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Glucagon-Like Peptide-1 Is Involved in Sodium and Water Homeostasis in Humans

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Key Words
Glucagon-like peptide-1 · Natriuresis · Thirst regulation

Abstract
In previous studies with glucagon-like peptide-1 (GLP-1) we have observed that this peptide modulates fluid intake and increases renal sodium excretion in healthy volunteers and in patients with diabetes mellitus type 2. The effect of GLP-1 on thirst, water intake and on osmoregulation has, however, not been examined in detail in humans. ***Methods:*** Seventeen healthy male subjects were enrolled in two double-blind, placebo-controlled studies. In study part A, 8 volunteers participated in a protocol with an intravenous salt load of 26.7 ± 0.9 g comparing the effect of an infusion of GLP-1 (1.5 pmol/kg × min) to isotonic saline (placebo). Sodium excretion and water intake were measured. In part B, 9 volunteers were challenged with an oral salt load of 27.7 ± 0.5 g; sodium excretion and water intake were determined comparing an infusion of GLP-1 (1.5 pmol/kg × min) to isotonic saline (placebo). In part C, intestinal biopsies along the gastrointestinal tract were obtained from 14 healthy subjects. Expression of human GLP-1 receptor mRNA was measured by real-time polymerase chain reaction. ***Results:*** In study part A, an increase in renal sodium excretion was demonstrated: FeNa rose from 1.6 ± 0.3 (placebo) to 2.7 ± 0.2% (GLP-1; $p = 0.0005$). There was no difference in water consumption between the two treatments: 1,291 ± 69 (saline) vs. 1,228 ± 74 ml (GLP-1; $p = 0.49$). In part B, an oral salt challenge of 27.7 ± 0.5 g led to an increased renal excretion of sodium during GLP-1: FeNa increased from 1.6 ± 0.2% (placebo) to 2.0 ± 0.2% (GLP-1; $p = 0.012$). In contrast to part A, oral water intake was reduced by 36% under GLP-1 treatment: 1,848 ± 331 ml (placebo) vs. 1,181 ± 177 ml (GLP-1; $p = 0.0414$). Three subjects in part B did not finish treatment with GLP-1 because of diarrhea. Human GLP-1 receptor mRNA expression was highest in the proximal human small intestine compared to terminal ileum and colon ($p < 0.02$). ***Conclusions:*** GLP-1 acts on renal tissue reducing sodium absorption, probably via similar sodium transporters, which also may be localized in the gastrointestinal tract. This hypothesis needs to be confirmed by further studies.

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Introduction

The pro-glucagon-derived glucagon-like peptide-1(7–36) amide (GLP-1) is a gastrointestinal hormone that is released from the distal small intestine in response to

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food [1, 2]. Its physiological effects include a glucose-dependent insulinotropic action on the pancreatic β cells, inhibition of gastric acid secretion and inhibition of gastric emptying. The latter effects can be interpreted as being part of the 'ileal break mechanism', an endocrine feedback loop that becomes activated when nutrients are present in the ileum [2, 3]. It is well accepted that nutrients in the ileum not only inhibit upper gastrointestinal functions, but also affect appetite and food intake [4, 5]. GLP-1 is one of the mediators of this effect and has been proposed to play a physiological regulatory role in controlling appetite and energy intake in health and disease [6–8]. When studying the effect of GLP-1 on appetite, we observed a reduction in water intake with concomitant GLP-1 treatment, both in healthy subjects and in moderately obese patients with type-2 diabetes mellitus [7, 8]. Furthermore, preliminary data in rats suggest a role for GLP-1 in regulating water and salt homeostasis [9]. Finally, we have recently demonstrated that GLP-1 is involved in the regulation of water and salt homeostasis in healthy males and obese subjects [10].

The homeostasis of body fluid can be divided in two major factors: the effective circulating volume and plasma osmolality. Maintenance of the effective circulating volume is largely related to the regulation of sodium balance. In contrast, maintenance of osmolality is largely associated with the regulation of water balance with the plasma Na^+ concentration and arterial blood pressure acting as modulating factors. Sodium is the major extracellular cation and has a strong influence on plasma osmolality. Thus, a hypertonic solution of NaCl increases water intake [11]. An increase in plasma osmolality appears to be detected by cerebral osmoreceptors which are located in the vascular organ of the lamina terminalis (OVLT) [12]. Surgical destruction of the OVLT eliminates water drinking in response to increased osmolality [13]. In this model, osmoregulation is envisioned as a single-loop negative-feedback system in which dehydration increases osmolality and acts as a stimulus for thirst. As a consequence, water intake serves to decrease osmolality back to normal. However, the control system for osmoregulation is more complicated: a substantial delay occurs for ingested water to produce a significant decrease in osmolality, whereas thirst and water intake are terminated more rapidly [14]. Thus an early signal is sent to the brain in anticipation of subsequent rehydration. Finally, thirsty rats drink increased amounts of water when the pylorus is ligated [15]. These observations suggest the existence of an osmoreceptor system, located after the pylorus, that detects ingested water before it enters the general circulation. The small intestine, the hepatic portal vein, and the liver are all potential sites for such receptors.

