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European Journal of Pharmacology 371 (1999) 113–122

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Effects of antidepressants and benzodiazepine treatments on the dendritic structure of CA3 pyramidal neurons after chronic stress

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Received 11 December 1998; received in revised form 25 February 1999; accepted 5 March 1999

Abstract

Both repeated stress and corticosterone administration induce remodeling of apical dendrites of hippocampal CA3 pyramidal neurons. Circulating glucocorticoids are involved in the mechanism that produces atrophy, along with excitatory amino acids and serotonin (5-hydroxytryptamine, 5-HT). We used 5-HT-related antidepressants and a benzodiazepine in order to explore indirectly the role of serotonin and $GABA_A$-benzodiazepine receptors in the stress-induced structural changes visualized by the Golgi impregnation of the rat hippocampus. The 5-HT reuptake enhancer (±)-tianeptine prevented the dendritic atrophy caused by repeated restraint stress in a non-stereoselective fashion and two 5-HT reuptake antagonists, fluoxetine and fluvoxamine, failed to block dendritic atrophy. Tianeptine also functions as a therapeutic tool since it reversed the already established hippocampal atrophy caused by treatment with corticosterone for 3 weeks. Finally, the benzodiazepine agonist adinazolam was effective in preventing the stress-induced dendritic atrophy. These findings suggest that the synaptic availability of 5-HT is involved in the mechanism leading to stress-induced dendritic remodeling and supports the idea that the hippocampal inhibitory GABAergic tone may play a regulatory role.

Keywords: Hippocampus; Dendritic atrophy; Stress; Tianeptine; 5-HT (5-hydroxytryptamine, serotonin) reuptake inhibitor, selective; Adinazolam

1. Introduction

Beyond development, neurons retain the ability to remodel themselves in response to changes in their environment. In particular, hippocampal neurons show a remarkable plasticity in the aftermath of deafferentation (Kelley and Steward, 1997) and focal chemical lesions that result in epileptogenic activity (Nadler et al., 1980; Pyapali and Turner, 1994). Less intrusive challenges such as exposure of rodents to complex environments or successful aging in rodents and humans can also result in the increased complexity or elongation of neuronal dendritic fields (see Turner et al., 1998 for review).

We have previously described a model of stress-induced morphological reorganization in the hippocampus, in which adrenal steroids and excitatory amino acids mediate the reversible remodeling of apical dendrites of CA3 pyramidal neurons (McEwen, 1997). Dendritic remodeling, which consists of a reduction of dendritic branching and length of the apical, but not of the basal, dendrites is produced by repeated restraint stress in rats and repeated psychosocial stress in tree shrews (Watanabe et al., 1992a; Magariños et al., 1996). In rats, the stress-induced atrophy is mimicked by both systemic and oral corticosterone administration (Woolley et al., 1990; Magariños et al., 1998) and can be prevented by inhibiting adrenal steroid synthesis (Magariños and McEwen, 1995b). The blockade of NMDA receptors and the administration of the antiepileptic phenytoin, a drug that interferes with the mechanism of action of excitatory aminoacids, also prevents the chronic stress-induced CA3 apical atrophy (Magariños and McEwen, 1995b; McEwen, 1997), suggesting that excitatory amino acids could, at least in part, mediate this dendritic remodeling.

Chronic stress has been associated with the onset of depression (Post, 1992) and dysregulation of the hypothalamic–pituitary–adrenal axis as well as disturbances in serotonergic transmission are commonly observed with both chronic stress and depression (López et al., 1998). We

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0014-2999/99/$ - see front matter
PII: S0014-2999(99)00163-6

have previously shown that the racemic form of the atypical antidepressant tianeptine, a serotonin (5-hydroxytryptamine, 5-HT) reuptake enhancer (see Wilde and Benfield, 1995 for review), effectively blocks chronic stress-induced hippocampal atrophy (Watanabe et al., 1992b), suggesting that manipulations of the serotonergic tone could counteract the effects of stress on CA3 dendritic morphology.

In the present study, we compared the efficacy of (±)-tianeptine, at a dose lower than previously tested, with that of two 5-HT reuptake antagonists, fluoxetine and fluvoxamine, in preventing stress-induced hippocampal atrophy. In addition, we further explored the actions of tianeptine by investigating if its enantiomers have a differential effect in counteracting stress effects and if the racemic compound could, in addition to preventing the atrophy, reverse it, once the CA3 apical dendrites were already remodeled. Finally, we tested the hypothesis that an indirect increase in γ-aminobutyric acid (GABA) inhibitory tone in the hippocampus, induced by the benzodiazepine agonist adinazolam, could counterbalance the increased excitatory transmission generated by the chronic stress paradigm. In fact, $GABA_A$ receptors play a major role in inhibiting excitatory processes in the hippocampus (Freund and Buzsaki, 1996); since apical dendritic atrophy involves a synergy between adrenal steroids, excitatory amino acids and NMDA receptors (Magariños and McEwen, 1995b), the role of agents that increase internal hippocampal inhibition might be to antagonize the stress-induced dendritic atrophy.

