

Differential Distribution of Neurotransmitter Amino Acids From the Limbic System of Aggressive and Non-Aggressive Bull Strains

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MUÑOZ-BLANCO, J., B. YUSTA AND F. CORDOBA. *Differential distribution of neurotransmitter amino acids from the limbic system of aggressive and non-aggressive bull strains.* PHARMACOL BIOCHEM BEHAV 25(1) 71-75, 1986.—The amino acid content of crude synaptosomal fractions from the limbic system and related CNS regions showed significant differences between the aggressive Spanish fighting-bull and the non-aggressive Friesian bull breeds. Neurotransmitter amino acids (glutamate, aspartate, GABA and glycine) were the most unequally distributed. A higher ratio of excitatory to inhibitory neurotransmitter amino acids was always found in all the CNS regions studied in the aggressive breed. The concentrations of five non-transmitter amino acids (threonine, alanine, serine, leucine and isoleucine) showed minor variations between both studied bull strains and cannot be ascribed to differences in central energy metabolism. The results are explained in terms of a possible relationship between the amino acid neurotransmitter levels and the innate aggressiveness of the Spanish fighting-bull.

Aggressive behaviour Aspartate Bull breeds GABA Glutamate Glycine Limbic system
Synaptosomal fractions

AGGRESSION is an ample concept and is composed of different, complex behavioural patterns, each of them with its own neuronal and endocrine basis (e.g., predatory, irritable, territorial, inter-male, fear induced, hostility towards lactating intruders, etc.) [9, 17, 31, 39].

Pharmacological studies suggest that there is no uniform neurobiological substrate of aggressiveness, and that different aggressive responses are controlled by various neurotransmitter systems, which probably interact [25]. However, Valzelli [50,51] has proposed that a common neuronal mechanism for all behavioural types of aggression should be considered. Although the neurochemical basis of the varying aggressive patterns is far from clear, recent findings support the theory that the neurotransmission aminoacidergic system plays a role in many forms of aggressive responses. Thus, increased levels of glutamate, aspartate and glutamine in the hippocampus-amygdala; glutamate and γ -aminobutyric acid (GABA) in the thalamus-hypothalamus, and a decrease in the levels of glutamate, GABA and glutamine in the caudate nucleus, have been reported in aggressive dogs kept in isolation in contrast with their non-aggressive counterparts [3]. Moreover, intracerebral microinjections of glutamic acid into the hypothalamus produce defensive and aggressive behaviour in cats [5]. Furthermore, biochemical and phar-

macological studies have recently suggested that GABA may exert a great inhibitory influence on aggressive behaviour.

In cats, GABA inhibits the aggressive responses induced by hypothalamic stimulation [18]. Early and Leonard [27] also showed that aggressive mice have lower GABA levels than their non-aggressive peers in several brain regions, including the olfactory bulb and striatal areas. Compared to their non-aggressive counterparts, aggressive mice kept in isolation have shown a decrease in the GABA content of the brain [34]. Indeed, it has been found that aggressive mice (DBA/2 breed) have lower regional levels than non-aggressive ones (e.g., C57B1/6). Thus, in conditions of isolation both breeds showed a decrease of GABA levels in the septum, striatum, olfactory bulb and posterior colliculus. Furthermore, only DBA mice showed an increase of GABA levels in the amygdala. Irrespective of differential housing conditions, DBA mice showed lower levels of GABA in the olfactory bulb and striatum, and higher levels in the posterior colliculus [46]. De Feudis [23,24] has found that grouped mice display a higher GABA binding capacity in crude synaptosomal fractions than aggressive mice. In addition, a lower GABA receptor density in aggressive mice than in non-aggressive ones (although with higher affinity) has been reported in several brain areas [49].

Similar results have been found in studies of high-affinity

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GABA binding to subcellular fractions obtained from the cerebral cortex of rats subjected to differential housing conditions [40]. A lower brain activity of the GABA synthetic enzyme (glutamic acid decarboxylase) has also been observed in crude synaptosomal fractions from the brains of aggressive mice, compared to those of their non-aggressive peers [10].

