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Effects of Co-Application of Corticosterone and Growth Hormone on Hippocampal Neurons Involve NMDA Receptor Upregulation of NR2B Protein Expression and Increasing NR2B/NR2A Ratio

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Growth hormone (GH); Corticosterone (CORT); Stress; Field excitatory postsynaptic potentials (FEPSPs); N-methyl-D-aspartate receptors (NMDARs); Hippocampus; Synaptic plasticity;

Abstract

Objectives to investigate the possible mechanism underlying the protective effect of growth hormone (GH) on hippocampal function during periods of acute stress. ***Methods*** the effects of co-application of GH and corticosterone (CORT) at different concentration on field excitatory postsynaptic potential (fFEPSPs) of hippocampal slices of rats at two different age groups were examined. Also, the protein expression of N-methyl-D-aspartate receptor (NMDARs) subunits; NR1, NR2B, and NR2A in hippocampal brain slices treated with artificial cerebrospinal fluid (ACSF) or low concentration of CORT alone or both CORT and GH for three hours were measured. ***Results*** We found an additive effect of co-application of CORT and GH on hippocampal synaptic transmission compared to CORT alone. Furthermore, we found that the combined use of low concentration of GH and CORT have significantly higher effects on enhancement of fFEPSPs in old rats compared to young ones. We showed that both GH and CORT enhanced protein expression of NR2A subunit of NMDARs. Meanwhile, we demonstrated that the coexposure to low concentration of GH and CORT significantly enhanced NR2B expression and increased the NR2B/NR2A. In contrast, perfusion with CORT alone caused significant suppression in NR1 and NR2B protein expression and decrease in NR2B/NR2A. ***Conclusion*** we suggest that NMDARs provide potential target for mediating GH potential protective effect against stress and age related memory and cognitive impairment.

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INTORDUCTION

Stress causes the release of large amounts of glucocorticoids (GCs) that affect the brain, particularly the hippocampus.[1] Corticosteroids released during stress induces memory retrieval impairment that could be abolished through lowering blood GC pool in corticosteroid binding globulin knock-out mice and restored by CORT infusion into the hippocampus.[2] Early exposure of immature hippocampus to traumatic experience is associated with human posttraumatic stress disorder, although there was no memory recalled of the past trauma.[3] Previous studies indicate that GH therapy have a protective effect on cognitive brain function. In earlier work we demonstrated that growth hormone (GH) has beneficial effect on synaptic transmission in CA1 area of rat hippocampus.[4] Aging and repeated stress could damage the hippocampus that is considered as a vulnerable CNS structure.[5] Overexpression of GH reversed stress-induced decrease in both acquisition and long-term storage of fear memory, thus, promoting the ability of hippocampus to combat stress.[6]

We wondered if co-exposure to low concentration of GH and CORT would have any beneficial effect on hippocampal synaptic transmission compared to CORT alone. Therefore, we tested the effects of co-exposure of GH and CORT at different concentration on fFEPSPs in hippocampal slices of rats at two different age groups. NMDA receptor antagonist 3-[(R)-2-carboxypiperazin-4-yl]-propyl-1-phosphonic acid (CPP) impairs both long term potentiation (LTP) and long term depression (LTD) in hippocampal dentate gyrus of rat.[7] We hypothesized that CORT would modulate hippocampal synaptic function through the NMDA receptors. Therefore, we tested the change of protein expression of NMDA receptor subunits; NR1, NR2B, and NR2A in hippocampal brain slices treated with ACSF or low dose of CORT alone or both CORT and GH for three hours.

MATERIALS AND METHODS

Overall classification of the studied groups

Field potential recording

To test if there is any additive effect could be gained by treatment with GH in slices pretreated with CORT; we either perfuse the slices from young adult rats with CORT only for two hours or with GH only for two hours or with CORT for one hour followed by combined CORT + GH at different concentration.

To test if there is any additive effect could be gained by treatment with CORT in slices pretreated with GH; we perfuse the slices from both young adult and old rats with either GH for 2 hours or for 1 hour followed

by combination of GH + CORT for another hour.

Western Blotting

To test for protein expression of NMDA receptor subunits (NR1, NR2A, and NR2B); hippocampal slices obtained from the same animals were separated into 3 interface chambers; the first chamber contained ACSF alone to serve as a control, the second chamber contained ACSF + CORT, while the third chamber contained a combination of ACSF + CORT + GH, each group was incubated for 3 hours.

Slice Preparation for In Vitro Electrophysiology

All experimental procedures were in accordance with the National Institute of Health Guide for the Use and Care of Lab. Animals (NIH Publications No. 80-23) and agreed on by the Institutional Animal care and Use Committee at Marshall University – Joan C. Edwards School of Medicine, Huntington, WV, USA. Hippocampal slices were prepared from two groups of rats 1.5 to 3 month old male Sprague-Dawley rats (considered as young adult), and 15 to 18 month old male Sprague-Dawley rats considered as old rats (Hilltop Laboratory Animals Inc., Scottdale, PA, USA).