2. Materials and methods

2.1. Experimental animals

Male Sprague–Dawley rats (CD strain, Charles River, Kingston, NY) weighing 290–300 g at the beginning of the experiments were housed in groups of three in hanging metallic cages with ad libitum access to food and tap water. Animals were maintained in a temperature and light controlled environment (12/12 h light/dark cycle, lights on from 0700 to 1900 h). The rats were adapted to daily handling during the week after delivery. Experimental animals were weighed and randomly assigned to experimental groups. Experiments were performed during the light period of the cycle. All animal experimentation was conducted in accordance with The National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Experimental treatment groups

2.2.1. Experiment 1

(1) *Unstressed control group* ($n = 6$): rats remained in their cages except for the daily administration of i.p. saline injections. (2) *Chronic restraint-stressed group* ($n = 6$): for 21 days, rats received daily i.p. injections of saline prior to restraint stress sessions, which consisted of 6 h/day (1000 to 1600 h) restraint in wire mesh restrainers secured at the head and tail ends with clips. During the restraint sessions, the rats were placed in their home cages. (3) *Chronic tianeptine-treated restraint-stressed group* ($n = 6$): these rats received i.p. injections of 10 mg/kg (±)-tianeptine (Servier, France) in saline prior to each restraint stress session. This dose is lower than that previously shown to effectively block stress-induced atrophy (15 mg/kg per day), Watanabe et al., 1992b). The tianeptine dose we chose has been shown to be effective in preventing stress-induced memory deficits (Conrad et al., 1996), reducing extracellular 5-HT levels in hippocampus (Koshikawa et al., 1998) and increasing 5-HT uptake in synaptosomes of rat cerebral cortex and hippocampus (Mocaër et al., 1988). (4) *Chronic fluoxetine-treated restraint-stressed group* ($n = 6$): these rats received i.p. injections of 10 mg/kg fluoxetine (Eli Lilly, Indianapolis) in saline prior to each restraint stress session. This dosage was chosen because it has been shown to produce effects on 5-HT synthesis, extracellular 5-HT concentrations and 5-HT metabolism in discrete regions of the rat brain (Hwang et al., 1980; Trouvin et al., 1993; Rutter et al., 1994; Mück-Seler et al., 1996).

2.2.2. Experiment 2

(1) *Unstressed control group* ($n = 6$): rats remained in their cages except for the daily administration of i.p. saline injections. (2) *Chronic restraint-stressed group* ($n = 6$): same as in Experiment 1 (see above). (3) *Chronic (−)-tianeptine-treated restraint-stressed group* ($n = 6$): these rats received i.p. injections of 10 mg/kg (−)-tianeptine (S16190, Servier) in saline prior to each restraint stress session. (4) *Chronic (+)-tianeptine-treated restraint-stressed group* ($n = 6$): these rats received i.p. injections of 10 mg/kg (+)-tianeptine (S16191, Servier) in saline prior to each restraint stress session.

2.2.3. Experiment 3

(1) *Unstressed control group* ($n = 6$): rats remained in their cages except for the daily administration of i.p. saline injections. (2) *Chronic restraint-stressed group* ($n = 6$): rats received restraint stress sessions as described above for 5 weeks. (3) *Chronic corticosterone treatment* ($n = 6$): these rats received 400 μg/ml corticosterone in tap water over 5 weeks. Corticosterone (Aldrich, Milwaulkee, WI) was dissolved in a small volume of 100% ethanol and then diluted with tap water. All other groups of rats received the same amount of ethanol in their drinking water (2.4%). We have previously demonstrated that the oral administration of corticosterone is as effective as repeated stress in inducing apical atrophy of CA3 principal neurons (Magariños et al., 1998). We chose this passive administration of corticosterone instead of the stress paradigm to test the therapeutic effect of (±)-tianeptine because corticosterone is a major mediator of the stress-induced atrophy and we

wanted to dissociate the corticosterone effects from the complex stress stimulus. (4) *Chronic corticosterone treatment plus (±)-tianeptine treatment during weeks 4 and 5* ($n = 6$): rats received the full 5 weeks of corticosterone treatment plus i.p. injections of 10 mg/kg (±)-tianeptine in saline during weeks 4 and 5. Rats receiving corticosterone in the drinking water (group 3) received saline as vehicle injections. Because apical atrophy is reversible after the termination of inducing treatment (Conrad, Magariños, LeDoux and McEwen, unpublished observations), it was necessary to continue the corticosterone administration while administering tianeptine. Repeated restraint stress for five weeks was used as a positive control for the induction of hippocampal atrophy.

