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Regulation of the Nicotinic Receptor Alpha7 Subunit by Chronic Stress and Corticosteroids

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Abstract

The α7 subunit of the nicotinic acetylcholine receptor (NAchRα7) is one of the principal brain receptors for nicotine and is thought to be a mediator of nicotine's pro-cognitive effects. While nicotine is known to interact with the stress axis, little is known about the effect of stress or corticosteroids on the expression in the hippocampus, a brain region important to both cognition and stress reactivity. We examined the effects of chronic (21 day) restraint stress (CRS) and adrenalectomy with hormone replacement with the selective mineralocorticoid receptor (MR) agonist aldosterone, the selective glucocorticoid receptor (GR) agonist RU28,362 or corticosterone for 7 days, on the hippocampal expression of NAchRα7 mRNA and protein, as measured by ^{125}I α-Bungarotoxin autoradiography. We found that CRS increase the levels of NAchRα7 mRNA in the CA1, CA3 and Dentate gyrus while levels of the protein were lowered by the same treatment. Corticosteroid replacement showed a GR specific increase in NAchRα7 mRNA, consistent with a corticosteroid mediated effect of CRS. While the mechanism behind these observations is as yet unclear, they may be neuroprotective against the damaging effects of CRS or an example of adaptation to the allostatic load produced by CRS.

1. Introduction

Nicotine is one of the most commonly used stimulants known to man, and it has a profound and well publicized negative impact upon public health. The interaction of nicotine with the stress axis is well known, and nicotine use shares a high co-morbidity with a number of psychiatric disorders (1,2). In addition to its pathogenic role, nicotine has also been shown to have pro-cognitive effects in humans and animals (3,4). Studies of the effects of adrenalectomy and sub-chronic corticosterone treatment have shown that both treatments alter nicotine tolerance and binding of the NAchRα7 selective ligand α-Bungarotoxin in the hippocampus and other brain regions (5–10). In addition to containing nicotinic receptors, the hippocampus is rich in both MR and GR receptors and is a nexus for many of the effects of stress on the brain, as well as being a center for spatial cognition and declarative memory formation. Chronic stress has been shown to have a number of effects on hippocampal structure and function, including dendritic remodeling and impairment of spatial and declarative memory in both man and animals (11,12).

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The present study examined the impact of chronic restraint stress and pharmacologic manipulation of corticosteroid levels, after removal of the source of endogenous corticosteroids by adrenalectomy, upon the expression of the α7 subunit of the nicotinic acetylcholine receptor in the hippocampus. Adrenalectomy allows the manipulation of corticosteroid levels without the confound of background levels of endogenous corticosteroids, while replacement in the drinking water produces a diurnal rhythm of corticosteroid levels approximating those occurring in the intact animal.

2. Results

Chronic restraint stress

As figure 1 shows, NAchR α7 mRNA was significantly elevated by CRS in CA1 (138±1.0%), CA3 (21 ±2.8%), and DG (118±4.6%) (n=8, $p<0.0001$, 0.02 and 0.0001 respectively).

Chronic steroid treatment

As can be seen in figure 2, The α7 NAchR subunit showed a main effect of treatment in CA1, CA3 and DG (n=7, F=3.512, 6.462, and 6.087 respectively; $p<.0001$, 0.007, 0.002 respectively). Adrenalectomized (ADX) animals co-treated with RU28,362 showed significant elevations in expression in CA1 (17±4.8%), CA3 (18±3.7%) and DG (15±2.3%) when compared to sham (n=7, $p<0.05$). RU28,362 co-treatment also produced significantly higher levels of NAchR α7 mRNA than ADX with aldosterone (n=7, $p<0.05$) and ADX with corticosterone (n=7, $p<0.05$) co-treatment in all three of the aforementioned regions. ADX + RU28,362 also induced mRNA expression significantly higher than ADX plus vehicle treatment in both the CA3 and DG (n=7, $p<0.05$).

^{125}I α-Bungarotoxin autoradiography

In contrast to the effect of adrenal steroids and stress on NAchRα7 mRNA levels, ^{125}I α-Bungarotoxin autoradiography showed a 20%±5.6% reduction in levels of NAchRα7 binding in the dentate gyrus after chronic restraint of (n=8, $p<0.025$, see figure 3.) and a trend towards a reduction in binding after adrenalectomy and replacement with RU28,362 (data not shown). No effect of any treatment was observed upon ^{125}I α-Bungarotoxin binding in the CA1 or CA3.

