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Experimental studies and theoretical aspects on A2A/D2 receptor interactions in a model of Parkinson's disease. Relevance for L-dopa induced dyskinesias

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Abstract

Dual probe microdialysis was used to study A2A/D2 receptor interactions in the striato-pallidal GABA pathway in a model of Parkinson's Disease. The A2A agonist CGS21680 and/or the D2-like agonist quinpirole were perfused via reverse microdialysis into the DA denervated striatum and the effects on globus pallidus (GP) extracellular GABA levels were evaluated. CGS21680 alone produced in the DA denervated striatum a transient rise of GP GABA levels. Quinpirole perfused alone into the DA denervated striatum reduced GP GABA levels, which was not only counteracted by coperfused CGS21680, but led to an enhancement of the GABA levels, which was larger than that seen with CGS21680 alone. These results may reflect existence not only of antagonistic A2A/D2 interactions but also of the appearance of D2/A2A interactions increasing the A2A signaling at the level of the adenylate cyclase. Such actions diminish the therapeutic efficacy of L-dopa and D2 agonists. L-dopa induced dyskinesias could be caused by changes in the balance of A2A/D2 heteromers vs A2A homomers expressed at the surface membrane, where A2A homomers dominate with abnormal increases in A2A signaling. This may lead to stabilization of abnormal receptor mosaics (high order hetero-oligomers) leading to formation of abnormal motor programs contributing to dyskinesia development.

Keywords: Microdialysis; A2A/D2 receptor interactions; DA denervated striatum; Pallidal GABA level; Parkinson's Disease

1. Introduction

In 1993 evidence was presented [1] that the D2 like agonist quinpirol after intrastriatal perfusion reduced the extracellular GABA levels in the globus pallidus in the rat using dual probe microdialysis in the awake rat. This D2 like action was counteracted by co-perfusion with an A2A agonist CGS 21680. CGS 21680 alone in increasing concentrations had no effect on extracellular GABA levels in the globus pallidus. These results suggested the in vivo existence of antagonistic A2A/D2 interactions in the striato-pallidal GABA pathways mediating motor inhibition [1–3]. It has therefore become of interest to study in vivo if these antagonistic A2A/D2 interactions are altered in the striato-pallidal GABA neurons after DA denervation. Previously it has been shown that studies in striatal membrane preparations indicate that the potency of CGS 21680 to reduce the affinity of the DA agonist binding sites is increased in striatal membrane preparations from the DA denervated striatum [4].

We have employed a well known hemiparkinsonian model in the rat involving unilateral 6-OH-DA induced lesions of the nigrostriatal DA pathways [5]. The experiments were carried out in the lesioned and sham operated, awake rat using a dual probe microdialysis technique with measurements of extracellular GABA levels in the dorsal striatum and the ipsilateral globus pallidus.

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2. Methods

2.1. Animals

Male adult Sprague-Dawley rats were housed in cages in groups of five animals at a constant room temperature (20 °C) and exposed to a 12:12 h light–dark cycle (lights on at 06.00 a.m.). Food and water were provided ad libitum. Following delivery, the animals were allowed to adapt to the environment for at least 1 week before the experiment started.

2.2. 6-OH-DA lesion

Male adult Sprague-Dawley rats weighing 150 g were used. 8 μg of 6-0H-DA (dissolved in 4 μl of saline containing 0.2% of ascorbic acid) was injected into the substantia nigra at a rate of 1 μl/min (stereotaxic coordinates from bregma: AP = − 4.4 mm, L = − 1.2 mm, V = − 7.8 mm below dura). Sham operated animals were unilaterally injected in the same way with the same saline/ascorbic acid solution.

2.3. Selection of 6-OH-DA lesioned rat

The rotational model [6] was employed to select the rats which had been successfully lesioned. 2 weeks after the 6-OH-DA or saline injection, rats were primed with a 50 μg/Kg s.c. dose of apomorphine and subsequently treated twice a week with a test dose of apomorphine (25 μg/Kg s.c.). Only animals showing turning behaviors >400 rotations/40 min were chosen for the experiments. This behavior has been correlated to a 95% depletion of dopamine in the striatum [7].

