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BIOPHYSICS AND BIOCHEMISTRY

Role of Proglucagon Peptides in Osmoregulation

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Glucagon-like peptide-1 (GLP-1), a product of partial proteolysis of proglucagon, is involved not only in regulation of carbohydrates, but also in water-salt metabolism. The study examined the role of proglucagon derivatives GLP-1, GLP-2, and oxyntomodulin in rat osmoregulation. Of them, only blood plasma GLP-1 increased in response to water load (20 ml/kg). Administration of glucose (1.5 g/kg) elevated GLP-1 and oxyntomodulin but did not change the level of GLP-2. GLP-1 accelerated excretion of excess water during hyperhydration, whereas GLP-2 decreased this parameter. No physiological effects of oxyntomodulin in the kidneys were revealed. Probably, the blood levels of proglucagon derivatives are independently regulated for each peptide. In contrast to GLP-2 and oxyntomodulin, GLP-1 is involved in osmoregulation.

Key Words: *glucagon-like peptide-1; glucagon-like peptide-2; oxyntomodulin; kidney; osmoregulation*

The gastrointestinal hormone glucagon-like peptide-1 (GLP-1) is known for a broad profile of physiological activity including postprandial stimulation of insulin secretion, the cardio- and neuroprotection, as well as the control of gastric secretory and motor activity, appetite, β-cells proliferation, diuresis, sodium reabsorption in kidneys, *etc*. [3,12]. Previously, we demonstrated that GLP-1 is implicated in osmoregulation [9]: its blood level increases when water enters the gastrointestinal tract [9,10], whereas blockade of GLP-1 receptors induces antidiuresis [9]. Administration of GLP-1 receptor agonists to hyperhydrated animals accelerates excretion of solute-free water by the kidneys and restoration of the normal level of blood osmolality [6,10]. The mechanism of the hydrouretic action of GLP-1 is based on a decrease of sodium reabsorption in proximal nephron and on an increase in the influx of tubular fluid into the distal segments and collecting tubes [6,9]. No direct effect of GLP-1 on the osmotic permeability of collecting tubes was found whereas the effect of GLP-1 mimetic on water excretion depended on vasopressin level and prostaglandin secretion [6]. GLP-1 is synthesized with oxyntomodulin (OXM) and GLP-2 in enteroendocrine L-cells by partial proteolysis of proglucagon [3]. It is assumed that OXM is an agonist at GLP-1 receptors producing insulinotropic effect [8,11]. Unlike GLP-1 or OXM, GLP-2 does not stimulate insulin secretion, but affects its receptors and exerts a trophic effect in intestinal mucosa, slows down the motility of the stomach and intestines, and enhances glucose absorption [1,7]. Secretion of OXM, GLP-1, and GLP-2 is elevated by income of the nutrients into gastrointestinal tract [3,7]. It can be also expected that hyperhydration can provoke the changes in secretion of regulatory peptides encoded by the same gene as GLP-1 leading to modulation of renal function by these peptides.

The aim of this study was to evaluate the role of proglucagon derivatives GLP-1, GLP-2, and OXM in osmoregulation.

MATERIALS AND METHODS

Experiments were carried out on 3-5-moth-old female Wistar rats obtained from Experimental and Biologi-

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cal Clinics, I. M. Sechenov Institute of Evolutionary Physiology and Biochemistry in accordance with Russian and International Guidelines on the Use of Experimental Animals. The rats had water and standard pellet food *ad libitum*. On the evening before the experiment (at 17.00), food was taken away, but the rats had free access to water.

GLP-1, GLP-2, and OXM (Bachem) or vehicle (0.9% NaCl) were administered intraperitoneally (1 ml/kg) in a dose of 1.5 nmol/kg. To enhance and prolong the effects of peptides, they were injected 30 min after preliminary intraperitoneal administration of a dipeptidyl peptidase-4 inhibitor vildagliptin (Matrix Scientific) in a dose of 1 mg/kg. The effects of GLP-1, GLP-2, and OXM on renal osmoregulatory function were studied under the normal conditions and after water load (WL; administration of 20 ml/kg water via a gastric tube). In control experiments performed with and without vildagliptin, the peptides were not injected. The urine samples were collected within 2 h. Concentrations of proglucagon derivatives in blood plasma and a number of biochemical serum parameters were determined 5 min after WL and oral glucose administration (1.5 g/kg in 50% solution). The changes in serum osmolality were evaluated 5, 15, and 30 min after WL. The intact animals served as the control. The blood was drawn from cervical vessels and collected in clean K_3-EDTA containing tubes from the neck vessels under general anesthesia (Zoletil, 50 mg/kg intramuscularly, Virbac); thereupon the animals were decapitated. Blood was centrifuged for 15 min in MIKRO 22R (Hettich) to obtain serum (8000 rpm, 22-25°C) or plasma (2000 rpm, 4°C).

