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Prevention of stress-induced morphological and cognitive consequences

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Abstract

Atrophy and dysfunction of the human hippocampus is a feature of aging in some individuals, and this dysfunction predicts later dementia. There is reason to believe that adrenal glucocorticoids may contribute to these changes, since the elevations of glucocorticoids in Cushing's syndrome and during normal aging are associated with atrophy of the entire hippocampal formation in humans and are linked to deficits in short-term verbal memory. We have developed a model of stress-induced atrophy of the hippocampus of rats at the cellular level, and we have been investigating underlying mechanisms in search of agents that will block the atrophy. Repeated restraint stress in rats for 3 weeks causes changes in the hippocampal formation that include suppression of 5-HT1A receptor binding and atrophy of dendrites of CA3 pyramidal neurons, as well as impairment of initial learning of a radial arm maze task. Because serotonin is released by stressors and may play a role in the actions of stress on nerve cells, we investigated the actions of agents that facilitate or inhibit serotonin reuptake. Tianeptine is known to enhance serotonin uptake, and we compared it with fluoxetine, an inhibitor of 5-HT reuptake, as well as with desipramine. Tianeptine treatment (10 mg/kg/day) prevented the stress-induced atrophy of dendrites of CA3 pycamidal neurons, whereas neither fluoxetine (10 mg/kg/day) nor desipramine (10 mg/kg/day) had any effect. Tianeptine treatment also prevented the stress-induced impairment of radial maze learning. Because corticosterone- and stress-induced atrophy of CA3 dendrites is also blocked by phenytoin, an inhibitor of excitatory amino acid release and actions, these results suggest that serotonin released by stress or corticosterone may interact pre- or post-synaptically with glutamate released by stress or corticosterone, and that the final common path may involve interactive effects between serotonin and glutamate receptors on the dendrites of CA3 neurons innervated by mossy fibers from the dentate gyrus. We discuss the implications of these findings for treating cognitive impairments and the risk for dementia in the elderly.

Keywords: Stress; Hippocampus; Glucocorticoids; Atrophy; Tianeptine

1. Introduction

The hippocampus is a vulnerable brain structure that is involved in aspects of learning and memory, especially those aspects related to spatial memory and 'working' memory [7]. Besides the actions of repeated stress, to be discussed below, the hippocampus is vulnerable to disruptive events such as seizures, head trauma and ischemia, as well as to degenerative changes accompanying aging [11,15,16,28,29]. An important factor in damage to the hippocampus is the action of glucocorticoid hormones, which potentiate damage produced by ischemia and excitotoxins and mimic the effects of aging and stress in causing hippocampal damage [28]. A second factor in hippocampal damage is the endogenous excitatory amino acids [28], but there is a paradox, in that excitatory amino acids are also involved in adaptive plasticity related to synapse formation [38], stabilization of neuronal populations [9], long-term potentiation [1] and memory [23]. Thus excitatory amino acids are beneficial under some circumstances and deleterious under others, and there must be other factors involved in the mechanisms for causing damage.

A third factor in producing hippocampal damage is serotonin. We have developed a model of stress-induced atrophy of the hippocampus of rats at the cellular level and have been investigating underlying mechanisms in search of agents that will block the atrophy. Repeated restraint stress of cats for 3 weeks causes changes in the hippocampal formation that include suppression of 5-HT1A receptor binding and atrophy of dendrites of CA3 pyramidal neurons [21,35]. Three weeks of 6 h/day restraint stress caused not only dendritic atrophy but also impairment of initial learning of a radial arm maze task [18]. Because

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serotonin is released by stressors and may play a role in the actions of stress on nerve cells, we investigated the actions of agents that facilitate or inhibit serotonin reuptake. Tianeptine is known to enhance serotonin uptake and thus remove it from extracellular interactions with serotonin receptors [22], and tianeptine treatment prior to daily restraint stress blocked stress-induced atrophy of CA3 pyramidal neurons [33] and prevented stress-induced impairment of radial maze learning [18]. This review will summarize these results in studies on the rat model and will then discuss the potential relevance to the human hippocampus and to human aging, where hippocampal atrophy occurs in some individuals and is a risk factor associated with later dementia [3,4,8].

