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Effects of Psychotropic Drugs Microinjected into the Hypothalamus on Muricide, Catalepsy and Cortical EEG in OB Rats

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HARA, C , S WATANABE AND S UEKI *Effects of psychotropic drugs microinjected into the hypothalamus on muricide catalepsy and cortical EEG in OB rats* PHARMACOL BIOCHEM BEHAV 18(3) 423–431, 1983 —In order to elucidate the mechanism of anti-muricide action of psychotropic drugs in the brain, the present study examined influences of these drugs microinjected into the hypothalamus on muricide Inhibition of muricide by chlorpromazine (CPZ) was found both in the lateral preoptic area (l-POA) and the posterior part of the lateral hypothalamus (p-LH), and that by chlordiazepoxide was seen only the mammillary body These effects were accompanied by a drowsiness of cortical EEG Anti-muricide action of tricyclic antidepressants was found in p-LH The effect was not accompanied by EEG drowsiness Effects of atropine injected into the hypothalamus on muricide and cortical EEG were similar to those of the antidepressants Norepinephrine and serotonin did not show selective muricide-suppression Although either CPZ in l-POA and p-LH or the antidepressants in p-LH showed cataleptogenic effect, these effects did not appear related to anti-muricide action The relationship between the anti-muricide action of psychotropic drugs and the physiological functions of the brain was discussed in comparison with these systemic administrations

Psychotropic drugs Atropine Biogenic amine OB rat Muricide Catalepsy Cortical EEG Hypothalamus Microinjection

THE present study was designed to elucidate the site and mode of actions of psychotropic drugs in the brain using a mouse-killing behavior (muricide) of the olfactory bulbectomized rat (OB rat) as an indicator of drug action The OB rat exerts hyperemotionality including muricide Malick [15] reported that the OB rat would appear to be a better model for screening psychotropic drugs Indeed, the hyperemotional syndromes including muricide are suppressed by peripheral treatments of these drugs In particular, antidepressants inhibited only muricide without changing the other emotional syndromes [10, 14, 19, 26] Accordingly, antidepressants seem to inhibit muricide selectively rather than neuroleptics and anxiolytics The difference between antidepressants and the other psychotropic drugs in the anti-muricide action, however, is unknown

On the other hand, muricide of the OB rat is inhibited by the amygdaloid lesion [6] When testing rats known to be innately muricidal, it was found that antidepressants injected into the amygdala blocked muricide immediately, while chlorpromazine (CPZ) had no effect until after 1 hr and the inhibition of muricide by the drug could have been due to the accompanying ataxia and genralized sedation [11] Watanabe *et al* [27] showed that CPZ and chlordiazepoxide (CDP) injected into the limbic structures of the OB rat did not show any effects, while antidepressants significantly inhibited muricide when the drugs were injected into the medial amygdaloid nucleus without causing any other behavioral changes Thus, a selective action of antidepressants on muricide of the OB rat in contrast to neuroleptics and anxiolytics may depend on different sites of action of these drugs in the brain However, there has been no report of study on the site of anti-muricide action of psychotropic drugs in the other brain areas with exception of the limbic structures, and on the physiological mechanism of the action in the brain

On the other hand, muricide relates with many physiological functions in the brain, i e , emotion, motion and arousal level, etc If one of these functions is deficient, it may lead to disappearance of muricide For instance, most psychotropic drugs administered peripherally can lead animals to sedation and drowsiness Moreover, neuroleptics treated peripherally induce catalepsy in animals Accordingly these effects may be components of anti-muricide action with psychotropic drugs Thus, there is difficulty in judging the drug specificity on muricide from behavioral tests

The present study has attempted to elucidate the site and

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mode of actions of psychotropic drugs using the microinjection technqiue in the hypothalamus which is known to play a very important role in regulating emotional behavior This study consists of 2 parts The first part examined the possibility of a site of anti-muricide action of these drugs in the hypothalamus The second part tested the EEG and cataleptogenic effects of these drugs administered either intrahypothalamically or peripherally in order to clarify the mechanism of antimuricide action of these drugs in the brain

EXPERIMENT 1

METHOD

Animals and Apparatus

Male Wistar-King A strain rats supplied by Kyushu University Institute of Laboratory Animals, weighing 200–250 g at the beginning of the experiment, were employed as subjects The animals were housed in groups of 4 animals each, maintained in an air conditioned room with an artificial 12 12 LD cycle (lights on 7 00–19 00) and given food and water ad lib After the olfactory bulbectomy, the animals were housed in individual cages (23×20×20 cm)

A sound-attenuated wooden box (126×37×45 cm) in which the individual cages were placed, was used in the experiment The box was illuminated with two 10 watt fluorescent lamps and forcedly ventilated during the experiment The behavior of each animal was observed through the window of the box

