Amino Acids (1998) 14: 197–203

Amino
Acids

Release of endogenous excitatory amino acids in the neostriatum of the rat under physiological and pharmacologically-induced conditions

M. Herrera-Marschitz[1], M. Goiny[1], Z.-B. You[1,4], J. J. Meana[1], E. Engidawork[1], Y. Chen[1], R. Rodriguez-Puertas[3], C. Broberger[3], K. Andersson[2], L. Terenius[4], T. Hökfelt[3], and U. Ungerstedt[1]

Departments of [1] Physiology and Pharmacology, [2] Internal Medicine, [3] Neuroscience, and [4] Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden

Accepted September 26, 1997

Summary. There is immunohistochemical evidence suggesting that glutamate (Glu) is released from nerve terminals and acts, via several receptor subtypes, as a major excitatory neurotransmitter in the cortico-striatal pathway of the rat. Aspartate (Asp) is also present in cortico-striatal neurons, but its role as a neurotransmitter has been questioned, since, in contrast to Glu, it has not been demonstrated in presynaptic vesicles. Glu and Asp can be found at subμM concentrations in the extracellular compartment of most areas of the basal ganglia. Their concentrations are largely regulated by transport mechanisms, but also by a synaptotagmin-dependent exocytotic release, and are sufficiently high to occupy junctional and extrajunctional receptors.

We have investigated whether Glu and Asp release in the neostriatum can be selectively modulated by different neuronal systems. Dopamine (DA) and cholecystokinin (CCK) selectively stimulate Asp release, via D_1 and CCK_B receptor subtypes, respectively. Also opioid κ-agonists increase Asp release. We propose that the selective modulation of Asp release by D_1-, CCK_B- and κ-agonists involves striatal neurons containing Asp, but not Glu. In contrast, local perfusion with the μ-opioid antagonist D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 (CTOP) increases both Glu and Asp release. This effect is probably exerted on cortico-striatal terminals, via presynaptic inhibitory μ-receptors. Thus, these results demonstrate that extracellular levels of Glu and Asp are modulated differentially by different neuronal systems, and suggest that in the neostriatum of the rat there are neuronal populations using Glu and/or Asp as messenger(s).

Keywords: Basal ganglia – Excitatory amino acids – Monoamines – Neuropeptides – Microdialysis – Immunocytochemistry – Parkinson's disease – Rat

On the origin of extracellular levels of glutamate and aspartate

Immunohistochemical studies have demonstrated the presence of glutamate (Glu) (Ottersen and Storm-Mathisen, 1984) and aspartate (Asp) (Dinopoulos et al., 1991) in the cortico-striatal pathway of the rat, suggesting that these amino acids are released from nerve terminals in the neostriatum of the rat and act as excitatory neurotransmitters via multiple, heterogeneously distributed, receptor subtypes (Hollman and Heinemann, 1994). So far, however, only Glu has been observed in presynaptic vesicles, leading to the conclusion that Glu is the excitatory neurotransmitter, and that Asp levels reflect rather general metabolism (Orrego and Villanueva, 1993; Fillenz, 1995).

When investigated with *in vivo* microdialysis (Ungerstedt et al., 1982), Glu and Asp are detected in sub μM concentrations in the extracellular compartment of many brain regions, without a clear regional distribution. As shown in Table 1, a similar result is obtained when the tissue content of Glu and Asp is analysed, suggesting a broad distribution of amino acid containing pathways.

It has been difficult to convincingly demonstrate a neuronal origin for Glu and Asp levels measured *in vivo* with microdialysis. In the neostriatum of the rat, extracellular Glu and Asp levels are increased at best ≈2 fold following depolarisation with 100 mM KCl, whereas simultaneously monitored substances such as dopamine, GABA or cholecystokinin are increased > 20 fold. Na^+-channel blockade with tetrodotoxin (TTX) has shown to be ineffective in decreasing, or rather increases Glu and Asp, but not the levels of other substances monitored simultaneously (Herrera-Marschitz et al., 1996; You et al., 1994a, b). Similarly removal of extracellular Ca^{2+} produces at best a ≈10% decrease (Morari et al., 1993), or a paradoxical increase of Glu and Asp levels, depending upon the extent of the Ca^{2+}-depletion (Herrera-Marschitz et al., 1996; You et al., 1994a, 1994b).

