ELSEVIER

European Journal of Pharmacology 275 (1995) R5–R6

ejp

Rapid communication

Adenosine release in the ventral striatum of the rat is modulated by endogenous nitric oxide

Hanspeter Fischer, H. Prast *, A. Philippu

Department of Pharmacology and Toxicology, University of Innsbruck, Peter-Mayr-Strasse 1, A-6020 Innsbruck, Austria

Received 2 February 1995; accepted 10 February 1995

Abstract

The influence of nitric oxide (NO) on adenosine release was investigated by the push-pull technique in the ventral striatum of the urethane-anaesthetized rat. Superfusion with the NO donor diethylamine-NO enhanced, whereas superfusion with the NO synthase inhibitor L-N^G-nitroarginine methyl ester decreased the output of adenosine. The effect of L-N^G-nitroarginine methyl ester was abolished by L-arginine methyl ester. These findings indicate that, in the ventral striatum of the rat, NO modulates adenosine release.

Keywords: Nitric oxide (NO); Adenosine release; Ventral striatum, rat

Adenosine plays a significant role in the regulation of striatal neurotransmitter release. It presynaptically suppresses the release of several neurotransmitters, and it influences postsynaptic effects of excitatory neurotransmitters (Williams, 1987). Adenosine is either released as such in the extracellular space, or it derives from an adenine nucleotide which is coreleased with other neurotransmitters from presynaptic terminals of the central nervous system (Williams, 1990).

It has been shown recently that nitric oxide (NO) modulates neurotransmitter release from neurons of the ventral striatum. Indeed, NO influences the in vivo release of acetylcholine (Prast and Philippu, 1992), amino acids and dopamine (Guevara-Guzman et al., 1994). We have now investigated the influence of NO on the release of adenosine in the ventral striatum.

In anaesthetized rats (Sprague-Dawley, male, 250–280 g; urethane 1.1 g/kg i.p.), a push-pull cannula (outer needle: O.D. 0.83 mm, I.D. 0.51 mm; inner needle: O.D. 0.31 mm, I.D. 0.16 mm) was inserted stereotaxically immediately before starting superfusion and the ventral striatum (coordinates as mm from bregma: AP + 1.0, L 2.5, V 8.0) was superfused with artificial cerebrospinal fluid (CSF) at a flow rate of 25 μl/min. The superfusate was collected in periods of 10 min at −20° C. Adenosine was determined in the superfusate by HPLC combined with UV detection at 254 nm using 0.01 mol/l $(NH_4)H_2PO_4$/methanol (4/1) as mobile phase at a flow rate of 0.45 ml/min. Adenosine was separated from interfering substances by a column filled with Nucleosil 100-5-C18 (250 × 4 mm). In parallel samples, identification of the adenosine peak was confirmed by enzymatic degradation of the purine with adenosine deaminase. The effect of NO on adenosine output was investigated by superfusing the ventral striatum either with the NO donor diethylamine-NO, or with the NO synthase inhibitor L-N^G-nitro-L-arginine methyl ester, or with L-N^G-nitro-L-arginine methyl ester in the presence of L-arginine methyl ester. Drugs were dissolved in CSF. Superfusions with drugs were started 100 min after insertion of the push-pull cannula and start of superfusion with CSF.

After the start of superfusion, the adenosine overflow declined gradually and reached a stable basal level after approximately 70 min. The mean basal output of adenosine in the ventral striatum was 204 ± 27 fmol/10 min (mean ± S.E.M., $n = 22$). Superfusion of the ventral striatum with 500 μmol/l diethylamine-NO for 10 min caused a pronounced increase (by about 400%) in adenosine release (Fig. 1). Decomposed diethylamine-NO (500 μmol/l) was ineffective (not shown). Superfusion with the NO synthase inhibitor L-N^G-nitro-L-

* Corresponding author. Tel. 43.512.507 5602, fax 43.512.507 2931.