Osmolality was determined on a 3300 Micro-Osmometer (Advanced Instruments), sodium concentration on a Sherwood-420 photometer (Sherwood Scientific), and chloride, creatinine and glucose concentrations on an Erba XL-200 analyzer (Erba-Lachema), hormone concentration — on an ELx808 microplate reader (Bio-Tek) using ELISA kits: total GLP-1 (Millipore), GLP-2 (Yanaihara) and OXM (Ansh Labs).

The data were processed statistically with Microsoft Excel 2016. In case of distribution normalcy (established with Shapiro—Wilk test available in https://nsu.ru/mmf/tvims/arkashov/calc/Stat/Shapiro/Shapiro.html), they were summarized as $m \pm SEM$, otherwise they were presented as Me (Q1; Q3). To assess equality of variances in the groups, the Fisher's test was used. The groups were compared with Student's t test or Mann—Whitney U test at $p<0.05$.

RESULTS

Oral water intake decreased serum osmolality in 5 min (Fig. 1), thereby generating the main stimulus to

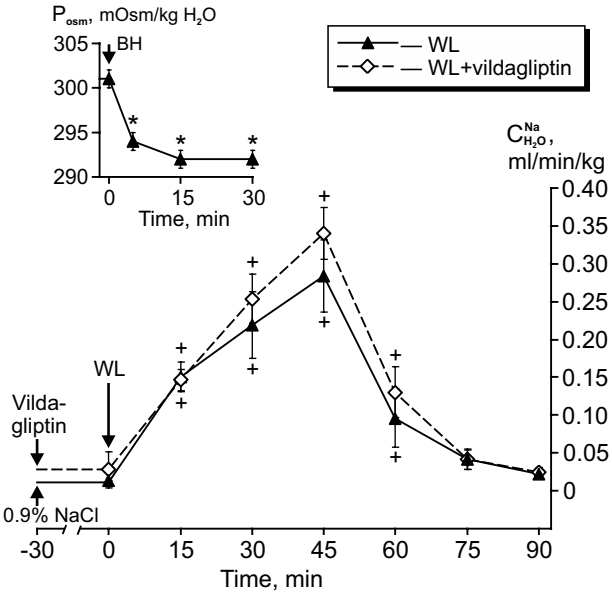

Fig. 1. The effect of WL in rats on blood serum osmolality (P_{Osm}) and clearance of sodium-free water (C^{Na}_{H2O}). Arrows show administration of vildagliptin (1 mg/kg) or its vehicle and WL (20 ml/kg). $^{*}p<0.001$ (t test), $^{+}p<0.05$ (Mann—Whitney U test) in comparison with the control.

down-regulate secretion of vasopressin and alter the processes of urine osmotic concentration. Therefore, this time point was chosen to analyze concentrations of proglucagon derivatives in response to hyperhydration. In reference group, oral administration of glucose solution was used as the standard stimulus for GLP-1 secretion. The glucose load increased osmolality of blood serum and elevated the concentrations of glucose, sodium ions, GLP-1 and OXM (Table 1). GLP-2 level did not change significantly. WL decreased the blood levels of sodium and chloride ions and elevated GLP-1 concentration, although it produced no effects on the levels of GLP-2 and OXM (Table 1). After hyperhydration, a 10-fold higher level of diuresis was observed in comparison with intact animals (Table 2).

