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Interaction of the Glucagon Receptor and a D64K Mutant with Position 12, 17, and 18 Replacement Analogs of Glucagon

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Introduction

Recently, Carruthers *et al.* synthesized and expressed a gene for the rat glucagon receptor [1]. COS-1 cells expressing the synthetic receptor bound glucagon with affinity and peptide hormone specificity similar to native receptors on liver cells. The transfected COS cells also increased levels of intracellular cAMP when stimulated with glucagon. In initial mutagenesis studies, the functional role of Asp^{64} in the extracellular N-terminal domain of the receptor was tested. A mutation at this site in the related growth hormone releasing factor receptor, was shown to be responsible for a genetic defect that results in mice of small size with hypoplastic pituitary glands [2]. Replacement of Asp^{64} resulted in a mutant receptor that did not bind glucagon and indicated that Asp^{64} played an important role in glucagon receptor binding [1]. This work was conducted to find the corresponding positively charged residue in the peptide hormone, which presumably could be involved with Asp^{64} of the receptor in an electrostatic interaction.

Results and Discussion

The hormone contains basic residues Lys^{12}, Arg^{17} and Arg^{18}, aside from His^{1} which are protonated at the physiological pH. We have previously shown that the amino terminal histidine contributes a determinant for both receptor recognition and the subsequent transduction of the hormone signal [3]. Nine glucagon analogs containing amino acid replacements at positions 12, 17, and 18 were synthesized by the solid phase method and tested for binding to receptors on liver membranes, and for the ability to stimulate adenylate cyclase. (Table 1).

Replacement of lysine at position 12 with a neutral alanine residue (analog #1) resulted in 83% loss in binding affinity for the glucagon receptor. Deletion of position 1 histidine (analog #2) reduced it further to less than 1%. Neutralization of the charge at Lys^{12} by acetylation in the analog ε-acetyl lysine glucagon amide (analog #3), resulted in about 50% loss in binding. Reversal of the charge at position 12, in the analogs Asp^{12} (analog #4), Glu^{12} (analog #5) and des-$His^{1}Glu^{12}$ (analog #6), resulted in 99% loss in binding. Interestingly, residual binding affinity in analogs # 1-6 was still sufficient to

Table 1. *Replacement analogs of positively charged residues in glucagon.*

Analog of glucagon amide	% Binding affinity	Cyclase activation	
		% Max. activity	% Rel. Potency
glucagon amide	100	100	15
1. Ala^{12}	17	60	17
2. des-$His^{1}Ala^{12}$	0.92	12	0.15
3. $AcLys^{12}$	47	90	32
4. Asp^{12}	0.6	78	10
5. Glu^{12}	1	80	50
6. des-$His^{1}Glu^{12}$	0.11	28	0.28
7. Ala^{17}	38	29	0.013
8. des-$His^{1}Ala^{17}$	2.3	29	0.28
9. Ala^{18}	13	94	71

transduce the signal, since all of these peptides were able to stimulate adenylate cyclase with relative potencies ranging from 10-50% with reduced activity for each of the *des*-His^{1} derivatives. Replacement of Arg^{17} with a neutral alanine (analog #7) resulted in an analog that retained 38% binding but whose relative potency in the cyclase assay was reduced to 0.013%. In contrast, replacement of Arg^{18} with alanine (analog #9) affected binding with a loss of 87% affinity but had only a slight effect on adenylate cyclase activity. At position 12 and 18, the positive charge appears to be important for binding only. However, at position 17, the positive charge influences both receptor recognition and activation.

The D64K mutant of the glucagon receptor was synthesized by replacement in the synthetic gene of a 74-bp *Bsi*WI-*Kpn*I restriction fragment with a duplex containing the desired codon alteration. COS-1 cells expressed the mutant receptor gene at normal levels, but failed to bind glucagon. However, upon incubation with Asp^{12} or Glu^{12} glucagon amide, cells expressing D64K caused a 32% and 43% increase in intracellular cAMP levels compared to cells expressing wild-type receptor when challenged with glucagon. This result might suggest that the opposite but complementary substitutions on both ligand and receptor restored some of the binding affinity, enough to partially allow transduction of the signal. Not one particular residue but all four positive residues in glucagon may contribute to binding affinity and interaction with Asp^{64} of the glucagon receptor. This study was supported by USPHS grant DK24039.

References

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