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The Effect of Lowering the Serotonin Content of the Rat Brain on Spontaneous Locomotor Activity

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Electrothermic lesions of the midbrain of rats were made in the region of the median or median plus dorsal raphe nuclei through an unipolar electrode. Concentration of serotonin (5HT) in the telencephalon decreased variously among rats and locomotor activity increased following lesions. No close correlation was obtained between locomotor activity and the 5HT concentration in the telencephalon 7 to 14 days after the lesion. The effect of *p*-chlorophenylalanine (PCPA, a total dose of 400 mg/kg, *i.p.*) was also investigated on locomotor activity, and on 5HT and 5-hydroxyindoleacetic acid (5HIAA) levels in the telencephalon of the rat. PCPA lowered the 5HT content to less than 35% of the control in the telencephalon, but did not significantly alter locomotor activity. Only an excess mounting behavior was observed. These results suggest that an increase in locomotor activity due to raphe lesion has no primary connection with lowering the 5HT content of the rat brain.

Electrical stimulation of the caudal midbrain raphe caused behavioral depression and a failure of habitution to repetitive sensory stimuli as manifested by a persistence of "startle" response in unanesthetized rats.[2,3] Kostowski, *et al.*[4] have reported an excitation of general behavior and shown a good correlation between behavioral excitation and a marked reduction in serotonin (5HT) and 5-hydroxyindoleacetic acid (5HIAA) levels after lesions in midbrain raphe. These facts suggest that 5HT in the forebrain may be associated with an inhibitory role in controlling behavioral pattern. Diminution of the 5HT content in the cat brain either by raphe lesion or by pretreatment with *p*-chlorophenylalanine (PCPA) yielded a marked decrease in slow-wave sleep time and the latter was proportional to the extent of the fall in 5HT.[5]

It is therefore of interest to examine various parameters concerned with 5HT level in the brain after midbrain lesion. The present paper describes an increase of locomotor activity caused by midbrain raphe lesion and by the pretreatment with PCPA in relation to the brain content of 5HT in rats.

Material and Method

Male Wistar rats weighing 200 to 250 g were used in all studies.

1) **Midbrain Raphe Lesion**——Rats were anesthetized with pentobarbital sodium (50 mg/kg, *i.p.*) and placed in a stereotaxic instrument (Todai Noken type). Electrothermic lesions in median raphe or median plus dorsal raphe were made by passing a high frequency current (<5 mA for 20 to 30 sec.) through an unipolar electrode with an indifferent electrode in the rectum. Coordinates according to the rat brain atlas of König and Klippel[6] were: for the median raphe-A, 350 μm; L, O; H, 1.6 mm, and for the dorsal raphe-A, 350 μm; L, O; H, 0.8 mm. Four to five rats were kept in a cage and used for the behavioral studies 7 to 14 days

1) Location: *3190, Gofuku, Toyama City, Toyama, 930, Japan.*
2) M. H. Sheard and G. K. Aghajanian, *Life Sci.*, **7**, 19 (1968).
3) W. Kostowski, E. Giacalone, S. Garattini and L. Valzelli, *Europ. J. Pharmacol.*, **7**, 170 (1969).
4) W. Kostowski, E. Giacalone, S. Garattini and L. Valzelli, *Europ. J. Pharmacol.*, **4**, 371 (1968).
5) M. Jouvet, *Science*, **163**, 32 (1969).
6) J. F. R. König and R. A. Klippel, "The Rat Brain," The Williams and Wilkins Company, Baltimore, 1963.

after the operation. In control rats the electrode was inserted into the same position as described, but current was not passed.

2) **Recording of Locomotor Activity**——Locomotor activity of the rat was tested in a field (60 cm square) with 43 cm high walls. The entire field was painted flat black and divided into 16 squares. A 100 watt bulb was suspended 80 cm above the center of a box in the quiet room. The temperature and humidity were kept constant. For testing, the animal was placed in a corner of the field and for 5 minutes the number of squares entered was recorded manually every hour 3 to 6 times.

