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Influence of Environment on GABA Receptors in Muricidal Rats

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DAVANZO, J. P., J. CHAMBERLAIN AND M. M. MCCONNAUGHEY. *Influence of environment on GABA receptors in muricidal rats.* PHARMACOL BIOCHEM BEHAV 25(1) 95–98, 1986.—The influence of environment (isolation) on GABA receptor numbers ([^{3}H]-muscimol binding sites) and affinities was investigated in specific limbic areas known to be involved with the development of muricide. Olfactory bulbs of isolated rats were found to have identical numbers of [^{3}H]-muscimol binding sites whether or not they were muricidal. However, in the olfactory bulbs of the aggregated animals a greater than two-fold increase was found in numbers of [^{3}H]-muscimol binding sites in those rats that were muricidal. In the amygdala muricidal rats had a 32–34% increase in [^{3}H]-muscimol binding sites over non-muricidal rats regardless of environment. In the septum non-muricidal rats had fewer [^{3}H]-muscimol binding sites than muricidal rats and although the trend was evident, statistical vigor was seen only in the aggregated animals. Neither muricide nor isolation significantly influenced [^{3}H]-muscimol binding numbers in the hypothalamus. GABA K_i values were examined in all brain regions and found to be the same in the isolated and aggregated animals whether or not they were muricidal. We concluded that environment appears to influence apparent GABA receptor numbers in the olfactory bulbs and septum whereas muricidal behavior correlates well with an increase in apparent number of GABA receptors in the amygdala. GABA receptors in the hypothalamus were not influenced by either environment or aggression.

GABA Muricide Receptors Brain Limbic system Muscimol

IT is generally accepted that GABA is an inhibitory modulator of various forms of aggressive behavior in rodents. In one commonly employed model of aggression, the muricidal rat, mouse-killing has been associated with a lower than normal level of GABA in olfactory bulbs [9]. Similar trends exist in other areas of the limbic system [2] but a distinction must be made between the development of muricidal behavior in rats housed in isolation from those housed in an aggregated environment. When GABAergic drugs are implanted in the olfactory bulbs of rats a reduction in mouse-killing occurs. Conversely GABA antagonists and inhibitors of GABA synthesis increase the incidence of muricide [10]. Muscimol, a GABA agonist, injected in the septum, however, facilitates muricide [12].

A similar pattern exists in mice in that there is an inverse relationship between GABA activity and aggression. Not all areas of the brain, however, show low GABA in aggression [5]. Furthermore, agents which affect GABA either inhibit or augment aggression depending on whether they are GABA agonists or antagonists and whether they inhibit synthesis or catabolism of GABA [3, 7, 9, 13]. It has been demonstrated that whole brain homogenates from isolated mice compared to aggregated mice display a reduction in protein content of certain synaptosomal fractions leading to lower total GABA binding [1,4].

To the best of our knowledge no study has appeared in the literature in which a direct measurement of [^{3}H]-muscimol binding (apparent GABA receptor numbers) and affinities has been studied in the brain of aggressive rats. Such a study has been conducted in hamsters, moreover and it was shown that these aggressive animals have a higher level of brain GABA binding [11]. In view of the above, we wondered what relationship GABA receptor binding in specific limbic areas known to be involved with aggression might have in the development of muricide, an example of interspecies aggression, and how the environment influences GABA receptor binding. The results of this study constitute the subject of this communication.

METHOD

Experimental Animals

Approximately 174 male Long-Evans rats (Blue Spruce Farms, Altamont, NY) weighing an average of 450 grams were randomly separated into two housing groups. Half were isolated in stainless steel self-cleaning cages measuring 24×18×18 cm. The other half were housed aggregated five per group in polypropylene cages measuring 56×51×23 cm for seven days. They were tested for muricide on the seventh day and again two days later. The muricide test consisted of putting each rat in a novel environment (a cage the same as the ones used for isolation) and quietly placing a male mouse (CD-1, Charles River, Wilmington, MA) with each rat for four hours. If a rat killed a mouse on both occasions it was considered muricidal. Rats that did not kill a mouse during either test were considered non-muricidal. All subjects had free access to food (Zeigler NIH 07 rodent chow) and water except during testing.

