Pharmacology Biochemistry & Behavior, Vol. 26, pp. 725–729. © Pergamon Journals Ltd., 1987. Printed in the U.S.A. 0091-3057/87 $3.00 + .00

Changes of Brain Monoamine Contents in Three Models of Experimentally Induced Muricide in Rats

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Received 18 October 1986

TANI, Y., Y. KATAOKA, Y. SAKURAI, K. YAMASHITA, M. USHIO AND S. UEKI. *Changes of brain monoamine contents in three models of experimentally induced muricide in rats.* PHARMACOL BIOCHEM BEHAV **26**(4) 725–729, 1987.—Monoamine levels were measured by high performance liquid chromatography with electrochemical detection in seven brain regions of three models of experimentally-induced muricide (mouse-killing behavior) in rats (bilateral olfactory bulbectomized rats: OB rats, midbrain raphe nuclei lesioned rats: Raphe rats and nucleus accumbens lesioned rats: Acc rats). Noradrenaline (NA) levels in the lateral hypothalamus (LH) and ventromedial hypothalamus (VMH), 3,4-dihydroxyphenylacetic acid (DOPAC) levels in LH and homovanillic acid (HVA) levels in the frontal cortex (FC) were increased in all three muricide models. In LH, serotonin (5-HT) levels increased in Acc rats and the 5-hydroxyindoleacetic acid (5-HIAA)/5-HT ratio was reduced in OB rats. But in VMH an increase in NA level was not accompanied by any changes of other amines in three muricide models except for 5-HT and 5-HIAA in Raphe rats. In the mamillary body (MB), NA level was increased and 5-HIAA/5-HT ratio was decreased in both OB and Acc rats. Monoamine changes in the amygdaloid nuclei were different in three muricide models, suggesting that the role of monoamines in various nuclei of the amygdala may be different in each muricide model. The present findings suggest that both noradrenergic (LH and VMH) and serotonergic function (LH and MB) may play an important role in exhibiting muricide of OB, Raphe and Acc rats, while dopaminergic function (LH and FC) may be related rather to hyperirritability elicited in these three muricide models.

Muricide Biogenic amines Olfactory bulbectomy Raphe lesion Accumbens lesion

MURICIDE of the rat has been known to be a useful experimental model for evaluating the properties of antidepressants [13,14]. There are various procedures producing muricide in rats [13,14]. Our interests have been concentrated on 3 models of muricide induced by olfactory bulbectomy, lesioning of the midbrain raphe and nucleus accumbens (OB, Raphe and Acc rats, respectively). These three types of muricide have been reported to have different behavioral and pharmacological characteristics. Our behavioral studies also suggest that different neural mechanisms may be involved in the exhibition of muricide in OB and Raphe rats [7,16].

Brain catecholamines (CA) and serotonin (5-HT) have been reported to mediate defensive and offensive aggression such as muricide [3]. There are few reports concerned with neurochemical studies on the exhibition of muricide in OB, Raphe and Acc rats. The present study was, therefore, designed to determine the dynamics of brain amines in various brain regions related to emotional behavior, in these three muricide models, in rats.

METHOD

Animals

Male Wistar King A rats, weighing 200–250 g at the beginning of the experiment, supplied by Seiwa Experimental Animals Ltd., were used. Before the experiment, all rats underwent a muricide test. Only animals not showing muricide were selected. After brain surgery, all animals except for intact nonkiller rats (28 rats) were housed in individual cages (17×17×21 cm) with food and water ad lib and maintained under standardized conditions of temperature (23±2°C) and light-dark cycle (light on 07:00–19:00) throughout the experiments.

