PHYSIOLOGY

Brain Contents of Substance P, Diazepam-Binding Inhibitor, and Neuropeptide Y in High- and Low-Anxiety Inbred Rats during Stress

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The content of substance P in the hippocampus, hypothalamus, and midbrain of WAG/G rats surpassed these in Fischer-344 rats. After a 15-min stay in a shuttle box, the level of substance P in the hypothalamus and especially in the hippocampus decreased only in WAG/G rats. The content of diazepam-binding inhibitor in the hippocampus and midbrain of WAG/G rats was higher than in Fischer-344 rats. Stress increased the content of diazepam-binding inhibitor only in Fischer-344 rats. Midbrain content of neuropeptide Y in intact and stressed WAG/G rats was significantly lower than in Fischer-344 rats. There were no interstrain differences in the initial hypothalamic levels of neuropeptide Y between WAG/G and Fischer-344 rats. However, 15-min stress in the shuttle box increased hypothalamic content of neuropeptide Y only in Fischer-344 rats. Thus, high-anxiety rats are characterized by a low density of benzodiazepine receptors, decreased levels of substance P and diazepam-binding inhibitor, and high brain content of neuropeptide Y.

Key Words: *anxiety; substance P; diazepam-binding inhibitor; neuropeptide Y; stress; inbred rats*

Some peptide systems of the brain are involved in the mechanisms of anxiety. Octapeptide cholecystokinin enhances anxiety in rats [5], while neuropeptide Y (NPY) reduces it [6]. Substance P (SP) causes similar antistress and anxiolytic effects [1]. Moreover, a dipeptide inhibiting diazepam binding (diazepam-binding inhibitor, DBI) was found in mammalian brain. DBI possesses a considerable anxiogenic activity, and immunization against this dipeptide increases the resistance to stress [2].

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Our previous experiments showed that WAG/G rats have a higher density of benzodiazepine receptors in the brain and display lower anxiety than Fischer-344 rats [3]. Here we studied the role of SP, DBI, and NPY in the mechanisms of individual differences in anxiety and stress-induced emotional reactivity.

MATERIALS AND METHODS

Experiments were performed on 52 male WAG/G and 52 male Fischer-344 rats weighing 180-200 g (Svetlye Gory Nursery, Russian Academy of Medical Sciences). The animals were kept in cages (7 rats per cage) at constant temperature and humidity and under conditions of 12:12 h light-dark cycle.

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The rats were placed for 15 min into a shuttle box divided with a door into 2 identical compartments. Thirty seconds later, the door opened and a loud signal sounded. Five seconds later, a current (1 mA) was passed through the floor of compartment 1 where the rat sat, while the floor of compartment 2 was not energized. The rat escaped and ran to compartment 2, and the procedure was repeated. The animals were decapitated 5 min after stress, and the brain was immediately removed. The midbrain, hypothalamus, and hippocampus were isolated [7] and frozen at -70°C. The frozen brain sample was weighted, put into a tube with hot 1 M acetic acid (1:10 w/v), and incubated in a water bath at 100°C for 15 min. After cooling, the samples were homogenized in a Potter glass homogenizer and centrifuged at 45,000*g* and 4°C for 20 min. The precipitate was homogenized and centrifuged under the same conditions. Both supernatants were pooled, neutralized with 4 N NaOH, and used for neuropeptide assay. Peptide contents in these extracts were determined by radioimmunoassay using DRG kits.

The results were analyzed by Student's *t* test.

RESULTS

WAG/G and Fischer-344 rats did not differ in the time of active avoidance conditioning and performed 18.4±1.8 and 21.2±0.5 transitions during 15 min, respectively, but the number of effective avoidance responses was low (2.1±0.9 and 0.8±0.9 for WAG/G and Fischer-344 rats, respectively). These data suggest that stress induces practically similar effects in these rat strains.

