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Zonation of the metabolic action of vasopressin in the bivascularly perfused rat liver

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Abstract

Predominance of the vasopressin binding capacity in the hepatic perivenous area leads to the hypothesis that the metabolic effects of the hormone should also be more pronounced in this area. Until now this question has been approached solely by experiments with isolated hepatocytes where an apparent absence of metabolic zonation was found. We have reexamined this question using the bivascularly perfused liver. In this system periportal cells can be reached in a selective manner with substrates and effectors via the hepatic artery when retrograde perfusion (hepatic vein → portal vein) is done. The action of vasopressin (1 – 10 nM) on glycogenolysis, initial calcium efflux, glycolysis and oxygen uptake were measured. The results revealed that the action of vasopressin in the liver is heterogeneously distributed. Glycogenolysis stimulation and initial calcium efflux were predominant in the perivenous area, irrespective of the vasopressin concentration. Oxygen uptake was stimulated in the perivenous area; in the periportal area it ranged from inhibition at low vasopressin concentrations to stimulation at high ones. Lactate production was generally greater in the perivenous zone, whereas the opposite occurred with pyruvate production. Analysis of these and other results suggests that at least three factors are contributing to the heterogenic response of the liver parenchyma to vasopressin: a) receptor density, which tends to favour the perivenous zone; b) cell-to-cell interactions, which tend to favour situations where the perivenous zone is amply supplied with vasopressin; and c) the different response capacities of perivenous and periportal cells.
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1. Introduction

Vasopressin (arginine–vasopressin) affects liver metabolism in several ways. It increases glycogen breakdown [1,2], stimulates oxygen uptake [3] and increases the flux through the tricarboxylic acid cycle [4]. As a general hypertensive agent it also increases resistance of the hepatic artery [5] but is without effect on portal resistance [6]. These actions in the liver are all directly or indirectly mediated by Ca^{2+} in the cytosol, whose concentration is increased under the influence of the hormone [7,8]. This increase takes the form of concentration spikes (oscillations) at subsaturating levels of vasopressin. The increased Ca^{2+} concentrations can also propagate intra- and intercellularly, creating apparent intercellular waves. Such coordinated and sequential signals elicited by vasopressin in the intact perfused liver originate waves of Ca^{2+} concentration increases running along the hepatocyte plates across the lobules, at a dose-dependent speed of 20–120 μm s^{-1} [9]. These waves propagate towards only one direction and coupling between adjacent cells is believed to be provided by gap junctions [10,11]. Most experimental data indicate that the starting area of the vasopressin-induced Ca^{2+} concentration waves in the liver lobule is the perivenous zone [12,13]. In one study waves initiating in the periportal region have been detected at low vasopressin concentrations (<1 nM, [14]). At these low concentrations, however, other investigators failed to detect coordinated Ca^{2+} waves [13]. The direction perivenous to

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periportal is opposite to the normal blood flow direction. It has been proposed that such retrograde waves are caused by the higher density of vasopressin receptors ($V1_a$) in the perivenous area of the liver [11]. In this respect, ratios of vasopressin binding capacity of isolated perivenous to periportal cells between 1.4 and 1.8 have been reported [11,15]. Consequently, there is also more vasopressin-induced IP_3 in perivenous than in periportal cells [11].

Predominance of the vasopressin binding capacity in the perivenous area leads to the hypothesis that the metabolic effects of the hormone should also be more pronounced in this area. Until now this question has been approached solely by experiments with isolated hepatocytes in which an apparent absence of metabolic zonation was found as far as vasopressin-mediated glycogenolysis and glycogenolytic potency is concerned [16]. In view of the heterogeneous distribution of vasopressin receptors in the liver, however, this is a surprising result, which deserves reanalysis by means of an experimental system that preserves cell-to-cell interactions and microcirculation. A suitable experimental system for this purpose is the bivascularly perfused rat liver in the antegrade and retrograde modes. When the rat liver is perfused bivascularly in the retrograde mode (hepatic vein → portal vein) periportal cells can be reached in a selective manner with substrates and effectors via the hepatic artery. This occurs because in the rat liver, the confluence of the ramifications of the hepatic artery and of the portal vein occur in presinusoidal as well as in intrasinusoidal regions [17–19]. This experimental system has already been used for studying zonation of several phenomena including, for example, drug metabolism [17], gluconeogenesis [20], alanine metabolism [21], the purinergic action of ATP [22] and the metabolic actions of glucagon [23,24] and ethanol [25]. Taking thus advantage of these characteristics of the rat liver, in the present work, zonation of the metabolic action of vasopressin was investigated by measuring initial calcium release, glycogenolysis, glycolysis and oxygen uptake.

