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Glucagon and Glucagon-like Peptide 1: Selective Receptor Recognition via Distinct Peptide Epitopes*

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Glucagon and glucagon-like peptide 1 (GLP-1) are homologous peptide hormones that are recognized by likewise homologous, but highly selective receptors. Analogs of glucagon and GLP-1, in which the divergent residues were systematically exchanged, were employed to identify the structural requirements for their selective receptor recognition. Substitutions in the NH_2-terminal part of the glucagon molecule with the corresponding GLP-1 residues, as for example in [Ala^2,Glu^3]-glucagon and [Val^{10},Ser^{12}]glucagon, reduced the binding affinity for the glucagon receptor several hundred-fold without increasing the affinity for the GLP-1 receptor. In contrast, introduction of GLP-1 residues into the far COOH-terminal part of the glucagon molecule, *e.g.* [Val^{27},Lys^{28},Gly^{29},Arg^{30}]glucagon, had a minimal effect on recognition of the glucagon receptor, but improved the affinity of the analog for the GLP-1 receptor up to 200-fold. Similarly, substitutions in especially the far COOH-terminal part of the GLP-1 molecule with the corresponding glucagon residues, *e.g.* des-Arg^{30}-[Met^{27},Asn^{28},Thr^{29}]GLP-1, decreased the affinity for the GLP-1 receptor several hundred-fold (IC_{50} = 0.4–190 nM) without increasing the affinity for the glucagon receptor. Conversely, substitutions in the NH_2-terminal part of the GLP-1 molecule impaired the affinity for the GLP-1 receptor only moderately. We conclude that the selective recognition of the glucagon and GLP-1 receptors is determined by residues located at opposite ends of the homologous peptide ligands. This conclusion is supported by the observation that a "chimeric" peptide consisting of the NH_2-terminal part of the glucagon molecule joined to the COOH-terminal part of the GLP-1 molecule was recognized with high affinity by both receptors.

Glucagon and glucagon-like peptide 1 (GLP-1)[1] are members of a family of structurally related peptide hormones, the glucagon/secretin family (1). Within this family, glucagon (29 amino acids) and GLP-1 (30 amino acids) constitute a highly homologous set of peptides. In addition, these two hormones originate from a common precursor, preproglucagon, which, upon tissue-specific processing, leads to the production of glucagon in the pancreas, while GLP-1 is predominantly secreted in the intestine (2, 3).

The cloning of cDNAs coding for glucagon and GLP-1 receptors, respectively, has recently demonstrated that these receptors belong to an interesting and separate branch of the superfamily of G-protein-coupled receptors. Like the peptides, the receptors are closely related, with an overall sequence homology of 58% (4–6).

Physiologically, both peptides play major roles in glucose homeostasis. Glucagon is directly implicated in the maintenance of plasma glucose concentrations via stimulation of glycogenolysis as well as gluconeogenesis, whereas GLP-1 is indirectly involved via stimulation of insulin and inhibition of glucagon secretion (7). In recent clinical studies, the infusion of GLP-1 into normal subjects as well as patients with non-insulin-dependent diabetes mellitus increased levels of insulin and normalized plasma glucose (8). These results hold promise for GLP-1 agonists as future therapeutic agents in the management of diabetes. Similar prospects have been proposed for glucagon antagonists (9, 10).

For both peptides, structure-function analyses involving truncated analogs have demonstrated the importance of residues at both ends of the molecules. An intact NH_2-terminal histidine is critically important in both peptides for receptor activation, although less so for receptor binding (11–14). Also, a number of studies aimed at the design of glucagon receptor peptide antagonists (15–18) have identified Asp^{15}, Ser^2, and Ser^8 as particularly important for high affinity receptor binding.