Animals were sedated by inhalation of a CO_2/air mixture and decapitated. The skull was opened and the brain was removed and submerged in chilled, oxygenated (95% O_2/5% CO_2), low Ca^{2+}/high Mg^{2+} artificial cerebrospinal fluid (ACSF), pH 7.35 and composed of: 124mM NaCl, 26mM $NaHCO_3$, 1.2mM NaH_2PO_4, 3mM KCl, 0.5mM $CaCl_2$, 5mM $MgSO_4$ and 10mM glucose. While submerged in chilled low Ca^{2+}/high Mg^{2+} ACSF the brain was trimmed to a block containing both hippocampi. The block was glued to the stage of a vibrating microtome (Campden Instruments Ltd., Lafayette, IN, USA), immersed in a bath of chilled, oxygenated, low Ca^{2+}/high Mg^{2+} ACSF, and 400 μm coronal sections were cut. Sections containing the hippocampus in transverse profile were selected and transferred to a small petri dish, where they were further dissected to free the hippocampus from its surrounding tissue. After dissection, hippocampal slices were transferred to a holding chamber, where they were stored for later use. Slices were maintained in a holding chamber at room temperature (20-22 ^oC) at the ACSF/atmosphere (95% O_2/5% CO_2) interface. The holding chamber was filled with standard ACSF, pH 7.35 and composed of 124mM NaCl, 26mM $NaHCO_3$, 3.4mM KCl, 1.2mM NaH_2PO_4, 2mM $CaCl_2$, 2mM $MgSO_4$, and 10mM glucose. Slices were incubated in the

holding chamber for a minimum of one hr prior to use. Slices were withdrawn from the holding chamber as needed and placed in a low volume (approximately 200 µl) interface recording chamber, where they were continuously perfused at a rate of 1-1.5 ml/min with standard ACSF. The recording chamber was kept at a temperature of 25±0.5 °C. A minimum 30 min period was allowed for recovery after transferring slices from the holding to the recording chamber.

To study the interactions of GH and CORT on synaptic transmission, we either perfuse the slices from young adult rats (5 to 6 animals in each group) with GH (0.1, 0.5, 2nM) for two hours or CORT (0.5, 5, 30nM) for two hours or CORT (0.5, 5, 30nM) for one hour followed by CORT (0.5, 5, 30nM) + GH (0.1, 0.5, 2nM). To test if there is any additive effect could be gained by treatment with CORT in slices pretreated with GH, we perfuse the slices from both young adult and old rats (8 animals) with either GH (0.5nM) for 2 hours or for 1 hour followed by GH (0.5nM) + CORT (5nM) for another hour.

Field Potential Recording

Extracellular potential were recorded through low impedance (3-4 MΩ) glass micropipettes filled with standard ACSF and placed into the stratum radiatum of area CA1 of rat hippocampus. Signals were amplified (gain 1000) and filtered (0.1 - 3,000 Hz) using a WPI DAM50 amplifier, then digitized (10 kHz; National Instruments Corporation, Austin, TX, USA) and stored on a personal computer.

Synaptic Stimulation

Postsynaptic potentials were evoked by delivery of constant voltage, stimuli through a bipolar stimulating electrode placed into stratum radiatum. Stimuli were delivered at a 15 sec interval. A 15 min baseline recording in standard ACSF is taken before the application of any drug or tetanus. Postsynaptic potentials evoked in standard ACSF were quantified by measuring the slope of the linear portion of the initial response. Changes in synaptic response caused by drug treatment were expressed as percentage of average baseline response prior to treatment.

Western Blotting

We examined effects of CORT application alone and the possible interaction of CORT and GH on the protein expression of NMDAR subunits'. Rat brain hippocampal tissues were removed from the animals, sliced and allowed to recover for 1 hour by incubating them in vitro in ACSF. After recovery, hippocampal slices obtained from the same animal were separated into 3 interface chambers; the first chamber contained ACSF alone to serve as a control, the second chamber contained ACSF + CORT 5nM, while the third chamber contained a combination of ACSF + CORT 5nM + GH 1nM, each group was incubated

for 3 hours. After the treatments, the three groups of slices were rapidly put on dry ice, frozen and stored at -80°C. The steps for protein isolation from hippocampal brain slices include homogenization in protein lysis buffer [composed of: 1% Nonidet P-40, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 0.15M NaCl, 0.01M sodium phosphate, pH 7.2, 2mM ethylenediamine tetra-acetic acid, and 1% protease inhibitor cocktail]. This was followed by burst sonication (< 10 sec), and hippocampal tissue proteins were centrifuged at a speed of 14,000 × g for 20 min at 4°C. The supernatant solution was obtained and total protein was estimated using the Bradford method.[8] Equal amounts of total protein from CORT (5nM) treated hippocampal slices and control hippocampal slices (200 μg) were separated on 8% polyacrylamide gels. The separated proteins were transferred to nitrocellulose membranes (Micron Separations Inc., Westboro, MA, USA).[9] The membranes were incubated for 1 hr at room temperature in 5% nonfat dry milk in T-T-S (0.5% Tween 20®, 10mM Tris-HCl, pH 8.0, 150mM NaCl, 0.2mM EDTA) to block non-specific binding sites.[10] The membranes were then incubated with primary antibody diluted in 5% non-fat dry milk in T-T-S overnight at 4 °C. To correct for possible loading differences, blots were probed with a primary antibody against neuron-specific enolase (NSE). Next, membranes were washed and incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies, diluted 1:10,000 in 5% non-fat dry milk in T-T-S for 1 hr at room temperature. Blots were washed again and proteins were detected on X-ray film (Fuji Medical System Inc., Stamford, CT, USA) using the ECL system (Amersham-Pharmacia), following the manufacturer's instructions. X-ray autoradiograms were scanned and their images were saved.

