activity has been obtained from secretin, the C-terminal octapeptide of cholecystokinin-pancreozymin shows a higher cholecystokinin and pancreozymin activity than the whole hormone, even on a molar basis (Ondetti, Rubin, Engel, Pluščec & Sheehan, 1970). In addition to the homology with the gastrins and with caerulein, cholecystokinin-pancreozymin shows some similarity to calcitonin (Bell *et al.* 1968; Potts, Niall, Keutmann, Brewer & Deftos, 1968). In the porcine hormones the amino acids are identical in positions 16, 20, 21 and 23. In position 17 there is an aspartyl residue in cholecystokinin-pancreozymin and an asparaginyl residue in calcitonin.

It has recently become evident that, in addition to secretin and cholecystokinin-pancreozymin, the wall of the upper intestine, and the polypeptide concentrates described above, contain several other biologically active polypeptides of probably hormonal nature. Two of these, one inhibiting gastric acid secretin (Brown, Mutt & Pederson, 1970), the other increasing blood flow to the splanchnic area, but also exhibiting several other activities (Said & Mutt, 1970), have been found to be chemically related to secretin and to glucagon (Said & Mutt, 1970; Brown, 1971).

Comparison of the amino acid sequences, when determined, of these four polypeptides, chemically related but with partly overlapping and partly different biological activities, may, it is hoped, shed some light on the structural requirements for these activities.

- Anastasi, A., Erspamer, V. & Endean, R. (1967). *Experientia*, **23**, 699.
- Bayliss, W. M. (1920). In *Principles of General Physiology*, 3rd ed., p. 712. New York: Longmans.
- Bell, P. H., Barg, W. F., jun., Colucci, D. F., Davies, M. C., Dziobkowski, C., Englert, M. E., Heyder, E., Paul, R. & Snedeker, E. H. (1968). *J. Am. chem. Soc.* **90**, 2705.
- Bodanszky, M., Ondetti, M. A., Levine, S. D. & Williams, N. J. (1967). *J. Am. chem. Soc.* **89**, 6753.
- Bromer, W. W., Sinn, L. G. & Behrens, O. K. (1957). *J. Am. chem. Soc.* **79**, 2807.
- Brown, J. C. (1971). *Can. J. Biochem.* **49**, 255.
- Brown, J. C., Mutt, V. & Pederson, R. A. (1970). *J. Physiol., Lond.*, **209**, 57.
- Fishman, L. (1959). *Fedn Proc. Fedn Am. Socs exp. Biol.* **18**, 226.
- Greengard, H. (1948). In *The Hormones*, vol. 1, p. 201. Ed. by Pincus, G., Thimann, K. V. & Astwood, E. B. New York: Academic Press Inc.
- Gregory, H., Hardy, P. M., Jones, D. S., Kenner, G. W. & Sheppard, R. C. (1964). *Nature, Lond.*, **204**, 931.
- Grossman, M. I. (1950). *Physiol. Rev.* **30**, 33.
- Harper, A. A. & Raper, H. S. (1943). *J. Physiol., Lond.*, **102**, 115.
- Ivy, A. C. & Oldberg, E. (1928). *Am. J. Physiol.* **86**, 599.
- Jorpes, J. E. & Mutt, V. (1961). *Acta chem. scand.* **15**, 1790.

- Jorpes, J. E. & Mutt, V. (1966). *Acta physiol. scand.* **66**, 196.
- Jorpes, J. E., Mutt, V., Magnusson, S. & Steele, B. B. (1962). *Biochem. biophys. Res. Commun.* **9**, 275.
- Jorpes, J. E., Mutt, V. & Toczko, K. (1964). *Acta chem. scand.* **18**, 2408.
- Legge, J. W., Morieson, A. S., Rogers, G. E. & Marginson, M. A. (1957). *Aust. J. exp. Biol.* **35**, 569.
- Mutt, V. & Jorpes, J. E. (1968). *Eur. J. Biochem.* **6**, 156.
- Mutt, V., Jorpes, J. E. & Magnusson, S. (1970). *Eur. J. Biochem.* **15**, 513.
- Mutt, V., Magnusson, S., Jorpes, J. E. & Dahl, E. (1965). *Biochemistry, Easton*, **4**, 2358.
- Newton, G. G. F., Love, J. W., Heatly, N. G. & Abraham, E. P. (1959). *Biochem. J.* **71**, 6P.
- Ondetti, M. A., Rubin, B., Engel, S. L., Pluščec, J. & Sheehan, J. T. (1970). *Am. J. digest. Dis.* **15**, 149.
- Potts, J. T., jun., Niall, H. D., Keutmann, H. T., Brewer, H. B., jun. & Deftos, L. J. (1968). *Proc. natn. Acad. Sci. U.S.A.* **59**, 1321.
- Said, S. I. & Mutt, V. (1970). *Science, N.Y.*, **169**, 1217.
- Smith, E. L. (1967). *Harvey Lect. Ser.* no. 62, p. 231.