2.2.4. Experiment 4

(1) *Unstressed control group* ($n = 6$): rats remained in their cages except for the daily administration of i.p. saline injections. (2) *Chronic restraint-stressed group* ($n = 6$): same as in Experiment 1 (see above). (3) *Chronic fluvoxamine-treated restraint-stressed group* ($n = 6$): these rats received i.p. injections of 10 mg/kg fluvoxamine (Upjohn, Kalamazoo, MI) in saline prior to each restraint stress session. Fluvoxamine was chosen as 5-HT reuptake inhibitor because of its high selectivity for the 5-HT transporter, with only low affinity for 5-HT and noradrenergic receptors (Hyttel, 1994). Doses and treatment durations similar to those used in this study have shown to enhance 5-HT turnover while having no effect on noradrenaline metabolism in frontal cortex (Ben Shachar et al., 1997). (4) *Chronic adinazolam-treated restraint stressed group* ($n = 6$): rats received i.p. injections of 10 mg/kg adinazolam (Upjohn) in saline prior to each restraint stress session. The dose used in this study has been shown to effectively suppress the stress-induced elevation in plasma corticosterone (Lahti et al., 1983).

2.3. Golgi staining procedure

At the end of the treatment period, the rats were deeply anesthetized with Metofane (Pitman-Moore, Mundelein, IL) and transcardially perfused with 150 ml 4% paraformaldehyde in 0.1 M phosphate buffer (pH: 7.4). Adrenals, thymus glands and spleens were removed, cleaned and weighed. Brains were post-fixed in the perfusate overnight. Sections, 100 μm thick, were cut with a Vibratome into a bath of 3% potassium dichromate in distilled water. Sections were then processed according to a modified version of the 'single' section Golgi impregnation procedure (Gabbott and Somogyi, 1984). Briefly, brain sections were incubated in 3% potassium dichromate in distilled water overnight. The sections were then rinsed in distilled water, mounted onto plain slides and a coverslip was glued over the sections at the 4 corners. These slides assemblies were incubated in 1.5% silver nitrate in distilled water overnight in the dark. The following day the slides assemblies were dismantled and the tissue sections were rinsed in distilled water and then dehydrated in 95% followed by absolute ethanol. The sections were then cleared in Histoclear (Americlear), mounted onto gelatinized slides and coverslipped under Permount (Fisher Scientific).

2.4. Data analysis

Slides containing brain sections were coded prior to quantitative analysis; the code was not broken until the analysis was complete. In order to be selected for analysis, Golgi impregnated neurons had to possess the following characteristics: (1) location in the CA3 subregion of the dorsal hippocampus; (2) dark and consistent impregnation throughout the extent of all of the dendrites; (3) relative isolation from neighboring impregnated cells that could interfere with analysis; and (4) a cell body in the middle third of the tissue section in order to avoid analysis of impregnated neurons which extended largely into other sections. For each brain, 6–8 pyramidal cells from CA3c were selected. Since this hippocampal subregion contains different subtypes of pyramidal neurons with different degrees of apical branching patterns (Fitch et al., 1989), special care was taken in including the same number of neuronal subtypes across experimental animals and experimental groups within each experiment. Each selected neuron was traced at 400× magnification using a light microscope with a camera lucida drawing tube attachment. From these drawings the number of dendritic branch (bifurcation) points within a 100-μm thick section of each dendritic tree was determined for each selected neuron. In addition, the length of the dendrites present in a 100-μm thick section was determined for each dendritic tree using a Zeiss interactive digitizing analysis system. Means were determined for both branch points and dendritic length for each brain and the resulting values were subjected to a one-way analysis of variance (ANOVA) followed by Tukey HSD post-hoc comparisons. A probability level of $P < 0.05$ was used as the criterion for statistical significance.

3. Results

3.1. Effects of (±)-tianeptine and fluoxetine treatments on chronic stress-induced atrophy of CA3 apical dendrites

Results depicted in Fig. 1 show that 21 days of daily restraint stress caused atrophy of apical dendrites of CA3 pyramidal neurons without causing any changes in the branching pattern and total length of basal dendrites. The lack of effect of stress and of drug intervention (see below) on basal dendrite morphology is an important control against non-specific effects of the treatments and suggests a selective action of repeated stress on apical dendritic morphology.