We have argued that these paradoxical results reflect rather unique mechanisms of clearance from the extracellular compartment, largely depending upon transport not only to presynaptic, but also to postsynaptic and glial elements (Nicholls and Attwell, 1990; Hansson and Rönnbäck, 1995). The effects of two transport inhibitors, dihydrokainic acid (DHKA) (Johnston et

Table 1. Tissue (μmol/g, wet weight) and extracellular (μM) levels of glutamate and aspartate in neocortex, neostriatum and substantia nigra of adult normal rats

Brain region	Glutamate		Aspartate	
	μmol/g	μM	μmol/g	μM
Neocortex	6–8	0.5–1	1–2	0.05–0.15
Neostriatum	8–10	0.5–1	2–3	0.05–0.15
Substantia nigra	4–5	0.6–1.2	2–3	0.06–0.12

For *ex vivo* tissue biochemistry see Tossman et al. (1986), and Chen et al. (1997). For *in vivo* microdialysis experiments see Godukhin et al. (1995), Herrera-Marschitz et al. (1996), You et al. (1996a, b).

al., 1979) and L-trans-pyrrolidine-2,4-dicarboxylic acid (PDC) (Bridges et al., 1991) on striatal Glu and Asp levels, measured under basal and K^+-depolarising conditions are shown in Table 2. PDC appears to be more potent than DHKA, and more importantly, it appears to selectively inhibit Glu transport, since following 10 μM of PDC, Glu, but not Asp, levels are significantly increased, both under basal and K^+-depolarising conditions (Herrera-Marschitz et al., 1996).

Thus, these results support the idea that high affinity transport is an important mechanism by which amino acids are removed from the extracellular compartment, and suggest that there is a selective transport system distinguishing between Glu and Asp.

α-Latrotoxin, which induces an irreversible blockade of exocytotic neurotransmitter release via binding to synaptotagmine, significantly decreases striatal Glu levels, both under basal and K^+-depolarising conditions, suggesting there is indeed an exocytotic release (Fig. 1). A similar effect has been observed on Asp (Herrera-Marschitz et al., 1996).

Functional interactions

Whatever their origin, Glu and Asp are found in the extracellular compartment of the neostriatum at concentrations sufficiently high to occupy junctional and extrajunctional receptors (Hollmann and Heinemann, 1994, Hansson and Rönnbäck, 1995). Furthermore, their levels are selectively modulated by several neuroactive drugs suggesting functional interactions with different neuronal pathways, including those containing DA, CCK and Dynorphin B (Table 3).

A unilateral 6-OHDA injection into the medial forebrain bundle (MFB) induces an almost complete depletion of extracellular levels of DA and its

Table 2. Effect of transport inhibition on extracellular glutamate and aspartate levels (μM) measured with *in vivo* microdialysis in the neostriatum of adult rats

Parameters	Basal	KCl (100 mM)	% of basal
CSF alone; N = 12			
Glutamate	0.6 ± 0.1	1.3 ± 0.4	220[a]
Aspartate	0.09 ± 0.02	0.2 ± 0.08	220[a]
DHKA (10 μM); N = 6			
Glutamate	0.9 ± 0.3[b]	2.7 ± 0.5[b]	300[a]
Aspartate	0.14 ± 0.0[b]	0.5 ± 0.02[b]	350[a]
PDC (10 μM); N = 6			
Glutamate	1.4 ± 0.1[b]	3.9 ± 0.1[b]	280[a]
Aspartate	0.1 ± 0.06	0.2 ± 0.01	200[a]

CSF alone refers to levels simultaneously analysed on the contralateral side. The drugs were added to the perfusion medium of the left neostriatum from the time when the probes were implanted [for details, see Herrera-Marschitz et al. (1996)]. [a] $p < 0.05$, KCl vs the corresponding basal level; [b] $p < 0.05$, compared with CSF condition.