TABLE 1

[^{3}H]-MUSCIMOL BINDING RECEPTOR NUMBERS IN BRAIN AREAS OF AGGREGATED AND ISOLATED RATS

	Muricidal	Non-muricidal
Aggregated		
Olfactory bulbs*	274 ± 46 (n = 6)	110 ± 18 (n = 12)
Septum*	202 ± 29 (n = 6)	147 ± 17 (n = 11)
Hypothalamus	98 ± 22 (n = 6)	81 ± 9 (n = 11)
Amygdala*	252 ± 53 (n = 6)	189 ± 19 (n = 12)
Isolated		
Olfactory bulbs	131 ± 6 (n = 13)	130 ± 19 (n = 12)
Septum	236 ± 44 (n = 8)	211 ± 21 (n = 9)
Hypothalamus	78 ± 4 (n = 12)	77 ± 9 (n = 13)
Amygdala*	231 ± 26 (n = 14)	174 ± 14 (n = 12)

[^{3}H]-Muscimol binding is expressed as fmol/mg protein and was determined as described in the Method section.

*Significant difference between muricidal and non-muricidal $p<0.05$ Student's t-test.

n = number of preparations assayed. Each preparation was comprised of 2–6 animals each.

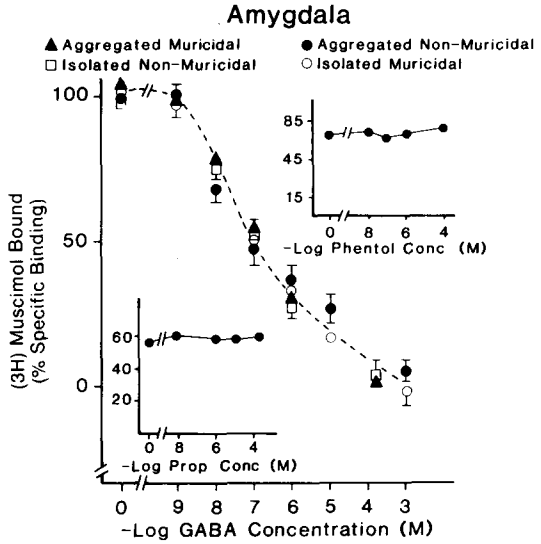

FIG. 1. Displacement of [^{3}H]-muscimol in amygdala brain preparations of aggregated and isolated muricidal and non-muricidal rats. Binding was determined in the presence of 1.6 nM [^{3}H]-muscimol with increasing concentrations of unlabeled GABA as described in the Method section. Each value is the mean±S.E. of 2–3 determinations (preparations) consisting of duplicate or triplicate incubations. K_i values from this group of displacement values in the amygdala as well as those determined in the other brain regions are expressed in Table 2. To assess specificity of binding neither phentolamine (top insert) nor propranolol (bottom insert) had any effect on [^{3}H]-muscimol binding even at the very high concentrations of 10^{-4} M. In the inserts the ordinates are expressed as fmol [^{3}H]-muscimol bound/mg protein. Each value is the mean of duplicate incubations.

Tissue Preparation

The animals were sacrificed by decapitation 24 hours after the last muricide test. The brains were rapidly removed and frozen in dry ice. Subsequently, various brain regions were dissected as described by Bolin and DaVanzo [2] including olfactory bulbs, septum, amygdala and hypothalamus. Brain regions were pooled into groups of two and then homogenized in 3 ml of ice-cold TRIS-citrate buffer (10 mM, pH 7.2) with a Brinkmann polytron PT-10 at a setting of six for 30 seconds. The homogenate was then centrifuged at 45,000 × g max for 20 minutes. The supernatant was discarded and the pellet resuspended in 3 ml of ice-cold TRIS-citrate buffer (10 mM, pH 7.2). The resuspended pellet was again centrifuged at 45,000 × g max for 20 minutes. Resuspension and centrifugation of the resulting pellet in fresh buffer were repeated twice more. The pellet was then frozen for 18 hours at −20°C. At the end of this period pellets were thawed and resuspended in 3 ml of ice-cold buffer, centrifuged at 45,000 × g max for 20 min and the resultant pellets resuspended in Tris-citrate buffer (50 mM, pH 7.2) to a final protein concentration of 1–2 mg/ml and frozen in (−20°C) small aliquots until the time of GABA receptor binding assays. Preliminary data suggest that this procedure reduces endogenous GABA levels to levels undetectable by current assay means. We therefore assume there to be no interference with endogenous GABA in the binding assay. Protein determination was by the method of Lowry *et al.* [8].

