GLUCAGON-LIKE PEPTIDE-1 7–36: A PHYSIOLOGICAL INCRETIN IN MAN

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Summary The physiological role of glucagon-like peptide-1 7–36 amide (GLP-1 7–36) in man was investigated. GLP-1 7–36-like immunoreactivity was found in the human bowel; its circulating level rose after oral glucose and after a test breakfast. When it was infused into seven volunteers at a rate to mimic its postprandial plasma concentration in the fasting state, plasma insulin levels rose significantly and glucose and glucagon concentrations fell. During an intravenous glucose load, it greatly enhanced insulin release and significantly reduced peak plasma glucose concentrations, compared with a control saline infusion, even inducing postinfusion reactive hypoglycaemia. By comparison, infusion of glucose-dependent insulinotropic peptide (GIP) to physiological levels was less effective in stimulating insulin release. These observations suggest that GLP-1 7–36 is a physiological incretin and that it is more powerful than GIP. The observation of greatly increased postprandial plasma GLP-1 7–36 levels in patients with postgastrectomy dumping syndrome suggests that it may mediate the hyperinsulinaemia and reactive hypoglycaemia of this disorder.

Introduction

SEQUENCING of mammalian glucagon messenger RNA has shown that the prohormone contains two separate glucagon-like peptides—GLP-1 and GLP-2.[1] The sequence of GLP-1, a 36-aminoacid peptide, is identical in various different mammals—man, ox, hamster, rat, and guineapig.[2] Such strong conservation suggests a biological role, but initial studies of complete GLP-1 did not identify any biological activity.[3] However, only the sequence after residue 7 shows similarities to glucagon and to other biologically active members of the secretin peptide family, particularly glucose-dependent insulinotropic peptide (GIP).[4] Moreover, this sequence has been especially well preserved, showing 66% nucleotide homology with GLP-1 in the proglucagon of the very primitive anglerfish.[5] This 7–36 sequence of GLP-1 is a potent insulin-releasing peptide in vitro.[6,7]

The plasma insulin response to a given rise in plasma glucose is much greater when glucose is given orally rather than injected intravenously. Indeed, the latter route results in a diabetic glucose curve. The important oral enhancement of insulin release is attributed to the "enteroinsular axis". In 1906 Moore postulated the presence of a hormonal factor in the gut that enhanced nutriment disposal; it was later named incretin.[8,9] The main candidate for incretin has been the gut-derived hormone GIP, which enhances glucose-stimulated insulin release in man.[10-13] However, administration of GIP antibodies does not abolish the incretin effect, implying that other factors operate.[14] Although many other hormones of the family of secretin, vasoactive intestinal peptide, and glucagon stimulate insulin release, their effect is seen only at supraphysiological concentrations.[15] The insulin secretagogue action of GLP-1 7–36 prompted us to investigate whether the peptide is present in the circulation in man and, if so, whether normal postprandial concentrations are sufficient to influence physiological insulin release.

Subjects and Methods

Plasma GLP-1 7–36 Responses to Oral Glucose and Eating

Five healthy subjects (mean age 31 [SEM 5] years, all within 15% of ideal body weight) drank 200 ml water containing 75 g glucose over 5 min after an overnight fast. The effects of oral glucose (50 g in 150 ml water) were similarly studied in six male patients who had undergone subtotal gastrectomy for carcinoma of the stomach 1–18 months previously. Their mean age was 62 (5) years and they were in apparently good health and within 10% of ideal body weight. All these patients had symptoms of the dumping syndrome (tachycardia, sweating, and faintness), which were also observed in each case during this test.[16] Blood samples were taken from an indwelling venous catheter before glucose administration and regularly up to 45 min thereafter in the healthy subjects and up to 120 min in the gastrectomy patients.

Six normal-weight subjects aged 25 (2) years ate a 2·2 MJ standard test breakfast (two eggs, toast, marmalade, and orange juice) after an overnight fast, and blood samples were taken at various times up to 120 min.

Infusion of GLP-1 7–36

Seven healthy normal-weight volunteers aged 24 (4) years, were studied after an overnight fast. After saline infusion for 30 min, sterile synthetic human GLP-1 7–36 (Peninsula Laboratories, Merseyside) was infused at a nominal rate of 0·5 pmol/kg/min for 70 min. On a separate occasion, and in random order, saline was infused throughout without GLP-1 7–36. Five of the subjects were studied a third time, when they received, instead of GLP-1 7–36, an

