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Mediation of ionotropic glutamate receptors in domoic acid-induced striatal dopamine release in rats

Begoña Arias Quintela *, Rafael Durán [1], Miguel Alfonso [2]

Department of Functional Biology and Health Sciences, Vigo University, Lagoas-Marcosende, 36200 Vigo, Spain

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Abstract

Our objective was to characterize the mechanism of action of intrastriatal infusion of domoic acid on extracellular dopamine levels, using in vivo dialysis in conscious and freely moving rats. The local infusion of domoic acid (500 μM) caused an increase (567.9 ± 142.5%, versus basal) in dopamine extracellular levels associated with a decrease in its metabolites: dihydroxyphenylacetate (DOPAC) and homovanillate (HVA) (47.3 ± 4.4% and 33.8 ± 4.2%, respectively, compared to basal). Infusion of the amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid/kainate (AMPA/kainate) receptor antagonist, 6,7-dinitroquinoxaline-2,3-dione (DNQX; 200 μM) reversed the effect of domoic acid infusion on striatal dopamine levels. However, the infusion of the selective non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist, dizocilpine (MK-801; 50 μM), did not change significantly the effect of domoic acid on dopamine extracellular levels. In conclusion, based on results with a microdialysis technique, we suggest that domoic acid may act through AMPA/kainate receptors in striatum.

Keywords: Domoic acid; DNQX; MK-801; Dopamine; Microdialysis; Striatum, rat

1. Introduction

Domoic acid is a phytoplankton-derived excitotoxin that was responsible for an outbreak of neurotoxicity that affected over 100 people in Canada in 1987 following the consumption of contaminated shellfish. Several survivors of the poisoning incident showed chronic neurological deficits including persistent memory impairment (Teitelbaum et al., 1990). Domoic acid structurally resembles glutamate (Glu) and kainic acid, both of which are excitatory amino acids, and produce lesions in brain tissue due to their excitotoxicity (Nijar, 1997). Evidence suggests that the release of striatal dopamine may be under glutamatergic control. Thus, stimulatory actions of agonists at glutamate receptors have been demonstrated both in tissue slices (Krebs et al., 1991) and cultured cells (Mount et al., 1990). In vivo studies using push–pull cannulae or microdialysis probes gave similar results in the intact animal (Chéramy et al., 1990). Glu and other excitatory amino acids can act through a large number of receptors; members of this family include metabotropic glutamate receptors and ionotropic glutamate receptors. The ionotropic receptors can be divided into three subgroups according to their affinities for *N*-methyl-D-aspartate (NMDA), amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainic acid (Imperato et al., 1990). It has been suggested that domoic acid may produce neurotoxicity by mechanisms similar to those of kainic acid, probably via interaction with AMPA/kainate receptors. domoic acid and kainic acid stimulated the release of glutamate from synaptosomes, being equipotent to stimulate the release of excitatory amino acids (Brown and Nijjar, 1995).

We have previously demonstrated that perfusion of domoic acid in the rat striatum increased the extracellular concentration of dopamine, and induced locomotor effects (Arias et al., 1998). However, the type of receptors mediating the action of domoic acid on striatal dopamine release is not yet known. The purpose of the present experiments was to characterize the in vivo release of striatal dopamine induced by domoic acid, using intracerebral microdialysis.

* Corresponding author. Tel.: +34-986-81-23-91; fax: +34-986-81-25-56.

E-mail addresses: barias@uvigo.es (B.A. Quintela), rduran@uvigo.es (R. Durán), pallares@uvigo.es (M. Alfonso).

[1] Tel.: +34-986-812392; fax: +34-986-812556.

[2] Tel.: +34-986-812391; fax: +34-986-812556.

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Thus, we infused different antagonists of glutamate receptors such as MK-801, a potent and selective non-competitive NMDA receptor antagonist, and 6,7-dinitroquinoxaline-2,3-dione (DNQX), a competitive AMPA/kainate receptor antagonist.

2. Materials and methods

Male Sprague–Dawley rats weighing 250–300 g were housed in a light- and temperature-controlled environment and provided with food and water ad libitum. Before surgery for implantation of a guide cannula (CMA/12), the animals were anesthetized with chloral hydrate (400 mg/kg, i.p.). They were placed in a Narishige stereotaxic frame for placement of a guide cannula at coordinates for the left striatum: A +2.0, L +3.0, and V +6.0 from the bregma (König and Klippel, 1963). The animals were allowed to recover from surgery for at least 24 h before experiments were performed. A CMA/12 microdialysis probe (ID: 0.5 mm and length: 3 mm) was introduced into the striatum through the guide cannula and connected to a CMA/102 infusion pump, to perfuse a Ringer solution (147 mM NaCl, 3.4 mM $CaCl_2$, 4 mM KCl, pH 7.4) at a flow rate of 2 μl/min. Dialysed samples were collected every 15 min using a CMA/142 fraction collector.

