Changes in CNS Levels of Serotonin and Its Metabolite in SART-Stressed (Repeatedly Cold-Stressed) Rats

Taeko Hata, Eiji Itoh and Atsufumi Kawabata

Department of Pharmacology, Faculty of Pharmacy, Kinki University, Kowakae, Higashi-Osaka 577, Japan

Received November 26, 1990 Accepted March 7, 1991

ABSTRACT—Central nervous system levels of serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in SART (specific alternation of rhythm in temperature)-stressed (repeatedly cold-stressed) rats were examined by HPLC-ECD. In SART-stressed rats, the levels of both 5-HT and 5-HIAA decreased in many brain areas. In the spinal cord, only the 5-HT level decreased. Therefore, the ratio of 5-HIAA to 5-HT increased only in the spinal cord. These results suggest that SART-stressed rats have some form of abnormality in the synthetic system of 5-HT.

Serotonergic and various other neural systems play important roles in pain sensation, behavior, learning and other activities. It has been reported that an increase in the brain level of serotonin (5-HT) coincides with decreases in spontaneous and feeding behavior and an increase in blood pressure in rats (1). It has also been reported that 5-HT levels increase in the rat hippocampus during acquisition trials in learning behavior (2). Decreases in rat brain 5-HT levels lead to increases in spontaneous and feeding behavior and hyperalgesia (3).

We previously reported that SART (specific alternation of rhythm in temperature)-stressed animals, repeatedly cold-stressed animals with dysautonomia, exhibit increased food intake and locomotor activity (4), decreased blood pressure (5), and hyperalgesia (6). These symptoms correspond to symptoms correlated with decreased levels in brain 5-HT in rats, as described above.

In the present study, we examined brain and spinal cord levels of 5-HT and its major metabolite, 5-hydroxyindoleacetic acid (5-HIAA), in SART-stressed rats, using high performance liquid chromatography (HPLC) with electrochemical detector. The relationship of 5-HT levels to some abnormalities such as impaired passive avoidance and hyperalgesia observed in SART-stressed animals is discussed.

Male Wistar rats (Japan SLC Inc.) weighing about 250 g were used throughout the experiments.

For the loading of SART stress (repeated cold stress), according to our previous report (7), rats were alternately placed into a cold cage set in a room maintained at -3°C and a normal temperature cage set in a room kept at 24°C at 1-hour intervals between 09:00 and 16:00. They were kept in the cold cage from 16:00 to 09:00 the next morning. On and after the 2nd day, animal positions were shifted between the two cages according to the same schedule on the 1st day, for five consecutive days. On the 6th day, the stressed animals were used for experiments.

For extraction of 5-HT and 5-HIAA from the brain and spinal cord, the rats were sacrificed by overhead irradiation with 2,450 MHz microwaves at 5 kW for 1.0 sec using a microwave applicator (Toshiba, TMW-6402 A) between 11:00 and 12:00, in consideration of the
circadian rhythm. The lumbar region of the spinal cord (L₁–L₆) was removed rapidly and irradiated with microwaves. After decapitation, the brain was excised and divided into the cerebral cortex, thalamus, hypothalamus, midbrain and pons plus medulla oblongata.

5-HT and 5-HIAA were extracted by the method of Kaneyuki et al. (8). Each of the above regions was homogenized in 20 volumes of 50 mM Tris-HCl buffer solution (pH 7.5), and the homogenate was allowed to stand in ice-water for 60 min to extract 5-HT and 5-HIAA. After centrifugation at 10,000 × g for 10 min, the supernatant was used as a sample for HPLC-ECD.

The HPLC apparatus consisted of a double plunger-type pump system (Irica, RP-530), reverse-phase ODS column (Irica, RP-18, 4 mm i.d. × 250 mm) and amperometric detector (Irica, E-308). The applied voltage was maintained at 750 mV throughout. The mobile phase was 0.1 M phosphate buffer (pH 3.4), containing 0.077% 1-octanesulphonic acid, 0.01% EDTA and 13% acetonitrile. The limit of detection for 5-HT and 5-HIAA was approximately 10 pg.

All data were expressed as means with S.E. Statistical significance between data was assessed by the unpaired Student's t-test with a significance level set at P < 0.05.

As shown in Fig. 1, SART-stressed rats exhibited significantly decreased levels of 5-HT in the hypothalamus, thalamus, midbrain, pons plus medulla oblongata and spinal cord, the values being 74.8, 74.0, 82.1, 77.6 and 74.4% of the respective non-stressed rat values. 5-HT levels in the cerebral cortex were not different between the non-stressed and SART-stressed groups.

