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In Vivo Changes in Brain Catecholamine Release From Rat Hypothalamus Following Olfactory Bulbectomy

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IWASAKI, K., M. FUJIWARA AND S. UEKI. *In vivo changes in brain catecholamine release from rat hypothalamus following olfactory bulbectomy*. PHARMACOL BIOCHEM BEHAV **34**(4) 879–885, 1989.—The mechanism eliciting mouse-killing behavior (muricide), induced by bilateral olfactory bulbectomy, has been shown to involve the brain noradrenergic system; this is because muricide is specifically inhibited by the drugs which potentiate the activity of catecholaminergic neurons such as tricyclic antidepressants. Our previous reports also demonstrated that the hypothalamic noradrenaline (NA) contents increased in the rats which exhibited muricide. To further examine the hypothalamic noradrenergic function in muricide, a push-pull perfusion technique was applied for direct measurement of NA release from the lateral (LH) and ventromedial (VMH) hypothalamus in freely moving rats. Subsequently, the perfusates, including catecholamines and their metabolites were measured by means of high-performance liquid chromatography with electrochemical detection (HPLC-ECD). Three days after olfactory bulbectomy, 67% of the rats elicited muricide and NA release from LH tended to decrease. Moreover, 7 days after olfactory bulbectomy, most of the rats elicited muricide and NA release from LH was significantly decreased, but not from VMH. On the other hand, dopamine (DA) release from VMH without LH conversely increased on the 7th day after olfactory bulbectomy. These results suggest that the dysfunction of the noradrenergic system caused by the decrease in NA release from LH played an important role for the incidence of muricide.

Olfactory bulbectomy Muricide In vivo catecholamine release Push-pull perfusion Hypothalamus

MOUSE-KILLING behavior (muricide) induced by olfactory bulbectomy, which is the widely used animal model for evaluating antidepressants, has shown a close association with the central noradrenergic systems. This is because muricide was markedly inhibited by tricyclic antidepressants, especially desipramine (DMI), well known to be a NA reuptake blocker (2,19) and was also inhibited by microinjections of DMI or NA into the lateral hypothalamus (6). Furthermore, our previous reports indicated that the electrical stimulation of locus coeruleus suppressed muricide in olfactory bulbectomized (OB) rats (22). These reports suggested that muricide in OB rats was suppressed by noradrenergic stimulation of the hypothalamus and a cell body which connected to the hypothalamus via the medial forebrain bundle (MFB).

Biochemical studies also indicated that NA levels increased in the medial amygdala (AME), ventromedial (VMH) and lateral hypothalamus (LH) in muricidal, but not in nonmuricidal OB rats, and NA levels in all these regions were normalized by the treatment of DMI in parallel with the suppression of muricide (7). These results also suggest that the mechanism eliciting muricide in OB rats involves the central noradrenergic systems such as the hypothalamus. However, it is still unknown whether these hypothalamic changes of the NA contents are related to the change of NA release from the nerve endings or for some other reasons. To further examine the hypothalamic noradrenergic function in muricide, the in vivo change of hypothalamic CA release was investigated because the hypothalamus is one of the NA-rich areas of the brain and CA release is easy to determine.

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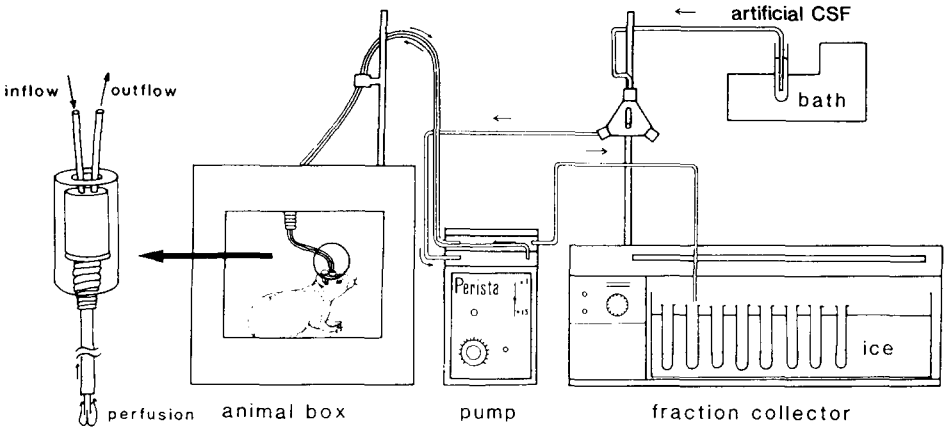

FIG. 1. Scheme of the push-pull perfusion system.