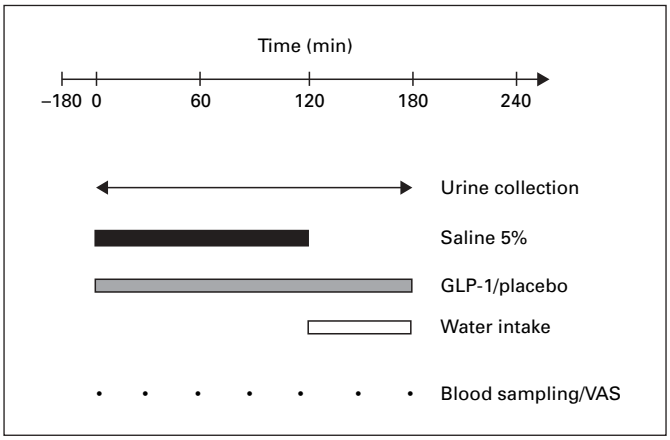

Fig. 1. Treatment flowsheet.

On the basis of this information, the present study was designed to investigate whether GLP-1 affects water intake and sodium excretion in humans both during an equal intravenous or an oral salt load, and second, whether GLP-1 could be a candidate in the postulated sodium regulation system in the gastrointestinal system distal to the pylorus.

Subjects and Methods

Eight healthy males (aged 25.5 ± 0.7 years) and nine healthy males (aged 26.4 ± 1.4 years) were recruited for study parts A and B. All subjects had a normal body mass index, normal blood pressure, and normal laboratory values. Also, 14 healthy subjects (7 males, 7 females, aged 59.8 ± 2.7 years) with no medication were included in part C of the study.

Each volunteer provided written informed consent. The Human Ethics Research Committee of the University Hospital of Basel approved the protocol. Before being enrolled in the study, participants were required to complete a medical interview, undergo a full physical examination, and participate in an initial laboratory screening. No subject was taking any medication or had a history of diabetes, hypertension or kidney disease.

Part A: Influence of Intravenous GLP-1 on Intravenous Sodium-Induced Thirst

For the purpose of the study, placebo and GLP-1 (1.5 pmol/kg/min) were infused in a random order, with infusions being separated by at least 7 days. All solutions were administered with a concomitant intravenous saline load (fig. 1).

On the day of each study, volunteers had fasted from midnight onwards before coming to the research unit 8 h later. The fasting state was assessed in the morning by an ultrasound examination

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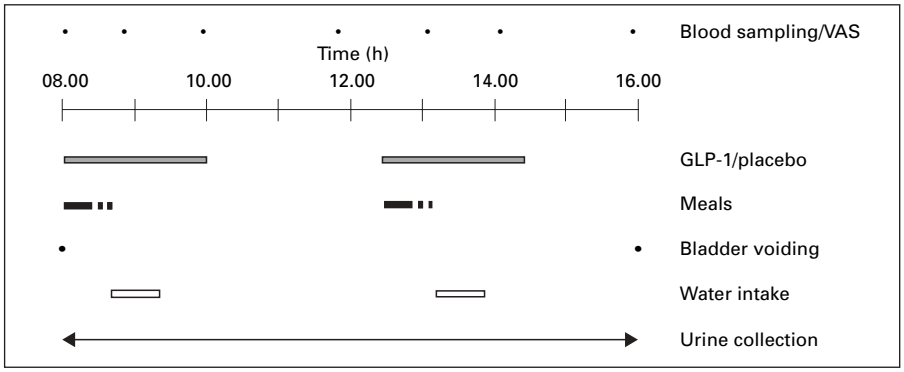

Fig. 2. Study part B.

of the gallbladder. At 8.00 a.m., volunteers voided their bladders, which was confirmed by ultrasound. Afterwards, subjects had to lie down for the duration of the experiment to avoid activation of the renin-angiotensin system. Two Teflon catheters were placed into each forearm: one for infusions, and the other for blood sampling. After the baseline blood sample had been drawn, an intravenous infusion of hypertonic saline (5% NaCl) was started at a rate of 0.06 ml/kg × min for 2 h. Simultaneously, a second infusion with 0.9% saline containing 0.5% albumin (placebo) or GLP-1, dissolved in 0.9% saline and 0.5% albumin, was started and continued for 3 h. The two solutions were indistinguishable in appearance, and were prepared by a pharmacist who was not directly involved in the study. The physician in charge was not aware of the respective treatment permitting a double-blind study design. During the first 2 h of each treatment, no fluid consumption was allowed; starting with the 3rd h, the volunteers were allowed to drink water ad libitum. Food intake was not permitted. At the end of each 180-min investigation period, water intake and the quantity of urine (ml) were measured; bladder emptying was confirmed by ultrasound. Through close observation of the participants, the attending physician assessed adverse effects.

Part B: Influence of GLP-1 on Thirst during Meals and Oral Salt Load

For the purpose of the study, placebo and GLP-1 (1.5 pmol/kg/min) were infused in a random order, with infusions being separated by at least 7 days. The protocol of this study part is depicted in figure 2. On the day of each study, volunteers fasted from midnight onwards before coming to the research unit 8 h later. The fasting state was assessed in the morning by an ultrasound examination of the gallbladder. At 8.00 a.m., volunteers voided their bladders, which was confirmed by ultrasound. One Teflon catheter was placed into each forearm: one for infusions, and the other for blood sampling.