Surgical Procedures

The animals were anesthetized with sodium pentobarbital (40 mg/kg IP) and placed on a stereotaxic instrument. Bilateral olfactory bulbectomy was performed by suctioning through a hole made in the skull (OB rats). Lesions of the raphe and nucleus accumbens were made by applying a di-

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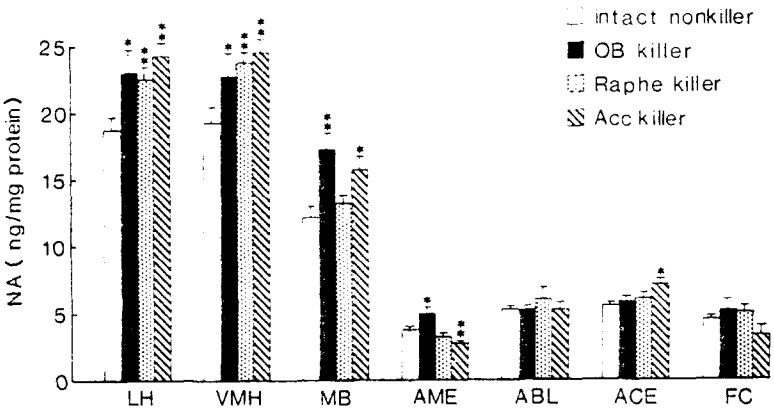

FIG. 1. Brain NA concentrations in OB, Raphe and Acc killer rats. Results expressed as the mean±S.E. for 28 rats (intact nonkiller), 19 rats (OB killer), 16 rats (Raphe killer), 17 rats (Acc killer). LH; lateral hypothalamus, VMH; ventromedial hypothalamus, MB; mamillary body, AME; medial amygdala, ABL; basolateral amygdala, ACE; central amygdala, FC; frontal cortex. $*p<0.05$, $**p<0.01$; significantly different from intact nonkiller (two-tailed Student's t-test). All these abbreviations and statistical analyses are the same as for Tables 1 and 2.

rect current of 3.0 mA for 15 sec through the electrode implanted as a cathode. The coordinates, anterior (A) from the lambda for raphe lesions and from the bregma for accumbens lesions, lateral (L) to the midline and horizontal (H) below the skull surface were selected with the aid of Paxinos and Watson [11]. Stainless steel electrodes (0.4 mm diameter) insulated except for 0.5 mm in length from the tip were inserted into the raphe (dorsal and medial) and accumbens (caudal and rostral). Coordinates for the raphe were A: 0.5 mm, L: 0 mm, H: 7.0 mm (dorsal) and A: 0.5 mm, L: 0 mm, H: 9.2 mm (medial). Those for the accumbens were A: 1.0 mm, L: ±1.7 mm, H: 7.7 mm (caudal) and A: 2.0 mm, L: ±1.0 mm, H: 6.9 mm (rostral).

Assay of Biogenic Amines

Two weeks after brain lesioning, the rats which exhibited muricide within 3 min after introducing a mouse into the rat's home cage were decapitated. The brain was quickly removed and dissected on an ice-cold glass plate into seven regions, i.e., the lateral hypothalamus (LH), ventromedial hypothalamus (VMH), mamillary body (MB), medial amygdala (AME), basolateral amygdala (ABL), central amygdala (ACE) and frontal cortex (FC) by the microknife according to the Paxinos and Watson rat brain atlas. Immediately after dissection, the brain tissues were homogenated in 300 μl of 0.5 M perchloric acid containing 0.1% w/v $Na_2S_2O_5$ and 0.1% w/v EDTA·2 Na, followed by the addition of internal standards; 3,4-dihydroxybenzylamine (DHBA) 10 ng and N-ω-methyl-5-hydroxytryptamine (Nω-Me-5-HT) 10 ng. Then homogenized tissues were centrifuged at 10,000 × g for 10 min and supernatants transferred to other tubes were stored at −80°C. Total protein was determined from 10 μl of the tissue homogenate before centrifugation by the method of Bradford [1].

