2-PYRROLIDONE AS A POSSIBLE ENDOGENOUS FACTOR INHIBITING MURICIDAL BEHAVIOR IN RATS

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Among the various forms of aggressive behavior in animals, predator aggression is the most widespread in nature and it has aroused considerable interest in investigators of the motivation systems of the brain. In laboratory practice, to study predator aggression an experimental model of stereotyped behavior of mouse-killing rats (muricidal behavior) is often used. It is characterized by the aggressive response of a rat to a mouse placed with it in the cage. A marked response of the rat terminates by attacking with biting the mouse in the region of the cervical spine, followed by biting it to death. First described in 1951, this model has been intensively studied in recent years [5]. It has been shown that keeping the rats in isolation and electrolytic destruction of certain brain structures, such as the olfactory bulbs, septum, lateral hypothalamus, and amydgala, has a marked activating effect on muricidal behavior [1, 4, 7, 9]. However, the neuronal mechanisms of this activation, like many other problems to do with regulation of aggressive predator behavior, have not yet been explained. Although data have now been obtained to show that the acetylcholinergic system of the amygdala is involved in the initiation of acts of aggressive behavior, many investigators at the same time ascribe great importance to inhibitory regulatory systems in the realization of muricidal behavior. These systems include serotoninergic and GABA-ergic conduction pathways running from the olfactory bulbs, septum, and raphe nuclei, destruction of which leads to activation of muricidal behavior [3, 6, 8, 10]. In recent investigations of the neuropharmacology of behavior great attention has been paid in the study of mechanisms of neuronal transmission not only to classical neurotransmitters, but also to endogenous analogs or ligands for receptors. For example, the following endogenous analogs are known: of serotonin — melatonin, tryptophol, etc., and of GABA — 2-pyrrolidone [2]. It can be tentatively suggested that these endogenous factors may be involved in the realization of aggressive behavioral responses.

Accordingly, the object of the present investigation was to search for endogenous neurochemical factors affecting the muricidal behavior of rats when activated by destruction of the olfactory bulbs.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 150-180 g. The animals were kept in groups of 10 per cage measuring 40 × 30 × 15 cm, and also isolated in single cages measuring 20 × 12 × 10 cm with no restriction of food and water. The rats underwent operative destruction of the olfactory bulbs (bulbectomy) by means of vacuum aspiration through the nose under ether anesthesia. The presence of muricidal behavior in the rats was tested after 20 and 40 days. Albino mice were placed in the cage with the rats for 1 min. An individual was considered to be muricidal if it killed the mouse within that time. Under ether anesthesia solutions of the different preparations in a volume of 30 µl were injected into the second ventricle of the muricidal rats. Material for injection was made up in physiological saline and 30 µl of physiological saline was injected into the control animals. Preservation of their murucidal behavior was tested in these animals after 1-3 h.

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TABLE 1. Effects of Isolation and Bulbectomy on Muricidal Behavior in Rats

Experimental conditions	Number of muricidal rats (%)	
	20th day	40th day
Keeping in group	0	0
Isolation	0	6
Keeping in group + bulbectomy	0	4
Isolation + bulbectomy	22	48

Legend. In each investigation 50 rats took part.

TABLE 2. Gel-Chromatography of Supernatant of Brain Homogenate from Nonmuricidal Rats on Column with Sephadex G-15 and Effect of Fractions Obtained Thereby on Muricidal Behavior in Recipient Rats

Fraction No.	A 280 nm	Number of muricidal rats
1	0,01	10/10
2	0,82	10/10
3	0,17	10/10
4	0,26	10/3*
5	0,21	10/4*
6	0,14	10/10
7	0,11	10/10
8	0,06	10/10

Legend. Numerator — before injection, denominator — 1-2 h after injection.
*P < 0.01.

To study endogenous factors blood was taken from the rats after decapitation in order to prepare plasma and the brain. Brain tissue was homogenized in distilled water (1:1 w/w) and centrifuged (100,000 g, 1 h) at 4°C. The supernatant was lyophilized and kept at −20°C. The freeze-dried preparation, weighing 100 mg, was dissolved in 0.5 ml of distilled water and applied to a column with Sephadex G-15 (30 × 1.5 cm). Elution was carried out with distilled water at the rate of 30 ml/h. Fractions of 10 ml were collected, freeze-dried, dissolved in distilled water, and subjected to thin-layer chromatography on silica-gel plates (Czechoslovakia) in systems of solvents: 1) isopropyl alcohol—acetic acid—water (6:1:1), 2) n-butyl alcohol—acetic acid—water (4:1:1), and 3) ether—acetone (3:2) twice.

After chromatography the plates were cut longitudinally into two parts, one of which was stained with 0.2% ninhydrin in acetone during heating to 100°C. The unstained part of the plate was cut into zones corresponding to the coefficient of chromatographic mobility (R_f) and eluted with physiological saline. The eluate from the plates and also the freeze-dried samples of supernatant of rat brain homogenates and fractions after gel-filtration, dissolved in physiological saline, were tested by intraventricular injection into the muricidal animals. The test solutions were injected into the recipient rats in a dose equivalent to material from two or three donor rats per injection. The following preparations were used: folic acid, nicotinic acid, serotonin creatinine-sulfate (from Reanal, Hungary), biopterin, betaine, melatonin, 5-hydroxytryptophan, acetyltryptophan (from Calbiochem, USA), kynurenine, kynurenic acid, putrescine, and 2-pyrrolidone (from Ferak, West Germany). The numerical results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Preliminary investigation of a large group of rats, kept 10 at a time in cages, showed the complete absence of spontaneous muricidal behavior. After 40 days individual muricidal rats appeared in a group of isolated animals and among bulbectomized rats kept in a group (Ta-