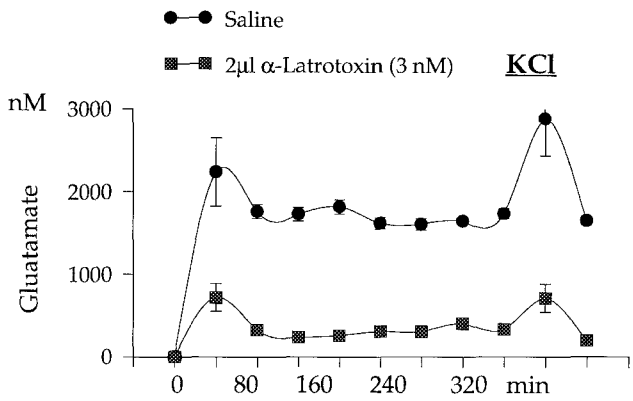

Fig. 1. Glu levels measured in left and right neostriatum of halothane-anaesthetised adult rats. α-Latrotoxin (2 μl, 3 nM) or saline (2 μl) was injected either into the left or the right neostriatum 30 min before microdialysis probe implantation (perfused with CSF or CSF + 100 mM KCl). Data are means ± S.E.M. (F-ANOVA 32, df = 9,70; $p < 0.05$) (Herrera-Marschitz et al., 1996)

metabolites in the neostriatum and substantia nigra (You et al., 1996a). This lesion leads to a significant decrease (≈40%) in striatal Asp levels, while Glu levels are increased (≈140%). In agreement, it has been found that striatal administration of the D1 receptor agonist SKF 38393 (10 μM), as well as of the dopamine precursor L-DOPA (25 mg/kg s.c.) induces a selective increase in striatal Asp levels (Broberger et al. in preparation). No such effect has been observed following treatments with the D_1 agonist, quinpirole.

Local administration of sulphated cholecystokinin-octapeptide (CCK-8S) (1–100 μM) produces a concentration-dependent increase in striatal Asp (You et al., 1994c). CCK-8S also increases Glu, but only following the highest concentrations. Furthermore, CCK-4 increases Asp but not Glu levels (You et al., 1996a). The effect of CCK-8 on striatal Asp is blocked by the selective CCK_B antagonist L-365,260 (20 mg/kg s.c.), but not by the selective CCK_A antagonist, L-364,718 (20 mg/kg s.c.) (You et al., 1996a).

Endogenous opioid peptides also selectively modulate the release of amino acids. Local infusion of the κ-receptor agonist, U-50,488 increases striatal Asp, but not Glu levels. In contrast, the selective μ-receptor antagonist, CTOP, but not the κ_1-receptor antagonist Nor-BNI, increases both Glu and Asp levels, probably via inhibition of a μ-receptor-dependent modulation of cortico-striatal terminals.

Taken together, these observations suggest that there are different neuronal pools of releasable Glu and Asp, which, in the neostriatum, may be modulated, under physiological and pharmacological conditions, by DA- and neuropeptide-containing pathways.