After the baseline blood sample had been drawn, a standardized, salty breakfast was served to the volunteers, water intake was allowed as much as they wanted. At 12.30 p.m., a standardized salty lunch was served. Simultaneously, an infusion with 0.9% saline containing albumin 0.5% (placebo) or GLP-1 (1.5 pmol/kg/min), dissolved in 0.9% saline and 0.5% albumin, was started and continued for 2 h during the meals. The two solutions were indistinguishable in appearance, and were prepared by a pharmacist who was not directly involved in the study. The physician in charge was not aware of the respective treatment permitting the double-blind study design. After they had finished the meal, volunteers were allowed to drink water ad libitum, but food was no longer permitted. At the end of each 480-min period, water intake and the quantity of urine (ml) were measured; bladder emptying was confirmed by ultrasound.

Volunteers scored their subjective feelings of thirst on visual analogue scales at defined intervals throughout the experiments. Through close observation of the participants, the attending physician assessed adverse effects.

Part C: mRNA Activity of GLP-1 Receptor of Duodenum, Ileum, Colon

Intestinal biopsies were obtained from 14 healthy subjects. The indication for combined upper and lower gastrointestinal tract endoscopy was a cancer-screening program. Biopsies were obtained from the duodenum, the terminal ileum and from different defined regions of the colon. Four biopsies were taken from each anatomic site.

Material

Synthetic human GLP-1 was obtained from Bachem (Bubendorf, Switzerland). The peptide content was used in the calculation of the doses infused. The University of Basel Hospital Pharmacy prepared the infusions according to GMP criteria. The solutions were tested for sterility and pyrogenicity.

Glucose and Electrolyte Analyses

At study begin and at 30-min intervals, blood samples were drawn for glucose and sodium determinations (fig. 1). Sodium excretion, pH, potassium and creatinine were measured in the urine collected at the end of 180 min. The same determinations were performed for part B.

pH was measured immediately from urine samples using an autoanalyzer (ABL 700; Radiometer, Copenhagen, Denmark).

Analysis of Human GLP-1 Receptor mRNA by Real-Time Polymerase Chain Reaction

Using standard pinch forceps, four intestinal biopsies were taken from each anatomic site (the distal part of the duodenum, terminal ileum, different segments of the colon) during gastroscopy and colonoscopy. Biopsies were immediately submerged in a tube with RNAlater (Ambion) and stored at –80°C until further

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Table 1. Baseline characteristics of study populations in parts A and B

	Part A	Part B	p
n	8	6	
Age, years	25.5 ± 0.7	26.4 ± 1.4	0.54
BMI, kg/m^2	21.8 ± 0.6	23.1 ± 0.9	0.21
BP systolic, mm Hg	121.9 ± 1.6	122.5 ± 2.8	0.84
BP diastolic, mm Hg	73.8 ± 2.1	75.0 ± 2.2	0.69
Glucose, mmol/l	5.0 ± 0.1	4.5 ± 0.1	0.003
Sodium, mmol/l	140.9 ± 0.9	140.3 ± 0.8	0.67
Potassium, mmol/l	3.9 ± 0.2	4.6 ± 0.2	0.03
Creatinine, μmol/l	84.2 ± 4.3	82 ± 4.9	0.74

Data are mean values ± SEM. p values were calculated using regression analysis between the two study populations. BMI = Body mass index; BP = blood pressure.

processing. For RNA isolation 2 biopsies from each intestinal region were homogenized for 30 s (Polytron PT 2100, Kinematika AG, Switzerland) and RNA was extracted using the RNeasy Mini Kit (Qiagen GmbH, Hilden, Germany) following the instructions provided by the manufacturer.

RNA was quantified with a GeneQuant photometer (Pharmacia, Uppsala, Sweden). After DNase I digestion (Gibco, Life Technologies, Basel, Switzerland) 1.5 μg of total RNA was reverse-transcribed by Superscript (Gibco Life Technologies) according to the manufacturer's protocol using random hexamers as primers.

TaqMan analysis was carried out on a 7900HT Sequence Detection System (Applied Biosystems, Rotkreuz, Switzerland). Polymerase chain reaction (PCR) cycling conditions were 10 min at 95°C followed by 40 cycles of 15 s at 95°C and 1 min at 60°C. Each TaqMan reaction contained 10 ng of cDNA in a total volume of 10 μl. TaqMan Universal PCR Mastermix from Applied Biosystems was used. The concentrations of primers and probes were 900 and 225 nmol/l, respectively. Primers and probes were designed according to the guidelines of Applied Biosystems with the help of the Primer Express 2.0 software (Villin, VIL1):

forward primer: 5′-CATGAGCCATGCGCTGAAC-3′;
reverse primer: 5′-TCATTCTGCACCTCCACCTGT-3′;
probe: 5′-TCATCAAAGCCAAGCAGTACCCACCAAG-3′;
GLP1 receptor forward primer:
5′-CGCCTCTGCGATCCTCC-3′;
reverse primer: 5′-GGTTCAGGTGGATGTAGTTCCTG-3′;
probe: 5′-CGGCTTCAGACACCTGCACTGCA-3′.

