent with our previous finding showing that increased corticosterone induced by chronic unpredictable stress can be prevented by chronic curcumin administration (Xu et al., 2006).

The dendritic abnormalities seen in the hippocampus during chronic stress and in patients with AD and depression supports the interpretation that changes in hippocampal circuitry are crucial for understanding of disorders involving dementia (Sapolsky et al., 1986). The hippocampus as a stress sensitive structure is critical in its role controlling the limbic-HPA axis and memory operations, including the formation of stable declarative (or explicit) memory in humans and spatial (or relational/contextual) memory in rodents. The role of stress, it’s impact on the hippocampus and the role that they play in the progression of depression and AD has recently received increasing attention. In the rat hippocampus, chronic stress and high concentrations of corticosterone adversely affect neuronal metabolism, cell survival, and dendritic morphology and functions (McEwen, 2000). The CA3 region is among the first hippocampal areas to show dendritic restructuring, involving a reversible shortening and debranching of dendrites, after restraint stress (Vyas et al., 2002). The current results showed that CA3 neurons are vulnerable to restraint stress (21 days) as evidenced by decreased apical and basal dendritic branching points and length. These abnormalities in CA3 morphology were prevented in rats treated with the 2 highest doses of curcumin during stress (10 and 20 mg/kg). Since changes in hippocampal neurons are closely linked to behavioral abnormalities in the water maze, curcumin may have exerted its beneficial effects on memory via restorative actions on dendritic restructuring in response to restraint stress.

Chronic stress has been linked to memory and other neurological disorders, however, little is known about the onset or development of memory impairment due to chronic stress and the therapeutic potential of curcumin. We hypothesize that oxidative damage to important brain regions after stress may be a result of corticosterone exposure, since excess corticosterone was detrimental to neuronal survival (Vyas et al., 2002). The reductionist power afforded by in vitro systems led to the decision to chose in vitro hippocampal cultures to study whether the endangerment of corticosterone to cultured neurons is directly related to oxidative stress and downstream receptor expression. We used the concentration of corticosterone in vitro (0.1 mM) which is equivalent to moderate to high stress levels in vivo (Sapolsky et al., 1995). The results confirm this hypothesis that corticosterone was neurotoxic to primary hippocampal neurons (Crochemore et al., 2005). Low concentrations of curcumin were used (0.62, 1.25 and 2.5 μM) and interpretation that changes in hippocampal circuitry are crucial for understanding of disorders involving dementia (Sapolsky et al., 1986). The hippocampus as a stress sensitive structure is critical in its role controlling the limbic-HPA axis and memory operations, including the formation of stable declarative (or explicit) memory in humans and spatial (or relational/contextual) memory in rodents. The role of stress, it’s impact on the hippocampus and the role that they play in the progression of depression and AD has recently received increasing attention. In the rat hippocampus, chronic stress and high concentrations of corticosterone adversely affect neuronal metabolism, cell survival, and dendritic morphology and functions (McEwen, 2000). The CA3 region is among the first hippocampal areas to show dendritic restructuring, involving a reversible shortening and debranching of dendrites, after restraint stress (Vyas et al., 2002). The current results showed that CA3 neurons are vulnerable to restraint stress (21 days) as evidenced by decreased apical and basal dendritic branching points and length. These abnormalities in CA3 morphology were prevented in rats treated with the 2 highest doses of curcumin during stress (10 and 20 mg/kg). Since changes in hippocampal neurons are closely linked to behavioral abnormalities in the water maze, curcumin may have exerted its beneficial effects on memory via restorative actions on dendritic restructuring in response to restraint stress.