Receptor Assay

GABA receptor binding was performed similarly to the procedure described by Enna and Synder [6] with some modifications. Briefly, [^{3}H]-muscimol (0.1–4 nM) was incubated in triplicate or quadruplicate with the various brain region homogenates (200 μl) in 500 μl of TRIS-citrate buffer (50 mM, pH 7.2) for 10 minutes at 4°C in the presence and absence of various concentrations of GABA (or displacing drug). Non-specific binding was determined by triplicate or quadruplicate incubations in the presence of 1 mM GABA. Reactions were terminated by dilution with 5 ml ice-cold distilled water and immediately filtered through Whatman GFC Glass fiber filters. The filters were then air dried overnight. Radioactivity remaining on the filter paper was determined by liquid scintillation spectroscopy using a Beckman LS 9000 scintillation counter. Binding saturation curves and Scatchard analyses [14] were initially done for [^{3}H]-muscimol binding in all brain regions of the 4 groups of rats used in this study. No significant differences were found in K_i values and saturation of the receptors appeared to be complete between 2 and 4 nM [^{3}H]-muscimol.

[^{3}H]-Muscimol binding was performed using centrifugation as well as Whatman GFC glass fiber filters to separate bound from free radioligand. Although non-specific binding was extremely high when using centrifugation to separate bound from free, both methods resulted in similar specific binding. Although differing concentrations of [^{3}H]-muscimol were used in the various experiments, the data expressed in the tables as total [^{3}H]-muscimol binding sites were calculated using a [^{3}H]-muscimol concentration of 3.2 n molar.

RESULTS

In olfactory bulb preparations from isolated rats, no differences were found in apparent [^{3}H]-muscimol binding sites whether the animals were muricidal or not (Table 1). However, in the aggregated animals there was a two and one-half fold increase in apparent [^{3}H]-muscimol binding sites in the muricidal animals, thus suggesting a correlation

TABLE 2

K_i VALUES FOR GABA (M)

	Amygdala	Olfactory Bulbs	Hypothalamus	Septum
Isolated muricidal	7.2×10^{-8}	1.3×10^{-7}	7.0×10^{-8}	8.3×10^{-8}
Isolated non-muricidal	8.5×10^{-7}	2.4×10^{-8}	8.1×10^{-8}	9.6×10^{-8}
Aggregated muricidal	7.2×10^{-8}	8.3×10^{-8}	8.3×10^{-8}	9.0×10^{-8}
Aggregated non-muricidal	9.1×10^{-8}	1.0×10^{-7}	7.2×10^{-8}	7.5×10^{-8}

K_i values were determined from displacement curves using increasing concentration of GABA as described in the Method section.

K_i values were calculated utilizing the formula $K_i = EC_{50}/1 + S/Km$, where S was the concentration of [^{3}H]-muscimol used in the binding assay and Km the dissociation constant for [^{3}H]-muscimol in our system (0.9) determined from Scatchard analysis.

No significant differences were found between any of the groups (Student's t-test).

between environment and receptor numbers in this brain area. Results were similar in the septum (Table 1) where in the aggregated rats [^{3}H]-muscimol binding sites were significantly higher in the muricidal animals. This trend was also seen in the isolated group but was not significant.

In the amygdala there seemed to be a correlation between aggression and non-aggression with regard to [^{3}H]-muscimol binding sites. As shown in Table 1 apparent numbers of [^{3}H]-muscimol binding sites in the amygdala of muricidal rats were significantly higher than those of non-muricidal rats regardless of the environmental status of the animals. Also shown in Table 1 are the apparent numbers of [^{3}H]-muscimol binding sites in the various brain preparations of the hypothalamus. It is interesting to note that of the four areas of the limbic system investigated in this study this is the only area where neither environment nor aggression had an influence on the apparent number of [^{3}H]-muscimol binding sites.

