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Glucagon-like peptides activate hepatic gluconeogenesis

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Piscine (anglerfish, catfish, coho salmon) glucagon-like peptides (GLPs), applied at 3.5 nM, stimulate (1.1–1.9-fold) flux through gluconeogenesis above control levels in isolated trout and salmon hepatocytes. Human GLP-1 and GLP-2 also activate gluconeogenesis, but to a lesser degree than their piscine counterparts. Minor increases of substrate oxidation are noticed at times of peak gluconeogenic activation through GLPs. These hormones, which are derived from the same precursor peptide as glucagon are more potent activators of gluconeogenesis than glucagon when applied at equimolar concentrations, and do not appear to employ cAMP or cGMP as the intracellular messenger in hepatic tissue.

Hepatocyte; Teleost; Gluconeogenesis; Lactate oxidation; cyclic AMP; Glucagon; Glucagon-like peptide

1. INTRODUCTION

In addition to glucagon, other functional peptide units are coencoded in the preproglucagon gene of vertebrates. Interest in these glucagon-like peptides (GLPs) has recently led to their isolation [1–4], determination of their amino acid sequences [1–3] and use of recombinant DNA techniques to elucidate homologies within the vertebrate line [3,4]. Less is known about any actual physiological function or the mechanism of action of these novel peptides, which are differentially processed by and secreted from vertebrate pancreas or gut [5,6]. On the one hand, mammalian GLP-1, GLP-2 and catfish GLP activate rat brain adenylate cyclase, but fail to activate the hepatic enzyme [7,8]. High-affinity GLP-1 receptors appear to be absent from rat liver membranes and GLP-1 does not alter the glucagon-dependent production of hepatocyte cAMP [9] which was interpreted to indicate that liver was not a target organ for GLPs [9]. The short form of GLP-$1_{(7\text{-}37)}$ exerts insulinotropic action on rat islets [10] and on perfused rat pancreas [11], while no such role could be attributed to mammalian GLP-$1_{(1\text{-}37)}$ or GLP-2 [10,11].

Since we and others have recently purified and characterized a number of vertebrate GLPs [1–4], and because isolated teleost hepatocytes are highly sensitive and responsive to hormones [13,14], we decided to analyze potential metabolic effects of GLPs on fish liver metabolism and investigate their possible mode of action.

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2. MATERIALS AND METHODS

Immature rainbow trout *(Salmo gairdneri,* 150–250 g) were maintained on a high protein diet at 9–13°C. Saltwater-adapted coho salmon (*Oncorhynchus kisutch,* 40–120 g) were kindly supplied (in March only) by Dr E.M. Donaldson, Department of Fisheries and Oceans, West Vancouver, and kept at 9°C for less than 6 days without feeding. Hepatocytes were isolated by in situ collagenase perfusion and subsequent dif-

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ferential centrifugation [12]. 20–45 mg packed cells were used per flask, containing 5 mM L-lactate and, after a 20 min preincubation, a total of 1.8–3.7 kBq L-[U-^{14}C]lactate were added (final volume 1.0 ml). Cells were incubated for 2 h at 15°C. Incorporation of label into glucose and CO_2 was assessed as described [15]. Hormones or vehicle were added after the preincubation period. Cells used for cAMP and cGMP radioimmunoassay (Amersham kits, Canada) were treated as above, but without radiolabel. Cells were incubated with hormones or vehicle for 15 min following a 20 min preincubation with 5 mM L-lactate. cAMP and cGMP were measured in neutralized perchloric acid extracts using internal and external standards. All experiments and analyses were done in duplicates in a block design and paired *t*-test was used for statistical analyses.

Coho salmon, catfish *(Ictalurus punctatus)* and anglerfish *(Lophius americanus)* GLPs were isolated as described [1–3]. Human GLP-1 (amidated) and GLP-2 were purchased from Bachem and Peninsula Labs, respectively. Calbiochem supplied bovine glucagon.

3. RESULTS

Rainbow trout (table 1) and coho salmon (table 2) hepatocytes respond to the addition of vertebrate GLPs with specific increases in the rate of flux through the gluconeogenic pathway. Piscine GLPs enhance (13–88%) the flux of carbon through the gluconeogenic pathway compared with untreated control cells and lead to an increased synthesis of glucose from radiolabelled lactate (tables 1,2). All piscine GLPs assayed are equally effective in their gluconeogenic action. A full metabolic response is elicited at the lowest hormone concentration applied (3.5 nM). Higher doses lead to only minor additional increases in gluconeogenic flux (statistically not significantly different from the 3.5 nM dose).

Piscine GLPs also tend to increase the amount of lactate carbon the cells funnel into oxidative pathways as judged by higher rates of CO_2 release (tables 1,2). This activation may reflect generally stepped-up cellular oxygen uptake concomitant with a higher rate of substrate consumption. In terms of actual carbon flow (cf. legend to table 1), the activation of oxidation is well below the increased flux through gluconeogenesis. We conclude that, at least in vitro, the activation of gluconeogenesis designates one important biological effect of GLP action.