Reagents

We used rat corticosterone and recombinant rat growth hormone (GH) (Purchased from, Cell sciences, 480 Neponset Street, Building 12A, Canton, MA 02021). The following primary antibodies were used: anti-NSE (AB951, Purchased from, Chemicon International, Millipore Corporation, Billerica, MA, USA, 1:6000 to 1:8000), anti-NMDA-R1 (60021A, Purchased from, PharMingen International, BD Biosciences 2350 Qume Drive, San Jose, CA 95131-1807, USA, 1:1000), anti-NMDA-R2A (AB1555P, Purchased from, Chemicon, Temecula, CA, USA, 1:500 to 1:2000), and anti-NMDA-R2B (N38120, Purchased from, Transduction Laboratories, 2222 Qume Drive, San Jose, CA 95131-1807, USA, 1:2000 to 1:4000).

Data Analysis

Field potentials data were collected and analyzed using WinWCP program (John Dempster, University of Strathclyde). The

western blotting bands representing different NMDA receptor protein expressions were quantified for their relative densities using the gel analysis densitometry tool of NIH image analysis software – Image J, version 1.31c [a public domain image processing and analysis program using optical density standards developed by Wayne Rasband at the Research Services Branch (RSB) of the National Institutes of Health (NIH) [http://rsb.info.nih.gov/ij/][11, 12,13]. Additional analysis was completed using GraphPad Prism 5 (GraphPad Software Inc., La Jolla, CA, USA) and Origin (OriginLab). All statistics are presented as mean ± one standard error of the mean. Statistical significance was assessed using paired and unpaired t-test, as appropriate; with *P* value < 0.05 is considered significant. Data were compared among groups using two-way ANOVA.

RESULTS

In Vitro Recordings From Rat Hippocampal Brain Slices:

Effect of application of CORT 0.5nM and GH 0.1nM in hippocampal slices pretreated with CORT.

CORT (0.5nM) causes significant enhancement of fEPSPs compared to baseline recording or control slices treated with standard ACSF for one or two hours. Continuous treatment of hippocampal brain slices with CORT (0.5nM) for the second hour causes insignificant change in the enhanced fFEPSP compared to the first hour. Insignificant difference between exposure to GH (0.1nM) for 2 hours and CORT(0.5nM)+GH (0.1nM) for 2 hours was found. Perfusion with GH (0.1nM)+CORT (0.5nM) respectively in the second hour causes significant additional enhancement of fFEPSP that is caused by CORT (0.5nM) alone for one or two hours. (Fig. 1). Significant interaction of GH 0.1nM and CORT 0.5nM was found by two way ANOVA (table 1).

Effect of application of CORT 5nM and GH 0.5nM in hippocampal slices primed with either drug alone.

Treatment with CORT (5nM), or GH (0.5nM), or CORT (5nM) + GH (0.5)nM after priming with GH or CORT alone cause significant enhancement of fFEPSPs compared to baseline recording or control slices treated with standard ACSF for one or two hours. Continuous treatment of hippocampal brain slices with CORT (5nM) for the second hour causes insignificant change in the enhanced fFEPSP compared to the first hour. Continuous treatment of hippocampal brain slices with GH (0.5nM) for the second hour causes moderately significant effect in enhancing fFEPSP compared to the first hour. Co-exposure to GH (0.5nM) and CORT (5nM)