Drugs

The drugs used in the present study and their abbreviations are as follows chlorpromazine hydrochloride (CPZ) chlordiazepoxide hydrochloride (CDP), imipramine hydrochloride (IMP) amtiriptyline hydrochloride (AMT), atropine sulfate (At), norepinephrine bitartrate (NE) and 5-hydroxytriptamine creatinine sulfate (5-HT) The doses which did not induce abnormal behaviors (e g , convulsion) in the preceding study [27] were used in this study and expressed in terms of the quantity of salt form The drugs were dissolved in distilled water, and proper quantity of sodium chloride was added to make an isotonic solution The drug treatment was performed on a randomized basis

Olfactory Bulbectomy

The day before the olfactory bulbectomy, the animals were transfered to the individual cages After the individual housing for 24 hr muricide test was performed by putting a white male mouse into the cage Only animals which did not show muricide within 30 min after introducing a mouse into the cage were selected and subjected to olfactory bulbectomy The olfactory bulbs of these animals were bilaterally removed by suctioning under pentobarbital anesthesia (45 mg/kg, IP) The animals which showed muricide within 2 weeks after the olfactory bulbectomy and killed a mouse within 1 min in the muricide test were subjected to the surgery for the guide-cannula implantation

Cannulation

The guide-cannula was constructed of stainless steel cannula of 0 7 mm in outer diameter and 15 mm in total length The guide cannulas were bilaterally implanted into one of the 11 regions within the hypothalamus for each animal under pentobarbital anesthesia according to the de Groot brain atlas [2], and fixed to the skull with dental cement together with two screws driven into the skull The stereotaxic coordinates and abbreviations of the cannula-implanted positions were as follows lateral preoptic area (l-POA, A 7 6, L 2 5 H −1 3), medial preoptic area (m-POA, A 8 2, L 2 0, H −1 0 and A 7 7, L 0 9 H −1 0), anterior part of the lateral hypothalamus (a-LH, A 7 0 L 1 7 H −1 5), middle part of the lateral hypothalamus (m-LH, A 6 0 L 1 7 H −1 5), posterior part of the lateral hypothalamus (p-LH, A 5 0, L 1 3 H −2 0), anterior hypothalamic area (AHA A 6 8, L 0 7 H −1 5) ventromedial hypothalamus (VMH, A 5 7, L 0 6, H −2 5) dorsomedial hypothalamus (DMH, A 5 4, L 0 6, H −2 0), posterior hypothalamus (PH A 4 5 L 0 7 H −2 0), and mammillary body (MB, A 4 0 L 0 5 H −3 1)

Drug Injections

Five days after the implanatation surgery, the animals were handled everyday with the same manner as the manipulation of the drug injection Ten days after the surgery, the muricide test for 1 min was performed again In the test animals which revealed muricide within 1 min were selected for the drug injection experiment The drug test was started at Day 14 after the implantation surgery In the drug test one μl drug solution was bilaterally injected through an injection cannula The injection cannula was made of the stainless steel cannula of 0 35 mm in outer diamter and protruded in a length of 0 5 mm from the tip of the guide-cannula The injection cannula was connected to polyethylene tubing (PE-10) attached to a 10 μl micro-syringe In case of repeating drug injection in the same animal, the interval between injections was more than 2 days On the experimental day, the animals received bilateral saline injections 1 hr before the drug test At 3, 5, 10 and 15 min after saline treatment, the muricide test was repeated Only the animals in which muricide was not inhibited by saline were used in the drug test In the drug test the muricide test was performed with the same manner as saline treatment When muricide was inhibited within 15 min after drug injection, the muricide test was continued at intervals of 30 min until reappearance of muricide Then, the same animals were injected unilaterally with the drug showing anti-muricide effect in order to confirm the drug effective site exactly Moreover because of the possibility for disruption of effectiveness of the drugs on muricide by repeating drug-injection the positive drugs were occasionally retested in the same animal during the experimental period When the drugs ceased to inhibit muridice the animals were suspended from the experiment Accordingly each animal was not always administered all of the drugs used in this study

Control Study

Since there is the possibility for non-specific stimulation effect of the experimental manipulation on the drug-injection site, both dose-response relationship and influence of local pH change were investigated The local pH change experiment was performed by using saline with pH 3–6

Histology

Terminating the experiment animals were injected with 10% Formalin solution through the carotid artery under ether anesthesia After the brain was removed and

