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Research article

Chronic restraint increases apoptosis in the hippocampus of rats with high responsiveness to fear stimuli

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HIGHLIGHTS

- High anxiety rats (HR) have increased stress-induced apoptosis in the rat hippocampus.
- Chronic corticosterone increases apoptosis of HR and low anxiety rats.
- Higher susceptibility to stress predisposes to increased apoptosis.

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ABSTRACT

This study examined the effects of chronic restraint stress and corticosterone treatment on the apoptosis-related processes in the dentate gyrus of the rat hippocampus. This study compared high (HR) and low anxiety rats (LR) (as defined by their behaviour during the contextual fear test, i.e., the duration of a freezing response was the discriminating variable). The results demonstrate that chronic restraint stress increased the number of caspase-3 immunoreactive cells in the HR group, whereas repeated corticosterone treatment increased the number of caspase-3 immunoreactive cells in both the HR and LR groups. This finding suggests that higher susceptibility to fear stimuli predisposes rats to increased apoptosis in the hippocampus after exposure to chronic stressors. This new animal model of HR and LR rats can be used to study the mechanisms underlying the relationship between higher levels of anxiety and greater vulnerability to stress.

1. Introduction

Both chronic exposure to stress and corticosterone treatment may negatively affect brain function, including emotional processes. The hippocampus serves a pivotal role in cognition, emotion regulation, and hypothalamus–pituitary–adrenal axis (HPA) control [14,24]. Many studies have demonstrated that the hippocampus, a structure enriched with corticosteroid receptors, is a brain region vulnerable to prolonged exposure to stress or corticosteroids [15,16,18]. Exposure to chronic stress induces dendritic atrophy and neuronal death, which cause behavioural impairments [19,25,26]. Hippocampal atrophy is considered to be one of the more reliable neuropathological findings in depression [15]. One of the primary mechanisms responsible for neuronal atrophy may be excessive apoptosis, i.e., programmed cell death [6,29]. Caspases belong to a large family of cysteine proteases, and caspase-3 is the key effector involved in the mitochondrial apoptotic pathway [22].

Stress is thought to induce apoptosis in many ways, including an increase in plasma corticosterone and local glutamate release [14,30]. Although the contribution of individual sensitivity to fear stimuli on the stress-induced behavioural and neuropathological effects appears obvious, there is a scarcity of relevant preclinical models for studying this phenomenon. Recently, we developed a model of high (HR) and low anxiety rats (LR) that are divided according to their behaviour during a contextual fear test (i.e., the duration of a freezing response was the discriminating variable). We demonstrated that HR rats under stressful conditions adopt an avoidance strategy more often than LR animals (which is expressed, for example, as a longer freezing time during the conditioned fear test and increased immobility time during the Porsolt test) and are more susceptible to stressful environmental challenges [9–11].

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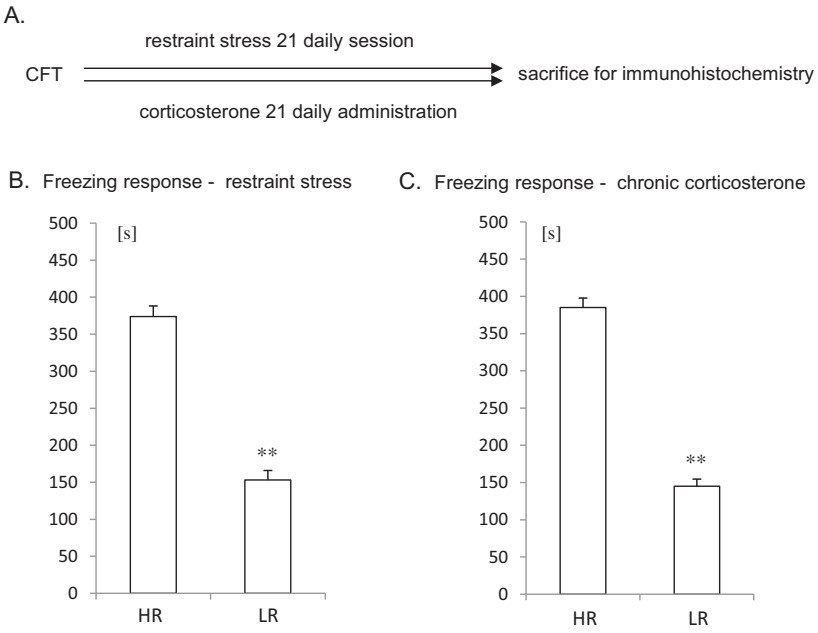

Fig. 1. (A) Experimental scheme. CFT – conditioned freezing test. The data are shown as means (+) SEM. (B) HR – high anxiety rats ($n = 15$); LR – low anxiety rats ($n = 16$) (test performed before restraint stress, qualification to experimental groups) (C) HR – high anxiety rats ($n = 14$); LR – low anxiety rats ($n = 14$) (test performed before chronic corticosterone, qualification to experimental groups).

