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Effect of Domoic Acid on Brain Amino Acid Levels

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R. DURÁN, M. C. ARUFE, B. ARIAS and M. ALFONSO. *Effect of Domoic Acid on Brain Amino Acid Levels.* Rev. esp. Fisiol. (J. Physiol. Biochem.), 51 (1), 23-28, 1995.

The administration of Domoic Acid (Dom) in a 0.2 mg/kg i.p. dose induces changes in the levels of amino acids of neurochemical interest (Asp, Glu, Gly, Tau, Ala, GABA) in different rat brain regions (hypothalamus, hippocampus, amygdala, striatum, cortex and midbrain). The most affected amino acid is the GABA, the main inhibitory amino acid neurotransmitter, whereas glutamate, the main excitatory amino acid, is not affected. The rat brain regions that seem to be the main target of the Dom action belong to the limbic system (hippocampus, amygdala). The possible implication of the amino acids in the actions of Dom is also discussed.

Key words: Domoic acid, Amino acids, Rat brain.

Glutamate is the main excitatory amino acid (EAA) in the Central Nervous System (CNS). Ionotropic glutamatergic receptors mediate direct cation fluxes elicited by selective ligands as N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and kainic acid (KA).

Domoic acid (Dom) is a neurotoxic secondary amino acid that interacts with the CNS glutamate receptors. Dom is a rigid tricarboxylic amino acid analogue of KA that has received a great deal of attention due to its lethal neurological effects, such as headache, confusion, disorientation and loss of memory.

It is generally accepted that KA exerts potent neuroexcitatory and neurotoxic effects, leading to selective neuronal damage. Dom is known as a potent neurotoxin and it is able to interact with non-NMDA glutamate receptors leading to neurotoxicity in cultures of hippocampus (3), cerebellum (6) and retina (9).

It has been proposed that both neurotoxins (KA and Dom) interact with a subtype of glutamate receptor named KA receptor, because an interaction of Dom was observed with high-affinity sites labelled by ^{3}H-kainic acid (4). Recent evidences suggest that kainate and domoate

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exert similar electrophysiological, biochemical and neurotoxic effects through identical mechamisms in hippocampus and retina.

The aim of our work is to study the effects of Dom on the brain levels of amino acids of neurochemical interest in order to determine if Dom could produce changes in these amino acids in different rat brain regions.

Materials and Methods

Domoic acid was obtained from Diagnostic Chemicals Ltd. (Canada). Pure amino acids (Asp, Glu, Gly, Tau, Ala, GABA) were obtained from Sigma (USA); o-pthalaldehyde was also obtained from Sigma (USA). Methanol for chromatography was HPLC grade. Water was obtained from a MilliQ system (Millipore). All other reagents were of analytical grade.

Stock solutions of the amino acids were prepared in pure water (60 nmol) and diluted daily to a final concentration of 1 nmol/10 µl.

OPA reagent was prepared as follows: 5 mg OPA, 250 µl ethanol, 250 µl mercaptoethanol, 4.5 ml boric acid.

In all the experiments, adult male Wistar rats kept in controlled conditions were used. The Dom dissolved in saline (NaCl 0.9 %) was administered via i.p. in a dose of 0.2 mg/kg. Control animals received an equivalent volume of saline.

One hour after administration, animals were decapitated and brains were removed and dissected in the regions studied (hypothalamus, hippocampus, amygdala, striatum, cortex and midbrain). The brain samples were weighed and homogenized in perchloric acid and kept during all night at room temperature for deproteinization. The following day, samples were microfuged during 10 min, neutralized with 1 N KOH, microfuged again and kept at –20 °C until assay.

Sample derivatization was made precolumn with the OPA reagent (1:1 v/v) 3 min and injected directly (20 µl) into the chromatograph.

Liquid chromatograph consisted in a Beckman Gold System coupled to a fluorescence detector. Separations were achieved with reversed-phase columns ODS 5 µm. Mobile phase was a mixture of (A) 0.1M acetate pH 5.5 and (B) methanol, pumped at 1.5 ml/min. In order to optimize the analytical conditions, a lineal gradient was made from 25 % to 75 % in (B) during 15 min. The components of the mobile phase were filtered through 0.22 µm Millipore filters and degassed by helium prior to use.

Results are expressed as means ± SEM of the indicated number of experiments. Statistical significance was determined using the Student's t test.

Results

In our analytical conditions, the separation and detection of six amino acids (Asp, Glu, Gly, Tau, Ala, GABA) were achieved with short run times and without interferences, using a gradient elution. In fig. 1 a typical chromatogram of the amino acid analysis is presented.