3) **Determination of 5HT, 5HIAA and Catecholamines**——The animals were sacrificed by decapitation at the end of the observation period (at 3:30 to 5:30 p.m.) and the brains were dissected into the telencephalon (cerebrum and basal ganglia), the diencephalon-midbrain and pons-medulla. Half of the telencephalon and the other parts were used for 5HT and 5HIAA assay. The other half of the telencephalon was used for catecholamine assay. The diencephalon-midbrain region of the lesioned rat was fixed in formalin and later processed for histological examination of lesion sites by means of paraffin preparation. 5HT and 5HIAA were extracted and determined by the method of Maickel, *et al.*[7] and of Curzon and Green.[8] Recoveries were checked in each experiment by adding 400 ng of 5HT and 5HIAA to cerebellum. Four cerebella were pooled, homogenized and divided into two portions. 5HT and 5HIAA were added to a portion and the second served as a tissue blank. Recoveries were $84 \pm 2.5\%$ (mean $\pm$ S.E.M., $n=10$) for 5HT and $81.4 \pm 2.3\%$ ($n=10$) for 5HIAA. Dopamine (DA) and noradrenaline (NA) were assayed according to a modification of Hogan's method quoted in ref. 9. Recoveries were $78.7 \pm 4.5\%$ (mean $\pm$ S.E.M., $n=10$) for DA and $68.7 \pm 3.4\%$ ($n=10$) for NA. Fluorescence was measured in Hitachi-203 spectrophotofluorometer. Values in the table are corrected for losses. Student's t-test was used to determine the statistical difference between control and lesioned or drug groups.

4) **Drugs**——DL-*p*-Chlorophenylalanine (Nakarai Chemicals, Ltd.) and L-5HTP (Ajinomoto Co., Inc.) were suspended in CMC-saline and were administered intraperitoneally. Ro 4-4602 was dissolved in saline and administered intraperitoneally.

Results

1) Increased Locomotor Activity and Reduction in the Brain 5HT Content after Midbrain Raphe Lesion

Midbrain raphe lesion produced rigid posture, stereotyped circling and an increase of locomotor activity immediately after pentobarbital anesthesia administered for making lesion had worn off. Rigid posture and stereotyped circling were ameliorated gradually during 14 days after lesion, although stimulated locomotor activity was still maintained through 6 weeks after lesion (the period observed). In Fig. 1 increased locomotor activity was shown for 6 hours on the 11th day after lesion. The degree of increase of locomotor activity varied markedly among lesioned rats. When rats became tame to the field, locomotor activity decreased moderately 5 to 6 hours after the initiation of the measurement of locomotor activity. There was no statistically significant correlation between the locomotor activity and 5HT level in the telencephalon 7 to 14 days after lesion. Locomotor activity and amine contents of the telencephalon are shown in the order of the degree of 5HT reduction in the Table I. In 7 out of 15 lesioned rats the 5HT contents of the telencephalon were lowered to less than 30% of the control, whereas those of the pons-medulla were approximately 85% of the control. The 5HT content of the pons-medulla was not altered in the remaining 8 rats. DA and NA contents in the telencephalon were at least 84 and 76% of the control, respectively. When the site of lesion was located between the median and dorsal raphe nuclei as a result of histological observation, locomotor activity was significantly increased but the 5HT content of the telencephalon was relatively unaffected. When the median and dorsal raphe nuclei were included in the lesion site, the 5HT content of the telencephalon was lowered to less than 50% of the control and locomotor activity was significantly increased. When the lesion site was localized in the median raphe region, however, locomotor activity was not increased though the 5HT content of the telencephalon was lowered to 70 to 60% of the control.

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2) Effect of L-5HTP with Decarboxylase Inhibitor on Increased Locomotor Activity

L-5HTP in a dose of 60 or 120 mg/kg in combination with 50 mg/kg of Ro 4-4602 suppressed increased locomotor activity for 3 or 5 hours after the injection (Fig. 2). A significant suppression was observed 2 hours after 120 mg/kg of 5HTP injection. The 5HT content of the telencephalon was more than 1.5 μg/g wet tissue weight 4 hours after 5HTP administration in lesioned as well as control rats. The locomotor activity in control rats was also suppressed with 5HTP plus Ro 4-4602.