Earlier structure-function studies indicated that the NH_2-terminal domain of glucagon was predominantly involved in receptor activation, while receptor selectivity primarily was determined by the COOH-terminal part of the peptide (19). More recent data have, however, weakened the concept of a clear segregation of receptor-binding *versus* receptor-activating peptide epitopes; rather, residues important for both functions appear to be found throughout the length of the peptide (17, 18). With the exception of a recent study involving systematic alanine substitutions (14), relatively little information exists on structure-function aspects for GLP-1 (13, 20, 21).

In this study, we have tested a series of glucagon/GLP-1 chimeric peptides for their ability to interact with the corresponding, likewise homologous receptors, addressing the identification of peptide residues that determine receptor binding affinity as well as receptor binding selectivity.

EXPERIMENTAL PROCEDURES

Tissue Cell Culture—CHL stable transfectants expressing the rat GLP-1 receptor were generously supplied by Bernard Thorens. The cells were grown at 10% CO_2 in Dulbecco's modified medium 1885 containing 5% fetal calf serum, 1% glutamine, 1% sodium pyruvate, 1% nonessential amino acids, and 0.1% gentamycin. The cells were seeded at 50,000 cells/well 24 h prior to the binding experiments.

Membrane Preparations—Membranes were prepared from freshly excised porcine liver according to a previously described method (22, 23) using the following protease inhibitors: phenylmethylsulfonyl fluoride (0.1 mM), aprotinin (5 kallikrein-inhibiting units/ml), bacitracin (100 μg/ml), and chymostatin (20 μg/ml). Membrane fragments were resuspended in 1 mM $NaHCO_3$, 0.5 mM $CaCl_2$, pH 7.4; quick-frozen; and stored at −80 °C.

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[1] The abbreviations used are: GLP-1, glucagon-like peptide 1; CHL, Chinese hamster lung; HPLC, high pressure liquid chromatography.

TABLE I

Binding affinities of glucagon, GLP-1, and peptide analogs for the glucagon and GLP-1 receptors

As described under "Experimental Procedures," the biologically active form of glucagon-like peptide 1, GLP-1-(7–36)-amide, is here referred to simply as GLP-1 and is numbered as the homologous glucagon peptide. The amino acid identities between glucagon and GLP-1 are shaded. The peptides are listed as either glucagon or GLP-1 analogs, with residues altered relative to the parent peptide indicated by black circles. The peptides were tested in binding assays with intact CHL cells stably transfected with cDNA encoding the rat GLP-1 receptor (4) and using porcine liver membranes for the glucagon receptor, employing as radioligands ^{125}I-GLP-1 and ^{125}I-glucagon, respectively. The two analogs des-Arg^{30}-[Asp^{15},Ser^{16},Arg^{17},Arg^{18},Gln^{20},Asp^{21},Val^{23},Gln^{24},Met^{27},Asn^{28},Thr^{29}]GLP-1 (same as [Ala^{2},Glu^{3},Val^{10},Ser^{12}]glucagon) and [Glu^{15},Gly^{16},Gln^{17},Ala^{18},Lys^{20},Glu^{21},Ile^{23},Ala^{24},Val^{27},Lys^{28},Gly^{29},Arg^{30}]glucagon (synonymous with [Ser^{2},Gln^{3},Tyr^{10},Lys^{12}]GLP-1) are listed twice, being considered both as glucagon analogs and as GLP-1 analogs. These analogs were abbreviated to GLP-1(N)-glucagon(C) and glucagon(N)-GLP-1(C), respectively. R, receptor. The single-letter code for amino acids is used to designate the peptide analogs presented in the table below.