The content of SP in the hippocampus, hypothalamus, and midbrain of WAG/G rats significantly surpassed that in Fischer-344 rats. After 15-min stress in the shuttle box, hippocampal level of substance P decreased only in WAG/G rats. However, the initial interstrain differences in hippocampal and midbrain contents of SP were retained (Fig. 1).

Hippocampal and midbrain contents of DBI in WAG/G rats surpassed those in Fischer-344 rats. Stress increased DBI content only in Fischer-344 rats and, therefore, the initial interstrain differences disappeared. We found no interstrain differences in the hypothalamic level of DBI between intact and stressed rats (Fig. 2).

Midbrain content of NPY in intact and stressed WAG/G rats was significantly lower than in Fischer-344 rats. There were no interstrain differences in the hypothalamic levels of NPY. However, after 15-min stress in the shuttle box NPY content increased only in Fischer-344 rats, which led to the appearance of interstrain differences. Hippocampal level of NPY was similar in WAG/G and Fischer-344 rats (Fig. 3).

Thus, our findings agree with the data showing that stress modulates SP contents in different brain areas and increases DBI and NPY levels in the majority of structures [4,8,9].

Our experiments showed that high-anxiety rats are characterized by a low density of benzodiazepine receptors, decreased levels of SP and DBI, and high brain content of NPY. The decrease in brain concentration of SP and a low density of benzodiazepine receptors probably determine high anxiety levels. Low DBI and high NPY contents are probably associated with compensatory processes reducing rat anxiety. This is confirmed by experiments showing stress-induced enhancement of anxiety. Stress decreased SP content (anxiety enhancement) in low-anxiety rats and increased DBI level (impairment of compensatory mechanisms) in high-anxiety rats. The more pronounced increase in the brain content of NPY in high-anxiety rats probably indicates intensification of compensatory reactions during stress.

These data demonstrate that the search for new methods for correction of emotional reactivity in stress requires individual behavioral characterization. The increase in brain content of NPY and SP induces stress-protective effects in high-anxiety and low-anxiety individuals, respectively.

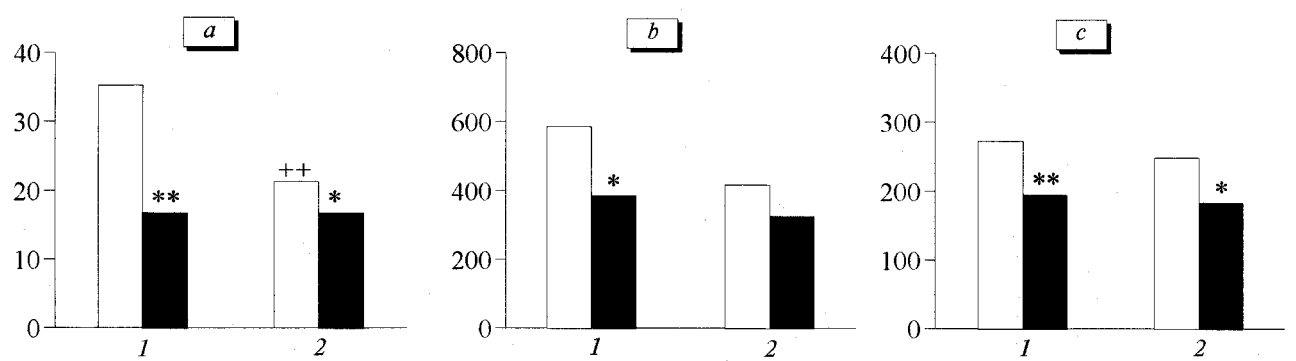

Fig. 1. Content of substance P (pg/mg tissue) in the hippocampus (*a*), hypothalamus (*b*), and midbrain (*c*) of WAG/G (light bars) and Fischer-344 (dark bars) rats before (*1*) and after 15-min stress in a shuttle box (*2*). Here and in Figs. 2 and 3: *$p<0.05$, **$p<0.01$, and ***$p<0.001$ compared with WAG/G rats; +$p<0.05$ and ++$p<0.01$ compared with the initial values in rats of the same strain.

