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Hormonal control of pancreatic growth during fetal, neonatal and adult life

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ABSTRACT

This review article has for major objective to summarize the old and latest developments on the hormonal controls of pancreatic growth. The article deals with hormonal controls during the fetal, neonatal and adult periods of pancreas development, growth and regeneration. During the fetal period, comparisons were made between studies performed with pancreatic explants and those designed *in vivo*. After birth, the effects of glucocorticoids, thyroxine, gastrin, bombesin, secretin, cholecystokinin alone or with secretin are reported. In the adults, similar studies were reported on hormones with addition of the effects of neuropeptides, the cell types targeted by hormones and the hormonal control after pancreatectomy and pancreatitis.

Key words: pancreas growth, hormonal control, pancreas regeneration, pancreatectomy, glucocorticosteroids, gastrointestinal hormones, cholecystokinin

INTRODUCTION

Investigation on the hormonal control of pancreatic exocrine secretion really started at the turn of the last century when Bayliss and Starling found in 1902 that acid in a jejunal loop was a strong stimulant of pancreatic exocrine secretion. They then proposed that this acid was releasing a substance from the gut mucosa into the blood stream which was stimulating the pancreas. This substance was named "secretin" [1]. The other partner in the control of pancreatic secretion, "cholecystokinin", was initially discovered as a substance able to contract the gallbladder [2]. Exactly fifteen years later, Harper and Raper found another substance in the intestinal mucosa with the property to stimulate enzyme secretion from the pancreas which they named "pancreozymin" [3]. Twenty five years later, two Swedish investigators, Mutt and Jorpes, purified pancreozymin and found that the 33-isolated amino-acid peptide possessed both cholecystokinetic and pancreozymic activities and named the peptide cholecystokinin-pancreozymin (CCK-PZ) [4]. Few years later, following a proposal by Grossman [5], CCK-PZ was changed for cholecystokinin (CCK), the originally described hormone.

With the discovery and purification of these two important gut hormones, secretin and cholecystokinin, research has been almost totally focused on establishing their physiological secretory properties. Such control studies were then performed on different gut organs but mostly on the pancreas of different species including man. During that early race to discover all the biological effects of secretin and CCK, gastrointestinal physiologists and gastroenterologists did not pay attention to the growth potential of these two hormones on the pancreas and other gut organs because their experimental efforts were concentrated on acute short-term secretory studies.

However, at the same time, nutritionists working in agriculture colleges and experimental farms made the initial observation that feeding a proteolytic inhibiting substance present in raw soybean resulted in chick growth inhibition concomitantly with inhibition of gut proteolysis. However, autoclaved raw soybean feeding caused increased body weight and gut proteolytic activities [6]. Few years later, it was clearly shown that this proteolytic-inhibiting substance found in raw soybean was able to increase the size of the chick pancreas as well as its tryptic content. It was then hypothesized that this growth resulted from stimulation of the pancreatic acinar tissue by either the inhibitor per se or by a product of incompletely

digested protein [7]. Years later, similar hypertrophy of the pancreas was also observed in rats fed a raw soybean meal for a month along with mitosis, a first sign of hyperplasia [8]. At that time, no gut hormone involvement was in the picture to explain the observed pancreatic hypertrophy in these animals. Now we know that these trypsin inhibitors prevent the hydrolysis of CCK and secretin releasing peptides, thus allowing endogenous release of CCK and secretin [132].

In this review article, the objective is to present data that demonstrate the implication of gastrointestinal, adrenal, thyroid and pancreatic hormones in the control of pancreatic growth during pre and postnatal periods, as well as after weaning, and pancreas regeneration after partial pancreatectomy or pancreas aggression.

Before we start this review, lets define some of the terms used to describe growth. Hypertrophy can refer to tissue and cells. Tissue hypertrophy represents an increase in total tissue mass measured as total tissue weight while atrophy describes a loss of tissue weight, always in comparison to controls. Cellular hypertrophy describes an increase in cell size established as cell mass (gland weight/DNA) along with concentrations of proteins, specific enzymes and RNA/DNA; cellular atrophy represents a decrease in these parameters always in reference to controls. Gland hyperplasia describes an increase in cell number and can be estimated by measuring total DNA contents, thymidine incorporation into DNA/DNA, and labeling or mitotic indices. Gland aplasia refers to decreases in all the above parameters, always in comparison to controls.

I. HORMONAL CONTROL OF FETAL PANCREAS GROWTH

Two different strategies have been used to evaluate the role played by different hormones in the control of fetal pancreas growth. Initially, fetal pancreatic explants isolated from chicks, mice or rats were kept in different culture media supplemented or not with various hormones of different glands origin. In such cases, differentiation of the pancreatic gland was mostly studied. The second approach, more physiological, kept the maternal-fetal interrelationship intact by treating the pregnant females with selected hormones and investigation of the fetal pancreas just before normal delivery.

A) Fetal Pancreatic Explant in Culture

Under well chemically defined culture conditions, it was clearly shown initially that the pancreatic chick explants could not increase their total DNA while augmenting their amylase synthesis until a plateau was reached after several days of culture. With hydrocortisone added to the explants, one can see an immediate burst in the amount of amylase produced by the explants in a dose-dependent manner [9]. The effect of the corticosteroid was not limited to amylase since the hormone also accelerated, dose-dependently, *de novo* synthesis of chymotrypsinogen with a maximal effect at 1 μM with no significant effect on total protein of the explants [10]. This last observation led the authors to put forward the hypothesis that corticosteroid hormones were involved mostly in the late stages of pancreas differentiation. In an attempt to determine whether glucocorticoids were the only hormones responsible to initiate and sustain pancreas cytodifferentiation *in vitro*, a synthetic glucocorticoid, prednisolone, was investigated alone and in combination with insulin and thyroxine on functional differentiation of the chick embryo pancreas, as evaluated by amylase and chymotrypsinogen accumulation in the gland [11]. It was observed that prednisolone had the same properties as those previously seen with hydrocortisone on both enzymes whereas neither thyroxine or insulin alone, nor both hormones together affected pancreas exocrine differentiation. However, thyroxine addition to prednisolone enhanced the effects of the steroid on both enzymes, while insulin did not. Maximum enzyme activities were surprisingly observed with the three hormones added together to the explants. These data clearly indicate that glucocorticoids are essential for normal chick pancreas differentiation with insulin and thyroxine as potential supporting agents for maximal differentiation.

In mouse and rat pancreas, studies using molecular biotechnology techniques have detected glucagon and insulin appearance in the pancreas before the pancreatic exocrine enzymes [12]. Thus it was pertinent to think that both hormones may influence pancreas differentiation in both species. However, as previously observed in the embryonic chick pancreas [11], no significant effect of insulin and glucagon or their specific antibodies were observed on accumulation of amylase and chymotrypsinogen by rat cultured embryonic pancreas [13]. In parabiotic culture with fetal adrenal glands, the fetal rat pancreas responded in sustaining its acinar cells proportion, in preventing islet cell proliferation and in maintaining its acinar cells amylase content. These results led to the potential recognition of an adrenal-exocrine pancreatic axis with adrenal secretions significantly involved in development of the exocrine pancreas [14]. It was later established that corticosterone was indeed the adrenal hormone associated with pancreas morphogenesis and differentiation previously observed in fetal rat pancreas in parabiotic culture with fetal adrenal glands [14], as the corticosteroid reproduced the same effects as the adrenals [15]. Furthermore, the adrenal gland content [16] and the circulating levels of corticosterone, the principal glucocorticoid of the rat, reached adult levels by day 17 of fetal life [17]. Normally, most of corticosterone in the fetus is of maternal origin, and maternal corticosterone can cross the placenta rapidly [18]. Anyway, corticosterone of embryonic origin is present at high levels when large accumulation of pancreatic exocrine enzymes occurred in the fetal pancreas. However, it is difficult to assess the contribution of glucocorticoids derived from the maternal circulation but the hormone is important at least early since the rat pancreas forms midway through gestation, two days before the appearance

of the adrenal cortex at a time when no embryonically synthesized adrenal cortical hormones can be present [19].

In this line of research, the synthetic glucocorticoid dexamethasone was shown to mimic the effects of corticosterone on rat pancreas differentiation with a concomitant reduction in cell number suggesting a modulatory role of glucocorticoids in pancreatic development rather than an inductive role [19]. In an effort to identify which steroids had the most positive effects on pancreatic acinar morphology and function, organ culture of fetal rat pancreas indicated that the mineralocorticoids (aldosterone and 11-deoxycorticosterone) as well as the glucocorticoids (cortisone and hydrocortisone) were the most potent while estrogens, androgens and progesterone had no effect [20]. More than fifteen years later, Sanvito et al. [21] showed no effect of bFGF, rhHGF, IGF-11, PDGF, or NGF on pancreatic bud cultures, while EGF tended to promote ductal development and TGF-β-1 the endocrine development.

