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MINI REVIEW

Discovery of gastric inhibitory polypeptide and its subsequent fate: Personal reflections

Raymond A Pederson*, Christopher HS McIntosh*

Diabetes Research Group, Department of Cellular & Physiological Sciences, University of British Columbia, Vancouver, British Colombia, Canada

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***Correspondence**
Raymond Pederson
E-mail address: raymond.pederson@gmail.com
and
Christopher McIntosh
E-mail address: mcintoch@mail.ubc.ca

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ABSTRACT

The present review focuses initially on experimental studies that were designed to identify acid inhibitory factors, referred to as 'enterogastrones,' that ultimately led to the isolation of gastric inhibitory polypeptide (GIP), a 42-amino acid polypeptide. GIP was shown to inhibit acid secretion in animal models, as well as stimulating gastric somatostatin secretion. However, its role in human gastric physiology is unclear. Further studies showed that GIP strongly stimulated the secretion of insulin, in the presence of elevated glucose, and this 'incretin' action is now considered to be its most important; an alternative for the GIP acronym, glucose-dependent insulinotropic polypeptide, was therefore introduced. In the 1970s, GIP purified by conventional chromatography was shown by high-performance liquid chromatography to consist largely of GIP_{1-42} and GIP_{3-42}. It was later shown that dipeptidyl peptidase 4 was a physiologically relevant enzyme responsible for this conversion, as well as the similar metabolism of the second incretin, glucagon-like peptide-1. Dipeptidyl peptidase-4 inhibitors are currently in use as type 2 diabetes therapeutics, and studies on islet transplantation in rodent models of type 1 diabetes have shown that dipeptidyl peptidase-4 inhibitor treatment reduces graft rejection. Additional studies on C-terminally shortened forms of GIP have shown that GIP_{1-30} and a dipeptidyl peptidase-4-resistant form ($D\text{-}Ala^2GIP_{1-30}$) are equipotent to the intact polypeptide *in vitro*, and administration of $D\text{-}Ala^2GIP_{1-30}$ to diabetic rodents greatly improved glucose tolerance and reduced apoptotic cell death in islet β-cells. There are probably therefore further clinically useful effects of GIP that require investigation.

The discovery of gastric inhibitory polypeptide (GIP) during the time-period 1969–1971 can be related historically to the recognition that food substances, when introduced into the small intestine, trigger a humoral reflex leading to the inhibition of gastric acid secretion. Acidic pH, hypertonic solutions and fat were the most potent stimuli for this inhibitory reflex. The term, 'enterogastrone,' was introduced by Kosaka and Lim[1] in 1930 to describe the blood-borne gastric inhibitory chemical messenger(s) released from the small intestinal mucosa by fat. With the isolation and chemical characterization of secretin and cholecystokinin (CCK), their possible roles as enterogastrones were investigated. Initial studies with a partially purified porcine CCK preparation showed inhibition of gastrin- and histamine-stimulated secretion from gastric pouches in dogs[2]. John Brown[3], while pursuing postdoctoral studies in Seattle (1967), observed that the same preparation inhibited acid secretion stimulated by endogenously-released gastrin in dogs, whereas it was stimulatory for acid secretion in the fasting state[4].

The initial evidence for the existence of GIP came from comparative studies by John Brown and Raymond Pederson on the gallbladder-stimulating and acid secretory effects of two different preparations of CCK, designated 10% and 40% pure on the basis of gallbladder-stimulating potency[5]. The animal model used in these studies was the dog, prepared with vagally and sympathetically denervated pouches of the stomach, and indwelling cannulae in the fundus of the gallbladder. In the fasting state, the 40% pure preparation produced a greater stimulatory effect on acid secretion than the 10% pure preparation. Two possible explanations for the uncoupling of gallbladder and acid stimulatory effects were proposed[5]: (i) either a gastric stimulant had been concentrated; or (ii) an inhibitor of acid secretion had been removed during the purification procedure.