A. C MOESSINGER AND OTHERS: REFERENCES—*continued*

6 Wigglesworth JS, Desai R. Effects on lung growth of cervical cord section in the rabbit fetus *Early Hum Dev* 1979; **3:** 51–65.
7. Wigglesworth JS. The effects of placental insufficiency on the fetal lung. *J Clin Pathol* 1976; **29** (suppl) 27–30.
8. Blott M, Greenough A, Nicolaides KH, Moscoso G, Gibb D, Campbell S Fetal breathing movements as predictor of favourable pregnancy outcome after oligohydramnios due to membrane rupture in second trimester *Lancet* 1987, ii: 129–31.
9. Fox HE, Moessinger AC Fetal breathing movements and lung hypoplasia Preliminary human observations. *Am J Obstet Gynecol* 1985, **151:** 531–33
10. Fox HE, Inglis J, Steinbrecher M Fetal breathing movements in uncomplicated pregnancies I: Relationship to gestational age. *Am J Obstet Gynecol* 1979; **134:** 544–46.
11 Wigglesworth JS, Desai R. Use of DNA estimation for growth assessment in normal and hypoplastic fetal lungs *Arch Dis Child* 1981; **56:** 601–05
12. Blanc WA, Apperson JM, McNally J. Pathology of the newborn and of the placenta in oligohydramnios. *Bull Sloane Hosp* 1962; **8:** 51–64.
13 Silver MM, Vilos GA, Silver MD, Shaheed WS, Turner KL Morphologic and morphometric analyses of muscle in the neonatal myotonic dystrophy syndrome. *Hum Pathol* 1984, **15:** 1171–82
14 Vintzileos AM, Campbell WA, Nochimson DJ, Connoly ME, Feunfer MM, Hoehn GJ The fetal biophysical profile in patients with premature rupture of the membranes. an early predictor of fetal infection. *Am J Obstet Gynecol* 1985, **152:** 510–16
15. Moessinger AC, Fewell JE, Stark RI, et al. Lung hypoplasia and breathing movements following oligohydramnios in fetal lambs In Jones C, Nathanielsz P, eds. The physiological development of the fetus and newborn London Academic Press, 1985. 293–98.
16 Harding R, Sigger JN, Wickham PDJ, Bocking AD. The regulation of flow of pulmonary fluid in fetal sheep. *Respir Physiol* 1984, **57:** 47–59.
17. Moessinger AC, Collins MH, Blanc WA, Rey HR, James LS Oligohydramnios-induced lung hypoplasia: the influence of timing and duration in gestation *Pediatr Res* 1986; **20:** 951–54.
18 Nimrod C, Davies D, Iwanicki S, Harder J, Persuad D, Nicholson S Ultrasound prediction of pulmonary hypoplasia. *Obstet Gynecol* 1986; **68:** 495–97
19. Johnson A, Callan NA, Bhutani VK, Colmorgen GHC, Weiner S, Bolognese RJ. Ultrasonic ratio of fetal thoracic to abdominal circumference: an association with fetal pulmonary hypoplasia *Am J Obstet Gynecol* 1987, **157:** 764–69

MEAN (SEM) PLASMA GLUCOSE CONCENTRATION AND HORMONE-LIKE IMMUNOREACTIVITY OF INSULIN, GLP-1 7–36, AND GIP

—	Glucose (mmol/l)	Insulin (pmol/l)	GLP-1 7–36 (pmol/l)	GIP (pmol/l)
Normal subjects (n=5): oral glucose				
0	4·9 (0·2)	55 (10)	15 (6)	47 (16)
10 min	5·6 (0·3)	116 (44)	30 (4)	104 (20)
15 min	6·4 (0·4)	282 (63)	39 (5)	141 (23)
20 min	7·0 (0·5)	242 (56)	35 (7)	149 (25)
30 min	7·6 (0·7)	245 (39)	48 (4)	174 (24)
45 min	7·5 (0·8)	291 (30)	41 (4)	168 (23)
Gastrectomy patients (n=6): oral glucose				
0	4·9 (0·2)	44 (10)	10 (3)	40 (17)
15 min	6·1 (0·4)	279 (62)	45 (12)	83 (21)
30 min	9·1 (0·7)	485 (55)	85 (17)	122 (22)
45 min	9·8 (1·0)	616 (77)	77 (15)	113 (22)
60 min	11·9 (0·9)	626 (99)	57 (9)	79 (26)
120 min	8·7 (0·8)	349 (69)	34 (11)	48 (18)
Normal subjects (n=6): standard test meal				
0	4·7 (0·2)	39 (3)	16 (2)	55 (15)
15 min	4·8 (0·3)	61 (11)	15 (3)	78 (12)
30 min	5·8 (0·4)	112 (46)	33 (3)	88 (14)
45 min	6·4 (0·3)	171 (23)	37 (3)	123 (13)
60 min	6·0 (0·4)	155 (24)	31 (3)	110 (12)
120 min	5·4 (0·4)	150 (16)	28 (2)	98 (12)

infusion of human synthetic GIP (Peninsula Laboratories) at a nominal rate of 1·5 pmol/kg/min. Glucose (as a 25% weight/volume solution) was infused intravenously at 1 g/min between 30 and 50 min of the test infusion. Blood pressure and pulse rate were monitored regularly throughout the infusions. At the end of the infusion, a sample was taken from the infusion cannula for assay of the infused peptide. Informed consent was obtained from each subject and approval received from the hospital ethical committee.

