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Gas-phase structural characterization of neuropeptides Y Y1 receptor antagonists using mass spectrometry: Orbitrap vs triple quadrupole

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ABSTRACT

Collision induced dissociation of triple quadrupole mass spectrometer (CID-QqQ) and high-energy collision dissociation (HCD) of Orbitrap were compared for four neuropeptides Y Y1 (NPY Y1) receptor antagonists and showed similar qualitative fragmentation and structural information. Orbitrap high resolution and high mass accuracy HCD fragmentation spectra allowed unambiguous identification of product ions in the range 0.04–4.25 ppm. Orbitrap mass spectrometry showed abundant analyte-specific product ions also observed on CID-QqQ. These results show the suitability of these product ions for use in quantitative analysis by MRM mode. In addition, it was found that all compounds could be determined at levels >1 $\mu g\,L^{-1}$ using the QqQ instrument and that the detection limits for this analyzer ranged from 0.02 to 0.6 $\mu g\,L^{-1}$. Overall, the results obtained from experiments acquired in QqQ show a good agreement with those acquired from the Orbitrap instrument allowing the use of this relatively inexpensive technique (QqQ) for accurate quantification of these compounds in clinical and academic applications.

1. Introduction

Neuropeptide Y (NPY) is a naturally occurring hormone that is expressed throughout the body, including the brain. It is one of the most abundant neuropeptides found in central nervous system and as a neurotransmitter or neuromodulator activates different NPY receptors in several brain regions [1,2]. Although NPY can produce a variety of biological effects this peptide has attracted widespread attention because of its pronounced cardiovascular effects [3–5], its association with neurodegenerative diseases [6–8], as well as its potential role in the regulation of feeding behavior [9–11] and energy homeostasis [12–14].

The rising prevalence of obesity, diabetes and their associated co-morbidities generated, in the last decade, an increasing need to find effective and safe therapies to treat these patients. All of the five different NPY receptors subtypes (Y1, Y2, Y4, Y5, and Y6) identified so far were implicated in the regulation of food intake and energy homeostasis. Nevertheless, there is growing pharmacological evidence reported in the literature that antagonists of the Y1 and Y5 receptors reduce food intake and body weight in a variety of animal models of obesity [15]. This fact prompted the development and the *in vivo* and *in vitro* studies of several structurally different families of Y_1 antagonists. BIBP 3226 (Fig. 1) was the first non-peptidic agent reported to be an inhibitor of NPY Y1 binding [16]. This antagonist is able to bind with the human Y1 receptor and exert its effect by mimicking the C-terminal region of the native ligand NPY [17].

Monitoring of new drugs or other chemical residues, namely metabolites, in *in vitro*, *in vivo* or clinical studies is often conducted using liquid chromatography coupled with mass spectrometry. These studies are frequently concerned only with monitoring ions that are related with high signal intensities totally dismissing structural interpretation.

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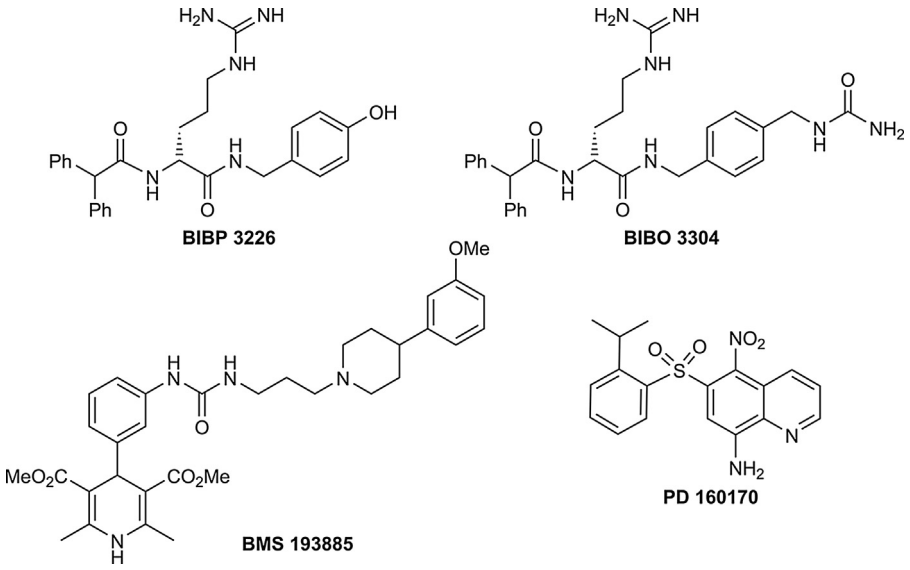

Fig. 1. Chemical structures of the studied NPY Y1 receptor antagonists.