5-HIAA levels were significantly decreased in the hypothalamus, thalamus, midbrain and pons plus medulla oblongata of stressed rats, being 72.2, 77.9, 88.5 and 84.8% of the respective levels in non-stressed rats. However, 5-HIAA levels in the cerebral cortex and spinal cord were not different between the stressed and non-stressed groups.

The ratio of the 5-HIAA level to 5-HT level has been used as an indicator of serotonergic neuronal activity, and the ratios were calculated from the above results in Fig. 1, and shown in Table 1. No significant differences were observed between the ratios in the cerebral cortex of non-stressed and stressed groups, in which no change was caused by SART stress in either 5-HT or 5-HIAA levels. There were also no differences in the ratios in the hypothalamus, thalamus, midbrain and pons plus medulla oblongata. However, the ratio in the spinal cord of the stressed group

| 5-HT (ng/g tissue) | 5-HIAA (ng/g tissue) |
|-------------------|---------------------|
| 0 | 200 | 400 | 600 | 800 | 0 | 200 | 400 | 600 |
| Cerebral cortex | | | | | | | | |
| Hypothalamus | | | | | | | | |
| Thalamus | | | | | | | | |
| Midbrain | | | | | | | | |
| Pons + Medulla oblongata | | | | | | | | |
| Spinal cord | | | | | | | | |

Fig. 1. 5-Hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) contents in non-stressed and SART-stressed rat CNS. □, Non-stressed; ■, SART stress. Data: Mean ± S.E. No. of animals: 11–14 rats/group (6/Cortex). *P < 0.05, **P < 0.01, ***P < 0.001 (t-test).
was significantly higher than that of the non-stressed group.

There are many reports on the relationship between stress and brain levels of 5-HT and 5-HIAA. Acute immobilization stress and cold stress cause an increase in 5-HT levels with or without increases in 5-HIAA (9, 10), although the degree of increase differs according to the brain area. Chronic stress in the form of repeated immobilization stress has been found to increase 5-HT and 5-HIAA levels simultaneously or cause no change in either (10–12). As for brain areas, Morgan et al. (13) reported cerebral cortex 5-HT and 5-HIAA levels to be most easily affected by stress, but Oomagari (9) reported that the hypothalamus was more easily influenced than the cerebral cortex. In this study, the cerebral cortex levels of 5-HT and 5-HIAA were hardly affected by SART stress, in contrast to the results of Morgan et al. In any case, it has rarely been reported that both 5-HT and 5-HIAA were decreased by stress. In the present study, both 5-HT and 5-HIAA decrease by SART stress in the hypothalamus, thalamus, midbrain and pons plus medulla oblongata. SART stress, due to sudden changes in environmental temperature, may probably cause decreased serotonergic neuronal activity. SART stress seems unique in this respect from other types of stress.

Thus, considering the data presented above, that 5-HT levels decrease by SART stress may be considered natural in view of the abnormal behavior (4), impairment of passive avoidance performance (14), hypotension (5) and hyperalgesia (6) caused by SART stress.

The relationship between the 5-HT system in the CNS and pain has often been noted. Morphine induces a slight increase in 5-HT and significant increase in 5-HIAA in rat brain stem and spinal cord. On the other hand, p-chlorophenylalanine, an inhibitor of 5-HT synthesis, and 5,7-dihydroxytryptamine, a neurotoxin of 5-HT neurons, induce a decrease in 5-HT in the rat spinal cord (15) and hyperalgesia (16). The present data showing that 5-HT decreased in the spinal cord in SART-stressed rats is consistent with the above reports, since SART-stressed animals are hyperalgesic (6). Also, the hyperalgesia observed in SART-stressed animals may involve decreases in 5-HT and 5-HIAA in the medulla oblongata and midbrain, where the centers of the descending inhibitory pain system are located.

The ratio of 5-HIAA/5-HT did not change in any brain regions of the SART-stressed rats. The contents of both 5-HT and 5-HIAA in brain areas, however, decreased, suggesting a decrease in serotonergic neuronal activity in these areas. The ratio changed only in the spinal cord. Increase in this ratio appears to result from an increased neuronal activity. However, in this case, the increase in the ratio is not due to an increase in 5-HIAA, but caused by a decrease in 5-HT. Therefore, serotonergic neuronal activity in this stressed

### Table 1. Ratio of 5-hydroxyindoleacetic acid to 5-hydroxytryptamine in non-stressed and SART-stressed rats

| Brain Region                  | Non-stress        | SART stress       |
|-------------------------------|-------------------|-------------------|
| Cerebral cortex               | 0.431 ± 0.040     | 0.450 ± 0.021     |
| Hypothalamus                  | 0.604 ± 0.035     | 0.605 ± 0.024     |
| Thalamus                      | 0.890 ± 0.040     | 0.935 ± 0.041     |
| Midbrain                      | 0.761 ± 0.036     | 0.834 ± 0.055     |
| Pons + Medulla oblongata      | 0.917 ± 0.043     | 0.964 ± 0.032     |
| Spinal cord                   | 0.577 ± 0.013     | 0.691 ± 0.030**   |

Data: Mean ± S.E. No. of animals: 11–14 rats/group (6/Cortex). **P < 0.01 (t-test).
animal is considered to decrease in the spinal cord. Hyperalgesia in this stressed animal may probably be a consequence of the decreased activity in serotonergic neurons that function in the inhibitory pain system at the spinal cord.