		Glucagon R			GLP-1 R		
	1 5 10 15 20 25 29	IC50 (nM)	(n)	Fold Decrease	IC50 (nM)	(n)	Fold Increase
Glucagon	HSQGTFTSDYSKYLDSRRAQDFVQWLMNT	1.5±0.2	(7)	=1	440±60	(7)	=1
Glucagon analogs							
[A^{2},E^{3}]-glucagon	HAEGTFTSDYSKYLDSRRAQDFVQWLMNT	>1,000	(8)	≥650	>1,000	(6)	-
[V^{10},S^{12}]-glucagon	HSQGTFTSDVSSYLDSRRAQDFVQWLMNT	290±70	(6)	193	>1,000	(4)	-
GLP-1(N)-glucagon(C)	HAEGTFTSDVSSYLDSRRAQDFVQWLMNT	>1,000	(5)	≥650	290±60	(6)	1.5
[E^{15},G^{16},Q^{17},A^{18}]-glucagon	HSQGTFTSDYSKYLEGQAAQDFVQWLMNT	41±13	(7)	27	330±80	(6)	1.3
[K^{20},E^{21}]-glucagon	HSQGTFTSDYSKYLDSRRAKEFVQWLMNT	2.8±0.8	(5)	1.9	210±50	(6)	2.1
[I^{23},A^{24}]-glucagon	HSQGTFTSDYSKYLDSRRAQDFIAWLMNT	41±11	(7)	27	79±22	(5)	5.6
[V^{27},K^{28},G^{29},R^{30}]-glucagon	HSQGTFTSDYSKYLDSRRAQDFVQWLVKGR	12.2±3.0	(4)	8.1	2.6±1.0	(5)	169
Glucagon(N)-GLP-1(C)	HSQGTFTSDYSKYLEGQAAKEFIAWLVKGR	47±11	(8)	31	1.7±0.8	(8)	258
				Fold Increase			Fold Decrease
GLP-1	1 5 10 15 20 25 30 HAEGTFTSDVSSYLEGQAAKEFIAWLVKGR	>1,000	(4)		0.4±0.1	(4)	=1
GLP-1 analogs							
[S^{2},Q^{3}]-GLP-1	HSQGTFTSDVSSYLEGQAAKEFIAWLVKGR	>1,000	(6)	-	5.0±2.2	(8)	13
[Y^{10},K^{12}]-GLP-1	HAEGTFTSDYSKYLEGQAAKEFIAWLVKGR	>1,000	(6)	-	1.0±0.3	(6)	2.5
Glucagon(N)-GLP-1(C)	HSQGTFTSDYSKYLEGQAAKEFIAWLVKGR	47±11	(8)	≥21	1.7±0.8	(8)	4.2
[R^{18}]-GLP-1	HAEGTFTSDVSSYLEGQRAKEFIAWLVKGR	>1,000	(1)	-	1.0±0.4	(3)	2.5
[Q^{20},D^{21}]-GLP-1	HAEGTFTSDVSSYLEGQAAQDFIAWLVKGR	>1,000	(5)	-	7.4±1.4	(5)	19
[V^{23},Q^{24}]-GLP-1	HAEGTFTSDVSSYLEGQAAKEFVQWLVKGR	>1.000	(1)	-	0.3±0.1	(3)	0.8
[M^{27},N^{28},T^{29},desR^{30}]-GLP-1	HAEGTFTSDVSSYLEGQAAKEFIAWLMNT	>1,000	(7)	-	190±60	(8)	475
[D^{15},S^{16},R^{17},R^{18},Q^{20},D^{21}]-GLP-1	HAEGTFTSDVSSYLDSRRAQDFIAWLVKGR	>1,000	(5)	-	31±9.0	(7)	78
[V^{23},Q^{24},M^{27},N^{28},T^{29},desR^{30}]-GLP-1	HAEGTFTSDVSSYLEGQAAKEFVQWLMNT	>1,000	(6)	-	110±30	(7)	275
GLP-1(N)-glucagon(C)	HAEGTFTSDVSSYLDSRRAQDFVQWLMNT	>1,000	(5)	-	290±60	(6)	725

Radioligands—^{125}I-GLP-1 (specific activity of 81 kBq/pmol) was prepared by the lactoperoxidase method (31) and purified by reverse-phase HPLC.^{125}I-Glucagon (specific activity of 41 kBq/pmol) was prepared by the chloramine-T method and purified by anion-exchange chromatography. Radioligands were generously supplied by U. D. Larsen (Novo Nordisk A/S).

