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Effect of intracerebral hydroxytyrosol and its nitroderivatives on striatal dopamine metabolism: A study by in vivo microdialysis

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ABSTRACT

Aims: The natural phenolic oil compound hydroxytyrosol (HTy) is widely studied because of its antioxidant and neuroprotective properties. Nitroderivatives of HTy have been studied in order to evaluate their putative effects on catechol-*O*-methyltransferase (COMT) activity.
Main methods: To study its effect on dopamine metabolism, nitrohydroxytyrosol and its lipophilic derivatives (nitrohydroxytyrosyl acetate and ethyl nitrohydroxytyrosyl ether), were administered into the rat corpus striatum through a microdialysis probe. Other catechols (HTy and the known COMT inhibitor Ro 41-0960) were also studied for comparison.
Key findings: The olive oil phenolic compounds (nitroderivatives and HTy) increased extracellular levels of 3,4-dihydroxyphenylacetic acid during the perfusion with similar maximum values to that of Ro 41-0960 when comparing to basal dialysate levels (approximately 140%). None of the compound series produced a decrease in the homovanillic acid extracellular levels below 75%. Among all novel compounds studied, both lipophilic nitrocatechols (nitrohydroxytyrosyl acetate and ethyl nitrohydroxytyrosyl ether) showed a long-acting effect over time once the perfusion through the microdialysis probe ended.
Significance: In accordance with the actual design of novel COMT inhibitors with a long profile, our results suggest a certain influence of the side chain substituent on the COMT activity that could provide new lipophilic COMT inhibitors.

1. Introduction

Parkinson's disease is characterized by the progressive and selective loss of dopaminergic neurons of the *substantia nigra pars compacta* and their terminals in the striatum [28], resulting in a deficit of dopamine (DA) content in the central nervous system [14,22]. DA does not cross the blood–brain barrier and, thus, parkinsonian patients are treated with the DA precursor, L-Dopa. Moreover, brain DA levels can increase as a result of catechol-*O*-methyltransferase (COMT) inhibitor activity [14,17]. The most commonly known second-generation COMT inhibitors are tolcapone [4] and entacapone [12], which contain a nitrocatecholic pharmacophore and, thus, belong to the nitroderivatives of catechol (NDCs) group. Nevertheless, many concerns have been raised regarding the clinical use of tolcapone and entacapone due to hepatotoxicity and short in vivo half-life, respectively [12,25]. Hence, the discovery and development of new nitrocatecholic COMT inhibitors with safer and long-acting profiles have been the goal for the last years [2,18,19].

In the group of NDCs, many phytochemical molecules have been reported to exert some interesting biological activities lately. Among olive oil components, hydroxytyrosol (HTy) has featured in a number of review articles relating to its biochemical and pharmacological roles [9,16,31]. In accordance with this, the capacity of HTy to scavenge radical species [13] and to induce antioxidant enzymes [24] has been widely described. These properties, considered as key markers in the protection against oxidative damage, are closely related to some neurodegenerative disorders, i.e., Parkinson's disease [7].

Nowadays, all efforts in the treatment of Parkinson's disease have been directed towards the development of novel drugs with single molecular entities that offer neuroprotection by having various CNS targets [33]. In accordance with this and considering the antioxidant properties of the new HTy nitroderivatives [30], these compounds have been hypothesized as a new class of lipophilic and potential COMT inhibitors.

Bearing this in mind, the aim of the present study was to evaluate the potential COMT inhibition capacity of the HTy nitroderivatives nitrohydroxytyrosol (NO_2HTy), nitrohydroxytyrosyl acetate (NO_2HTy-A) and ethyl nitrohydroxytyrosyl ether (NO_2HTy-E) (Fig. 1). For this reason, we have measured the striatum extracellular concentration of DA metabolites, such as 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), as well as 5-hydroxyindolacetic acid (5-HIAA) as a control metabolite, by in vivo microdialysis. This technique allows monitoring of local drug and metabolite concentrations at specific sites in the brain under physiological and pathological conditions [1]. We

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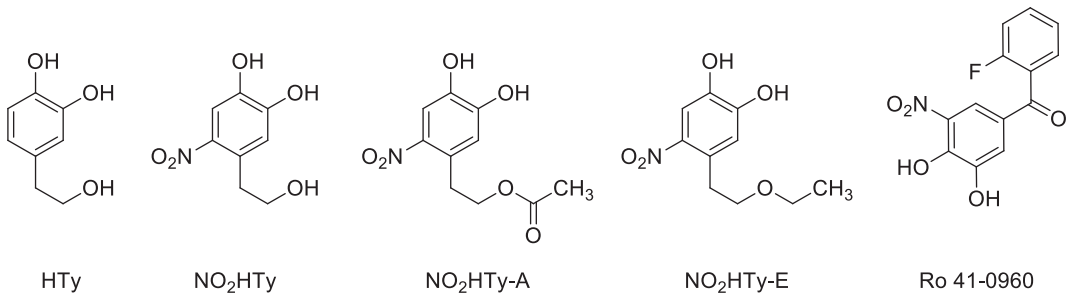

Fig. 1. Chemical structure of hydroxytyrosol (HTy), nitrohydroxytyrosol (NO_2HTy), nitrohydroxytyrosyl acetate (NO_2HTy-A), ethyl nitrohydroxytyrosyl ether (NO_2HTy-E) and Ro 41-0960.

have also included the natural precursor, HTy, and a commercial inhibitor, Ro 41-0960, in our study in order to compare the results with a catecholic compound and a known COMT inhibitor, respectively.