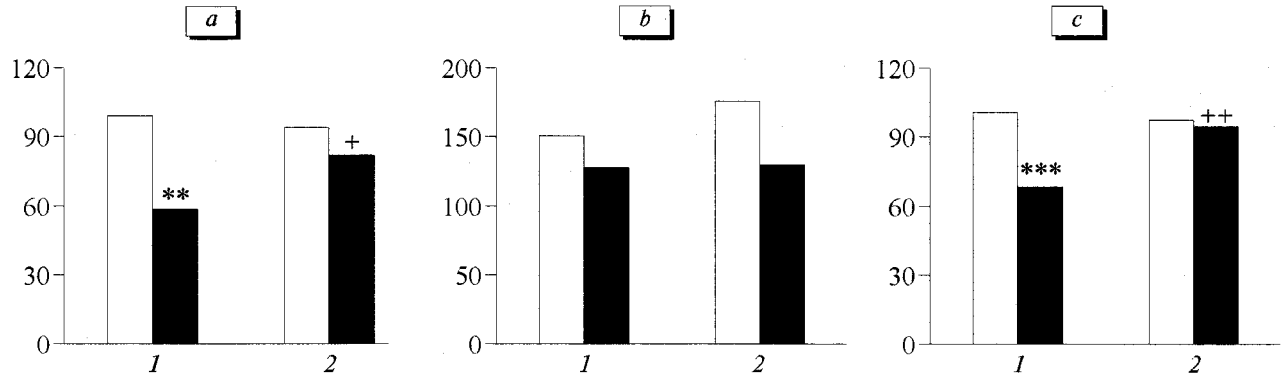

Fig. 2. Diazepam-binding inhibitor contents (pg/mg tissue) in the hippocampus (*a*), hypothalamus (*b*), and midbrain (*c*) of WAG/G (light bars) and Fischer-344 (dark bars) rats before (*1*) and after 15-min stress in a shuttle box (*2*).

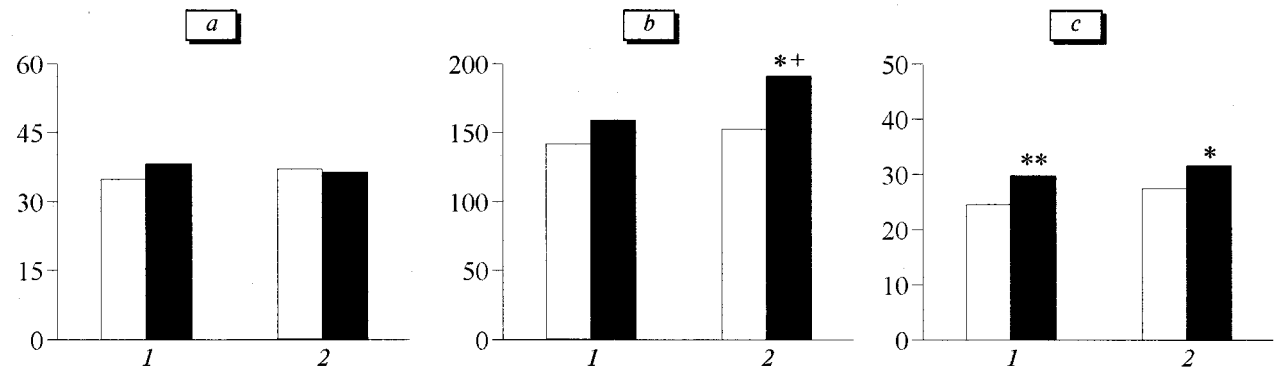

Fig. 3. Neuropeptide Y contents (pg/mg tissue) in the hippocampus (*a*), hypothalamus (*b*), and midbrain (*c*) of WAG/G (light bars) and Fischer-344 (dark bars) rats before (*1*) and after 15-min stress in a shuttle box (*2*).

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