Primers were synthesized by Invitrogen (Basel, Switzerland), probes by Eurogentec (Seraing, Belgium). For quantification we used external standard curves. All samples were run in triplicates. For each sample, the number of GLP-1 receptor transcripts and the number of villin transcripts were determined. By calculating the ratio of GLP-1 receptor/villin mRNA, the gene expression was normalized to the enterocyte content. Determination of villin, an enterocyte-specific, constitutively expressed protein, can be used to control for the variation of enterocyte content in biopsy [16, 17]. Not reverse transcribed RNA served as a negative control. No significant amplification was observed in these samples.

For quantification we used external standard curves. Standards were gene-specific cDNA fragments that cover the Taqman primer/probe area and were obtained by PCR amplification. As template cDNA from duodenal tissue was used. Gene-specific PCRs were performed with components from Applied Biosystems (AmpliTaq Gold, 10× PCR buffer, dNTPs, $MgCl_2$). Each reaction contained 25 ng cDNA and 300 nmol/l of each primer in a total volume of 25 μl. Thermal cycling was conducted using a Mastercycler personal from Eppendorf (Hamburg, Germany). All PCR products were purified by running a 1.5% agarose gel and a subsequent gel extraction (gel extraction kit, Qiagen). The standards were quantified using the PicoGreenreagent (Molecular Probes, Eugene, Oreg., USA).

Calculations

The fractional excretion of sodium (FeNa) was calculated as:

$$FeNa = [UNa \times PCr \times 100]/[PNa \times UCr],$$

where UNa is urine sodium in millimoles per liter; PCr is plasma creatinine in micromoles per liter; PNa is plasma sodium in millimoles per liter, and finally UCr is urine creatinine in millimoles per liter.

Statistical Analysis

Data are given as mean ± SEM unless stated otherwise. Analysis of variance for repeated measures was used to assess the statistical significance of differences between the treatments using STATA vs. 6.0 for Windows (Stata Corporation, College Station, Tex., USA). This approach was justified since QQ plots showed that the distributions of residuals were close to normal. In case that normal distribution could not be demonstrated, data were logarithmically transformed before statistical testing.

Results

The principal baseline characteristics of both study groups are shown in table 1. Six of nine volunteers completed part B; 3 volunteers had to be excluded from the analysis because of diarrhea during treatment with GLP-1. There was a slight difference in the fasting glucose and plasma potassium concentrations.

Part A

Effect of GLP-1 on Blood Glucose, Water Intake, Thirst and Plasma Sodium

Figure 3 shows the physiological effect of GLP-1 on blood glucose. As expected, GLP-1 significantly reduced glucose concentrations and the glucose/time area under the curve (AUC). Water consumption was not significantly affected by the two treatments (fig. 4A). There was

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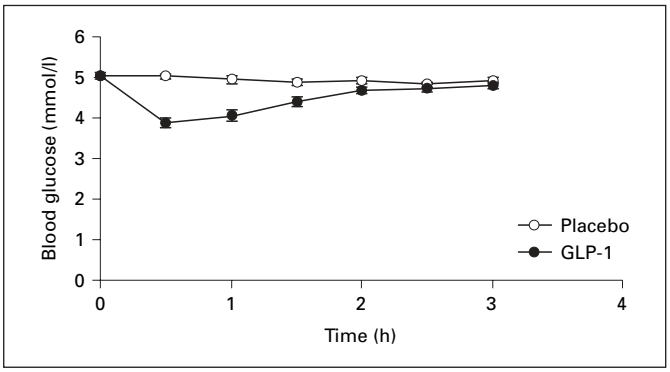

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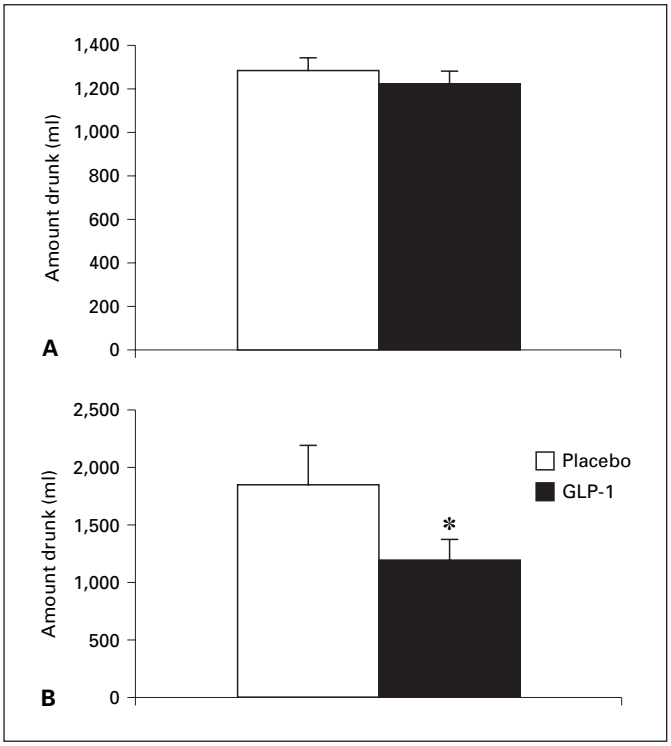

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Fig. 3. Plasma glucose. Glucose/time area under the curve are statistically significantly different: 139.6 ± 2.2 mmol × h/l (placebo) vs. 101.8 ± 3.6 mmol × h/l (GLP-1; $p < 0.0001$).