In in vivo studies, the release of NA from brain tissue has been studied extensively in recent years. Radiolabeled NA was widely used by means of tissue preloading methods (1,13). More recently, the release of endogenous NA from tissue slices has been made possible by the advent of radioenzymatic assays (15) and high-performance liquid chromatography (HPLC) (17).

On the other hand, the monitoring changes in endogenous monoamines and their metabolites in a small sample were established by the marked improvement in the sensitivity of assays, especially based on high performance liquid chromatography combined with electrochemical detection (HPLC-ECD). In addition, in vivo push-pull studies have been utilized for the measurement of endogenous monoamine release from brain perfusates accompanied with observing behavioral changes of the unanesthetized, freely moving rats (11, 14, 16, 24).

In the present study, we investigated the change of endogenous NA and DA release from the hypothalamus following olfactory bulbectomy of rats using the push-pull perfusion method.

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). All other reagents were reagent grade from E. Merck (Darmstadt, G.F.R.).

Animals and Surgery

Male Wistar King A rats, weighing 220–260 g, supplied by the Kyushu University Experimental Animal Institute were used. The rats were allowed to adapt to the individual home cages and were kept on a constant light-dark cycle (light 0700–1900). Before olfactory bulbectomy, all rats underwent one muricide test (14). Only animals not showing muricide were selected for this experiment.

Rats were anesthetized with sodium pentobarbital (40 mg/kg IP) and were implanted with a guide cannula (stainless steel, 21 gauge, Plastic Products Co., Roanoke, VA) in the left lateral hypothalamus (LH; A: anterior from the bregma; −3.0, L: lateral to the midline; 1.7, H: horizontal below the skull surface; 9.0) or ventromedial hypothalamus (VMH; A: 2.7, L: 0.6, H: 9.0) according to the coordinates of König and Klippel (9). Until the beginning of the experiment, a dummy cannula (stainless steel) was inserted into the guide cannula.

Olfactory Bulbectomy

Bilateral olfactory bulbs were suctioned through a hole made in the skull under pentobarbital sodium (40 mg/kg, IP) anesthesia. Immediately after the olfactory bulbectomy, isolation housing was commenced. Sham operations were performed by the same procedure with the exception of olfactory bulb suction.

Push-Pull Perfusion

For 1 week of the postoperative care period, the inner cannula (stainless steel, 28 gauge) was extended 0.5 mm beyond the end of the implanted guide cannula and each rat was connected to a two-channel peristaltic perfusion pump (Atoh, Japan) through polyethylene tubing and placed in an animal box (50 × 50 × 50 cm) for observation. The artificial cerebrospinal fluid (CSF; Merlis' solution of the following composition: 119.0 mM NaCl, 3.3 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM $MgCl_2$, 0.5 mM Na_2HPO_4, 21.0 mM $NaHCO_3$, 3.4 mM glucose, pH 7.4) was warmed to 37°C and bubbled with O_2 before use and push-pull perfusion was performed at the rate of 30 μl/min of CSF (Fig. 1). Samples of tissue perfusate were collected into ice-cold 1.5 ml test tubes containing 10 μl of perchloric acid (PCA) containing 0.1% (w/v) $Na_2S_2O_4$ and 0.1% (w/v) Na_2 EDTA. Successive 10-min (300 μl) samples of the perfusate were collected and stored at −20°C for later analysis.

Push-pull perfusion was performed before as well at 3 and 7 days after olfactory bulbectomy. The incidence of muricide was observed during the perfusion. Muricide was assessed as positive if the rat bit and killed a mouse within 3 minutes after introducing it into the rat's home cage.