The experiments were carried out for 180 min using freely moving animals. After a 60-min collection of samples under basal conditions, the animals were perfused for 30 min with domoic acid (500 μM). In other groups of animals, DNQX (200 μM) was co-perfused with domoic acid for 30 min, or perfused for 150 min before its coadministration with domoic acid; MK-801 (50 μM) was perfused for 150 min before its coadministration with domoic acid, 500 μM (during 30 min).

Following collection of samples, 20 μl of dialysates was immediately analyzed using a high-performance liquid chromatography (HPLC) system with electrochemical detection, according to the procedure of Durán et al. (1998). The chromatographic system consisted of a Hewlett-Packard 1050 isocratic system coupled to an ESA Coulochem 5100A electrochemical detector.

The mean substances levels in the three samples before domoic acid administration were considered basal levels. The basal levels were taken as 100% in order to compare the response of substances after drug administration. The statistical analysis of the results was performed using a one-way analysis of variance (ANOVA) with Mann–Whitney post-hoc analysis. $P < 0.05$ was considered statistically significant.

In vitro recoveries for dialysis probes were 11–12%, 18–23%, and 21–26% for dopamine, dihydroxyphenylacetate (DOPAC) and homovanillate (HVA), respectively. The day after surgery, the levels of dopamine and its metabolites were stable in control rats throughout all the experiment (data not shown). The basal output for dialysate dopamine and its metabolites from the striatum was as follows (mean ± S.E.M.): dopamine = 0.22 ± 0.05, DOPAC = 8.51 ± 0.46 and HVA = 6.24 ± 0.39 (pmol/20 μl).

3. Results

Intrastriatal perfusion of 500 μM domoic acid caused a significant increase (567.9 ± 142.5%) in the extracellular dopamine levels, 15 min after the infusion of domoic acid, while its metabolites showed a significant decrease (47.3

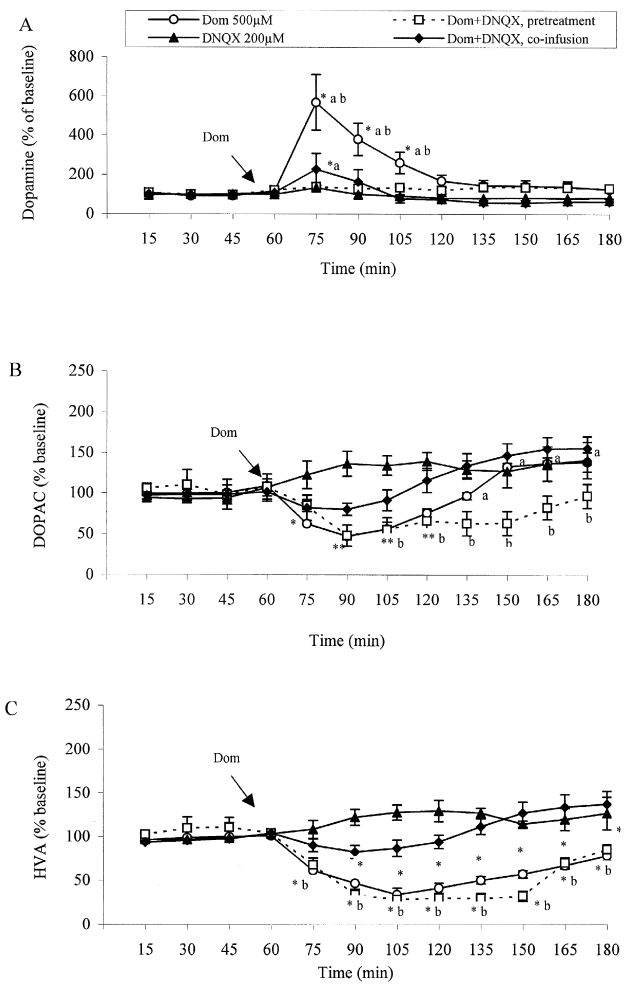

Fig. 1. Effect of the infusion of domoic acid (DOM; 500 μM) and DNQX (200 μM) on the release of striatal dopamine (A), DOPAC (B) and HVA (C). After collection of basal samples for 60 min, DOM was infused for 30 min as indicated by the arrow. DNQX was co-infused with DOM or infused for 150 min before its co-infusion with domoic acid (denoted as ''pretreatment''). Symbols denote DOM (open circles), DNQX (filled triangles), co-infused of DOM + DNQX (filled diamonds) and pretreatment (open squares). Data are means ± S.E.M. for 4–5 animals per group. $^{*}P < 0.05$, with respect to basal, $^{a}P < 0.05$, with respect to pretreatment group and $^{b}P < 0.05$, with respect to co-infused group.