Fig. 4. Water intake. **A** Amount of fluid drunk: 1,291 ± 69 ml (placebo) vs. 1,228 ± 74 ml (GLP-1; $p = 0.49$). **B** Amount of fluid drunk (relevant difference): 1,848 ± 331 ml (placebo) vs. 1,181 ± 177 ml (GLP-1; * $p = 0.0414$).

Table 2. Effect of salt load with and without intravenous GLP-1 on kidney parameters (mean ± SEM)

Parameter	Placebo	GLP-1 1.5 pmol/kg × min	p
A Intravenous salt load in 8 healthy subjects			
Urine volume, ml/180 min	349 ± 47	650 ± 92	0.0029
FeNa, %	1.6 ± 0.3	2.7 ± 0.2	0.0005
Urine pH	6.99 ± 0.36	7.44 ± 0.32	0.038
Glomerular filtration rate, ml/min	177.8 ± 10.1	198.4 ± 21.5	0.23
Filtered Na load, mmol/min	25.8 ± 1.5	29.1 ± 3.1	0.37
B Oral salt load in 6 healthy subjects			
Urine volume, ml/480 min	879 ± 160	998 ± 171	0.55
FeNa, %	1.6 ± 0.2	2.0 ± 0.2	0.0117
Urine pH	6.84 ± 0.24	7.40 ± 0.11	0.010
Glomerular filtration rate, ml/min	127.5 ± 8.7	120.6 ± 6.3	0.18
Filtered Na load, mmol/min	17.8 ± 1.0	17.1 ± 1.0	0.58

With both oral and intravenous salt loads, there is a rise in the fractional excretion of sodium (FeNa) and a statistical significant increase in urinary pH.

no difference in plasma sodium: the mean sodium concentration was 145.3 ± 0.4 mmol/l (placebo) compared to 146.6 ± 0.4 mmol/l (GLP-1; table 3). GLP-1 infusions did not have any influence on the visual thirst analogue scales (data not shown).

Renal Handling of Sodium, Hydrogen, Potassium and Urine Volume (table 2A)

With GLP-1, urinary sodium excretion increased from 70 ± 9 to 146 ± 27 mmol ($p = 0.008$, paired t test) during the 180-min recollection period, and the fractional

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Table 3. Comparison of plasma sodium concentrations with and without GLP-1 infusion after either an intravenous or an oral salt load (mean ± SEM)

Parameter	Mean ± SEM	95% Confidence interval
Salt infused, g (part A)	26.7 ± 0.9	24.7–28.6
Salt ingested, g (part B)	27.9 ± 0.5	26.8–29.0
Plasma sodium, mmol/l		
Part A		
Placebo	145.3 ± 0.4	144.4–146.2
GLP-1	146.6 ± 0.4	145.8–147.4
Part B		
Placebo	140.7 ± 1.0	138.8–142.7
GLP-1	141.7 ± 0.8	140.1–143.4

There is no statistical difference in the plasma sodium concentrations between treatments with GLP-1 or placebo, both for parts A and B.

However, a significant difference in the plasma sodium levels was seen between parts A and B despite a comparable salt load ($p < 0.0001$; multiple linear regression).

sodium excretion improved from 1.6 ± 0.3 to 2.7 ± 0.2% ($p = 0.0005$, paired t test). The urine volume also increased from 349 ± 47 to 650 ± 92 ml/180 min ($p = 0.0029$, paired t test). Urinary pH rose from 6.99 ± 0.36 to 7.44 ± 0.32 ($p = 0.038$, paired t test) and potassium excretion remained unchanged, urinary potassium was 31.0 ± 3.3 mmol/180 min with placebo and 35.6 ± 4.3 mmol/180 min with GLP-1 ($p = 0.09$). Glomerular filtration was enhanced with both infusions, consequently, the filtered sodium load was increased (table 2A).

Part B

Six volunteers were analyzed. The average salt amount eaten was comparable to the average intravenous salt load (table 3).

Effect of GLP-1 on Water Intake, Thirst, Plasma Sodium

GLP-1 reduced postprandial glucose concentrations (data not shown).

In contrast to part A, volunteers significantly reduced water consumption from 1,848 ± 331 (placebo) to 1,181 ± 177 ml/8 h (GLP-1; $p = 0.0414$, paired t test; fig. 4B). There was no difference in plasma sodium concentrations: mean plasma sodium was 141 ± 1 (placebo) compared to 142 ± 1 mmol/l (GLP-1). If the effect of an intravenous salt load (part A) on plasma sodium concentrations is compared to a similar oral salt load, a relevant difference in mean sodium plasma concentrations is apparent despite similar salt loads (table 3).