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The latter hypothesis was pursued, and a similar study was carried out by Ray Pederson[6], in which the two CCK preparations used earlier were tested for acid inhibitory activity in the same animal model. The less pure preparation of CCK (10%) was a more potent inhibitor of pentagastrin-stimulated acid secretion than the purer preparation (40%), supporting our hypothesis that the CCK preparations contained an inhibitor of gastric acid secretion.

In 1969–1970, John Brown spent a sabbatical year in the laboratory of Professor Viktor Mutt at the Karolinska Institute, Stockholm, Sweden. Viktor Mutt has been recognized as a pioneer in the identification and purification of gut hormones. Biochemical strategies for the isolation of this inhibitory substance from the impure CCK preparations were refined by John Brown and Viktor Mutt at the Karolinska, while parallel physiological studies were carried out by Raymond Pederson at the University of British Columbia (UBC), Vancouver, British Colombia, Canada. It became evident that, as the purification of CCK preparations progressed, CCK (gallbladder stimulating) activity decreased and acid inhibitory activity became more potent[7,8]. A purification procedure was described that resulted in the isolation of GIP, with a degree of homogeneity suitable for amino acid analysis to be carried out[7,8]. The amino acid sequence of GIP was reported in 1971[9] and later revised[10]. A radioimmunoassay was subsequently developed in John Brown's laboratory in 1974[11], allowing the measurement of GIP release into the circulation in response to ingested nutrients. A number of investigators reported that fat, in the form of triglycerides, was a potent stimulant of GIP release in humans and dogs[12]; adding support to our hypothesis that GIP was a component of the enterogastrone activity originally described by Kosaka and Lim[1] in 1930.

During 1974–1975, John Brown took a further sabbatical, with Werner Creutzfeldt in Gottingen, West Germany, to study the secretion of GIP in various clinical conditions. During this period, he met Christopher McIntosh, who was studying gastric and pancreatic islet somatostatin at the time, and recruited him to UBC. One of the questions targeted by Christopher McIntosh, Raymond Pederson and John Brown was whether the enterogastrone actions of GIP could be mediated through increased gastric somatostatin release. GIP was indeed found to be a very powerful stimulator of somatostatin release in an isolated perfused rat stomach model, and vagal stimulation was antagonistic to this effect[13]. Alison Buchan and Kenny Kwok later joined the laboratory, and the 'Medical Research Council Regulatory Peptide Group' was established in 1986, with John Brown as director. During this time, our group, and others, showed that a number of neuropeptides were involved in modulating somatostatin secretion in rodents[12,14]. However, GIP has not been shown to exert strong enterogastrone effects in humans, and it is still unclear as to which of these pathways are relevant in our species.

Concurrent with the search for the elusive enterogastrone, several groups were investigating the existence of gut endocrine factors that were released by nutrient ingestion and stimulated insulin secretion; a signaling pathway that Roger Unger termed the 'enteroinsular axis'[15]. Werner Creutzfeldt subsequently resurrected and anglicized the term 'incrétine' that La Barre[16] had earlier introduced to describe the hormonal component of this axis[17]. In collaborative studies with John Brown, John Dupré showed that intravenous infusion of GIP during a glucose tolerance test potentiated insulin secretion and increased disposal of an intravenous glucose load in normal humans[18]. Radioimmunoassay studies showing that oral glucose was a potent stimulant of GIP release in humans[19] strongly supported such a role for GIP in the enteroinsular axis. Animal studies were carried out at UBC to further characterize the actions of GIP and, in a perfused rat pancreas model, Ray Pederson established that the effect of GIP on insulin secretion was glucose-dependent, a critical characteristic of incretin action[20]. As this was now considered to be its more important function, an alternative for the GIP acronym, glucose-dependent insulinotropic polypeptide, was decided on in discussions at the UBC Faculty Club. During the 1980s, a second incretin, glucagon-like peptide-1 (GLP-1), was identified as a product of the intestinal processing of proglucagon[21–24]. Collectively, GIP and GLP-1 appear to account for the 'incretin-effect,' or greater stimulation of insulin release by oral versus intravenous glucose.

