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BIOLOGICAL ACTIVITIES OF CATFISH GLUCAGON AND GLUCAGON-LIKE PEPTIDE

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Summary: The ability of catfish glucagon and glucagon-like peptide to bind and activate mammalian glucagon receptors was investigated. Neither catfish peptide binds to glucagon receptors of rat liver, hypothalamus or pituitary. Neither stimulates adenylate cyclase activity in liver membranes. Catfish glucagon fails to activate adenylate cyclase in hypothalamic or pituitary membranes in contrast to mammalian glucagon. However, catfish glucagon-like peptide does stimulate hypothalamic and pituitary adenylate cyclase (EC_{50} ~ 1 pM) possibly through mammalian glucagon-like peptide receptors.

The mammalian gene encoding glucagon codes also for two glucagon-like peptides, GLP-1 and GLP-2 (1-3). Glucagon and a single GLP have been isolated from catfish endocrine pancreas (4) and other sources (5,6). Because of the high degree of sequence homology of the catfish peptides with each other and with their mammalian counterparts we have investigated their binding and adenylate cyclase activity in rat liver and brain, tissues which contain glucagon and/or GLP receptors (7,8).

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Abbreviations: GLP:glucagon-like peptide.

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MATERIALS AND METHODS

Catfish glucagon and GLP were highly purified as described by Andrews and Ronner (4). Porcine glucagon (Eli Lilly and Co.) was purified, iodinated with Iodogen and repurified (9) to produce [^{125}I]iodoTyr^{10}glucagon (10). Rat liver plasma membranes were prepared and stored frozen as described previously (7). Rat hypothalamus and pituitary were macroscopically dissected from male Sprague Dawley rats (200-250 g), weighed, and kept on ice for 20 min before being gently homogenized at low speed in 60 volumes of a solution containing 1 mM dithiothreitol, 0.1% bovine serum albumin and 30 mM Tris-HCl, pH 7.5 at 4°C in a loose-fitting Teflon-glass homogenizer with 10-12 slow up-and-down strokes. Homogenates were used immediately in competitive binding assays or adenylate cyclase assays as described (7). For adenylate cyclase assays with hypothalamic and pituitary membranes, 500 μg wet weight in a total incubation volume of 100 μL was assayed. Theophylline (1.0 mM) was used to inhibit phosphodiesterase. GTP was present at a concentration of 10 μM.

RESULTS

In three separate experiments both catfish glucagon and catfish GLP failed to inhibit [^{125}I]iodoTyr^{10}glucagon binding to liver plasma membranes to any extent (Fig.1-1). They also failed to compete with labeled glucagon for binding to hypothalamic and pituitary membranes at concentrations as high as 5×10^{-6}M (data not shown). The half-maximal concentration of porcine glucagon required for inhibition is 1 nM with liver membranes (Fig.1-1) compared with 5 nM for brain membranes (7).

Catfish glucagon failed to activate cyclase in any of the three preparations (Fig.1-1), although mammalian glucagon stimulates cyclase activity in rat liver (9), and hypothalamic and pituitary membranes (7) at nanomolar concentrations.

Catfish GLP also failed to stimulate adenylate cyclase in liver membranes at concentrations of 10^{-6} - 10^{-12} M (Fig. 1-2). However, catfish GLP stimulates adenylate cyclase activity in hypothalamic and pituitary membranes at very low concentrations even though it does not compete for glucagon binding. The half-maximal concentration for GLP stimulation is ~ 1 pM for both tissues (Fig.1-2) in contrast to the 3.7 nM concentration required for mammalian glucagon (7). In four assays, at concen-