B. Fetal Pancreas Growth in situ

Fetal pancreas growth *in situ* remains the most physiological approach to study pancreas development because the gland is exposed to all the natural endogenous and necessary hormones involved throughout its development either from its mother or from itself at the appropriate timely concentrations. Furthermore, treatments to the mothers with different hormones are expected to reach their fetuses.

The interest in this *in vivo* model of maternal-fetal relationship came upon from the previous observations that the responses of the fetal and newborn pancreas of rats [22,23] and humans [24] to secretagogues were immature. So, it was believed that we should be able to speed up fetal pancreas development as well as the receptors and intracellular pathways responsible for the induction of enzyme secretion by treating the pregnant females with specific hormones previously shown to favor fetal pancreas differentiation and maturation. By doing so, we should thus be able to initiate prematurely maturation of the exocytosis process, a potentially beneficial event in the overall development of premature babies' digestion process.

The implication of endogenous corticosterone in maturation of fetal pancreas was initially put forward from its positive effects observed in fetal pancreas culture [15] and then from the observation that fetal pancreatic amylase activity reached its maximum *in vivo* in the perinatal period. At the same time serum corticosteroid was very high in the later part of fetal life [25].

Treatments of pregnant dams with intraperitoneal hydrocortisone and CCK-8, a CCK analogue, for 4 days at the end of their pregnancy, days 16 to 19, did not alter their fetus pancreatic weight nor their pancreatic protein content. However, CCK-8 was associated with significant decreases in total DNA contents at the three selected doses of 2.5, 5 and 10 μg kg^{-1} while hydrocortisone had no effect at 5, 10 and 20 mg kg^{-1}. These data suggest that CCK inhibited growth of the fetal rat pancreas [26]. However, if the hydrocortisone treatment is given to pregnant rat at a higher dose of 50 mg kg^{-1}, IP, for 10 days, the pancreatic gland weight of the newborns was significantly increased by almost three fold along with their total DNA content and modest but significant increases in total amylase and its concentration [27]. Under this regimen, it is clear that hydrocortisone given to a pregnant rat can cause fetal pancreas hyperplasia with associated targeted hypertrophy focused on enzymes but not on total protein or RNA.

All agree that little is known about the role of gastrointestinal hormones in the regulation of gut organs formation during fetal development. Although the effect of gastrin on fetal pancreas growth has never been reported, one might expect some positive effects since rat newborn pancreas has more gastrin than its gastric antrum [27,28] and expresses preferentially the CCK-2 receptor as mRNA and protein [28]. Similarly, although not tested on fetal pancreas growth, bombesin could have some potential because of its reported involvement in pancreas growth in suckling rats [29] and rabbits [30].

In an initial study with CCK-8 given to pregnant rats at relatively high doses causing pancreatitis in a normal animal [31], aplasia of the pancreatic gland was observed in the pancreas of the fetuses [26], if not destruction. Therefore, some assumed that administration of such peptide hormones may provide only pharmacological but not physiological data as plasma hormone levels resulting from such treatments could be above normal values. To circumvent this problem, some investigators decided to play the inhibition of these potential growth factors to reveal whether they can have a physiological role in organ growth. However, the selection of such inhibitors has been done sometimes arbitrarily as was the case for CCK. In guinea pig, pregnant sows had mini-osmotic pumps implanted sc, three weeks before delivery. These pumps released either saline or the CCK-1 receptor antagonist MK-329 (L364,718) at a dose of 25 nmol $kg^{-1}h^{-1}$. In newborns, no significant changes in pancreatic growth parameters were observed in the CCK receptor antagonist group when compared to saline-controls [32]. The authors concluded that the CCK-1 receptors were not essential for fetal pancreas growth and that the treatment failure was not because of the dose used since a similar dose totally abolished pancreatic growth induced by exogenous CCK [33]. This lack of effect may however depend on the presence of relatively low levels of CCK-1 receptors as shown in rat fetal pancreas [34]. Whether or not we are dealing with species difference, another study, performed on pregnant rats with the same CCK-1 receptor antagonist, delivered by the same technique, the osmotic mini-pumps, at a dose of 138 μg $kg^{-1}h^{-1}$, indicated that the CCK-1 receptor antagonist caused a slight reduction in fetal pancreatic protein concentration and a huge decrease in their DNA content [35]. The authors also established clearly that the receptor antagonist reached the fetal pancreas with an estimated concentration of 3.5 ng/g of free devazepide. In contrast, in the litter, treatment with a monoclonal antibody rose against gastrin and CCK resulted only in a reduction of proteins without affecting total DNA content.

The only consensus obtained from the above studies indicates that hydrocortisone given at a relatively high dose to pregnant animals can favor growth and maturation of the fetal exocrine pancreas. Inhibition of the CCK-1 receptors had either negative or a positive effects depending on the dose given, while CCK seemed to cause growth inhibition. In a more recent study [36], effects of some gastrointestinal hormones have been revisited or assayed for the first time during a whole pregnancy. The role of gastrin-releasing peptide (GRP) was investigated because of its high plasma levels observed in the fetus during pregnancy in sheep [37] and its known effect on gastrin release [38]. Similarly, the high prevalence of CCK-2 receptors, also known as the gastrin receptor, over that of CCK-1 receptors in rat fetal pancreas [28,34] and gastrin high pancreatic concentrations [39] pledge for gastrin involvement.

In that study [36], hydrocortisone, serving as a positive control, was the only hormone causing fetal pancreas hyperplasia when given at its highest dose, 20 mg day^{-1}. Caerulein, a CCK analog given at 6 μg day^{-1}, was the only agent causing fetuses' pancreas aplasia associated with pancreatic hypertrophy as previously reported [26]. Furthermore, such growth inhibition by CCK was also observed in human pancreatic cancer cells transfected with both CCK receptor subtypes [40]. A potential explanation for this aplastic effect of caerulein on fetal pancreas may reside in its potential secretagogue action on pancreatic somatostatin release, already present in fetal mouse pancreas at around the 8^{th} day of fetal life [12]. This secretory effect of caerulein on somatostatin release has recently been demonstrated in a somatostatin pancreatic cell line with both CCK receptor subtypes present [41]. Also somatostatin infusion in adult rats reduced pancreas growth [42].

GRP failed to induce fetal pancreas growth in contrast to its trophic effects on those of suckling rats [29] and rabbits [30]. A relatively smaller dose used in the pregnant rats (4.3 μg) compared to those given to rats (40 μg) and rabbit (12.5 μg) may explain the difference. Data obtained with gastrin however pledge for an important role for this hormone in fetal rat pancreas development. As indicated earlier, gastrin averaged 2,2 to 4 ng in rat pancreas term fetus [27] and it may not come from the mother, as pregnancy did not appreciably influence levels of serum or antral gastrin [43]. The major effect of gastrin, given at the daily dose of 57 μg to the mother, was not fetal pancreas hyperplasia but mostly pancreas hypertrophy. It would seem that gastrin operates through its CCK-2 receptor since fetal pancreatic atrophy was observed in response to L-365,260, a specific CCK-2 receptor antagonist [36].

In conclusion, this model of mother-fetus relationship seems to be an excellent tool to study interactions of hormones with fetal pancreas and any other gut organs. It should be used more often in future research to determine hormonal combinations and their most efficient concentrations to eventually be able to favor development of the gut and pancreas in premature human babies and thus increase their digestive capacity. This would be the ultimate objective after extensive studies in other animal species, especially the pig, whose pancreas function control is very close to human.

II. HORMONAL CONTROL OF NEONATAL PANCREAS GROWTH

It is quite evident, at least in some rodents, that responsiveness to secretagogues, absent at birth, rapidly develops few days later [22,23]. In some mammalian species, several hormones, by virtue of their developmental changes in the circulation, have been considered to have an important role in controlling pancreas development and growth. Upon exogenous hormonal treatment, a sequential order of events has been observed with hormones related to enzyme secretion. Under experimental conditions, a hypersecretory state has been documented, followed by gland hypertrophy leading to hyperplasia under sustained treatment [44]. Therefore, an interrelation seems to exist between secretion and growth: an increased and sustained demand for secretory products necessitates a larger factory to supply the need. During the neonatal period, several hormones have been involved in pancreas development and growth with glucocorticoids, thyroxine and some gastrointestinal hormones among the most active.