To further investigate our binding data, GABA receptor affinities were determined. A typical displacement curve is shown in Fig. 1 using increasing concentrations of GABA. As can be seen in this figure the apparent GABA EC_{50} values in the amygdala preparations of the four various groups of rats were similar. Also shown in Fig. 1 in the two inserts are displacement curves generated using the alpha-adrenergic antagonist phentolamine and the beta-adrenergic antagonist propranolol. This was done to demonstrate the specificity of our assay since these agents would not be expected to displace the radioligand from the GABA receptor. GABA K_i values were generated for the other brain regions by displacement curves as in Fig. 1 and are represented in tabular form in Table 2. When K_i values were compared in the various preparations of isolated, aggregated, muricidal or non-muricidal animals no significant differences were found between any of the groups in any brain area studied. This rules out the hypothesis that housing or aggression may change the receptor affinity for GABA and lends more strength to the idea that receptor numbers are directly related to environmental conditions and/or muricidal behavior.

DISCUSSION

It is generally accepted that GABA modulates various forms of aggression in rodents. In particular an association has been made between low levels of GABA and mouse-killing in certain parts of the limbic system of muricidal rats. In the present study, we confirmed previous work (not reported here) showing this association between low GABA and muricide and extended it to include a determination of [^{3}H]-muscimol binding sites (GABA receptor numbers) and affinities. Initial Scatchard plot analyses of [^{3}H]-muscimol binding demonstrated no significant differences in K_i values; therefore, this study was directed mainly toward determining changes in receptor numbers due to isolation or muricidal behavior in the limbic brain areas. Our results confirm the work of Potegal *et al.* [11] who found that aggressive hamsters had significantly higher levels of GABA binding in a large area of the brain which included the limbic system, striatum and diencephalon. Since the rat is one species that is commonly used to study aggression we thought it appropriate to concentrate on this species. We confined our studies to the areas of the limbic system known to be involved in aggression, i.e., olfactory bulbs, septum, hypothalamus and amygdala. Needed in future studies is an examination of GABA binding in other parts of the limbic system and a study in which $GABA_A$ and $GABA_B$ subtypes are estimated.

Of interest in our study is the finding that the hypothalamus appears to be uninfluenced by either muricide or isolation. Whereas resting levels of GABA are highest (5^{-6} μM/g) in the hypothalamus compared to other areas, receptor binding is lowest in the hypothalamus compared to other areas and neither muricide nor isolation influences binding. Perhaps this is suggesting that the hypothalamus is not directly involved in aggression but merely serves as a relay station for the transmission of this particular neurotransmitter or perhaps that a negative feedback system is involved.

This is the first study, to our knowledge, to demonstrate a relationship between the number of [^{3}H]-muscimol binding sites and muricidal behavior in specific areas of the limbic system. When isolated muricidal rats were compared to isolated non-muricidal rats, thus eliminating the influence of environment, apparent GABA receptor numbers ([^{3}H]-muscimol binding sites) in the olfactory bulbs were identical. In the isolated environment there is no correlation between aggression and receptor numbers. However, when the rats were placed in an aggregated environment this aggregation allowed a correlation to become manifest between olfactory bulb binding site increase and aggression. These muricidal rats exhibited a 2.5 fold increase in number of [^{3}H]-muscimol binding sites with aggression.

GABA receptors in the septum follow a very similar pattern to those of the olfactory bulbs. However, in the amygdala, aggression can be correlated with increased number of [^{3}H]-muscimol binding sites. This is true regardless of environment.

The results of this study are directly related to the integrity of the muricidal rat model. It must be pointed out that although we observed an increased incidence of muricidal behavior in the isolated animals and the opposite in the aggregated group, results using this paradigm are known to be variable and we must consider the possibility of having some inherent muricidal animals that fail to kill for some unknown reason and therefore are categorized incorrectly. With a large population we hope to negate any major effects caused by a few non-responders. It should be mentioned that this entire experimental protocol was performed on three separate occasions over a two year time span with similar findings each time.