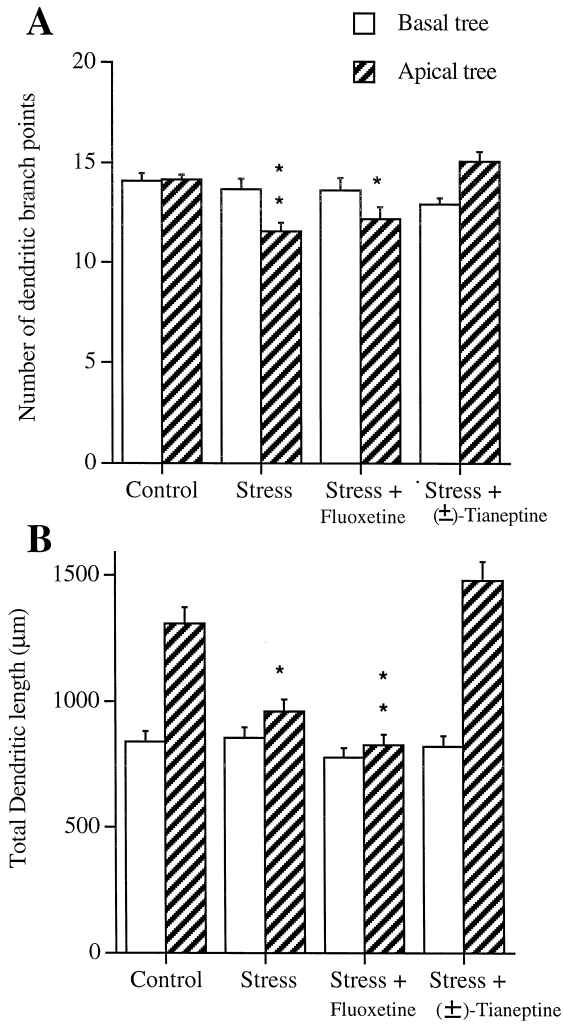

Fig. 1. Effect of repeated restraint stress and cotreatment with fluoxetine and (±)-tianeptine on the number of dendritic branch points (Panel A) and total dendritic length (Panel B) of CA3 pyramidal neurons. After 21 days of daily restraint stress, a decrease in the number of branch points and total dendritic length of CA3 apical dendritic trees was observed. While the antidepressant fluoxetine, a serotonin reuptake antagonist, was without effect, the serotonin reuptake enhancer (±)-tianeptine prevented the hippocampal atrophy induced by stress. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with controls. One-way ANOVA, Tukey post-hoc test. Bars represent means + S.E.M.

Treatment with (±)-tianeptine (10 mg/kg, i.p.) prior to daily restraint stress prevented stress-induced apical dendritic atrophy, whereas a similar course of fluoxetine treatment (10 mg/kg, i.p.) did not have any effect. Interestingly, fluoxetine did not exacerbate the atrophic effect of stress (Fig. 1). The basal dendritic morphology was not affected by stress nor by drug interventions.

3.2. Tianeptine enantiomers

Because tianeptine is available in two stereoisomeric forms, we conducted Experiment 2 to test if either stereoisomer was able to produce the effect found with the racemic mixture in the tianeptine preparation used in Experiment 1. As shown in Fig. 2, 21 days of restraint stress once again reduced apical dendritic branching and dendritic length for CA3 pyramidal neurons, without affecting basal dendritic branching or length. Both the (+)- and (−)-tianeptine enantiomers, at doses of 10 mg/kg, were equally effective at blocking the atrophy caused by repeated restraint stress without affecting the morphology of the basal dendritic tree.

3.3. Ability of (±)-tianeptine to reverse established atrophy

Experiment 3 was designed to explore the therapeutic capability of (±)-tianeptine in the already established

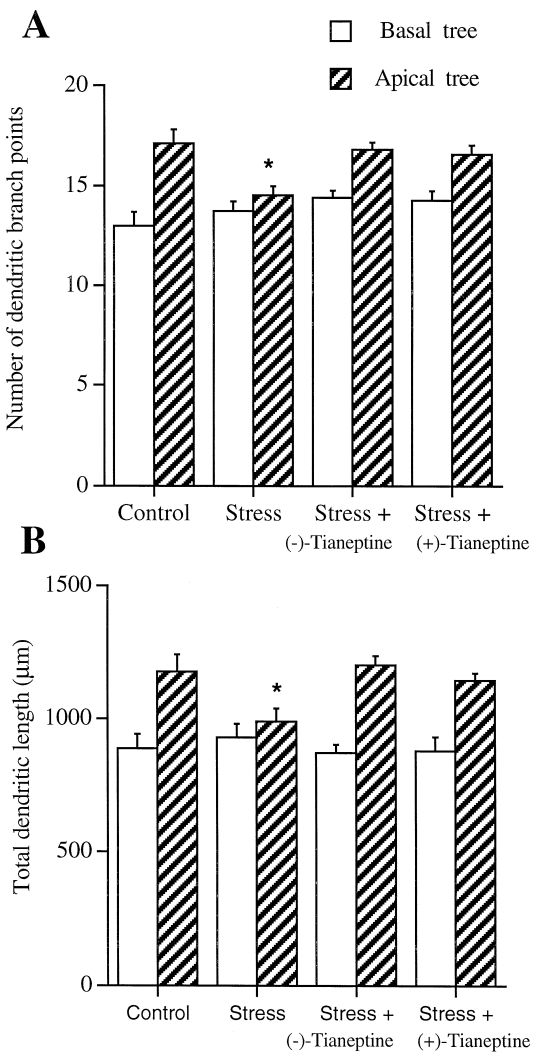

Fig. 2. Effect of tianeptine enantiomers on the number of dendritic branch points (Panel A) and total dendritic length (Panel B) of CA3 pyramidal neurons from rats subjected to repeated restraint stress. Both enantiomers prevented, with a similar strength, the stress-induced reduction in the number of branch points and dendritic length of CA3 apical trees. $^{*}P < 0.01$, compared with controls. One-way ANOVA, Tukey post-hoc test. Bars represent means + S.E.M.