TABLE 1

INCIDENCE OF ANIMALS SUPPRESSED MURICIDE BY VARIOUS DRUGS MICRO-INJECTED INTO THE HYPOTHALAMUS

Brain Regions	Drugs							
	Saline	CPZ (50 μg)	CDP (50 μg)	IMP (30 μg)	AMT (30 μg)	At (20 μg)	NE (30 μg)	5-HT (20 μg)
l-POA	0/7	6/7†	0/7	2/5	0/5	2/5	2/7	0/5
m-POA	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
a-LH	0/7	1/7	0/7	2/5	0/5	0/5	0/5	0/5
m-LH	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5
p-LH	0/14	8/14‡	0/7	4/8†	4/10*	4/12*	4/10*	0/5
AHA	0/5	0/5	0/5	1/5	0/5	1/5	3/5	1/5
VMH	0/6	0/6	2/5	0/5	0/6	2/5	1/5	0/5
DMH	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
PH	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
MB	0/9	0/5	7/9†	2/5	0/5	2/8	0/5	1/5

*$p<0.05$, †$p<0.01$, ‡$p<0.001$ significantly different from saline treated group (Fisher exact probability test, one-tailed)

Numbers represent the number of the animal with disappearance of muricide/the number of the tested animal

preserved in the Formalin solution for 7 days, the frozen frontal sections were sliced at 50 μ throughout the implantation site and stained with cresyl violet The location of the cannula tips and extent of the olfactory bulbectomy were verified histologically The data from the animals in which the cannula was not located at the appropriate region was not adopted

Data Analysis

The statistical evaluation was based on Fisher exact probability test [22] in comparison between drug and saline treated animals

RESULTS

The effects of drugs injected into the hypothalamic regions on muricide are shown in Table 1 Inhibition of muricide by CPZ (50 μg) was found both in l-POA and p-LH The inhibitory effect appeared within 5 min after the treatment and lasted for 0 5 or 1 hr These animals became sedated and kept still in their cages following the treatment They also revealed ptosis Since the animals could easily escape the touch of the experimenter's hand when muricide was inhibited, CPZ did not appear to induce ataxia

Inhibition of muricide by CDP (50 μg) was found only when injected into MB When muricide was inhibited, the animals became sedated and kept still in their cages similar to CPZ Their cannula tips were located more closely to the mammillothalamic tract CDP did not appear to produce ataxia similar to CPZ

Inhibition of muricide by IMP and AMT (30 μg) was found in p-LH When muricide was inhibited, the animals became hyperactive and showed rearing and sniffing in contrast to CPZ and CDP

The inhibition by At (20 μg) was found in p-LH When muricide was suppressed, the animals became hyperactive and revealed rearing and sniffing similar to the antidepressants They also showed exophthalmos The positive regions both on muricide and the behavioral changes in the At treatment corresponded well to those of the antidepressants

Inhibition of muricide by NE (30 μg) was found in p-LH When muricide was inhibited, the animals showed paralysis of their hind limbs and hyperventilation Accordingly, the muricide inhibition by NE did not appear to be specific on muricide

The inhibition by 5-HT (20 μg) was not found in any hypothalamic regions

In order to investigate the possibility for nonspecific stimulation effect with the experimental manipulation, dose-response relationships of the positive drugs for muricide and influence of local acid-base composition on muricide were examined in p-LH where many drugs showed antimuricide action In the experiment, CPZ, IMP and At were used The results are shown in Table 2 These drugs appear to show dose-dependent inhibition on muricide However, animals injected with CPZ at doses over 70 μg

TABLE 2

DOSE-RESPONSE RELATIONSHIPS OF DRUGS MICRO-INJECTED INTO THE POSTERIOR PART OF THE LATERAL HYPOTHALAMUS ON MURICIDE OF OB RATS

Drugs	Incidence of Animals Suppressed Muricide			
	Dose			
	5 μg	10 μg	20 μg	50 μg
CPZ		0/5	2/5	5/5
IMP	0/5	3/5	5/5	*
At	1/4	4/4		

*Was not tested because of motor seizure

developed motor seizure within a few min after the injection Similarly, IMP and At injections at doses over 40 μg produced hyperactivity which was followed by a slowly developed motor seizure

To control for changes of the local acid-base composition in the drug-injected regions, saline with pH 3–6 were used The treatment did not affect muricide in any hypothalamic regions

EXPERIMENT 2

The first experiment showed that there was a site of anti-muricide action of psychotropic drugs in the hypothalamus Therefore, in order to elucidate the mechanism of anti-muricide action in the hypothalamus, EEG and cataleptogenic effects of these drugs were examined in Experiment 2 This study consists of two parts as follows the first part is on relationship between muricide and EEG, and the second part is on relationship between muricide and catalepsy