Peptide Synthesis—The peptides were synthesized using an ABIMED 422 multiple synthesizer according to a previously described method (14). The deprotected peptides were precipitated in *tert*-butylmethyl ether, washed twice in diethyl ether, and lyophilized from 10% acetic acid. The crude peptides were subsequently purified by reverse-phase HPLC in a gradient of acetonitrile (15–40% acetonitrile in 0.01 M HCl; Superpak Pep column, Pharmacia Biotech Inc.). The peptides were of >95% purity as characterized by analytical HPLC and high performance capillary electrophoresis, and their molecular mass was within the calculated value ± 2 mass units as determined by mass spectroscopy.

Binding Assays—GLP-1 receptor binding was determined by a whole cell assay on stably transfected CHL cells using 50,000 cells/well. Glucagon receptor binding was determined using 25 μg of liver membrane suspension/tube. Nonspecific binding was determined in the presence of 1 μM of GLP-1 or glucagon. The whole cells assay was performed at 4 °C for 6 h, and the membrane assay at room temperature for 2 h, both in a buffer composed of 25 mM Hepes, pH 7.4, 1 mM $MgCl_2$, 2.5 mM $CaCl_2$, 0.1% bovine serum albumin, and 100 μg/ml bacitracin. Peptides were present in concentrations ranging from 10^{-12} to 10^{-6} M.

Nomenclature—Biologically active GLP-1 corresponds to a truncated form of the initially identified GLP-1 segment that is derived from the combined glucagon and GLP-1 precursor, preproglucagon (24–26). The peptide has been referred to as proglucagon-(78–107), truncated GLP-1, GLP-1-(7–37), and GLP-1-(7–36)-amide, the latter two representing naturally occurring and seemingly biologically equivalent forms of the hormone (27). "Truncated GLP-1'" is also the molecule that has the highest homology to glucagon as well as the other peptides from the glucagon/secretin family (1). For a more logical comparison, especially in the present report, where residues corresponding to equivalent positions have been exchanged between glucagon and GLP-1, we here refer to GLP-1-(7–36)-amide simply as GLP-1. Hereby, the NH_2-terminal His of fully processed GLP-1 is residue 1, exactly as in glucagon, and all other residues at equivalent positions in the two peptides are conveniently referred to by identical numbers. A nomenclature such as the one proposed here was furthermore recently agreed upon at a recent international meeting, convening a large number of GLP-1 investigators (28).

RESULTS

Design of Peptide Analogs—The glucagon and GLP-1 peptides are composed of an NH_2-terminal homologous domain and a COOH-terminal divergent domain. The divergent residues were substituted, individually or in sets, with the amino acid residue(s) found at the equivalent position in the homologous peptide (Table I). For convenience, some peptides were considered as *glucagon analogs* and others as *GLP-1 analogs*, although all