Renal Handling of Sodium, Hydrogen, Potassium and Urine Volume (table 2B)

In this part of the study, sodium excretion only increased slightly from 156 ± 27 (placebo) to 215 ± 42 mmol/480 min (GLP-1; $p = 0.1268$, paired t test) and the fractional sodium excretion rose from 1.6 ± 0.2 to 2.0 ± 0.2% ($p = 0.0117$, paired t test) during placebo and GLP-1 infusion periods, respectively. As in part A, urinary pH rose from 6.84 ± 0.24 to 7.40 ± 0.11 with GLP-1 ($p = 0.010$, paired t test) and the potassium excretion in urine achieved similar values, amounting to 64.1 ± 9.5 (placebo) and 80.3 ± 10.8 mmol/480 min (GLP-1, $p = 0.21$). In contrast to part A, urinary volume was not affected by GLP-1, achieving 879 ± 160 ml/480 min with placebo and 998 ± 171 ml/480 min with GLP-1 ($p = 0.55$, paired t test). Another major difference with part A of the study was noted, in this group of volunteers the glomerular filtration rate and the filtered sodium load were not increased.

Part C

Biopsies from the different anatomic segments of the 14 subjects were analyzed in part C. Figure 5 depicts mRNA expression of the GLP-1 receptor in the duodenum, the terminal ileum and the colon. A higher expression of the GLP-1 receptor was seen in the duodenum compared to the other gut localizations ($p < 0.02$, nonparametric test).

Discussion

The present study demonstrates the potential role of the gastrointestinal hormone GLP-1 in the regulation of sodium and water homeostasis in men. Extracellular volume expansion induced by an intravenous infusion of hypertonic saline is partially compensated by an increase in urinary sodium and water excretion with GLP-1. However, the oral ingestion of the same salt load does not produce a significant expansion in extracellular volume; in this setting, GLP-1 contributes to avoiding the increase in volume by means of a slight enhancement in urinary

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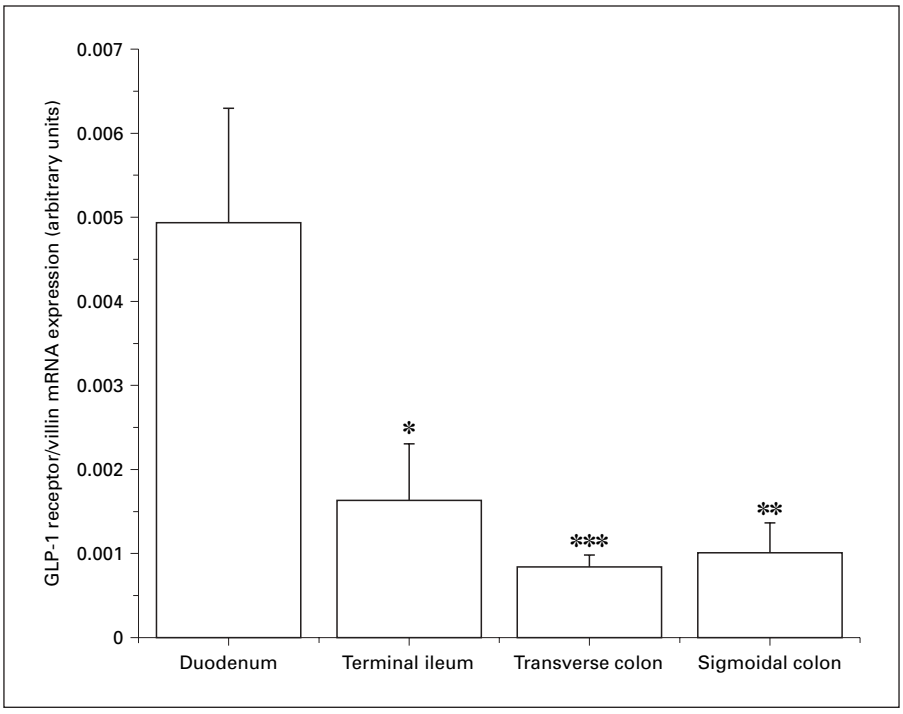

Fig. 5. mRNA expression of human GLP1 receptor in the gut. Expression of GLP-1 receptor mRNA in different gut segments (normalized to villin). Data represent means (± SEM) of biopsies from 14 healthy subjects, except for the terminal ileum where only 13 biopsies could be used. Mann-Whitney U test: * $p = 0.011$; ** $p < 0.01$; *** $p < 0.001$.

sodium elimination and through a significant reduction in water ingestion.

Volume expansion, as with intravenous hypertonic saline, is associated with increased renal perfusion and a rise in glomerular filtration and the filtered sodium load as shown in part A of the study. Reduced angiotensin II and norepinephrine and enhanced release of dopamine and natriuretic peptide may all contribute to this response. In this setting, GLP-1 is able to dramatically increase renal sodium excretion by 69% and urine volume by 86%. Simultaneously, proton excretion is reduced as shown by an increase in urinary pH; potassium excretion remains unchanged and the high glomerular filtration rate is maintained.