2.4. Microdialysis surgery

The sham operated and the lesioned animals, weighing 250–300 g, were kept under halothane anaesthesia (1.5% mixture of halothane and air) and mounted in a David Kopf stereotaxic frame with the upper incisor bar set at − 2.5 mm below the interaural line. Two microdialysis probes of concentric design (2 mm dialysing membrane length) were implanted. One probe was implanted into the lesioned or sham operated striatum (St) and the other into the ipsilateral globus pallidus (GP). The coordinates relative to the bregma were: St: AP: + 0.3; L: ± 3.1; V: − 8.5; GP: AP: − 1.3; L: ± 3.3; V: − 8.0.

2.5. Experimental protocol

On the day of the release experiment, the probes were perfused with Ringer's solution (in mM: Na^+ 147; K^+ 4; Ca^{++} 1.4; Cl^- 156; glucose 2.7) at a constant flow rate (2 μl/min) by using a microinfusion pump. Perfusates were collected every 20 min and to achieve stable dialysate GABA levels collection of samples started 300 min after the onset of perfusion. After three stable basal values were obtained, the probe was perfused with an isotonic Ringer solution containing quinpirole and/or CGS21680 when required. This medium was then replaced with the original one and a further three samples were collected. To assess basal GABA levels, the mean of the first three samples was calculated.

The St probe was employed for local perfusion with quinpirole and/or CGS21680. The GP probe was employed for the recording of extracellular GABA levels. At the end of each experiment, the brain was removed from the skull and the location of the probe was carefully verified in 30-μm-thick coronal cryostat sections. Only those animals in which the probe was correctly located were included in this study.

The experimental procedures in vivo were approved by local Ethic Committee and by Italian Ministero della Sanita' (licence n° 111/94).

2.6. GABA analysis

Endogenous GABA levels were quantified using high performance liquid chromatography (HPLC)/electrochemical detection system, including precolumn derivatization with an *o*-phtaldialdehyde/*t*-butylthiol reagent and a re-

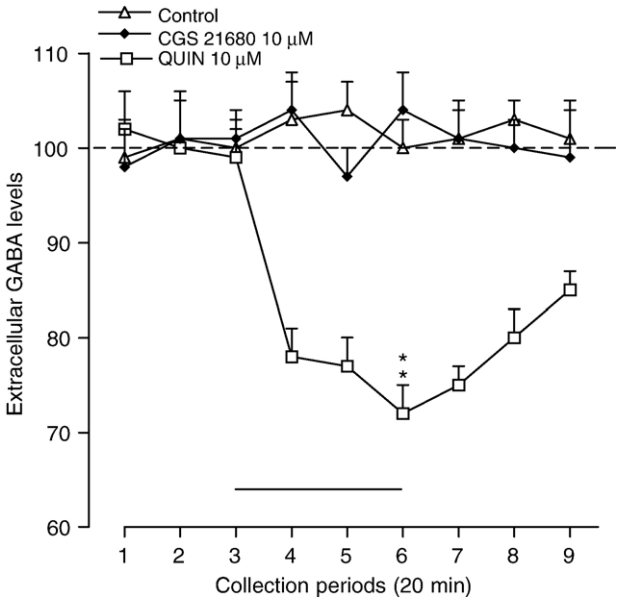

Fig. 1. Effects of intrastriatal perfusion with quinpirole (10 μM) and CGS 21680 (10 μM) alone on pallidal extracellular GABA levels in sham operated rat. The solid bar indicates the period of perfusion (60 min). The results are expressed as percentage of the mean of the three basal values before treatment and the significance with regard to the peak effects (maximal responses) is shown. Basal pallidal GABA levels were 9.03 ± 0.93 nM (n = 11). Each point represents the mean ± SEM of 5–6 animals. Control rats were perfused with normal Ringer perfusion medium throughout the experiment. **P < 0.01 significantly different from control or CGS 21680 according to one-way ANOVA followed by the Newman–Keuls test for multiple comparisons.