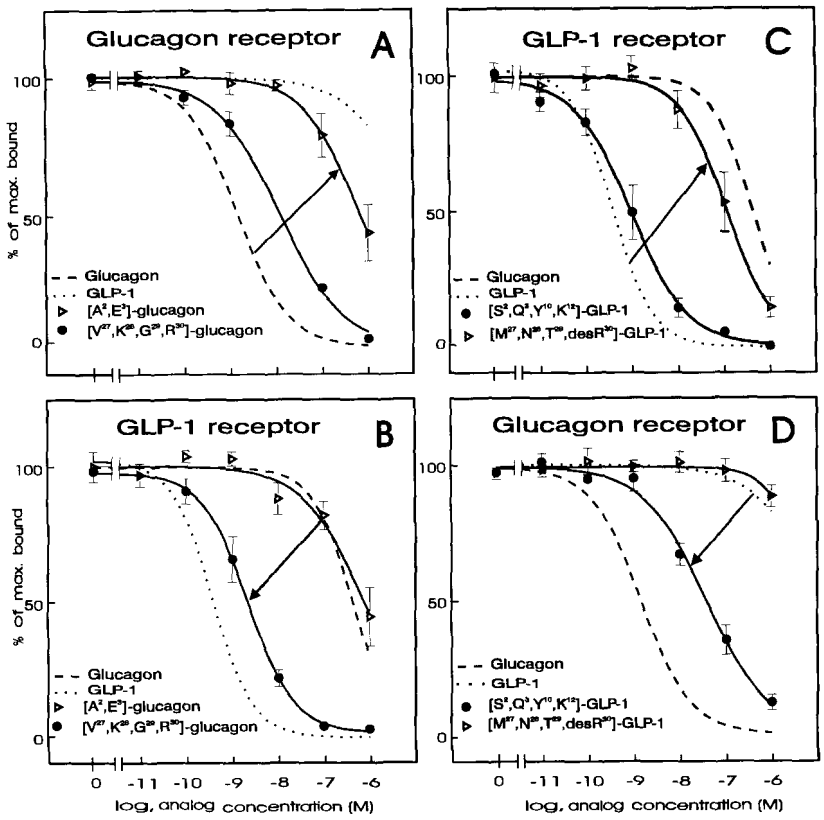

FIG. 1. **Competitive binding analysis of selected peptide analogs to the glucagon and GLP-1 receptors.** Binding profiles are shown for selected glucagon analogs (*A* and *B*) and selected GLP-1 analogs (*C* and *D*) on glucagon receptors (*A* and *D*) and GLP-1 receptors (*B* and *C*), respectively. Binding assays were performed on intact cells for the GLP-1 receptor and on liver membranes for the glucagon receptor using ^{125}I-peptide ligand, ^{125}I-glucagon (*A* and *D*), and ^{125}I-GLP-1 (*B* and *C*). The structures of the peptide analogs are shown in Table I.

peptides could be considered to belong to both categories.

Receptor Binding Studies—The peptides were tested for binding to the cloned GLP-1 receptor present on stably transfected CHL cells and to glucagon receptors present on liver membranes. The cloned glucagon receptor was not used in this study as its apparent binding affinity, when expressed in COS or baby hamster kidney cells, was significantly lower than that of the natural glucagon receptor present on liver membranes.

Glucagon Analogs on the Glucagon Receptor—The two glucagon analogs with GLP-1 substitutions in the NH_2-terminal part of the molecule, [Ala^2,Glu^3]glucagon and [Val^{10},Ser^{12}]-glucagon, bound to the glucagon receptor with ≥666- and 193-fold reduced affinities, respectively, as compared with glucagon itself (Fig. 1 and Table I). When the substitution of all 4 divergent residues was combined, resulting in the GLP-1(N)-glucagon(C) analog (or [Ala^2,Glu^3,Val^{10},Ser^{12}]glucagon), an affinity comparable to that resulting merely from the substitution Ala^2,Glu^4 was observed. This result fully agrees with conclusions from a previous analysis (17), suggesting that the exact chemical nature of the residue present at position 2 highly influences the binding affinity of glucagon analogs. In comparison, substitutions affecting the COOH-terminal domain of glucagon reduced the receptor affinity considerably less (between 1.9- and 31-fold) (Table I).

Glucagon Analogs on the GLP-1 Receptor—The affinity of the analog in which the entire NH_2-terminal part of the glucagon molecule was GLP-1-like was only 1.5-fold higher than that of glucagon itself on the GLP-1 receptor: IC_{50} = 440 nM for glucagon and 290 nM for GLP(N)-glucagon(C), respectively (Table I). In contrast, substitutions with GLP-1 residues in the COOH-terminal part of glucagon, which only slightly impaired binding to the glucagon receptor, improved the affinity of the molecule for the GLP-1 receptor. The gain in GLP-1 receptor affinity was most pronounced with substitutions located at the far COOH-terminal end of the glucagon molecule. Thus, [Val^{27},Lys^{28},Gly^{29},Arg^{30}]glucagon, in which only the 3 COOH-terminal residues of glucagon were substituted with the corresponding 4 GLP-1 residues, binds with a 169-fold higher affinity to the GLP-1 receptor compared with glucagon.