2. Atrophy of hippocampal neurons under stress and glucocorticoid treatment

Repeated stress for 21 days in young adult rats causes atrophy of apical dendrites of CA3c neurons in the hippocampal formation without causing permanent cell loss or other signs of permanent damage [20,33] (see Fig. 1). CA1 pyramidal cells and dentate granule neurons are not affected in this way; and within CA3, only CA3c neurons have been studied, although an analysis of the distribution of atrophy within the CA3 region is ongoing along with an electron microscopic study of the ultrastructure of this region (Magarinos, in progress). The atrophy is only seen on the apical dendritic tree, and it is reversible within 7–10 days of the termination of stress. Thus the change in dendritic structure that accompanies reductions in length and branching must involve asymmetric alterations in the apical dendritic cytoskeleton, and possible underlying mechanisms include depolymerization of the cytoskeleton, limited proteolysis or decreased biosynthesis of cytoskeletal proteins.

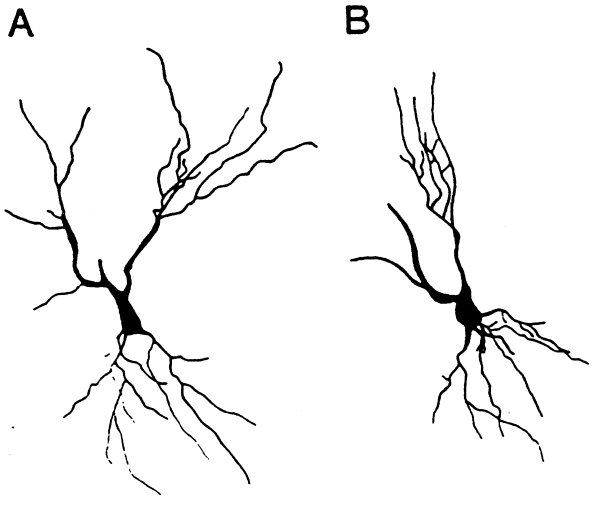

Fig. 1. Camera lucida drawings of representative Golgi-impregnated CA3 pyramidal neurons from control (A) and stress (B). Note decrease in dendritic length and branchpoints in (B) compared to (A). Reprinted from [33] by permission.

3. Pharmacological manipulation of atrophy

Corticosterone treatment mimics the effects of stress to produce dendritic atrophy in CA3 pyramidal neurons [39], and yet the mechanism for stress-induced atrophy is more complex than being caused solely by stress-induced release of corticosteroids. Rather, pharmacological manipulations have shown that a number of neurotransmitter systems are involved, including excitatory amino acid, the GABAa-benzodiazepine system and serotonin. First of all, stress-induced atrophy does involve endogenous glucocorticoid secretion, since the atrophy caused by 21 days of daily restraint stress is blocked by an adrenal steroid synthesis inhibitor [20]. But it is also blocked by phenytoin and by an NMDA receptor blocker, implicating excitatory amino acids, and by a benzodiazepine, implicating the GABAa benzodiazepine receptor ([20]; Magarinos and McEwen, unpublished). These findings indicate that adrenal steroids work in concert with excitatory amino and in opposition to the GABAa-benzodiazepine system in inhibitory interneurons.

As noted above, repeated stress causes changes in the hippocampal formation that include oppression of 5-HT1A receptor binding [21]. Because serotonin is released by stressors [13] and may play a role in the actions of stress on nerve cells, we investigated the actions of agents that facilitate or inhibit serotonin reuptake. Tianeptine is known to enhance serotonin uptake [22] and thus remove it from extracellular interactions with serotonin receptors. We compared tianeptine with fluoxetine, an inhibitor of 5-HT reuptake, as well as with desipramine. Tianeptine treatment (10 mg/kg/day and 15 mg/kg/day) prevented the stress-induced atrophy whereas neither fluoxetine (10 mg/kg/day) nor desipramine (10 mg/kg/day) had any effect ([34], Watanabe, Magarinos and McEwen, unpublished) (see Fig. 2). Tianeptine treatment also prevented the stress-induced impairment of radial maze learning [18]. Fluoxetine, desipramine and imipramine were not tested in the learning paradigm.

