Dynamic Alterations in Growth Hormone Receptor mRNA Levels in Rat Brain During Stress Tolerance

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IN THE RAT, GH receptor (GHR) [1] and GH-binding protein (GHBP) [2] are known to be encoded by alternatively spliced messenger RNA (mRNA) species expressed in various regions of the brain [3]. In addition to GHR mRNA, Somatostatin (SS) and GH-releasing factor (GRF) neurons are also detected in the arcuate (ARC), periventricular (PeV) and paraventricular nuclei of the rat [4]. Several laboratories have reported that various kinds of stress decrease the circulating level of GH in the rat [5–7]. This repressive effect of stress on GH secretion is proposed to occur via stress-induced hypothalamic corticotropin-releasing factor (CRF) stimulating the inhibitory somatostatin release into portal blood [7]. A recent study by Martin *et al.* [8] has shown that the acute stress response of GH may not be correlated with the activity of peripheral sympathetic nerve terminals, which is exerted on hypophysiotropic hormone release rather than its synthesis, in the rat. It is not clear yet, however, whether or not the stress can alter the gene expression of GHR/GHBP in rat brain.

Here we report that a severe stress, restraint stress in water (RSW), increases the levels of GHR/GHBP mRNAs in the brain of the rat.

Materials and Methods

At 10 weeks of age, male Sprague-Dawley rats were put into individual restraining wire net cage-

es, and immersed in water (23 ± 0.5 °C) to the chest. At 0.5, 1, 2, 4 or 7 h of RSW, or at 3 or 6 h of resting after 7 h of RSW, rats were taken out from restraining cages and decapitated as described previously [9]. Total RNA was isolated from rat brain (cerebrum), which was isolated from the cerebellum and pituitary gland, and RNA was diluted serially from 40 to 2.5 μ g. Each sample was subjected to reverse transcription-polymerase chain reaction (RT-PCR) as described by Sakaguchi *et al.* [10]. Autoradiograms of the Southern blots were analyzed by a Machintosh-based image analyzing system with the NIH image 1.44 software. The correlations between log RNA input and densitometric measurements of autoradiograms of amplified cDNA for GHR/GHBP mRNAs were linear (Fig. 1). Ten μ g total RNA from the brain (cerebrum) was used in the subsequent experiment for RT-PCR. The mean and SEM of the data were determined and presented. The significance of differences between the values was determined by Dunnett's *post-hoc* procedure test after performing the Bartlett test, and $P < 0.05$ was considered significant.

Results

With the RT-PCR method, the mRNA expression levels for GHR and GHBP in rat brain were determined during the course of RSW. The GHR mRNA level was started to increase at 2 h of RSW (Fig. 2). The increase in the GHR mRNA level was maintained during RSW and reached about three times the control level before RSW. The mRNA expression level was quickly decreased when the animals were released from the stress. It returned to the initial level in 3 h of rest after 7 h of RSW.

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Fig. 1. A: Schematic representation and detection of cDNAs for GHR/GHBP by RT-PCR and densitometry. The open boxes indicate the extracellular regions common to GHR/GHBP, and the solid, shaded and dotted boxes indicate the transmembrane, cytoplasmic region unique to GHR and hydrophilic tail unique to GHBP, respectively. Arrowheads and P indicate the positions for the primers in RT-PCR and the probe for Southern blot, respectively. B: Autoradiograms of Southern blotting of the amplified cDNA fragments. C: Plots of the densitometrically determined signals in the autoradiograms for both GHR (closed circle) and GHBP (open circle).

The brain GHBP mRNA expression was similarly altered during RSW, but the expression levels were somewhat lower than that for GHR mRNA (Fig. 2).

The animals released from 7 h of RSW quickly recovered physically and emotionally.

Discussion

The present study has shown that the severe stress, RSW, induces obvious enhancement of GHR/GHBP gene expressions in the rat brain. In a previous study [9], we observed that the mRNA expression of long form PRL receptor was remarkably enhanced in rat brain during RSW. The result of the present study suggests that GH as well as PRL may act directly on the brain during tolerance of stress. Recently, several studies have provided convincing evidence that GH can pass the blood-brain barrier (BBB) via a receptor-mediated

mechanism in the choroid plexus [11, 12] and may affect central nervous system eliciting the well-being feeling [13]. In the rat tissues, the correlations between GHR gene expression and GHR/GHBP protein production are not clear yet. A recent study on dwarf rat by Gabrielsson *et al.* [14] has shown that continuous hGH infusions induce GHR1 transcripts in the liver, resulting in increases in GHR/GHBP proteins. They also reported that dexamethasone, which was given in the drinking water for 12 days, reduced both protein and mRNA levels of hepatic GHR. Our present results, however, showed that the expression of brain GHR mRNA was increased in 2 h of dexamethasone treatment (20 μ g, i.v.), and the elevated mRNA level was then decreased after 4 h of the treatment, probably by down-regulation (data not shown). Further studies are need to elucidate the molecular mechanism inducing mRNA expressions in the brain for GHR and GHBP.

Fig. 2. Autoradiograms and densitometric determinations of GHR and GHBP mRNAs during RSW. The mRNAs for GHR (closed bars) and GHBP (open bars) were determined before RSW (0 h), during the course of RSW (0.5–7 h), and after 3 and 6 h of rest following 7 h of RSW (10 and 13 h). The data were the means of triplicate experiments. *, $P < 0.05$; **, $P < 0.005$ (compared with the values at time zero, respectively).

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