3) Locomotor Activity and the Brain 5HT Content after PCPA

When 100 mg/kg of PCPA was administered 51 and 27 hours before sacrifice, both 5HT and 5HIAA contents in the telencephalon were lowered to less than 40% of the control. The 5HT content in the diencephalon-midbrain and pons-medulla was lowered to 50% of the

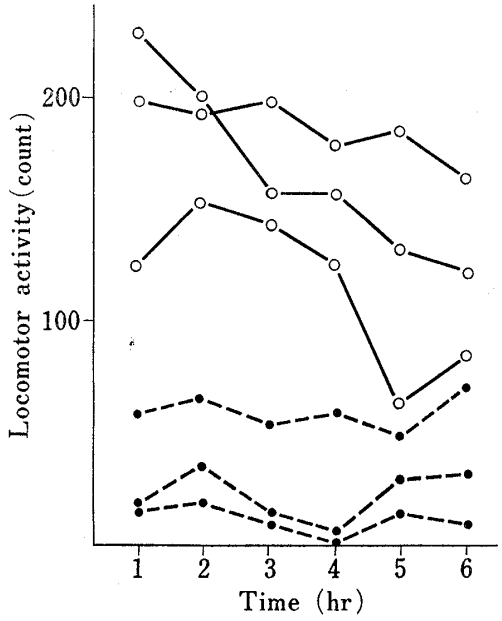

Fig. 1. Locomotor Activity on the 11th Day after Raphe Lesion (---○---) and Sham Lesion (---●---) in the Rat

Each line represents response of a single animal. Locomotor activity in the ordinate is shown as a count of squares of which a rat traversed on the field in 5 minutes. Locomotor activity was measured from 1:00 to 6:00 pm

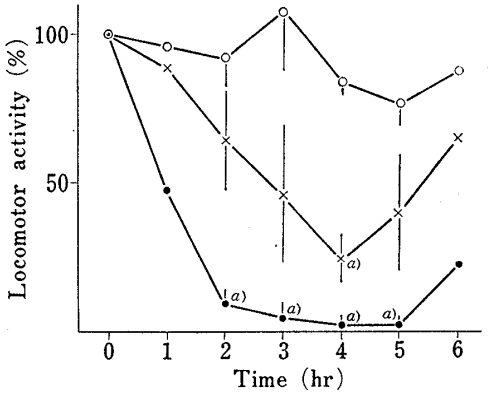

Fig. 2. Suppressive Effect of L-5HTP combined with Ro 4-4602 on increased Locomotor Activity on the 11th Day after Raphe Lesion in the Rat

L-5HTP in the dose of 60 (---×---, $n=4$) or 120 mg/kg (---●---, $n=3$) was injected intraperitoneally 30 min after an intraperitoneal administration of 50 mg/kg of Ro 4-4602. Saline and CMC-saline were administered instead of drugs into the lesioned rat as a control (---○---, $n=6$). Since locomotor activity level varies markedly among rats, change in locomotor activity was expressed as percent of the initial locomotor activity of each rat (ordinate). Vertical bar shows a standard error of mean.
a) $p<0.01$

TABLE I. Locomotor Activity and Concentrations of 5HT, 5HIAA, DA and NA (ng/g Wet Tissue, Mean±S.E.M.) in the Telencephalon after Midbrain Raphe Lesion

Site of lesion	No. of rats	Locomotor activity[a)]	5HT	5HIAA	DA	NA
Control	10	32± 5	477±13	419±13	831±52	240± 9
Median raphe	3	176	356	366	874	182
Median raphe or Median plus dorsal raphe	5	135±46[b)]	251±18[b)]	191±26[b)]	701±82	216± 5
Median plus dorsal raphe	7	141±21[b)]	<150[b)]	<150[b)]	776±69	206± 5[c)]

The rat was sacrificed between 7 and 14 days after the lesion. The concentration of amine was expressed in <150 ng/g when a fluorescence peak was not obtained with the same wevelength as 5HT's in the brain extract.
a) Locomotor activity is a mean of three values observed at 1:00, 2:00 and 3:00 pm
b) $p<0.01$ *c*) $p<0.05$

Table II. Locomotor Activity and Concentrations of 5HT, 5HIAA, DA and NA (ng/g Wet Tissue, Mean±S.E.M.) in the Telencephalon after *i.p.* Injection of 400 mg/kg of PCPA

Treatment	No. of rats	Locomotor activity[a)]	5HT	5HIAA	DA	NA
CMC-saline	6	32±8	444±21	394±23	1034±100	226±10
PCPA	4	49±5	<150[b)]	<150[b)]	636± 42[b)]	124±10[b)]