in the second hour causes significant additional enhancement of fFEPSP that is

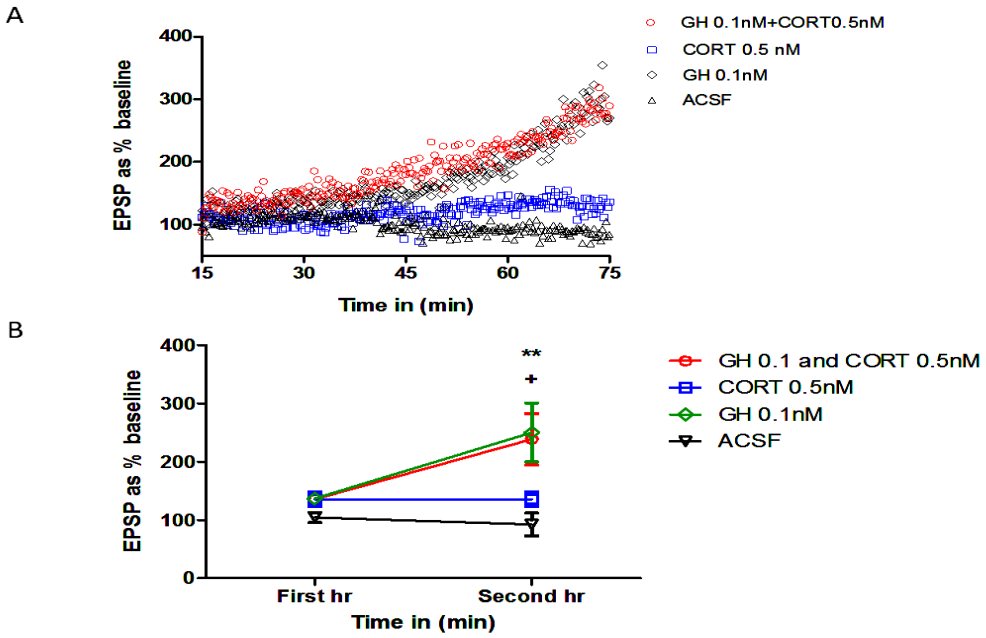

Fig. (1): Effect of co-application of CORT 0.5nM and GH 0.1nM in hippocampal slices pretreated with CORT 0.1nM. (A) Effect of one hour treatment with; ACSF, or CORT 0.5nM, or GH 0.1nM or CORT 0.5nM + GH 0.5 nM. (B) Interaction of CORT 0.5nM and GH 0.1nM. (n=5). (** $P< 0.01$ GH+CORT compared to CORT; $^{+}P< 0.05$ compared to first hour

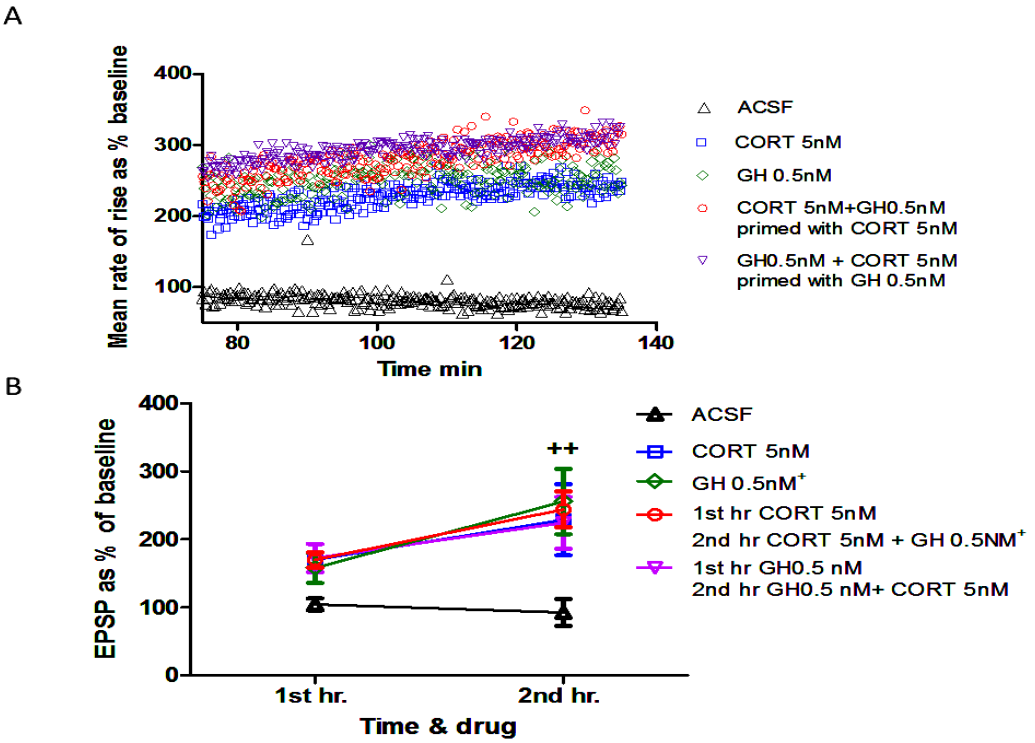

Fig. (2): Effect of co-application of CORT 5nM and GH 0.5nM in hippocampal slices pretreated with CORT or GH alone. (A) One hour treatment with; ACSF, or CORT 5nM, or GH 0.5nM, or CORT 5nM + GH 0.5nM after priming with GH or CORT. (B) Interaction of CORT 5nM and GH 0.5nM. (**$^{++}$** $P< 0.01$ compared to the first hour). (n=5).

Table (1): Two-way analysis of effect of interaction of CORT 0.5nM and GH 0.1nM in hippocampal slices pretreated with CORT 0.1nM.

Source of Variation	DF	Sum of square	Mean square	F	P value
Interaction	3	22946	7649	3.6	**0.037**
Time & drug	1	4075	4075	5.3	**0.036**
Treatment combination	3	63037	21012	6.5	**0.005**
Subjects (matching)	16	79491	4978	1.3	**0.326**
Error	16	47851	2991		
Total	39	217400			

Table (2): Two-way analysis of effect of co-application of CORT 5nM and GH 0.5nM in hippocampal slices pretreated with CORT or GH alone.