Sample Treatment and Assays

Blood was taken at each time point into fluoride-oxalate tubes for blood glucose measurement by means of a glucose-oxidase-based autoanalyser (Beckman Instruments Inc, Fullerton, USA). 10 ml blood samples collected into lithium heparin tubes containing 4000 kIU aprotinin ('Trasylol R', Bayer, Haywards Heath) were immediately centrifuged, and the plasma was stored at −20°C until assay of peptide hormones. 2 ml plasma samples were extracted on 'Sep-Pak' cartridges (C. A. T. Walters Associated, Milford, Massachusetts, USA), eluted with 60% acetonitrile, freeze-dried, and reconstituted with assay buffer for the measurement of GLP-1 7–36 immunoreactivity. With this extraction procedure, the recovery of synthetic GLP-1 7–36 added to fasting plasma was 62 (2)% (n=5) and this factor was used to correct the measured concentration of unknown samples of GLP-1 7–36.

Insulin, glucagon, and GIP were measured by established radioimmunoassays,[17-19] except that synthetic human GIP was used instead of porcine GIP for the standard.

GLP-1 7–36 was measured by means of an antibody raised in a rabbit immunised with the peptide conjugated to bovine serum albumin by carbodi-imide.[20] The antibody crossreacted 100% with synthetic entire GLP-1 1–36NH_2 (Peninsula) but did not crossreact with glucagon, GIP, or other gut or pancreatic peptides. Iodine-125-labelled GLP-1 7–36 was prepared by the chloramine T method[21] and purified by high-performance liquid chromatography. The mixture was incubated for 5 days at 4°C and free and antibody-bound peptide were separated by means of dextran-coated charcoal. Concentration differences of 1·5 fmol per tube could be detected with 95% confidence. For all four radioimmunoassays the interassay and intra-assay coefficients of variation were under 10%.

Fresh human ileum was obtained at operation and human pancreas at necropsy. Tissues were diced and immediately extracted by boiling in 0·5 mol/l acetic acid.[22] Tissue extracts were subjected to chromatographic analysis on a 'Mono Q HR 515' column on a Pharmacia FPLC system (Uppsala, Sweden) with a linear elution buffer gradient of 0–20% buffer B (0·02 mol/l ethanolamine with 1·0 mol/l sodium chloride, pH 9·25) in buffer A (0·2 mol/l ethanolamine, pH 9·25) over 40 min at a flow rate of 1 ml/min. The same procedure was applied to sep-pak-extracted samples taken before and 30 min after oral glucose administration from both the healthy subjects and the postgastrectomy patients.

Results were expressed as mean and standard error of the mean and statistical significance analysed by Student's *t* test (unpaired or paired, as appropriate).