A. Glucocorticoids

One of the first studies performed on pancreas development and growth in suckling rats was performed in 1962 [45] when 9 days old rats were given a pharmacological dose of cortisone (2.5 mg/100 BW) for six days. Although body weights of the cortisone animals were significantly reduced by 20%, their pancreas exhibited impressive growth with a 58% increase in gland weight, hypertrophy as evaluated by increased concentrations of amylase (118%), proteolytic activity (346%) and RNA (35%) and hyperplasia documented by a 53% increase in total DNA. It has been established and accepted [46] that doses of hydrocortisone of 0.125 and 0.5 mg/100 g body wt were physiological because 0.3 mg hydrocortisone/100g body weight per day was equivalent to at most about twice the basal secretory rate of corticosterone, the principal glucocorticoid in the rat. In mice 6 days old given hydrocortisone sc for 5 days at a pharmacological dose of 10 mg/100g body wt, amylase concentration doubled; in this study, insulin alone had no effect but it drastically reduced the effect of hydrocortisone as did thyroxine, also given at a physiological dose [47].

Although hydrocortisone has been shown to increase levels of rat pancreatic hydrolases during its newborn period [48], a dose-response assessment of this glucocorticoid at physiologic and pharmacologic doses revealed that a treatment of three days on 9 days old rats, at doses of 0,125, 0,5 and 2,0 mg/100g body wt, caused pancreas hypertrophy and hyperplasia in a dose-dependent fashion. Under these conditions, amylase concentrations remained the most sensitive to the treatment with increases of 360, 850 and 1339% above

control values in response to the 0,125, 0,5 and 2,0 mg doses, respectively, as compared to increases of 27, 132 and 225% in chymotrypsinogen concentrations in response to the same doses. Hyperplasia was also evident with increases in total DNA contents of 28, 36 and 26% when compared to control values [49]. Similar results were obtained also with hydrocortisone given at comparable doses during the first five days of life [50]. The role of glucocorticoids in neonatal pancreas development is very important, although not absolute as suppression of corticosterone production in rats by adrenalectomy [51] or by an inhibitor of steroidgenesis [52], delayed but did not prevent developmental accumulation of pancreatic exocrine enzymes. This role of glucocorticoids in pancreas development is also strongly supported by the observation that the glucocorticoid binding capacity in pancreatic cytosol of neonatal rats gradually increased postnatally and coincided with the developmental patterns for total corticosterone in rat sera [53]. Ultrastructural morphometric studies indicated that hydrocortisone at the pharmacological dose of 20 mg/kg/day induced exocrine cell atrophy with decreased cell size and zymogen granules combined with pancreatic hyperplasia suggested by increased pancreatic weight, an effect not observed at a lower dose [54].

In larger mammals, hydrocortisone given to 3-day old calves for 4 days at a dose of 50 mg/kg BW, caused pancreatic hypertrophy and hyperplasia but only pancreatic hyperplasia when the same treatment was given to calves of 17 days. These data also point out that the calf pancreas seems much less sensitive to glucocorticoid than the rat pancreas during the neonatal period [55]. In neonatal piglets, glucocorticoids caused a stimulating effect on the maturation of pancreatic enzymes. Unfortunately, data on DNA were missing in this study so that it is impossible to determine whether the treatment caused growth of the pancreatic gland in this species [56]. It has been previously shown that exogenous glucocorticoids have only marginal effect on enzyme development in the postnatal period of the pig [57].

B. Thyroxine

An early study comparing the effects of thyroxine and cortisone on pancreas development of 9 days-old rats, indicated that thyroxine, given at 0.5 mg/100g/day for 6 days, had its major effect on pancreatic DNA content with an increase of 126% compared to the 53% increase in response to cortisone. However, the hypertrophic effect also resulting from the thyroxine treatment was much less important than that of cortisone [45]. In rats, thyroxine resulted in increased contents of pancreatic enzymes [58] and to explain these effects, a temporal relationship has been documented in this species between the appearance of serum thyroxine peak and a serum corticosterone peak during the suckling period [59] with the possibility that thyroxine could lead to increases in plasma corticosterone level [60]. In suckling rat pups, thyroxine administration caused increases in pancreatic weight, protein and DNA contents, and enzyme activities. These data suggest that thyroxine is an important modulator of the development of the rat exocrine pancreas when acting alone [51] and it may also have an indirect action via glucocorticoids as previously suggested [61]. Its direct action on pancreas development is further supported by the finding of specific T_3 receptors found and characterized in rat pancreas nuclei [62].

C. Gastrin and Bombesin

With gastrin absent from the normal adult rat pancreas but found at high concentrations in glands from fetus and newborns [63], one may wonder if the trophic effect of gastrin on the pancreas should not be observed only during fetal life as previously observed [36] and during the early postnatal period with pancreas still expressing gastrin [63]. To test the role of gastrin early on pancreas growth, a selective CCK-B/gastrin receptor antagonist, CI-988, was given to newborn rat pups intraperitoneally twice daily at a dose of 1 mg/kg for 5 days [64]. The data indicate that this treatment had no effect on all the pancreatic parameters examined: pancreas weight, total protein, amylase, RNA and DNA. The authors concluded that inhibition of the pancreatic CCK-B/gastrin receptor at birth did not modify normal pancreas development and growth. A previous study performed in infant rabbits showed that pentagastrin given intraperitoneally also failed to induce pancreatic weight and morphology [65]. To emphasize this lack of effects by gastrin, hydrocortisone which caused pancreatic growth did so while decreasing the pancreatic gastrin contents of rats in their second week of life [28,66]. These data seem to indicate that gastrin is involved in neonatal pancreatic growth although more studies are needed to ascertain this statement since the hormone had positive effects on fetal pancreas development at least in the rat.

Eventhough it is not clear yet if fetal and neonatal pancreas exhibit functional bombesin receptors, it has been characterized biochemically on pancreatic dispersed acini from adult guinea pig [67]. The trophic effects of bombesin have also been investigated on the pancreas of suckling rabbits [30] and rats [29,68]. In 4 day old rabbits [30], a 13 day treatment with increasing doses of bombesin (1.25, 12.5 and 30 µg/kg) led to pancreatic hyperplasia at the two highes doses. Although total pancreas weight and protein contents were significantly increased at all doses, pancreatic hypertrophy did not develop in response to this treatment. Thus it seems that bombesin has its major effect on increased cellularity in these young rabbits rather than cellular hypertrophy. In the rat, two studies [29,68] with bombesin given at the same dose of 20 µg/kg, twice a day in gelatin for 9 days, indicated that the peptide caused pancreatic hyperplasia with a selective effect on trypsinogen and chymotrypsinogen concentrations [68]. It was also clearly established that the growth promoting effect of bombesin was direct on the pancreatic cells without interference from CCK or glucocorticoids as evaluated with specific receptor antagonists [29].

D. Secretin

The potential of secretin as a pancreatic growth factor is supported by early observations that basal serum concentrations of secretin were elevated relative to adult values in newborn human [69] and pigs [70]. The few studies published indicate that secretin, given at 25 µg/kg every 12h for five days to six days old rats, produced hyperplasia and hypertrophy of the pancreatic gland [71]. The pancreas of suckling rat seems however more sensitive than the adult's as the 35% increase in DNA content obtained with 25 µg/kg of secretin, twice a day for 5 days was reproduced in the adult with the dose of 100 µg/kg of the hormone given each 8h for 10 days [72]. Also of importance, pancreatic hyperplasia caused by secretin in suckling rats is identical to that induced by hydrocortisone but higher than that of caerulein by 8% [73]. When secretin was administered at a dose of 100 µg/kg for 7 days to rats aged 3, 6, 13 and 24 days, a significant increase in DNA content (a criteria for hyperplasia) was observed only in 20-day-old rats while increases in pancreatic weight, protein and enzymes were observed in rats older than 13 and 20 days of age [74]. The discrepancy between the two studies with regards to DNA content may indicate that the pancreas of suckling rats is refractory to high doses of secretin. It is clear from the data presented that secretin can be considered as a trophic factor for the pancreas of suckling rats but whether these effects are physiological remains to be demonstrated until we can show plasma secretin levels comparable to those seen after a meal.