Pyknotic Cell Counts

adrenalectomy produced a 46% (n=8, $p< 0.05$) increase in the number of pyknotic cells in the dentate gyrus relative to sham adrenalectomized animals, whereas other treatments showed no significant effect on pyknotic cell number and in no treatment was the number of pyknotic cells higher than 5% (data not shown).

3. Discussion

We have shown that chronic stress produces substantial increases in NAchRα7 mRNA while decreasing binding to the receptor in the hippocampal formation, changes which suggest that the NAchRα7 may be important to hippocampal adaptations to stress or allostatic overload (defined as an attempted adaptation to an environmental challenge which has the capacity to produce pathophysiology (13)). This appears to be the case with CRS and NAchRα7, as mRNA is up-regulated while protein levels are reduced, suggesting increased turnover and, perhaps “wear” on the system, although alternative explanations are not excluded by the data presented here. For example, stress may alter trafficking or post-translational modification of NAchRα7 protein, or, mRNA may be upregulated indirectly due to the level

of stimulation of the receptors during stress, though the RU28,362 data argue against the latter interpretation.

That adrenalectomy and treatment with the selective GR agonist RU28,362 recapitulates some of this effect seems to indicate that corticosterone is at least partially responsible for the effects we observed with chronic restraint stress. Corticosterone is the principal endogenous ligand for both the GR and the mineralocorticoid receptor (MR) in rodents, and has a higher affinity for the latter. That a selective activation of the GR has a similar effect to CRS suggests that balanced activation of MR and GR in the hippocampus maintains homeostatic levels of NAchRα7 whereas excess GR activation produces up-regulation of the sub-unit mRNA. It is also possible that the differing effects of RU28,362 and corticosterone are due to differing activity at non-genomic GR sites (14). Other groups have observed a corticosterone mediated reduction in α-bungarotoxin binding in the hippocampus using corticosterone pellets (10) in mice. That we did not may be explicable due to species differences or by the fact that our animals received corticosterone in their drinking water and therefore showed a behaviorally induced circadian rhythm in corticosterone levels, which is likely to produce different effects than chronic steady state levels of the steroid. Similarly, though it has been reported that ADX results in an increase in α-bungarotoxin binding in the hippocampus of mice (6) this effect was not present in all mouse strains (15), and our results suggest it is absent in the Sprague-Dawley rat.

That CRS produces such a substantial increase in NAchRα7 mRNA, while reducing labeling with the NAchRα7 selective ligand ^{125}I α-bungarotoxin, implies a higher turnover of receptors. This may be a result of failed adaptation to the allostatic overload produced by CRS. Since NAchRα7 activation is associated with improved cognition (4), the reduction in NAchRα7 levels may contribute to the cognitive deficits observed after chronic stress (11), though further studies will be required to assess this hypothesis. The NAchRα7 gene, CHRNA7, contains a glucocorticoid response element (GRE) (16) and we found that selective agonism of the GR with RU28,362 produced the expected increase in NAchRα7 mRNA. That said, the difference in the magnitude of the stress and RU28,362 effects admits of mechanisms in addition to direct activation of GR. It is quite possible that some of the effect is due to increases in glutamate signaling, which increases in the hippocampus during stress (17). The CHRNA7 gene contains multiple SP1 elements (16) and SP1 family transcription factors are highly regulated by glutamatergic signalling in neurons (18).

Given that NAchRα7 is expressed in most inhibitory interneurons in the hippocampus (19), its levels would have an impact on GABA tone in the region. The NAchRα7 is also directly neuroprotective of hippocampal neurons (20–22); these two observations demonstrate that intact NAchRα7 function may be important to the ability of the hippocampus to resist excitotoxic insult and the damaging effects of stress.

Our results are of broader interest given the effects of nicotine and the NAchRα7 on learning and memory, dementia (4,23) and schizophrenia (24) as well as the role of stress and hypothalamic-pituitary axis activity in nicotine addiction and relapse (3,25). The emerging neuroprotective role of NAchRα7 (26) also adds important context to our observations.