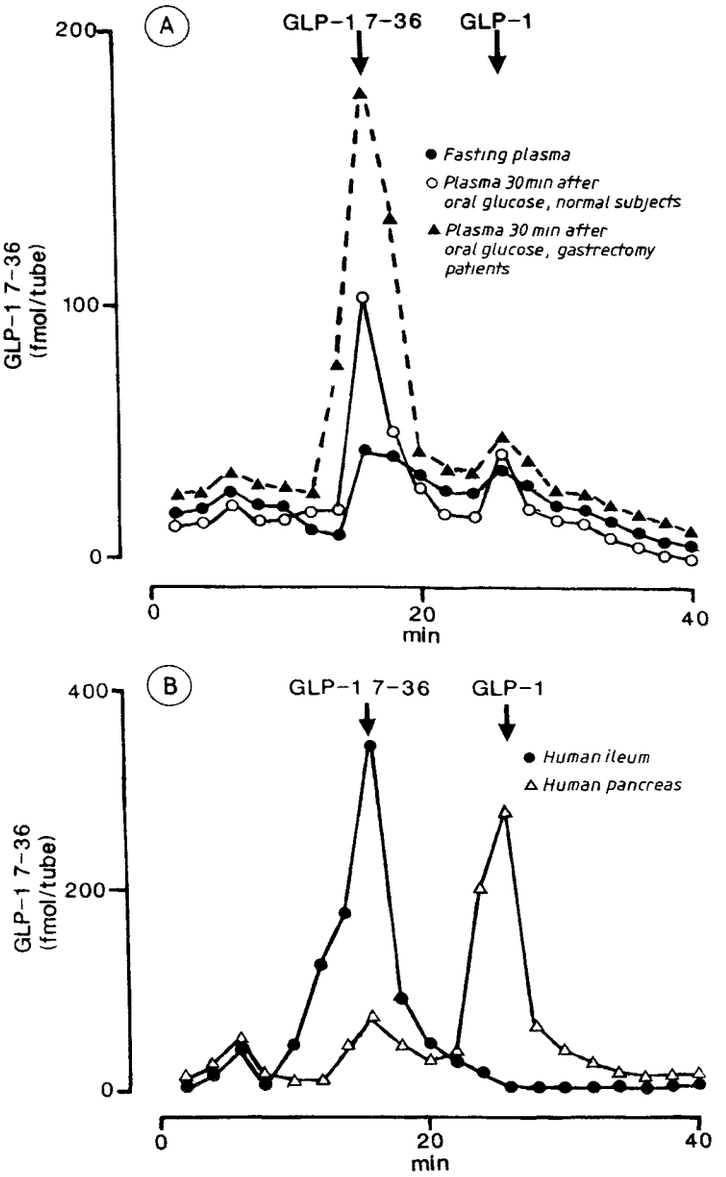

Fig 1—Ion-exchange chromatography profiles.

Arrows indicate elution positions of synthetic GLP-1 7–36 and GLP-1. Recovery of loaded peptide immunoreactivity was 65–70%.

Results

Glucose and Test Meal Ingestion

Responses of plasma GLP-1 7–36 immunoreactivity, glucose, insulin, and GIP to oral glucose and to the test breakfast in healthy volunteers are shown in the table. Much greater rises were seen in the postgastrectomy patients (table). On chromatography most of the GLP-1 7–36 immunoreactivity in plasma after a stimulus showed a similar pattern to that of the gut extract (fig 1).

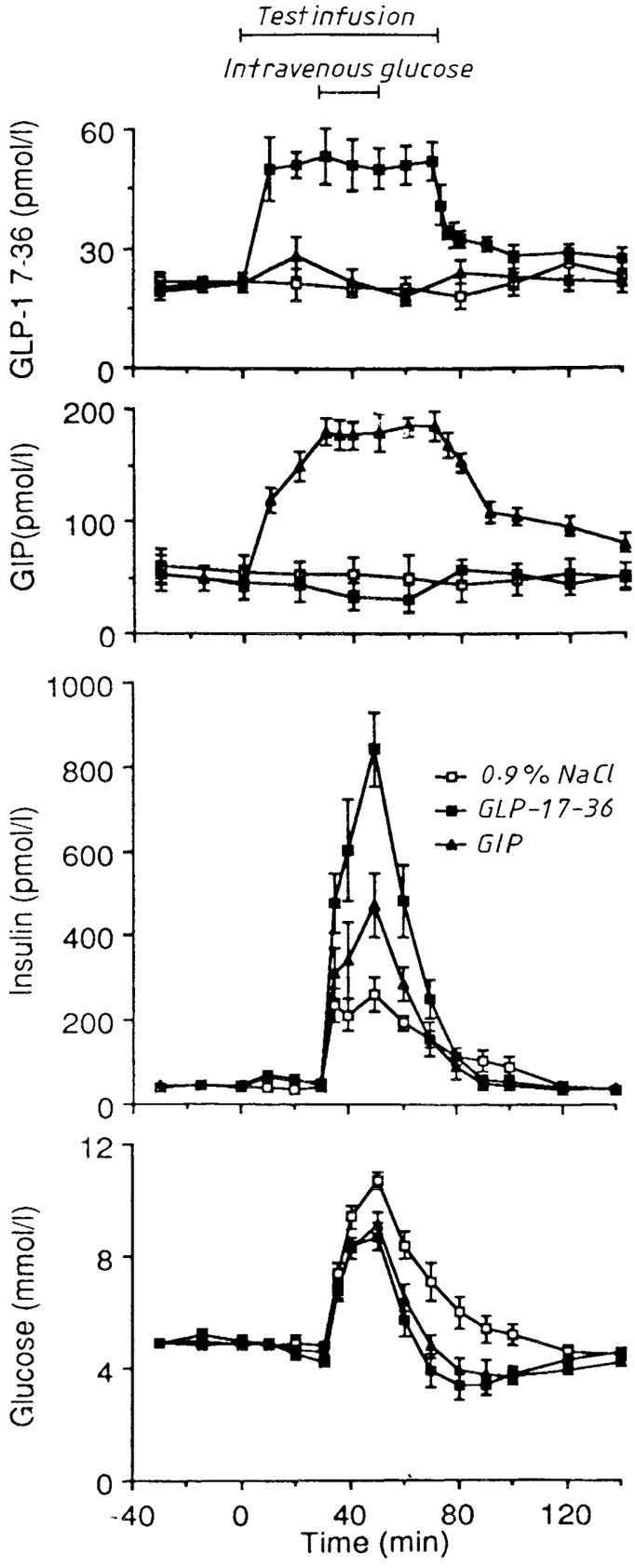

Fig 2—Venous plasma concentrations of glucose and hormone-like immunoreactivity of GLP-1 7–36, GIP, and insulin during infusion in normal subjects of GLP-1 7–36, GIP, or 0·9% saline.