Characteristics of Glucagon Action on the Hepatic Adenylate Cyclase System

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Hormone-sensitive adenylate cyclase systems are multimolecular communication devices integrated into the plasma membranes of target cells (Birnbaumer & Rodbell, 1969). In the rat hepatic system glucagon reacts specifically through non-covalent forces with a component, termed 'discriminator', that functions to regulate the activity of adenylate cyclase (Rodbell, Krans, Pohl & Birnbaumer, 1971a). Lipids play an essential role in the binding of glucagon and the regulatory function of the discriminator. Treatment of hepatic membranes with phospholipases and detergents results in concomitant losses of the binding and regulatory functions without affecting the activity and response of adenylate cyclases to fluoride ion, which acts directly on the enzyme (Birnbaumer, Pohl & Rodbell, 1971). Addition of phospholipids to treated membranes results in partial restoration of the binding and functional properties of the discriminator (Pohl, Krans, Kozyreff, Birnbaumer & Rodbell, 1971). The precise function of lipids in the binding process is unknown, but they may participate in the hydrophobic forces involved in binding of glucagon to the discriminator, as evidenced by the reversible inhibitory effects of low concentrations of urea and the marked sensitivity of binding to temperature (Rodbell, Krans, Pohl & Birnbaumer, 1971b).

Studies of the binding and activity of enzymically and chemically produced fragments of glucagon have provided some clues to the structural-functional relationships in the glucagon molecule (Rodbell, Birnbaumer, Pohl & Sundby, 1971d). Removal of the *N*-terminal histidine residue by a one-step Edman degradation procedure results in loss of biological activity of the hormone. The product, des-histidine-glucagon, binds to the discriminator and competitively inhibits both the binding of glucagon and the response of adenylate cyclase to the hormone. Removal of six of eight of the amino acids from the intensely hydrophobic *C*-terminal region results in complete loss of both binding and activity of glucagon. Thus nearly the entire molecule is required for biological activity, the *N*-terminal histidine residue and the *C*-terminal hydrophobic region being essential for activity and for interaction with the discriminator respectively. Evidence (Bornet & Edelhoch, 1971) that the detergent cetyltrimethylammonium bromide 'binds' to the hydrophobic *C*-terminal region of glucagon and induces tertiary structure into the essentially structureless molecule, taken together with the evidence that glucagon binds to the discriminator via hydrophobic forces, suggests that the environment of the discriminator determines the conformation of glucagon required for biological activity, i.e. the 'message' contained within the molecule is only revealed at its site of action.

Glucagon activates instantaneously the adenylate cyclase system at all concentrations of the hormone tested (Pohl, Birnbaumer & Rodbell, 1971). In the presence of substrate, MgATP, addition of des-histidine-glucagon causes the response of adenylate cyclase to decay to basal activity within 45s (L. Birnbaumer, S. L. Pohl & M. Rodbell, unpublished work). These findings indicate that the components of the system exist in rapidly reversible states of activity and that occupation of the discriminator by glucagon is transient; the system requires the continuous presence of glucagon for maintenance of activity. It has also been found that glucagon does not activate adenylate cyclase at low concentrations of ATP (below 0.1mM) unless GTP (or GDP) at concentrations as low as 50nM is present (Rodbell, Birnbaumer, Pohl & Krans, 1971c). The guanyl nucleotides (or much higher concentrations of ATP or ADP) change binding of glucagon from a slow, essentially irreversible, process to one that exhibits both rapid binding and dissociation of the hormone (Rodbell *et al.* 1971b). Only in the presence of the purine nucleotides does des-histidine-glucagon exchange with glucagon at the discriminator and block the action of the hormone. However, measurements of the amount of bound glucagon exchanged with des-histidine-glucagon indicated that only a fraction of the

binding sites need be occupied by the competitive analogue for complete cessation of hormone action. Other studies indicated that 10%, at most, of the binding sites can represent the active species of the discriminator.

The findings related above are compatible with the reaction scheme:

where (s) indicates the inactive state and (r) indicates the active state, and cAMP represents cyclic AMP (adenosine 3':5'-cyclic monophosphate). In this scheme the discriminator and the enzyme exist in at least two states, the equilibrium between these states being shifted, in the case of the discriminator, to the active state ($D_{(r)}$) by GTP or ATP, and to the active state of the enzyme ($E_{(r)}$) by interaction of this state with the complex formed between glucagon and $D_{(r)}$. According to this reaction scheme the extent of activation of adenylate cyclase by glucagon is determined by the ratio $D_{(r)}/D_{(s)}$ and the concentration of free hormone and nucleotides available for binding at their respective allosteric sites in the system.

- Birnbaumer, L., Pohl, S. L. & Rodbell, M. (1971). *J. biol. Chem.* **246**, 1857.
 Birnbaumer, L. & Rodbell, M. (1969). *J. biol. Chem.* **244**, 3477.
 Bornet, H. & Edelhoch, H. (1971). *J. biol. Chem.* **246**, 1785.
 Pohl, S. L., Birnbaumer, L. & Rodbell, M. (1971). *J. biol. Chem.* **246**, 1849.
 Pohl, S. L., Krans, H. M. J., Kozyreff, V., Birnbaumer, L. & Rodbell, M. (1971). *J. biol. Chem.* **246**, 4447.
 Rodbell, M., Birnbaumer, L., Pohl, S. L. & Krans, H. M. J. (1971c). *J. biol. Chem.* **246**, 1877.
 Rodbell, M., Birnbaumer, L., Pohl, S. L. & Sundby, F. (1971d). *Proc. natn. Acad. Sci. U.S.A.* **68** (in the Press).
 Rodbell, M., Krans, H. M. J., Pohl, S. L. & Birnbaumer, L. (1971a). *J. biol. Chem.* **246**, 1861.
 Rodbell, M., Krans, H. M. J., Pohl, S. L. & Birnbaumer, L. (1971b). *J. biol. Chem.* **246**, 1872.