Evidence for aspartate immunoreactive neurons in the neostriatum of the rat

Using an antiserum raised against Asp conjugated to keyhole-limpet hemocyanin, Asp-positive neurons have been occasionally seen in the neostriatum of

Table 3. Modulation of glutamate and aspartate release by dopamine, cholecystokinin and opioid mechanisms in the neostriatum of the rat

Treatment		Glutamate	Aspartate
Dopamine	6-hydroxydopamine/MFB	≈(⇑)	⇓
	SKF 38393 (1–100 μM)	∅	⇑
	Quinpirole (1–100 μM)	∅	∅
Cholecystokinin	CCK_B	≈(⇑)	⇑
	CCK_A	≈(⇑)	∅
Opioids	U-50, 88H (10 μM)	∅	⇑
	Nor-BNI (1–10 μM)	∅	∅
	Morphine (1 mg/kg s.c.)	∅	∅
	CTOP (1–10 μM)	⇑	≈(⇑)

∅ No effect; ⇑ increase; ⇓ decrease; ≈(⇑) occasionally increased, or only at the highest examined doses (for details, see You et al., 1994c, 1996a, b).

the rat under normal conditions (Snyder et al., 1993). We have found that the amount of Asp-positive neurons is increased by systemic treatment with metamphetamine, and SKF 38393, in particular when this D_1-agonist is administered to animals with a unilateral 6-OHDA lesion. The effect of SKF-38393 is dose-dependent, ipsilateral to the lesion, probably mediated through DA receptors rendered supersensitive, and selectively inhibited by the D_1 antagonist, SCH 23390. Double staining experiments have demonstrated that there is a minor overlap between Asp- and NPY-positive neurons, but an almost complete lack of overlap with DARPP-32-, dynorphin- and substance P-positive neurons, suggesting that Asp is contained in intrinsic interneurons (Pettersson et al., 1996).

Clinical implications

The fact that Asp can be released in large amounts following CCK and/or DA stimulation makes it likely that it has a functional role in the neostriatum, independent from that of Glu. Elevated Asp levels may convey desirable functional responses, but also lead to overstimulation of NMDA-receptor subtypes, with excitotoxic consequences. It is probable that this striatal Asp neuronal system is also up-regulated under chronic treatment with L-DOPA in Parkinson's disease.

Acknowledgements

This study was supported by grants from the Swedish Medical Research Council, the Swedish Society of Medicine, and the Karolinska Institute Funds.

References

Bridges R, Stanley MS, Anderson MW, Cotman CW, Chamberlin R (1991) Conformationally defined neurotransmitter analogues. Selective inhibition of glutamate uptake by one pyrrolidine-2,4-dicarboxylate diastereomer. J Med Chem 34: 717–725

Chen Y, Engidawork E, Loidl F, Dell'Anna E, Goiny M, Lubec G, Andersson K, Herrera-Marschitz M (1997) Short- and long-term effects of perinatal asphyxia on monoamine, amino acid and glycolysis product levels measured in the basal ganglia of the rat. Dev Brain Res 103

Dinopoulos A, Dori I, Parnavelas JG (1991). Immunohistochemical localization of aspartate in corticofugal pathways. Neurosci Lett 121: 25–28

Fillenz M (1995) Physiological release of excitatory amino acids. Behav Brain Res 71: 51–67

Godukhin O, You Z-B, Herrera-Marschitz M, Goiny M, Pettersson E, Hökfelt T, Ungerstedt U (1995) Effect of local cholecystokinin-8 administration on extracellular levels of amino acids and glycolytic products monitored by *in vivo* microdialysis in the fronto-parietal cortex of the rat. Neurosci Lett 194: 29–32

Hansson E, Rönnbäck L (1995) Astrocytes in glutamate transmission. FASEB J 9: 343–350

Herrera-Marschitz M, Goiny M, Meana JJ, Silveira R, Godukhin O, Chen Y, Espinoza S, Pettersson E, Loidl F, Lubec G, Andersson K, Nylander I, Terenius L, Ungerstedt U (1996) On the origin of extracellular glutamate levels monitored in the basal ganglia by *in vivo* microdialysis. J Neurochem 66: 1726–1735

Hollman M, Heinemann S (1994) Cloned glutamate receptors. Annu Rev Neurosci 17: 31–108