Source of Variation	DF	Sum of square	Mean square	F	P value
Interaction	4	16754	4188	1.5	**0.24**
Time & drug	1	36568	36568	13.1	**0.002**
Treatment combination	4	88248	22062	3.9	**0.02**
Subjects (matching)	20	114059	5703	2.05	**0.06**
Error	20	55639	2782		
Total	49	311268			

caused by CORT (0.5nM) alone for one or two hours ($^{++}$ $P< 0.01$). compared to the first hour. Co-exposure to GH (0.5nM) and CORT (5nM) in the second hour causes significant additional enhancement of fFEPSP that is caused by CORT (0.5nM) alone for one or two hours ($^{++}$ $P< 0.01$).

Conversely, Co-exposure to GH (0.5nM) and CORT (5nM) in the second hour causes insignificant additional enhancement of fFEPSP that is caused by GH (0.5nM) alone for one or two hours (Fig. 2). Highly significant interaction of GH 0.5nM and CORT 5nM was found by two way ANOVA (table 2).

Effect of application of CORT 30nM and GH 2nM in hippocampal slices pretreated with CORT.

Treatment with CORT (30nM) causes significant enhancement of fFEPSPs compared to baseline recording or control slices treated with standard ACSF for one or two hours. Continuous treatment of hippocampal brain slices with corticosterone (30nM) for the second hour causes insignificant change in the enhanced fFEPSP compared to the first hour. Insignificant difference between perfusion with GH 2nM for 2 hours and CORT (30nM)+GH (2nM) was found. Perfusion with GH (2nM) + CORT (30nM) in the second hour causes significant additional enhancement of fFEPSP that is caused by CORT (30nM) alone for 1 or 2 hours. Insignificant interaction of GH 2nM and CORT 30nM was found by two way ANOVA (table 3).

Table (3): Two-way analysis of co-application of CORT 30nM and GH 2nM in hippocampal slices pretreated with CORT 30nM.

Source of Variation	DF	Sum of square	Mean square	F	P value
Interaction	3	15523	5174	9.2	**0.0005**
Time & drug	1	10573	10573	18.7	**0.0003**
Treatment combination	3	93786	31262	4.27	**0.02**
Subjects (matching)	20	146424	7321	12.9	**<0.0001**
Error	20	11283	5642		
Total	47	277589			

Interaction of CORT 30nM and GH 2nM in hippocampal slices pretreated with CORT.

Treatment with CORT (30nM) for 2 hours causes significant enhancement of fEPSPs compared to baseline recording. No additional effect of continuous treatment of hippocampal brain slices with corticoterone (30nM) for a second hour.

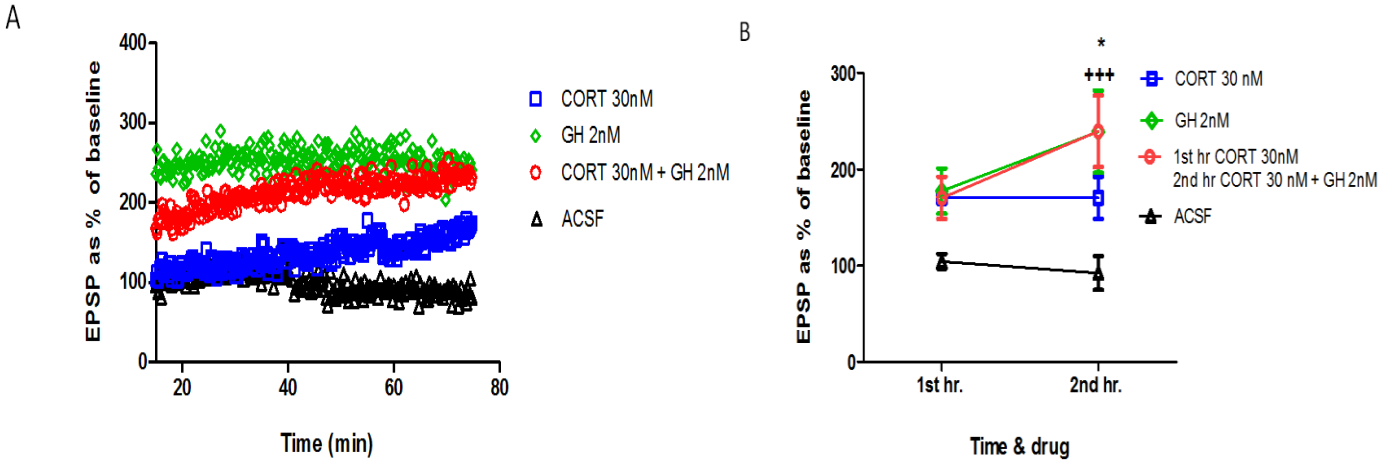

Fig. (3) : Effect of co-application of CORT 30nM and GH 2nM in hippocampal slices pretreated with CORT 30nM. (A) One hour perfusion with ACSF, or CORT 30nM, or GH 2nM, or CORT 30nM + GH 0.5 nM. (B) Interaction of CORT 30nM + GH 2nM. (* P< 0.05 CORT + GH compared to CORT; $^{+++}$ P< 0.005 compared to first hour treatment). (n=6).

Co-exposure to GH (2nM) and CORT (30nM) respectively in the second hour causes significant additional enhancement of fEPSP that is caused by CORT (30nM) alone in the first hour. (Fig. 3).