E. Cholecystokinin and analogues

The initial study looking at the trophic effect of CCK in suckling rats was performed with the purified CCK-PZ preparation of Jorpes and Mutt [75]. The CCK-PZ hormone was given IP at a dose of 20 IVY units/kg, twice a day for 5 days to rat pups of 6 days old. This treatment had no effect on pancreas growth with regards to hyperplasia and hypertrophy. The only affected parameter was total chymotrypsin content with a slight increase of 24%. The same treatment given to adult rats led to pancreas hyperplasia and hypertrophy [76]. This lack of effect of CCK-PZ on pancreas growth in suckling rats may have depended on the hormone purity because, with the availability of synthetic CCK analogues such as CCK-8 and caerulein, comparable studies indicate that caerulein given at a dose of 1 µg/kg every 12h for 5 days led to pancreatic hyperplasia in suckling rats accompanied however with atrophy as the cellular mass and concentrations of amylase and protein were decreased [71]. In a similar study with the treatment initiated on the first day of life with CCK-8 at 2.5, 5 and 10µg/kg for 5 days, the hormone led to significant increases in total pancreas DNA with no difference between doses. Similarly, comparable significant increases were also observed in pancreatic weight and in amylase, chymotrypsin and lipase total contents with no difference between doses [50]. These significant increases in pancreatic weight and enzyme contents seen in this study [50] were not observed in pups five days older [71]. One might argue that the CCK peptides may have a variety of effects on rats which differ with age; however, it is important to point out that in the latest study, CCK-8 was used at doses 2.5 to 10 times higher than that of caerulein [71]. It is also known that in suckling rat pups, their pancreas is much less sensitive to excessive doses of caerulein than those of weaned animals [77]. This phenomenon can be explained by the observation that the capacity of the high-affinity CCK binding sites gradually increased with age whereas that of the low-affinity sites remained relatively constant [78]. The pancreatic growth response to CCK analogues in suckling rats seems to be physiological since it has been reproduced by feeding suckling rats with soybean trypsin inhibitor [79], a treatment known to release endogenous CCK [80].

In suckling rats, the efficacy of the CCK analogue caerulein in promoting pancreatic hyperplasia and atrophy remains a question of treatment length. Indeed, as pancreatic hyperplasia and atrophy were observed after 5 days of caerulein treatment [71], atrophy can be already seen after 3 days while hyperplasia had not yet occurred [81]. Although it is clear that exogenous CCK can exert a trophic effect on the pancreas of suckling rats [71], and that endogenous CCK release can also reproduce these growth effects [79], it remains to be demonstrated that normal physiological variations in serum endogenous CCK can be associated with pancreas growth in suckling animals. The answer to this question was addressed with the use of L-364,718, a specific CCK-1 receptor antagonist [82]. Even though the CCK receptor antagonist succeeded in inhibiting pancreas growth induced by exogenous caerulein, it failed to affect pancreas development when given orally to suckling rats at the relatively high doses of 2.5 and 5 mg/kg. These data suggest that endogenous CCK might not be a dominant factor in growth regulation of neonatal rat pancreas [83]. In guinea pigs, the same CCK receptor antagonist given by infusion through osmotic minipumps [32] instead of gavage [83], also failed to prevent normal pancreas development in animals aged 4 or 15 days at the end of treatment. It was also strongly suggested that occupation of the CCK receptors was not essential for pancreas growth and development in this species as in rats.

F. Combination of hormones

Two hormones association has been investigated in suckling rats: secretin-caerulein and caerulein-hydrocortisone. The first one [71], the secretin-caerulein combination reduced hyperplasia induced by secretin while improving atrophy observed with caerulein alone. The tandem caerulein-hydrocortisone was much more efficient [81] as caerulein potentiated the growth-promoting effect of the steroid on total DNA as well as on intracellular pancreatic enzymes and protein concentrations.

In summary, pancreas development and growth of suckling rats can be accelerated physiologically by hydrocortisone and thyroxine as plasma levels of these two hormones gradually increase during this preweaning period. On the contrary, endogenous CCK does not seem to be involved

since blockade of its specific receptor did not interfere with normal development. The role of secretin is more difficult to evaluate because of lack of information and tools. Indeed, we do not know if serum concentrations increase with age, although its basal concentration was found at around 1-2 pM in many species [84]. Also, we know that basal serum concentrations of secretin are elevated relative to adult values in newborn humans [69] and pigs [70]. On the other hand, in human infants, plasma secretin levels do not increase in response to milk until 24 days of age [69] while in piglets, a large increase was observed in response to duodenal acid [70]. Furthermore, specific secretin receptor inhibitors are not available as they are for the CCK receptors [82]. In these suckling animals, data strongly suggest that glucocorticoid, thyroxine and secretin are the most potent agents when given exogenously with the response to CCK developing with age. Finally, the best hormonal combination to speed up growth would be caerulein-hydrocortisone. In this area of research, human remains a total unknown.

III. HORMONAL CONTROL OF ADULT PANCREAS GROWTH

The experimental approaches to investigate the trophic action of hormones on adult pancreas are quite similar to those used in animals of younger ages. They include cell culture, endogenous release of the hormones, their exogenous application and their implication during the regenerative processes.

A. Acinar and duct cells in culture

Although *in vivo* model systems have provided numerous information on the systemic control of pancreatic acinar cells and duct cells secretion, isolation of dispersed acinar cells and of intact segments duct systems has provided valuable information on the characterization of enzyme release and electrolyte transport components. However, as we will see below, cells in culture had their limitations while evaluating factors involved in growth control.

One of the initial studies using mouse pancreatic acini in short-term culture looked at the effects of EGF, carbachol, insulin, corticosterone, secretin, growth hormone, glucagon and thyroxine on protein synthesis [85]. Among these factors, only EGF caused a significant increase in leucine incorporation into total protein when added alone to the culture medium, hydrocortisone led to a 50% inhibition. In combination, EGF, carbachol and insulin almost tripled the rate of leucine incorporation. Using the same mouse acinar cells in monolayer culture, the same authors were able to keep these acinar cells to increase their DNA synthesis for up to 14 days [86]. Over a 9 day period, caerulein significantly increased total DNA and protein contents of these cells up 2 fold with a maximal effect at the relatively high dose of 10 nM; this study showed that the trophic effects of the CCK analogue was exerted directly on the acinar cells in a medium supplemented with fetal bovine serum, EGF, carbachol, insulin and corticosterone. In a more elaborate study designed to evaluate the growth potential of many factors alone or in combination, it was found that CCK was the only secretagogue exhibiting trophic effects at concentrations within the physiological range. Hormones of the secretin family failed to induce growth as well as all the steroid hormones tested. Among the known growth factors, EGF was more efficient than IGF-1 and insulin. Finally, in combination, the effects of CCK, EGF and insulin were additive [87]. In all the above studies, growth of the acinar cells in culture was always estimated from evaluation of their rate of DNA synthesis. To date, no study has yet established any growth effect of hormones using acinar cells in culture by measuring increases in total cell counts, even the most recent one using human fetal pancreatic acini [88].

Investigators have been more successful in studying growth effects of hormones by using pancreatic cancer cell lines. As an example, in a rat acinar pancreatic cell line, the AR4-2J cells, growth, evaluated by DNA synthesis, was shown to be stimulated by gastrin and not by CCK-9, indicating a growth-promoting effect through CCK-2 receptor occupation [89]. Similarly, these same cells were used to evaluate the growth potential of glycine-extended progastrin [90].

The physiological mechanisms through which pancreatic duct epithelial cell turnover is regulated have not been extensively investigated. Pancreatic duct cells of the Syrian hamster were evaluated for their ability to increase their DNA synthesis [91]. At 1 μM, secretin (178%), bombesin (153%), VIP (138%) and gastrin (126%) stimulated DNA synthesis above their respective control with CCK without any effect. Whether these hormones can induce growth of pancreatic duct cells *in vivo* remains to be seen. Contrary to the duct cells isolated from the Syrian hamster, those of the guinea pig responded only to EGF as measured by monolayer growth; caerulein, secretin, VIP, carbachol and bombesin had no detectable effects [92].

B. Hormonal treatments in vivo

1. Muscarinic agonists

With the discovery that efferent vagal nerves to the pancreas release not only acetylcholine but also other neurotransmitters such as GRP and VIP and that CCK may act on pancreatic acinar cell secretion via a vago-vagal reflex [93], one may ask whether CCK and other hormones can cause pancreatic growth through some neurotransmitter release such as acetylcholine as final mediator. The involvement of acetylcholine was rendered plausible with the initial finding that atropine, given to pigeons four up to six hours, caused decreased protein synthesis, oxygen uptake and oxidation of glucose and fatty acids [94]. Even though acetylcholine seems involved in some metabolic activities of the pancreatic gland, its implication in its growth control is much less evident. Indeed, treatment of rats with bethanechol, an acetylcholine agonist, twice a day for 5 days at a dose of 6 mg/kg, caused

pancreatic hypertrophy with no hyperplasia [76]. With DNA synthesis measured *in vivo* following ^{3}H-thymidine injection, carbachol at 1 mg/kg induced pancreatic DNA synthesis maximally 27h after the drug administration; labeling was dose-dependent and acinar and connective cells were the most active from autoradiography evaluation [95]. In another study in rats in which bethanechol was given once a day at doses of 2, 6 and 12 mg/kg for 7 and 14 days, only hypertrophy occurred after 7 days of treatment at the highest dose. After 14 days of treatment, the lowest dose effect was limited to an increase in amylase concentration. The 6 mg dose resulted in pancreatic hypertrophy while it took the highest dose to obtain both hypertrophy and hyperplasia of the pancreatic gland [96]. This 12 mg/kg dose of bethanechol was also associated with significant elevations of serum gastrin for the 4 hours time period looked at. The question still unanswered remains: "are the trophic effects of the cholinergic agonist due to its direct effect on the pancreatic cells or due to the secondary effects of other substances released by bethanechol?" Gastrin could be one of them and its effects will be examined later.