Determination of biogenic amines in the brain tissues were performed using HPLC with electrochemical detection. The HPLC system (Waters Company) utilized a Yanapak-ODS-A column (7 μm, Ø4.6×250 mm) coupled with a glassy carbon detector (VMD-501, Yanaco) at a potential of 0.73 V versus the reference electrode. The mobile phase contained 0.6% v/v tetraethylamine (TEA), 8% v/v acetonitrile, 0.01% w/v EDTA·2 Na, 11 mM heptanesulfonic acid, adjusted pH to 2.85 with ortho-phosphoric acid. Buffer was degassed under vacuum before use. The flow-rate of HPLC was maintained at 1.0 ml/min. The concentrations of biogenic amine were quantified by calculating the area under the curves using an integrator (730 data module, Waters).

Histology and Statistical Analysis

After the dissection, the rest of the rat brain was stored in 10% Formalin at least for 10 days. Brain sections of 50 μm thick were made and stained with cresyl violet. The site and extent of the lesions were verified histologically. The animals in which lesions of the aimed area were incomplete were excluded from the data. The results were statistically analyzed using Student's t-test.

RESULTS

The extent of lesions in OB, Raphe and Acc rats were almost the same as those previously reported [6, 9, 15].

Fourteen days after the surgery, OB, lesions of the raphe and accumbens produced muricide in 100% (19 rats), 84% (16 rats) and 85% (17 rats) of rats, respectively.

Figure 1 shows brain NA concentrations in three muricide models of the rat. Significant increases in NA content of LH and VMH were observed in all three muricide models. In addition, NA concentrations in MB and AME also significantly increased in OB rats. In Acc, rats NA content increased in MB and ACE and decreased in AME.

Changes in 5-HT and 5-HIAA concentrations as well as in the ratio of 5-HIAA/5-HT following brain lesioning were shown in Table 1. In Raphe rats, 5-HT content was decreased by 27–72% in all brain regions studied except FC, and 5-HIAA concentration was also significantly decreased by

TABLE 1

BRAIN 5-HT, 5-HIAA CONCENTRATIONS AND THE RATIO OF 5-HIAA/5-HT IN OB, RAPHE AND Acc KILLER RATS

	Frontal Cortex	Hypothalamus LH	Hypothalamus VMH	Mamillary Body	Amygdala AME	Amygdala ABL	Amygdala ACE
				5-HT			
Intact	4.64 ± 0.34	8.21 ± 0.53	5.33 ± 0.43	13.45 ± 1.11	7.00 ± 0.20	6.92 ± 0.43	6.35 ± 0.47
OB	4.95 ± 0.52	9.43 ± 0.52	5.70 ± 0.24	17.20 ± 1.21*	7.55 ± 0.53	6.86 ± 0.75	6.87 ± 0.63
Raphe	3.39 ± 0.57	4.92 ± 0.58‡	3.01 ± 0.80*	6.74 ± 0.97‡	1.93 ± 0.29‡	3.06 ± 0.45‡	2.43 ± 0.35‡
Acc	4.19 ± 0.25	10.62 ± 0.58†	6.34 ± 0.50	15.84 ± 1.14	6.64 ± 0.38	7.50 ± 0.69	8.03 ± 0.60*
				5-HIAA			
Intact	2.22 ± 0.11	4.14 ± 0.30	3.23 ± 0.33	8.03 ± 0.63	3.66 ± 0.19	3.55 ± 0.26	4.07 ± 0.25
OB	1.68 ± 0.10*	3.59 ± 0.28	3.30 ± 0.35	8.09 ± 0.67	3.74 ± 0.25	3.24 ± 0.35	3.66 ± 0.35
Raphe	1.51 ± 0.18†	2.78 ± 0.23†	2.05 ± 0.18†	4.02 ± 0.44‡	1.58 ± 0.15‡	1.80 ± 0.25‡	1.96 ± 0.21‡
Acc	1.82 ± 0.26	4.70 ± 0.32	3.41 ± 0.37	6.76 ± 0.66	3.02 ± 0.24*	3.49 ± 0.41	3.97 ± 0.33
				5-HIAA/5-HT			
Intact	0.51 ± 0.03	0.50 ± 0.03	0.63 ± 0.06	0.62 ± 0.03	0.56 ± 0.04	0.55 ± 0.04	0.73 ± 0.07
OB	0.37 ± 0.05*	0.38 ± 0.02†	0.65 ± 0.09	0.47 ± 0.02*	0.52 ± 0.04	0.49 ± 0.04	0.56 ± 0.06
Raphe	0.54 ± 0.06	0.63 ± 0.05*	0.80 ± 0.11	0.72 ± 0.10	1.05 ± 0.27	0.70 ± 0.09	0.97 ± 0.10
Acc	0.43 ± 0.04	0.45 ± 0.02	0.53 ± 0.05	0.43 ± 0.03‡	0.47 ± 0.04	0.48 ± 0.04	0.52 ± 0.04*

