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Changes in Brain Catecholamine Levels Following Olfactory Bulbectomy and the Effect of Acute and Chronic Administration of Desipramine in Rats

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IWASAKI, K., M. FUJIWARA, S. SHIBATA AND S. UEKI. *Changes in brain catecholamine levels following olfactory bulbectomy and the effect of acute and chronic administration of desipramine in rats.* PHARMACOL BIOCHEM BEHAV 24(6) 1715–1719, 1986.—Bilateral olfactory bulbectomy of the rat caused marked changes of noradrenaline level in several brain regions accompanied with the development of mouse-killing behavior (muricide). Noradrenaline level increased in the medial amygdala, ventromedial and lateral hypothalamus in muricidal olfactory bulbectomized rats (OB rats) but not in non-muricidal OB rats, while dopamine level decreased in the lateral hypothalamus in muricidal OB rats. Acute administrations of desipramine not only suppressed muricide of OB rats but normalized noradrenaline change in ventromedial hypothalamus and dopamine change in lateral hypothalamus. Chronic administration of desipramine also suppressed muricide and normalized noradrenaline changes in ventromedial and lateral hypothalamus and medial amygdala. These findings suggest that the increase in noradrenaline levels in the medial amygdala, ventromedial and lateral hypothalamus may be important for the induction of muricide in OB rats, and muricide was suppressed by desipramine in accordance with the normalization of increased noradrenaline levels, and that the change in dopaminergic function in the lateral hypothalamus may also be important for this muricide.

Brain catecholamine HPLC-EC Hypothalamus Amygdala Desipramine Olfactory bulbectomy Muricide

CERTAIN brain lesions can alter the levels of biogenic amines in remote areas from the lesioned site. For example, transection of bilateral olfactory bulbs is associated with a decrease of noradrenaline (NA) level in the telencephalon [6] and an increase in the hypothalamus [15]. Unilateral lesion of the olfactory bulb caused a significant decrease of NA level in the ipsilateral telencephalon and an increase in the brainstem [4].

Bilateral olfactory bulbectomy has been well known to elicit characteristic hyperemotionality including mouse-killing behavior (muricide) in the rat. This muricide is selectively inhibited by tricyclic antidepressants, especially desipramine (DMI), well known to have a potent blocking action on NA uptake in the central nervous systems [1,8]. Furthermore, muricide is similarly inhibited by NA or DMI microinjected into the medial amygdala but not in the central and basolateral amygdala. These facts also suggest that the mechanism eliciting muricide in olfactory bulbectomized rats (OB rats) involves the central noradrenergic system, especially in the medial amygdala. However, none of the studies have so far proposed the relationship between muricide and neurochemical changes of NA in small regions of the brain following olfactory bulbectomy.

Recently developed procedures of high-performance liquid chromatography (HPLC) are now available and have an advantage of allowing endogenous levels of a number of neurotransmitters to be measured simultaneously in the same small sample [7]. In the hope of elucidating the role of catecholamines (CA) in muricide in this study, CA levels in small brain areas of the hypothalamus and amygdaloid complex were measured in OB rats using an HPLC with electrochemical detection (HPLC-EC), and the effects of acute and chronic administrations of DMI on CA levels were also investigated.

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METHOD

Chemicals

The catechol standards noradrenaline HCl (NA), adrenaline bitartrate (AD), 3,4-dihydroxyphenylalanine (L-DOPA), 3,4-dihydroxyphenylacetic acid (DOPAC), dopamine HCl (DA) and 3,4-dihydroxybenzylamine (DHBA) were purchased from Sigma (St. Louis, MO). Desipramine HCl (Pertfran) was obtained from CIBA-GEIGY. Activated alumina was purchased from Bioanalytical Systems. All other reagents were reagent grade from E. Merk (Darmstadt, G.F.R.).

Animals and Surgery

Male Wistar King A rats weighing 250–280 g at the beginning of the experiment, supplied by Kyushu University Institute of Experimental Animal were used. Before olfactory bulbectomy, all 131 rats underwent one muricide test. Only animals (88 rats) not showing muricide were selected for the experiment. The olfactory bulbs were removed bilaterally by suction as described previously [10]. Immediately after olfactory bulbectomy, isolation housing was commenced. Rats displaying muricide within 14 days after the surgery were used for OB killer group and remainders for OB non-killer group. For control group (intact non-killer) rats not displaying muricide in group housing were used and the rats displaying muricide in group housing for intact killer group. All animals were given food and water ad lib in an air conditioned room (23±1°C) with a 12 hr light-dark cycle (lights on at 07:00 a.m.).