The effect of application of CORT and GH on the fFEPSP of young and old rats:

To examine if the additive effect of CORT and GH in young adult rat will also be seen in old rats. The additive effect of co-exposure to small dose of CORT 5nM and GH 0.5nM in the second hour on the enhanced fFEPSP by GH 0.5nM alone in the first hour is significantly higher compared to baseline recording, compared to the first hour in old rats only, and in old rats compared to young rats in both first and second hour (Fig. 4). Significant interaction of GH 0.5nM and CORT 5nM in young adult and old rats was found by two way ANOVA (table 4).

Table (4): Two-way analysis of interaction of CORT and GH on the fFEPSP of young and old rats:

Source of Variation	DF	Sum of square	Mean square	F	P value
Interaction	2	48709	24354	3.7	**0.043**
Time & drug	1	72791	72791	10.97	**0.003**
Treatment combination	2	362269	181135	26.05	**<0.0001**
Subjects (matching)	21	146036	6954	1.048	**0.4578**
Error	21	139364	6636		
Total	47	769169			

Western Blotting:

Effect of CORT and GH on protein expression of NMDA receptor subunits in hippocampal brain slices

To test for the role of NMDA receptors in mediating the additive effect of GH and CORT on enhancing hippocampal synaptic transmission, we perfused hippocampal brain slices with CORT 5nM alone or CORT 5nM and GH 1nM in standard ACSF for three hours. Then, we looked for protein expressions of NMDA receptor subunits NR1, NR2A, NR2B, and NR2B/NR2A ratio in control hippocampal brain slices treated with (ACSF) or CORT 5nM alone or CORT 5nM + GH 1nM. We found that CORT treatment 5nM for 3 hours significantly decreased the expression of NR1 and NR2B subunits of NMDA receptors and increased the NR2A subunit compared to control slices treated with ACSF alone for 3 hours (Fig. 5 a, a´). Treatment of slices with CORT 5nM + GH 1nM for 3 hours significantly increased the expression of NR2A and NR2B and did not change NR1 protein expression compared to control slices treated with ACSF for 3 hours (Fig. 5 b, b´). Exposure of hippocampal brain slices to CORT 5nM alone caused significant suppression in the NR2B/ NR2A ratio compared to control (ACSF). Co-exposure of hippocampal brain slices to CORT 5nM and GH 1nM for 3 hours significantly increased the NR2B/ NR2A ratio compared to control (ACSF) as well as compared to CORT 0.5nM exposure alone (Fig. 5 c).

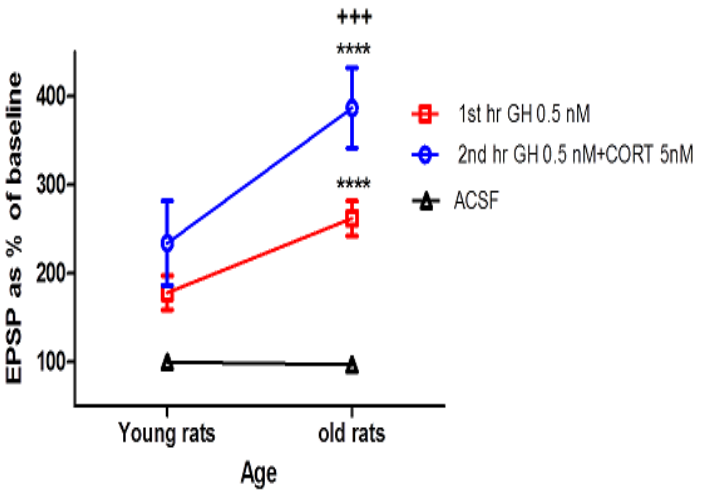

Fig. (4): The interaction of CORT and GH on the fFEPSP of young and old rats: ($P< 0.01$) compared to baseline recording, ($^{+++}$ $P< 0.005$) compared to the first hr in old rats only, and (**** $P< 0.001$) old rats compared to young rats (n=8).

DISCUSSION

Normal aging-induced decrease in GH is associated with a decrease in learning and memory function of the hippocampus and its glutamatergic function in rats and humans.[14] We reported in a previous work that GH has beneficial effect on hippocampal synaptic function.[4] It was reported that adrenal steroids given at low to moderate doses over hours enhanced synaptic and memory function, while hippocampal fFEPSPs that is concentration and age dependent. This additive effect was significantly higher in old rats compared to young aged rats suggesting a protective effect of GH against stress especially in old age. The beneficial effect of GH in both young and old rats is supported by previous study showing that