2. Glucocorticoids

The role of corticosteroids on pancreatic growth after weaning has been reported only in few studies. Its potential effects were initially speculative from the observation that hydrocortisone increased serum and antral gastrin levels in the rat with gastrin the assumed trophic factor [97]. Effects on pancreatic growth were also postulated when hydrocortisone treatment was associated with increased concentrations and outputs of proteins both in basal and stimulated pancreatic secretion in human [98] and rats [99]. These increases in enzyme secretion in response to steroid treatment may result from increases in pancreatic enzyme contents as observed in the rat. Indeed, hydrocortisone given to adults rats for three days at the doses of 0.125, 0.5 and 2.0 mg/100 g body wt resulted in significant increases in pancreatic weight, pancreatic cellular mass and in protein, amylase and chymotrypsinogen concentrations at the highest dose given. However, total DNA contents remained unaffected in response to the three doses. Contrary to what was observed in suckling rats where pancreatic hypertrophy and hyperplasia occurred simultaneously in response to the same treatments, adult pancreas exhibited only hypertrophy [81]. These data explain the increased enzyme release following steroid treatment in man [98] and rats [99].

3. Gastrin and bombesin

In spite of several published studies on the effects of exogenous and endogenous gastrin on pancreatic growth, its role as an accepted pancreatic growth factor is not yet well defined. In favor of a potential effect of the hormone, its receptor has been characterized biochemically on guinea pig pancreatic acini [100], and localized on rat pancreatic acini by RT-PCR, Western blot and immunofluorescence [101]. One of the early studies on the trophic action of gastrin on the pancreatic gland indicated that rats injected with pentagastrin for 15 days at the daily high dose of 20 mg/kg saw significant increases in their pancreatic weight without any effect on DNA content [102]. Few years later, Majumdar and Goltermann [103] found a dose-related (0.5 to 2 mg/kg/day pentagastrin for 14 days) increase in pancreatic weight and a 50% increase in total DNA content at the highest dose. The growth-promoting effect of gastrin was confirmed later when pentagastrin was given every 8h for 7 days at a dose of 250 μg/kg; this treatment resulted in increased DNA and RNA concentrations (μg/100 mg tissue) without any data on pancreas weight [104]. In a more extensive study, pentagastrin was given every 8h sc in gelatin for 5 days at doses of 0.25, 1 and 4 mg/kg. Over that period, pentagastrin produced dose-dependent increases in pancreatic weight of 6, 10 and 28% over control with only the highest dose giving a significant effect. When DNA synthesis was looked at, there were no significant effect at any doses; this lack of effect of pentagastrin was also reflected on total pancreatic DNA contents at all doses [105]. In this study, the growth response was one of hypertrophy with no change in DNA content. It is possible that the pancreas would be more sensitive to the trophic effect of pentagastrin under conditions in which circulating levels of gastrin are low. This possibility is supported by studies showing dramatic effects of exogenous pentagastrin on pancreatic weight and DNA content in rats with very low levels of plasma gastrin observed after antrectomy [106]. It remains to be seen however that exogenous gastrin at concentration present in the circulation can induce pancreatic growth.

In studies in which hypergastrinemia can be compared to exogenous pentagastrin given at relatively high doses, contradictory data appeared. In one study [107], hypergastrinemia was produced either by constant gastrin infusion, fundectomy, or by omeprazole. As expected, gastrin influsion, omeprazole treatment and fundectomy, all induced comparable increases in serum gastrin concentrations. However, with comparable serum gastrin levels, gastrin infusion and omeprazole treatment were without effect on the weight and DNA content of the pancreas while fundectomy increased both. It would seem that fundectomy acts by another mechanism than hypergastrinemia to cause the observed pancreatic growth, and CCK does not seem to be the alternative since its serum level was not increased under these conditions [107]. Omeprasol given to hamster, guinea pig and chicken, once daily at a dose of 400 μmol/kg, caused hypergastrinemia in all three species but did not have any effect on the pancreas of these animals [108].

In summary, the fact that pentagastrin had trophic effects on the pancreas only at very high doses and not at those considered physiological [105], and that hypergastrinemia obtained through different strategies was not involved in growth of the pancreas in almost all studies, it seems appropriate to say that gastrin, if effective in some cases, is not the primary pancreatic growth factor, nor the most potent.

Gastrin releasing peptide (GRP) is the mammalian counterpart of bombesin, the 14 amino acid peptide discovered

in extracts of the skin of Bombina bombina [109]. This peptide has been located in pancreatic nerve fibers [110] and may thus operate on pancreatic acinar cells via paracrine action. The observation that GRP can induce release of CCK and gastrin, known to influence pancreatic growth, most often led the investigators to believe that the neurohormone may act on the pancreas indirectly through the release of the hormone listed above [111]. In a comparative study, both bombesin and GRP exhibited comparable growth-promoting effects on rat pancreas [112]. The direct action of bombesin on pancreas growth was clearly established when the peptide exhibited its trophic action in situations where plasma gastrin levels were minimal after antrectomy and also after blockade of the CCK receptor by a specific antagonist [113]. A direct effect of bombesin on the pancreatic gland was confirmed later when bombesin, added to mouse pancreatic acini in culture for 4 days, was able to significantly increase DNA synthesis in a dose-dependent fashion as did EGF and caerulein [114]. Finally, using one of the most potent CCK-1 receptor antagonist, L-364,718, it was possible to establish that bombesin, at the lowest effective doses, had direct effects on pancreatic growth while the CCK-1 receptor antagonist was able to reduce the growth response of bombesin when given at higher doses, those able to release endogenous CCK [115]. It thus seems that GRP can be classified among the physiological growth factors to the pancreas but a study involving its endogenous release associated with pancreatic growth would confirm its trophic status.

4. Secretin

Although secretin has been shown to be a very potent trophic hormone to the pancreas in suckling rats [71], a similar treatment at the same dose of 25 μg/kg, three times a day in gelatin, failed to induce increases in DNA synthesis and total pancreatic DNA contents in adult rats; it also caused a slight significant pancreatic weight increase [105] not associated with hypertrophy [116]. At the equivalent of 5 μg/kg (20 CU/kg), secretin given for 21 days also failed to induce pancreas growth [117]. In smaller rats, given half the previous dose of secretin, 12.5 μg/kg (50 units: 1 μg = 4U) every 8h for 7 days, the hormone caused slight significant increases in DNA synthesis and a more important increase in total DNA contents. These data confirm a previous observation that pancreas of younger rats are more sensitive to the growth effects of secretin than that of older animals [71, 104]. In further support of this hypothesis, if the dose of secretin is increased to 100 μg/kg for up to 5 days, the labeling index increased by 30% at day 1, total DNA content by 30% at day 5 (an increase comparable to that after caerulein) and total pancreatic weight and RNA content by 50% [118]. Such increases in pancreatic weight, DNA and RNA contents are quite comparable to those observed in the pancreas of suckling rats treated with secretin also for 5 days, at the dose of 25 μg/kg, under the same experimental conditions [71].

5. Cholecystokinin

Control of pancreas growth has been investigated since the end of the 60s and the beginning of the 70s. The initial study performed with pancreozymin, 20 Units/kg 3 x a day for 4 days, resulted in a significant increase in pancreas weight accompanied also by significant increases in trypsinogen, chymotrypsinogen and amylase concentrations. Microscopic examination confirmed the hypertrophic state of the gland but there was no observable increase in the number of mitosis [119]. When DNA synthesis and total DNA contents were measured for the first time, it was shown that the previously described pancreatic hypertrophy in response to pancreazymin [119] was also accompanied by pancreatic hyperplasia with pancreozymin also given at the same dose for one more day [76].