With the availability of small-scale high-performance liquid chromatography systems, it became possible to study in more detail the preparations of GIP classified as enterogastrone IV, produced by classical chromatography. Christopher McIntosh and John Brown purified, on C18 high-performance liquid chromatography columns, two major GIP peptides that were sequenced in Viktor Mutt's laboratory[10]. The larger of these peaks was shown to be GIP_{1-42}, and the second GIP_{3-42}. We speculated that GIP_{3-42} was formed from the intact peptide by amino- or dipeptidyl peptidase hydrolytic activity, but it was unknown as to whether this occurred physiologically within the intestine or pathologically during the extraction process. We eventually returned to this question, when Timothy Kieffer, then a graduate student, undertook the challenge of evaluating the potential physiological significance of GIP_{1-42} and GLP-1 N-terminal metabolism by dipeptidyl peptidase-4 (DPP4). Using ^{125}I-labeled peptides, he showed that, in agreement with Mentlein *et al.*[25], DPP4 cleaved both incretins *in vitro* and, importantly, that such degradation occurred physiologically, after peptide administration to rats[26]. Such degradation was absent in DPP4-deficient rats[26]. In a subsequent long-term collaboration with Hans Ulrich Demuth in Halle, Germany, extensive mass spectroscopic studies were carried out on the kinetics of both GIP and GLP-1 degradation by DPP4 and inhibition by selective DPP4 inhibitors[27]. Andrew Pospisilik *et al.*[28,29], in our group, showed that administration of the DPP4 inhibitor, isoleucine thiazolidide (P32/98), in the Vancouver diabetic Zucker rat resulted in the potentiation of circulating levels of insulin and improved glucose tolerance. Such beneficial effects were subsequently shown in a number of animal models of

type 2 diabetes, and these findings contributed to the development of DPP4 inhibitors for clinical use[30,31], in parallel with the development of incretin mimetics. Results from rodent studies have shown that DPP4 inhibitors might also be beneficial in type 1 diabetes treatments. In collaboration with Doris Doudet and Chris McIntosh, Su-Jin Kim[32] established a positron emission tomography imaging system that allowed quantitative tracking of the fate of islets after transplantation, and showed that treatment of streptozotocin-induced diabetic or non-obese diabetic mice before and post-transplantation with DPP4 inhibitors prolonged graft survival significantly and prolonged longevity[33,34]. This indicates that DPP4 administration could be beneficial in human islet transplant recipients.

We have also been intrigued by the question as to whether 42-amino acids are required for GIP action, as there is considerable N-terminal sequence similarity with the 30-amino acid peptide, GLP-1. Over many years, we have examined the biological actions of a large number of truncated GIP peptides and convincingly shown that C-terminally shortened GIP (GIP_{1-30}) exerts equivalent activity to GIP_{1-42} in stimulating cyclic adenosine monophosphate production in GIP receptor-transfected Chinese hamster ovary cells and, when protected from DPP4 metabolism, strongly reduces glucose excursions in tolerance tests *in vivo*[35–37]. Twice daily injections of DPP4-resistant D-Ala^2GIP_{1-30} resulted in marked improvements in morning glucose and glucose tolerance in obese Zucker diabetic fatty rats[38]. Additionally, there was an increase in β-cell area in the pancreata from the obese rats, with improved structural integrity of the islets, mainly resulting from a promotion of survival as a result of a reduced apoptosis[38]. Of interest is that GIP_{1-30} appears to be a naturally produced variant of GIP, both in the gut and pancreas[39]. Clearly, there is still potential for additional beneficial clinical effects of GIP to be identified.

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DISCLOSURE

The authors declare no conflict of interest.

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