Procedure

Muricide was assessed as positive if the rat bit and killed a mouse within 3 min after introducing it into the rat's home cage. In order to elucidate a relationship between brain catecholamine changes and muricide, we used the following 4 experimental groups: intact non-killer (n=12), intact killer (n=6), OB non-killer (n=5) and OB killer (n=12). In the next experiment we examined the effect of DMI on brain catecholamines in OB killer rats. DMI (20 mg/kg) was intraperitoneally administered once in an acute study (n=24) and once a day for 7 days in a chronic study (n=6). Physiological saline was also intraperitoneally administered once in an acute study (n=7) and once a day for 7 days in OB killer (n=6) and intact non-killer (n=10) rats.

Assay of Catechols

The rat was sacrificed by decapitation and the brain was immediately removed. The brain was cut into 1 or 2 mm coronal sections using a micro knife. Individual regions, i.e., the ventromedial hypothalamus (VMH), lateral hypothalamus (LH), medial amygdala (AME), central amygdala (ACE), basolateral amygdala (ABL) from 2 mm section and preoptic area (POA) from 1 mm section, were dissected on an ice-cold glass stage. The frontal cortex (FC) was used at the central part. The regional samples were homogenized in 300 μl 0.5 M PCA containing 0.05% Na_2EDTA and 0.1% $Na_2S_2O_5$, and added DHBA (20 ng) as the internal standard. Following centrifugation, the supernatant was added to 20 mg activated alumina and buffered to pH 8.6 with 1.5 M Tris EDTA buffer. The vials were shaken for 20 min on a reciprocal shaker, the supernatant was removed and the activated alumina was washed three times with 1 ml of distilled water.

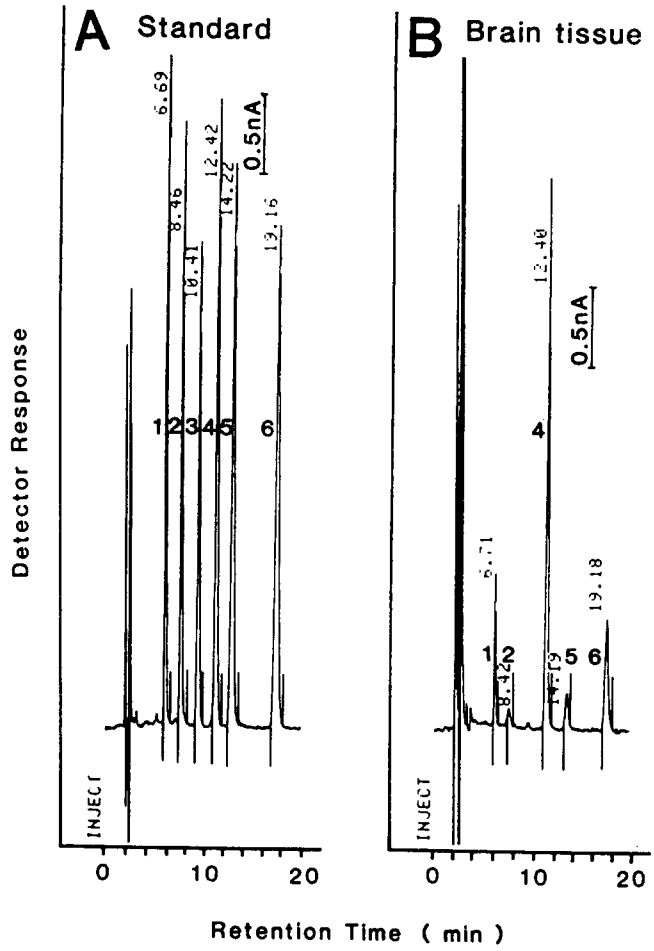

FIG. 1. Chromatograms of catecholamines. (1) Noradrenaline (NA), (2) adrenaline (AD), (3) 3,4-dihydroxyphenylalanine (L-DOPA), (4) 3,4-dihydroxybenzylamine (DMBA), (5) 3,4-dihydroxyphenyl acetic acid (DOPAC), (6) dopamine (DA). Part A is a profile of standards. Parts B is a profile of a tissue sample from VMH.

Activated alumina was replaced to microfilter and water was filtered off. Finally, the catechols were eluted from the alumina by agitation for 20 min with 100 μl of 0.2 M HCl. The supernatant was filtered off and transferred into microvials (250 μl size) of the automatic injector.

Determination of catechols in the tissue samples was performed using HPLC-EC. A standard curve was generated by taking varying amounts of the compounds.

