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Terlipressin, a vasoactive prodrug recommended in hepatorenal syndrome, is an agonist of human V1, V2 and V1B receptors: Implications for its safety profile

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ABSTRACT

Terlipressin is recommended as a gold standard to treat hepatorenal syndrome complicating liver cirrhosis. It is presented as a specific V1A receptor agonist, beyond its enzymatic conversion into lysine[8]-Vasopressin (LVP), able to counteract the splanchnic vasodilation. However, the complete pharmacological characterization of this drug with respect to the different vasopressin receptor subtypes is missing. We studied terlipressin intrinsic properties, focusing not only on V1A, but also on other vasopressin receptor subtypes. The experimental studies were conducted on rat and human cellular models. Binding experiments were performed on rat liver membranes and CHO cells transfected with the different human vasopressin receptor subtypes. Agonist status was assessed from inositol phosphate or cyclic AMP assays, and measurement of intracellular calcium variations, performed on cultured vascular smooth muscle cells from rat aorta and human uterine artery and CHO cells. Terlipressin binds to the rat and human V1A receptors with an affinity in the micromolar range, a value 120 fold lower than that of LVP. It induces a rapid and transient intracellular calcium increase, a robust stimulation of phospholipase C but with reduced maximal efficiencies as compared to LVP, indicating a partial V1A agonist property. In addition, terlipressin is also a full agonist of human V2 and V1B receptors, with also a micromomolar affinity.

Conclusions: Terlipressin is a non-selective vasopressin analogue, exhibiting intrinsic agonist properties. Its full V2 receptor agonism may result in renal effects potentially aggravating water retention and hyponatremia of cirrhosis.

1. Introduction

Terlipressin is currently the treatment of reference for patients with hepatorenal syndrome (HRS) complicating liver cirrhosis, especially for HRS type 1 a particular form with acute functional renal failure due to splanchnic and systemic vasodilatation [1,2]. Terlipressin is a synthetic peptide composed of one molecule of [Lysine[8]]Vasopressin (LVP) and three additional glycine residues associated to its N-terminal end. It is metabolized *via* exopeptidases into LVP, the natural vasopressin porcine hormone. Terlipressin is presented as an inactive pro-drug able to release sustained amounts of LVP, the active form providing prolonged biological effect [3,4]. The vasoactive properties of LVP decreases splanchnic blood flow in order to prevent oesophageal varices haemorrhage or the hepatorenal syndrome complicating liver cirrhosis [5–8]. In this specific indication, terlipressin may improve renal perfusion and may pro-

Abbreviations: AVP, [arginine[8]]vasopressin; LVP, [lysine[8]]vasopressin; HRS, hepatorenal syndrome; VSMCs, vascular smooth muscle cells; hV1A-R, hV1B-R and hV2-R, human V1A V1B and V2 vasopressin receptor; BSA, bovine serum albumin; PLC, phospholipase C; IPs, total inositol lipids; AC, adenylate cyclase; ATP, adenosyl triphospshate; cAMP, cyclic adenosyl monophosphate; $[Ca^{2+}]_I$, cytosolic Ca^{2+}; K_i, affinity constant of peptide; K_{act}, concentration of peptide leading to half maximal stimulation; E_{max}, peptide maximal efficiency.

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long survival. It is considered as a standard of care in Europe [9]. Recent studies suggest that its beneficial effect may be extended in case of HRS associated with sepsis [10].

The terlipressin vasopressor effects are thought to result from the slow enzymatic conversion into LVP and a potentiation of adrenergic vasoconstriction [11]. However, some studies suggest that terlipressin exhibit intrinsic vasoconstrictive properties [12]. In a previous report [13], we have shown that terlipressin triggers a strong vasocontriction of both rat and human arteries *via* the activation of V1A receptor. This effect develops quickly, suggesting that terlipressin, besides its pro-LVP status, could also be considered as a direct vasoconstrictor drug *per se*. Terlipressin is presented as a V1A receptor agonist [14], but it is known that vasopressin analogues and especially LVP could interact with other receptor subtypes (V1B and V2). If a direct effect of terlipressin on arteries has been evidenced, the complete pharmacological properties of terlipressin for the different human vasopressin receptor subtypes have never been established so far.

The aim of this work was to characterize the full intrinsic pharmacological properties of terlipressin for all the human vasopressin receptor subtypes. Improved knowledge of these pharmacological properties is necessary to understand complete mode of action of the drug and to unveil possible therapeutic limitations.

2. Materials and methods

2.1. Animals and human tissues

Male Wistar rats (225–250 g) were provided by Janvier (Le Genest-St-Isle, France). Experiments were conducted in accordance with national law and approved by the committee on animal care of Montpellier-Languedoc-Roussillon (No. CEEA-LR-12075).