Each value represents the mean ± S.E. (ng/mg protein) for 28 rats (intact nonkiller), 19 rats (OB killer), 16 rats (Raphe killer), 17 rats (Acc killer).

Significantly different from intact: *$p<0.05$, †$p<0.01$, ‡$p<0.001$.

TABLE 2

BRAIN DA, DOPAC, AND HVA CONCENTRATIONS IN OB, RAPHE AND Acc KILLER RATS

	Frontal Cortex	Hypothalamus LH	Hypothalamus VMH	Mamillary Body	Amygdala AME	Amygdala ABL	Amygdala ACE
				DA			
Intact	0.28 ± 0.04	1.37 ± 0.10	3.65 ± 0.44	3.69 ± 0.64	0.64 ± 0.08	2.83 ± 0.19	6.13 ± 0.47
OB	0.29 ± 0.06	1.56 ± 0.13	3.48 ± 0.45	5.07 ± 0.72	1.16 ± 0.26	3.15 ± 0.45	6.70 ± 1.03
Raphe	0.40 ± 0.05	2.00 ± 0.14†	4.45 ± 0.74	2.50 ± 0.43	0.79 ± 0.22	3.00 ± 0.48	5.54 ± 0.70
Acc	0.38 ± 0.04	2.09 ± 0.16†	4.98 ± 0.78	2.19 ± 0.29	0.77 ± 0.18	2.13 ± 0.29	7.91 ± 1.63
				DOPAC			
Intact	0.10 ± 0.02	0.36 ± 0.05	0.69 ± 0.14	1.52 ± 0.20	0.36 ± 0.06	0.86 ± 0.14	1.22 ± 0.23
OB	0.21 ± 0.04*	1.00 ± 0.24*	0.80 ± 0.17	1.80 ± 0.43	0.72 ± 0.09†	1.61 ± 0.38	0.92 ± 0.27
Raphe	0.14 ± 0.04	0.59 ± 0.05†	0.86 ± 0.15	1.61 ± 0.24	0.39 ± 0.08	0.69 ± 0.17	1.17 ± 0.18
Acc	0.16 ± 0.05	0.59 ± 0.09	0.89 ± 0.12	1.49 ± 0.15	0.43 ± 0.06	0.40 ± 0.08*	1.00 ± 0.35
				HVA			
Intact	0.23 ± 0.03	n.d.	n.d.	1.53 ± 0.21	n.d.	0.73 ± 0.05	1.08 ± 0.09
OB	0.53 ± 0.11*	n.d.	n.d.	1.51 ± 0.18	n.d.	0.97 ± 0.21	1.01 ± 0.10
Raphe	0.65 ± 0.12†	n.d.	n.d.	1.39 ± 0.10	n.d.	0.95 ± 0.11	0.89 ± 0.13
Acc	0.75 ± 0.10‡	n.d.	n.d.	1.35 ± 0.15	n.d.	0.84 ± 0.12	1.27 ± 0.16

Each value represents the mean ± S.E. (ng/mg protein) for 28 rats (intact nonkiller), 19 rats (OB killer), 16 rats (Raphe killer), 17 rats (Acc killer).

Significantly different from intact: *$p<0.05$, †$p<0.01$, ‡$p<0.001$.

n.d.; not detectable.

