MATTERS ARISING

Does the C-terminal tetrapeptide of gastrin and CCK exist as an entity?

IT was reported by Rehfeld *et al.*[1] that the COOH-terminal tetrapeptide (Trp-Met-Asp-Phe-NH_2, abbreviated to G4 or CCK4), which is common to gastrin and cholecystokinin (CCK), exists in free form in pancreatic nerves, and may control islet hormone release. This report extends previous studies from the same group in which G4 was said to occur in pyloric antral mucosa in concentrations up to twice those of heptadecapeptide gastrin (G17), and in intestine and brain in concentrations considerably higher than those of the 33-residue form of CCK (CCK33) or its COOH-terminal octapeptide (CCK8). In these studies, particular importance was attached to the observation that after gel filtration of tissue extracts, a peak of material eluted in the position of G4 and reacted in radioimmunoassays using antisera specific for the COOH-terminus of gastrin and CCK. However, recent studies in our laboratory cast doubt on the interpretation of these data, and suggest instead that free G4 does not exist in significant amounts in pancreas or gut.

We have raised a rabbit antiserum to G4 conjugated through its NH_2-terminal amino group to thyroglobulin by glutaraldehyde. When used in radioimmunoassays with an ^{125}I-CCK8 label, this antiserum reacts almost equally with G4, G17 and CCK8, and can detect 10–20 pmol l^{-1} of G4. This system offers a clear advantage for the estimation of G4 over the one used by Rehfeld *et al.*, because in the latter system G4 has a 30-fold lower immunochemical potency than CCK8 and CCK33. To estimate the true molar concentration of G4, it was, in effect, necessary for Rehfeld *et al.* to multiply the peak of activity eluting in the position of G4 by 30 compared with the other peaks. There is an obvious disadvantage in this method, for errors are also greatly magnified. Such a correction is unnecessary, of course, when G4, CCK8 and G17 and the other principal forms of CCK and gastrin have equal immunoreactivity. We have found that when hog pancreas is extracted according to the method of Rehfeld *et al.*, the total concentration of immunoreactivity measured with the G4 antiserum was 3.5 ± 0.8 pmol per g (mean, ± s.e., $n = 4$; G4 standard). Closely similar estimates (2.1 ± 0.4 pmol per g) were obtained with a second radioimmunoassay system using an antibody (L48, CCK8 standard) that, like Rehfeld's, reacts about 50 times less well with G4 than with CCK8. Fractionation on Sephadex G-50 indicated that over 80% of immunoreactivity in the extracts had the properties of CCK8. These data indicate that G4 cannot occur in more than trace amounts in pancreas. However, when synthetic G4 was added to the tissue early in the extraction procedure in amounts comparable to those reported by Rehfeld *et al.*, the peptide was recovered in a yield of $73 \pm 6\%$. In parallel studies we have also failed to confirm the presence of G4 in hog, rat or human antral mucosal extracts, which according to Rehfeld *et al.* contain G4 at about 15 nmol per g; instead, we have consistently found a major peak of immunoreactivity corresponding to G17, confirming numerous previous studies.

We conclude from our work that free G4 does not occur in significant amounts in pancreas or gut. The high concentrations of G4 reported by Rehfeld *et al.* reflect the fact that a minor component of gastrin–CCK immunoreactivity is interpreted as G4 and estimates of its molar concentration corrected to allow for the low potency of G4 in the assay system. This correction might conceivably be justified if G4 was proven to exist naturally by isolation and full chemical characterization; this has not been the case. Until the material described by Rehfeld *et al.* in pancreas and elsewhere is isolated and characterized, its true concentration in these tissues cannot be determined. The issues raised here serve to emphasize the very great caution that needs to be exercised in identifying and determining substances by gel filtration and radioimmunoassay, when the material in question has not been isolated.

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1. Rehfeld, J. F. *et al. Nature* **284**, 33–38 (1980).

REHFELD *ET AL.* REPLY—First, we note that the total CCK immunoreactivity in pancreatic extracts measured by the G4 antiserum, 3.5 pmol per g, is of the same order of magnitude as that reported by us[1] (6.8 pmol equiv. CCK8 per g). It is interesting also that the L48 antiserum, which reacts less well with G4, measures lower concentrations (2.1 pmol per g) than the G4 antiserum, suggesting that G/CCK4 is indeed present in the pancreas.

Second, Dockray has previously reported[2] that the mammalian brain contains significant amounts of a third immunoreactive component (BP III) with a size corresponding to G/CCK4 or G/CCK6. He measured this BP III peak by four different C-terminal specific antisera (including our ab. 2716). Using CCK8 as standard, the BP III peak constituted 12% of the CCK8-like peak in hog, 31% in dog and 18% in rat cerebral cortex (Table 2 in ref. 2). The BP III peak should be easily measurable by the new G4 antiserum. We are surprised that Dockray has not discussed these earlier results in the light of his new assay findings.

Third, we can only speculate on the reason for the discrepancy between our data (supported by Dockray's earlier data[2]) and those of Dockray and Gregory. (1) Microheterogeneity of the natural tetrapeptide (in analogy with the microheterogeneity of CCK octapeptide[3]) might explain[4,5] the failure of the new assay to measure G/CCK4-like material. (2) Several samples of synthetic G4 received by us from other laboratories have been found to be grossly impure, and even the purest contain minor impurities—might the new G4 antiserum of Dockray and Gregory have specificity for these impurities? (3) In the raising of antibodies, we question the use of G4 conjugates prepared by means of glutaraldehyde. The presence in G4 of N-terminal Trp complicates the normal reaction with aldehydes, and we would anticipate that conjugation would be through a carboline rather than the terminal amino group. If this is so, the specificity of antibodies raised to such conjugates might be abnormal.

In conclusion, we consider the evidence to be in favour of the presence of a tetrapeptide in brain, gut and pancreas, but final confirmation must come from isolation and sequence work.

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1. Rehfeld, J. F. *et al. Nature* **284**, 33–38 (1980).
2. Dockray, G. J. *Nature* **264**, 568–570 (1976).
3. Dockray, G. J., Gregory, R. A., Harris, J. I., Hutchison, J. B. & Runswick, M. J. *Nature* **274**, 711–713 (1978).
4. Rehfeld, J. F. in *Hormone Receptors in Digestion and Nutrition* (eds Rosselin, G., Fromageot, P. & Bonfils, S.) 45–53 (Elsevier, Amsterdam, 1979).
5. Rehfeld, J. F. & Morley, J. S. in preparation.

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