ELSEVIER

European Journal of Pharmacology 416 (2001) 69–73

ejp

www.elsevier.nl/locate/ejphar

The effect of *N*-acetyl-L-aspartic acid dilithium salt on dopamine release and synthesis in the rat striatum in vivo

Vladimir I. Petrov, Vladimir S. Sergeyev, Natalya V. Onishchenko *

Laboratory of Psychopharmacology, Research Institute of Pharmacology, Volgograd Medical Academy, Pavshih Bortsov sq. 1, Volgograd 400131, Russian Federation

Received 24 August 2000; received in revised form 16 February 2001; accepted 23 February 2001

Abstract

The effect of the dilithium salt of *N*-acetyl-L-aspartic acid on release and synthesis of dopamine in the striatum was investigated using microdialysis in freely moving rats. Intrastriatal infusion of 1 mM *N*-methyl-D-aspartate, an NMDA receptor agonist, augmented extracellular dopamine to 215% of baseline, while 1 mM dilithium *N*-acetyl-L-aspartate increased dopamine release to 190% of baseline in rat striatum. Infusion of DL-2-amino-5-phosphonopentanoic acid, a competitive NMDA receptor antagonist, prior to infusion of dilithium *N*-acetyl-L-aspartate did not significantly alter basal levels of dopamine, but reversed the dilithium *N*-acetyl-L-aspartate-evoked elevation in extracellular dopamine. Intrastriatal perfusion with 6-cyano-7-nitroquinoxaline-2,3-dione, an AMPA/kainate receptors antagonist, altered neither basal levels of dopamine nor dilithium *N*-acetylaspartate-induced dopamine release. When the striatum was continuously perfused with the inhibitor of L-aromatic amino acid decarboxylase, 3-hydroxybenzylhydrazine dihydrochloride (100 μM), both dilithium *N*-acetylaspartate and NMDA added to the perfusate increased extracellular 3,4-dihydroxyphenyl-L-alanine, reflecting the effect of the compounds on the biosynthesis of dopamine. The data suggest that availability of dilithium *N*-acetyl-L-aspartate to activate dopamine turnover and release in the rat striatum may be mediated by presynaptic NMDA heteroreceptors located at dopaminergic neurons. © 2001 Published by Elsevier Science B.V.

Keywords: *N*-acetylaspartate; Dopamine; Striatum; Microdialysis

Introduction

The study of physiological and pathological aspects of excitatory amino acid neurotransmission is currently one of the most exciting areas of basic and clinical neuroscience (Heresco-Levy and Javitt, 1998). One of the mechanisms that may influence the activity of monoamine transmission is mediated by excitatory amino acid heteroreceptors located on monoaminergic neurons. Particularly, glutamatergic and dopaminergic neurotransmission are closely interconnected in various brain regions such as prefrontal cortex, striatum, nucleus accumbens and several others. Ionotropic excitatory amino acids receptors (NMDA, AMPA and kainate) are primarily considered to participate in the modulation of dopamine neurotransmission (Westerink et al., 1992).

The importance of interactions between endogenous excitatory amino acids and the dopamine system in the pathological processes underlying schizophrenia, depression, Parkinson's disease and alcoholism has become increasingly appreciated (Carlsson and Carlsson, 1990; Heresco-Levy and Javitt, 1998). Disturbances in excitatory amino acid derivatives are also believed to play a role in these and several other disorders. *N*-acetylaspartate (NAA) is one of the most abundant amino acids in the brain, but the role of this amino acid in physiology and pathology is unclear. That NAA functions as a neurotransmitter has been suggested, but current evidence does not support this notion (Tsai and Coyle, 1995). It is thought that NAA may participate in brain osmoregulation, acid–base homeostasis (Taylor et al., 1994; Davies et al., 1998) and glial cell-specific signaling (Baslow, 2000). Most of the endogenous extracellular NAA seems to be derived from *N*-acetylaspartylglutamate (NAAG) (Zollinger et al., 1986), which is an endogenous NMDA receptor ligand. At the same time, NAA may also serve as a precursor of NAAG (Tyson and Sutherland, 1998).

* Corresponding author. Tel.: +7-8442-97-81-78; fax: +7-8442-97-81-60.

E-mail address: onishchenko@vlink.ru (N.V. Onishchenko).