2. Materials and methods

2.1. Animals and drug treatment

Animals used in this study were male albino Wistar rats weighing 270–320 g at the time of probe implantation. The rats were kept, three or four rats per cage, at constant room temperature (22 ± 2 °C), 60% relative humidity with a 12-h light–dark cycle and food and water ad libitum. Experiments were carried out in accordance with the Guidelines of the European Union Council (2010/63/EU), following the Spanish regulations (BOE 34/11370-421, 2013) for the use of laboratory animals and approved by the Scientific Committee of the University of Sevilla.

The following drugs were used: 2′-fluoro-3,4-dihydroxy-5-nitrobenzophenone (Ro 41-0960, Sigma Chemical Co., MO, USA). HTy was extracted and purified from olive oil wastewaters as previously described Fernández-Bolaños et al. [8]. NO_2HTy and NO_2HTy-A were obtained in our lab according to the synthetic procedures described by Trujillo et al. [30]. NO_2HTy-E was obtained in a similar way from ethyl hydroxytyrosyl ether [23]. All drugs were dissolved in Ringer's solution.

2.2. Surgery and brain dialysis

Animals were anesthetized with isoflurane (minimum of 1.5 alveolar concentrations) and mounted in a stereotaxic apparatus (David Kopf Instruments) with the nose positioned 3.3 mm below the horizontal bar. Following a midline incision, the skull was exposed and 2 burr holes were drilled, through which 2 probes were implanted in both corpus striata with coordinates based on the bregma and dura (A/P +0.6, L/M ± 2.5, V/D −6.0; [26]). Following surgery, the animals were housed individually in plastic cages (35 × 35 × 40 cm) and allowed to recover overnight, with food and water ad libitum.

Microdialysis in the corpus striatum was performed with an I-shaped cannula [27]. The exposed tip of the dialysis membrane was 4 mm. The dialysis tube (ID: 0.22 mm; OD: 0.31 mm) was prepared from polyacrylonitrile/sodium methallyl sulfonate copolymer (AN 69, Hospal, Barcelona, Spain).

Microdialysis and subsequent chemical analyses were performed using an automated on-line sample injection system [32]. The corpus striatum was perfused at a flow rate of 3.0 μL/min, using a microperfusion pump (model 22, Harvard Apparatus, South Natick, MA, USA), with a Ringer's solution containing (in mM): NaCl, 140; KCl, 4.0; $CaCl_2$, 1.2; and $MgCl_2$, 1.0. With the help of an electronic timer, the injection valve was held in the load position for 15 min, during which the sample loop (40 μL) was filled with dialysate. The valve then switched automatically to the injection position for 15 s. This procedure was repeated every 15 min — the time needed to record a complete chromatogram. After establishing a steady baseline level in four consecutive samples, drugs were perfused for 1 h and sampling was continued for 2.5 h thereafter.

2.3. Chemical assays

3,4-Dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindolacetic acid (5-HIAA) levels in dialysates were analyzed by HPLC with electrochemical detection. A Merck L-6200A intelligent pump was used in conjunction with a glassy carbon electrode set at 550 mV (DECADE II, ANTEC, The Netherlands). A Merck Lichrocart cartridge (125 mm × 4 mm) column filled with Lichrospher reverse-phase C18 5 μM material was used. The mobile phase consisted of a mixture of 0.05 M of sodium acetate, 0.4 mM of 1-octanesulfonic acid, 0.3 mM of Na_2EDTA and 70 mL methanol/L, adjusted to pH 4.1 with acetic acid. All reactive agents and water were of HPLC grade. The flow rate was 1.0 mL/min.

Concentrations of striatal DOPAC, HVA and 5-HIAA samples were calculated with the aid of the eDAQ PowerChrom 280 software.

2.4. Statistics

The last four stable samples, which showed less than 10% variation, before drug treatment were considered as the basal values, which were expressed as percentage of controls. The difference between dialysate concentration of basal values and drug treatments was compared by One-Way ANOVA and, where appropriate (P value greater than the 95% confidence level), followed by LSD for post hoc multiple range comparisons. The same statistical method was used when comparing different drug concentrations at the same collection time. A P value <0.05 was considered statistically significant. The Statgraphics Centurion XVI statistical package was used for the analyses.

3. Results

3.1. Basal values

Average basal values of DOPAC, HVA and 5-HIAA did not differ between the various experimental groups. Basal extracellular outputs, before drug perfusion, were as follows (in fmol/min): DOPAC, 1892.45 ± 176.23; HVA, 1302.03 ± 122.58; and 5-HIAA, 1297.61 ± 95.72 (mean ± SEM, N = 23).

3.2. Hydroxytyrosol

The natural precursor, HTy, showed a significant increase in DOPAC extracellular output (approximately 140.0%) between 75 and 135 min perfusion, and thereafter returned to basal values (Fig. 2). However, HVA showed a significant decrease (approximately 80.0%) from 105 min to 180 min (Fig. 2), with a delay of 30 min with respect to the effect of HTy on DOPAC extracellular levels. 5-HIAA analysis did not show any statistical difference with respect to basal extracellular levels (Fig. 2).