The HPLC system (Waters Assoc., Milford, MA.) utilized a Yanapak ODS-A reverse phase column (25 cm × 40 mm, Yanaco, Kyoto, Japan) coupled with a glassy carbon electrode (VMD-501, Yanaco, Kyoto, Japan) set at a potential of +0.65 V versus reference electrode. The electronic controller was set at 4 nA/V. The HPLC buffer was 0.1 M phosphate pH 2.85 containing 1.5 mM sodium octyl sulphate and 11% methanol with 20 μM Na_2EDTA. The flow rate of the HPLC was maintained at 0.9 ml per min. Catechols were quantified by calculating the area under the curves using an integrator (Model 730, Waters Assoc.) and their contents determined from standard curves.

RESULTS

Chromatograms of NA, AD, L-DOPA, DOPAC, DA with

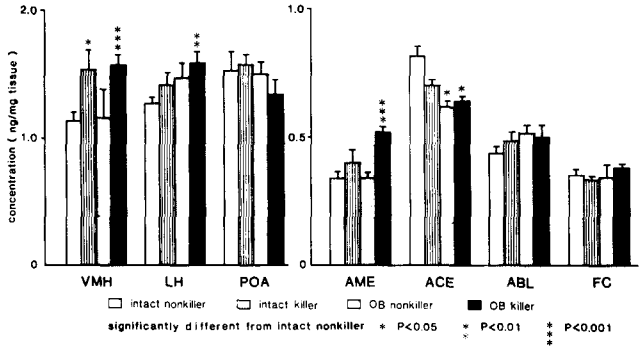

FIG. 2. Changes in brain noradrenaline levels following olfactory bulbectomy in rats. Mean±S.E. statistical values are evaluated by means of the Student's *t*-test. VMH: ventromedial hypothalamus, LH: lateral hypothalamus, POA: preoptic area, AME: medial amygdala, ACE: central amygdala, ABL: basolateral amygdala, FC: frontal cortex. Results were evaluated statistically by means of the Student's *t*-test. All these abbreviations and statistical analysis are the same in the following Figs. 3, 4, 5, 6 and 7.

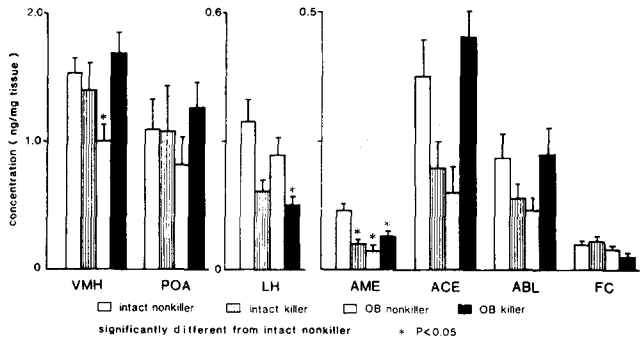

FIG. 3. Changes in brain dopamine levels following olfactory bulbectomy in rats. Mean±S.E. statistical values are evaluated by means of the Student's *t*-test.

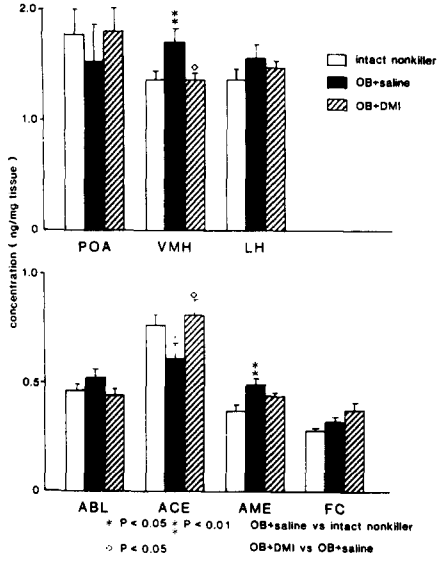

FIG. 4. Effects of acute administration of desipramine on brain noradrenaline levels in rats. Mean±S.E. statistical values are evaluated by means of the Student's *t*-test.