TABLE 3. Effect of Biologically Active Substances (2 mg/kg) on Muricidal Behavior of Rats when Injected into the Second Ventricle

Substance	Muricidal behavior, Time of determination, h			
	0	1	2	3
Biopterin	+	+	+	+
Folic acid	+	+	+	+
Kynurenic acid	+	+	+	+
Nicotinic acid	+	+	+	+
Indolylacetic acid	+	−	+	+
Kynurenine	+	−	+	+
Melatonin	+	+	+	+
5-Hydroxytryptophan	+	−	+	+
N-acetylserotonin	+	−	+	+
Serotonin creatinine-sulfate	+	−	−	+
Betaine	+	+	+	+
Putrescine	+	−	+	+
GABA	+	−	−	+
2-Pyrrolidone	+	−	−	+
Physiological saline	+	+	+	+

Legend. +) Muricidal response in five animals, −) inhibition of muricidal behavior in three of five animals.

ble 1). Their number in each group did not exceed 6%. In turn, the operation combined with isolation sharply increased their number (48%). The muricidal rats exhibited all the essential patterns of aggressive behavior: pursuing the fleeing mouse, attacking with blows with the paws, biting in the region of the cervical spine, until complete motionlessness and death of the mouse.

The next series of experiments was devoted to the search for endogenous factors influencing muricidal behavior. For this purpose blood plasma and supernatent of brain homogenates of muricidal rats, obtained by a combination of bulbectomy and keeping in isolation, were injected intraperitoneally and intracerebrally into the second ventricle of nonmuricidal rats, in a dose of material from three donors into one recipient. Analogous preparations from nonmuricidal rats were injected in the same way into muricidal individuals. It was found that injection of the preparations intraperitoneally, and also intraventricular injection of the supernatant of brain homogenate from murcidal rats into nonmuricidal individuals caused no changes in the original behavior of the recipient animals. However, intraventricular injections of brain preparations from nonmuricidal rats inhibited aggressive behavior in six of 10 muricidal recipient rats for 1-2 h. The results thus indicate that the brain of nonaggressive rats contains endogenous factors which inhibit muricidal behavior.

An attempt was next made to establish the nature of these endogenous factors. The supernatant of the brain homogenate from nonaggressive rats was fractionated by gel-filtration on a column with Sephadex G-15 and the material of these fractions was injected into the second ventricle of muricidal rats (Table 2). The results showed that material from fractions Nos. 4 and 5, containing low-molecular-weight substances with mol. wt. of under 15,000 daltons, had an inhibitory effect on the muricidal behavior of the rats. Injection of physiological saline and of material of the remaining fractions did not affect the animals' behavior.

The combined lyophilized material of fractions Nos. 4 and 5 was subjected to thin-layer chromatography in silica-gel in solvent system 1. Material eluted with physiological saline from different zones of the chromatograph was tested for ability to weaken muricidal behavior. It was found that substances with R_f = 0.1 (subfraction I) and R_f = 0.6 (subfraction II) possessed this ability. Material from these zones introduced into the quantities which correspond to the brain content of 3 mice-donors, retard muricidal behavior in the flow 1–2 h at 60–70 % of the receptors. Injection of physiological saline and eluates from the other zones of the chromatogram had no such effect.

We know that certain endogenous neurotransmitters and biologically active compounds, such as serotonin, 5-hydroxytryptophan, and GABA, may have an inhibitory action on muricidal behavior [6, 8]. We tested 14 compounds present in the rat brain and which are structural analogs of serotonin and GABA, as well as certain other biologically active compounds which, depending on the conditions of isolation, could be present in eluates from TLC (Tables 3 and 4). The following were used: serotonin creatinine-sulfate, melatonin, 5-hydroxytryptophan, N-acetyl-

TABLE 4. R_f for Thin-Layer Chromatography of Subfractions I and II and Biologically Active Compounds on Silica-Gel

Substance	System 1	System 2	System 3
Subfraction I	0,1	0,1	0,0
Subfraction II	0,6	0,4	0,2
Kynurenine	0,7	0,5	0,3
Indolylacetic acid	0,7	0,5	0,5
Acetylserotonin	0,7	0,6	0,5
5-Hydroxytryptophan	0,8	0,7	0,5
Serotonin creatinine-sulfate	0,5	0,2	0,0
GABA	0,1	0,1	0,0
2-Pyrrolidone	0,6	0,4	0,2

serotonin, indolylacetic acid, GABA, putrescine, 2-pyrrolidone, biopterin, folic, nicotinic, and kynurenic acids, kynurenine, and betaine. The test subfractions showed coincidence in all three solvent systems with GABA (subfraction I) and with 2-pyrrolidone (subfraction II). On the basis of these experimental results it can thus be tentatively suggested that the ability of brain supernatant from nonmuricidal rats to inhibit the muricidal behavior of recipient rats after intraventricular injection is due to the presence of GABA or 2-pyrrolidone in it.

Interaction between activating and inhibitory stimuli in the brain plays an important role in the realization of different types of behavior, including aggressive. It can be suggested that one of these inhibitory systems for the regulation of aggressive behavior is the GABA-ergic system of the olfactory bulbs. The hypothesis of the inhibitory role of endogenous GABA and 2-pyrrolidone in muricidal behavior may be an important addition to the investigation of the role of the GABA-ergic system in the CNS.

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