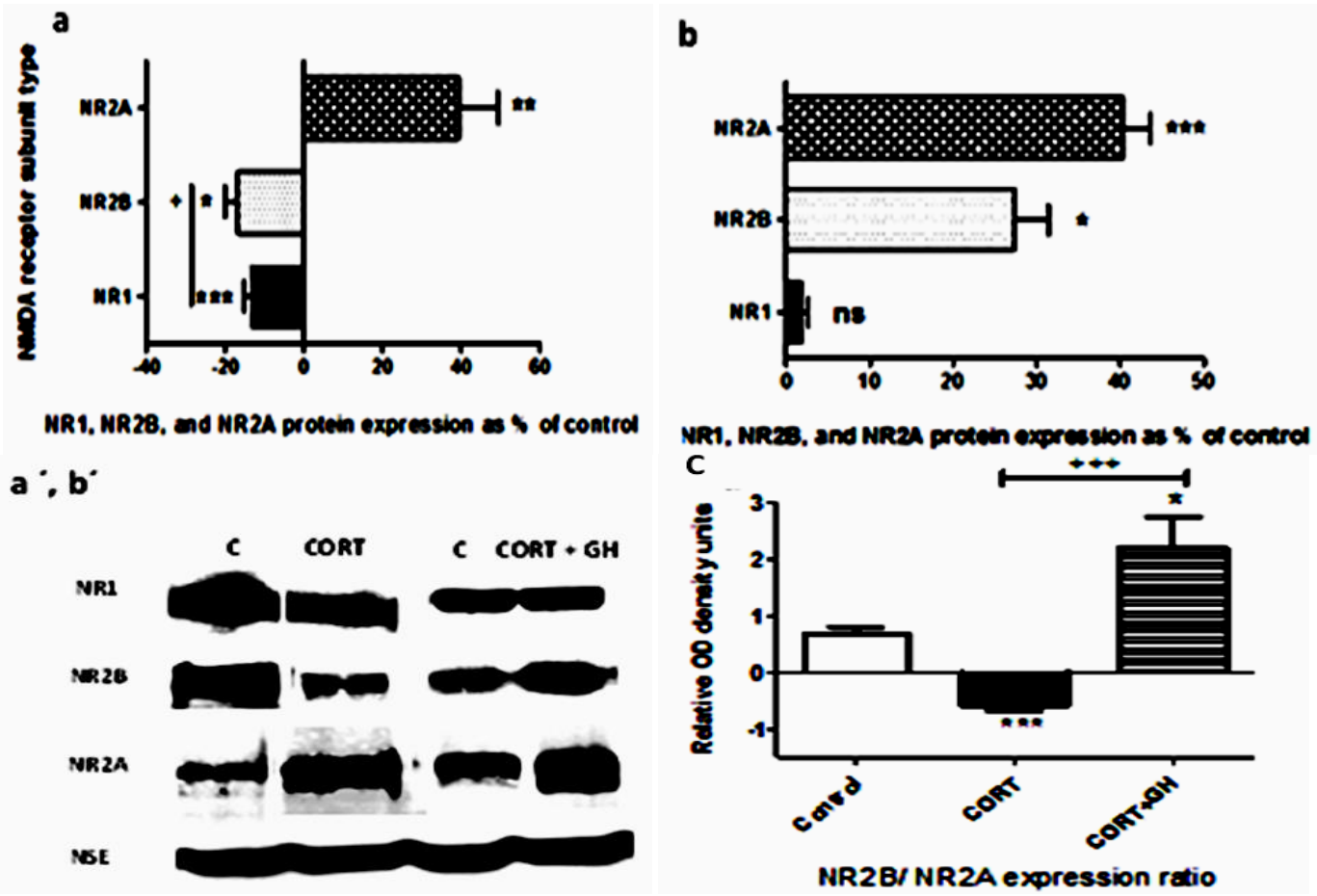

Fig. (5): Effect the interaction of CORT and GH on protein expression of NMDA receptor subunits in hippocampal brain slices.
(a, a´) CORT 5nM ((n=9), (b, b´) CORT 5nM + GH 1nM (n=7), (c) NR2B/NR2A ratio (*P< 0.05, **P< 0.01, *** P< 0.005, ^{+++}P< 0.005).

higher doses had the opposite effect.[15] We wondered whether co-exposure to GH and CORT would have any additive or subadditive effect on hippocampal synaptic transmission and if this effect differs with age. In this study, we demonstrated an additive effect of coexposure to GH and CORT compared to CORT alone on hippocampal fFEPSPs that is concentration and age dependent. This additive effect was significantly higher in old rats compared to young aged rats suggesting a protective effect of GH against stress especially in old age. The acute growth hormone treatment improved spatial learning in the radial maze compared to the control group in both young and old rats.[16] In agreement with us another study reported that both GH and IGF-I enhanced fFEPSPs of in vitro CA1 hippocampal slice preparation to a similar extent in slices from both young and old aged rats that is mediated via a postsynaptic mechanism.[14]

GH exerted a direct effect in restoring aging-induced NMDAR-mediated changes in basal synaptic function of hippocampus in old aged rats to the levels of young adult ones.[14]

Therefore, we tested the effects of coexposure to GH and CORT on the protein expression of NMDARs compared to CORT alone. In our study, we showed that perfusion of hippocampal slices with CORT alone significantly increased the expression of NR2A, decreased NR2B and NR1 protein expression, and decreased NR2B/ NR2A ratio. However, the co-exposure of hippocampal slices to small concentration of both CORT and GH for 3 hours significantly increased NR2A and NR2B subunit expression compared to control slices and increased the NR2B/ NR2A ratio that was significantly suppressed by exposure to CORT alone while no change in NR1 protein expression was obtained. While the NR1 subunit is obligatory for channel function, the NR2 composition plays an important functional modulatory role, affecting channel kinetics and pharmacology.[17]