0014-2999/01/$ - see front matter © 2001 Published by Elsevier Science B.V.
PII: S0014-2999(01)00872-X

Substances modulating excitatory amino acid neurotransmission are of great interest currently as novel avenue in the search for potential pharmacological means to treat the conditions mentioned above. One of the most promising trends in the development of new effective drugs is chemical modification of the endogenous molecule structures. In this case, glutamate and aspartate as endogenous excitatory amino acid receptors ligands are potentially most interesting.

It is thought that NAA, administered peripherally, cannot cross the blood–brain barrier (Tsai and Coyle, 1995). In an attempt to develop a derivative, which would be able to cross the blood–brain barrier, the phosphonic derivatives and salts of *N*-acetyl-L-aspartic acid were prepared in our laboratory (Sazhin et al., 1991; Ozerov et al., 1993). Dilithium *N*-acetyl-L-aspartate (lab code: AKF-94) was identified as the most potent compound exerting a psychotropic action. In preliminary investigations, it was found that this compound, administered peripherally, may exert antidepressant-like and antiamnestic effects in animal models (Petrov and Grigoryev, 1998).

Since interplay between glutamate and dopamine systems may be involved in the pathogenesis of several mental disorders (Carlsson and Carlsson, 1990), an effect of the drug on dopaminergic transmission represents an attractive potential site of action of dilithium *N*-acetyl-L-aspartate. The purpose of the present study was to investigate the effect of the aspartic acid derivative on release and synthesis of dopamine in the striatum of freely moving rats.

Materials and methods

2.1. Animals and surgery

Male Wistar rats, weighing between 250 and 300 g, were used. For surgery, the rats were anaesthetised with Nembutal (40 mg/kg, i.p.) and placed in a stereotaxic apparatus (David Kopf Instruments, USA). The animals were implanted with probes for microdialysis in the right striatum. Hollow dialysis fibers (Gambro, Germany; mol wt cutoff 5000 Da) with 250 μm outer diameter were used to prepare the microdialysis probes. The exposed length of dialysis membrane was 5.0 mm. Co-ordinates of the implantation were AP 0.5, L 3.0, V 7.4 from bregma point and dura according to the atlas of Paxinos and Watson (1986).

2.2. Microdialysis procedure

Microdialysis experiments were carried out 24 h after implantation of the probes. The probes were perfused with a Ringer's solution at a flow rate 2.0 μl/min (Syringe Pump, Razel Scientific instruments, USA). Sample collection began following a 60-min stabilization period. Dialysate, as 40-μl fractions was collected at 20-min intervals into microtubes that contained 5 μl 1 M perchloric acid, and was injected directly into the high performance liquid chromatography (HPLC) system. At least three samples were collected before drug infusion to determine the basal level of dopamine in dialysates. To assess the dopamine synthesis rate in vivo, an inhibitor of L-aromatic amino acid decarboxylase, 3-hydroxybenzylhydrazine dihydrochloride (NSD-1015, 100 μM), was infused into the striatum for at least 2 h before the drugs were tested and dialysate levels of 3,4-dihydroxyphenyl-L-alanine (L-DOPA) were measured (Westerink et al., 1990). Baseline L-DOPA was determined as described for dopamine, after the extracellular L-DOPA level had become stable after inhibition of L-aromatic amino acid decarboxylase by NSD-1015.

2.3. Neurochemical assay

Dopamine and L-DOPA were quantified by HPLC with electrochemical detection. A reverse-phase column (3 × 150 mm, C18, 5 μm, ELSICO) was used. The mobile phase was a phosphate buffer (0.1 mM) containing EDTA (0.1 mM), octane sulphonic acid (0.3 mM) and acetonitrile (7%) with pH 3.6 (Petrov and Grigoryev, 1998). The electrochemical detector, with glassy carbon electrode, was set at 800 mV against an Ag/AgCl reference electrode.

2.4. Drugs and chemicals

All reagents for the HPLC mobile phase were analytical grade. *N*-methyl-D-aspartate, DL-2-amino-5-phosphonopentanoic acid (AP-5), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 3-hydroxybenzylhydrazine dihydrochloride were obtained from Sigma (USA). *N*-acetyl-L-aspartic acid dilithium salt was synthesized by Prof. Ozerov A.A. (Research Institute of Pharmacology, Volgograd, Russia). All drugs were dissolved in perfusion fluid and administered via the dialysis probe.