The demonstration of the growth promoting effects of CCK on the pancreatic gland has been done in traditional fashions, first with impure preparations of the isolated hormone, pure preparations and then with active synthetic agonists such as CCK-8, CCK-9 and caerulein. The initial studies were performed with the hormone given exogenously by different routes of application, intraperitoneally, subcutaneously in saline or gelatine to prolong its absorption and finally in mini-pumps delivering the product at a constant output either SC or IV for various period of time depending on the size of the pump. Later, to clearly establish that the effects observed in response to the hormone given exogenously were physiological, ways were found to show that comparable effects could be obtained with endogenously released hormone. Three different strategies were used to increase plasma cholecystokinin: feeding a diet rich in proteins, add to the diet soybean trypsin inhibitor and divert pancreatic juice from the gut.

a) Exogenous CCK

The initial dose of pancreozymin given to rats was 20 Units/kg and the treatment lasted 5 days [76]. When given for 9 days to rats, once a day at the dose of 12.5 Units/kg, pancreozymin increased pancreas weight by 31% and caused gland hypertrophy and hyperplasia with a 17% increase in total DNA content [120]. In the first comparative study in which purified CCK-PZ and synthetic CCK-8 were given at equipotent dose (CCK-PZ: 2 Units, CCK-8: 60 ng) three time a day for 10 days, the two preparations led to comparable increases in pancreas weight and concentrations of amylase, lipase and trypsinogen while insulin contents were not affected [121]. Treatments for up to 20 days in rats with CCK-PZ given at 15 and 60 Units/kg also led to pancreatic hypertrophy and hyperplasia with no difference between the two doses. Again, total insulin contents remained stable [117]. In a more detailed study using increasing doses of caerulein (0.2, 1 and 5 μg/kg) and length of time of treatment (5, 10 and 15 days), the data indicate that the effect of caerulein on pancreatic weight was dose related and twice the controls at 15 days. The smallest dose of caerulein had no effect on total DNA content after 15 days while those in the groups receiving 1 and 5 μg/kg caerulein

increased at a significantly greater rate than control. Similar changes were observed for total RNA and protein contents. In this study, pancreatic hypertrophy and hyperplasia were observed only in response to the 5 µg dose of caerulein [116]. The trophic effect of CCK-8 was also demonstrated in the pancreas of Syrian hamster with dose related increased in pancreatic weight and contents of amylase, RNA and DNA [122]. The trophic effects of CCK-8 were also observed in another rodent, the mice [123]. Indeed, when treated sc every 8 hr for 10 days at a dose of 1 µg/kg of CCK-8, their pancreas weight was significantly increased along with their protein and DNA contents while protein concentration was significantly decreased. The direct effect of CCK-8 on the acinar cells was demonstrated by a complete inhibition of the CCK-8 response by a CCK receptor antagonist, CR1409. It therefore seems that a prolonged CCK-8 treatment in this species was able to initiate pancreas hyperplasia without hypertrophy, which is different from the response in the rat [118].

With the discovery of specific CCK-1 receptor (CCK-A) agonists and antagonists, it was possible to clearly ascertain that the growth promoting effect of CCK on the pancreas resulted from occupation of the CCK-1 receptors. It was initially demonstrated that CCK-JMV-180, a potent CCK-1 agonist, was able to differentiate the high-affinity CCK receptor from its low affinity [124]. Its infusion (0.3 mg/kg/h) for 48 h in the rat caused significant increases in pancreatic weight and total contents of protein, RNA and DNA and DNA synthesis measured *in vivo* and *in vitro*. These effects were totally comparable to those in response to infusion of caerulein at 0.3 µg/kg/h [125], a physiological dose, since comparable to that of an infusion of 300 ng/kg/h of CCK-8, giving plasma levels of CCK at 6 pM, similar to physiological postprandial concentrations in the rat [126]. The trophic effect of infused CCK-JMV-180 on rat pancreas was dose-dependent from 50 to 300 µg/kg/h for 4 days with maximal increases in pancreatic weight obtained at the 150 µg dose while those of total protein, DNA and RNA were obtained at the 300 µg dose, giving responses identical to those obtained with 1 µg of caerulein also in infusion [127].

b) Endogenous CCK

Three strategies have been used to induce increased serum CCK: feeding diets rich in protein, feeding raw soya flour and bile-pancreatic or pancreatic juice diversion.

Raw soy flour has been shown to cause pancreatic hyperplasia [128-129]. Whether the trypsin inhibitor comes from egg white or soy protein isolate, its feeding to rats led to higher pancreatic enzymes, sustained gland enlargement and hyperplasia as evidenced by higher pancreatic DNA contents [130]. The growth promoting effect of trypsin inhibitor on the pancreas seems to occur in selected species: the rat is very sensitive, the hamster much less and the guinea pig not at all [131]; the response of the human pancreas is still uncertain and controversial [132]. In this model of trypsin inhibitor feeding, changes in the pancreatic gland occurred sequentially; indeed, the first significant variations happened in total RNA (day 4), in total proteins (day 7) and finally in total DNA (day 14) [133]. This slow development is quite different from what happened in response to exogenous CCK with significant increases in total pancreatic weight and RNA already after 2 days of treatment [118].

Diet manipulation also resulted in changes in pancreas growth. Indeed, rats adapted to 5% casein diet and then switched to 75% casein diet, saw their plasma CCK increased from 2.5 pM to 8.5 pM within the first day. Their pancreas increased steadily to reach a maximum after 7 days while hyperplasia was also definitive after a week [134]. Growth of the pancreas was dependent on the % of protein in the diet as 60% was associated with greater hypertrophy and hyperplasia that the 30% regimen. Also of interest is the observation that caerulein failed to induce growth in 5% casein fed rats probably because of a deficiency in dietary nitrogen and essential amino acids and/or a lack of endogenous CCK release [135]. This role of endogenous CCK as the trophic factor for pancreatic growth was later confirmed as feeding 70% casein resulted in pancreatic hyperplasia and hypertrophy, effect totally blocked by the CCK-1 receptor antagonist, MK-329 (L-364,718) [136].

Another way to show that increased food intake is also associated with pancreatic growth would be the condition of hyperphagia observed during pregnancy and lactation, the best physiological model. In these circumstances, pregnant and lactating rats exhibited pancreatic hypertrophy and hyperplasia with a maximal effect after the 3rd week of lactation [137]. The observed increases in total pancreatic DNA content would be equivalent to the following treatments given to adult rats: 14 days of pentagastrin, 0.5 mg/kg /day [103], 5 days of CCK, 20 U/kg, twice a day [75] or 10 days of a combination of caerulein (1 µg/kg) and secretin (25 µg/kg) 3 times a day [116].

It is clear from these data that feeding high protein regimen or protease inhibitors, both associated with increased CCK plasma levels, can lead to pancreas growth. It is not yet clear however, if this process occurs in human. One study showed that the human pancreas enlarged after camostate feeding [138] while another failed to reproduce pancreas hypertrophy after 3 months of ximelagatran feeding, a new protease inhibitor [139]. In the first study, increases in plasma CCK levels were observed after 4 weeks of camostate feeding while ximelagatran did not elevate human plasma CCK levels after 3 months of consumption.

Pancreaticobiliary diversion (PBD) to the ileum in rats makes the upper half of the small intestine completely free of pancreatic proteases and results in a marked increase of the concentration of plasma CCK with no effect on plasma gastrin [140]. In a 20 days study, PBD caused more than a two fold increase in pancreatic weight after 10 days, with no further increase thereafter. This weight increase was associated with hypertrophy and hyperplasia, effect completely abolished by the CCK-1 receptor antagonist, L-364,718, supporting CCK as the major trophic factor. The observation that the CCK

receptor antagonist resulted in pancreas atrophy and aplasia when given alone for 7 weeks support the view that CCK is not only involved in induction of pancreas growth but also participates to maintain normal growth of the gland [141]. This maintenance of normal pancreas growth is further emphasized by the observation that PBD in 3 day fasted rats kept pancreatic weight and DNA content at the level of fed sham control rats as well as expression of mRNA for amylase, chymotrypsinogen and procarboxypeptidase A with plasma CCK levels four fold higher (4.2 vs 0.9 pmol/l) [142]. A modification of the PBD model consisting of pancreatic juice diversion 8h/day for 4 days with bile reinfused into the duodenum also led to increased plasma CCK and pancreas hypertrophy and hyperplasia [126]. In such models, how critical is the return of pancreatic juice to the animal to obtain pancreatic hypersecretion and pancreas growth induction? It was shown that constant pancreatic juice drainage to the exterior failed to cause protein hypersecretion when compared to its reinfusion into the ileum or to caerulein infusion, despite the fact that the three procedures caused comparable increases in plasma CCK. While pancreatic hypertrophy and hyperplasia resulted from pancreatic juice diversion to the ileum and caerulein infusion, its total drainage outside also failed to induce pancreatic growth [143]. The severe protein losses caused by pancreatic juice drainage may explain the absence of pancreatic growth although plasma CCK levels were elevated. In a comparable situation, caerulein failed to induce pancreas growth in rats fed a low-protein diet [135].