4. Experimental Procedures

Animals

Male Sprague-Dawley rats were obtained from Charles River (Kingston, NY) at 70 days of age. Animals were housed 2–3 per cage (same age cage mates) in clear polycarbonate cages with wood chip bedding. All animals were maintained on a 12 h light-dark schedule (lights on at 0800 h) and the temperature was kept at 21±2°C. All animals had *ad libitum* access to

food and water. All procedures were carried out in accordance with the guidelines established by the NIH Guide for the Care and Use of Laboratory Animals.

Chronic restraint stress

To assess the effect of CRS on NAchRα7 levels we subjected rats to a 6 hour a day restraint for 3 weeks. Animals were left undisturbed after arrival for one week after delivery. Animals were restrained in wire mesh restrainers, secured at the head and tail ends with clips. Chronic stress was administered for 6 hours daily for 21 days. These animals were sacrificed 18 hours after the last stress to ensure that neither circulating corticosteroids (18 hours later being close to the circadian nadir in corticosterone secretion) nor the acute effects of stress contaminated the results. Animals were returned to their home cages immediately after termination of the stressor, unless immediately sacrificed. Brains were removed and flash frozen on dry ice then stored at −80°C until processing. All animals were killed between 1300 and 1700 h.

Steroid treatments

In order to determine the contribution of adrenal steroids to the regulation of NAchRα7 mRNA in the hippocampal formation we adrenalectomized rats and treated them with several corticosteroid receptor agonists. These treatments followed those administered in (27,28) with some modification. Animals were anesthetized using ketamine and xylazine and the adrenal glands removed, save for one group which received a sham surgery. During the same surgery, osmotic mini-pumps (Alzet, Cupertino, CA) were implanted between the scapula. These pumps delivered either vehicle, the mineralocorticoid receptor agonist aldosterone at 10mg/hour or the glucocorticoid receptor agonist RU28,362 at 10μg/hour. Animals who underwent ADX received 0.9% saline in their drinking water and one group received 400μg/ml corticosterone in addition to the saline. Seven days after the completion of the surgeries the animals were sacrificed and their brains frozen as described above.

In Situ Hybridization

Brain sections were cut at 20 μm on a cryostat and placed on Fisher Biotech ProbeOn Plus slides (Fisher, Pittsburgh, PA). In situ hybridization began with a tailing reaction to radioactively label the oligonucleotide probes with ^{35}S. The probe sequences are those described by Ryan and Loiacono (29) (α7 accession number: L31619, mRNA sequence 701–746, 1031–1076, 1161–1206) . Processing of the slides followed methods as previously described (30) with some modification as described in (31). Anatomical locations were determined with the assistance of the atlas of Paxinos and Watson (32). Optical density was determined using MCID 5.0 (Imaging Research, St. Catharine's, OT, Canada).

^{125}I α-Bungarotoxin autoradiography

We followed the procedure of (33) and (34) with some modification. 20 μm thaw mounted sections were brought to room temperature in a desiccator and pre-incubated in 1%BSA and 50mM Tris pH 7.4 for 30 minutes, followed by a one hour RT incubation with 5nM ^{125}I α-Bungarotoxin (Perkin Elmer, Waltham, MA, USA) with or with out 1mM (−) nicotine to determine non-specific binding. Sections were then washed 4 times for 5 minutes in ice cold 50mM Tris, pH7.4, dried and placed on Kodak BioMax MR film for 3 days.

Pyknotic Cell Counts

Numbers of pyknotic cells were assessed following the method of Frye and McCormick (35). Sections were serial to those used for autoradiography and in situ. Slides containing these sections were processed to reveal Nissl substance beginning with a brief fixation in 4% paraformaldehyde in 0.1M PB for 15 minutes after which they were washed in distilled

water three times for 2 minutes per wash. Sections were then dipped in 0.1% Cresyl Violet for 2 minutes and then dehydrated in ascending concentrations of ethanol prior to clearing in xylenes for 4 minutes. After drying the slides were coverslipped with permount. Pyknotic cells in the granule cell layer and subgranule zone of the dentate gyrus were identified in a 100× visual field as those having a small volume, membrane blebbing, and dark condensed nucleus and chromatin.

Statistics

Optical density measurements were analyzed by a one way ANOVA for the chronic steroid study and by Student's t-test for the chronic stress study. Significant main effects and interactions in ANOVA were further analyzed using Tukey's test, respectively. Differences are considered significant at $p<0.05$. All data are presented as mean ±SEM.

