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In vivo striatal measurement of hydroxytyrosol, and its metabolite (homovanillic alcohol), compared with its derivative nitrohydroxytyrosol

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HIGHLIGHTS

- In vivo measurement of polyphenols by HPLC with electrochemical detection.
- Hydroxytyrosol produces high levels of homovanillic alcohol in the brain.
- Hydroxytyrosol crosses the blood brain barrier better than nitrohydroxytyrosol.

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ABSTRACT

Phenolic compounds were measured by in vivo brain microdialysis in rat striatum. Basal extracellular levels of hydroxytyrosol, homovanillic alcohol and nitro-hydroxytyrosol were not detectable by HPLC with electrochemical detection. However, systemic administration of hydroxytyrosol (20 and 40 mg/kg, i.p.) showed a clear increase in the extracellular level of this compound. This increase was accompanied by an increase in the extracellular level of homovanillic alcohol, a metabolite of hydroxytyrosol formed by catechol-O-methyltransferase activity. Perfusion of hydroxytyrosol (20 μM) through the microdialysis cannula also produced an increase in the extracellular level of homovanillic alcohol. Systemic administration of nitro-hydroxytyrosol (20 and 40 mg/kg, i.p.) produced a small increase in the extracellular level of this compound. Our data show that hydroxytyrosol is a more brain penetrant phenolic compound than nitro-hydroxytyrosol. Accordingly, there is high cerebral metabolism of hydroxytyrosol to produce homovanillic alcohol by catechol-O-methyltransferase activity, that is saturated at the higher administered dose of hydroxytyrosol.

1. Introduction

Adherence to the so-called Mediterranean diet [1] is believed to provide protection against neurodegenerative disorders [2]. Olive oil is the main source of fat in the Mediterranean diet. It is rich in oleic acid, but also contains a wide range of 'minor constituents', like polyphenols that contribute to the stability of the oil and exhibit potent antioxidant properties [3,4]. Moreover, the beneficial effects of virgin olive oil consumption have been attributed in part to the presence of phenolic compounds [5]. Owing to the properties of olive oil phenolic compounds, research should be directed not only towards the characterization and study of the bioactivity, but also towards revealing the bioavailability in order to gain new insight into their in vivo physiological behaviour. Therefore, the determination of virgin olive oil phenolic metabolites in brain samples is of relevance in order to understand how they cross the blood brain barrier and how they are metabolized. Hydroxytyrosol (HTy) that is presented as a secoiridoid derivative and to a lower extent as glucosylate and acetate derivatives is the most active phenolic compound in the olive oil [6].

In the present study we have used an in vivo microdialysis technique, which allows drug molecule sampling without depriving the subject of biological fluids, and subsequently involves minimal disturbance of physiological functions [7,8]. For brain drug pharmacokinetic studies, intracerebral microdialysis is the only

Abbreviations: HTy, hydroxytyrosol; HVAL, homovanillic alcohol; NO-HTy, nitro-hydroxytyrosol; COMT, catechol-O-methyl transferase; Ip, intraperitoneal.

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technique offering the possibility to continuously monitor the local blood–brain barrier transport of unbound drugs in tested animals under physiological and pathological conditions [9]. We have measured striatal extracellular concentration of HTy, its metabolite homovanillic alcohol (HVAL), and nitrohydroxytyrosol (NO-HTy) after peripheral administration of HTy and NO-HTy.

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 to four rats per cage, at constant room temperature ($22 \pm 2\,^{\circ}C$), 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. The study was approved by the Scientific Committee of the University of Sevilla.

The following drugs were used: 2-(3,4-dihydroxyphenyl)-ethanol (HTy), NO-HTy and HVAL. HTy was extracted and purified from olive oil wastewaters as previously described [10], whereas NO-HTy was prepared according to Napolitano et al. [11]. HVAL was purchased from Aldrich (Madrid, Spain).