2.5. Data analysis

The average of the last three stable samples before drug treatment was considered as the baseline and was defined as 100%. Values obtained during drug infusions were expressed as percentages of basal levels. Statistical comparisons were carried out by analysis of variance followed by Student's *t*-test. Values of $P < 0.05$ were considered as significant.

Results

Intrastriatal infusion of 1 mM *N*-methyl-D-aspartate, an agonist at the NMDA receptor, caused augmentation in

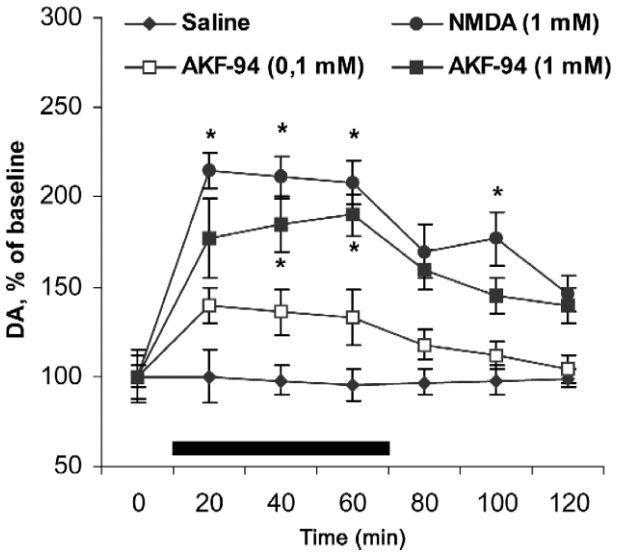

Fig. 1. Effect of local infusion of NMDA (1 mM) or dilithium *N*-acetyl-L-aspartate (AKF-94, 0.1 and 1 mM) (black bar) on the level of extracellular dopamine in the striatum of freely moving rats. The data are expressed as percentages of dopamine basal level ± S.E.M. ($n = 4–5$). * $P < 0.05$ vs. saline-treated controls.

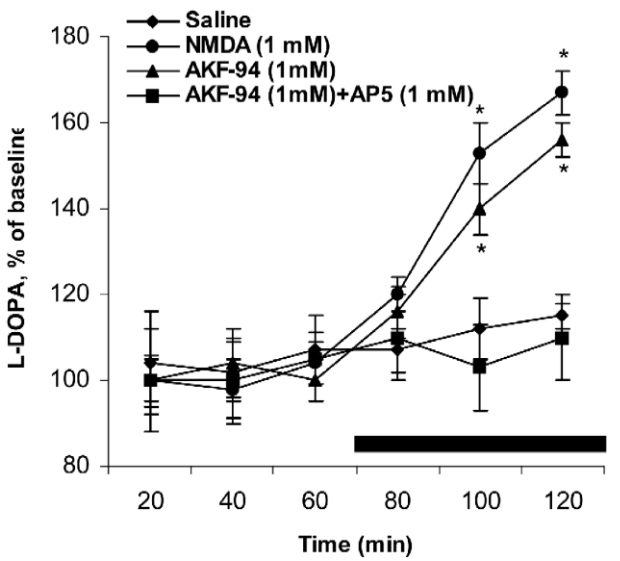

Fig. 3. The effect of local infusion of NMDA (1 mM) or dilithium *N*-acetyl-L-aspartate (AKF-94, 1 mM) (black bar) on striatal 3,4-dihydroxyphenyl-L-alanine (L-DOPA) accumulation following perfusion with NSD-1015 (100 μM). The data are expressed as percentages of L-DOPA basal level (average of three samples prior to infusion of NMDA or dilithium NAA) ± S.E.M. ($n = 4–5$). * $P < 0.05$ vs. saline-treated controls.

extracellular dopamine to 215% of baseline in this region. Infusion of 1 and 10 μM dilithium *N*-acetyl-L-aspartate did not lead to changes in dopamine level. No significant effect of infusion of 0.1 mM dilithium *N*-acetyl-L-aspartate was observed, whereas infusion of 1 mM dilithium *N*-acetyl-L-aspartate increased dopamine to 190% of baseline in the rat striatum. The effect was maximal 20–40 min after beginning of the infusion of *N*-acetylaspartate (Fig. 1).