32–57% in all brain regions examined. In OB rats, 5-HT content was significantly increased in MB, 5-HIAA content was significantly decreased in FC, and a ratio of 5-HIAA/5-HT was significantly reduced in LH, MB and FC. In Acc rats, 5-HT content was significantly increased in LH and ACE, 5-HIAA concentration was decreased in AME, and the 5-HIAA/5-HT ratio was decreased in MB and ACE.

As shown in Table 2, DOPAC and HVA concentrations of LH and FC, respectively, increased in all three muricide models, accompanied by an increase in DA content of LH in Raphe and Acc rats. OB rats showed an increase of DOPAC content in FC and AME. Acc rats displayed a decrease of DOPAC content in ABL.

DISCUSSION

The present study demonstrated that NA content in LH and VMH increased in OB, Raphe and Acc rats exhibiting muricide. The turnover rate of NA has been reported to decrease in LH and AME of OB rats, and in vivo NA release from LH was significantly decreased after OB [4]. These evidences suggest that an increased NA content means lowered NA function in LH, VMH and AME of OB rats. It may, therefore, be speculated that the lowering of NA function in LH and VMH has an important role in the exhibition of muricide in OB, Raphe and Acc rats. NA level in MB was also significantly increased in OB and Acc rats but not in Raphe rats. NA level in AME was increased in OB rats but decreased in Acc rats. These results suggest that the noradrenergic mechanisms in MB and AME are differently involved in the mediation of muricide in these three muricide models. The functional role of MB has remained unclear. There is some evidence suggesting an involvement of MB in the regulation of emotional behavior [5, 10, 12]. Taking the present results suggesting the lowered serotonergic function in MB in all three muricide models into consideration, MB may have an important role in the exhibition of muricide. An increase of NA content in VMH was accompanied by no change of other monoamines in OB and Acc rats. The alteration of NA activity in VMH thus seems to be important in the mediation of muricide in OB and Acc rats.

It is of interest that changes in the dynamics of indoleamines of LH and MB was observed in all three muricide models. In Raphe rats, of course, 5-HT and 5-HIAA contents decreased in LH and MB, and a ratio of 5-HIAA/5-HT tended to increase in all brain areas. This may reflect the activity of 5-HT neurons protected from raphe lesions. OB rats showed a decrease in a ratio of 5-HIAA/5-HT in LH and MB. Acc rats displayed a decrease in 5-HIAA/5-HT ratio in MB and an increase of 5-HT content resulting in a tendency to decrease in 5-HIAA/5-HT ratio of LH. These findings suggest that the serotonergic function may be lowered in LH and MB of OB, Raphe and Acc rats. This extended to FC and ACE in OB and Acc rats, respectively. The serotonergic mechanisms have been known to be involved in the exhibition of muricide. Therefore, the reduced serotonergic function in LH and MB may mediate the exhibition of muricide in these three muricide models.

An increase of HVA and DOPAC content was found in FC and LH of all three muricide models. This increase of DA metabolites led us to speculate that the dopaminergic activity may be increased in LH and FC. Broderick *et al.* [2] suggested that the dopaminergic system in the amygdala and hypothalamus was not predominantly involved in the appearance of muricide. It has also been reported that DA may mediate defensive aggression such as hyperirritability [8]. Therefore, increased activity of DA neurons in LH and FC may be related to hyperirritability manifested by OB, Raphe and Acc rats.

In conclusion, the present study demonstrated that the changes in the noradrenergic (LH and VMH) and serotonergic functions (LH and MB) were well correlated with the manifestation of muricide of OB, Raphe and Acc rats. Different mechanisms in various amygdaloid nuclei and FC mediate the exhibition of muricide in three muricide models. In OB rats, the noradrenergic function of MB and AME and the serotonergic function of FC seem to participate in the mediation of muricide, while the exhibition of muricide in Acc rats seems to be mediated by the noradrenergic system in MB and both the serotonergic and noradrenergic systems in AME and ACE.

ACKNOWLEDGEMENTS

This investigation was supported by the Grant-in-Aid for Scientific Research and the Grant-in-Aid for Encouragement of Young Scientists from the Japanese Ministry of Education, Science and Culture.

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