Exogenous corticosterone treatment also produced atrophy of dendrites of CA3 pyramidal neurons, and this effect was also blocked by tianeptine (15 mg/kg/day) treatment [34] as well as by phenytoin [33] (see Fig. 3). Thus the effects of tianeptine on CA3 morphology during repeated restraint stress is not due to its reported effects to reduce corticosterone secretion [5], but may instead be related to its reported effects to enhance the reuptake of serotonin with the hippocampus [34]. Because corticosterone- and stress-induced atrophy of CA3 dendrites is also blocked by phenytoin [33], an inhibitor of excitory amino acid release and actions, these results suggest that serotonin released by stress or corticosterone may interact pre- or post-synapti-

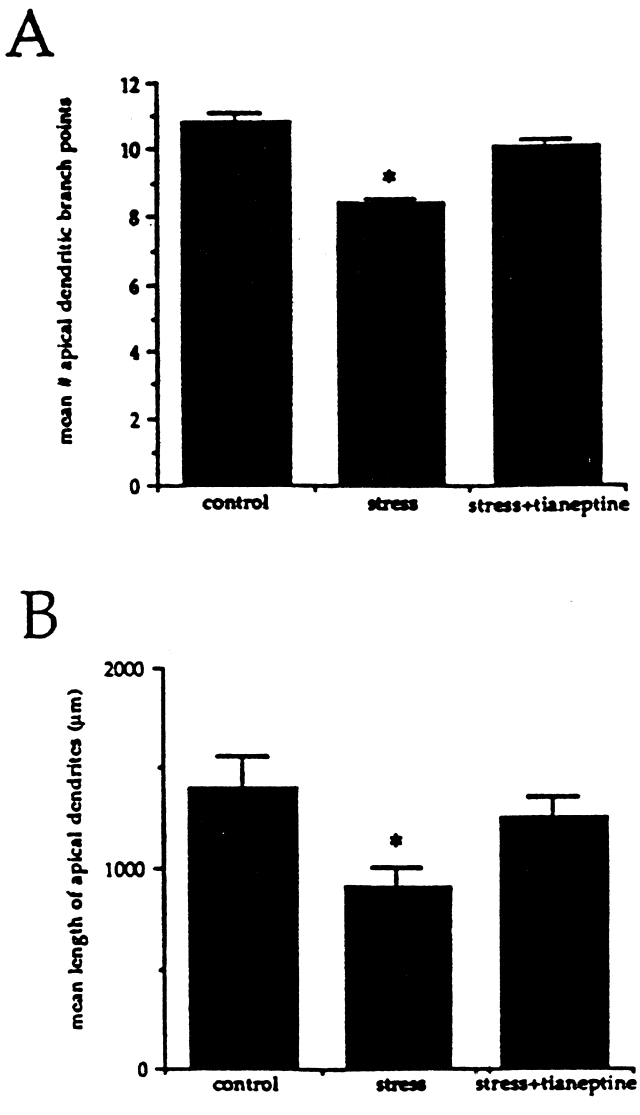

Fig. 2. Effects of tianeptine and restraint stress on number of branch points (A) and total length (B) of apical CA3c dendrites. The stress group was significantly different from the two other groups. Reprinted from [34] by permission.

