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Determination of the dopamine agonist rotigotine in microdialysates from the rat brain by microbore column liquid chromatography with electrochemical detection

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Abstract

Rotigotine, an investigational dopamine agonist formulated as a patch, is being studied in Parkinson's disease. A microdialysis technique, in combination with microbore column liquid chromatography and electrochemical detection, was developed to monitor rotigotine levels in the brain. Microdialysis probes were inserted into the striata of anesthetized rats, and samples were collected during perfusion with Ringer's solution. Rotigotine was separated using a C18 reversed-phase column. The mobile phase consisted of 50 mM $Na_2HPO_4 \cdot 2H_2O$, 2.5 mM sodium octyl sulfonate, and pH 4.5; 35% volume to volume acetonitrile. The flow rate was 30 μl/min, and the potential of the glassy carbon electrode was set to +850 mV. The method allowed monitoring of the time course of brain extracellular rotigotine levels with a detection limit of 1 nM following either intravenous (0.5 mg/kg) or subcutaneous (5.0 mg/kg) rotigotine injection.

Keywords: Rotigotine; Microdialysis; Electrochemical detection; Liquid chromatography

1. Introduction

Rotigotine is a new, non-ergolinic dopamine receptor agonist currently in development for the treatment of Parkinson's disease and restless leg syndrome. From a pharmacokinetic point of view, characterizing rotigotine levels in the brain is of interest.

With in vivo *microdialysis*, the extracellular concentrations of neurotransmitters, neuromodulators, and other endogenous molecules [1], as well as exogenously administered compounds, can be determined in the extracellular space of the brain or other organs following their systemic administration [2]. With the microdialysis probe inserted into the area of interest in the brain and with suitable analytical techniques, the extracellular dynamics of small molecules in the extracellular space can be monitored without the need of complex purification procedures, thus allowing evaluation of pharmacokinetic/pharmacodynamic profiles of drugs more or less continuously [3]. So far, rotigotine and its pharmacokinetics have never been determined in animal or human tissues using this approach. Considering the potential use of rotigotine as an antiparkinsonian drug, the development of a suitable analytical methodology that would allow for its determination in the brain extracellular space was of interest.

The molecular structure of rotigotine, (−)-5,6,7,8-tetrahydro-6-[propyl-[2-(2-thienyl) ethyl] amino]-1-naphthalenol hydrochloride (Fig. 1), includes a tetrahydronaphthalenol moiety. This structural characteristic suggests that rotigotine can undergo anodic oxidation in a 3-electrode electrochemical cell [4]. This reaction would afford electrochemical detection of the drug in combination with high-performance liquid chromatography (LCEC). Earlier, several studies described LCEC methods for separating 5-hydroxytetralins from biological samples such as plasma and urine [4,5] or the use of HPLC with UV detection [6]. Halogenated or substituted phenols can be measured using electrochemical and fluorescence detection [7] or using fluorescence detection following postcolumn derivatization, such as with Ce^{3+} ions [8]. Spectrophotometric detection modes such

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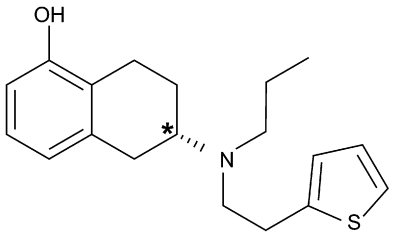

Fig. 1. Rotigotine formula.

as photodiode-array detection generally do not provide enough sensitivity for quantitative analysis of trace levels of rotigotine in biological samples [6]. Liquid chromatography and mass spectroscopy (LC/MS) technology represents a possible alternative for highly specific detection of rotigotine in biological tissues; however, the high concentrations of inorganic salts (physiological perfusion media) can interfere with its sensitivity, and therefore LC/MS may be a poor choice for use with microdialysate samples.

The objectives of the present study were to define and optimize the chromatographic conditions for determination of rotigotine by LCEC that would allow continuous measurement of the drug in the extracellular space of the rat striatum following its systemic administration at clinically relevant doses.

2. Experimental

2.1. Animals and drug administration

Male Sprague-Dawley rats (B&K Universal, Sollentuna, Sweden) weighing between 200 and 350 g were used for the experiment. The rats were fed lab chow (Ewos R36; Ewos, Sweden) and water ad libitum. Animals were housed in pairs at an ambient temperature of 20 °C with a 12 h light-dark cycle and were approximately 7–8 weeks in age at the time of experiment.