± 4.4% DOPAC and 33.8 ± 4.2% HVA), 30 min after the infusion of domoic acid. Local perfusion of 200 μM DNQX for 30 or 180 min and of MK-801 (50 μM) for 180 min had no significant effects on the extracellular dopamine, DOPAC and HVA levels in the striatum (Figs. 1 and 2).

Intrastriatal perfusion of the AMPA/kainate receptor antagonist, DNQX (200 μM), or the NMDA receptor antagonist, MK-801 (50 μM), with domoic acid (for 30 min) was applied to pharmacologically characterize the receptor specificity of the domoic acid effect on striatum dopamine release. Local co-perfusion of DNQX together with domoic acid (500 μM) partially counteracted the domoic acid facilitatory effect on dopamine release and inhibited the release when the antagonist was preadministered 150 min before domoic acid (Fig. 1A). The decrease in DOPAC and HVA levels induced by infusion of 500 μM domoic acid was partially reversed by co-infusion with DNQX (Fig. 1B and C). However, this effect of domoic acid on metabolite levels was not reduced by pretreatment with DNQX 150 min before domoic acid coadministration.

Pretreatment with 50 μM MK-801 for 150 min did not antagonize the domoic acid-induced increase in dopamine release (Fig. 2A). The combination of domoic acid and MK-801 did not inhibit the effect of domoic acid on extracellular levels of DOPAC and HVA (Fig. 2B and C).

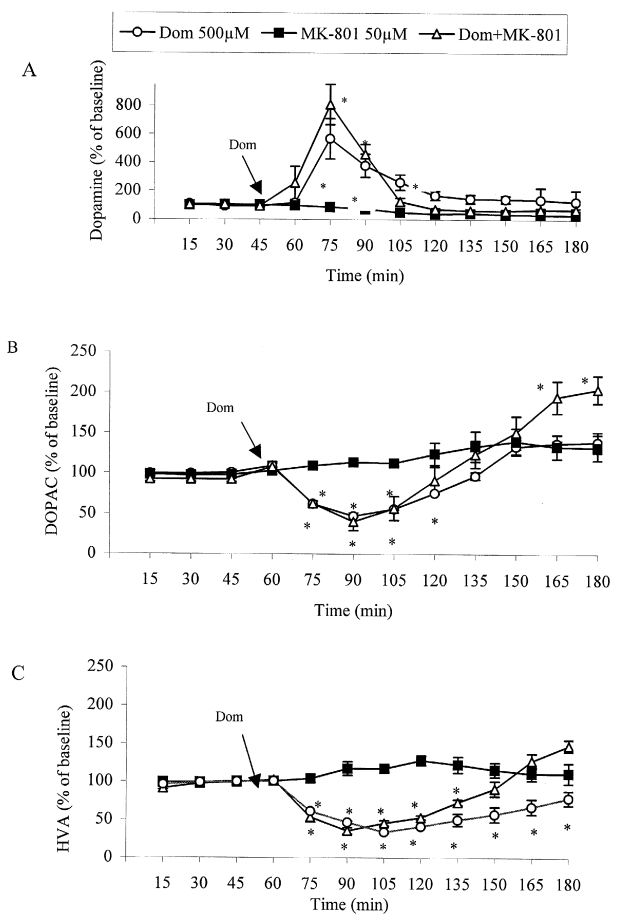

Fig. 2. Effect of the infusion of domoic acid (500 μM) and MK-801 (50 μM) on the release of striatal dopamine (A), DOPAC (B) and HVA (C). After collection of basal samples for 60 min, domoic acid was infused for 30 min as indicated by the arrow. MK-801 was infused for 150 min before its co-infusion with DOM. Symbols denote domoic acid (open circles), MK-801 (filled squares) and MK-801 + domoic acid (open diamonds). Data are means ± S.E.M. for 4–5 animals per group. * $P < 0.05$, with respect to basal.

4. Discussion

The present results provide the first evidence in vivo that DNQX, a specific antagonist of AMPA/kainate receptors, reduces the domoic acid-induced increase in dopamine release.