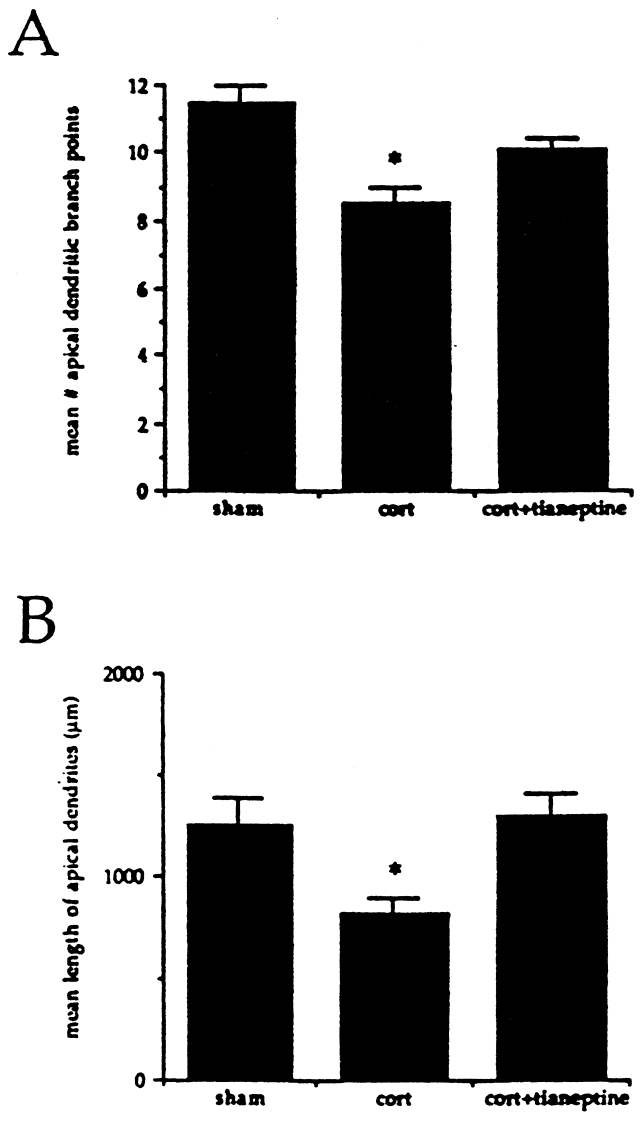

Fig. 3. Effects of tianeptine and corticosterone treatment on number of branch points (A) and total length (B) of apical CA3c dendrites. The corticosterone group was significantly different from the two other groups. Reprinted from [34] by permission.

cally with glutamate released by stress or corticosterone, and that the final common path may involve interactive effects between serotonin and glutamate receptors on the dendrites of CA3 neurons innervated by mossy fibers from the dentate gyrus. The GABAa-benzodiazepine system plays an opposing role to excitatory amino acid actions through the actions of inhibitory interneurons. A schematic summary of these relationships is provided in Fig. 4.

4. Molecular markers and mechanisms of atrophy

Adrenal steroids regulate many aspects of hippocampal gene expression, and they do so via Type I and Type II receptors that are found in Ammons horn and the dentate gyrus, usually co-expressed in the same neurons ([10]; de Kloet, personal communication). We have found that Type I receptors regulate expression of dynorphin mRNA in dentate gyrus [36], NPY mRNA in dentate hilus [32], kainate receptor binding in the mossy fiber terminals [36], and 5HT1A receptors in the dentate gyrus and CA3 [14]. Although Type II receptor involvement is not precisely defined, it appears likely that it is involved in the glucocorticoid induction of NMDA receptor subunit mRNA in Ammons horn and dentate gyrus [37] and in the up- and down-regulation of different subunits of the GABAa-benzodiazepine receptor [24]. In addition to these gene products involved in neurochemical and pharmacological responses, adrenal steroids also participate in the regulation of GAP43 mRNA [2] and several neurotrophins, NT-3 and BDNF [2,30], and they probably do so through Type I

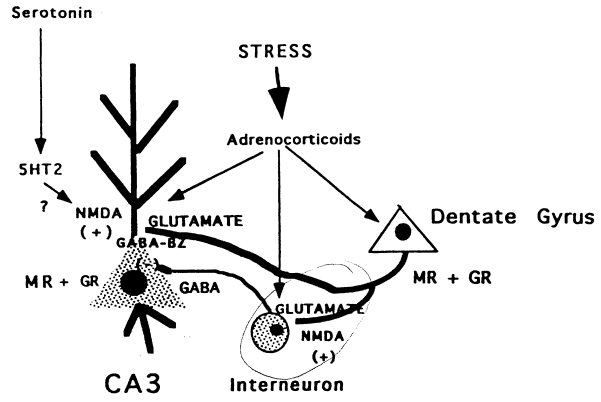

Fig. 4. Schematic summary of the mechanism of stress-induced neuronal atrophy, showing the role of adrenal steroid hormones, excitatory amino acids, serotonin and inhibitory input via the GABAa-benzodiazepine receptor via inhibitory interneurons. The final pathway leading to dendritic atrophy appears to be excitatory amino acids acting via NMDA receptors. GABAa-benzodiazepine receptors provide inhibitory tone that opposes these effects, and serotonin is hypothesized to enhance NMDA receptor function, possibly via a 5HT2 receptor [34]. Adrenal steroids, acting on the two types of adrenal steroid receptors, enhance NMDA transmission, facilitate the release of glutamate and reduce inhibitory tone via the GABAa-benzodiazepine receptor [20].

and Type II receptors, which may mediate effects in opposite directions in some cases and in concert in others.