GLP-1 7–36 Infusions

Infusions of GLP-1 7–36 and GIP were free of any side-effects or changes in pulse or blood pressure. The measured infusion rates achieved were 0·32 (0·03) pmol/kg/min for GLP-1 7–36 and 1·05 (0·06) pmol/kg/min for GIP. GLP-1 7–36 immunoreactivity rose to a steady plateau (increment 29 [5] pmol/l over baseline) which was maintained during the last 60 min of the infusion. The calculated metabolic clearance rate was 12 (2) ml/kg/min and the half-time of decay from the incremental plateau was 3·9 (0·5) min.[23] During the GIP infusion, a peak increment of 137 (8) pmol/l was achieved at 70 min but, since its half-life exceeded 30 min, this probably did not represent a steady state. During the GLP-1 7–36 infusion plasma insulin concentrations before glucose administration were significantly greater than baseline values at 10 and 20 min (65 [10] pmol/l, $p < 0·05$, and 70 [8] pmol/l, $p < 0·01$, compared with saline control; fig 2). A smaller but still significant rise in insulin was also seen at 10 min before glucose administration with the GIP infusion (63 [7] pmol/l, $p < 0·05$ compared with control). After 30 min of GLP-1 7–36 infusion the plasma glucose concentration was significantly lower than that during the control saline infusion. Glucagon was significantly suppressed during GLP-1 7–36 infusion at 20 and 30 min ($p < 0·02$), contrasting with a significant rise at 30 min during GIP infusion ($p < 0·05$).

Both GLP-1 7–36 and GIP significantly enhanced the release of insulin after stimulation by intravenous glucose from 30 min (total integrated area under the plasma insulin curve between 30 min and 70 min: saline 181 [28] pmol/l/min, GLP-1 7–36 504 [78] pmol/l/min, GIP 301 [63] pmol/l/min). Mean insulin increments were significantly greater than during the control infusion at all time points between 35 min and 60 min during GLP-1 7–36 infusion, whereas with GIP a significant increase over the control was seen only at 50 min. Plasma glucose concentrations were significantly lower at 50 min during both peptide infusions than during the control infusion. After the glucose infusion stopped glucose concentrations fell to half the peak level in 16 (3) min during GLP-1 7–36 infusion, 22·5 (5) min during GIP infusion, and 37·5 (7) min ($p < 0·01$ compared with GLP-1 7–36) during the saline infusion. During both GLP-1 7–36 and GIP infusions, glucose concentrations fell significantly below basal values (GLP-1 7–36 lowest 3·0 [0·3] mmol/l, GIP 3·3 [0·2] mmol/l).

Tissue Content of GLP-1 7–36

The chromatographic profiles of GLP-1 7–36 immunoreactivity in tissue and plasma are shown in fig 1. Only pancreas contained a significant peak of immunoreactivity in the position of synthetic whole GLP-1. The tissue concentration in fresh ileum was 127 (25) pmol/g wet weight of tissue ($n = 4$).

Discussion

Our finding that GLP-1 7–36 is a major product of the glucagon gene in the gut but not the pancreas of man accords with previous animal data.[7] Although the alpha cell of the pancreas and the enteroglucagon cell of the intestinal mucosa appear to have identical mRNAs coding for the preprohormone, they differ in their post-translational enzymic processing.[24] We have also shown (unpublished) that, as expected, postprandial rises in peripheral plasma GLP-1 7–36 immunoreactivity parallel those of another gut-specific product of the glucagon gene, enteroglucagon. We have now shown that infusion of exogenous GLP-1 7–36 enhances insulin release, both basal and glucose-stimulated, confirming previous observations in animals

that it could stimulate the beta cell.[6,7] The presence of a GLP-1 7–36-like peptide in human gut, its postprandial release into the circulation, and its ability to stimulate endogenous insulin secretion at physiological postprandial concentrations all suggest that this peptide may function as an incretin in man. This possibility invites comparison of GLP-1 7–36 with GIP, which hitherto has been accepted as the most likely candidate for incretin,[10] despite reported inconsistencies in the evidence.[25]

Previous studies on endogenous GIP release have used assays with porcine GIP as the standard. Since the aminoacid composition of these peptides differs slightly, true human GIP levels were not measured.[26] However, the availability of synthetic human GIP allowed us to compare accurately changes in the concentrations of endogenous human GIP with those produced by infusing exogenous human GIP. We have shown that human GIP is likely to be an insulinn secretagogue in man, being more powerful than the porcine peptide (Fuessl HS, Bloom SR, et al, unpublished) and that GIP is likely to have a physiological effect on insulin release. The earlier rise in GIP compared with that in GLP-1 7–36 after the test breakfast suggests that it may affect the early insulin response which is also mediated by the nervous system.[15]

In comparing the importance of endogenous GLP-1 7–36 and GIP in enhancing postprandial insulin release, both their observed plasma concentrations and insulinotropic potency should be taken into account. On a molar basis, the GIP increment after oral glucose or a standard test breakfast was nearly four times greater than that of GLP-1 7–36. However, when their respective postprandial concentrations were mimicked by exogenous infusion, GLP-1 7–36 elicited a greater insulin response. Thus, while both peptides may contribute to the incretin effect, GLP-1 7–36 appears to be physiologically more important.