To assess the changes in renal osmoregulatory function, we calculated the sodium-free water clearance remembering that sodium is the main osmotically active substance of the blood. This indicator reflects implication of the kidneys in effective osmoregulation aimed to maintain the cell volume [9]. Over an 1 h, WL increased the sodium-free water clearance (Fig. 1). Vildagliptin-induced inhibition of degradation of proglucagon-derived peptides 30 min before the loading test increased sodium clearance by 2.5 times (Table 2) while dynamics of sodium-free water clearance (Fig. 1) and other examined parameters of renal function (Table 2) did not change. Administration of GLP-1 under these conditions (vildagliptin+WL) accelerated excretion of excess water. Diuresis increased sharply, so half of introduced volume was excreted

TABLE 1. Plasma Concentrations of Glucagon Derivatives and Biochemical Parameters in Blood Serum 5 Min after Oral Administration of Water or Glucose Solution (*m*±*SEM*)

Parameter	Intact control	WL	Glucose load
Osmolality, mOsmol/kg H_2O	299±1	292±1**	302±1**
Sodium, mM	145.9±0.2	142.7±0.4**	147.2±0.3**
Chlorides, mM	102.8±0.5	98.8±0.4**	102.9±0.3
Glucose, mM	5.6±0.1	5.7±0.1	7.7±0.2**
GLP-1, pg/ml	197±14	359±36*	273±19**
GLP-2, pg/ml	520±8	500±13	484±12
OXM, pg/ml	161±25	153±18	309±34**

Note. *p<0.05, **p<0.01 in comparison with control (t test).

23 min earlier than in control (Table 2), and the peak of sodium-free water clearance was observed 30 min earlier than in control (Fig. 2). The total amount of sodium-free water excreted was the same as observed without GLP-1 (Table 2). OXM did not affect amount of access water (Table 2) and the rate of its excretion (Fig. 2). GLP-2 demonstrated an antidiuretic and antinatriuretic effects by significantly reducing sodium-free water clearance and sodium clearance (Table 2) and increasing 2-fold the excretion time of 50% load volume (Table 2). The examined peptides did not affect the glomerular filtration rate; in all groups, the creatinine clearance was 3.2±0.1 ml/min/kg.

Reabsorption of solute-free water (T^C_{H2O}) in the kidneys was assessed under the standard conditions of water-salt balance. At this, neither vildagliptin nor GLP-1, GLP-2, and OXM affected this parameter (Table 3). Vildagliptin increased sodium clearance 3-fold compared to animals not injected with dipeptidyl peptidase-4 inhibitor. The combined administration of GLP-2 and OXM with vildagliptin did not affect diuresis and sodium clearance (Table 3), while injection of GLP-1 led to a significant increase of diuresis and a 12-fold increase in sodium clearance (Table 3), while the glomerular filtration rate did not change.

TABLE 2. The Effect of GLP-1, GLP-2 and OXM in a Dose of 1.5 nmol/kg on Rat Renal Functions Against the Background of WL (20 ml/kg) of Administration of Vildagliptin (Me (Q1; Q3); m±*SEM*))

Parameter	0.9% NaCl+WL (*n*=10)	Vildagliptin, 1 mg/kg			
		WL (control; *n*=19)	WL+GLP-1 (*n*=10)	WL+GLP-2 (*n*=10)	WL+OXM (*n*=10)
Diuresis, ml/h/kg	11.6±1.0	14.2±0.8	24.8±1.1*	8.4±1.2*	14.0±1.0
$T_{50\%}$, min	45 (40; 51)	38 (32; 51)	15 (13; 17)*	81 (54; 100)*	38 (35; 42)
C_{Na}, ml/h/kg	0.4±0.1*	1.1±0.2	11.3±0.6*	0.4±0.1*	1.6±0.3
$C^{Na}_{H_2O}$, ml/h/kg	11.2±1.0	13.1±0.8	13.5±1.0	8.0±1.1*	12.5±0.9

Note. C_{Na} — sodium clearance, $C^{Na}_{H_2O}$ — clearance of sodium-free water. $T_{50\%}$ — excretion time of 50% WL (*i.e.* 10 ml/kg). *p<0.05 in comparison with control (Mann—Whitney U test).

TABLE 3. Effects of GLP-1, GLP-2 and OXM in a Dose of 1.5 nmol/kg on Renal Functions in Rats under Standard Conditions Against Background Administration of Vildagliptin (*m*±*SEM*; *n*=10)

Parameter	0.9% NaCl	Vildagliptin, 1 mg/kg			
		0.9% NaCl (control)	GLP-1	GLP-2	OXM
Diuresis, ml/h/kg	1.1±0.1	1.6±0.4	10.7±1.6*	0.9±0.1	1.3±0.3
$T^C_{H_2O}$, ml/h/kg	4.0±0.2	4.4±0.5	4.3±0.5	3.8±0.3	4.2±0.5
C_{Na}, ml/h/kg	0.3±0.1*	0.9±0.2	10.6±1.4*	0.7±0.1	1.0±0.2

Note. $T^C_{H_2O}$ — reabsorption of osmotically free water, C_{Na} — sodium clearance. *p<0.05 in comparison with control (Mann—Whitney U test).