After a gastric salt load, several signals activate a complex system of channels, carriers and pumps located in the cells of different intestinal segments to reduce sodium absorption, preventing severe volume expansion. Channels, presumably close to the duodenum, decrease the pass of sodium through the apical cellular membrane and pumps, e.g. Na^+/H^+-ATPase reduces the active transport of sodium. Carriers, such as the Na^+/H^+ antiporter which exchanges extracellular sodium for intracellular hydrogen (NHE3), are inhibited to decrease the Na influx to the intestinal cell. Sodium flux provides the principal driving force for absorption of fluid, and a reduction in sodium absorption is coupled with a reduction in water absorption. For this reason, volume expansion after an oral salt load must be of a lesser degree than that produced directly by an intravenous saline infusion. Accordingly, glomerular hyperfiltration is not necessary to enhance sodium filtration. In fact, glomerular filtration and the filtered sodium load were near normal in part B of the study. As in part A, GLP-1 infusions augment the excretion of sodium, however the effect is much more modest compared to part A, the enhancement reaching only 25%; the urine volume remained constant. As in part A, an increase in sodium excretion comes in parallel with a reduction in proton excretion suggesting a reduction in the sodium–hydrogen exchange rate in the proximal tubule. Whereas GLP-1 infusions enhance sodium excretion, urinary potassium remains constant indicating that the ratio of sodium reabsorption to potassium secretion is not affected in the cortical collecting tubule. On the other hand, GLP-1 had no effect on glomerular filtration.

Based on these findings, we suggest that GLP-1 inhibits sodium reabsorption and proton secretion in the proximal tubule. In fact, this could be done by a reduction in the activity of the Na^+/H^+ exchanger in the proximal renal tubule. This exchanger is the main determinant of proximal sodium reabsorption [18].

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In contrast to the intravenous salt load in part A, the equivalent amount of ingested salt in part B, induced a reduced water intake during GLP-1 infusion (reduction over 36%). This is indirect evidence of a reduction in intestinal sodium absorption. If an excess of sodium is absorbed, the primary protective mechanism against hypernatremia is the stimulation of thirst to increase water intake thereby returning plasma sodium concentrations to normal. Therefore, less water intake necessarily reflects an intestinal inhibition of sodium absorption in the phase of GLP-1 infusion compared to the absorption rate during placebo. Indirect evidence is derived from the fact that 3 volunteers dropped out of the study due to osmotic diarrhea during GLP-1 infusion. The oral salt load administered is three times more than the average daily salt intake of a liberal Western diet [19]. It must be mentioned, however, that gastric emptying should be considered as a possible factor which can bias the results obtained in our study. As it is known that GLP-1 slows gastric emptying, the observed effect in part B, resulting in a lower water intake by GLP-1, may be due to a lower gastric emptying rate, resulting in a lower absorption of sodium. An argument against this criticism may be data obtained with the better known gut peptide cholecystokinin (CCK) and saline intake in rats. Green et al. [20] showed that the gastric emptying of isotonic and hyperosmolar saline solution was not changed by the administration of either CCK or the CCK antagonist devazepide, in contrast to the gastric emptying of solid food. In addition, in dogs given saline 3 h after solid food, Tanaka et al. [21] found that saline was discharged similar to when it was ingested at the beginning of a meal. Both observations indicate that CCK does not influence the gastric emptying rate of saline. These observations could be extended to the gut peptide GLP-1, which is secreted into the circulation at a time similar to CCK.

What could be the mechanism of GLP-1 inducing a reduction in intestinal sodium absorption? Because GLP-1 changes the concentrations of sodium and H^+ in excreted urine, transporters such as NHE3 are candidates for involvement in this GLP-1-mediated effect. It could be demonstrated that NHE3 is the major absorptive sodium-hydrogen exchanger, both in the kidney and intestine, and that the lack of the exchanger impairs sodium and fluid homeostasis [22]. GLP-1 could reduce sodium absorption in the kidney, leading to a higher urinary output of sodium and, in addition, reduce sodium absorption in the gut leading to diarrhea. All 3 volunteers who stopped the study because of diarrhea were exposed to GLP-1 and high-salt intake. Diarrhea developed 7–8 h after initiation of the experiment. These observations suggest an influence of GLP-1 in the regulation of sodium uptake into the gut and kidney. These observations must be confirmed in studies with duodenal tubes and perfusion with different concentrations of saline.

The results presented in part C indicate the possible presence of the GLP-1 receptor in the small intestine: GLP-1 receptor mRNA expression was higher in the proximal small human intestine compared with the distal small intestine and colon (fig. 5). The methodology used does not fulfill the criteria to prove GLP-1 receptors in the small intestine, and unfortunately specific GLP-1 receptor antibodies to demonstrate the presence of a GLP-1 receptor in the gut are not available. However, using mRNA expression we were able to demonstrate that the GLP-1 receptor may be localized in the gut. This is an important finding because thirst receptors, called 'hepatic osmoreceptors', have been suggested to be due to the findings that, first, thirst can be stimulated in rats by gastric loads of hypertonic NaCl solution before systemic increases in pOsm are detected [23], and second, thirst due to an overnight fast in rats can be increased by a gastric load of hypertonic saline [24]. A putative GLP-1 receptor in the gut could be a candidate for part of this system of hepatic osmoreceptors.

In conclusion, GLP-1 increases sodium excretion by the kidneys to control extracellular volume expansion, and may reduce sodium absorption by the gastrointestinal tract to prevent volume expansion. In this way, this incretin hormone seems to play a significant role in sodium and water homeostasis.

