Pancreas: A Sex Steroid-Dependent Tissue*

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Abstract

Sex steroid hormones (estrogens, progestagens and androgens) have been associated with healthy and neoplastic pancreatic biology, although the precise significance of the findings has not been well established. Receptors for the three different types of SSH are expressed in normal and tumoral pancreatic tissue with varying profiles related to cell origin (exocrine or endocrine), to type of neoplasm, and probably even to tumoral behavior. The activity of specific enzymes involved in the synthesis and transformation of SSH are increased in some neoplastic pancreatic tissues, which may influence the circulating concentrations of these hormones, such as the low serum testosterone:dihydrotestosterone ratio described in male patients with pancreatic carcinoma. Different patterns of age and gender-related incidence and growth of neoplasms have been identified. Experimental studies have shown that pancreatic carcinogenesis is promoted or inhibited by SSH. At present, the data supporting hormonal manipulation for the treatment of these tumors are non-conclusive. Normal and tumoral pancreatic tissues may be regarded as a target for SSH and an additional site of biosynthesis. The influence of these hormones on physiological activities is not well known but should be further explored. The study of SSH in pancreatic neoplasms will provide clues about its origin, development, tumoral behavior, prognosis and more specific hormonal therapy. We review here the evidence favoring the role of SSH and their possible clinical implications in pancreatic function.

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The pancreas is an unusual organ because of its dual function as an exocrine and an endocrine gland. The interaction between these two functions plays a central role in nutrition, such as digestion (exocrine function) and regulation of blood glucose levels (endocrine function) [1]. Pancreatic exocrine tissue is composed of acinar and duct cells that secrete mainly digestive enzymes, water and electrolytes. The secretion is regulated by neurohormonal interactions and hormonal-hormonal interactions.

The exocrine pancreas is a physiological target for gut and pancreatic peptides that stimulate or inhibit the secretion, such as secretin, cholecystokinin, gastrin, neurotensin and motilin, or pancreatic polypeptide, peptide YY, somatostatin and glucagon, respectively [1]. The endocrine pancreas consists of groups of hormone-secreting cells called islets of Langerhans, which are dispersed throughout the exocrine tissue. There are several markers suggesting that these cells may belong to a family of endocrine and neuronal cells that constitute the APUD system (amine precursor uptake and decarboxylation). In humans, pancreatic endocrine cells are highly differentiated into four major types that synthesize insulin, glucagon, somatostatin, and pancreatic polypeptide. These hormones are involved in the glucose utilization and digestive processes [1].

Sex steroid hormones comprise estrogens (estradiol, estrone and estriol), progestagens (pregnenolone, progesterone) and androgens (testosterone, dihydrotestosterone, dehydroepiandrosterone, androstenedione), and are mainly produced in the ovary, placenta, testis and adrenal gland. These hormones are responsible for sexual differentiation, and their major target tissues are the mammary gland, urogenital tract, bone, cardiovascular and central nervous systems [2].

The involvement of SSH in pancreatic function came to light after epidemiological, clinical and biochemical findings – such as the relationship between gender and prevalence of some tumors of the pancreas, the effect of pregnancy on insulin and glucose metabolism, as well as the identification of SSH receptors in the pancreatic tissue [1,3]. Studies *in vivo* and *in vitro* have investigated the effect and mechanisms of action of these hormones on animal and human pancreas. However, due to the experimental designs and the possibility of species-specific differences, the clinical applications of the results are limited. A precise role for SSH in pancreatic tissue has not been found, but according to the studies reviewed in this work, they may participate in different physiological and pathological conditions, suggesting that the pancreas is a target organ for SSH. This knowledge allows new opportunities for diagnosis and therapy.

The normal pancreas

In normal pancreatic tissue, the presence of receptors for the three different types of SSH, as well as the activity of specific

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SSH = sex steroid hormones

enzymes involved in their synthesis, have been identified. Also, there is evidence that SSH modify the normal exocrine and endocrine pancreatic function.