Human uterine arteries were obtained from patients undergoing hysterectomy. The procedures were approved by national ethic Committee and the French Ministry of Research (DC-2008-488) and patients gave informed consent.

2.2. Smooth muscle cell isolation and culture

Vascular smooth muscle cells (VSMCs) were isolated from rat aorta and from human uterine artery. The descending thoracic aorta was collected from six week-old male Wistar rats anesthetized with intra-peritoneal pentobarbital (1.5 ml/kg) and uterine arteries were excised from the parametrium, connective tissues and adjacent myometrium immediately after surgery. Arteries were aseptically immersed in isolation isotonic buffer and media layers were dissected. Cells were enzymatically isolated and grown in primary culture as previously described [15]. Cells were used during 5 passages.

2.3. Analysis of terlipressin purity

Purity of terlipressin was evaluated by mass spectrometry as previously described [16] using an UltraFlex TOF/TOF mass spectrometer (Bruker-Daltonik, Bremen, Germany) The sensibility of the MALDI-TOF spectrometer used in the study was about 1 femtomole.

2.4. Binding experiments

The affinity of terlipressin was determined by competition binding experiments using [^{3}H]AVP as radioligand on two biological models: rat liver membranes known to naturally express high level of V1A vasopressin receptor isoform and membranes from CHO cells stably transfected with the human V1A, V1 B and V2 receptor (hV1A-R, hV1B-R, and hV2-R, respectively) as previously described [17]. Note that our experimental conditions included 0.1% Bovine Serum Albumin (BSA) and Leupeptin (0.1 mg/ml) preventing noticeable terlipressin conversion into LVP.

2.5. Second messenger measurements

The ability of terlipressin to stimulate Phospholipase C (hV1A-R, hV1B-R) or Adenylate Cyclase activities (hV2-R) was tested on rat and human cultured VSMCs naturally expressing V1A-R and on CHO cell transfected and stably expressing hV1A-R or hV2-R.

Phospholipase C (PLC) activity was assessed as described previously [17] by labelling cellular Phosphoinositol lipids during preincubation with myo-[2-^{3}H] inositol, then measuring total inositol phosphates (IPs) generated under hormonal stimulation.

Adenylate cyclase (AC) activity was assessed as described previously [18] by labelling intracellular Adenosyl Triphosphate (ATP) by pre incubation with [^{3}H] Adenine, then measuring cyclic Adenosyl Monophosphate (cAMP) generated under hormonal stimulation.

2.6. Intracellular calcium variation measurements

Changes in cytosolic Ca^{2+} ($[Ca^{2+}]_i$) were measured using the ratiometric fluorescent Ca^{2+} indicator Fura-2 as previously described [19]. Results were normalized to the effect of 100 nM LVP. The changes in $[Ca^{2+}]_i$ were deduced from the variations of the fluorescence ratio after correction for background and dark currents. Data were averaged (at least 20–25 cells per recorded field/coverglass). One field, chosen randomly, was recorded in each coverglass, n represented the number of cell cultures.

2.7. Chemicals

Commercial terlipressin was obtained from Ferring A. B. (Limhamn, Sweden), Arginine^{8}Vasopressin (AVP) and LVP from Bachem (Bubendorf, Switzerland) and SR49059 from Sanofi-Aventis (Sanofi-Aventis, Toulouse, France). Most standard chemicals were purchased from Sigma (St Louis, MO), Roche Molecular Biochemicals (Mannheim, Germany) or Merck and Co (Darmstadt, Germany) unless otherwise indicated. [^{3}H]AVP (60–80 Ci/mmol), myo- [2- ^{3}H]inositol (10–20 Ci/mmol) were obtained from Perkin-Elmer Life Sciences (Courtaboeuf, France).

2.8. Data analysis

The radioligand binding data were analyzed by GraphPad Prism (GraphPad Software, Inc., San Diego, CA) as previously described [18]. Results are expressed as mean ± SEM. Statistic analysis consisted in ANOVA and Student's *t*-test, and p value < 0.05 considered as statistically significant.

3. Results

3.1. Terlipressin purity

The mass spectrum obtained for 0.1 pmole terlipressin showed a major peak at a molecular mass of 1227 that corresponded to the theoretical mass of terlipressin and of two minor peaks at molecular mass corresponding respectively to terlipressin associated with one Na^+ cation and one K^+ cation (1250 and 1256 respectively) (Fig. 1 panel A). Analysis of this spectrum also revealed the presence of other peaks of very low amplitude and lower molecular mass. According to their molecular weight, these peaks corresponded to shorter forms of terlipressin respectively deprived of 1–3 glycine residues: desGly1-terlipressin, desGly2-terlipressin and desGly3-terlipressin representing LVP (Fig. 1 panel B).