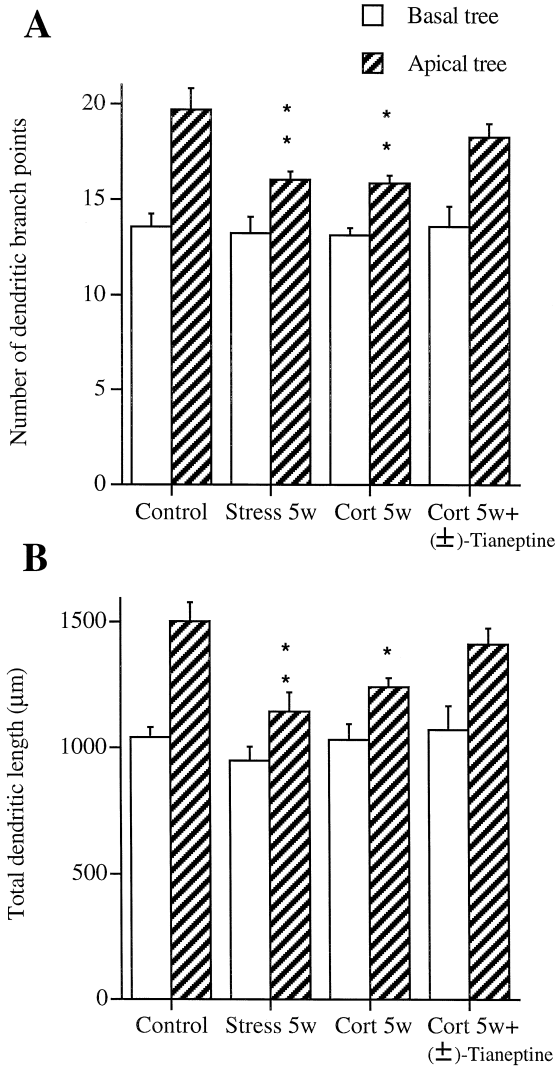

Fig. 3. Effect of repeated stress, corticosterone administration and cotreatment with (±)-tianeptine on the number of dendritic branch points (Panel A) and total dendritic length (Panel B) of CA3 pyramidal neurons. After 5 weeks of daily administration of exogenous corticosterone in the drinking water (400 μg/ml, Corticosterone 5w), animals showed apical dendritic atrophy of CA3 principal neurons similar in magnitude to that obtained after 5 weeks of restraint stress (Stress 5w). The antidepressant (±)-tianeptine administered for weeks 4 and 5 (Corticosterone 5w + (±)-tianeptine) reversed the hippocampal atrophy induced by corticosterone. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with controls. One-way ANOVA, Tukey post-hoc test. Bars represent means + S.E.M.

hippocampal atrophy induced by oral, non-invasive administration of corticosterone. As shown in Fig. 3, both daily restraint stress for 5 weeks as well as corticosterone in the drinking water for 5 weeks caused significant atrophy of apical dendrites of CA3 pyramidal neurons, as measured by the number of branch points. The total dendritic length of the apical arbors were also significantly decreased by both treatments (Fig. 3). In fact, the effects of corticosterone on these parameters did not differ from those of repeated stress. Moreover, as predicted in the therapeutic model, (±)-tianeptine treatment for the last two weeks of corticosterone administration reversed the atrophy that, based upon past work and the studies described herein, is already evident by the end of 3 weeks of either chronic stress or corticosterone treatment (Fig. 3). The morphology of basal CA3 dendrites remained unaffected by restraint stress, corticosterone treatment or cotreatment with (±)-tianeptine (Fig. 3).

3.4. Effects of fluvoxamine and adinazolam treatments on stress-induced hippocampal atrophy

As observed in Experiments 1, 2 and 3, chronic restraint stress produced reduced apical dendritic branching and

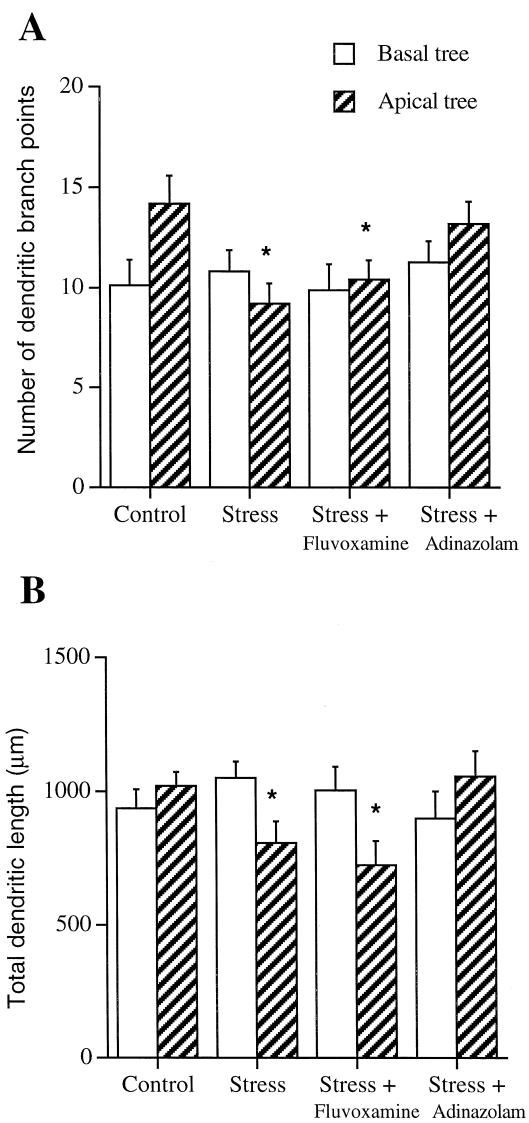

Fig. 4. Effect of adinazolam and fluvoxamine on the branching pattern (Panel A) and total dendritic length (Panel B) of CA3 pyramidal neurons from rats subjected to repeated restraint stress. Only adinazolam prevented the stress-induced reduction in the number of apical dendrites and total dendritic length of CA3 principal neurons. $^{*}P < 0.05$, compared with controls. One-way ANOVA, Tukey post-hoc test. Bars represent means + S.E.M.