Assay of Catecholamines

Endogenous catecholamine release from the hypothalamus was measured using HPLC-ECD. Brain perfusate (200 μl) was purified by the aluminum absorption method, which we previously described (5), and injected into HPLC-ECD. The HPLC system (Waters Assoc., Milford, MA) utilized a Yanapak ODS-A reverse phase column (25 cm × 46 mm, Yanako, Kyoto, Japan) coupled

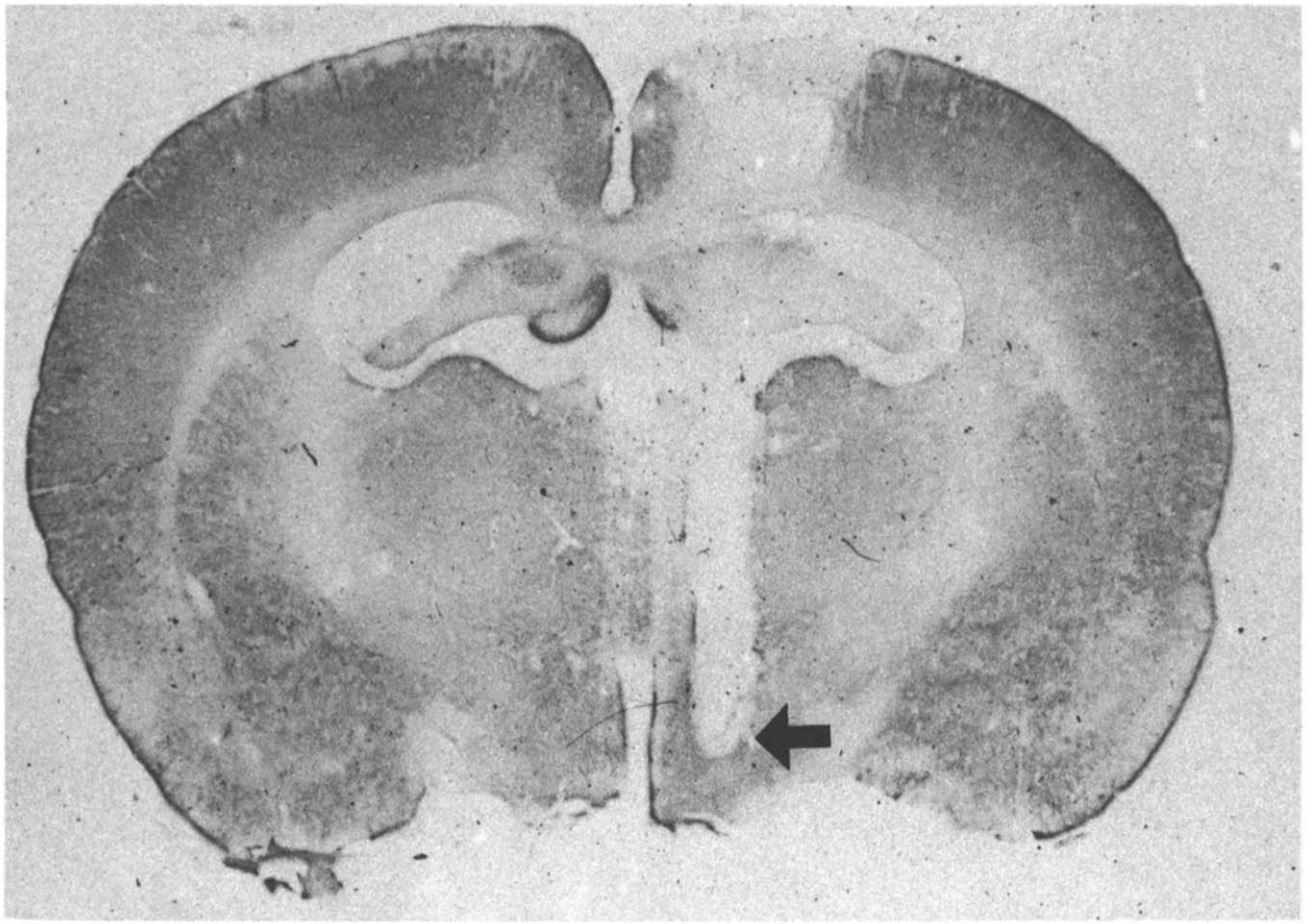

FIG. 2. The photograph of a typical placement of cannula in LH. The arrow represented the placement of cannula tip.

with a glassy carbon electrode (VMD-501, Yanako, Kyoto, Japan) set at a potential of +0.65 V versus reference electrode. The HPLC buffer was 0.1 M phosphate, pH 2.85 containing 1.5 M sodium octyl sulfate (PIC B_8, Waters Assoc.) and 11% methanol with 20 μM Na_2 EDTA. The flow rate was maintained at 0.9 ml/min. Catecholamines in the perfusate were quantified by calculating the area under the curves using an integrator (Model 730, Waters Assoc.) and their contents determined from the standard curves.