METHOD

Animals

Male Wistar-King A strain rats were used as subjects similar to Experiment 1

EEG Electrode and cannulation

The animals which showed muricide within two weeks after the olfactory bulbectomy and killed a mouse within one min, were implanted with the cortical electrode and guide-cannula The bipolar electrode for cortical EEG recording consists of a twisted pair of insulated stainless steel wires (0 2 mm in diameter) The guide-cannula was constructed of an insulated stainless steel cannula of 0 7 mm in diameter and 15 mm in total length, and was attached to a EEG lead wire The cannula was insulated, with the exclusion of 0 5 mm from the cannula tip in order to record EEG from the drug-injection site The cortical electrode was placed on the frontal cortex The cannula was bilaterally implanted into l-POA, p-LH and MB according to the de Groot atlas [2] Electrode and wires from the cannulas were connected to the pins of a small socket They were fixed to the skull with dental cement together with two screws driven into the skull EEG both from the frontal cortex and the injection site were recorded on a polygraph (Nihon Koden) EEG from the cannula tip was monopolarly recorded against the screw driven into the skull as the ground electrode Effect of drugs on EEG was judged on a basis of appearance of the drowsy pattern in the cortical EEG (high voltage slow waves or spindle burst with intermingled high-voltage slow waves)

Procedure

The procedure in this study was similar to that outlined for Experiment 1 The experiment was performed in the observation box (20×20×25 cm) which was placed in a sealed box The behavioral observation was performed through the window of the box Animals were adapted to the observation box for at least one hr The animals were injected with saline bilaterally one hr before the drug-injection At 3, 5, 10 and 15 min after saline treatment, both the muricide test and EEG recording were simultaneously performed Only animals that were not affected by saline and for which EEG could be stably recorded, were used in the drug test Drugs were used in this study as followed CPZ, CDP, IMP, AMT and At Doses were determined in reference to Experiment 1 The drug test was carried out according to the same procedure as saline treatment When muricide was suppressed by the drug treatment, the muricide test and EEG recording were successively performed at intervals of 30 min until reappearance of muricide In order to clarify the relationship between the anti-muricide and EEG effects of the drugs, the animals which exhibited suppressed muricide by the drug treatment, were exposed to only the EEG recording without the muricide test Terminating the microinjection experiment, influence by the IP treatment of the drugs on muricide and cortical EEG were examined Doses used in this study were ED_{50} of each drug showing muricide-inhibition [4, 14, 19, 26]

Catalepsy

In the experiment examining relationship between muricide and catalepsy, the animals with guide-cannulas in l-POA or p-LH were newly prepared The procedure was similar to the EEG experiment The catalepsy test was performed just after the muricide test The presence of catalepsy was estimated as cataleptic if animals retained for 30 sec the imposed abnormal position placing both frontal limbs over the horizontal bar

At termination of the experiment, the location of the cannula tips were verified histologically The data from the animals in which the cannula was not located in the three hypothalamic regions was discarded The statistical evaluation was based on Fisher exact probability test [2] similar to Experiment 1

RESULTS

EEG Experiment

Comparison between the intrahypothalamic and peripheral administrations of psychotropic drugs on muricide and cortical EEG is shown in Table 3 In the case, the effects on EEG were expressed as incidence of rats which showed a drowsy pattern of cortical EEG accompanied by muricide suppression Table 4 shows effects of drugs administered intrahypothalamically on cortical EEG without the muricide test In the table, numbers represent incidence of rats with drowsiness when the EEG recording was performed according to the same manner as the muricide test without introducing a mouse into the box

Saline did not evoke either muricide suppression or induction of the EEG drowsiness In this case, the cortical EEG revealed the arousal pattern with low voltage waves during the test period from 3 to 15 min after the saline treatment

Chlorpromazine

CPZ (50 μg) revealed anti-muricide action when injected into l-POA and p-LH as mentioned in Experiment 1 The anti-muricide action by CPZ was accompanied by drowsiness in the cortical EEG (Table 3) The typical EEG record from a rat with CPZ in p-LH is shown in Fig 1 The cortical EEG observed in most animals was the drowsy pattern with spindle burst intermingled with high voltage slow waves This EEG pattern appeared corresponding to the duration of the muricide suppression from one min after the drug injection When EEG recording was performed without the muricide test, CPZ obviously evoked the drowsy pattern (Table 4) In addition, CPZ (5 mg/kg, IP) also induced the

TABLE 3

INCIDENCE OF THE ANIMALS WITH MURICIDE SUPPRESSION AND THE DROWSINESS IN THE CORTICAL EEG INDUCED BY PSYCHOTROPIC DRUGS MICROINJECTED INTO THE HYPOTHALAMUS OR ADMINISTERED PERIPHERALLY

		Intrahypothalamic Administration				
Drugs (μg/μl)	Saline	CPZ (50)	CDP (50)	AMT (10)	IMP (10)	At (10)
l-POA						
Muricide suppression	0/6	6/6†	1/4	0/5	2/5	0/5
EEG drowsiness	0/6	5/6†	1/1	4/5*	2/2	—
p-LH						
Muricide suppression	0/9	9/9‡	2/7	5/6‡	3/8	5/7†
EEG drowsiness	0/9	8/9†	2/2*	2/5	1/3	1/5
MB						
Muricide suppression	0/6	1/5	6/6†	3/4*	2/4	1/4
EEG drowsiness	0/6	1/1	5/6†	2/4	2/2	1/1
		Peripheral Administration				
Drugs (mg/kg, IP)	Saline	CPZ (5)	CDP (10)	AMT (20)	IMP (10)	At (5)
Muricide suppression	0/10	8/8‡	6/8†	6/9†	7/12†	8/11†
EEG drowsiness	0/10	7/8‡	5/6†	2/6	0/7	7/8‡