2. Materials and methods

2.1. Materials

The liver perfusion apparatus was built in the workshops of the University of Maringá. Radioactive calcium ([^{45}Ca]$CaCl_2$) was purchased from NEN™ Life Sciences Products, Inc., (Boston, USA). Vasopressin, enzymes and coenzymes used in the assay procedures were purchased from Sigma Chemical Co. (St. Louis, USA). All other chemicals were from the best available grade.

2.2. Liver perfusion

Male albino rats (Wistar), weighing 190–220 g, were fed ad libitum with a standard laboratory diet (Purina®). For the surgical procedure, the rats were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg).

Hemoglobin-free, non-recirculating bivascular liver perfusion was done either in the antegrade mode (entry via the portal vein plus hepatic artery and exit via the hepatic vein) or in the retrograde mode (entry via the hepatic vein plus hepatic artery and exit via the portal vein). In situ perfusion was carried out, the flow perfusate being provided by two peristaltic pumps. The perfusion fluid was Krebs/Henseleit-bicarbonate buffer (pH 7.4) containing 25 mg% bovine-serum albumin, saturated with a mixture of oxygen and carbon dioxide (95 : 5) by means of a membrane oxygenator with simultaneous temperature adjustment (37 °C). The composition of the Krebs/Henseleit-bicarbonate buffer is the following: 115 mM NaCl, 25 mM $NaHCO_3$, 5.8 mM KCl, 1.2 mM Na_2SO_4, 1.18 mM $MgCl_2$, 1.2 mM NaH_2PO_4 and 1.3 mM $CaCl_2$.

The surgical procedure described by Suzuki-Kemmelmeier et al. [26] was adopted. The final flow through both entry vessels was adjusted to definitive values, i.e., 28–32 ml/min for the portal vein and 2–3 ml/min for the hepatic artery. All perfusion experiments were initiated in the antegrade mode. Retrograde perfusion was established by changing the direction of flow at 15–20 min before initiating sampling of the effluent perfusate.

For measuring Ca^{2+} efflux, the cellular stores were labelled with $^{45}Ca^{2+}$ [27]. At approximately 20 min after the surgical procedure, [^{45}Ca]$CaCl_2$ was added to 50 ml of the perfusion fluid (0.25 μCi/ml) and the system was switched to a recirculating one with a dual entry and single exit. In antegrade perfusion entry was composed by portal vein + hepatic artery and exit was the hepatic vein; in retrograde perfusion entry was composed by hepatic vein + hepatic artery and exit was the portal vein. Recirculation was continued for 40 min. After this time the perfusion fluid was switched to Ca^{2+}-free Krebs/Henseleit-bicarbonate buffer containing 1 mM ethylene diamine tetraacetic acid (EDTA) and perfusion was continued in an open system in the desired mode (antegrade or retrograde). Samples for the measurement of the outflowing radioactivity were collected and vasopressin was infused in the desired vessel.

2.3. Perfusion pressure

The perfusion pressures of the portal and arterial beds were monitored simultaneously by means of pressure transducers (Hugo Sachs Elektronic-Harvard Apparatus GmbH, March-Hugstetten, Germany). Two sensors were positioned near to the entry of the portal vein and hepatic artery and the transducers were connected to a two-channel recorder. The pressure changes were computed from the recorder tracings and expressed as millimeters of mercury (mm Hg).

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