ELSEVIER

European Journal of Pharmacology 420 (2001) R3–R4

ejp

www.elsevier.nl/locate/ejphar

Rapid communication

Accelerated functional recovery after neuronal injury by P2 receptor blockade

Ute Krügel *, Holger Kittner, Heike Franke, Peter Illes

Rudolf-Boehm-Institute of Pharmacology and Toxicology, University of Leipzig, Härtelstrasse 16-18, D-04107 Leipzig, Germany

Received 12 April 2001; accepted 18 April 2001

Abstract

The effect of the P2 receptor antagonist pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS) on changes of the quantitative electroencephalogram (EEG) after injury of rat brain tissue was investigated. PPADS accelerated the functional recovery from microdialysis probe-induced disturbances in the nucleus accumbens by a decrease of the ratio of absolute slow (0.6–4 Hz) to fast (8–30 Hz) power, mainly caused by a decrease in the delta frequency power. These data provide evidence for a possible neuroprotective effect of P2 receptor antagonists in vivo. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Neuroprotection; P2 receptor; PPADS

Previous studies suggest that P2 receptors are involved in de- and regeneration mechanisms of brain tissue. Scattered evidence has shown that different cell types may undergo a necrotic and apoptotic cell death after sustained exposure to high concentrations of extracellular ATP released following injury (Gordon, 1986; Di Virgilio, 1998). The stimulation of neuronal P2 receptors may cause an influx of Ca^{2+} directly via P2X receptors (Nörenberg and Illes, 2000). Furthermore, there is evidence that the stimulation of P2 receptors in the nucleus accumbens increased the release of glutamate in vivo (Krügel et al., 2001). Under the conditions of injury-induced high ATP concentrations, both mechanisms may induce a cascade of potentially neurotoxic mechanisms contributing to degenerative processes.

Furthermore, it has been shown that an injury-induced astrogliosis in vivo is enhanced by P2 receptor agonists in the nucleus accumbens, whereas the P2 receptor antagonist pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS) diminishes the formation of the glial scare when given alone, and in addition, counteracts the effects of the respective agonists (Franke et al., 1999). Proliferation of astrocytes is thought to be a limiting factor in the neuronal regeneration following cellular damage (Abbracchio and Burnstock, 1998).

Therefore, the question arises whether the inhibition of P2 receptors by PPADS can at least partly diminish functional impairments in the brain in vivo. Disturbance of neuronal tissue inevitably occurs when a microdialysis probe is inserted into the brain. In the present study, microdialysis was used for a twofold purpose, firstly to model a damage of neuronal tissue and secondly to locally apply the P2 receptor antagonist PPADS.

Disturbances of brain function can be recognised by changes in the spectral pattern of the quantitative electroencephalogram (EEG). A shift from the activity in the higher frequency bands to generalised delta band activity occurs in correlation to the severity of the damage (for review, see Facco, 1999). In the present study, the functional changes in the neuronal activity were recorded by telemetric EEG (TSE, Bad Homburg, Germany). The obtained data were transformed into real-time by means of fast Fourier analysis and displayed as continuous spectra of power density (for details, see Kittner et al., 2000). EEG electrodes were fixed on the surface of microdialysis guides (CMA11, CMA, Solna, Sweden) and implanted together into the nucleus accumbens of male rats (WIST/Lei, body weight 250–300 g) at coordinates relative to bregma—A: 1.7 mm, L: 1.5, V: 6.2 mm (Krügel et al., 2001).

* Corresponding author. Tel.: +49-341-971-3007; fax: +49-341-972-4609.

E-mail address: krueu@medizin.uni-leipzig.de (U. Krügel).

0014-2999/01/$ - see front matter © 2001 Elsevier Science B.V. All rights reserved.
PII: S0014-2999(01)01001-9