*$p<0.05$, †$p<0.01$, ‡$p<0.001$ significantly different from saline treated group (Fisher exact probability test, one-tailed)

In muricide suppression, numbers represent the number of the animal with disappearance of muricide/the number of the tested animal, and, in EEG the numbers express the number of the animal with the drowsiness/the number of the animal with disappearance of muricide

TABLE 4

INCIDENCE OF ANIMALS WITH THE DROWSINESS IN THE CORTICAL EEG INDUCED BY PSYCHOTROPIC DRUGS MICRO-INJECTED INTO THE HYPOTHALAMUS WITHOUT THE MURICIDE TEST

Drugs Dose (μg/μl)	Saline	CPZ (50)	CDP (50)	AMT (10)	IMP (10)	At (10)
l-POA	0/6	5/6*	1/4	4/5*	1/5	4/4*
p-LH	0/6	10/10‡	2/9	4/8*	2/10	1/9
MB	0/6	2/3	5/6†	2/4	3/4*	2/4

*$p<0.05$, †$p<0.01$, ‡$p<0.001$ significantly different from saline treated group (Fisher exact probability test, one-tailed)

Numbers represent the number of the animal with the drowsiness/ the number of the tested animal

EEG drowsy pattern accompanied by muricide inhibition (Table 3)

Chlordiazepoxide

CDP (50 μg) significantly inhibited muricide in MB as mentioned in Experiment 1 The inhibition of muricide by CDP was accompanied by the EEG drowsy pattern with high voltage slow waves (Table 3) The duration of muricide suppression corresponded to that of the EEG drowsiness When EEG recording was carried out without the muricide test, CDP could induce the drowsiness in MB (Table 4) Moreover, CDP (10 mg/kg, IP) showed the anti-muricide action and simultaenously evoked the drowsiness in the cortical EEG (Table 3)

Tricyclic Antidepressants

AMT significantly inhibited muricide when injected into p-LH and MB, whereas IMP inhibited only in p-LH, but the effect was not statistically significant (Table 3) When muricide was inhibited by antidepressants, the animals did not exert the drowsiness in the cortical EEG A typical EEG record from the animals is shown in Fig 2 When EEG recording was performed without the muricide test, AMT in l-POA and p-LH, and IMP in MB significantly induced the drowsiness (Table 4) In the peripheral administration, these drugs (10 mg/kg, IP) did not induce th drowsy pattern even though muricide was inhibited by the drugs similar to the intrahypothalamic administration (Table 3) The animals with antidepressants exerted exploratory behaviors similar to Experiment 1 when muricide was inhibited by antidepressants

Atropine

At (10 μg) revealed the anti-muricide action when injected into p-LH When muricide was inhibited by At, the animals did not show the drowsiness in the cortical EEG (Table 3) In addition, only the cortical EEG of the animal with At in l-POA showed the drowsiness when the EEG recording was carried out without the muricide test (Table 4)