GLP-1 Analogs on the GLP-1 Receptor—Substitutions at the NH_2-terminal end of GLP-1, as in [Ser^2,Gln^3]GLP-1 and [Tyr^{10},Lys^{12}]GLP-1, had a relatively minor effect on the binding affinity for the GLP-1 receptor, yielding decreases in affinity of 13- and 2.5-fold, respectively (Table I). This is in contrast to the result obtained with substitutions at the corresponding position in glucagon.

The loss in GLP-1 receptor affinity was most pronounced when glucagon residues were introduced at the very COOH-terminal end of GLP-1, as in des-Arg^{30}-[Met^{27},Asn^{28},Thr^{29}]GLP-1 (IC_{50} = 190 nM, corresponding to a 475-fold decrease; Fig. 1*C* and Table I). The importance of these residues for selective GLP-1 receptor recognition is in agreement with the increased affinity for the GLP-1 receptor, which was observed when the corresponding GLP-1 residues were introduced into glucagon, as in [Val^{27},Lys^{28},Gly^{29},Arg^{30}]glucagon (Fig. 1*B*).

GLP-1 Analogs on the Glucagon Receptor—Substitutions with glucagon residues in the NH_2-terminal part of GLP-1 did not, when performed individually, increase the affinity of the molecule for the glucagon receptor, *e.g.* [Ser^2,Gln^3]GLP-1 and [Tyr^{10},Lys^{12}]GLP-1. However, when combined as in the analog glucagon(N)-GLP-1(C) ([Ser^2,Gln^3,Tyr^{10},Lys^{12}]glucagon), the affinity for the glucagon receptor increased from undetectable to 47 nM (Fig. 1*D* and Table I). In contrast, substitutions with glucagon residues in the COOH-terminal part of the GLP-1 molecule, which led to a dramatic loss in GLP-1 receptor reactivity, did not increase glucagon receptor affinity appreciably (IC_{50} > 1000 nM). Not even substitution of the entire COOH-terminal part of the GLP-1 molecule with the sequence of glucagon made the molecule react detectably with the glucagon receptor (Table I).

Combined Glucagon and GLP-1 Receptor Ligands—From the above results, it is clear that the affinity of the glucagon mol-

ecule for the GLP-1 receptor can be increased by COOH-terminal substitutions without loss of glucagon receptor reactivity and that the affinity of the GLP-1 molecule for the glucagon receptor can be increased by NH_2-terminal substitutions without a major loss of GLP-1 receptor affinity. Consequently, peptides consisting of the NH_2-terminal part of the glucagon molecule and the COOH-terminal part of the GLP-1 molecule react well with both receptor types, as exemplified by the glucagon(N)-GLP-1(C) analog and especially [Val^{27},Lys^{28},Gly^{29},Arg^{30}]glucagon, which binds with similar and high affinity to both the glucagon and GLP-1 receptors (Table I).

DISCUSSION

In homologous peptides, selective receptor recognition is often determined by a few residues that dictate whether the hormone is recognized by either one or the other selective receptor. Receptor selectivity may be reversed, *i.e.* the affinity increased on one and decreased on the other by exchange of a few residues. For example, residues located in the COOH-terminal part of neuropeptide Y and pancreatic peptide determine whether the peptide is recognized by either the neuropeptide Y or pancreatic peptide receptor (29). In the tachykinin family of peptides, the identical COOH-terminal hexapeptide binds equally well, albeit with a somewhat reduced affinity, to all receptor subtypes, and the divergent NH_2-terminal sequences of substance P, NKA, and NKB then determine the high affinity recognition with their respective receptors (30). In analogy with these examples, we would have expected that the receptor selectivity of glucagon and GLP-1 would be determined by corresponding residues in the two homologous peptides, for example by residues located in the rather divergent COOH-terminal part of the molecules. If this had been the case, we should have been able to exchange the high affinity receptor binding by the substitution of some particular residues. Unexpectedly, we have found instead that by NH_2-terminal peptide modifications, the selective recognition by the glucagon receptor can be destroyed in the glucagon molecule and built into the GLP-1 molecule without appreciably changing the reactivity of the peptides on the GLP-1 receptor. Conversely, by COOH-terminal modifications, the selective reactivity on the GLP-1 receptor can be destroyed in the GLP-1 molecule and built into the glucagon molecule without a significant effect on binding of the glucagon receptor.