Pharmacological investigations have moreover provided support for GABAergic inhibition of aggressive behaviour in rats. Thus, dipropylacetate, an inhibitor of GABA transaminase (GABA-T) reduces aggressive responses in isolated mice and increases the GABA levels in some brain areas [47]. A selective and dose-dependent reduction in defensive fighting behavior was observed in rats which had been injected with gamma-vinyl-GABA, a specific inhibitor of GABA catabolism [41]. Similarly injections of aminooxyacetic acid (AOAA) and gamma-acetylenic-GABA (GAG), a non-specific inhibitor of GABA-T, suppress aggressive behaviour, induced by isolation, in rats, increasing the levels of GABA in several parts of the limbic system [11,22]. The aforementioned reports contrast with the results obtained with intraventricular injections of GABA agonist 4,5,6,7-tetrahydroisoxazolo (5,4-c)-pyridin-5-ol (THIP), since this treatment incites mouse killing in killer rats, and elicited this behaviour in non-killer rats [26].

In general, all these authors have established a correlation between a decrease in GABA levels in some brain areas and a deficiency of GABA-mediated inhibition in different types of aggressive behaviour [18, 27, 36, 46, 47].

In the present report, a comparison between two breeds of *Bos taurus* L. is studied in terms of the differential distribution of transmitter and non-transmitter amino acids in crude synaptosomal fractions from several areas of the limbic system and related CNS regions.

The aggressiveness of the fighting-bull breed has been well defined and studied. It is constituted of a number of fixed action patterns, found in both males and females of the breed (being more accused in the females). These patterns are similar to fixed action patterns observed in predatory, territorial, irritable and defensive-aggressive responses, in cats, mice and rats. This particular aggressiveness has been named "bravura" (ferocity) [4, 19, 32, 43, 44]. This strain of *Bos taurus* L., only differs in its general behaviour with respect to Friesian strain in this aggressive behaviour [4], that is not present in the Friesian strain [4, 13-16].

In the present study we have selected those areas of CNS, hypothalamus, medial thalamus, caudate nucleus, corpus striatum, septum, hippocampus and occipital cortex, that have, in previous investigations, been related to aggressive behaviour [1, 6, 8, 28].

The results obtained suggest a possible relationship between neurotransmitter amino acids and the aggressive behaviour observed in the fighting-bull strain.

METHOD

Animal and Tissue Preparation

Ten females, ranging from two to three years old, of Spanish fighting-bull and the Friesian bull breeds were killed by decapitation. Their brains were then quickly removed and divided into six parts by one longitudinal and two transversal abscissions. They were placed until the moment of dissection in a continuously shaken ice-cold saline solution. This process was accomplished in <60 sec. Tissue samples from the hypothalamus, medial thalamus, caudate nucleus, corpus

striatum, septum, hippocampus and occipital cortex, were excised according to González [30] and Yoshikama [53]. The samples were homogenized in an ice-cold 0.32 M sucrose solution, buffered with 0.01 M, pH 7.4 Tris-HCl, applying seven strokes per sample in a borosilicate glass homogenizer (0.10-0.15 mm radial clearance). The above dissection was performed at 0-4°C, within 90-120 sec.

Subcellular Fractionation and Analytical Procedures

The isolation of crude synaptosomal fractions and the extraction of amino acids were carried out as previously described [38]. The amino acid pool of crude synaptosomal fractions was partially purified according to Adams [2]. The purified amino acids were desiccated at 60°C, then redissolved in 0.1 M, pH 2.0, citrate buffer and finally filtered through a Millipore PHWP 1300 filter.

The amino acid analysis was performed in a Technicon Amino Acid Analyzer (TSM-1). Norleucine (2.5 μmole/ml) was used as an internal standard. The protein content in the crude synaptosomal fractions was determined by the method of Lowry *et al.* [35]. Lactate dehydrogenase (LDH) and acetylcholinesterase (AChE) activities were determined according to Booth and Clark [12]. They were used as indicators of the integrity of crude synaptosomal fractions.

Data Analysis

Results are expressed as nmole/mg protein. The figures are mean average ± ninety five percent limit of confidence for a sample size of ten animals. Statistical significance between the average means was calculated by using the Student's *t*-test (two tailed assay).

RESULTS

The activities of LDH and AChE were 0.49 and 0.12 U/mg synaptosomal protein, respectively. A ratio LDH/AChE ≅ indicates a suitable integrity (wholeness) for the crude synaptosomal fractions used in this work. Determination of the amino acid levels was carried out on brains both frozen in liquid nitrogen or maintained in ice-cold saline solution, in order to evaluate the possibility of post-mortem changes during the tissue preparation, prior to the isolation of synaptosomal fractions. Only statistically non-significant differences were observed between the two procedures, which concurs with previous work following the same procedures [42,52].