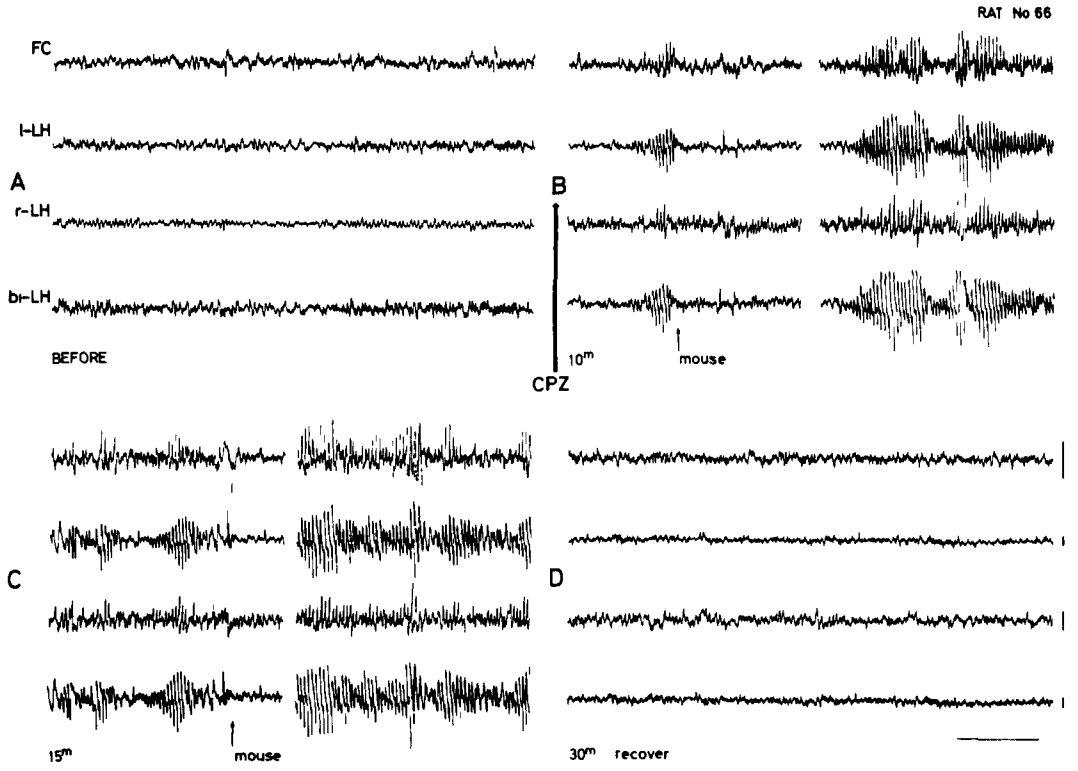

FIG 1 Effect of chlorpromazine (CPZ, 50 μg) injected into the posterior part of the lateral hypothalamus (p-LH) on EEG A EEG just after muricide in the saline treatment B and C EEG at 10 and 15 min after the CPZ treatment when muricide was inhibited EEG showed the arousal pattern just after introducing a mouse and changed to the drowsy pattern immediately D EEG just after muricide at 30 min after the treatment when muricide reappeared FC EEG from the frontal cortex, bi-LH EEG from the bilateral p-LH l-LH EEG from p-LH in the left hemesphere, r-LH EEG from p-LH in the right hemesphere Vertical bar 200 μV horizontal bar, 3 sec

In the peripheral treatment, most of animals with At (5 mg/kg, IP) showed the drowsiness in the cortical EEG when muricide was inhibited by the drug (Tables 3 and 4)

Catalepsy

Table 5 shows effects of CPZ, IMP and At microinjected into the hypothalamus on muricide and catalepsy, and their dose-response relationships Injection of CPZ into l-POA and p-LH induced both anti-muricide and cataleptogenic effects (Table 5) In l-POA, the anti-muricide effect by CPZ was dose-dependent, but the cataleptogenic effect was not Incidence of rats with catalepsy was higher than that of rats with muricide suppression In p-LH, both anti-muricide and cataleptogenic effects by CPZ showed dose-dependency The duration of catalepsy did not correspond with that of muricide suppression both in l-POA and p-LH, and the duration of catalepsy was longer than that of muricide suppression Injection of IMP into p-LH showed both anti-muricide and cataleptogenic effects (Table 5) The anti-muricide effect of IMP was dose-dependent, but the cataleptogenic effect was not The duration of catalepsy did not correspond with that of muricide suppression In addition, incidence of rats showing disappearance of muricide was higher than that of rats showing catalepsy Injection of At into p-LH showed anti-muricide effect, but not cataleptogenic effect (Table 5) The anti-muricide effect showed dose-dependency

TABLE 5

RELATIONSHIP BETWEEN MURICIDE SUPPRESSION AND CATALEPSY INDUCED BY DRUGS MICROINJECTED INTO THE HYPOTHALAMUS

Drugs (μg/μl)	Saline	CPZ 20	CPZ 50	IMP 10	IMP 20	At 5	At 10
Muricide suppression							
l-POA	0/6	2/6	4/6	—	—	—	—
p-LH	0/6	2/5	5/5	3/5	5/5	1/4	4/4
Catalepsy induction							
l-POA	0/6	5/6	5/6	—	—	—	—
p-LH	0/6	3/5	4/5	2/5	2/5	0/4	0/4

GENERAL DISCUSSION

In order to elucidate the site and mode of action of psychotropic drugs in the brain, the present study examined