2.2. Surgery and brain dialysis

Animals were anesthetized with isofluorane (1.5 minimum 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 bregma and dura (A/P +0.6, L/M ±2.5, V/D −6.0) [12]. Following surgery, animals were housed individually in plastic cages (35 cm × 35 cm × 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 [13]. 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 methalyl sulfonate copolymer (AN 69, Hospal, Barcelona, Spain). The in vitro recovery of the membrane ($N = 4$) was: 8.6 ± 0.22%, for HTy; 7.7 ± 0.89%, for NO-HTy; and 4.9 ± 0.44%, for HVAL.

The perfusion experiments were carried out at 24 h and at 48 h after implantation of the probe. Microdialysis and subsequent chemical analysis were performed using an automated on-line sample injection system [14]. 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, U.S.A.), 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 (in nM), drugs were systemically administered and sampling was continued for 2.5 h. HTy and NO-HTy were dissolved in saline.

2.3. Chemical assays

HTy, NO-HTy and HVAL 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, Netherlands). A Merck Lichrocart cartridge (125 mm × 4 mm) column filled with Lichrospher reverse-phase C_{18} 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 HPLC grade. The flow rate was 1.0 ml/min.

2.4. Mathematical methods and Statistics

The trapezoidal rule was used to calculate the area under the curve (AUC) of dialysate concentrations vs time, for HTy, HVAL and NO-HTy. For statistical comparisons, AUC values were normalized by the administered dose. The unpaired Student's *t*-test was used when comparing different drug concentrations at the same collection time and normalized AUC (nM min/mg) values.

3. Results and discussion

In the present study we were able to measure polyphenols by HPLC with electrochemical detection in the brain. It is to our knowledge the first cerebral measurement of polyphenols reported with HPLC, coupled to an electrochemical detector, in conscious freely moving rats. This possibility to measure HTy, its metabolite HVAL, and NO-HTy is depicted in Fig. 1A. These compounds were not detected in basal dialysate samples as shown in Fig. 1B. However, when HTy was systemically administered a peak of HTy and HVAL appeared in the chromatogram (Fig. 1C). A similar chromatogram was found when NO-HTy was systemically administered and measured by microdialysis (Fig. 1D).

The systemic administration of HTy produced a strong increase in the striatal level of the same compound (Fig. 2A). The increase, however, was not linear with the administered dose. Thus, the normalized AUC values showed statistically significant differences (20 mg/kg: 232.4 ± 12.8, $N = 4$; and 40 mg/kg: 356.3 ± 34.1, $N = 4$), suggesting that catechol-O-methyltransferase (COMT), the enzyme that metabolize HTy, is saturated at the administered dosis of this phenolic compound. The striatal concentration of HTy decreased very rapidly to undetectable levels after systemic administration (Fig. 2A). Similarly, systemic administration of NO-HTy produced an increase in the striatal level of this compound, with a similar shape to that of HTy, but one order of magnitude lower (Fig. 2B). This increase was linear with the administered dose. Thus, the normalized AUC values did not show any statistically significant differences (20 mg/kg: 22.6 ± 3.7, $N = 4$ and 40 mg/kg: 17.6 ± 4.8, $N = 5$).

Taking into account that we intraperitoneally administered the same quantity of HTy and NO-HTy; these data could suggest that the quantity of NO-HTy that reaches the brain is lower than that of HTy. After intraperitoneal administration, HTy and NO-HTy shortly reached the brain. This is in agreement with Orozco-Solano et al. [15], who reported that the maximum concentration of olive oil phenols in plasma is generally reached within 30–60 min after oral intake of virgin olive oil. Furthermore, we detected levels of the parent compounds, HTy and NO-HTy, after intraperitoneal administration suggesting an acceptable penetrability in the brain; although it has been reported that extensive first-pass metabolism occurs in the small intestine after uptake of polyphenols [16]. Nevertheless, phenolic derivatives from olive oil have been demonstrated to be slowly metabolized by intestinal cells in vitro. Evidence supports that over 80% of the precursor HTy reaching the bloodstream remains as non-metabolized compound [17]. Moreover, it has been demonstrated that NO-HTy is also