Rotigotine was obtained from Schwarz Biosciences (Monheim, Germany; Batch number WE11471), stored at −20 °C as a 10^{-2} M stock solution in deionized water, and thawed immediately prior to final dilution and administration. Two treatment groups received rotigotine, either infused intravenously ($n = 5$; 0.5 mg/kg; 150 μl/min) into the femoral vein or injected subcutaneously ($n = 3$; 5 mg/kg; 1 ml/min) into the scruff of the neck at a concentration of 0.5 mg/l ml. All animal experiments were approved by the local ethical committee (Stockholm Norra Forsoksdjurs Etiska Kommittee) following the directives of the "Principles of Laboratory Animal Care" (NIH publication No. 85-23) and the Council of the European Communities (86/809/EEC).

2.2. Surgery and brain microdialysis

Microdialysis experiments were carried out on anesthetized rats using the protocol developed by Kehr [9]. Briefly, the rats were anesthetized with enflurane (3% N_2O:O_2 [1:1] at 0.8 l/min) and placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA) using a flat skull position with incisor bar set to −3.2 mm. The body temperature of the animal was controlled by a rectal thermometer and maintained at 37 °C, using a CMA/105 temperature controller (CMA/Microdialysis, Stockholm, Sweden). An incision was made in the middle of the scalp, approximately 2–3 cm long, and the skin flaps were kept aside using the homeostatic forceps. After exposing the skull, a hole was made using a fine trephine drill for insertion of a microdialysis probe. The CMA/12 microdialysis probe (4 mm membrane length; 20,000 Da) was implanted into the right striatum at the coordinates AP + 1.2 mm; L −3.0 mm; V −4.2 mm (from bregma and the dural surface) according to the atlas of Paxinos and Watson, 1997 [10]. The dialysis probe was perfused with Ringer's solution (NaCl 147 mM, KCl 4 mM, $CaCl_2$ 2.3 mM) at a flow rate of 1.0 μl/min, using a CMA/100 microinjection pump. The dialysates were collected every 20 min with a CMA/170 refrigerated fraction collector. The fractions corresponding to the first 60 min after the probe insertion were discarded; thereafter, two microdialysis samples (blank) were collected prior to drug administration, and sampling continued for 240 min after the drug was administered. Thus, a total of 15 samples were collected, each containing 20 μl. After finalizing the experiment, the rats were sacrificed by carbon dioxide, followed by dislocation of the neck.

2.3. Chromatographic conditions

Rotigotine (Fig. 1) was detected by microbore column liquid chromatography with electrochemical detection. The chromatographic apparatus consisted of a LC100 micropump (ALS, Inc., Tokyo, Japan), a CMA/260 degasser (CMA/Microdialysis, Stockholm, Sweden), and a CMA/200 refrigerated microsampler (CMA/Microdialysis). An electrochemical detector LC4B (Bioanalytical Systems, West Lafayette, IN, USA) was equipped with a radial flow cell (ALS, Inc., Tokyo, Japan). At optimal conditions, the potential of a glassy carbon electrode (6 mm in diameter) was set to +850 mV, versus the Ag/AgCl/3 M NaCl reference electrode. Data were collected using an EZChrom data acquisition system (Scientific Software, Pleasanton, CA, USA). A microbore column (Luna C18 silica; 3 μm particle size; 150 mm × 1 mm I.D.; Phenomenex, Torrance, CA, USA) was maintained at room temperature (22–25 °C). The mobile phase was pumped at a flow rate of 30 μl/min.

Liquid chromatography calibration standards were prepared daily by diluting stock solutions in Ringer's solution to concentrations ranging between 1 and 100 nM. Standard solutions of dopamine, noradrenaline, and serotonin, and their major metabolites (3,4-dihydroxyphenylacetic acid [DOPAC]; homovanillic acid [HVA]; and 5-hydroxyindoleacetic acid [5-HIAA]) were assessed for rotigotine interference LCEC studies. Molecular standards were prepared as 10 mM stock solutions in deionized water and kept frozen at −20 °C. Fifteen microliters of standard solution or microdialysis sample was injected into the chromatographic column. All chemicals and solvents were of analytical or HPLC purity grade and purchased from Sigma-RBI (St. Louis, MO, USA), Merck (Darmstadt, Germany), or Fluka Chemie (Buchs, Switzerland).