Johnston GAR, Sue M, Kennedy E, Twitchin B (1979) Action of the neurotoxin kainic acid on high affinity uptake of l-glutamic acid in rat brain slices. J Neurochem 32: 121–127

Morari M, O'Connor WT, Ungerstedt U, Fuxe K (1993) N-Methyl-D-aspartic acid differentially regulates extracellular dopamine, GABA, and glutamate levels in the dorsolateral neostriatum of halothane-anesthetized rat: an *in vivo* microdialysis study. J Neurochem 60: 1884–1893

Nicholls DG, Attwell D (1990) The release and uptake of excitatory amino acids. Trends Pharmacol Sci 11: 462–468

Orrego F, Villanueva S (1993) The chemical nature of the main central excitatory transmitter: a critical appraisal based upon release studies and synaptic vesicle localization. Neuroscience 56: 539–555

Ottersen OP, Storm-Mathisen J (1984) Glutamate- and GABA-containing neurons in the mouse and rat brain as demonstrated with a new immunohistochemical technique. J Comp Neurol 229: 374–392

Pettersson E, Herrera-Marschitz M, Rodriguez-Puertas R, Xu Z-Q, You Z-B, Hughes J, Elde RP, Ungerstedt U, Hökfelt T (1996) Evidence for aspartate immunoreactive neurons in the neostriatum of the rat: modulation by the mesencephalic dopamine pathway via the D_1-subtype of receptor. Neuroscience 74: 51–66

Snyder GL, Fisone G, Morino P, Gundersen V, Ottersen, OP, Hökfelt T, Greengard P (1993) Regulation by the neuropeptide cholecystokinin (CCK-8S) of protein phosphorylation in the neostriatum. Proc Natl Acad Sci USA 90: 11277–11281

Tossman U, Jonsson G, Ungerstedt U (1986) Regional distribution and extracellular levels of amino acids in rat central nervous system. Acta Physiol Scand 127: 535–545

Ungerstedt U, Herrera-Marschitz M, Jungnelius U, Ståhle L, Tossman U, Zetterström T (1982) Dopamine synaptic mechanisms reflected in studies combining behavioural recordings and brain dialysis. Adv Biosci 37: 219–231

You Z-B, Herrera-Marschitz M, Brodin E, Meana JJ, Morino P, Hökfelt T, Silveira R, Goiny M, Ungerstedt U (1994a) On the origin of striatal cholecystokinin release: studies with *in vivo* microdialysis. J Neurochem 62: 76–85

You Z-B, Nylander I, Herrera-Marschitz M, O'Connor WT, Goiny M, Terenius L (1994b) The striatonigral dynorphin pathway of the rat studied with *in vivo* microdialysis-I. Effects of K^+-depolarization, lesions and peptidase inhibition. Neuroscience 63: 415–425

You Z-B, Pettersson E, Herrera-Marschitz M, Hökfelt T, Terenius L, Nylander I, Goiny M, Hughes J, O'Connor WT, Ungerstedt U (1994c) Modulation of striatal aspartate and dynorphin B release by cholecystokinin (CCK-8) studied *in vivo* with microdialysis. Neuroreport 5: 2301–2304

You Z-B, Herrera-Marschitz M, Pettersson E, Nylander I, Goiny M, Shou H-Z, Godukhin O, Hökfelt T, Terenius L, Ungerstedt U (1996a) Modulation of neurotransmitter release by cholecystokinin in neostriatum and substantia nigra of the rat: regional and receptor specificity. Neuroscience 74: 793–804

You Z-B, Herrera-Marschitz M, Nylander I, Goiny M, Kehr J, Ungerstedt U, Terenius L (1996b) Effect of morphine on dynorphin B and GABA release in the basal ganglia of rats. Brain Res 710: 241–248

Authors' address: Dr. M. Herrera-Marschitz, Department of Physiology and Pharmacology, Karolinska Institutet, S-171 77 Stockholm, Sweden.

Received August 25, 1997