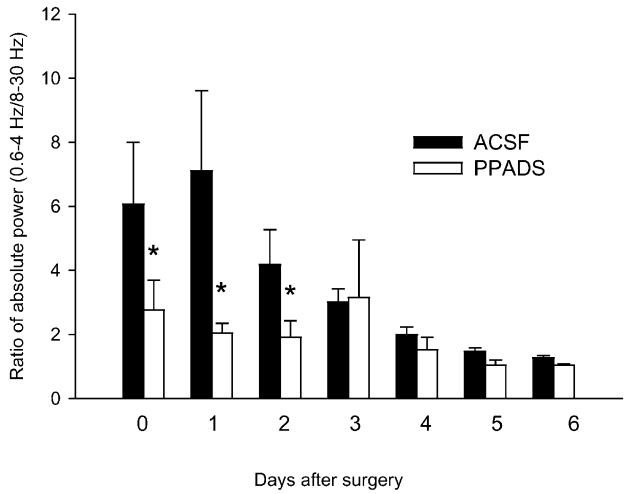

Fig. 1. Effect of PPADS on the time course of EEG changes caused by brain injury of rats. Artificial cerebrospinal fluid (ACSF) alone (open columns) or together with PPADS (50 μM, closed columns) were applied by reversed microdialysis immediately and on days 1 and 2 after guide implantation. The pattern of the quantitative EEG of freely moving animals was recorded telemetrically. Each column represents the mean ± S.E.M. of eight rats calculated from the ratios of the absolute power of the delta frequency band (0.6–4 Hz) versus the absolute power of the alpha and beta frequency bands (8–30 Hz) of each animal. $^{*}P < 0.05$; significant differences from ACSF-treated animals.

Immediately after surgery, microdialysis probes (CMA11, 2 mm) perfused either with artificial cerebrospinal fluid (ACSF; NaCl 126 mM, KCl 2.5 mM, NaH_2PO_4 1.2 mM, $MgCl_2$ 1.3 mM and $CaCl_2$ 2.4 mM, pH 7.4) or with ACSF containing PPADS (50 μM; Biotrend, Köln, Germany) ($n = 8$ each) were inserted into the nucleus accumbens for 30 min. The microdialysis was repeated on days 1 and 2 after surgery. The pattern of the quantitative EEG of the freely moving animals was recorded until day 6 after surgery once a day for 15 min each. Whereas, on the day of surgery (day 0) the EEG was performed 4 h after the first microdialysis, on the 2 following days (days 1 and 2) the EEG recording preceded the microdialysis procedure.

The treatment with PPADS in the injury model reduced the absolute power of the delta band (0.6–4 Hz) in the first 48 h after surgery ($P < 0.05$, data not shown). The estimation of the ratio of the absolute power in the delta frequency band to the absolute power in alpha and beta frequency bands (0.6–4 and 8–30 Hz) for each animal revealed that the treatment with PPADS accelerates the regression of changes in the EEG power spectra compared to the ACSF-treated animals ($F_{1,14} = 6.54$; $P = 0.039$; analysed by two-way analysis of variance (ANOVA) with repeated measures followed by the Student–Newman–Keuls test) (Fig. 1).

The EEG monitoring provided a real-time image of neuronal tissue function after injury. The present data indicate that the P2 receptor antagonist PPADS facilitates the recovery of EEG power spectra deteriorated by the insertion of the microdialysis probe. It remains to investigate whether the accelerated recovery of the EEG pattern causes enduring benefit for the brain after injury.

In summary, the blockade of P2 receptors and subsequently, the inhibition of the neurotoxic consequences of injury-induced high extracellular ATP provide a potential novel neuroprotective mechanism.

Acknowledgements

The authors gratefully acknowledge the excellent technical assistance of Mrs. K. Krause, Mrs. M. Noack and Mr. K. Weigand. We are grateful to Dr. A. Reichenbach for helpful discussions. This work was supported by the Deutsche Forschungsgemeinschaft (KI 677/2-2).

References

Abbracchio, M.P., Burnstock, G., 1998. Purinergic signalling: pathophysiological roles. Jpn. J. Pharmacol., 78, 113–145.

Di Virgilio, F., 1998. ATP as a death factor. BioFactors, 8, 301–303.

Facco, E., 1999. The role of EEG in brain injury. Intensive Care Med., 25, 872–877.

Franke, H., Krügel, U., Illes, P., 1999. P2 receptor-mediated proliferative effects on astrocytes in vivo. Glia, 28, 190–200.

Gordon, L., 1986. Extracellular ATP: effects, sources and fate. Biochem. J., 33, 309–319.

Kittner, H., Krügel, U., Hoffmann, E., Illes, P., 2000. Effects of intra-accumbens injection of 2-methylthio ATP: a combined open field and electroencephalographic study in rats. Psychopharmacology, 150, 123–131.

Krügel, U., Kittner, H., Illes, P., 2001. Mechanisms of adenosine 5′-triphosphate-induced dopamine release in the nucleus accumbens in vivo. Synapse, 39, 222–232.

Nörenberg, W., Illes, P., 2000. Neuronal P2X receptors: localisation and functional properties. Naunyn-Schmiedeberg's Arch. Pharmacol., 362, 324–339.