SSDI 0014-2999(95)00097-6

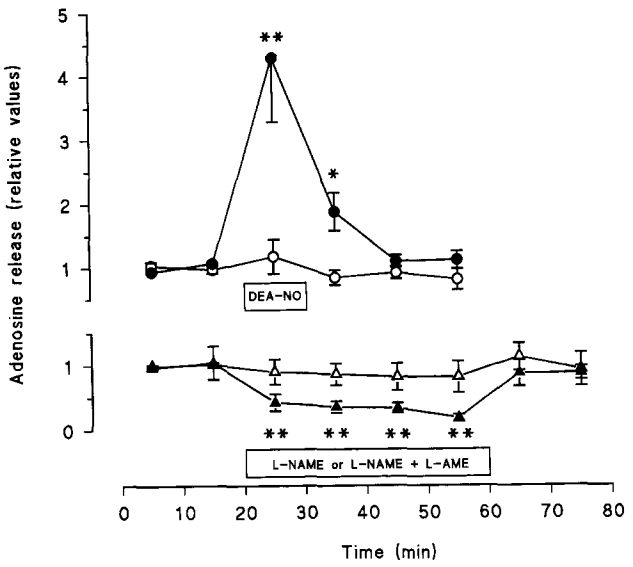

Fig. 1. Effects of diethylamine-NO (DEA-NO), L-N^G-nitro-L-arginine methyl ester (L-NAME), or L-NAME in the presence of L-arginine methyl ester (L-AME) on adenosine output in the ventral striatum. Open circles: basal release ($n = 5$). Superfusion with drugs: solid circles: 500 μmol/l DEA-NO ($n = 7$), solid triangles: 100 μmol/l L-NAME ($n = 9$), open triangles: 100 μmol/l L-NAME and 500 μmol/l L-AME ($n = 7$). Horizontal bars indicate begin and duration of superfusion with drugs. Release rates are shown as relative values. Means ± S.E.M. The mean release rates in the two samples before superfusion with drugs were taken as 1.0. Friedman's analysis of variance, followed by Wilcoxon's signed rank test. $^*P < 0.05$, $^{**}P < 0.02$

arginine methyl ester (100 μmol/l) for 40 min caused a 50% decrease in adenosine release. After termination of superfusion with L-N^G-nitro-L-arginine methyl ester the adenosine output gradually increased to the basal level, indicating that the effect of L-N^G-nitro-L-arginine methyl ester on NO synthase was reversible. The L-N^G-nitro-L-arginine methyl ester-induced decrease was abolished by simultaneous superfusion with 500 μmol/l L-arginine methyl ester. L-Arginine methyl ester per se had no effect on the adenosine output (not shown).

These results show that, in the ventral striatum, endogenous NO increases the extracellular concentration of adenosine, very probably by enhancing its release and not by inhibiting its metabolism. Indeed, the inosine concentration in the superfusate was not reduced by diethylamine-NO (not shown). The decrease of the adenosine output by L-N^G-nitro-L-arginine methyl ester confirms the previous finding that endogenous NO is continuously synthesized in the ventral striatum (Prast and Philippu, 1992; Prast et al., 1995) and indicates that the extracellular adenosine concentration is continuously enhanced by this modulator.

In slices from rat cortex, adenosine release is not influenced either by the NO donor sodium nitroprusside, or by L-N^G-nitroarginine (Craig and White, 1993). Though results on NO-evoked transmitter release obtained in slices have to be interpreted cautiously, it might be possible that NO does not modulate adenosine release in the cortex.

Our findings together with recent in vitro and in vivo studies support the idea that the functional significance of NO might be the acute enhancement in the release of neurotransmitters (Prast and Philippu, 1992; Guevara-Guzman et al., 1994; Meffert et al., 1994; Montague et al., 1994; Prast et al., 1995). It remains to be elucidated, whether the elevation of the extracellular adenosine level is partly due to ATP coreleased with neurotransmitters.

Acknowledgements

This work was supported by the Fonds zur Förderung der wissenschaftlichen Forschung.

References

Craig, C.G. and T.D. White, 1993, NMDA-evoked adenosine release from rat cortex does not require the intermediate formation of nitric oxide, Neurosci. Lett. 158, 167.

Guevara-Guzman, R., P.C. Emson and K.M. Kendrick, 1994, Modulation of in vivo striatal transmitter release by nitric oxide and cyclic GMP, J. Neurochem. 62, 807.

Meffert, K.M., B.A. Premack and H. Schulman, 1994, Nitric oxide stimulates Ca^{2+}-independent synaptic vesicle release, Neuron 12, 1235.

Montague, P.R., C.D. Gancayco, M.J. Winn, R.B. Marchase and M.J. Friedlander, 1994, Role of NO production in NMDA receptor-mediated neurotransmitter release in cerebral cortex, Science 263, 973.

Prast, H. and A. Philippu, 1992, Nitric oxide releases acetylcholine in the basal forebrain, Eur. J. Pharmacol. 216, 139.

Prast, H., H. Fischer and A. Philippu, 1995, Nitric oxide modulates the release of acetylcholine in the ventral striatum of the freely moving rat, Naunyn-Schmied. Arch. Pharmacol. (in press).

Williams, M., 1987, Purine receptors in mammalian tissues: pharmacology and functional significance, Annu. Rev. Pharmacol. Toxicol. 27, 315.

Williams, M., 1990, Purine nucleosides and nucleotides as central nervous system modulators, Ann. NY Acad. Sci USA 603, 93.