This study examined the effects of chronic restraint stress and corticosterone treatment on apoptosis-related processes in the dentate gyrus of the rat hippocampus and compared rats with different fear-conditioned response strengths. The behavioural data obtained from the same groups of animals have been previously published [23,28]. These publications demonstrate that HR rats exposed to chronic restraint and corticosterone had increased immobility times during the Porsolt test and enhanced anxiety-like behaviour (a decreased anti-thigmotactic index in the open field test) compared with LR rats.

2. Materials and methods

All experiments were performed in accordance with the European Communities Council Directive of November 24, 1986 (86/609 EEC). The Local Committee for Animal Care and Use at the Medical University of Warsaw, Poland approved all of the experimental procedures.

Male Wistar adult rats (6–8 weeks old) purchased from the stock of the Centre for Experimental Medicine, Medical University of Białystok, Poland, were housed under standard laboratory conditions with a 12 h light/dark cycle (lights on at 7 a.m.) at a constant temperature (21 ± 2 °C). The fear-conditioning test was performed in experimental cages (36 cm × 21 cm ×20cm, w/l/h) with constant white noise conditions (65 dB). The experiment was performed on three consecutive days. On the 1st day, the animals were individually placed in a training box for 2 min to adapt to the experimental conditions. On the 2nd day, after 5 min of habituation to the training box, the animals underwent a fear conditioning procedure that consisted of each animal receiving three foot shocks (stimulus: 0.7 mA, 1 s, repeated every 59 s). The conditioned fear was tested on the 3rd day (test day) by re-exposing rats to the testing box and recording their freezing response for 10 min. Freezing was measured by infra-red photo beams with a 10 Hz detection rate controlled by fear conditioning software (TSE, Bad Homburg Germany) [23,28]. According to the duration of their context-induced freezing responses during the Conditioned Freezing Test (10 min long) [28], the animals were divided into a low anxiety (LR) group (comprised of rats with a total freezing response duration of one SEM or more below the mean (260.17–21.8 s, i.e., <238.37)) and a high-anxiety (HR) group (comprising rats with a total freezing response duration of one SEM or more above the mean (260.17 + 21.8 s, i.e., >281.97)) (Fig. 1B).

The rats were then exposed to 21 sessions of chronic restraint stress (29 days, with a break on the weekends) in restraint hemicylinders for 3 h per session with one session per day as previously described in detail [28]. The control groups remained in their home cages, and these rats were only handled for 5 min per day (Fig. 1A). Chronic restraint stress decreased rat activity in the Porsolt test and reduced body weight more in the HR group [28]. On the last day, 48 h after the last immobilisation session and 45 min after the test session of the Porsolt test was conducted, the animals were decapitated and their brains were removed and frozen at −70 °C for immunochemistry.

Separate groups of animals were used for chronic corticosterone administration. Male Wistar adult rats (6–8 weeks old) from the stock of the Centre for Experimental Medicine, Medical University of Białystok, Poland, were housed under standard laboratory conditions. The rats were also divided into LR (265.04–24.95, i.e., <240.09) and HR groups (265.04 + 24.95, i.e., >289.99) [23] according to the duration of their context-induced freezing responses (Fig. 1C). The rats received one s.c. injection of corticosterone (20 mg/kg) per day for a total of 21 injections (29 days, with a break on the weekends) (Fig. 1A). The control groups received vehicle injections (1 ml/kg sesame oil). Chronic corticosterone increased rat immobility in the Porsolt test and reduce body weight more in the HR group [23]. On the last day, 48 h after the last corticosterone injection and 45 min after the test session of the Porsolt test was conducted, the animals were decapitated and their brains were removed and frozen at −70 °C for immunochemistry.

Immunocytochemical staining for cleaved caspase-3-expressing cells was performed on hippocampal slide-mounted frozen brain sections: AP (−) 3.14 mm. Coronal 18-μm cryostat sections identified with the rat brain atlas [21] were cut, mounted on silane-coated slides and fixed in methanol for 7 min. Two slices from each section were utilised for cleaved caspase-3 immunostaining. After blocking the activity of endogenous peroxidases and non-specific binding, the primary rabbit polyclonal antibody