All these studies support the claim that CCK is the major gastrointestinal hormone responsible for the maintenance of normal pancreas functions as well as induction of its growth after weaning. This status of prime pancreatic growth factor has been observed whether CCK was provided exogenously or release endogenously and was obtained under normal nutritional conditions.

6. Neuropeptides

a. **Substance P:** Found around ganglion cells in the pancreas [144] and with specific species effects on enzyme secretion [145], there is no indication so far that this peptide has trophic effect on the pancreas.

b. **Neurotensin:** This peptide has been identified in pancreas nerves [146] and its long term infusion at a relatively high dose induced increases in pancreatic weight and DNA content with no effect on protein, RNA nor enzyme contents [147]. It remains to be determined whether this represents a physiological effect of the neuropeptide. Its status as a weak trophic agent for growth of the pancreas was confirmed later [148].

c. **Vasoactive intestinal polypeptide (VIP):** When given alone for a week in rats, this neuropeptide, whose fibers terminate around the acinar cells, ducts and blood vessels [149], did not stimulate pancreatic growth but potentiated the effect of caerulein on pancreatic DNA content [150].

d. **Pituitary adenylate cyclase-activating polypeptide (PACAP):** PACAP-like immunoreactivity was observed in both exocrine and endocrine parts of rat and mouse pancreas [151-152]. To my knowledge, the trophic effects of PACAP has been so far demonstrated uniquely on the pancreatic cancer cells AR42J through its high affinity type 1 receptor [153-155].

7. Combination of hormonal treatments

a. **Caerulein and hydrocortisone:** In adult rat, hydrocortisone (0.5 mg/100 g body wt, once a day for 3 days) did not add to the growth promoting effect of caerulein (1 µg/kg, twice a day, for 3 days) on rat pancreatic weight and DNA content, while the steroid potentiated the caerulein effect on amylase, chymotrypsin, and protein concentrations [81].

b. **Pentagastrin and secretin:** When given to rats every 8h for 5 days, pentagastrin (0.25, 1 and 4 mg/kg) and secretin (25 µg/kg) exhibited an additive effect on pancreatic weight; this was the only significant modification induced by this hormone combination [105].

c. **Caerulein and secretin:** The effect of the two hormones given to rats for 7 days, 3 times a day (secretin: 50 U/kg; Cae: 320 ng/kg) caused potentiation on total pancreatic RNA, DNA and pancreatic weight [104]. This same combination (secretin, 25 µg/kg and caerulein, 0.2, 1 and 5 µg/kg) for up to 15 days, significantly increased weight, protein and trypsinogen more than the sum of the effects of both hormones given singly. However, the increase in DNA content induced by caerulein was not amplified by secretin [116]. However, in another study also in rats, potentiation was found when caerulein and secretin were injected together for pancreatic weight on day 2, labeling index of non acinar cells also on day 2, thymidine incorporation in acinar and non acinar cells on day 3 along with DNA content also on day 3 [118].

Of all these hormone combinations, that of caerulein and secretin was the most potent for all the growth parameters examined.

IV. CELL TYPES TARGETED BY HORMONES

Acinar cells and other cell types of the exocrine pancreas were originally classified as cells showing a low activity regarding cell replication [156]. These cells however were able to enter the cell cycle under appropriate conditions.

In studies on growth promoting effects of gastrointestinal hormones on the pancreas, no one has provided information to determine whether or not there was metabolic uniformity among segments regarding rates of DNA synthesis. A striking uniformity has been shown between the head, body and tail of the pancreas regarding content and basal rates of DNA, RNA and protein synthesis in an unstimulated gland but such data do

not exist for growing pancreas under the influence of trophic factors [157]. In a study where rats were treated with caerulein (1 µg/kg, 3 times a day for 2 days), DNA synthesis was measured either *in vivo* or *in vitro* from thymidine incorporation into DNA. Interestingly, increases in DNA synthesis initiated by this 2 day caerulein treatment were identical percentage wise whether rates were estimated *in vivo* or *in vitro*. Furthermore, more importantly, the growth promoting effect of caerulein was uniform in all pancreatic segments. Such data plead for uniform growth of the pancreatic gland when stimulated to grow in response to CCK [158].

In normal mice, given ^{3}H-thymidine in a continuous manner for up to 60 days without further treatment, labeling was observed in small and large ducts one hour after its initial injection and reached a level of about 67% cells labeled after 60 days. Interestingly, the distribution of labeled cells in the acini population did not appear to be random and reached between 1.4 and 4.2% after 60 days while the labeling index in the islet cells varied between 8.4 and 13.4%. It thus seems that the duct cells are a lot more active than the two other populations regarding cell division. These data suggest that the added ductal cells could replace those washed away by pancreatic juice flow [159]. Upon stimulation by CCK in mice, the ductal cell population seems to be more sensitive than the acinar cell population to long-term stimulation [160]. With endogenous elevated plasma CCK following pancreaticobiliary diversion in the rat, administration of ^{3}H-thymidine 1h before sacrifice, indicated that the labeling index of the acinar cell in the control was 0.42% reached 2% after 5 day of diversion and was back to normal by day 40. Comparable increases were observed in the labeling index of the ductal cells after 5 days at 2.6% and still significantly elevated at 40 days. The only increased labeling in centroacinal cells was observed at day 10 with a labeling index less than that of the two other cell populations [161]. When given at a relatively high dose equivalent to 5 µg kg^{-1} h^{-1} for 7 days, CCK-8S induced pancreatic weight increases significantly only 18h after the beginning of treatment and the decrease in total DNA content observed at 36h is indicative of pancreas destruction. During this treatment, maximum labeling indices of acinar cells (4%) and of centroacinar cells (6.5%) were observed at 36h while that of the ductal cells reached its maximum closed to 3.5% at 18h and then declined [162]. What is interesting in that study is the finding that devazepide, a CCK-1 receptor antagonist, given alone reduced significantly the labeling index of the acinar cells at 18h, 36h, 3 and 7 days, that of the centroacinar cells at 18h, 36h and 3 days and that of the ductal cells at 18h, 36h and 3 days. The effects of this CCK-1 receptor antagonist were expected on the acinar cells because they have CCK-1 receptors while the two other cell populations are known to be deprived of them until now [34]. Then, how do these ductal and centroacinar cells respond to CCK still remains an unanswered question so far.

One possibility would be the presence of an intermediate cell with both exocrine and endocrine potentials [163,164]. If such intermediate or precursor cells exist, their transformation into the other cell types of the exocrine pancreas has to be a relatively fast phenomenon. Indeed, when the rat pancreatic gland is stimulated to grow by a physiological dose of caerulein [116] for 4 days with constant labeling of all the pancreatic cells after each treatment with ^{3}H-thymidine, it is quite interesting to see the stability of the labeling of each cell type in the control group as in the caerulein-stimulated group for up to 50 days after the end of the 4 day treatment. In the control group, the labeling index (LI) of the acinar and ductal cells remained at around 6% and that of the endothelial and interstitial cells stabilized at 4%. Under caerulein, each cell type increased its rate of proliferation with the acinar cells being the most active with a LI of 30%, followed by the interstitial cells at 16%, the ductal cells at 9% and the endothelial cells at 8 to 9% [165]. These data indicate that the newly formed cells remained in place for at least 50 days after the end of treatment. The only population with an important decrease in its labeled cells is that of the interstitial cells; one may then suggest that this could be the intermediate or precursor cell population previously described [163, 164]. Another interesting observation indicates that whether CCK is given exogenously [118] or release endogenously from feeding raw soya flour [133], the labeling index or thymidine incorporation picked always one day earlier in the acinar cells than in the duct cells.

A fundamental difference seems to exist between pancreatic growth induced by caerulein as opposed to feeding raw soya flour containing soya bean trypsin inhibitor known to release endogenous CCK [166]. Indeed, after four days of soya flour feeding [133], pancreatic tissue increased by 75% in size with its RNA and protein contents almost doubled without any change in total DNA. Continuous feeding for 4 weeks almost doubled the size of the pancreas and DNA content. However, immediately upon changing this soya flour diet to a normal diet, a rapid fall in pancreatic DNA was observed that reached control levels within 48h of the changeover. However, it took 7 days for RNA, pancreatic weight and protein to regain control levels [167]. Furthermore, this tremendous loss in DNA content was accompanied by cell death through apoptosis with a maximal incidence of apoptotic bodies observed 24h after the switch to a normal diet. Such drastic changes were never observed after caerulein treatment. In fact, losses of pancreatic tissue and DNA content as well as differences in apoptosis between control and caerulein groups were never observed. As an explanation, a programmed cell death was postulated after withdrawal of the soya diet [169], which certainly did not occur after caerulein [168]. Another possibility remains that the constant presence of soya bean trypsin inhibitor in the gut may have exhausted and/or desensitized the CCK cells in the intestine as shown with feeding a high protein diet [134]. Such a phenomenon would not occur under or after caerulein treatment since the stimulus was exogenously given leaving the normal endogenous release of CCK unimpaired.