The distribution of synaptosomal transmitter and non-transmitter amino acids in both the Spanish fighting-bull and the Friesian bull breeds is shown in Table 1. Higher concentrations of glutamate in the medial thalamus ($t=14.16$; $p<0.001$), caudate nucleus ($t=7.61$; $p<0.001$), septum ($t=7.27$; $p<0.001$) and hippocampus ($t=12.75$; $p<0.001$) were found in synaptosomal fractions of the Spanish fighting-bull strain. Similarly, aspartate levels were higher in the hypothalamus ($t=19.80$; $p<0.001$), caudate nucleus ($t=9.30$; $p<0.001$), corpus striatum ($t=2.85$; $p<0.05$), septum ($t=2.33$; $p<0.05$), and the hippocampus ($t=23.10$; $p<0.001$).

Glycine presented higher levels in the hypothalamus ($t=10.46$; $p<0.001$), septum ($t=4.16$; $p<0.001$) and hippocampus ($t=9.77$; $p<0.001$) of Spanish fighting-bull breed, whereas it diminished in the caudate nucleus ($t=4.83$; $p<0.001$) and occipital cortex ($t=2.47$; $p<0.05$). The concentration of GABA was also significantly higher in the medial

TABLE 1
TRANSMITTER AND NON-TRANSMITTER AMINO ACIDS CONTENT IN CRUDE SYNAPTOSOMAL FRACTIONS FROM SEVERAL CNS REGIONS OF AGGRESSIVE AND NON-AGGRESSIVE BULLS

		Hypothalamus	M. thalamus	Caudate N.	C. striatum	Septum	Hippocampus	O. cortex
Aspartate	NA	14.4±0.8	20.2±0.8	16.6±0.9	24.7±0.7	16.0±0.4	12.2±0.5	20.5±1.1
	A	27.5±1.3‡	36.0±1.7‡	23.2±1.3‡	26.4±1.2*	17.1±1.0*	22.5±0.9‡	21.8±1.2
Glutamate	NA	29.9±1.4	33.7±2.3	40.7±1.9	35.7±1.3	36.2±1.6	33.7±1.5	41.1±2.2
	A	29.5±0.9	43.9±1.0‡	49.4±1.7‡	35.3±1.2	43.4±1.6‡	44.4±1.2‡	40.3±1.4
Glycine	NA	9.1±0.4	12.2±0.5	12.1±0.4	12.6±0.7	9.6±0.4	7.8±0.4	9.4±0.4
	A	13.3±0.8‡	12.6±0.7	10.6±0.6‡	13.1±0.7	10.8±0.5‡	10.6±0.6‡	8.8±0.5*
GABA	NA	30.7±0.6	22.6±0.8	33.9±1.1	32.3±1.0	20.0±0.4	20.4±0.8	20.4±0.7
	A	21.2±1.1‡	27.5±1.1‡	22.5±0.9‡	26.6±0.8‡	20.5±0.9	21.4±0.8	19.9±0.9
Threonine	NA	1.6±0.1	1.9 ± 0.1	1.8±0.1	1.8±0.1	2.1±0.1	2.2±0.2	1.7±0.1
	A	1.9±0.1‡	2.3±0.1‡	2.0±0.1	1.9±0.1	1.9±0.1*	2.4±0.2*	1.6±0.1
Serine	NA	5.7±0.2	6.2±0.3	6.4±0.2	6.1±0.2	8.0±0.4	6.2±0.2	6.4±0.4
	A	6.3±0.4‡	5.9±0.3	6.6±0.3	6.4±0.2	7.9±0.3	6.3±0.3	6.2±0.3
Alanine	NA	8.2±0.5	8.4±0.4	8.7±0.4	9.8±0.5	8.7±0.4	8.5±0.3	9.3±0.3
	A	8.2±0.4	4.3±0.4	9.0±0.4	9.1±0.4*	8.8±0.3	9.4±0.3‡	8.4±0.4‡
Leucine	NA	1.5±0.1	2.4±0.1	1.8±0.1	1.7±0.1	1.9±0.1	1.7±0.1	1.8±0.1
	A	1.4±0.1	2.3±0.1	1.8±0.1	1.6±0.1	1.9±0.1	1.8±0.1	1.9±0.1
Isoleucine	NA	1.1±0.1	1.3±0.1	1.1±0.1	1.1±0.1	1.2±0.1	1.0±0.1	1.0±0.1
	A	1.1±0.1	1.3±0.1	1.1±0.1	1.0±0.1	1.2±0.1	1.0±0.1	1.1±0.1

Results are expressed as nmole/mg synaptosomal protein ± 95% C.L. (n=10 animals for each strain). Statistical significance between means for Spanish fighting-bull and Friesian strains was calculated by using the Student's *t*-test.