Regarding the mechanism of dendritic atrophy (Fig. 4), it appears that adrenal steroids act at several places in a multicellular array: (a) permissively regulating glutamate release from presynaptic terminals, especially mossy fibers [17]; (b) up-regulating the NMDA receptor system [37]; (c) impairing the GABAa system by changing the subunit composition in a direction that reduced GABAa-benzodiazepine inhibition [24]. In addition, one possibility currently under investigation is that modulation of neurotrophin expression may play a role, and that agents which block dendritic atrophy may also prevent stress-induced suppression of neurotrophin expression in hippocampus.

5. Electrophysiology

Adrenal steroids biphasically modulate long-term potentiation or primed-burst potentiation in the dentate gyrus [6], enhancing it via Type I receptors and inhibiting it via Type II receptors [26]. The excitatory effects are particularly interesting because, in awake, freely moving adrenalectomized (ADX) rats, Type I receptor stimulation enhances LTP in dentate gyrus for several days, whereas LTP in the ADX animal decays in a matter of hours [25]. The inhibitory actions mediated by glucocorticoid receptors mimic the effects of acute and chronic treatment with corticosterone at high doses [27] and this suggests that there may be electrophysiological consequences of the stress-induced atrophy in the CA3 region. These are currently under investigation.

It remains to be seen how these biphasic actions of adrenal steroids are distributed within the hippocampal neural circuitry and, in particular, which effects operate within the mossy fiber and commissural input to CA3, where the dendritic atrophy occurs. Thus far it appears that the entire hippocampus is responsive to adrenal steroids: e.g. it is apparent from studies on the CA1 pyramidal neurons that glucocorticoids produce biphasic effects on excitability [6,12]. However, the specific characteristics and sensitivity of the mossy fiber input to CA3 neurons deserve separate study because of their putative role in stress-induced dendritic atrophy.

6. Effects of stress on cognitive function

Repeated stress for 21 days impairs initial learning on a radial maze task, which is known to depend on hippocampal function; and the impairment is reversible, like the dendritic atrophy [18,18a]. Moreover, like the dendritic atrophy it can be blocked by the same agents – phenytoin and tianeptine [18,33,34]. Tianeptine is an atypical tricyclic antidepressant that enhances 5HT uptake. Whereas tianeptine treatment blocks dendritic atrophy, fluoxetine treatment does not (Magarinos, Watanabe, McEwen, unpublished). This suggests that 5HT release during stress also participates in atrophy, and one possibility is that 5HT acts to enhance NMDA receptor efficacy, as has been demonstrated in some electrophysiological studies.

7. Conclusion: significance for human aging and risk for dementia

Atrophy and dysfunction of the human hippocampus is a feature of aging in some individuals; moreover, hippocampal atrophy is a risk factor that predicts later dementia [3,4,8]. Several types of evidence suggest that glucocorticoids may be involved in the mechanism of human hippocampal atrophy. In Cushing's syndrome and during normal aging, glucocorticoid levels are negatively correlated with hippocampal volume and with short-term verbal memory impairment ([8,31]; de Leon, personal communication). In parallel studies that have not involved MRI analysis of the hippocampus, there are individual differences in human aging that are reflected in elevated cortisol levels in individuals at risk for cognitive impairment as they age ([19]; T. Seeman, Yale University, personal communication).

These results are consistent with the model of dendritic atrophy described above in the rat, and they suggest that therapeutic strategies that work to block CA3 dendritic

atrophy in the rat hippocampus might be useful to try on human subjects. Insofar as agents we are finding effective in blocking stress-induced atrophy of hippocampal neurons in rats and which may be able to reverse atrophy of the human hippocampus associated with elevated cortisol levels, then it will be important to see whether the progression towards dementia is also slowed or arrested in these subjects.

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