All these studies point out that all cells of the exocrine pancreas are sensitive to the trophic effects of CCK and its

analogues. Furthermore, each specific cell type seems to have its own level and length of response and the presence of a precursor cell still remains to be discovered. Also, standard protocols should be designed in the future to get a better image of the cell cycle response of each cell population in the exocrine pancreas when stimulated to grow.

V. REGENERATION OF THE PANCREATIC GLAND

Pancreas regeneration has been studied in small animals such as rats, mice and hamsters. However, our knowledge of its regeneration in humans and in large animals is quite limited. In order to investigate pancreas regeneration, two strategies were used, that of partial pancreatectomy and of partial gland destruction after induced pancreatitis.

A. Pancreatectomy

In a well controlled experiment on rats, Lehv and Fitzgerald [170] found that after 55% pancreatectomy, the residual pancreas grew to be about 50% heavier than the corresponding control segments. In another study, a greater than linear increase in regeneration of the residual pancreas was observed with increasing degree of resection from 50, 70 to 90% [171]. After 90% pancreatectomy (PX) in rats, the growth of the exocrine tissue and of the endocrine β-cells was discordant during the early weeks after pancreatectomy. Initially, at 3 and 7 days after PX, mitotic indices in both cell types were comparable at 3 to 4 fold above the sham operated animals. Later on, at 14 and 21 days, the β-cells of the PX animals had a mitotic index still double that of the shams while no difference was observed in the exocrine tissue [172]. We do not know yet which factors are responsible for this prolonged growth of the β cells but CCK does not seem involved because it failed to increase the mitotic index of the endocrine cells after a 4 day treatment [165].

If CCK does not seem involved in regeneration of the β cells, its endogenous release by the trypsin inhibitor FOY-305 after a 66% distal pancreatectomy led to significant increases in pancreatic weight and contents of protein, RNA and DNA after 27 days when compared to PX animals. Furthermore, the increases of all parameters observed in PX and FOY rats were higher than those observed in the sham and FOY animals. Hypertrophy was already observed 13 days post PX while it took until 27 days to detect hyperplasia [173]. The interesting point with this specific trypsin inhibitor is that pancreatic regeneration occurred rapidly, hypertrophy after 13 days and both hypertrophy and hyperplasia after 27 days. Such rapid growth contrasts with significant increases in pancreatic mass of the remnant after distal PX reported only at around 60 days after resection with growth plateaured after 6 months [170,171,174]. It is evident that FOY-305, a chemically pure trypsin inhibitor, was more efficient than raw soya flour, containing varying amount of trypsin inhibitor, in stimulating pancreas regeneration, 13-27 days compared to 45 to 160 days [173,174]. Finally, feeding raw soya flour after PX to an already enlarged pancreas resulted subsequently only in hypertrophy while after PX alone, raw soybean flour feeding caused both hypertrophy and hyperplasia of the remnant [174]. Does the pancreas have a limited capacity for additional growth? Such a possibility has been explored after 60% pancreatectomy in young rats [175] and indicates that the time-course of pancreatic regeneration corresponded to the elevation of basal plasma CCK levels with the highest levels observed 45 days after PX followed by a significant decrease at 60 days. It was also demonstrated that a one month treatment with CCK-8 accelerated regeneration in the first month with almost no effect for the next two months.

Of concern is the fact that almost all studies on pancreatic growth have been done using rodents as the experimental animals. Few experiments were performed in pigs and even in humans. In the pig, it has been shown that its pancreas can regenerate after 50% PX and the road leading to remnant regrowth involved cellular processes including inflammation, apoptosis, acinar cell atrophy followed by recovery of the pancreas size and increased total DNA contents 30 days post-resection [176]. Also in the pig, an Italian group has documented that bombesin, 5 µg sc three times daily for 4 weeks, stimulated pancreatic regeneration after 70% pancreatectomy as evaluated by pancreatic weight, labeling index and markers of the cell cycle [177].

Although controversy exists on the potential of human pancreas to grow in response to endogenous CCK release [138,139], one study clearly indicated that the human pancreas do not regenerate after partial pancreatectomy without any hormonal treatment [178]. If however the human pancreas has any potential to regenerate after hormonal treatment, CCK would not be the factor since it was recently shown that the human pancreatic acinar cells lack functional responses to CCK and gastrin [179] and do not express any CCK receptors [180]. With the recent finding that the pig pancreas can accelerate its regeneration in response to bombesin [177], this peptide should be given a trial as a human pancreatic growth factor.

B. Pancreatitis

Pancreatitis induction in animals has been another approach to study pancreas regeneration and the effects of various hormonal treatments. Various procedures, duct ligation [181], ethionine treatment [182] or supramaximal doses of caerulein [183] were used to induce pancreatitis.

After such a treatment with caerulein, infused at a dose of 5 µg/kg/h for 12 h, pancreatic inflammation occurred concomitant with progression of acinar cell destruction [184]. While still under the degenerative process, the pancreatic gland exhibited signs of regeneration with thymidine incorporation into total DNA showing a biphasic pattern with a initial peak at day 1 and a second extended one between days 4 and 7. Labeling indices indicated incorporation into intercalated duct

cells and interstitial cells within the first 24h with the second major peak located in the acinar cells. Such regeneration was associated with increased rates of collagen synthesis in day 1.5 and 2.5 after pancreatitis induction and remained elevated for as long as 10 days [185].

Using another model of caerulein-induced pancreatitis consisting of SC injections of caerulein in gelatin at a dose of 12 µg/kg three time a day for 2 days [186], it was shown that pancreatic weight recovery to control values was not obtained 20 days after pancreatitis induction while total DNA contents had reached control values after 10 days as well as total contents of chymotrypsinogen and RNA. Regeneration of the pancreatic gland to control values can be accelerated by feeding the rats a 50% protein rich diet, a normal diet supplemented by 1% soybean trypsin inhibitor (SBTI) and by sc injections of caerulein 3 times a day at a dose of 1 µg/kg. Of these treatments, caerulein was the most efficient, followed by SBTI feeding and then the 50% protein diet [187]. The importance of endogenous CCK in the regeneration process of the pancreas after caerulein-induced pancreatitis was demonstrated when L-364,718, a CCK-1 receptor antagonist, given at 1 mg/kg twice a day for 13 days, strongly inhibited spontaneous regeneration of the pancreatic gland [188]. In the model of pancreatic duct occlusion in which plasma CCK levels remained high [189], the absence of the CCK-1 receptor (CCK-A), because of a genetic abnormality in the rat, did not modify the acute phase of pancreatitis but significantly retarded pancreas regeneration [190]. These data point out the importance of the CCK-1 receptors in pancreas regeneration and also indicate that CCK is not the only growth factor involved.

In the rat model of pancreas destruction by ethionine in which pancreatic weight, DNA and protein contents were significantly decreased by 62, 47 and 80% respectively, infusion of caerulein at 600 ng/kg/h via a mini-pump, accelerated the pancreatic regeneration process via hypertrophy and hyperplasia [191].

CONCLUSIONS

The main features of this review are the following:

1. The rat fetal pancreas can be stimulated to grow with glucocorticosteroids the most potent factors, gastrin seems the most efficient among the gastrointestinal hormones and CCK was associated with acinar cell maturation but not growth;
2. The pancreas of suckling rats are more sensitive to secretin than CCK as a trophic factor with no potentiation between the two hormones; hydrocortisone caused hyperplasia and hypertrophy;
3. The adult pancreas of rodents remains insensitive to gastrin, CCK exhibits its strongest trophic effects with combined hypertrophy and hyperplasia with great potentiation by secretin; the hydrocortisone effect is centered on hypertrophy;
4. Regeneration of the pancreatic gland has been observed in rodents and pigs after partial pancreatectomy and induced-pancreatitis in rodents; CCK seems the most potent factor involved in pancreas regeneration in rodents while bombesin could be the actor in the pig;
5. With regards to human pancreas development, growth and regeneration, most of the research remains to be done and the new findings in the pig strongly support that this species could be the model of choice as a substitute for research directly performed on humans.

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