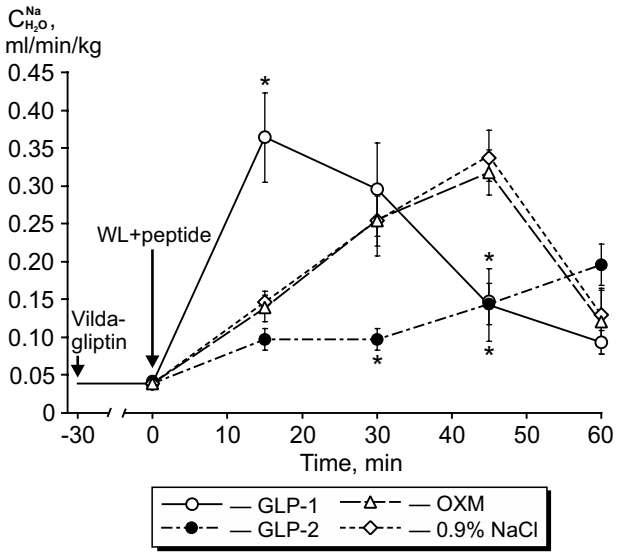

Fig. 2. The effect of GLP-1, GLP-2 and OXM on clearance of sodium-free water ($C^{Na}_{H_2O}$) in hyperhydrated rats. Arrows show administration of vildagliptin (1 mg/kg), WL (20 ml/kg), or peptides (1.5 nmol/kg). *p<0.05 in comparison with control (Mann—Whitney U test).

Many physiological processes are regulated by correcting the deviating parameters; in the system of water-salt balance such a parameter is a shift in blood osmolality. Specifically, if the blood osmolality deviates from stable normal value, secretion of vasopressin varies to tune the osmotic permeability of the collecting ducts. Therefore, a change in water reabsorption/excretion leads to normalization of blood osmolality. However, there is another mechanism that works in "anticipation" of the changes in blood osmolality. *In vivo*, the cause of a decrease in blood osmolality is drinking of excess water producing the signals from gastrointestinal tract, which can modulate the osmoregulatory function of the kidneys. An example of such signals is incretin GLP-1. The universality of regulatory effect of GLP-1 is based on the fact that the final result of its action (the magnitude and duration of the excretion of excess water) depends both on secretion and renotropic action of GLP-1 with deviation of the main adjustable parameter (osmolality) and the effects of antidiuretic hormone. Here, we demonstrated that GLP-2 and OXM are not implicated in osmoregulation. WL did not change the level of GLP-2 and OXM in the blood but increased the concentration of GLP-1, which attests to independent regulation of secretion of individual proglucagon-derived peptides. The changes in GLP-1, GLP-2, and OXM levels evoked by food intake were also not always synchronous [1,3,7]. Unlike GLP-1, injection of GLP-2 during hyperhydration did not facilitate but made it difficult to excrete an excess water. OXM also did not exert an effect similar to renotropic GLP-1 action. No effects were revealed either on sodium clearance or solute-free water reabsorption and sodium-free water clearance either under normal conditions or after WL. The effects of OXM can differ from those of GLP-1 due to the differences in activation of intracellular signal transmission pathways triggered by binding of GLP-1 to its receptor. Actually, OXM *in vitro* is a full agonist of not only at GLP-1 receptors, but also at those of glucagon [2]. In comparison with GLP-1, OXM binds to GLP-1 receptor with smaller affinity [2]; it demonstrates smaller preference for signal transduction via cAMP relative to phosphorylation of extracellular signal-regulated kinase (ERK1/2) [5] and acts as a partial agonist for activation of β-arrestin and G-protein-coupled receptor kinase 2 [4]. The results obtained indicate that *in vivo* OXM is not a physiologically significant agonist at renal GLP-1 receptors. Probably, it exerts its effect in proximal nephrons hosting GLP-1 receptors [12]; this effect is very weak and can be counterbalanced by the changes in ion reabsorption in more distal nephrons. Thus, the level of proglucagon derivatives in the blood is probably regulated independently for each peptide. In contrast to GLP-2 or OXM, GLP-1 is specifically involved in osmoregulation.

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