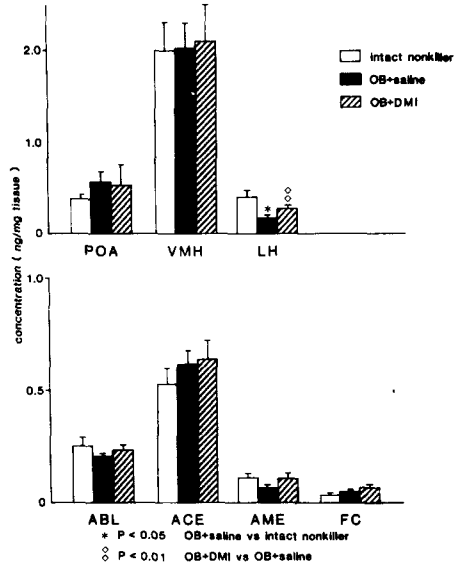

FIG. 5. Effects of acute administration of desipramine on brain dopamine levels in rats. Mean±S.E. statistical values are evaluated by means of the Student's *t*-test.

internal standard DHBA are shown in Fig. 1. Part A and part B are profiles of standards and tissue sample from VMH respectively. Calibration curves for standards were found to be linear from 0.05 to 100 ng (result not shown).

Following olfactory bulbectomy, the rats elicited muricide. Fourteen days after lesion, NA level significantly increased in VMH, LH and AME of OB killer and in VMH of intact killer rats (n=6–8) in comparison with intact non-killer rats (n=5–9), while NA level did not change in OB non-killer rats (n=6) (Fig. 2). In ACE, NA level decreased in both OB killer and OB non-killer rats (n=9). There was no change of NA levels in all other brain regions. DA levels decreased in LH of OB killer (n=7), and decreased in AME of both OB killer (n=7) and OB non-killer rats (n=5) (Fig. 3). Other compounds (AD, L-DOPA, DOPAC) were not changed after olfactory bulbectomy in all areas.

Single Administration

Single administration of saline not only had no effect on muricide of OB rats but did not induce muricide in intact non-killer rats. In OB killer rats treated with saline, NA level significantly increased in VMH and AME, but decreased in ACE in comparison with that of intact non-killer rats with saline treatment. A single administration of DMI 20 mg/kg suppressed muricide of OB rats. At the time of peak effect, 30 min after administration, the incidence of muricide was 50%. At this time, the rats with suppressed muricide (12/24) were killed and taken for assay. DMI significantly decreased NA levels in VMH and increased in ACE of OB killer rats compared with saline treated rats (n=7), and NA levels returned to the level in intact non-killer rats (n=10) (Fig. 4). There is no significant difference between OB killer rats with DMI and intact rats with saline. Dopamine level in LH was also normalized by DMI 20 mg/kg administration (Fig. 5).

Chronic Administration

Chronic administration of saline also did not affect the

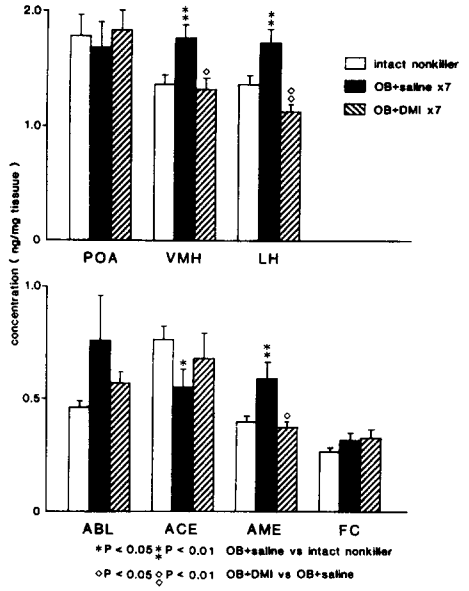

FIG. 6. Effects of chronic administration of desipramine on brain noradrenaline levels in rats. Mean±S.E. statistical values are evaluated by means of the Student's *t*-test.

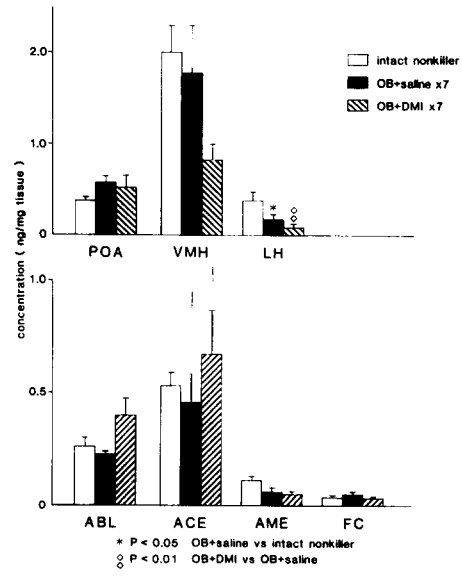

FIG. 7. Effects of chronic administration of desipramine on brain dopamine levels in rats. Mean±S.E. statistical values are evaluated by means of the Student's *t*-test.

incidence of muricide in both OB killer and intact non-killer rats, while chronic administration of DMI 20 mg/kg for 7 days strongly suppressed muricide, as mentioned in our previous report [12]. Catecholamine (CA) levels were determined 24 hr after the last administration of saline or DMI. In OB killer rats with saline treatment, NA level increased in VMH, LH and AME, but decreased in ACE. Catecholamine levels were determined 24 hr after the last drug administration in the rats (n=5) with suppressed muricide by DMI (5/6). Noradrenaline levels in VMH, LH and AME were decreased by DMI and returned to those of intact non-killer rats except LH (n=4–5) (Fig. 6). However, DA level of LH did not return to the control level (Fig. 7).

