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An Animal Model for Studying Therapeutic Drugs against Post-Traumatic Stress Disorder

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An animal model for the evolvement of post-traumatic stress disorder (PTSD) was developed by simulating the hormonal consequences of prolonged stress via the continuous administration of corticosterone by subcutaneously implanted sustained-release pellets. Behavioral, morphological, and biochemical effects were recorded and analyzed. This model has shown cognitive deficits as well as hippocampal damage in the rat similar to those found in PTSD patients. The model was also used to test a therapeutic treatment against stress-induced brain damages. Concomitant treatment with the L-type calcium channel blocker, nimodipine, protected young rats from corticosterone-induced morphological brain changes but not cognitive impairments. The proposed animal model may be useful for testing the efficacy of various neuroprotective drugs. Development of an effective drug treatment for use after a traumatic event and through the trauma period might prevent permanent brain damage and the development of PTSD.

Introduction

Post-traumatic stress disorder (PTSD) develops in approximately 18% of trauma victims during the trauma period and the prolonged stress that follows. No preventive treatment currently exists for PTSD. The development of an animal model is necessary for the study of therapeutic and prophylactic treatments. Historically, effective therapeutic treatments have been developed for diseases only if adequate animal models were available. Unfortunately, no such animal model for PTSD has been validated and tested.

During stress, plasma levels of glucocorticoid hormones are elevated both in animals and in humans. Prolonged high levels of these hormones or long-term stressful conditions have been shown to accelerate age-dependent degeneration of hippocampal neurons.[1,2] Magnetic resonance imaging volumetric analysis studies of PTSD patients have also found diminished hippocampal volume.[3] Hippocampal changes have been shown in many studies to be correlated with cognitive impairments.[4] In an attempt to develop an animal model for the development of PTSD, we simulated the hormonal consequence of prolonged stress by the continuous administration of corticosterone, using subcutaneously implanted sustained-release (SR) pellets. Behavioral (cognitive impairment tests), morphological (brain histology), and biochemical (brain acetylcholine levels) effects of this treatment were subsequently recorded and analyzed. The neuroprotective potential of the L-type calcium-channel blocker nimodipine was examined when given concomitantly with the glucocorticoid treatment.

The rationale for these studies was the following. Under stress, plasma levels of glucocorticoid hormones are elevated. Prolonged exposure to stress (or glucocorticoids) leads to morphological brain changes at the hippocampal formation (hippocampal deterioration is also found in PTSD patients). These changes correlate with cognitive impairments (cognitive impairments are also found in PTSD patients). Altered calcium homeostasis plays a major role in brain neuronal damage. A connection was established between glucocorticoids and voltage-activated calcium influx in aged hippocampal neurons.[5]

Various aspects of our studies, which were designed and performed according to the above rationale, have been published during the last 7 years.[6–11] The hypothesis of the present manuscript, advocating the validity of the procedure as an animal model for PTSD, is based on the following arguments. Sustained high levels of glucocorticoids may simulate the hormonal state under prolonged stress. Because prolonged stress might be the basis for the development of PTSD, any therapeutic drug that might exhibit neuroprotective effect during the simulated "stress" period might be of value in preventing the development of PTSD. Because the last stage of neurodegeneration involves high influx of calcium,[5] the L-type calcium-channel blocker nimodipine was our first choice for testing this hypothesis. In the current manuscript, the methods for establishing and testing the model will be specified along with part of the data that is relevant to the animal model and its validity.

Materials and Methods

Male Fischer 344 rats, 3 months old upon arrival to the laboratory, were used. Four 200-mg corticosterone SR pellets (Innovative Research of America, Toledo, OH), which released over 90 days, or placebo pellets were implanted subcutaneously 4 cm lateral of the median line under general anesthesia in each rat. Nimodipine SR 20-mg pellets, which released over 21 days, or placebo pellets were implanted at the nape of the neck in each rat every 3 weeks (four times).

Thirty-two rats were randomly assigned into two treatment groups: (1) corticosterone-treated (four SR pellets to each rat) or (2) placebo-treated (same pellets without drug). Each of the above groups was further subdivided into (a) Nimodipine-treated (four consecutive implantations every 3 weeks) or (b) placebo-treated (same pellets without drug). Thus, four treatment groups ($N = 8$ in each) were obtained.

Behavioral Testing

Four weeks following termination of the corticosterone/nimodipine treatments, rats participated for 6 days in a radial-arm-

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maze (RAM) learning test. Time to complete the task and number of errors were recorded. The histology went as follows: rats were anesthetized and perfused (saline followed by a formaldehyde mixture). Brains were fixed, dehydrated, and paraffin embedded. Six-micrometer coronal sections, at the hippocampal level, were stained with hematoxylin and eosin. Quantitative analysis of morphological changes was carried out by counting the number of damaged cells, as well as the total number of cells, in various hippocampal regions.