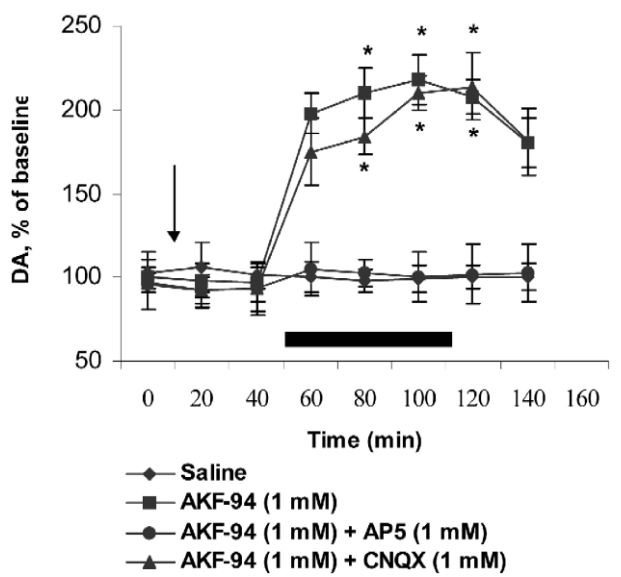

Fig. 2. Effect of local infusion of dilithium *N*-acetyl-L-aspartate (AKF-94) in combination with glutamate receptor antagonists on the striatal dialysate levels of dopamine. In two groups, AP-5 (1 mM) or CNQX (1 mM) was added to the perfusate (arrow) 60 min before the dilithium *N*-acetyl-L-aspartate (1 mM) infusion (black bar). The data are expressed as percentages of dopamine basal level (average of three samples prior to infusion of antagonist) ± S.E.M. ($n = 4–5$). * $P < 0.05$ vs. saline-treated controls.

Infusion of 1 mM AP-5, a competitive NMDA receptor antagonist, prior to infusion of 1 mM dilithium *N*-acetyl-L-aspartate, did not significantly alter basal levels of dopamine, but reversed the *N*-acetylaspartate-evoked elevation in extracellular dopamine. Intrastriatal perfusion with 1 mM CNQX, an antagonist of AMPA/kainate receptors, altered neither basal levels of dopamine nor *N*-acetylaspartate-induced dopamine release (Fig. 2).

Extracellular L-DOPA after inhibition of L-aromatic amino acid decarboxylase by NSD-1015 provided a measure of dopamine synthesis (Westerink et al., 1990; Castro et al., 1996). Extracellular L-DOPA before the infusion of NSD-1015 was under the detection limit. When the striatum was perfused with NSD-1015 (100 μM) dialysate, L-DOPA was detected from the first 20-min sample onward. Dialysate L-DOPA contents were increasing and became stable over 60 min after the beginning of NSD-1015 perfusion (data not shown). After this, both dilithium *N*-acetyl-L-aspartate and NMDA added to the perfusate caused a significant increase of extracellular L-DOPA. This effect was reversed by co-perfusion with AP-5 (Fig. 3).

Discussion

There are several hypotheses concerning the functions of endogenous acetylaspartate and its role in brain pathophysiology. In particular, disturbances in the NAA and

NAAG contents in the brain are described in patients with schizophrenia (Lim et al., 1998; Parsons et al., 1998; Deicken et al., 2000). Moreover, it has been suggested that pathology of the prefrontal cortex, measured as local decrease of NAA concentration, and a decrease of striatal dopaminergic neuron activity can be associated with the pathophysiology of schizophrenia (Bertolino et al., 1999).

Taylor et al. (1994) reported that basal extracellular concentration of endogenous NAA in the rat striatum measured using microdialysis in vivo was 83.7 μM. It was markedly lower than the tissue content. The authors hypothesized that high tissue/extracellular fluid concentration ratios might be due to the low release of NAA under basal conditions, and that there exist potent re-uptake mechanisms for NAA in the cellular membrane of neurons (Taylor et al., 1994). In our study, infusion of 100 μM NAA salt led to only a slight augmentation of dopamine release in the striatum, and this effect did not reach significance. However, at a concentration of 1 mM, the drug effectively elevated extracellular levels of dopamine. It is worth mentioning that in vivo recovery of the drug was not tested under the microdialysis conditions we used and the concentration of the drug in brain tissue may have been significantly lower than the one infused. Nevertheless, future investigation is required to determine whether this ability of NAA to modulate the dopamine system is physiologically relevant.