SSH receptors

The uptake and retention of estrogens within human and animal pancreatic tissue discovered several decades ago have suggested the presence of a binding protein and/or receptor for estrogens. Pousette et al. [4] described the estrogen-binding protein in different subcellular compartments of normal human pancreas, mostly within zymogen granules. This protein, which constitutes 2–4% of the total protein content of pancreatic cytosol, is secreted into the duodenum following stimulation of the pancreas by food, suggesting that it may be related to the secretion of digestive enzymes [4]. Expression of a nuclear estrogen receptor, distinct from the estrogen-binding protein [5], has been found in normal fetal [3,6] and adult [7] human pancreas. The presence of some estrogen-induced genes may provide evidence for a functional estrogen receptor, even though the expressed levels are low [7]. Two other kinds of estrogen receptors have also been identified: a plasma membrane bound in mice and an estrogen receptor-related receptor-gamma in humans. The first is involved in rapid non-genomic effects of estrogens in pancreatic-beta cells [8], while the latter is a member of the so-called estrogen orphan nuclear receptors (for which no ligands have been identified), which are capable of activating DNA transcription although they are unaffected by estradiol and are thought to be involved in tissue differentiation and organ development [9].

Expression of progesterone receptors has also been found in normal human pancreas [6,7,10]. In endocrine tissue, this receptor was present in 75% of glucagon-producing cells but in only 5–20% of the insulin-producing ones [10]. Similarly, expression of androgen receptors has been demonstrated in fetal and adult normal human pancreas [6], as well as androgen receptor mRNA in rat pancreas [11].

Sex steroid biosynthesis and biotransformation

The fact that pancreatic tissue is capable of transforming SSH through specific enzymes involved in their biosynthesis and biotransformation provides further evidence that the pancreas is a SSH-dependent organ. Morales et al. [12] demonstrated that mitochondria from dog pancreas has the ability of transforming cholesterol into pregnenolone, a reaction catalyzed by the cytochrome P450scc, and also confirmed the transcription of the gene responsible for this enzyme in pancreas homogenate. Given that this enzyme catalyzes the first and rate-limiting reaction in the biosynthesis of steroid hormones, this finding is strong evidence that the pancreas is a steroidogenic tissue, as are other classic tissues such as the ovary, placenta, testis and adrenal gland. Furthermore, the enzyme 3β-hydroxysteroid dehydrogenase, which converts pregnenolone into progesterone, an initial step in steroid biosynthesis, has been localized in the canine pancreas [13]. Another enzyme in the steroid synthesis pathway, 17β-hydroxysteroid dehydrogenase, responsible for the interconversion of estrone and estradiol, and of androstenedione and testosterone, has been identified in the dog pancreas [14]. In agreement with this finding, we have shown that the isolated perfused canine pancreas rapidly converts testosterone mainly into androstenedione, and to a lesser degree into DHT and estradiol, thus supporting the high activity of this enzyme in the pancreas [15]. Another study also showed the presence of the enzymes aromatase and 5α-reductase in normal pancreatic tissue, which are involved in the transformation of testosterone into estradiol and estrone, and testosterone into DHT, respectively [16].

SSH and pancreatic function

Effects of SSH on exocrine and endocrine pancreatic secretion have been demonstrated in animal and human studies under different experimental conditions, resulting sometimes in distinct and non-conclusive findings.

• *Exocrine pancreas*

Sexual dimorphism in the genetic expression of pancreatic secretory proteins has been shown in mice (higher amylase and lower trypsinogen and lipase in females), but not in rat pancreas. Although genetic expression was dimorphic, there was no difference at the protein expression level [17]. Estradiol increases zymogen granules in rat acinar cells as it decreases responsiveness to cholecystokinin stimulus, thus reducing amylase secretion. This was related to a decrease in the number of cholecystokinin receptors and to an increase in an inhibitor of secretion, $G_{q/11}$ [18]. Studies in post-menopausal women have shown a decreased pancreatic secretion after a secretin-stimulation test, and that estrogen administration for short periods increases this pancreatic secretion, whereas prolonged administration (3 to 4 months) abolishes this effect [19]. Progesterone and estradiol antagonize the Cl^- transport mediated by the cystic fibrosis transmembrane conductance regulator in a human pancreatic duct cell line (PANC-1), thus diminishing fluid secretion. Also, progesterone has been shown to decrease the synthesis of CFTR mRNA and protein in rabbit acini. These findings may explain the clinical deterioration in female cystic fibrosis patients after puberty, and the shorter mean life span when compared with their male counterparts [20].