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References

1 Drucker DJ: Glucagon-like peptides. Diabetes 1998;47:159–169.
2 Holst JJ: Enteroglucagon. Annu Rev Physiol 1997;59:257–271.
3 Layer P, Holst JJ, Grandt D, Goebell H: Ileal release of glucagon-like peptide-1 (GLP-1). Association with inhibition of gastric acid secretion in humans. Dig Dis Sci 1995;40: 1074–1082.
4 Welch I, Saunders K, Read NW: Effect of ileal and intravenous infusions of fat emulsions on feeding and satiety in human volunteers. Gastroenterology 1985;89:1293–1297.
5 Welch I, Sepple CP, Read NW: Comparisons of the effects on satiety and eating behaviour of infusion of lipid into the different regions of the small intestine. Gut 1988;29:306–311.
6 Flint A, Raben A, Astrup A, Holst JJ: Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. J Clin Invest 1998;101:515–520.
7 Gutzwiller JP, Goke B, Drewe J, Hildebrand P, Ketterer S, Handschin D, et al: Glucagon-like peptide-1: a potent regulator of food intake in humans. Gut 1999;44:81–86.
8 Gutzwiller JP, Drewe J, Goke B, Schmidt H, Rohrer B, Lareida J, et al: Glucagon-like peptide-1 promotes satiety and reduces food intake in patients with diabetes mellitus type 2. Am J Physiol 1999;276:R1541–R1544.
9 Tang-Christensen M, Larsen PJ, Goke R, Fink-Jensen A, Jessop DS, Moller M, et al: Central administration of GLP-1-(7–36) amide inhibits food and water intake in rats. Am J Physiol 1996;271:R848–R856.
10 Gutzwiller JP, Tschopp S, Bock A, Zehnder CE, Huber AR, Kreyenbuehl M, Gutmann H, Drewe J, Henzen C, Goeke B, Beglinger C: Glucagon-like peptide 1 induces natriuresis in healthy subjects and in insulin-resistant obese men. J Clin Endocrinol Metab 2004; 89:3055–3061.
11 Gilman A: The relation between blood osmotic pressure, fluid distribution, and voluntary water intake. Am J Physiol 1937;120: 323–328.
12 Oldfield BJ, Badoer E, Hards DK, McKinley MJ: Fos production in retrogradely labelled neurons of the lamina terminalis following intravenous infusion of either hypertonic saline or angiotensin II. Neuroscience 1994;60: 255–262.
13 Buggy J, Johnson AK: Preoptic-hypothalamic periventricular lesions: thirst deficits and hypernatremia. Am J Physiol 1977;233:R44–R52.
14 Huang W, Sved AF, Stricker EM: Water ingestion provides an early signal inhibiting osmotically stimulated vasopressin secretion in rats. Am J Physiol 2000;279:R756–R760.
15 Blass EM, Hall WG: Drinking termination: interactions among hydrational, orogastric, and behavioral controls in rats. Psychol Rev 1976;83:356–374.
16 Lown KS, Mayo RR, Leichtman AB, Hsiao HL, Turgeon DK, Schmiedlin-Ren P, Brown MB, Guo W, Rossi SJ, Benet LZ, Watkins PB: Role of intestinal P-glycoprotein (mdr1) in interpatient variation in the oral bioavailability of cyclosporine. Clin Pharmacol Ther 1997;62:248–260.
17 Taipalensuu J, Tornblom H, Lindberg G, Einarsson C, Sjoqvist F, Melhus H, Garberg P, Sjostrom B, Lundgren B, Artursson P: Correlation of gene expression of ten drug efflux proteins of the ATP-binding cassette transporter family in normal human jejunum and in human intestinal epithelial Caco-2 cell monolayers. J Pharmacol Exp Ther 2001;299:164–170.
18 Knepper MA, Brooks HL: Regulation of the sodium transporters NHE3, NKCC2 and NCC in the kidney. Curr Opin Nephrol Hypertens 2001;10:655–659.
19 Stamler J: The INTERSALT Study: background, methods, findings, and implications. Am J Clin Nutr 1997;65:626–642.
20 Green T, Dimaline R, Peikin S, Dockray GJ: Action of the cholecystokinin antagonist L364,718 on gastric emptying in the rat. Am J Physiol 1988;255:G685–G689.
21 Tanaka T, Mizumoto A, Muramatsu S, Mochiki E, Haga N, Suzuki H, Itoh Z: Postprandial normal saline intake delays gastric emptying of solids in conscious dogs. Dig Dis Sci 1999;44:1516–1524.
22 Schultheis PJ, Clarke LL, Meneton P, Miller ML, Soleimani M, Gawenis LR, Riddle TM, Duffy JJ, Doetschman T, Wang T, Giebisch G, Aronson PS, Lorenz JN, Shull GE: Renal and intestinal absorptive defects in mice lacking the NHE3 Na+/H+ exchanger. Nat Genet 1998;19:282–285.
23 Kraly FS, Kim Y, Dunham LM, Tribusio RA: Drinking after intragastric NaCl without increase in systemic plasma osmolality in rats. Am J Physiol 1995;269:R1085–R1092.
24 Stricker EM, Callahan JB, Huang W, Sved AF: Early osmoregulatory stimulation of neurohypophyseal hormone secretion and thirst after gastric NaCl loads. Am J Physiol Regul Integr Comp Physiol 2002;282:1710–1717.

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