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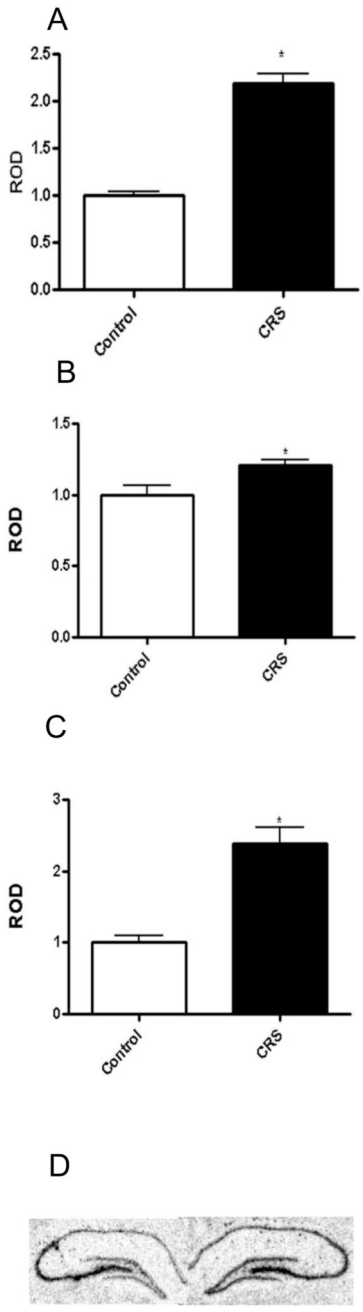

Figure 1.

A) shows relative levels of NAchRα7 mRNA in the CA1 of control rats and those subjected to CRS. B) shows mRNA levels in the CA3 and C) shows levels in the dentate gyrus. D) shows representative autoradiograms of the hippocampus of control (left) and CRS (right) treated rats. *-$p<0.05$, n=8.

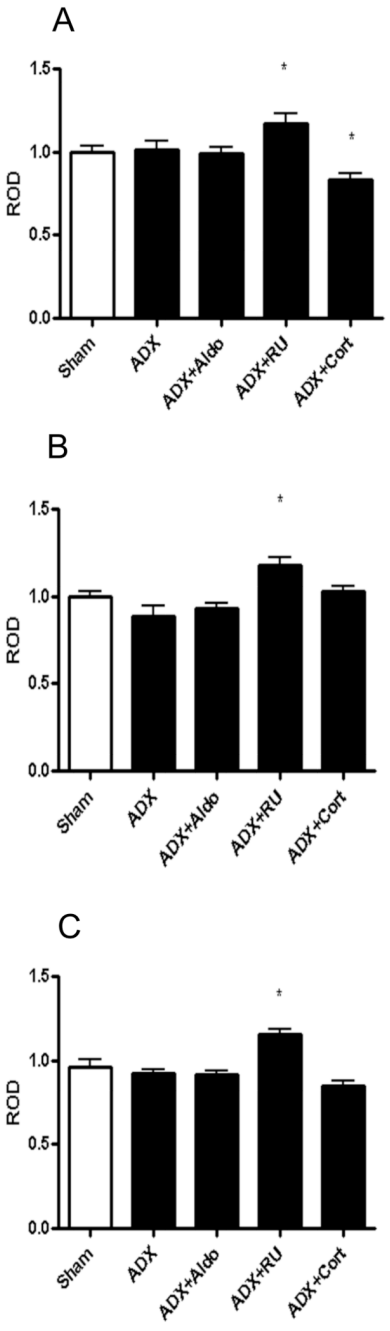

Figure 2.

A) shows relative levels of NAchRα7 mRNA in the CA1 of rats given either sham surgery, adrenalectomy (ADX), ADX plus aldosterone replacement, ADX plus RU28,362 replacement, or ADX plus corticosterone replacement. B) shows mRNA levels in the CA3 and C) shows levels in the dentate gyrus. *-$p<0.05$, n=8.

Figure 3.

A) shows relative levels of ^{125}I α-Bungarotoxin binding in the dentate gyrus in control and CRS treated rats. B) shows a representative autoradiogram of the rat hippocampus. *- $p<0.05$, n=8.