A number of residues located in the NH_2-terminal part of GLP-1 were identified in a recent alanine scan as being important for receptor binding (14). These positions, His^1, Gly^4, Phe^6, Thr^7, and Asp^9, all correspond to residues conserved between GLP-1 and glucagon, residues that were not probed in this study, which was exclusively directed at divergent residues. Each of the 4 COOH-terminal residues, which in this study were found to determine the selective recognition of GLP-1 by the GLP-1 receptor, only gave a 5–17-fold reduction in affinity when substituted with alanine. Alanine substitution of each of the most important residues in the NH_2-terminal end of the GLP-1 molecule reduced the affinity several hundred-fold (14). Thus, although selective recognition is determined by residues located in the far COOH-terminal end of GLP-1, the NH_2-terminal conserved part of the molecule also appears to be very important for receptor recognition of the peptide.

An interesting consequence of the differentially located selective recognition epitopes is the possibility of developing peptides that bind with high affinity to two receptor types in this receptor family. In this paper, we describe analogs like glucagon(N)-GLP-1(C) and [Val^{27},Lys^{28},Gly^{29},Arg^{30}]glucagon that have almost the same high affinity toward the glucagon and GLP-1 receptors (Table I). It will be interesting to determine in the other members of this peptide family which part of the molecules may dictate their receptor selectivity. It could be envisioned that other *chimeric peptides* between members of the peptide family could be constructed in such a way that they would similarly be capable of reacting with more than one receptor type, for example glucagon/gastric inhibitory peptide chimeras, vasoactive intestinal peptide/GLP-1 chimeras, glucagon/secretin chimeras, etc.

When expressed either transiently in COS cells or stably in baby hamster kidney cells, the affinity of the glucagon receptor for the natural glucagon ligand was significantly lower (data not shown) than seen for the high affinity form of liver membranes. Presumably, this was caused by inefficient coupling to G-protein, either due to stoichiometry or perhaps an inappropriate subtype of G-protein.

We are currently constructing a series of chimeric glucagon/GLP-1 receptors as a complementary, alternative approach to identify essential epitopes for selective receptor-ligand recognition. From these studies, we hope to be able to determine residues of the receptors that correspond to the epitopes identified in this analysis using chimeric peptides.