• *Endocrine pancreas*

In endocrine pancreas estrogens, progesterone and androgens have been shown to be involved in insulin secretion. A study in mice demonstrated that 17β-estradiol at physiological concentrations increased the secretion of insulin in the presence of glucose through a non-genomic process mediated by a membrane receptor that closes K_{ATP} channels. This finding may explain previous reports of the success of estrogen

DHT = dihydrotestosterone
CFTR = cystic fibrosis transmembrane conductance regulator

administration in preventing the development of diabetes mellitus [8]. Progesterone-treated mouse pancreatic islets release more insulin in response to glucose, probably by increasing the glucose sensitivity, an important mechanism for altered glucose and insulin metabolism in pregnancy [21]. The androgens epiandrosterone and DHT have been reported to diminish the deleterious effects of interleukin-1β on insulin secretion and nitric oxide-mediated damage in rats [22]. Another study performed in rats similarly demonstrated that testosterone administration was able to increase insulin mRNA and its serum levels, as well as cell insulin content and release (even in the absence of glucose), however the mechanism was not investigated [23].

Progesterone has been implicated as an inhibitor of mouse islet cell proliferation in *in vitro* studies [21]. Although in a more recent *in vivo* study in rats, progesterone increased islet cell proliferation (both α and β cells) of already differentiated cells, an effect that was dependent on some gonadal factor since it was not observed in gonadectomized male and female rats or in gonadectomized estradiol-supplemented females. Given that pregnancy is accompanied by high circulating levels of progesterone, it was suggested that under this condition progesterone may prepare the pancreas for increased metabolic activity by stimulating cell proliferation [24].

The neoplastic pancreas

Pancreatic tumors originate from exocrine or endocrine cells. Exocrine neoplasms, mainly ductal adenocarcinoma, are the most frequent pancreatic tumors and one of the most common causes of cancer death. There are other tumors, such as some cystic neoplasms with a strong female preponderance, whose cellular origin and phenotypic relationship to the pancreas have remained enigmatic. Different patterns of age and gender-related incidence, as well as specific SSH profiles, have been linked to different types of pancreatic neoplasms, suggesting that the development and growth of these tumors may result from an SSH-driven process.

Role of age and gender

Adenocarcinoma of the pancreas is an uncommon disease in young females, while in the post-menopausal period its prevalence progressively increases to equal that of men [1,25]. This finding has assumed a protective role of estrogens in this neoplasm. However, there is no conclusive evidence that menstrual and reproductive factors or estrogen replacement therapy have a protective or adverse effect on the risk of pancreatic cancer [26].

Mucinous cystadenoma or cystadenocarcinoma, papillary cystic neoplasm and serous microcystic adenoma belong to a group of rare pancreatic tumors with a strong female predilection [27,28]. Serous microcystic adenoma is a tumor that affects mainly post-menopausal women [1], whereas the other two neoplasms occur in fertile women and an increased growth may be observed during pregnancy, suggesting estrogen and/or progesterone dependency [29,30]. Histogenesis of these tumors is uncertain, and mucinous and papillary tumors might originate from cells of the female genital tract that were attached or incorporated into pancreatic tissue during the seventh week of gestation [27,28].

SSH receptors

SSH receptors, mainly ER and PR, have also been found in neoplastic tissue. High affinity estrogen binding was identified in human ductal pancreatic adenocarcinoma over two decades ago [3]. However, immunocytochemical research on ER and PR has not been successful in this pancreatic cancer, despite positive PR staining of some endocrine and papillary-cystic pancreatic tumors investigated in the same studies [7,10]. Singh et al. [7] used different techniques and found very low and variable amounts of ER, however estrogen-inducible genes and PR were expressed suggesting the presence of a functional ER. Human cancerous pancreatic duct cell line CAPAN-1 expresses ER α and at a lower level PR, which showed a twofold increase after addition of estradiol [31]. The presence of specific binding proteins for androgen in malignant tissues is controversial [3,6] and no definitive identification of androgen receptor has been provided.

Progesterone receptors have been the most consistent SSH receptors with the strongest immunoreactivity in mucinous cystadenoma and cystadenocarcinoma [27,30], papillary cystic neoplasm [28,29] and endocrine pancreatic tumors [10,32]. The absence of PR correlated with a worse outcome in mucinous and endocrine neoplasms [27,32]. Since the malignant potential of these pancreatic tumors might be somewhat unpredictable but lower than that of adenocarcinomas, and express PR more consistently, this receptor could be useful as a marker of low malignancy.