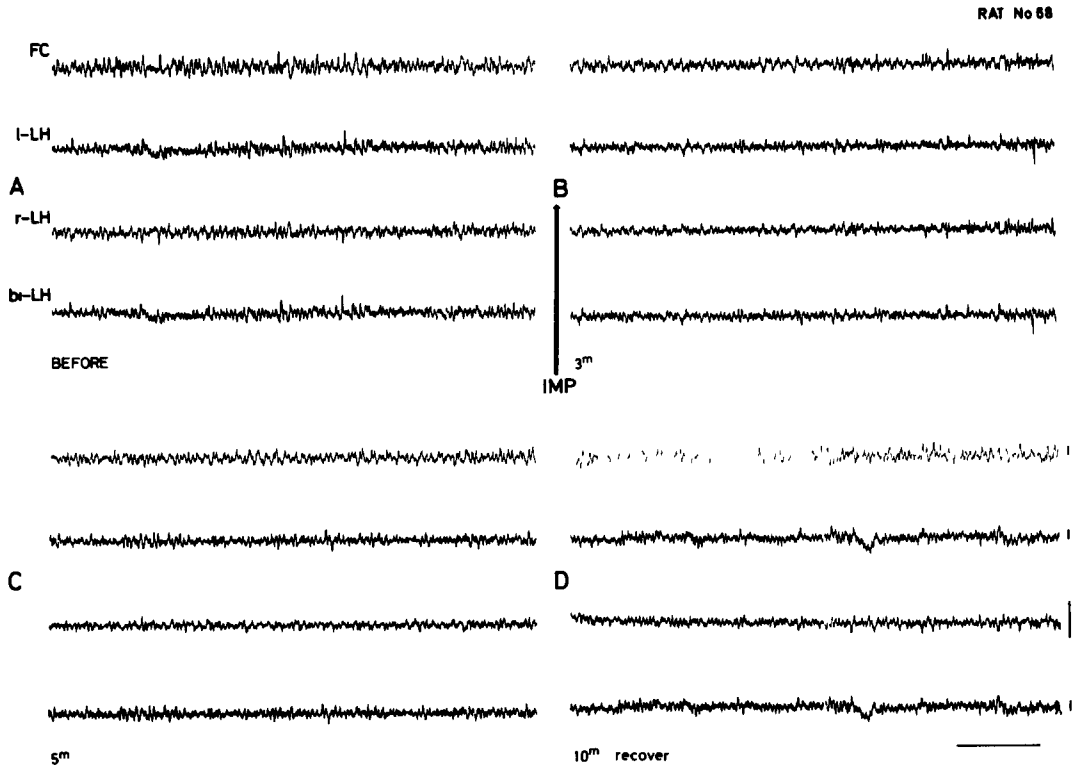

FIG 2 Effect of imipramine (IMP, 10 μg) injected into p-LH on EEG A EEG just after muricide in the saline treatment B and C EEG at 3 and 5 min after the IMP treatment when muricide was inhibited EEG continued to show the arousal pattern after the IMP treatment D EEG just after muricide at 10 min after the treatment See Fig 1 legend for Abbreviations

effects of the drugs on muricide of the OB rat by microinjecting the drugs into the hypothalamus

Experiment 1 showed that inhibition of muricide by CPZ was found both in l-POA and p-LH, and that by CDP was found in MB In addition, the inhibition by AMT and IMP was found in p-LH (Table 1) The interesting fact is that these positive sites on muricide correspond to hypothalamic regions known to be implicated with predatory aggression That is, chemical and electrical stimulations of LH can evoke muricide in the rat [1, 20, 23, 28] In the cat, quiet-biting attack results from electrical stimulation (ES) of l-POA similar to that from LH [5] In addition, this attack elicited from LH is activated by ES of l-POA [5] Thus, the positive sites of CPZ and antidepressants on muricide in the present study correspond to these hypothalamic regions The role in emotional behavior of MB in which inhibition of muricide by CDP was found in this study is poorly understood Since this region is contained within the emotional circuit of Papez [21] and MB lesion induce the animals to be gentle [6], MB may relate with aggressive behavior On the other hand, the results from the control study showed that these actions depend on neither the non-specific mechanical stimulation effect nor local change of the acid-base composition in the injection site Furthermore, muricide of rats injected with saline was never prevented by repeated exposure to the muricide test This fact indicates that the anti-muricide action of the drugs observed in Experiment 1 did not depend on tolerance to killing Moreover, in spite of the data only from p-LH, the anti-muricide action appears to show a dose-response relationship and to be an inverted U-shape on muricide because higher doses induced motor seizure (Table 2) Therefore, the anti-muricide actions of the drugs observed in Experiment 1 appear to be due to the direct action on these hypothalamic regions and to be implicated in the local physiological functions of the regions on the base of pharmacological properties with the drugs

In contrast to antidepressants, inhibition of muricide by CPZ and CDP has been considered to be due to the accompanying ataxia and generalized sedation The results from Experiment 2 showed that, both in the intrahypothalamic and peripheral treatments, anti-muricide action of CPZ and CDP is closely related to the drowsiness of the cortical EEG, whereas that of antidepressants is related to arousal (Table 3 and 4) On the other hand, the peripheral treatment of CPZ can induce catalepsy In the present study, inhibition of muricide by CPZ and IMP was accompanied by catalepsy (Table 5) The cataleptogenic effect, however, did not appear to relate with the anti-muricide action because the duration of catalepsy was different from that of muricide suppression and, additionally, the cataleptogenic effect did not show dose-dependency in contrast to the anti-muricide effect (Table 5) In addition, it is known that sensory stimulation can exhibit locomotor responses in cataleptic rats with haloperidol [16] Therefore, anti-muricide action by CPZ and IMP in the present study is not considered to depend on catalepsy The cataleptogenic effect observed in the present

study may reflect the blockade of dopaminergic neurons ascending through the hypothalamus because both CPZ and IMP possess dopamine receptor blocking action [3,18] and p-LH lesion induces catalepsy [12]