The effects of Dom (0.2 mg/kg) administration on the levels of the six amino acids studied in the different rat brain regions are presented in table I. In hypothalamus, no significant changes were observed in the case of the different amino acids. The levels of Asp and GABA were significantly increased in hippocampus. The amygdala was the rat brain region in which the most significant changes were detected, so that the levels of Asp, Gly, Ala and GABA were increased in this region. No significant changes were

Table I. *Effect of Dom 0.2 mg/kg i.p. on the levels of the amino acids studied in the different rat brain regions.* Values are the mean ± S.E.M. of 4 determinations. Signification levels by the Student's *t* test: * p<0.01, ** p<0.001.

Region	Amino Acid	Asp	Glu	Gly	Tau	Ala	GABA
Hypothalamus	Control	0.24 ± 0.01	0.11 ± 0.02	0.21 ± 0.01	0.28 ± 0.04	0.74 ± 0.06	0.17 ± 0.01
	Treated	0.25 ± 0.02	0.10 ± 0.01	0.19 ± 0.02	0.26 ± 0.05	0.71 ± 0.09	0.19 ± 0.02
Hippocampus	Control	0.27 ± 0.02	0.09 ± 0.02	0.23 ± 0.03	0.13 ± 0.03	0.55 ± 0.10	0.23 ± 0.01
	Treated	0.39 ± 0.03 *	0.10 ± 0.01	0.28 ± 0.03	0.18 ± 0.02	0.71 ± 0.09	0.37 ± 0.04 **
Amygdala	Control	0.43 ± 0.06	0.14 ± 0.01	0.40 ± 0.06	0.23 ± 0.03	1.01 ± 0.14	0.41 ± 0.07
	Treated	0.59 ± 0.06 *	0.16 ± 0.03	0.55 ± 0.07 *	0.33 ± 0.06	1.47 ± 0.22 *	0.66 ± 0.05 **
Striatum	Control	0.66 ± 0.04	0.22 ± 0.06	0.56 ± 0.13	0.30 ± 0.09	1.46 ± 0.36	0.66 ± 0.03
	Treated	0.74 ± 0.08	0.21 ± 0.04	0.51 ± 0.09	0.29 ± 0.06	1.36 ± 0.19	0.65 ± 0.04
Cortex	Control	0.36 ± 0.05	0.14 ± 0.02	0.36 ± 0.04	0.22 ± 0.02	0.98 ± 0.09	0.53 ± 0.04
	Treated	0.51 ± 0.08	0.15 ± 0.02	0.41 ± 0.05	0.28 ± 0.03	1.26 ± 0.18	0.86 ± 0.08 **
Midbrain	Control	0.34 ± 0.04	0.19 ± 0.02	0.24 ± 0.01	0.36 ± 0.06	1.13 ± 0.05	0.33 ± 0.03
	Treated	0.57 ± 0.06 **	0.26 ± 0.05	0.35 ± 0.07	0.68 ± 0.19	1.84 ± 0.30 *	0.60 ± 0.13 *

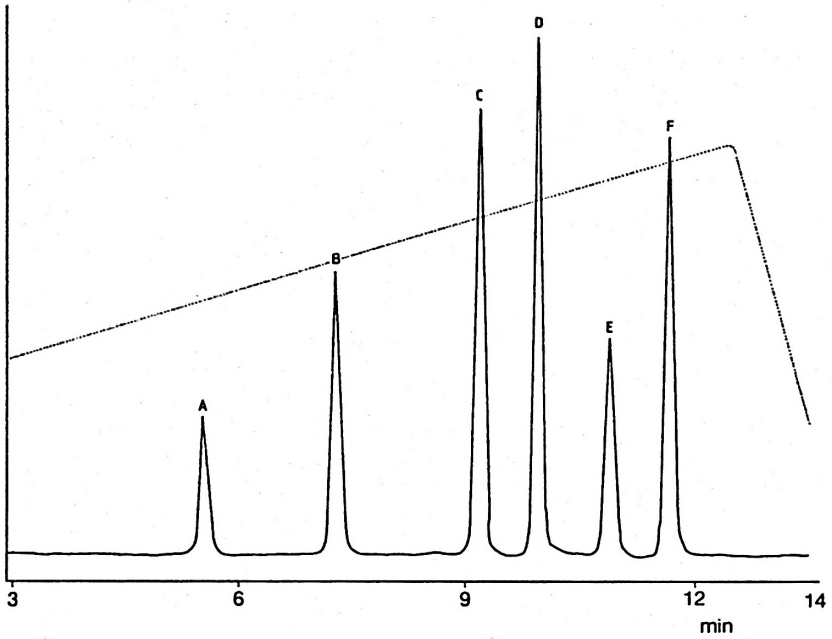

Fig. 1. *Representative chromatogram of the analysis of the six amino acids.* A: Asp; B: Glu; C: Tau; E: Ala; F: GABA. The chromatographic conditions are presented in the text. The gradient of elution is also shown in the figure.

detected in striatum. In cortex, only a significant increase in the case of GABA was detected. The Asp and GABA levels were increased significantly in midbrain.