We have previously demonstrated that the intracerebral administration of domoic acid produced a dose-dependent increase in striatal dopamine levels, as well as a decrease in extracellular levels of its metabolites DOPAC and HVA (Arias et al., 1998) and this could suggest that the striatal dopaminergic system is sensitive to the action of domoic acid, a glutamatergic agonist. Previous studies have concluded that there must be glutamatergic control exerted on striatal dopaminergic terminals (Maione et al., 1995). Moreover, some authors (Carroza et al., 1992; Herrera-Marschitz et al., 1990; Jedema and Moghaddam, 1996) have suggested that there is modulation of dopamine release via NMDA and/or via AMPA/kainate receptors, showing that Glu and its analogues (AMPA, kainic acid and NMDA) stimulate striatal dopamine release in vivo (Jedema and Moghaddam, 1994) and from striatal slices (Clow and Jhamandas, 1989). There are a few reports of microdialysis studies of the effects of domoic acid on dopamine release. Some studies have shown effects of domoic acid on neurotransmitter systems. Thus, Alfonso et al. (1994) showed that domoic acid stimulated γ-aminobutyric acid (GABA) release in cultured chick retina cells, while Brown and Nijjar (1995) recently showed that domoic acid enhanced Glu release from rat brain synaptosomes and Durán et al. (1995)observed that the administration of domoic acid induced changes in the levels of Glu and GABA in various brain regions (hippocampus, amygdala, cortex and midbrain) in the rat.

In this study, the total inhibition of domoic acid-induced dopamine release was observed 150 min after pretreatment with the AMPA/kainate receptor antagonist, DNQX. In this regard, Jedema and Moghaddam (1996) have observed that the increase of the extracellular dopamine levels induced by local application of AMPA or kainic acid in prefrontal cortex was attenuated by the infusion of CNQX (50 μM) 1 h before the infusion of glutamatergic agonists. Furthermore, Imperato et al. (1990) found an increase of dopamine release in caudate and

accumbens nuclei after 50 μM KA infusion. This effect was reduced, but not completely antagonized, by DNQX applied 1 h before kainic acid.

Our results obtained with the NMDA receptor antagonist, MK-801, showed that this antagonist did not reduce domoic acid-stimulated dopamine release. Because MK-801 is a very selective antagonist of the NMDA receptor, the results suggest that the action of domoic acid on in vivo dopamine release may be not mediated by the activation of NMDA receptors. In addition, Alfonso et al. (1994) observed that MK-801 did not affect the release of [^{3}H]GABA evoked by domoic acid in cultured chick retina cells. Moreover, Tasker et al. (1996) have reported that the toxic actions of domoic acid in vivo were generally insensitive to NMDA receptor antagonists.

Finally, the treatment with domoic acid decreased the extracellular level of DOPAC and HVA. This effect could have been due to a reduction of the cytoplasmic dopamine pool available for deamination to DOPAC. HVA could be considered as a ''second metabolite'' of dopamine which is formed from extracellular DOPAC (Westerink, 1985), and changes in HVA levels could reflect smaller extracellular levels of DOPAC. Other studies showed a similar profile in which the application of exogenous excitatory amino acids to neostriatum decreased the extracellular concentrations of DOPAC and HVA (Keefe et al., 1993). However, the results for DOPAC and HVA levels in the presence of domoic acid and DNQX are difficult to explain. It could be argued that these levels of extracellular dopamine induced by domoic acid, despite the inhibitory effect of DNQX, signify a reduction of the cytoplasmic dopamine pool available for deamination, which leads to a decrease in extracellular metabolite content (Ichikawa and Meltzer, 1992).

One interesting observation was that the behavioural stimulation induced by the local application of domoic acid is consistent with the results of other behavioural studies (Tryphonas et al., 1990). In the present study local co-infusion of MK-801 did not change the behavioural stimulation induced by the intracerebral application of domoic acid, but the infusion of DNQX reduced the behavioural effects produced by the perfusion of domoic acid alone. These results, combined with results of a study by Boldry et al. (1991) showing that the increase in extracellular dopamine and locomotor activity produced by AMPA can be inhibited by DNQX, could suggest that the effects of domoic acid on endogenous dopamine and behavioural activation in striatum are mediated through the activation of AMPA/kainate receptors.

In conclusion, this study demonstrated that pretreatment with DNQX significantly inhibited in vivo domoic acid-induced striatal dopamine release in rats. The results obtained with the quinoxalinedione AMPA/kainate receptor antagonist were consistent with the assumed mechanism of domoic acid action.

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