The present study showed that the anti-muricide action by CPZ and CDP was due to the suppression of the brain arousal system In fact, both POA and LH in which inhibition of muricide by CPZ was found in this study are well-known to play a very important role in the brain arousal system [7,25] On the other hand, the mechanism of anti-muricide action of CDP in MB, however, is unclear because relation between the emotional behavior and EEG in MB is unknown However, it is known that ES of MB induces the behavioral excitation, i e , the reactions of altertness, orientation, searching and ' flight" in the rabbit [9] In addition, MB is suggested to make a behavioral contribution different from other limbic structures [24] In this study, the cannula tips with animals showing muricide suppression by CDP in MB were closely placed to the mammillothalamic tract (MTT) Comparing with the report that lesions of MTT disrupt the behavior motivated by shock [24], relationship between the anti-muricide and EEG effects of CDP in MB observed in this study is very interesting Accordingly, the results from this study appear to suggest that a part of the hypothalamic activating system may ascend through MTT and inhibition of muricide by CDP may be due to inhibition of this pathway or suppression of MB function per se, although the activation of the neocortex is produced not directly from the hypothalamus to the neocortex, but via the midbrain reticular formation [25]

In contrast to CPZ and CDP, the antidepressants did not affect the cortical EEG when muricide was suppressed (Table 3 and 4) The report that antidpressant selectively inhibit muricide appear to be supported by this event On the other hand, the effect of antidepressants in the present study resembled well that of At It has been known that LH contains a cholinoceptive component of innate system activating muricide in the rat [1,23] Accordingly, anti-muricide action of antidepressants may depend on the anticholinergic property with the drugs This appears to be supported by the result of Experiment 2 (Table 3) that inhibition of muricide by AMT was more potent than that of IMP Because, anticholinergic effect of AMT is stronger than that of IMP [17] However, muricide of the OB rat was inhibited more potently by desipramine inhibiting NE re-uptake than by chlorimipramine inhibiting 5-HT re-uptake [29] In addition, ES of the locus coeruleus suppressed muricide, whereas that of the raphe nucleus did not inhibit it [30] In this study, injection of NE into p-LH inhibited muricide (Table 1) The effect, however, did not appear to be selective because it was accompanied by limb paralysis and hyperventilation On the other hand, 5-HT did not affect muricide Therefore, although the monoamine re-uptake inhibition effect with antidepressants does not appear to be excluded completely from the anti-muricide components, the results from this study suggest that the anticholinergic effect with the drugs implicated in the anti-muricide action

It is important to notice that the intrahypothalamic and peripheral treatments of antidepressants as well as At induced exploratory behaviors when muricide was inhibited by these drugs Rather, inhibition of muricide by these drugs appears related to the arousal in the cortical EEG On the other hand, ES of p-LH [12,16] as well as that of the reticular formation [8] induce such behavioral arousal These stimulations also induce the EEG arousal and are accompanied by impairment of the goal oreinted behavior [8, 12, 16] Therefore, inhibition of muricide by antidepressants and At may attribute to the hyperarousal accompanied by attention deficit, although the relationships between the cholinergic system and muricide and between the cholinergic system and behavioral arousal are unclear The behavioral arousal observed in this study may attribute to not only the elevation of dopaminergic function based on suppressing the cholinergic pathway through the hypothalamus, but also the hyperarousal based on activating the hypothalamic arousal system

Another interesting fact is that the peripheral treatment of At induced the behavioral arousal, but showed the drowsiness in the cortical EEG (Table 3) This phenomenon is well-known as dissociation between EEG and behavior [13] On the other hand the intrahypothalamic treatment of At exhibited the behavioral arousal as well as the EEG arousal These events may suggest that the behavioral change in the dissociation phenomenon by At attributes to the anticholinergic effect in the subcortex, while the EEG change is due to the effect on the reticular activating system Thus, the dissociation phenomenon of At appears to be able to explain the difference of the site of action in the brain

In conclusion, the anti-muricide actions of CPZ and CDP closely relate to the suppression of the hypothalamic activating system, although the pharmacological mechanism remains unclear On the other hand, the result of antidepressants in the present study appears to support the reports that antidepressants as well as At selectively inhibit muricide However the present study proposed the possibility that inhibition of muricide by antidepressants and At attributes to attention deficit based on the behavioral and EEG arousal The pharmacological mechanism of anti-muricide action of the antidepressants appears to depend on the anticholinergic property, although the monoamine re-uptake inhibition effect with the drugs can not be excluded completely from the anti-muricide components

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