The kinetic data demonstrated no significant effect on receptor affinities by either environment or muricidal behavior. This agrees with the work of Potegal *et al.* [11] who demonstrated no GABA receptor affinity changes in "mid region" of aggressive and non-aggressive female hamsters.

It appears from these data that only apparent GABA receptor numbers ([^{3}H]-muscimol binding sites) and not affinities can be correlated with either environment or aggression in three of the four areas of the limbic system studied.

Muricidal behavior is probably influenced by a number of receptors and transmitters and it would be inappropriate to assume this one particular receptor-transmitter system as the only mechanism involved. However, we feel that the GABA system plays a key role in muricidal behavior and we now have evidence at the biochemical level for alterations in number of [^{3}H]-muscimol binding sites which correlate with aggression and with isolated or aggregated housing conditions.

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REFERENCES

1. Blindermann, J. M., F. V. DeFeudis, M. Maitre, R. Misslin, P. Wolff and P. Mandel. A difference in glutamate decarboxylase activity between isolated and grouped mice. *J Neurochem* **32:** 1357–1359, 1979.
2. Bolin, P. and M. P. DaVanzo. The influence of isolation and aminooxyacetic acid (AOAA) on GABA on muricidal rats. *Psychopharmacology (Berlin)* **76:** 367–370, 1982.
3. DaVanzo, J. P. and M. Sydow. Inhibition of isolation-induced aggressive behavior with GABA transaminase inhibitors. *Psychopharmacology (Berlin)* **62:** 23–27, 1979.
4. DeFeudis, F. V., P. Madtes, A. Ojeda and P. A. DeFeudis. Binding of γ-aminobutyric acid and glycine to synaptic particles of the brains of differentially-housed mice: evidence for morphological changes. *Exp Neurol* **52:** 285–294, 1976.
5. Early, C. J. and B. E. Leonard. The effect of testosterone and cyproterone acetate on the concentration of γ-aminobutyric acid in brain areas of aggressive and non-aggressive mice. *Pharmacol Biochem Behav* **6:** 409–413, 1977.
6. Enna, S. J. and S. H. Snyder. Properties of γ-aminobutyric acid (GABA) receptor binding in rat brain synaptic membrane fractions. *Brain Res* **100:** 81–97, 1975.
7. Krsiak, M., A. Sulcova, Z. Tomasikova, N. Dlohzkova, E. Kosar and K. Masek. Drug effects on attack, defense and escape in mice. *Pharmacol Biochem Behav* **14:** Suppl 1, 47–52, 1981.
8. Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. Protein measurement with the folin phenol reagent. *J Biol Chem* **193:** 265–275, 1951.
9. Mandel, P., G. Mack and E. Kempf. Molecular bases of some models of aggressive behavior. In: *Psychopharmacology of Aggression*, edited by M. Sandler. New York: Raven Press, 1979, 95–110.
10. Mandel, P., G. Mack, E. Kempf, A. Ebel and S. Simber. Molecular aspects of a model of aggressive behavior. In: Interactions Between Putative Neurotransmitters in the Brain, edited by S. Garattini, T. F. Pujol and R. Samanin. New York: Raven Press, 1978, pp. 285–303.
11. Potegal, M., A. S. Perumal, A. I. Barkai, G. E. Cannova and A. D. Blau. GABA binding in the brains of aggressive and non-aggressive female hamsters. *Brain Res* **247:** 315–324, 1982.
12. Potegal, M., B. Toburne and M. Glusman. Effects of intraseptal GABAergic and serotonergic agents on muricide. *Soc Neurosci Abstr* **7:** 203, 1981.
13. Puglesi-Allegra, S. and P. Mandel. Effects of sodium-n-dipropyl-acetate, muscimol hydrobromide and (R, S) nipecotic acid amide on isolation-induced aggressive behavior in mice. *Psychopharmacology (Berlin)* **70:** 287–290, 1980.
14. Scatchard, G. The attractions of proteins for small molecules and ions. *Ann NY Acad Sci* **51:** 660, 1948.