Table 1
Effects of chronic restraint stress, oral corticosterone (400 μg/ml) and cotreatment with (±)-tianeptine (10 mg/kg) on final body weights and organ weights

Treatment	Final body weight (g)	Spleen (mg/100 g body weight)	Thymus (mg/100 g body weight)	Adrenals (mg/100 g body weight)
Control	449.67 ± 3.99	175.74 ± 5.56	105.03 ± 7.54	13.36 ± 0.84
Stress 5w	408.00 ± 9.27	167.98 ± 6.05	103.26 ± 11.55	14.46 ± 1.01
Corticosterone 5w	304.17 ± 19.81[a]	124.00 ± 8.69[a]	32.22 ± 5.07[b]	5.61 ± 0.25[b]
Corticosterone 5w + (±)-tianeptine 2w	324.00 ± 10.69[a]	127.99 ± 6.46[a]	49.82 ± 4.58[b]	4.24 ± 0.46[b]

Each value represents the mean ± S.E.M.
[a,b]Significantly different from control group, $P < 0.01$ and 0.005, respectively; one-way ANOVA, Tukey post-hoc test.

length of CA3 pyramidal neurons (Fig. 4). Whereas fluvoxamine mimicked fluoxetine in that it failed to block or exacerbate atrophy, adinazolam treatment prevented apical dendritic atrophy (Fig. 4). Basal dendritic branching and length were unaffected by stress or by drug treatments.

3.5. Stress, corticosterone and drug effects on body and adrenal weights

Body and organ weight data from Experiments 1, 2 and 4 revealed a somewhat lower body weight gain in stressed animals that was not significantly modified by drug treatments (data not shown). Daily restraint stress for three weeks did neither affect adrenal to body weight ratio nor thymus or spleen to body weight ratios (data not shown). In the case of Experiment 3, Table 1 shows that 5 weeks of daily restraint stress did not affect the organ weights corrected for body weight. Chronic treatment with corticosterone significantly decreased final body weights and (±)-tianeptine cotreatment did not have any additional effect (Table 1). Moreover, as shown in Table 1, corticosterone treatment significantly decreased spleen, thymus and adrenal weights, corrected for body weights, compared to controls.

4. Discussion

The results of these experiments confirm and extend our previous findings that chronic administration of (±)-tianeptine, a reputed in vivo 5-HT reuptake enhancer, prevents the dendritic atrophy of CA3 pyramidal neurons induced by repeated stress (Watanabe et al., 1992b). Since hippocampal 5-HT release is increased in response to stress (see Chaouloff, 1993; McKittrick and McEwen, 1996 for reviews) and repeated stress leads to sensitization of some 5-HT-mediated functions (Kennett et al., 1985; Ohi et al., 1989), these findings would support the hypothesis that the enhancement of 5-HT uptake and the consequent decreased availability of synaptic 5-HT could contribute to the prevention of the stress-induced atrophy. Chronic administration of (±)-tianeptine counteracts certain biochemical and behavioral consequences of stress such as the evoked activation of the hypothalamic–pituitary–adrenal axis (Delbende et al., 1991) and the memory impairment that accompanies the 21 days restraint stress paradigm used in the present report (Luine et al., 1994; Conrad et al., 1996). These findings make tianeptine a potentially useful agent in situations where there may be a temporary cognitive impairment due to hippocampal dendritic remodeling. The atrophy of hippocampal dendrites in animal models of stress may be related to atrophy of the human hippocampus associated with recurrent depressive illness (Sheline et al., 1996), post-traumatic stress disorder (Bremner et al., 1995; Gurvits et al., 1996) and mild cognitive impairment in aging (Convit et al., 1997). Insofar as any of these forms of atrophy represent potentially reversible changes in neuronal structure (McEwen, 1997), and not neuronal loss (Sapolsky, 1996), an agent like tianeptine may have therapeutic benefits. In testing this hypothesis, we found that (±)-tianeptine not only prevents but also reverses the structural remodeling when given daily for two weeks while the atrophy-inducing stimulus (i.e., corticosterone in the drinking water) is continued.