In short, the most important increases were detected in the Asp and GABA levels. Midbrain, amygdala and hippocampus were the rat brain regions most affected by Dom.

Discussion

The increases observed in the brain levels of most amino acids studied could be related to increases in the metabolism and/or the release of these amino acids in the rat brain regions studied. In this sense Dom had previously been found to stimulate the release of ^{3}H-GABA in cultures of chick retina cells in a dose-dependent way (1). The GABA released upon activation of glutamate receptors could modulate the glutamatergic activity (10).

Five µM Dom was previously found to induce neurotoxicity in cell cultures of chick retina, being mediated by non-NMDA receptors and dependent on external calcium. Other endogenous amino acids could be involved in the toxic effect of Dom observed *in vitro* (6).

The most affected rat brain regions by the Dom administration were hippocampus and amygdala, which belong to the limbic system. This is consistent with the intoxication effects by Dom in the case of ingestion of contaminated mussels (11), especially with disorientation and loss of memory.

NOVELLI *et al.* have observed that subtoxic Dom concentrations are sufficient to potentiate glutamate and aspartate neurotoxicity (7). Excitatory amino acids of potential neurotoxicity can be pro-

duced in some rat brain regions by the effect of the Dom administration, which agrees with the increases observed by us in the case of Asp in the limbic system (hippocampus, amygdala).

The systemic KA administration has been shown to produce some decrease in the hippocampal concentration of glutamate and aspartate (5), as well as seizures and neuropathological alterations similar to those observed in temporal epilepsy in man (2). These facts support the implication of some amino acids in the mediation of KA and Dom effects on different neurochemical functions.

The relevant effects induced by a low dose of Dom on the brain amino acid levels can underline the potential risk for the human health represented by some amino acids as envirommental neurotoxins.

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Se estudia el efecto de una dosis única de 0,2 mg/kg i.p. de ácido domoico (Dom) sobre los niveles de diversos aminoácidos de interés neuroquímico (Asp, Glu, Gly, Tau, Ala, GABA) en varias regiones del cerebro de rata (hipotálamo, hipocampo, amígdala, estriado, corteza y cerebro medio). Los resultados muestran que el contenido en GABA, principal aminoácido neurotransmisor inhibitorio, resulta el más afectado mientras que el glutamato, neurotransmisor excitatorio, no se modifica. Las regiones cerebrales que parecen ser más sensibles a la acción del Dom pertenecen al sistema límbico (hipocampo y amígdala). También se discute la posible implicación de los aminoácidos en las acciones neuroquímicas del Dom.

Palabras claves: Acido domoico, Aminoácidos, Cerebro de rata.

References

1. Alfonso, M., Durán, R., Duarte, C. B., Ferreira, I. L. and Carvalho, A. P. (1994): *Neurochem. Int.*, 24, 267-274.
2. Arias, C., Montiel, T. and Tapia, R. (1990): *Neurochem. Res.*, 15, 641-645.
3. Debonnel, G., Beauchesne, L. and De Montigny, C. (1989): *Can. J. Physiol. Pharmacol.*, 67, 29-36.
4. Hampson, D. R., Huang, X., Wells, J. W., Walter, J. A. and Wright, J. L. C. (1992): *Eur. J. Pharmacol.*, 218, 1-8.
5. Nicoletti, F., Perciavalle, V., Berretta, S., Speciale, C., Polizzi, M., Patti, F., De Simone, D. (1984): *Pharmacol.*, 103, 133-137.
6. Novelli, A., Fernández, M. T., Kispert, J., Torreblanca, A., Gascón, S. and Zitko, V. (1992): *Amino Acids*, 2, 233-244.
7. Novelli, A., Kispert, J., Fernández, M.T., Torreblanca, A. and Zitko, V. (1992): *Brain Res.*, 577, 41-48.
8. Perl, T. M., Bedard, L., Kosatsky, T., Hockin, J. C., Todd, E. C. D. and Remis, R. S. (1990): *N. Engl. J. Med.*, 322, 1775-1780.
9. Stewart, G. R., Zorumski, C. F., Price, M. T. and Olney, J. W. (1990): *Exp. Neurol.*, 110, 127-138.
10. Travagli, R. A., Ulivi,,M. and Wojcik,,W. (1991): *J. Pharmacol. Exp. Ther.*, 258, 903-909.
11. Wright, J., Bird, C., Freitas, A. S., Hampson, D., McDonald, J. and Quilliam, M. (1990): *Can. Dis. Wkly. Rep.*, 16, 21-26.