Histological Analysis

After completion of the push-pull study, the animals were anesthetized with ether and their brains were perfused with saline and 10% formalin through the carotid. The brain was then removed, frozen sections, 50 μm thick, were made and stained with cresyl violet. The extent of olfactory bulbectomy and the placement of guide cannula were verified histologically. If the extent of olfactory bulbectomy and the placement of guide cannula were not appropriate, the results in these rats were discarded from the data.

Statistical Analysis

Student's *t*-test was used to determine the significant differences in CA release from the brain.

RESULTS

In push-pull studies, the recovery of monoamines through the cannula implanted into brain tissues is influenced by the distance between the end of inner and guide cannula and the flow rate of the perfusate. In the initial preliminary study, therefore, we measured the in vitro recovery of the perfusate in varying tip extension (0.25–0.75 mm) of the inner cannula beyond the guide cannula using 0.6% agar gel containing ^{3}H-noradrenaline (data not shown). Tip extension of 0.5 mm was most efficient for the recovery of the perfusate. In the second preliminary study, the optimum flow rate proved to be 30 μl/min, which showed the adequate measurable material to be collected within the shortest sampling time for 10 min. In the present study, brain damage due to the guide cannula implantation was mild (Figs. 2 and 3) and did not influence the general behavior of the rats either, for example, open-field activity, motor coordinations and incidence of muricide. Figure 4A shows the typical chromatograms of NA, AD, L-DOPA, DOPAC and DA with internal standard DHBA. Figure 4B is a perfusate from LH. The determination limit of noradrenaline (NA) was about 10 pg from calibration curves (data not shown), and is enough to detect the content of NA in brain perfusate. Normal release of CA from LH is shown in Fig. 5. Constant release of NA and DA were seen in successive 10-min perfusates for 10 to 60 min.