Thus, SART-stressed rats may have a dysfunction in the serotonergic system, particularly with respect to 5-HT synthesis rather than metabolism.

REFERENCES

1. Grahame-Smith, D.G.: Studies in vivo on the relation between brain tryptophan, brain 5-HT synthesis, and hyperactivity in rats treated with a monoamine oxidase inhibitor and L-tryptophan. J. Neurochem. 18, 1053–1066 (1971)

2. Ramakers, F., Righter, H. and Leonard, B.E.: Parallel changes in behavior and hippocampal serotonin metabolism in rats following treatment with desglycynamide lysine vasopressin. Brain Res. 120, 485–492 (1977)

3. Berge, O.G., Fasmer, O.B. and Flatmark, T.: Time-course of changes in nociception after 5,6-dihydroxytryptamine lesions of descending 5-HT pathways. Pharmacol. Biochem. Behav. 18, 637–643 (1983)

4. Hata, T., Nishimura, Y., Kita, T., Itoh, E. and Kawabata, A.: The abnormal open-field behavior of SART-stressed rats and effects of some drugs on it. Japan. J. Pharmacol. 48, 479–490 (1988)

5. Hata, T., Kita, T., Namimatsu, A., Itoh, E. and Oda, Y.: Changes of blood pressure and regional blood flow in SART rats and drug actions on these changes. Folia Pharmacol. Japon. 79, 335–342 (1982) (Abs. in English)

6. Kita, T., Hata, T., Iida, J., Yoneda, R. and Ishida, S.: Decrease in pain threshold in SART stressed mice. Japan. J. Pharmacol. 29, 479–482 (1979)

7. Hata, T., Kita, T., Itoh, E. and Harada, N.: Experimental studies on optimal conditions of loading SART stress (repeated cold stress) upon animals. Japan. J. Psychosom. Med. 29, 651–658 (1984)

8. Kaneyuki, T., Morimasa, T. and Shohmori, T.: Effects of malnutrition on the monoamine levels in the adult rat brain nuclei. Neurosciences 12, 202–203 (1986) (Abs. in English)

9. Oomagari, K.: Effects of morphine and diazepam pretreatment on immobilization stress-induced increase of serotonin metabolism in discrete brain areas of the rat. Fukuoka Acta Med. 80, 81–94 (1989) (Abs. in English)

10. Roth, K.A., Mefford, I.M. and Barchas, J.D.: Epinephrine, norepinephrine, dopamine and serotonin: Differential effects of acute and chronic stress on regional brain amines. Brain Res. 239, 417–424 (1982)

11. Culman, J., Kiss, A. and Kvetnansky, R.: Serotonin and tryptophan hydroxylase in isolated hypothalamic and brain stem nuclei of rats exposed to acute and repeated immobilization stress. Exp. Clin. Endocrinol. 83, 28–36 (1984)

12. Adell, A., Garcia-Marquez, C., Armario, A. and Gelpi, E.: Chronic stress increases serotonin and noradrenaline in rat brain and sensitizes their responses to a further acute stress. J. Neurochem. 50, 1678–1681 (1988)

13. Morgan, W.W., Rudeen, P.K. and Pfeil, K.A.: Effect of immobilization stress on serotonin content and turnover in regions of the rat brain. Life Sci. 17, 143–150 (1975)

14. Nishimura, Y., Hata, T., Kawabata, A., Itoh, E. and Kita, T.: Impairment of passive avoidance performance in SART-stressed mice and the action of drugs. Japan. J. Pharmacol. 49, 111–117 (1989)

15. Naranjio, J.R., Arnedo, A., Molinero, M.T. and Del Rio, J.: Involvement of spinal monoaminergic pathways in antinociception produced by substance P and neurotensin in rodents. Neuropharmacology 28, 291–298 (1989)

16. Berge, O.G. and Ogren, S.O.: Selective lesions of the bulbospinal serotonergic pathways reduce the analgesia induced by p-chlorophenylalanine in the hot-plate test. Neurosci. Lett. 44, 25–29 (1984)