The rat was sacrificed 52 hours after the first PCPA injection. The concentration of amine was expressed in <150 ng/g when a fluorescence peak was not obtained with the same wavelength as 5HT' s in the brain extract.
a) Locomotor activity is a mean of three values observed at 1: 00, 2: 00 and 3: 00 pm
b) $p<0.01$

control. The DA and NA contents in the telencephalon were not affected by the dose of PCPA. Locomotor activity in PCPA-treated rats was not significantly different from the control. PCPA in the dose of 200 mg/kg yielded a slightly significant difference in locomotor activity (50±6, mean ±S.E.M., $n=8$) from the control (23±7, mean±S.E.M., $n=8$) 24 hours after the administration. As shown in Table II, however, PCPA in the total dose of 400 mg/kg (200, 100 and 100 mg/kg; 48, 24 and 12 hours before the test) produced no statistically significant increase of locomotor activity, while it caused excess mounting behavior and hyperactivity not recorded as locomotor activity in the field. 5HT and 5HIAA levels in three parts of the brain were less than 35 and 40% of the control, respectively. The DA and NA contents in the telencephalon were lowered to 61.1 and 68.4% of the control, respectively (Table II).

Discussion

Several behavioral abnormalities have been shown to be produced by midbrain raphe lesion, such as excitation of general behavior,[4,10] muricide behavior,[11,12] and a decrease in normal sleep pattern.[5] These effects were attributed to loss of cerebral 5HT caused by destruction of the ascending serotonergic neurons innervating the forebrain. In the present study we observed locomotor activity as a parameter of behavioral changes produced by raphe lesion. Increased locomotor activity caused by raphe lesion seems to be due to not only the lowered content of 5HT in the telencephalon but also the degeneration of a factor other than the serotonergic nervous system in view of the facts that: 1) there was no direct correlation between the 5HT content in the telencephalon and locomotor activity in lesioned rats, 2) increase of locomotor activity was observed in rats that have the lesion site between the median and dorsal raphe nuclei. An administration of L-5HTP in combination with Ro 4-4602 produced a reversal of increased locomotor activity. The mechanism by which 5HTP can replenish the 5HT content of the telencephalon may be explained as follows. Catecholaminergic and survived serotonergic neurons in lesioned rats could take up 5HTP and decarboxylate it to 5HT. The reversal of increased locomotor activity produced by 5HTP may be due to the accumulation of 5HT in serotonergic as well as catecholaminergic neurons where it may act as a false transmitter. 5HTP plus decarboxylase inhibitor suppressed locomotor activity in the control as well as lesioned rats, suggesting that the suppressive effect of 5HTP on locomotor activity is not selective in raphe lesioned rats. The effect of 5HTP on spontaneous locomotor activity in the control rats contrasts with the results of Modigh[13] in which

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5HTP with MK 486 produced a marked increase of locomotor activity in mice. The contradictory results obtained in rats and mice remain unexplained.

Some views in the literature on changes of motor activity after PCPA seem to conflict because of the parameters which have been used to record motor activity.[14–16] Telemetric recordings by Borbely, *et al.*[17] have shown that motor activity during the light periods was significantly increased 24 and 48 hours after 300 mg/kg of PCPA in the rat. In the present study the 5HT and 5HIAA contents of the telencephalon were lowered to less than 40% of the control after 400 mg/kg of PCPA, but locomotor activity was relatively unaffected. This fact may support the idea that increased locomotor activity has no primary connection with lowering the 5HT content of the telencephalon. There seem to be some differences between effects of raphe lesion and those of PCPA on the regional monoamine levels in the brain. The degree of diminution of the 5HT content in the pons-medulla was relatively slight after lesion. DA and NA contents of the telencephalon were slightly lowered when 5HT was lowered to less than 50% of the control. On the other hand, PCPA keeps all neurons alive but lowered the 5HT contents of all parts of the brain to less than 40% of the control. 40% loss in DA and NA was seen in the telencephalon. However, we need further studies to know how those differences of monoamine levels contribute to behavioral variations produced by raphe lesion or PCPA. Furthermore, cholinergic mechanisms may be involved in the cause of increased locomotor activity in lesioned rats since a decrease in the brain acetylcholine has been shown in the raphe lesioned rat with a complex behavioral picture.[18]

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