Following olfactory bulbectomy, rats with perfusion began to

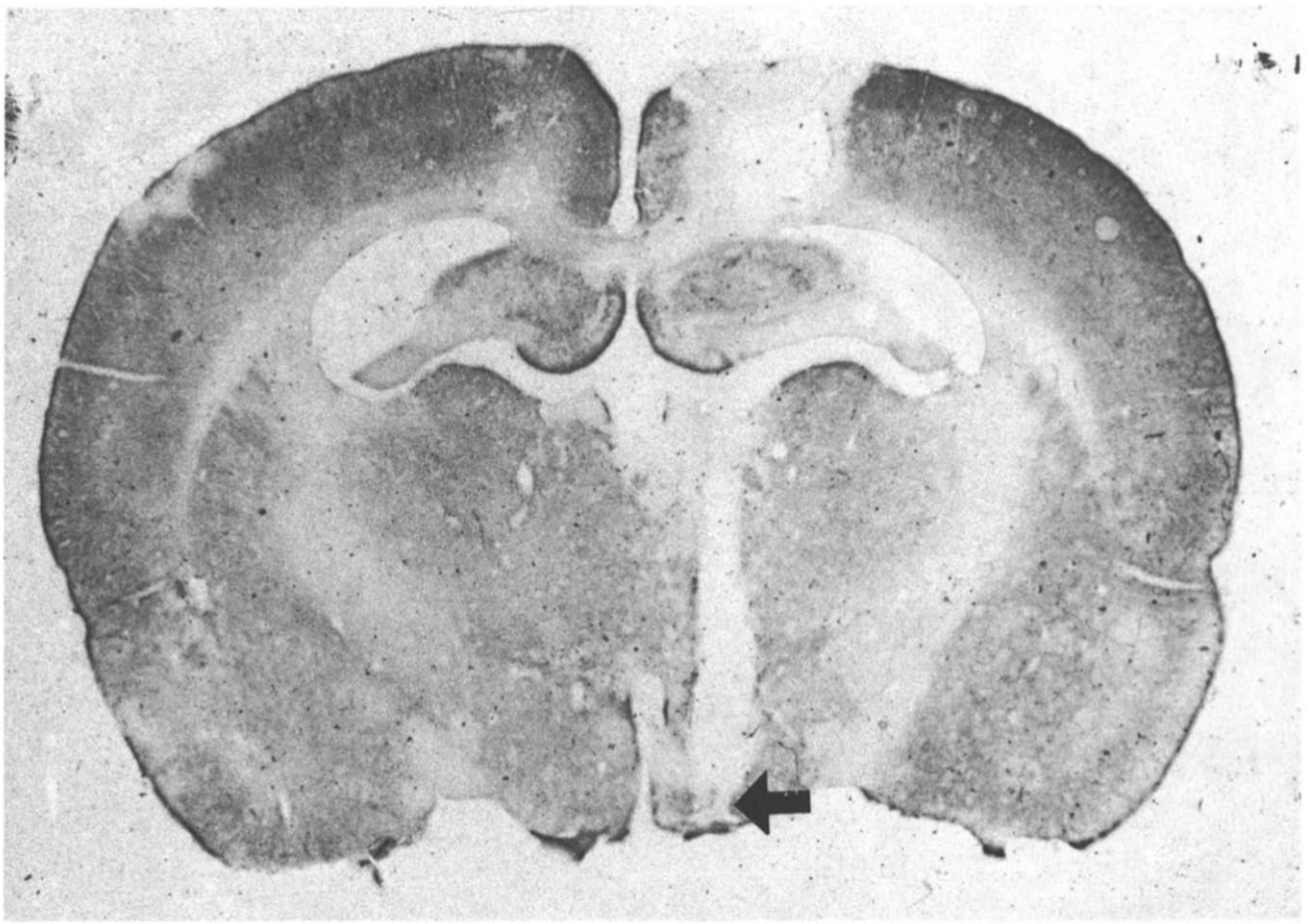

FIG. 3. The photograph of a typical placement of cannula in VMH. The arrow represented the placement of cannula tip.

elicit muricide 3 days after the surgery (67%), and the incidence of muricide was 89% at 7 days after the surgery, while sham rats with perfusion elicited no muricide. The mean value of NA release from LH decreased significantly 7 days after olfactory bulbectomy (sham group: N=5, 58.83±5.07 pg/100 μl/10 min; OB group: N=5, 38.00±3.09 pg/100 μl/10 min. $p<0.05$ by means of Student's *t*-test). There was, however, no change in the dopamine (DA) release from LH at eiher 3 days or 7 days after olfactory bulbectomy (sham group: 14.81±2.52 pg/100 μl/10 min; OB group: 13.76±3.21 pg/100 μl/10 min, Fig. 6).

On the other hand, NA release from VMH remained unchanged even 7 days after olfactory bulbectomy (sham group: N=5, 42.2±3.79 pg/100 μl/10 min), but DA release from VMH increased significantly 7 days after olfactory bulbectomy (sham group: N=5, 12.98±3.24 pg/100 μl/10 min; OB group: N=5, 35.38±8.83 pg/100 μl/10 min, Fig. 7).

DISCUSSION

The purpose of the present investigation was to determine the endogenous catecholamine release from the hypothalamus using the dynamic utility of push-pull perfusion technique, and to elucidate the relationship between behavioral events and biochemical change in unanesthetized freely moving rats.

A number of studies on the NA release from brain tissue have been demonstrated in vitro using radioactivity from preloading tissue slices with radiolabeled NA (1,13). Earlier studies demonstrated catecholamine release from the brain using push-pull perfusion (11). However, these studies did not measure endogenous bioamines, but rather the efflux of exogenously administered radiolabeled amines.

Recently, development of separation and assays based on high-performance liquid chromatography combined with electrochemical detection (HPLC-ECD) has prompted the increased use of push-pull perfusion methods for both studying and directly monitoring endogenous monoamine release in the brain (5,10). More recently, a similar procedure has been reported using a brain dialysis method which involves the implantation of a small dialysis tube into the brain area (3,24). However, most of the reports which described CA release using brain dialysis succeeded in the determination of DA release from the striatum, but not in that of NA from the hypothalamus.