Although a dissociation between the actions of tianeptine enantiomers has been described recently, we found that the effects of the (+) and (−) enantiomers were indistinguishable, at the dose employed, in terms of their potency at preventing repeated stress-induced apical atrophy. The attenuation of 5-hydroxytryptophan (5-HTP)-induced behavior by (±)-tianeptine in rats has been mimicked by acute administration of the (−) enantiomer alone (Oluyomi et al., 1997), while the antidepressant effects of (±)-tianeptine in the rat model of learned helplessness is also substantially dependent on the (−) isomer (Lacroix et al., 1996). However, neither of these studies investigated the effects of chronic administration of tianeptine enantiomers, and it is possible that the (+) and (−) forms might have different profiles of both 5-HT- and non-5-HT-related actions following chronic administration. For example, the inhibitory effects of acute (±)-tianeptine on the 5-HTP induced 'serotonin syndrome' is not maintained after chronic treatment in spite of the persisting inhibition of the hippocampal 5-HT efflux evoked by 5-HTP administration (Koshikawa et al., 1998). In addition, chronic, but not acute, treatment with (±)-tianeptine antagonizes the

behavioral manifestations of the olfactory bulbectomized rat model of depression (Kelly and Leonard, 1994). The relevance of studies using chronic tianeptine treatments is emphasized by the fact that the clinical efficacy of this compound in depressed patients is evident only after several weeks of treatment (Wilde and Benfield, 1995).

Chronic stress causes a very selective atrophy that is confined to the apical dendritic tree of the hippocampal CA3 pyramidal cells and is not accompanied by signs of reactive astrocytosis or cell death (Watanabe et al., 1992a,b; Magariños and McEwen, 1995a,b; Sunanda et al., 1995). Within the CA3 subregion, the stratum radiatum and the lacunosum moleculare are the layers to which the distal apical dendritic trees of the pyramidal neurons project and where the stress-induced atrophy is detected. These strata are heavily innervated by 5-HT terminals and possess high concentration of 5-HT transporters (see Mongeau et al., 1997 for review). Interestingly, McKittrick et al. (1996) have found that in a rat model of psychosocial stress that leads to CA3 apical atrophy in both dominant and subordinate animals, stress also induces a downregulation of 5-HT transporters in the stratum pyramidale, stratum radiatum and stratum lacunosum moleculare of the CA3 subregion. If the downregulation of 5-HT transporters is associated with decreased reuptake of 5-HT, (±)-tianeptine could be acting by reversing this effect of chronic stress.

Tianeptine is effective at blocking other consequences of chronic stress as we have recently shown that the 5-HT reuptake enhancer partially reverses the presynaptic ultrastructural rearrangement caused by chronic restraint at the level of the mossy fiber terminals (Magariños and McEwen, 1997; Magariños et al., 1997). These fibers carry the major excitatory input from the dentate gyrus to the proximal apical dendrites of the CA3 pyramidal neurons. The clustering of clear synaptic vesicles near active sites and the mitochondrial hypertrophy observed in mossy fiber terminals after chronic stress could be a morphological indication of an increased excitatory tone that is compensated by a retraction of the distal dendritic field of the apical tree. The partial reversal induced by (±)-tianeptine argues in favor of a role for containing the excitatory exacerbation induced by stress.

While considering the possible mechanisms involved in the stress-induced atrophy a complex interaction among glucocorticoids, excitatory amino acids and serotonin is suggested by this and our previous studies (McKittrick et al., 1996; Magariños and McEwen, 1995b, 1997; Magariños et al., 1998). Evidence from the literature supporting these interactions include the glucocorticoid-induced facilitation of 5-HT turnover in rat midbrain and cortex and the 5-HT_{1A} receptor-mediated hyperpolarization of hippocampal CA1 pyramidal neurons (Azmitia and McEwen, 1969; Singh et al., 1990; De Kloet et al., 1998). Adrenal steroids have also been shown to increase hippocampal NMDA receptor subunit expression (Weiland et al., 1997), induce the enhancement of glutamate release in hippocampus during stress (Lowy et al., 1993; De Bruin et al., 1994; Moghaddam et al., 1994) and activate presynaptic kainate receptors on mossy fiber terminals (Watanabe et al., 1995). Furthermore, adrenal steroids cause a reduction of the inhibitory 5-HT_{1A} receptor expression in hippocampus (Mendelson and McEwen, 1992; Meijer et al., 1997). In vitro electrophysiological studies have demonstrated that hippocampal slices from chronically adrenalectomized rats subjected to a chronic overexposure to corticosterone show a decreased 5-HT_{1A} receptor-mediated hyperpolarization in CA1 pyramidal neurons (Beck et al., 1996). Taken together, these actions of adrenal steroids may contribute to a stress-induced enhancement of excitation and a reduction of inhibition in hippocampus. In fact, we have shown that excitatory amino acids acting through NMDA receptors constitute one of the mechanisms involved in the atrophy of CA3 apical dendrites (Magariños and McEwen, 1995b). A possible mechanism connecting 5-HT to NMDA receptors is the reported enhancement of NMDA receptor currents produced by 5-HT_{2A} receptor-mediated phosphorylation of NMDA receptor subunits (Rahman and Neuman, 1993). We are currently investigating this possibility.