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REFERENCES

1. Jacobsen, C. R., and Schwartz, T. W. (1990) in *Neuropeptides and their Receptors* (Schwartz, T. W., Hilsted, L., and Rehfeld, J. F., eds) pp. 403–446, Munksgaard International Publishers Ltd., Copenhagen
2. Mojsov, S., Heinrich, G., Wilson, I. B., Ravazzola, M., Orci, L., and Habener, J. F. (1986) *J. Biol. Chem.* **261,** 11880–11889
3. Ørskov, C., Holst, J. J., Knuhtsen, S., Baldissera, F. G. A., Poulsen, S. S., and Nielsen, O. V. (1986) *Endocrinology* **119,** 1467–1475
4. Thorens, B. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89,** 8641–8645
5. Jelinek, L. J., Lok, S., *et al.* (1993) *Science* **259,** 1614–1616
6. Segre, G. V., and Goldring, S. R. (1993) *Trends Endocrinol. Metab.* **4,** 309–314
7. Ørskov, C. (1992) *Diabetologia* **35,** 701–711
8. Nauck, M., Kleine, N., Ørskov, C., Holst, J. J., Wilms, B., and Creutzfeldt, W. (1993) *Diabetologia* **36,** 741–744
9. Brand, C. L., Jørgensen, P. N., and Holst, J. J. (1994) *Diabetes* **43,** 172a (Abstr. 535)
10. Brand, C., Rolin, B., Jørgensen, P. N., Svendsen, I., Kristensen, J. S., and Holst, J. J. (1994) *Diabetologia* **37,** in press
11. Lin, M. C., Wright, D. E., Hruby, V. J., and Rodbell, M. (1985) *Biochemistry* **14,** 1559–1563
12. Unson, C. G., Macdonald, D., and Merrifield, R. B. (1993) *Arch. Biochem. Biophys.* **300,** 747–750
13. Gefel, D., Hendrick, G. K., Mojsov, S., Habener, J. F., and Weir, G. C. (1990) *Endocrinology* **126,** 2164–2168
14. Adelhorst, K., Hedegaard, B. B., Knudsen, L. B., and Kirk, O. (1994) *J. Biol. Chem.* **269,** 6275–6278
15. Kofod, H., Andreu, D., Thams, P., Merrifield, R. B., Hedeskov, C. J., Hansen, B., and Lernmark, Å. (1988) *Am. J. Physiol.* **254,** E454–E458
16. Unson, C. G., Macdonald, D., Ray, K., Durrah, T. L., and Merrifield, R. B. (1991) *J. Biol. Chem* **266,** 2763–2766
17. Unson, C. G., Wu, C.-R., Fitzpatrick, K. J., and Merrifield, R. B. (1994) *J. Biol. Chem.* **269,** 12548–12551
18. Unson, C. G., Wu, C.-R., and Merrifield, R. B. (1994) *Biochemistry* **33,** 6884–6887
19. Hruby, V. J., Krstenansky, J., Gysin, B., Pelton, J. T., Trivedi, D., and McKee, R. L., (1986) *Biopolymers* **25,** 135–155
20. Gallwitz, B., Schmidt, W. E., Conlon, J. M., and Creutzfeldt, W. (1990) *J. Mol. Endocrinol.* **5,** 33–39
21. Kawai, K., Watanabe, Y., Suzuki, S., Ohashi, S., Mukai, H., and Yamashita, K. (1991) *Diabetes* **40,** 71a (abstr.)
22. Neville, D. M. (1968) *Biochim. Biophys. Acta* **154,** 540–552
23. Holst, J. J. (1975) *Diabetologia* **11,** 211–219
24. Holst, J. J., Ørskov, C., Nielsen, O. V., and Schwartz, T. W. (1987) *FEBS Lett.* **211,** 169–174
25. Schwartz, T. W. (1990) in *Molecular Biology of the Islets of Langerhans* (Okamoto, H., ed) pp. 153–205, Cambridge University Press, Cambridge, Great Britain
26. Ørskov, C., Holst, J. J., Poulsen, S. S., and Kirkegaard, P. (1987) *Diabetologia* **30,** 874–881
27. Ørskov, C., Bersani, M., Johnsen, A. H., Højrup, P., and Holst, J. J. (1989) *J. Biol. Chem.* **264,** 12826–12829
28. Göke, B., Göke, R., Holst, J. J., and Ørskov, C. (1993) *Digestion* **54,** 339–340
29. Jørgensen, J. C., Fuhlendorff, J., and Schwartz, T. W. (1990) *Eur. J. Pharmacol.* **186,** 105–114
30. Cascieri, M. A., Huang, R. R., Fong, T. M., Cheung, A., Sadowski, S., Ber, E., and Strader, C. D. (1992) *Mol. Pharmacol.* **41,** 1096–1099
31. Thorell, J. I., and Johansson, B. G. (1971) *Biochim. Biophys. Acta* **251,** 363–369