In our preliminary studies, the brain dialysis method was less sensitive to the assay of NA release from the hypothalamus in freely moving rats. There seemed to be a lower recovery of amines across the dialysis membrane from brain tissue than in push-pull perfusion.

The present study, therefore, used the push-pull perfusion method for measuring extracellular NA and DA in the various brain areas.

It is also important to mention that in studies using chronic cannula implanting, it is generally necessary to consider the potential for brain tissue damage due from the cannula. In our present experiment, the histological results indicated some mild destruction of the brain tissue site by the implanting of a guide cannula. Therefore, there was no evidence at the site of the

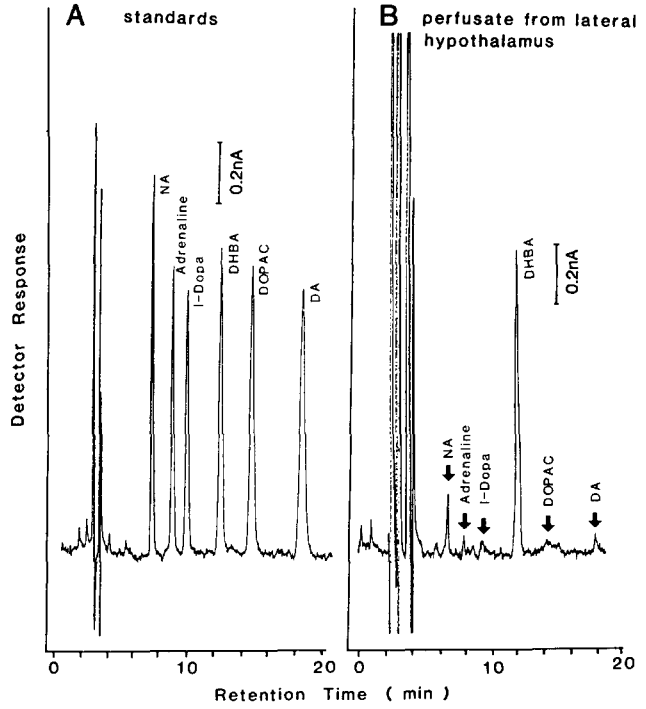

FIG. 4. HPLC chromatograms of catecholamines (1 ng) (A) and brain perfusate from the lateral hypothalamus (B).

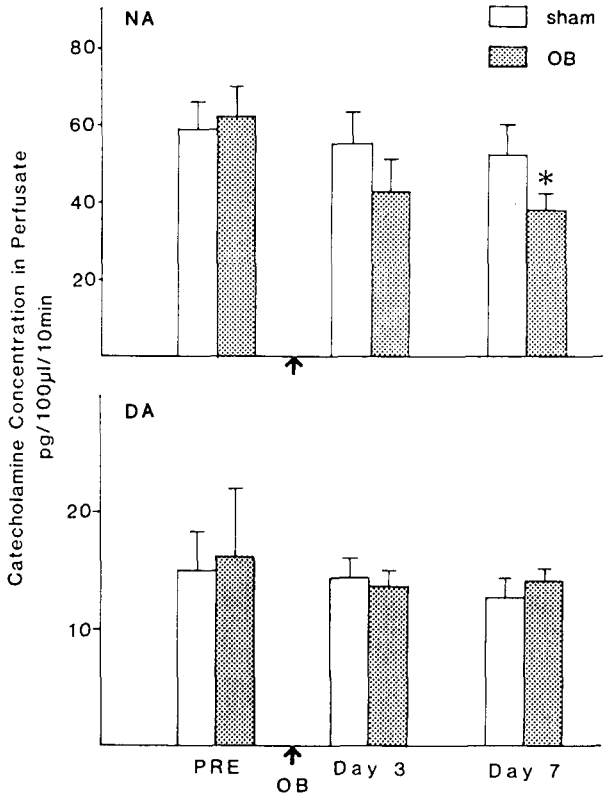

FIG. 6. Changes in brain noradrenaline and dopamine release from the lateral hypothalamus following olfactory bulbectomy in rats. Mean ± S.E. Statistical values are evaluated by means of Student's *t*-test.

cannula tip, and no changes in the rat's general behavior such as motor activity and/or motor coordination by this method of implantation. Furthermore, no significant differences in NA contents were observed between the implanted site and the intact site of LH (implanted site: 1.58 ± 0.07 pg/mg, N = 4; intact site: 1.60 ± 0.07 pg/mg, N = 4). These facts suggested that our cannula implantation had no affect on the behavior of the rats and did not destroy any brain tissue of the target site of perfusion such as LH or VMH.