The mechanism of the stimulatory action of NAA on dopamine transmission is unclear at present. One of the possibilities may include the role of NAA as a precursor of NAAG, an endogenous ligand of NMDA receptors (Baslow, 2000). At the same time, it cannot be totally excluded that NAA can exert a direct influence on the neurotransmitter receptors. Budygin et al. (1997) showed that *N*-acetylaspartate is able to increase dopamine release from the rat striatum during perfusion in vitro. Co-perfusion with 3-(2-carboxypiperazin-4-yl)-propil-1-phosphonic acid (CPP), a NMDA receptor antagonist, markedly reversed the effect of NAA (Budygin et al., 1997). It has been suggested that NAA can exert a stimulatory effect on dopamine transmission via excitatory amino acid receptors, particularly the NMDA type. Nevertheless, the present results establish firmly that NAA salt can influence dopamine transmission in the striatum in vivo and that this effect can be blocked by an NMDA receptor antagonist.

Within the basal ganglia, interactions between dopamine and excitatory amino acids occur at different anatomical levels (Calabresi et al., 1997). There is extensive evidence from experiments with slices and synaptosomes that dopaminergic nerve terminals in the striatum have NMDA receptors, which can regulate both release and synthesis of dopamine (Krebs et al., 1991; Antonelli et al.,1997). Experiments in which both release and synthesis have been measured in vivo showed that, under conditions of low extracellular dopamine, endogenous excitatory amino acids may influence dopamine release, as well as dopamine synthesis, by an intrastriatal action (Castro et al., 1996; Zigmond et al., 1998). Dopamine release in vivo, evoked by local infusion of NMDA into the striatum, can occur through different mechanisms, one of them being a direct effect on presynaptic receptors (Wheeler et al., 1995).

Our results indicated that dilithium *N*-acetyl-L-aspartate can enhance dopamine release in vivo. This effect was qualitatively similar to the effect of NMDA but augmentation in catecholamine release, caused by dilithium *N*-acetyl-L-aspartate, was less expressed. Both the effect on release and the influence on synthesis (the former evaluated as increase in extracellular L-DOPA after inhibition of aromatic amino acid decarboxylase) were reversed by the NMDA receptor antagonist, AP-5. Although these data suggest strongly that high concentrations of NAA salt can modulate dopamine transmission, a physiological role of this modulation remains to be established.

In conclusion, these data indicate that NAA dilithium salt can influence dopamine release and synthesis in the rat striatum. It could be suggested that these effects are apparently mediated by NMDA heteroreceptors located on the presynaptic dopamine terminals.

Acknowledgements

We would like to thank Prof. A.A. Ozerov for the compound provided for research. We thank Dr. R.R. Gainetdinov for the helpful comments.

References

Antonelli, T., Govoni, B.M., Bianchi, C., Beani, L., 1997. Glutamate regulation of dopamine release in guinea pig striatal slices. Neurochem. Int. 30, 203–209.

Baslow, M.H., 2000. Functions of *N*-acetyl-L-aspartate and *N*-acetyl-L-aspartylglutamate in the vertebrate brain: role in glial cell-specific signaling. J. Neurochem. 75, 453–459.

Bertolino, A., Knable, M.B., Saunders, R.C., Callicott, J.H., Kolachana, B., Mattay, V.S., Bachevalier, J., Frank, J.A., Egan, M., Weinberger, D.R., 1999. The relationship between dorsolateral prefrontal *N*-acetylaspartate measures and striatal dopamine activity in schizophrenia. Biol. Psychiatry 15, 660–667.

Budygin, E.A., Gainetdinov, R.R., Titov, D.A., Kovalev, G.I., 1997. Effect of aspartic acid derivatives, *N*-acetyl-aspartate and its phosphonic analog PIR-87-6-0, on dopamine release from the rat striatum during perfusion in vitro. Biul. Eksp. Biol. Med. 123, 57–60, in Russian.

Calabresi, P., Pisani, A., Centonze, D., Bernardi, G., 1997. Synaptic plasticity and physiological interactions between dopamine and glutamate in the striatum. Neurosci. Behav. Rev. 21, 519–523.

Carlsson, M., Carlsson, A., 1990. Interactions between glutamatergic and monoaminergic systems within the basal ganglia-implications for schizophrenia and Parkinson's disease. Trends Neurosci. 13, 272–276.

Castro, S.L., Sved, A.F., Zigmond, M.J., 1996. Increased neostriatal tyrozyne hydroxylation during stress: role of extracellular dopamine and excitatory amino acids. J. Neurochem. 66, 824–833.