Since all hepatocyte experiments were performed at a constant temperature of 15°C throughout the year, we conclude that the differential response of the cells to added GLPs (cf. table 1) is due to the altered circannual state of the experimental animals. The largest response is elicited during the summer, while cells isolated from winter fish fail to respond (Mommsen and Plisetskaya, unpublished). This type of cell behaviour is by no means restricted to GLPs but also applies to glucagon [17].

Although the control rates for gluconeogenic flux and for lactate oxidation show considerable differences between individual fish (cf. tables 1,2), no significant differences are noticed over the course of a year (ANOVA, oxidation: D.F. = (4,26), $F = 2.04$, $P > 0.11$; glucose formation: D.F. = (4,26), $F = 0.94$, $P > 0.45$), resulting in the mean control rates given in the tables. However, our random block experimental design allows us to focus our analysis specifically on the effectiveness of GLP addition.

With respect to the possible mechanism of action of GLPs, additional experiments with fish hepatocytes reveal that cAMP may not be involved as an intracellular messenger. When we tested the effects of GLPs on intracellular cAMP and cGMP in a series of block design experiments (table 3) no alterations were apparent in the amount of intracellular messengers, while glucagon caused the expected increase in cellular cAMP.

4. DISCUSSION

Our identification of a pronounced metabolic function of GLPs adds a new member to the list of hormones regulating gluconeogenesis in vertebrates. In addition, the physiological effect in piscine in vitro system is stronger than the response elicited by glucagon. If this phenomenon also holds true for the in vivo situation, it will have important implications on the general understanding of the regulation of glucose metabolism in vertebrate animals. Interestingly, teleost fish possess one GLP, which is homologous to mammalian GLP-$1_{(7\text{-}37)}$, the only mammalian GLP with

reported biological activity [1,2,18].

Large differences also exist in the amounts of GLPs available in vertebrate tissues. In mammals, GLP concentrations in pancreas or gut fluctuate around 100 pmol/g [20], which dwarfs in comparison with values exceeding 120 nmol/g in salmon pancreas [2]. The lowest concentration of GLP (3.5 nM) applied in our experiments falls into the plasma concentration range for salmonids (Plisetskaya, unpublished).

Table 1

Effects of peptide hormones on gluconeogenesis and CO_2 release from lactate in isolated trout hepatocytes

	Oxidation (μmol CO_2/g per h)	Gluconeogenesis (μmol glycose/g per h)
Control	9.99 ± 0.70 (31)	2.28 ± 0.29 (31)
Salmon GLP		
(3.5 nM, March)	+ 6.4% ± 4.3 (7)	+20.0% ± 5.9 (7)[a]
(April)	+10.8% ± 5.8 (4)	+21.8% ± 6.1 (4)[a]
(June)	+27.5% ± 15.3 (4)[b]	+65.3% ± 22.9 (4)[a]
(Aug.)	+ 4.0% ± 5.0 (2)	+12.5% ± 0.5 (2)
(15.5 nM, March)	+ 5.9% ± 5.1 (6)	+25.1% ± 8.9 (6)[a]
(April)	+14.0% ± 4.3 (4)[a]	+16.3% ± 3.9 (4)[a]
(Sept.)	+ 4.6% ± 1.9 (14)	+34.2% ± 5.1 (14)[a]
Anglerfish GLP		
(3.5 nM, March)	+ 1.2% ± 3.6 (6)	+23.4% ± 8.6 (6)[a]
(15.5 nM, March)	+ 0.8% ± 4.0 (5)	+31.9% ± 11.1 (5)[a]
Catfish GLP		
(3.5 nM, June)	+37.0% ± 11.7 (4)[a]	+87.7% ± 21.8 (4)[a]
Human GLP-1		
(3.5 nM, June)	+18.8% ± 8.8 (4)[a]	+33.5% ± 15.6 (4)[a]
Human GLP-2		
(3.5 nM, June)	+ 9.8% ± 13.1 (4)	+19.3% ± 21.9 (4)
Bovine glucagon		
(10 μM, Sept.)	+ 3.2% ± 3.7 (10)	+31.5% ± 7.0 (10)[a]
Salmon glucagon		
(15 nM, Sept.)	+ 3.2% ± 1.8 (6)	+ 7.3% ± 2.2 (6)[a]

[a] Significantly different from untreated control at $p<0.01$

[b] Significantly different from untreated control at $p<0.05$

Hormones were added after a 20 min preincubation. Control rates are given as μmol product formed in 60 min per g packed cells ± SE. See text for seasonal variation in control rate. In salmonid hepatocytes CO_2 production reflects true substrate oxidation [26]. Therefore, division of oxidation values by 3 (carbon atoms in lactate) and multiplication of values for gluconeogenesis by 2 (theoretical incorporation of lactate into glucose) will yield the total substrate utilization. Rates after hormone exposure are given as percent change from the control rate ± SE. In parentheses: number of independent determinations