Neither fluoxetine nor fluvoxamine, both selective 5-HT reuptake inhibitors, blocked the stress-induced dendritic remodeling at the doses employed. Surprisingly, neither drug exacerbated the dendritic atrophy, which might have been expected if increased extracellular 5-HT plays a critical role in mediating the morphological changes. One explanation might be that the stress-induced 5-HT release is sufficient to cause the hypothesized changes in NMDA receptor activity described above, and that further enhancement of 5-HT levels by blocking reuptake does not have any additional effect. The fact that we did not observe an enhanced apical dendritic atrophy after fluoxetine treatment could be considered in light of clinical studies showing that chronic administration of fluoxetine for 3 weeks to depressive patients is associated with a less hyperactive hypothalamic–pituitary–adrenal axis since reductions in cerebrospinal fluid concentrations of corticotropin-releasing hormone and arginine vasopressin have been described (De Bellis et al., 1993). Furthermore, recent findings show that 21 days of fluoxetine administration blocks the increased secretion of corticosterone in response to an acute fluoxetine challenge but not the swim stress-induced corticosterone secretion in rats (Duncan et al., 1998). In fact, the corticosterone surge in response to stress has been identified as one critical mediator of the stress-induced atrophy of CA3 pyramidal dendrites (Magariños and McEwen, 1995b). These observations suggest that, while not affecting corticosterone release induced by stress, chronic fluoxetine administration does not seem to produce a further increase in stress-induced corticosterone secretion. Therefore, a potentiation of hippocampal atrophy associated with the steroid hypersecretion would not be expected.

Besides inhibiting 5-HT reuptake, chronic fluoxetine administration also affects the biochemistry of catecholamines, i.e., enhances the hippocampal norepinephrine release in response to stress and stimulates the responsivity of catecholaminergic neurons in the locus coeruleus to environmental stimuli (Page and Abercrombie, 1997). Although a role for catecholamines in the stress-induced atrophy has not yet been investigated, it is interesting to note that a more selective serotonin reuptake inhibitor like fluvoxamine was also ineffective in either blocking or potentiating dendritic atrophy at the dose used. This drug has a more selective profile than fluoxetine as a 5-HT reuptake inhibitor, with negligible affinity for noradrenaline receptors (Hyttel, 1994).

Our finding that adinazolam is effective in preventing stress-induced atrophy suggests that enhancing activity of the inhibitory GABA-benzodiazepine system in hippocampus might counteract the increased excitation produced by stress and glucocorticoids which leads to dendritic atrophy. Adinazolam is a triazolobenzodiazepine that possesses anxiolytic as well as antidepressant properties (Amsterdam et al., 1986). The effectiveness of adinazolam to prevent stress-induced atrophy may be related to its ability to suppress the stress-induced increases in plasma corticosterone in rats (Lahti et al., 1983), since our previous studies have demonstrated that cyanoketone, which also inhibits stress-induced corticosterone surges, can similarly prevent the hippocampal atrophy induced by stress (Magariños and McEwen, 1995b).

The antidepressant component of adinazolam actions is believed to be mediated by sites other than the GABA receptor complex, however. While adinazolam has no effect on 5-HT or noradrenaline reuptake, chronic administration of the drug downregulates 5-HT_2, but not β-adrenoceptors in rat cerebral cortex (Lahti et al., 1983). Recent microvoltammetric studies in freely moving rats show that acute adinazolam treatment is associated with increased serotonergic release and a biphasic response (immediate increase and then decrease) in extracellular noradrenaline levels in the hippocampal CA1 region (Broderick et al., 1998). Earlier reports also associated the antidepressant activity of a sustained, very low, dose of adinazolam administration to anesthetized rats with sensitization of pyramidal cells to iontophoretically applied 5-HT, and hence to a heightened 5-HT neurotransmission (Turmel and De Montigny, 1984). However, chronic adinazolam treatments similar to the one used in this report failed to affect 5-HT turnover in the rat brain (O'Connor et al., 1985). It remains to be determined if adinazolam prevention of stress-induced atrophy is associated with changes in serotonergic and/or noradrenergic neurotransmission.

In summary, tianeptine is an effective agent in both blocking atrophy of dendrites of hippocampal neurons produced by repeated restraint stress and reversing atrophy produced by continuous glucocorticoid administration, thus emphasizing once again that dendritic atrophy is a reversible process that is not synonymous with neuronal damage. Whereas, the mechanism for dendritic atrophy involves multiple neurochemical systems as well as circulating glucocorticoids, the findings of the present study are consistent with a role for increased synaptic reuptake of 5-HT in the actions of tianeptine. However, the role of other neurotransmitter systems in actions of tianeptine, as well as those of adinazolam, must be considered as well. Future studies employing 5-HT receptor antagonists will address specifically the direct or indirect role of 5-HT in causing stress-induced dendritic atrophy.

Acknowledgements

We thank Dr. Christina McKittrick for her critical reading of this manuscript. This study was supported by NIH Grant MH 41256 to BMc and by Servier, France and Upjohn, Kalamazoo, MI, USA. We also acknowledge Eli Lilly (Indianapolis, IN) for generously providing fluoxetine and Upjohn for providing fluvoxamine and adinazolam.

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