Infusion in volunteers of higher concentrations of GLP-1 7–36 produced even greater basal and glucose-stimulated insulin release, and glucagon suppression, leading subsequently to symptomatic reactive hypoglycaemia (unpublished). In the postgastrectomy patients with dumping syndrome, postprandial release of GLP-1 7–36 was exaggerated, achieving plasma increments more than twice those obtained in our original infusion study. In view of the unopposed insulinotropic action of GLP-1 7–36, it seems possible that this peptide may contribute to the late reactive hypoglycaemia seen in some individuals after gastrectomy. The conventional explanation that reactive hypoglycaemia is due merely to the very rapid rise in plasma glucose concentrations leading to overstimulation of the beta cell is unconvincing, since intravenous glucose boluses do not cause such reactive hypoglycaemia.[27] Rehfeld et al[28] implicated increased enteroglucagon release as a pathogenetic factor in hypoglycaemia in the dumping syndrome 14 years ago. Another possible link between GLP-1 7–36 and human disease is in the low blood glucose concentrations associated with disorders such as infective diarrhoea and jejunoileal bypass.[29,30] Enteroglucagon levels are high in these disorders and the expected parallel rises in GLP-1 7–36 may contribute importantly to such hypoglycaemia through its insulinotropic action.

Finally, we can speculate that if the high enteroglucagon concentrations seen in high-viscous-fibre diets[31] are also associated with high plasma levels of GLP-1 7–36 immunoreactivity this peptide could have a tonic action on the beta cell and partly cause the improved glucose insulin ratio.

Further studies in the insulinotropic action of GLP-1 7–36 in health and disease will be of great interest. It is even possible that the insulin-stimulating, glucagon-suppressing effects of the peptide or a more active synthetic analogue may be useful in the treatment of non-insulin-dependent diabetes, in which both impaired insulin release in response to glucose[32] and persistent hyperglucagonaemia[33] are thought to be key pathophysiological abnormalities.

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REFERENCES

1. Bell GI, Sanches-Pescador R, Laybourn PJ, Najarian RC Exon duplication and divergence in the human preproglucagon gene *Nature* 1983; **304:** 368–71.
2. Seino S, Welsh M, Bell GJ, Chan SJ, Steiner DF Mutations in the guinea pig preproglucagon gene are restricted to a specific portion of the prohormone sequence *Febs Lett* 1986; **203:** 25–30.
3. Ghiglione M, Uttenthal LO, George SK, Bloom SR. How glucagon-like is glucagon-like peptide-1? *Diabetologia* 1984; **27:** 599–600
4. Schmidt WE, Siegel EG, Creutzfeldt W. Glucagon-like peptide-1 but not glucagon-like peptide-2 stimulates insulin release from isolated rat pancreatic islets *Diabetologia* 1985, **28:** 704–07.
5. Lund PK, Goodman RH, Montminy MR, Dee PC, Habener JF. Anglerfish islet preglucagon II *J Biol Chem* 1983; **258:** 3280–84
6. Mojsov S, Weir GC, Habener JF Insulinotropin: glucagon-like peptide 1 (7–37) co-encoded in the glucagon gene is a potent stimulator of insulin release in the perfused rat pancreas. *J Clin Invest* 1987; **79:** 616–19.
7. Holst JJ, Orskov C, Nielsen VO, Schwartz TW Truncated glucagon-like peptide 1, an insulin-releasing hormone from the distal gut *Febs Lett* 1987, **211:** 169–74.
8. Moore B, Edie ES, Abram JH On the treatment of diabetes mellitus by acid extraction of duodenal mucous membrane *Biochem J* 1906; **1:** 28–38
9 Zunz E, La Barre J. Contributions a l'étude des variations physiologique de la secretion interne du pancreas *Arch Int Physiol Biochem* 1929; **31:** 20–44
10. Creutzfeldt W, Ebert R. New developments in the incretin concept *Diabetologia* 1985; **28:** 565–73
11 Dupre J, Ross SA, Watson D, Brown JC Stimulation of insulin secretion by gastric inhibitory polypeptide in man *J Clin Endocrinol Metab* 1973, **37:** 826–28
12 Sarson DL, Wood SM, Kansal PC, Bloom SR Glucose-dependent insulinotropic polypeptide of insulin. Physiology or pharmacology? *Diabetes* 1984, **33:** 389–93
13. Ehlai D, Andersen DK, Brown JC, et al Pancreatic alpha and beta-cell responses to GIP infusion in normal man *Am J Physiol* 1979; **237:** E185–91.
14 Ebert R, Creutzfeldt W. Influence of gastric inhibitory polypeptide antiserum on glucose-induced insulin secretion in rats *Endocrinology* 1982, **111:** 1601–06
15 Wood SM, Bloom SR Physiology of the islets of Langerhans. In Howard JM, Jordan GL, Reber HA, eds Surgical diseases of the pancreas Philadelphia Lea & Febiger, 1987: 789–800.
16 Sagor SM, Gatei MA, McGregor GP, Mitchenere P, Kirk MR, Bloom SR. The influence of an intact pylorus on postprandial enteroglucagon and neurotensin release after upper gastric surgery *Br J Surg* 1981; **68:** 190–93
17 Albano JDM, Elkins RP, Maritz G, et al A sensitive, precise radioimmunoassay of serum insulin relying on charcoal separation of bound and free hormone moieties *Acta Endocrinol* 1972, **70:** 487–509
18 Christofides ND Pancreatic glucagon In: Bloom SR, Long RG, eds. Radioimmunoassay of gut regulatory peptides Eastbourne WB Saunders, 1982: 74–79
19 Sarson DL. Gastric inhibitory polypeptide In: Bloom SR, Long RG, eds Radioimmunoassay of gut regulatory peptides. Eastbourne: WB Saunders, 1982: 101–11
20. Goodfriend TL, Levine L, Fasman GD. Antibodies to bradykinin and angiotensin. a use of carbidiimide in immunology *Science* 1964, **144:** 1344–46.
21 Hunter WH, Greenford FC. Preparation of iodine-131 labelled human growth hormone of high specific activity. *Nature* 1962, **194:** 495–96
22. Bryant MG, Bloom SR. Measurement in tissue. In: Bloom SR, Long RD, eds. Radioimmunoassay of gut regulatory peptides Eastbourne WB Saunders, 1982 36–42
23 Goldstein A, Aronow L, Kalman SM In Principles of drug action, 2nd ed. New York John Wiley, 1974 311
24. Mojsov S, Heinrich G, Wilson IB, Ravazzola M, Orci L, Habener JF. Preproglucagon gene expression diversifies at the level of post-translational processing *J Biol Chem* 1986; **261:** 11 880–88
25 Lauritsen KB. GIP, incretin and gastrointestinal disease. *Dan Med Bull* 1983, **30:** 205–14
26. Jorde R, Burhol PG, Schulz TB Fasting and postprandial plasma GIP values in man measured with seven different antisera. *Regulatory Peptides* 1983, **7:** 87–94.
27 Smith JR Hypoglycemia. In Joslin's Diabetus mellitus Philadelphia. Lea & Febiger, 1985. 867–81