Results

During the 90 days of treatment, corticosterone levels in the two corticosterone-treated groups were kept in the range of 150 to 350 ng/ml, corresponding to the range found normally in rats under mild stress. The two control groups were shown to have normal corticosterone levels.[8] Under these conditions, the corticosterone without nimodipine group exhibited severe morphological changes in district hippocampal areas (Table I). Nimodipine, administered concomitantly, provided almost complete protection against the hormonal-induced brain damages. Damage in the CA3 region was less pronounced, and the difference between groups was not statistically significant.

In the behavioral tests, the cognitive parameter (number of correct entries out of the first eight in the RAM) improved similarly during the 6 training days in all four groups. Significant differences were found between groups only in the time parameter (Fig. 1, $p = 0.025$, $F_{1,25} = 5.67$, ANOVA). Using simple main effect contrast, a statistically significant difference was found between groups not treated with nimodipine (placebo versus corticosterone: $t(88) = 3.75$, $p < 0.001$). It seemed that nimodipine treatment abolished this difference (by slowing down the placebo group and speeding up the corticosterone-treated group).

Discussion and Conclusions

Our data show that inducing sustained high levels of glucocorticoids in rats may lead to an animal model for PTSD, which can be used for the study of potential therapeutic drugs. The model produces five effects that resemble PTSD in humans: first, hippocampal morphological damage, induced by prolonged high corticosteroid levels, imitates injuries found in PTSD patients. Second, cognitive impairments correlated with the morphological changes.[6] Third, selective injury was found in various hippocampal sub-regions.[8] Fourth, the injury was enhanced in old rats as compared with young ones.[7] Finally, cholinergic hypofunction was measured in corticosterone-treated rats.[10,11]

TABLE I

PERCENTAGE OF DAMAGED CELLS IN CA1, CA3, AND CA4 HIPPOCAMPAL SUBFIELDS, FOLLOWING 3 MONTHS OF PLACEBO/CORTICOSTERONE/NIMODIPINE TREATMENTS IN THE FOUR EXPERIMENTAL GROUPS OF YOUNG RATS

Treatment Group	CA1	CA3	CA4
Placebo + placebo	3.7 ± 1.4	3.1 ± 1.0	5.8 ± 3.2
Placebo + nimodipine	2.2 ± 0.5	8.6 ± 4.1	7.1 ± 2.1
Corticosterone + placebo	22.6 ± 7.9[a]	22.7 ± 8.9	28.3 ± 8.4[b]
Corticosterone + nimodipine	3.2 ± 0.8	5.9 ± 2.9	2.1 ± 1.9

[a] $p < 0.04$ compared with all three groups.
[b] $p < 0.03$ compared with all three groups.

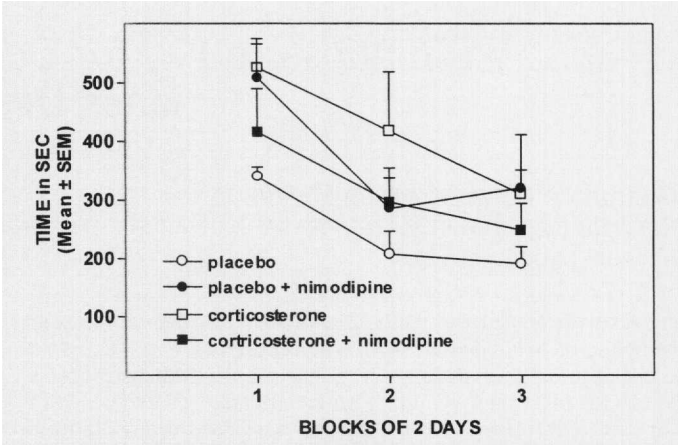

Fig. 1. Total time to finish the RAM task (average of 6 days training) in the four experimental groups following 3 months of treatment ($N = 8$ in each group). $P < 0.001$ only for the comparison between placebo and corticosterone without nimodipine.

The L-type calcium channel blocker nimodipine protected the hippocampal formation but did not prevent the cognitive impairments caused by the prolonged exposure to corticosteroids. The intervention of the calcium channel blocker at a late stage during the degeneration cascade might be enough to preserve the morphology of the neurons but not their function. Other drugs with potential neuroprotective action should be tested using the present animal model. Any drugs exhibiting encouraging results in this animal model might be considered for further testing as a therapeutic agent following traumatic events. Such treatment might be able to prevent the development of PTSD.

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