The beneficial effects of upregulation of NR2B subunit of NMDARs on memory and synaptic transmission is proved by several previous studies. One of them showed that enhancement of the NMDAR subunit NR2B expression by administration of D-cycloserine enhanced retention and recall of fear extinction memory in the dentate gyrus, CA1 and CA3 areas of the hippocampus.[18] Another study demonstrated that NR2B upregulation enhances LTP in CA1 synapses of hippocampus, while NR2B inhibition in tissue from epileptic animals significantly increased epileptic activity and susceptibility to hyperexcitability.[19] It was demonstrated that chronic restraint stress or acute CORT treatment or glucocorticoid receptor agonist seriously deteriorated contextual fear memory function of the hippocampus through an effect that involved the transcription of protein.[20,21] Acute and chronic stress-induced rise of GCs in the prefrontal cortex and hippocampus caused changes in glutamate transmission and cognitive function that underlie stress-induced mental illness.[22]

Taken together, we may speculate that the ability of GH to reverse the effect of CORT through upregulating the NR2B subunits of NMDARs and increasing the NR2B/NR2A ratio is considered to be of great importance in management of age related disorders in memory function. Added to that, the use of GH may also have implication in management of stressor related disorders, particularly depression and anxiety disorders. Further in vitro as well as in vivo studies are needed to demonstrate other mechanisms explaining the beneficial effects of GH in combating stress.

CONCLUSIONS

The present study showed an additive effect of co-exposure to small doses of GH and CORT on hippocampal synaptic transmission that was significantly higher in old age compared to young aged rats suggesting a protective effect of GH against stress and memory impairment that was mainly manifested in old age. Although short term treatment with small dose of CORT for 2 hours enhanced fEPSPs in CA1 area of in vitro rat hippocampus,

it decreased the NR2B expression that is known to increase the synaptic excitability, susceptibility to epilepsy especially in old age. The co-exposure to both GH and CORT reversed these effects by upregulation of NR2B subunits and increasing the NR2B/NR2A ratio that is suppressed by CORT alone. The present study also demonstrated a direct role for GH in the reversing CORT related changes in NMDA receptor subunit component of the hippocampus.

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المستخلص العربى

إن أستجابة الغدد الصماء و الخلايا العصبية لنوبات التوتر الحاد مهم من أجل البقاء ولكن ما إذا كان هذا الضغط له تأثير مفيد أو ضار على التعلم و التخزين الطويل للمعلومات فى المخ لا يزال لغزا. ان أنسجة الهيبوكامبس بشكل خاص عرضة للإجهاد مع العجز المعرفي و الادراكى عند تعرض الانسان لفترات طويلة من الضغط و التوترالنفسى و العصبى الشديد. هناك أدلة قوية على أن العلاج بهرمون النمو (GH) له دور في تحسين الوظيفة الإدراكية وكذلك يمارس تأثير وقائي على الجهاز العصبي المركزي. في هذه الدراسة تم اختبار التفاعل التبادلى بين الجرعات المنخفضة من هرمونى النمو والكورتيكوستيرون (CORT) على سرعة انتقال الاشارات العصبية عبر نقاط الاشتباك العصبى فى الهيبوكامبس فى مختلف الفئات العمرية لفئران التجارب. درسنا التعبيرالبروتينى عن مستقبلات NMDA (NR1، NR2A ، و NR2B) في شرائح الهيبوكامبس التى يتم معالجتها لمدة ثلاث ساعات بهرمون الكوتيكوستيرن أو هرمون الكوتيكوستيرن مع هرمون النمو فى ان واحد أو السائل السحائى. وجدنا أن إضافة هرمون النمو لهرمون الكوتيكوستيرن له تأثير أقوى على زيادة سرعة انتقال الاشارات العصبية عبر نقاط الاشتباك العصبى فى الهيبوكامبس فى جميع الجرعات التى استخدمت . وعلاوة على ذلك ، وجدنا أن الجمع بين استخدام جرعة منخفضة من هرمون النمو و هرمون الكوتيكوستيرن لها آثار أعلى بكثير على تعزيز انتقال الاشارات العصبية في الفئران الكبيرة فى السن مقارنة مع صغارالسن. أظهرنا أن كلا من هرمون النمو و هرمون الكوتيكوستيرن يزيد من البروتين NR2A لمستقبلات NMDA . كما أثبتنا أن التعرض المشترك لجرعات منخفضة من هرمون النمو و الكوتيكوستيرن يزيد من بروتين NR2B ويؤدى الى زيادة نسبة NR2B/NR2A مقارنة بهرمون الكوتيكوستيرن وحدة . وجدنا أن العلاج بهرمون الكورتيكوستيرون وحدة يسبب نقص كبير في بروتينات NR1 و NR2B لمستقبلات NMDA وكذلك انخفاض في نسبة NR2B/NR2A . وأظهرت دراسات سابقة أن تثبيط بروتينNR2B يؤدى الى زيادة التعرض إلى الاضطرابات العصبية والنفسية . لذا تدل نتائج هذا البحث على أن هرمون النمو له تأثيروقائى من خلال تأثيره على مستقبلات NMDA ضد ضعف الذاكرة و الإدراك المتعلقة بتقدم العمر و التوتر النفسى و العصبى.