Chronic stress has been linked to memory and other neurological disorders, however, little is known about the onset or development of memory impairment due to chronic stress and the therapeutic potential of curcumin. We hypothesize that oxidative damage to important brain regions after stress may be a result of corticosterone exposure, since excess corticosterone was detrimental to neuronal survival (Vyas et al., 2002). The reductionist power afforded by in vitro systems led to the decision to chose in vitro hippocampal cultures to study whether the endangerment of corticosterone to cultured neurons is directly related to oxidative stress and downstream receptor expression. We used the concentration of corticosterone in vitro (0.1 mM) which is equivalent to moderate to high stress levels in vivo (Sapolsky et al., 1995). The results confirm this hypothesis that corticosterone was neurotoxic to primary hippocampal neurons (Crochemore et al., 2005). Low concentrations of curcumin were used (0.62, 1.25 and 2.5 μM) and interpretation that changes in hippocampal circuitry are crucial for understanding of disorders involving dementia (Sapolsky et al., 1986). The hippocampus as a stress sensitive structure is critical in its role controlling the limbic-HPA axis and memory operations, including the formation of stable declarative (or explicit) memory in humans and spatial (or relational/contextual) memory in rodents. The role of stress, it’s impact on the hippocampus and the role that they play in the progression of depression and AD has recently received increasing attention. In the rat hippocampus, chronic stress and high concentrations of corticosterone adversely affect neuronal metabolism, cell survival, and dendritic morphology and functions (McEwen, 2000). The CA3 region is among the first hippocampal areas to show dendritic restructuring, involving a reversible shortening and debranching of dendrites, after restraint stress (Vyas et al., 2002). The current results showed that CA3 neurons are vulnerable to restraint stress (21 days) as evidenced by decreased apical and basal dendritic branching points and length. These abnormalities in CA3 morphology were prevented in rats treated with the 2 highest doses of curcumin during stress (10 and 20 mg/kg). Since changes in hippocampal neurons are closely linked to behavioral abnormalities in the water maze, curcumin may have exerted its beneficial effects on memory via restorative actions on dendritic restructuring in response to restraint stress.

Chronic stress has been linked to memory and other neurological disorders, however, little is known about the onset or development of memory impairment due to chronic stress and the therapeutic potential of curcumin. We hypothesize that oxidative damage to important brain regions after stress may be a result of corticosterone exposure, since excess corticosterone was detrimental to neuronal survival (Vyas et al., 2002). The reductionist power afforded by in vitro systems led to the decision to chose in vitro hippocampal cultures to study whether the endangerment of corticosterone to cultured neurons is directly related to oxidative stress and downstream receptor expression. We used the concentration of corticosterone in vitro (0.1 mM) which is equivalent to moderate to high stress levels in vivo (Sapolsky et al., 1995). The results confirm this hypothesis that corticosterone was neurotoxic to primary hippocampal neurons (Crochemore et al., 2005). Low concentrations of curcumin were used (0.62, 1.25 and 2.5 μM) and interpretation that changes in hippocampal circuitry are crucial for understanding of disorders involving dementia (Sapolsky et al., 1986). The hippocampus as a stress sensitive structure is critical in its role controlling the limbic-HPA axis and memory operations, including the formation of stable declarative (or explicit) memory in humans and spatial (or relational/contextual) memory in rodents. The role of stress, it’s impact on the hippocampus and the role that they play in the progression of depression and AD has recently received increasing attention. In the rat hippocampus, chronic stress and high concentrations of corticosterone adversely affect neuronal metabolism, cell survival, and dendritic morphology and functions (McEwen, 2000). The CA3 region is among the first hippocampal areas to show dendritic restructuring, involving a reversible shortening and debranching of dendrites, after restraint stress (Vyas et al., 2002). The current results showed that CA3 neurons are vulnerable to restraint stress (21 days) as evidenced by decreased apical and basal dendritic branching points and length. These abnormalities in CA3 morphology were prevented in rats treated with the 2 highest doses of curcumin during stress (10 and 20 mg/kg). Since changes in hippocampal neurons are closely linked to behavioral abnormalities in the water maze, curcumin may have exerted its beneficial effects on memory via restorative actions on dendritic restructuring in response to restraint stress.
were shown to significantly prevent the corticosterone-induced deleterious effects notably neuronal death. Studies on the toxicity have revealed the biphasic behavior of curcumin, which are concentration dependent. Lower concentrations of curcumin (up to 1 µM for 24 h) protect against cell death, but higher concentrations of curcumin (more than 10 µM) induce cell apoptosis in vitro (Salvioni et al., 2007; Ringman et al., 2005). These results agree with the present in vivo studies that suggest that small doses of curcumin are needed to produce the neuroprotective effect.