References continued at foot of next page

Preliminary Communication

REDUCED IN-VITRO SUSCEPTIBILITY TO MEFLOQUINE IN WEST AFRICAN ISOLATES OF PLASMODIUM FALCIPARUM

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Summary West African isolates of *Plasmodium falciparum* were more susceptible to chloroquine but less susceptible to mefloquine than were Southeast Asian isolates. The West African isolates were more sensitive to halofantrine than to mefloquine. Since neither mefloquine nor halofantrine has been used in West Africa, the findings suggest that *P falciparum* may be inherently resistant to mefloquine and that mefloquine should be introduced cautiously to West Africa. Moreover, halofantrine may be of greater value than mefloquine for controlling multidrug-resistant falciparum malaria in West Africa.

INTRODUCTION

CHLOROQUINE-RESISTANT strains of *Plasmodium falciparum* in Africa have been identified with increasing frequency since such a strain was first reported in Kenya in 1979.[1] Resistant parasites have now been reported as far west as the Republic of Cameroon,[2] Ghana,[3] Benin,[4] and Nigeria.[5] Resistant cases in semi-immune residents have also been suspected in other West African countries, including Nigeria.[6]

The new antimalarial drug mefloquine, a 4-quinoline carbinol, is a well-tolerated drug and potentially a drug of choice for the treatment of chloroquine-resistant and multidrug-resistant parasites.[7] It is rapidly absorbed after an oral dose,[8] and its elimination half-life ranges from 6 to 33 days.[8,9] In the early clinical trials, single oral doses of 1·0 and 1·5 g were effective in curing 83% and 100%, respectively, of chloroquine-resistant *P falciparum* infections in non-immune patients,[10] and 97–100% cure rates were obtained in field studies in Thailand.[7] Peak plasma concentration after a single 250 mg dose in Thai patients were three to five times (0·71 μg/ml to 1·44 μg/ml) higher than in non-infected volunteers (0·22 μg/ml).[8,9] Although how mefloquine acts on *P falciparum* is not known, chromosomal changes have been associated with acquisition of resistance in a cloned parasite in vitro.[11] The drug also interferes with the pigment clumping associated with 4-aminoquinolines[12] but it does not intercalate with DNA.[13]

IC_{50} (ng/ml)