The use of high resolution mass spectrometry for the generation of MS^n product ions allows, in most cases, the assignment of logical structures to the monitored ion transitions derived from correct elemental formula. When necessary, isotopically labelled compounds or hydrogen/deuterium exchange experiments can be carried out to assure the proposed structures. This is extremely important since it provides not only accurate quantification but also qualitative identification and confirmation, essential for the correct analysis of biological phenomena. One could argue that the use of high-purity compounds and standards would diminish the chances of incorrect selection of ions. Nevertheless, these are not always available and analyte degradation or gross errors can occur during the experiments and hence, the ion transitions detected must relate and be consistent with the chemical structure of the analyte under study to avoid incorrect assignments.

In the course of our research, the group came across the need to quantify BIBP 3226 in samples obtained from cellular internalization assays. During the development of the analytical method for quantification of this drug, it was recognized the lack of information concerning the structural characterization of the mass transitions to be used for quantification and identification of BIBP 3226. Also, no internal standard was available for use during the HPLC–MS/MS quantification experiments. To overcome these issues the use of BIBO 3304 (Fig. 1), another argininamide based NPY Y_1 receptor antagonist, as internal standard for the quantification of BIBP 3226 was considered. Likewise, no structural characterization of the mass transitions for BIBO 3304 was found in the literature. An additional literature review revealed that no gas-phase behavior study was ever performed for BMS 193885 and PD 160170 (Fig. 1), two other selective Y1 receptor antagonists with demonstrated anti-orexigenic properties [18–20].

Hence, in the present study, the gas-phase structural characterization of these drugs was performed using a high-mass-resolving ESI–MS and higher-energy collision dissociation-tandem mass spectrometry (HCD-MS/MS) conducted in Q-Exactive Orbitrap system and also a low-mass-resolving ESI–MS and collision-induced dissociation-tandem mass spectrometry (CID-MS/MS) conducted in a triple quadrupole (QqQ) system. The data obtained by these two commonly used mass detectors, namely the structural characterization of the most abundant product ions, are herein compared and discussed. The comparison of the data obtained for each of the studied compounds was also performed in terms of limits of detection (LOD) and quantification (LOQ) for the triple quadrupole instrument.

2. Experimental

2.1. Materials and reagents

BIBP 3226, BIBO 3304, BMS 193885 and PD 160170 (Fig. 1) were purchased from Tocris (Bristol, UK). Acetonitrile (LiChrosolv LC–MS grade) and formic acid were acquired from Merck (Darmstadt, Germany). Water from Arium water purification system (resistivity >18 MΩ cm, Sartorius, Goettingen, Germany) was used for the preparation of all solutions. BIBP 3226, BIBO 3304 and BMS 193885 stock solutions were prepared in water at 1 mg mL^{-1} whereas PD 160170 was dissolved in a water:acetonitrile mixture (40:60, *v/v*) at a concentration of 0.4 mg mL^{-1}. All stock solutions were stored at −20 °C.

2.2. Instrumentation and conditions

High-mass-resolving ESI–MS and HCD-MS/MS were conducted in a Q-Exactive® hybrid quadrupole Orbitrap® mass spectrometer (Thermo Fisher Scientific, Bremen, Germany).

The instrument was operated in positive mode, with a spray voltage at 3.0 kV and interfaced with a HESI II ion source. Samples of the selected antagonists were diluted from stock solutions using MeOH with 1% (*v/v*) formic acid to a final concentration of 0.1 μg mL^{-1} for BIBP 3226, BIBO 3304 and BMS 193885, and 0.04 μg mL^{-1} for PD 160170. The analyses were performed through direct infusion of the prepared solutions at a flow rate of 10 μL min^{-1} into the ESI source, and the operating conditions were as follows: sheath gas (nitrogen) flow rate 5 (arbitrary units); auxiliary gas (nitrogen) 1 (arbitrary units); capillary temperature 320 °C, and S-lens rf level 50.

The acquisition method was set with a full scan and resolution was set to 140,000, the *m/z* ranges were set to 50–750 in the normal mass range during full-scan experiments. The automatic gain control (AGC) target was set at 5×10^6 and the maximum injection time (IT) was 250 ms. The Q-Exactive system was tuned and calibrated using peaks of known mass from a calibration solution (Thermo Sci-