Davies, S.E., Gotoh, M., Richards, D.A., Obrenovitch, T.P., 1998. Hypoosmolarity induces an increase of extracellular *N*-acetylaspartate concentration in the rat striatum. Neurochem. Res. 23, 1021–1025.

Deicken, R.F., Johnson, C., Pegues, M., 2000. Proton magnetic resonance spectroscopy of the human brain in schizophrenia. Rev. Neurosci. 11, 147–158.

Heresco-Levy, U., Javitt, D.C., 1998. The role of NMDA receptor-mediated neurotransmission in the pathophysiology and therapeutics of psychiatric syndromes. Eur. Neuropsychopharmacol. 8, 141–152.

Krebs, M.O., Desce, J.M., Kemel, M.L., Gauchy, C., Godeheu, C., Cheramy, A., Glowinski, J., 1991. Glutaminergic control of dopamine release in the rat striatum: evidence for presynaptic *N*-methyl-D-aspartate receptors of dopaminergic nerve terminals. J. Neurochem. 56, 81–85.

Lim, K.O., Adalsteinsson, E., Spielman, D., Sullivan, E.V., Rosenbloom, M.J., Pfefferbaum, A., 1998. Proton magnetic resonance spectroscopic imaging of cortical gray and white matter in schizophrenia. Arch. Gen. Psychiatry 55, 346–352.

Ozerov, A.A., Brel, A.K., Petrov, V.I., Grigoryev, I.A., Gaevaya, L.M., Gunger, A.A., 1993. Synthesis and pharmacological activity of 2-dimethoxyphosphorylethyl esters of excitatory amino acids *N*-acylderivatives. Khim.-Farm. Zh. 27, 39–42, in Russian.

Parsons, C.G., Danysz, W., Quack, G., 1998. Glutamate in CNS disorders as a target for drug development: an update. Drug News & Perspect. 11, 523–569.

Paxinos, G., Watson, C., 1986. The Rat Brain in Stereotaxic Coordinates. Academic Press, New York.

Petrov, V.I., Grigoryev, I.A., 1998. EAA modulation of dopaminergic neurons as a basis for designing new nootropic and stress-protective agents. Vestn. Ross. Akad. Med. Nauk 11, 29–35, in Russian.

Sazhin, V.A., Shcherbakov, A.A., Breslaukhov, A.G., Ozerov, A.A., Petrov, V.I., 1991. *N*-acyl derivatives of aspartic acid: psychotropic activity-electron structure relationship in molecules. Khim.-Farm. Zh. 8, 50–53, in Russian.

Taylor, D.L., Davies, S.E., Obrenovitch, T.P., Urenjak, J., Richards, D.A., Clark, J.B., Symon, L., 1994. Extracellular *N*-acetylaspartate in the rat brain: in vivo determination of basal levels and changes evoked by high K^+. J. Neurochem. 62, 2349–2355.

Tsai, G., Coyle, T., 1995. *N*-acetylaspartate in neuropsychiatric disorders. Prog. Neurobiol. 46, 531–540.

Tyson, R.L., Sutherland, G.R., 1998. Labeling of *N*-acetylaspartate and *N*-acetylaspartylglutamate in rat neocortex, hippocampus and cerebellum from [1-^{13}C]glucose. Neurosci. Lett. 251, 181–184.

Westerink, B.H.C., De Vries, J.B., Duran, R., 1990. Use of microdialysis for monitoring tyrosine hydroxylase activity in the brain of conscious rats. J. Neurochem. 54, 381–387.

Westerink, B.H.C., Santiago, M., De Vries, J.B., 1992. The release of dopamine from nerve terminals and dendrites of nigrostriatal neurons induced by EAAs in the conscious rat. Naunyn-Schmiedeberg's Arch. Pharmacol. 345, 523–529.

Wheeler, B., Boutelle, M.G., Fillenz, M., 1995. The role of *N*-methyl-D-aspartate receptors in the regulation of physiologically released dopamine. Neuroscience 65, 767–774.

Zigmond, M.J., Castro, S.L., Keefe, K.A., Abercrombie, E.D., Sved, A.F., 1998. Role of excitatory amino acids in the regulation of dopamine release in the neostriatum. Amino Acids 14, 57–62.

Zollinger, M., Amsler, U., Do, K.Q., Streit, P., Cuenod, M., 1986. Release of *N*-acetylaspartylglutamate on depolarization of rat brain slices. J. Neurochem. 51, 1919–1923.