Isolate (date)	Chloroquine diphosphate	Mefloquine hydrochloride	Quinine sulphate	Halofantrine
Sierra Leone I/CDC[1] (1980)	4·73 (0 46)	24·5 (1·3)	53·60 (5 6)	3·9 (0 7)
Sierra Leone I/WRAIR (1986)	6 39 (1·07)	18·71 (1·71)	40·09 (3 18)	3·6 (0·1)
Nigeria ST/WRAIR (1986)	6 91 (0·85)	15 03 (0·09)	40 33 (5 6)	1 26 (0 15)
Nigeria I/CDC (1980)	7·20 (1 57)	11·83 (1 77)	40 80 (3 01)	0 9 (0 02)
Nigeria I/UNC–CH (1984)	8·41 (1 83)	17·06 (2 52)	77·99 (5·75)	4·7 (1·03)
Liberia I/UNC–CH (1981)	8 89 (1·83)	13·80 (2·60)	40 63 (6·27)	ND
Nigeria S/CDC* (1984)	13 20 (1·06)	32·29 (1·50)	91 15 (0 83)	2 4 (0·8)
Sudan I/WRAIR (1985)	15·83 (0·16)	1·57 (0·11)	10·65 (0 44)	0·33 (0·01)
CAR (1984)	20·62 (2·46)	3 24 (0·9)	34 67 (6 67)	ND
Kenya I/UNC–CH (1983)	25·06 (5 05)	2·45 (0·35)	35·2 (7 8)	ND
Clone W2 (1980) (Indochina III/ CDC)	72 03 (3·79)	4·3 (0 2)	90·75 (5·83)	0·45 (0 1)
Thailand RIII–MEF† (1982)	35 7 (2·53)	10·7 (0 36)	73·3 (9·6)	ND

Findings given as mean (SD); n = 3.
*Isolates obtained from Dr Nguyen-Dinh, Malaria Branch, Centers for Disease Control, Atlanta, USA.
†Clinical mefloquine-resistant isolate reported from Thailand by E. F. Boudreau et al.[14]

P falciparum strains resistant to mefloquine (R-I and R-II types of resistance) have now been reported from endemic areas, including areas where clinical trials were conducted.[14,15] In recent studies, mefloquine-treatment failure of falciparum malaria in Thailand was associated with parasites having 50% inhibitory concentrations (IC_{50}) of mefloquine of 12 ng/ml or greater in vitro.[14] Resistance to the drug has also been induced in vitro in a previously sensitive cloned strain[16] and selected in a sensitive uncloned isolate of *P falciparum*.[17] We now report isolates of *P falciparum* from West African countries which exhibit reduced susceptibility to mefloquine.

DRUG SUSCEPTIBILITIES

In the past six years we have isolated and cultured *P falciparum* from patients who had acquired falciparum malaria in West or East African countries. The parasites were maintained in continuous culture and evaluated against antimalarial drugs by the use of standard culture procedures and a semiautomated microdilution technique.[18] The concentration of each drug required to produce 50% inhibition of ^{3}H-hypoxanthine by an isolate was compared with the effect on a chloroquine-resistant clone (W2) from Indochina and on an isolate from a case of clinical mefloquine-treatment failure from Thailand (CH-12).

Parasites obtained from West Africa—consisting of two *P falciparum* isolates from Sierra Leone, one from Liberia, and four from Nigeria—were more susceptible to chloroquine (lower IC_{50}) but less susceptible to mefloquine than were the two isolates from Southeast Asia (table). In contrast, parasites from patients who had acquired malaria in East Africa were less susceptible to chloroquine but more susceptible to mefloquine than were isolates from either

B KREYMANN AND OTHERS REFERENCES—*continued*

28 Rehfeld JF, Heding LG, Holst JJ. Increased gut glucagon release as a pathogenetic factor in reactive hypoglycaemia *Lancet* 1973, i: 116–18.
29 Bestermann HS, Christofides ND, Welsby PD, Adrian TE, Sarson DL, Bloom SR. Gut hormones in acute diarrhoea *Gut* 1983, **24:** 665–71.
30. Rehfeld JF, Lauritsen KB, Holst JJ. Increased glucose disposal after jejuno-ileostomy. *Diabetologia* 1979; **16:** 31–34
31 Jenkins DJA, Thorne MJ, Taylor RH, et al Slowly digested carbohydrate food improves impaired carbohydrate tolerance in patients with cirrhosis. *Clin Sci* 1984; **66:** 649–57
32. Ward KW, Beard CM, Halter JB, Pfeiffer MA, Porte D Pathophysiology of insulin secretion in non-insulin-dependent diabetes mellitus *Diabetes Care* 1984, **7:** 491–502.
33. Baron AD, Schaeffer L, Shragg P, Koltermann OG Role of hyperglucagonaemia in maintenance of increased rates of hepatic glucose output in type II diabetics. *Diabetes* 1987, **36:** 274–83