In this experiment, we applied this push-pull pefusion for OB rats. Sixty-seven and 89 percent of the rats showed signs of muricide with aggressive behavior such as rod attack and resistance to handling at 3 and 7 days after olfactory bulbectomy, respectively. But the sham rats with no olfactory bulbectomy never showed aggressive behavior. When compared with the extracellular NA levels of sham rats, a 15% and 35% decrease in extracellular NA levels in LH was observed 3 days ($p<0.1$) and 7 days ($p<0.05$) after surgery, respectively. There seemed to be a very close correlation between the incidence of muricide and the decrease of NA in the perfusate from LH.

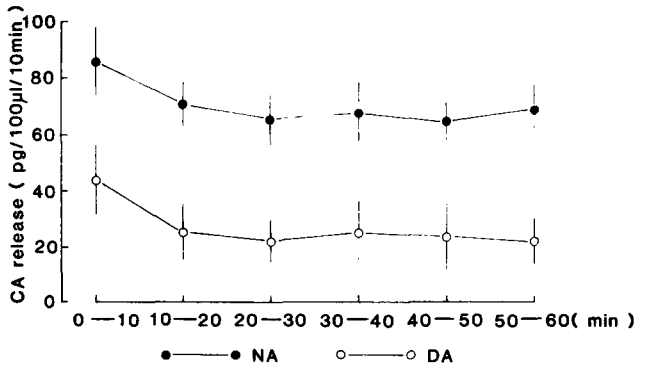

FIG. 5. In vivo release of noradrenaline and dopamine from the lateral hypothalamus in successive 10-min perfusate in normal rats.

There have been many reports which provide behavioral, physiological and biochemical support for the involvement of noradrenergic systems in the development and maintenance of muricide. Desipramine (DMI), which is known to be a selective NA reuptake blocker (19) in nerve endings, markedly suppressed the muricide of OB rats (20). Electrical stimulation of the locus coeruleus, which is a cell body-rich area of noradrenergic fiber, inhibited muricide (22). Moreover, microinjection of NA into the lateral hypothalamus (6) and medial amygdala (AME) was also shown to suppress muricide (20). These reports suggest that a noradrenergic mechanism plays a role in the incidence of muricide of OB rats.

Furthermore, our recent biochemical studies also demonstrated that brain NA levels in VMH, LH and AME significantly increased in muricidal, but not in nonmuricidal OB rats 7 days after olfactory bulbectomy (7,8). On the other hand, NA turnover rate decreased in LH and AME of muricidal OB rats (8). Interestingly, all these changes were normalized to control levels by acute and chronic administration of DMI accompanied with the suppression of muricide in OB rats (7). These facts also suggested a relationship between muricide incidence and brain noradrenergic function, especially in the hypothalamus or amygdala.

In analyzing the mechanisms involved in muricide following olfactory bulbectomy, Nakamura and Nakamura (12) suggested