The -calcium/calmodulin-dependent kinase II (CaMKII) is intricately involved in memory formation and synaptic plasticity in the hippocampus (Fukunaga et al., 1992; Colbran, 2004). The phosphorylation of CaMKII at Thr286 switches the kinase into an active biochemical state required for synaptic plasticity and learning, including spatial learning (Irvine et al., 2006). However, CaMKII over phosphorylation may produce some degree of neurotoxicity to the cells and alter some biochemical pathways involved in memory processing (Cao et al., 2008). It should be noted that mice that expressed a constitutively active CaMKII lacked low frequency LTP and were not able to form stable place cells within the hippocampus (Rotenberg et al., 1996). The present molecular experiments suggested that the pCaMKII levels in hippocampal neurons were significantly increased in response to corticosterone exposure, though no changes were found in total CaMKII levels. These results were similar to the changes elicited by the immobilization stress, which was previously reported (Suenaga et al., 1998).

The phosphorylation of CaMKII at Thr286 reverses by curcumin administration (0.62, 1.25 and 2.5 mM), a finding that has not been previously reported. The phosphorylation of CaMKII at Thr286 switches the kinase into an active biochemical state required for synaptic plasticity and learning, including spatial learning (Irvine et al., 2006). However, CaMKII over phosphorylation may produce some degree of neurotoxicity to the cells and alter some biochemical pathways involved in memory processing (Cao et al., 2008). It should be noted that mice that expressed a constitutively active CaMKII lacked low frequency LTP and were not able to form stable place cells within the hippocampus (Rotenberg et al., 1996). The present molecular experiments suggested that the pCaMKII levels in hippocampal neurons were significantly increased in response to corticosterone exposure, though no changes were found in total CaMKII levels. These results were similar to the changes elicited by the immobilization stress, which was previously reported (Suenaga et al., 2004). This elevation of pCaMKII was reversed by curcumin administration (0.62, 1.25 and 2.5 mM), a finding that has not been previously reported.

NMAD-R2B has been previously proposed to be an important postsynaptic density docking site for CaMKII (Leonard et al., 1999). Phosphorylated CaMKII translocates to the postsynaptic density where the enzyme binds to the NMDA receptor, particularly to the NMDA-R2B subunit and activates different postsynaptic proteins implicated in memory formation (Moyano et al., 2005). In addition to the pCaMKII level changes, our preliminary study using gene expression microarray analysis suggested that some glutamate receptors, including NMAD-R2B sub-receptor, were also altered in the presence of increased corticosterone levels after chronic stress (W.O. Ogle and K.A. Goosens unpublished work). The present in vitro study shows that stress levels of corticosterone increased NMAD-R2B mRNA by 181% in hippocampal neurons relative to the normal control. Curcumin decreased NMAD-R2B levels when introduced to neuronal cells 1h before corticosterone treatment. It is hypothesized that down-regulation of NMAD-R2B after corticosterone treatment may suppress the induction of over-phospho-CaMKII in response to corticosterone exposure. Thus, curcumin may have ameliorated the cognitive impairment induced by chronic stress, at least in part, via regulating pCaMKII expression and its downstream target NMAD-R2B mRNA level in the hippocampus.

The data presented here suggest that curcumin may be effective in treating cognitive difficulties and the neuronal structural abnormalities that accompany chronic stress. The neuroprotective effect of curcumin is possibly mediated by its modulatory effect on the LHPA axis function and its ability to decrease in pCaMKII and NMAD receptor subunit in hippocampal neurons. Studies are currently being conducted in our laboratory to further investigate the molecular mechanisms underlying curcumin’s neuroprotective effects and to examine whether curcumin regulates the post-receptor signal transduction in chronic stress-induced LHPA dysfunction. Notably it has been seen the curcumin can have an affect on the phosphorylated state of the glucocorticoid receptor (Chen et al., 2008), which suggest that one of the mechanisms of curcumin could be the direct regulation of the GR response. In all, curcumin may be an effective therapeutic for the cognitive dysfunction related to chronic stress and may also be beneficial in other related disorders such as depression and AD.

Acknowledgments

This work was supported by an Ellison Medical Foundation New Scholar Award to William O. Ogle. The authors do not have financial or personal conflicts of interest associated with this work.

References


Cacucci, F., Wills, T.J., Lever, C., Giese, K.P., O’Keefe, J., 2007. Experience-dependent increase in CA1 place cell spatial information, but not spatial reproducibility, is dependent on the autophosphorylation of the alpha-isofrom of the calcium-/calmodulin-dependent protein kinase II. Journal of Neuroscience 27, 7854–7859.