**p*<0.05, †*p*<0.01, ‡*p*<0.001.

NA=non-aggressive Friesian bull strain.

A=aggressive Spanish fighting-bull strain.

thalamus ($t=8.53$; $p<0.001$) and lower in the hypothalamus ($t=17.47$; $p<0.001$), caudate nucleus ($t=18.49$; $p<0.001$) and corpus striatum ($t=9.89$; $p<0.001$) of the Spanish fighting-bull breed.

Non-reputedly neurotransmitter amino acids showed minor differences between the non-aggressive and the aggressive breeds, with the exception of threonine and alanine. In the aggressive fighting bull strain, the threonine content was higher in the hypothalamus ($t=4.50$; $p<0.001$), medial thalamus ($t=6.86$; $p<0.001$) and hippocampus ($t=2.13$; $p<0.05$), and lower in the septum ($t=2.41$; $p<0.05$). Alanine concentration was higher in the hippocampus ($t=4.88$; $p<0.001$) and diminished in the corpus striatum ($t=2.47$; $p<0.05$) and occipital cortex ($t=4.57$; $p<0.001$) of the aggressive fighting-bull strain. The levels of serine were higher only in the hypothalamus ($t=3.22$; $p<0.001$) of the Spanish fighting-bull strain. The levels of leucine and isoleucine however remained unaltered.

In the present report, the ratio of excitatory amino acids to inhibitory amino acids has been considered as an indicator of the relationship between the aminoacidergic excitatory capacity and the aminoacidergic inhibitory capacity of each area studied [33]. This is due to the fact that the amino acids levels have been determined in nerve endings where the neurotransmitter compartments are located [7, 29, 37].

Data for different regions are plotted (Fig. 1) as a percentage difference between the Spanish fighting-bull and the Friesian breeds: the excitatory/inhibitory ratio in the fighting-bull was significantly higher in the hypothalamus ($t=11.75$; $p<0.001$), medial thalamus ($t=10.83$; $p<0.001$), septum ($t=4.82$; $p<0.001$), and hippocampus ($t=7.71$; $p<0.001$). In the occipital cortex, this ratio was not significant ($t=1.71$).

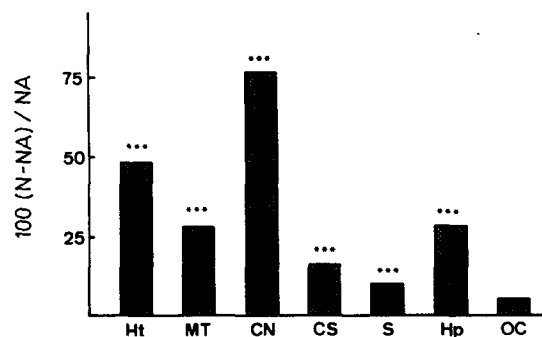


FIG. 1. Percent differences in the excitatory/inhibitory amino acid ratio between Spanish fighting-bull and Friesian bull strains. The excitatory/inhibitory amino acid relation was calculated by using the formula $(\text{Glu} + \text{Asp})/(\text{GABA} + \text{Gly})$ for each region of both aggressive fighting-bull (A) and non-aggressive Friesian bulls (NA). Data were plotted as a percentage differences between fighting-bull and Friesian strains (i.e., $100(\text{N-NA})/\text{NA}$). Ht: Hypothalamus; MT: Medial Thalamus; Hp: Hippocampus; CN: Caudate Nucleus; CS: Corpus Striatum; S: Septum and OC: Occipital Cortex. Symbols as in Table 1.

DISCUSSION

We chose to use crude synaptosomal fractions due to the following advantages: (1) They provide high and reproducible yields of synaptosomes. (2) The loss of low molecular compounds, due to leakage or shrinking in conditions of isolation, is small. (3) The isolation procedure is rapid [42].