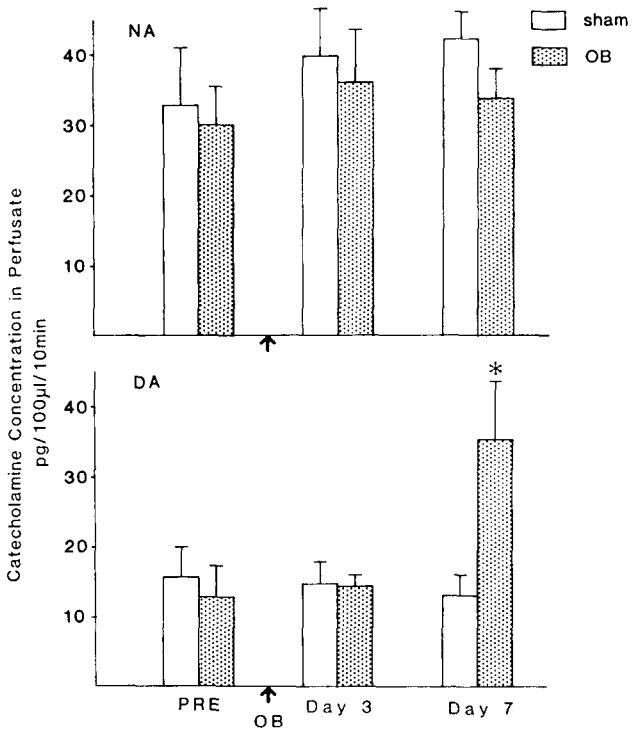

FIG. 7. Changes in brain noradrenaline and dopamine release from the ventromedial hypothalamus following olfactory bulbectomy in rats. Mean ± S.E. Statistical values are evaluated by means of Student's *t*-test.

that the destruction of olfactory bulbs caused retrograde interferences in the NA axonal flow in medial forebrain bundle (MFB) and successively produced a decrease in cortical NA content and an increase in pons-medulla and midbrain NA contents. If there were some retrograde interferences in NA fiber of MFB by olfactory bulbectomy, our present result of the decreased release of NA in LH and our previous report of the increased level NA content of LH (7) might also be responsible for the accumulation of presynaptic amine in the hypothalamus.

However, in our experiment, it is still unknown whether the decrease of extracellular NA release due to the interference of NA axonal flow occurred only in LH or not. Therefore, NA terminal rich areas such as LH might be easily influenced by axonal interference as well as the small change of NA function can be easily detected.

It was also necessary to clarify whether the incidence of muricide was really responsible for the monoamine change of the hypothalamus or whether it was responsible for the loss of sensory information from the vomeronasal organ. Next, we tried to use zinc sulfate treatment which disrupted the nasal mucosa (4) and make an experiment to determine whether these rats exhibited muricide or not. By the loss of sensory information of olfactory bulbs, zinc sulfate-treated rats could not easily discover the food pellets which were concealed from rat's view. The average deviation of elapsed time until the food was discovered was over 300 sec as compared with the intact control rats which discovered the food within 30 sec. Similar results were observed in OB rats. These facts suggested that both zinc sulfate and OB rats impaired sensory information. Nevertheless, only the OB rats elicited muricide while the zinc sulfate-treated did not. These results also supported the report of Cain *et al.*, which demonstrated that damage to the nasal mucosa with zinc sulfate had no change on either the copulatory effectiveness or mouse killing (4). Considering these results, the incidence of muricide of OB rats may not depend on the sensory information from olfactory bulbs, but on the change of brain noradrenaline in the brain.

In this present study, by using push-pull perfusion in a small portion of the hypothalamus, we demonstrated the significant decrease of NA release from LH which was 15% at 3 days and 35% at 7 days after olfactory bulbectomy. Furthermore, this change of NA release was closely paralleled with the incidence of muricide. This present conclusion is also supported by the works of Nakamura and Nakamura (12), Yoshimura (23) and Pohorecky (18) who suggest that changes in behavior of the rats is not due to the sensory function of olfactory bulbs, but instead related to central nervous tissue ablation. Moreover, nomifensine (10 mg/kg, IP) also suppressed muricide of OB rats and markedly increased NA release from LH to control levels (unpublished data). These facts suggest that decreased NA release is closely related to the incidence of muricide in OB rats.

It is also interesting to note that DA release from VMH increased 7 days after olfactory bulbectomy. Therefore, our previous reports indicated that the DA level and its turnover rate in VMH neither changed following olfactory bulbectomy (7,8), nor did the DA level change by DMI. These facts suggest that the increased DA release in VMH may not directly reflect the incidence of muricide.

In conclusion, the push-pull perfusion technique with HPLC-ECD allowed the monitoring of the neurotransmitter release from a small brain portion under the behavioral change in freely moving rats. The present results showing a decrease of NA release from LH appear to prove that NA function in LH is the critical structure mediating the development in muricide of OB rats.

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