DISCUSSION

In order to elucidate the role of brain catecholamines in the development of muricide following olfactory bulbectomy in rats, we investigated the contents of these amines in the hypothalamus, amygdaloid complex and frontal cortex using a high sensitive HPLC-EC, since this method can detect a picogram of many catecholamines simultaneously from mg-range samples [7]. The NA level increased in VMH, LH and AME but not in POA, ABL and FC in OB killer rats. In ACE, NA level decreased in both OB killer and OB non-killer rats. This suggests that the NA mechanism of ACE is not involved in the induction of muricide of OB rats. These results strongly suggest that an increase of NA level in VMH, LH and AME plays an important role in the induction of muricide following olfactory bulbectomy.

A number of studies have reported that olfactory bulbectomy caused an increase of NA in the hypothalamus [15]. The high NA level in the brainstem and hypothalamus can be explained by the assumption that the olfactory bulb exerts an inhibitory influence on NA storage in these brain regions [4]. Several anatomical and physiological studies have shown a close relationship between the olfactory bulb and hypothalamus and amygdaloid complex [3,5]. Moreover, there are direct and indirect projections from the olfactory bulb to the amygdaloid complex and hypothalamus [5]. Although these reports are in agreement with our present results, our present results strongly suggest that within the hypothalamus and amygdala, the increase of NA level in VMH, LH and AME is very important for the development of muricide.

Our recent study demonstrated that the in vivo NA release in LH decreased in OB killer rats 7 days after olfactory bulbectomy (unpublished result). As the olfactory bulbs exert the inhibitory influence on NA storage but the facilitatory influence on NA release, the increase of NA level of VMH, LH and AME in OB killer rats may indicate the reduction of NA function in these brain regions.

Meanwhile, Uchimura *et al.* suggested that brain HVA level increased in the amygdala in isolated muricidal rats [14]. Therefore, the decreased level of DA in LH may be due to the increase of DA function in this area. But we have no idea at the moment about the relationship between DA function and the muricide induction.

From the behavioral studies we have suggested that the increased NA function in AME and LH but not in ACE and ABL is important for muricide inhibition in OB rats, since microinjection of NA into AME and LH but not into ABL, ACE and POA strongly inhibited muricide [2,11]. The present results also strongly indicate that the induction of muricide following olfactory bulbectomy is resulted from the reduction of NA mechanisms in VMH, LH and AME, since NA level at storage increased and became functionally decreased in OB rats.

We previously reported that DMI, which predominatly blocks the NA uptake into nerve ending [8], inhibited muricide and this effect was potentiated by its chronic treatment [12]. In the present experiment, therefore, we examined whether the increase of NA in VMH, LH and AME was normalized by acute or chronic administration of DMI.

The increase of NA in VMH of OB rats was reversed to the control level but that of NA in LH and AME remained

unchanged by a single administration of DMI 20 mg/kg IP. However, the increase of NA in VMH, LH and AME of OB killer rats was remarkably decreased and returned to the control level except LH by daily administrations of DMI 20 mg/kg for 7 days. The decreased level of DA in LH was also normalized by a single administration of DMI 20 mg/kg IP.

From neurochemical studies it was revealed that chronic administration of DMI potentiated the NA release mediated by blockade of presynaptic α_2-receptor and its NA uptake blocking action [9] and resulted in the increase of turnover rate of NA [13]. Our behavioral studies have demonstrated that the increase in brain NA activity but not in serotonin activity may be more important in the mechanism of muricide inhibition by chronic administration of antidepressant drugs in OB rats, since the order of potency of antimuricide effect was DMI > amitriptyline > clomipramine [12]. Our present finding, together with those behavioral and neurochemical studies, also suggest that the reduction of NA function in VMH, LH and AME after olfactory bulbectomy may be reversed by chronic administration of DMI.

In conclusion, the present results at least indicate that the NA mechanism in VMH and LH and AME plays an important role in the development of muricide by olfactory bulbectomy as well as in the muricide suppression by DMI, and the DA mechanism in LH may also play some role in muricide induction.

ACKNOWLEDGEMENTS

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