The results showed that glutamate and/or aspartate con-

centrations were always significantly higher in all CNS regions of the aggressive Spanish fighting-bull strain, with the exception of the occipital cortex. GABA content, however, only present higher levels in the medial thalamus. These data are partly consistent with those of Agrawal *et al.* [3] who, in their studies on isolated dogs, found an increase of glutamate levels in the thalamus and hippocampus, an increase of aspartate content in the hippocampus, and a decrease of GABA levels in the caudate nucleus. Our data, however, are at variance concerning the increased levels of glutamate observed in the caudate nucleus of the Spanish fighting-bull breed. In the fighting-bull breed, the generalized increase by two aminoacidergic excitatory neurotransmitter [29] indicate a possible direct relationship between glutamate and aspartate levels, and aggressive behaviour in the fighting-bull. This has been previously proposed in dogs [3] and cats [5].

The decrease of GABA levels in the hippocampus and striatum in aggressive fighting-bull is consistent with previous reports on isolated aggressive mice [27,46] and spontaneously aggressive ones [46]. With regard to GABA content in the septum, we have not observed changes in the fighting-bull strain as opposed to Friesian strain. However, a decrease of GABA levels in this area has been reported in isolated aggressive mice [46]. Many studies have recently pointed out an inverse relationship between GABA levels in the brain and various forms of aggression in rats, mice and cats [10, 18, 27, 40, 45, 46]. Our results concerning GABA content agree with these reports, except for the medial thalamus.

Glycine, a reputedly inhibitory neurotransmitter at higher levels of CNS morphofunctional organization [29], showed no uniform variations between both breeds. An interpretation of these results is rather difficult in view of the controversy concerning to role of glycine in the forebrain. However, a relevant modulatory function can be attributed to glycine [29]. Thus, nalorphine and mephensine, drugs that increase central glycine levels, and glycine, injected intraperitoneally, reduced aggressiveness caused by water deprivation or forebrain lesions in rats [48]. Our results relative to occipital cortex and caudate nucleus are in agreement with the above mentioned report [48], although the results obtained in this study of the other five areas are in opposition. Besides, these non-uniform variations in glycine levels between both breeds concur with our previously reported modification of the glycine content in several CNS areas of mice subjected to differential housing conditions [54]. However the higher glycine levels observed in several areas of aggressive bulls do not modify the higher excitatory to in-

hibitory amino acid ratio for these areas in the aggressive breed.

In our opinion, the similarities encountered, could support the Valzelli hypothesis [50,51] concerning the presence of a common neural background for all behavioural types of aggression. The differences observed with respect to previous reports can be related to the specific aggression pattern studied, or they may depend on species differences and different experimental procedures.

Thus, we have determined amino acid levels of the pool contained in the crude synaptosomal fractions. In contrast, the aforementioned studies on changes in amino acid levels and aggressive behaviour have been made on homogenates of total cerebral tissue, which contains the metabolic and neurotransmitter amino acid pool: this material is perhaps not completely appropriate for studies on neurotransmitter amino acid levels and behaviour.

If we consider the ratio (glutamate + aspartate)/(GABA + glycine) as an index of the balance between excitatory and inhibitory neurotransmitter amino acids, the higher values observed for this ratio in the Spanish fighting-bull strain support the assumption that the aggressive responses showed by this breed are related to the increased excitatory amino acid levels (glutamate and aspartate), and to the decrease of GABA levels in several CNS areas.

The content of non-transmitter amino acids in Spanish and Friesian breeds showed minor variations. The differences were also irregular, suggesting the involvement of specific mechanisms related to neurotransmission, other than those dependent on cerebral energy metabolism [54].

It must be taken into account that differences observed between the strains in the levels of neurotransmitter amino acids could be related to changes in other neurotransmission systems in the brain areas studied, and to some regulatory mechanism which might be related to other neurotransmitter systems. Thus, an increase in serotonin levels and a decrease in noradrenaline and dopamine content have been reported, in the same areas studied, in Spanish fighting-bull respect to Friesian strains [20,21].

Further investigations are necessary in order to clarify the interactions between different amino acid neurotransmitters and other neurotransmitters in various brain areas, and also the role that these neurotransmitters play in the regulation of aggressive responses in the Spanish fighting-bull breed.

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