Table 2

Effects of glucagon-like peptides on gluconeogenesis and CO_2 release from lactate in isolated coho salmon hepatocytes

	Oxidation (μmol CO_2/g per h)	Gluconeogenesis (μmol glycose/g per h)
Control	8.37 ± 1.46 (6)	2.54 ± 0.40 (6)
Coho salmon GLP		
(3.5 nM)	+11.3% ± 12.5 (3)	+18.4% ± 7.7 (3)[b]
(15.5 nM)	− 3.3% ± 1.2 (3)	+16.7% ± 8.2 (3)[b]
(70.0 nM)	+ 2.7% ± 3.2 (3)	+39.3% ± 12.7 (3)[b]
Anglerfish GLP		
(3.5 nM)	− 1.9% (1)	+22.9% (1)
(15.5 nM)	+ 1.0% ± 4.4 (2)	+33.9% ± 3.0 (2)

All experiments were performed during March. See table 1 for explanation of experimental procedure and symbols

Full activation of gluconeogenesis is achieved at 3.5 nM GLP and is not accompanied by increases in cAMP (table 3). At higher concentrations (> 30 nM GLP) minor increases in cAMP are detectable (Ottolenghi, C., personal communication). Our conclusion that GLPs exert their metabolic action without activating the hepatic adenylate cyclase system is in line with the observation that GLPs fail to compete for glucagon-binding sites on the hepatocyte surface [7,8]. The additional finding

Table 3

Intracellular messengers in trout hepatocytes after hormonal treatment

Treatment	cAMP (pmol/g)	cGMP (pmol/g)	Flux (% increase)
Control	386 ± 42 (10)	10.3 ± 0.7 (4)	n/a
Salmon GLP (15 nM)	396 ± 44 (10)	11.2 ± 0.4 (4)	31.6 ± 6.5 (10)[a]
Bovine glucagon (10 μM)	1120 ± 316 (6)[a]	–	31.5 ± 7.0 (10)[a]
Salmon glucagon (15 nM)	–	11.7 ± 1.6 (4)	7.3 ± 2.2 (6)[b]

[a] Significantly different from untreated cells at $p < 0.01$

[b] Significantly different from control and treatment with salmon GLP ($p < 0.05$, Student-Newman-Keuls-test)

Values are given in pmol nucleotide per g packed hepatocytes ± SE after a 15 min incubation with or without the respective hormone. (——) not determined. Flux: refers to the increase in flux through gluconeogenesis measured over a 2 h period as outlined in table 1. Experiments were carried out in September in a block design

that the brain, but not the hepatocyte membrane, contains specific GLP receptors [8,9] can only be reconciled with our results on hepatic action of GLPs if we postulate intracellular recognition sites for the GLPs. As a consequence, GLPs cannot interfere directly with the glucagon-dependent production of cAMP.

GLPs also differ from glucagon in their effect on glycogenolysis. In coho salmon in vivo and in liver slices, salmonid GLPs appear to exert much less of a glycogenolytic action than either salmon or mammalian glucagon ([19] and Plisetskaya and Ottolenghi, unpublished). This observation supplies preliminary evidence that the preferred target for GLPs is indeed the gluconeogenic pathway.

The physiologically interesting outcome of our study is that such closely related peptide hormones (glucagon and GLP), forming part of the same precursor peptide (preproglucagon), encoded by the same gene [3,4,18], and similar in amino acid sequence [2] activate gluconeogenesis, albeit by differing modes of action. While glucagon binds to receptors on the hepatocyte surface, no outer membrane receptors have been found for GLPs [9]. Alternatively, intracellular receptors for peptide hormones, including glucagon [21], have been described, although it has been suggested that these may represent receptor molecules in the process of recycling (cf. [21]).

Flux through hepatic gluconeogenesis is known to be stimulated hormonally by two distinct mechanisms. Glucagon and β-adrenergic agonists lead to transient increases in intracellular cAMP, followed by protein phosphorylation [16], a mode of action which appears to be identical in mammals and in teleosts [12,14]. α-Adrenergic agonists and vasopressin act through altering the intracellular Ca^{2+} distribution mediated by phosphoinositols, at times also resulting in protein phosphorylation [22]. While the exact regulatory sites causing altered flux through gluconeogenesis still await identification, the above distinction has been challenged by the recent description for hepatocytes of two-signal transduction systems for glucagon (adenylate cyclase and phosphodiesterase, [23,24]). Also, a cAMP-independent activation of fructose-1,6-bisphosphatase has been noticed [25], possibly involving two membrane receptor populations. Our report on the potent physiological action of GLPs in conjunction with the alleged lack of GLP membrane receptors will make the sorting out of the control of gluconeogenesis and the interaction of hormones, receptors and their intracellular messengers an even more challenging task.

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