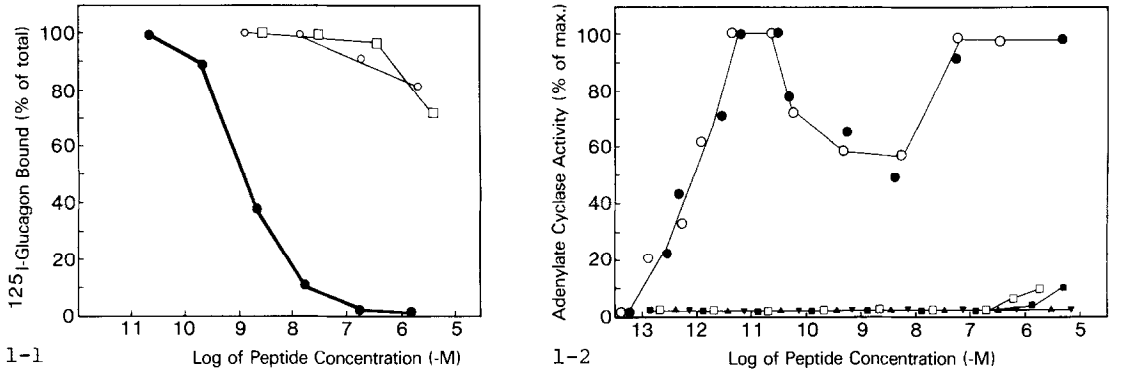

Figure 1-1. Displacement of [^{125}I]iodoTyr^{10}glucagon binding from rat liver membranes. Results with mammalian glucagon (●), catfish glucagon (○) and catfish GLP (□) are plotted for partially purified rat liver plasma membranes. Nonspecific binding measured in the presence of 1 μM unlabelled glucagon was 25-30% of total and was subtracted from the total to give specific binding. Assays were repeated at least three times with similar results. Figure 1-2. Dose-response curves for stimulation of adenylate cyclase activity by catfish peptides. Activation of hypothalamic (○), pituitary (●) and liver (■) adenylate cyclase by catfish GLP. Catfish glucagon shows insignificant stimulation in liver (□), hypothalamic (▲) and pituitary homogenates (▼). Results are expressed as percent maximal activity attained by porcine glucagon (1 μM). Basal activity was approximately 0.5 nmol and 0.15 nmol cAMP formed per mg wet weight of tissue for hypothalamic and pituitary homogenates, respectively. Maximal activation over basal levels was ~ 2-fold. In liver membranes basal enzyme activity was approximately 0.95 nmol cAMP formed per mg protein and maximal stimulation was ~ 3-fold over basal levels. Standard deviation of each point performed in triplicate was < 5%. Each assay performed three additional times gave similar results.

trations of catfish GLP of 0.05 -.05nM, enzyme activity was reduced. High dose inhibition of cyclase has also been reported with human GLPs (8) as well as with glucagon, secretin, β-adrenergic agonists and prostaglandins (11,12).

DISCUSSION

Catfish glucagon has 23 of 29 residues in common with mammalian glucagon (Table 1). Despite the high degree of sequence homology and the conservative nature of the substitutions, catfish glucagon does not compete for rat liver glucagon receptors (Fig. 1-1), or for hypothalamus or pituitary receptors (data not shown); further, it does not stimulate adenylate cyclase activity in the three tissues (Fig. 1-2). It

has been shown that His-1 of mammalian glucagon is critical for full hepatic receptor binding as well as cyclase activation whereas the carboxyl terminal region contributes high receptor affinity (9,11,13-15). Wright and Rodbell (16) have suggested that Gln-3 in mammalian glucagon is important for its recognition by the hepatic receptor so that Asp instead of Gln at this position in the related peptides, secretin and vasoactive intestinal polypeptide, may be responsible for their lack of cross-reaction with glucagon receptors. Stabilization of the β-turn in the amino terminal region by substitution of D-Phe at position-4 produces partial agonism in cyclase assays (17) but superagonism in vivo (18). It is therefore interesting that the replacement of Gln-3, a β-turn former, with Glu, a β-turn breaker is associated with the failure of catfish glucagon to interact with the mammalian glucagon receptor.

Although none of the GLPs bind to glucagon receptors in the three tissues examined and do not activate hepatocyte cyclase, catfish GLP, like human GLP-1 and GLP-2 (8), does activate hypothalamic and pituitary cyclase at low (30-50 pM) concentrations (Fig. 1-2). Because of the close sequence homology

Table 1

Primary structures of catfish and mammalian glucagon and glucagon-like peptides

GLP-1[1] human	HAEGT	FTSDV	SSYLE	EQAAK	EFIAW	LVKGR(NH_2)
GLP-2[2] human	HADGS	FSDEM	NTILD	DLAAR	DFINW	LIQTK
GLP[3] catfish	HADGT	YTSDV	SSYLQ	EQAAK	DFITW	LKSGQ
Glucagon mammalian	HSQGT	FTSDY	SKYLD	SRRAQ	DFVQW	LMNT
Glucagon catfish	HSEGT	FSNDY	SKYLE	TRRAQ	DFVQW	LM(NS)

[1] The first six residues of human GLP-1 are HDEFER
[2] Residues 31-34 of GLP-2 are ITDR
[3] Residues 31-34 of catfish GLP are PKPE

between catfish GLP and mammalian GLP-1 (68% identity) it is possible that catfish GLP stimulates cyclase from interaction with GLP-1 receptors. The higher biopotency of catfish GLP (EC_{50} ~ 1 pM) relative to mammalian GLPs (EC_{50}, 30-50 pM) in mammalian tissue is reminiscent of the situation with insulin whose receptors are more highly conserved over the course of vertebrate evolution than are the insulins themselves. Insulin receptors of all species tested bind chicken insulin > pork insulin > guinea pig insulin (19).

GLPs are present in lower vertebrate pancreas and mammalian intestinal tissues (6,20,21); furthermore, glucagon and peptide fragments derived from the preproglucagon molecule have been identified in brain tissue (22-24). While the GLPs are devoid of hepatic activity (8,25), glucose-dependent insulin release from pancreatic islets by GLPs has been reported (26).

The high potency with which GLPs stimulate hypothalamic adenylate cyclase suggests that they may be novel vertebrate neurotransmitters and/or neuromodulators. Their activation of pituitary adenylate cyclase suggests a releasing factor role (27). Further study of their role(s) is warranted.

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