The synthesis of PR is induced by estrogens, thus the lack of ER in some pancreatic neoplastic tissues in the presence of PR suggested that the latter could be expressed in an estrogen-independent manner. However, the recent discovery of a type II or β-isoform of ER [33], not identified using methods directed to the "classical" type I or α-isoform, offers an explanation for the previously reported indirect evidence of a functional ER [7,16] and suggests that PR expression may result from ER-β stimulation. According to this premise, our group has shown, through immunohistochemical methods performed in pancreatic tissues of seven cases of papillary cystic neoplasm, that the β-isoform of ER is preferentially expressed over the "classical" α-isoform (unpublished data). The preferential expression of ER-β suggests that ER may be more commonly present in these tumors and that the low frequency of positive results may be due to a limited search for the α-isoform. The presence of ER-β in a cystic pancreatic tumor with reduced malignancy may have clinical prognostic significance as recently established in breast cancer [33].

ER = estrogen receptors
PR = progesterone receptors

Sex steroid biosynthesis and biotransformation

There is increased activity of aromatase and 5α-reductase in a malignant pancreas as compared to fetal and normal pancreas [16]. Both enzymes are involved in the transformation of testosterone, the latter reducing testosterone into DHT, its more active metabolite.

The activities of sex steroid biosynthetic enzymes in pancreatic carcinoma may influence the circulating concentration of SSH. In fact, low serum testosterone and DHT levels have been described in these cases, and a striking difference was noticed in male patients when comparing the proportion of serum DHT by the T:DHT ratio. Moreover, we proposed that a low T:DHT ratio was an early tumor marker for pancreatic cancer in male patients even in stage I of the disease, and that a ratio below 5 was as sensitive but more specific than the pancreatic tumor marker CA 19-9 at a cutoff point of 100 U/ml. This alteration in serum androgen profile distinguished pancreatic cancer from chronic pancreatitis and tumors from the lower biliary tract and papilla in men [34]

Indirect evidence of testosterone conversion in tumoral pancreatic tissue also resulted from the increased serum androstenedione concentration, another major circulating steroid with androgenic activity [35]. A higher activity of the enzyme 17β-hydroxysteroid dehydrogenase, which converts testosterone into androstenedione, may be responsible for this finding [14]. The role of the neoplastic pancreas in this process is supported by the fact that tumoral resection led to androstenedione reduction and testosterone increase [39].

Sex steroid and carcinogenesis

Several experimental studies on pancreatic carcinogenesis have stated that androgens and estrogens may play key roles as promoters and inhibitors, respectively. Tumor growth rate of human pancreatic adenocarcinoma xenografts in nude mice was consistently stimulated by testosterone and inhibited by the anti-androgen cyproterone acetate [36]. However, the androgen DHT-sulfate, which has been shown to reduce the incidence of carcinogen-induced tumors in animal models, inhibited *in vivo* and *in vitro* growth of human pancreatic cancer cell lines. This decreasing carcinogenetic effect is probably related to the potent non-competitive inhibition of glucose-6-fosfate dehydrogenase, the rate-limiting step of the hexose monophosphate shunt, a biochemical pathway that provides substrate for DNA synthesis in neoplastic tissue [37]. Similar delay in the growth of some human pancreatic carcinoma cells *in vitro* was obtained with medroxyprogesterone acetate by inducing apoptosis probably via the PR pathway in association with decreased bcl-2 function (inhibition of apoptotic cell death) [38].

Acinar preneoplastic lesions in rats treated with azaserine occurred in greater number in males than females. Castration notably reduced preneoplastic lesions to a similar amount in ovariectomized females, and was further inhibited in a dose-dependent way by the addition of estrogens [39,40]. Pretreatment of rats with castration alone or in combination with estradiol also inhibited the growth of transplanted rat acinar cell carcinoma [41]. These effects on pancreatic tumorigenesis were not found in hamsters treated with different carcinogenetic agents more specific for the induction of ductal carcinoma. [40,42]. The varying effects of SSH in development and growth of pancreatic neoplasm may be related to the mechanism affecting carcinogenesis, which is unclear, and may be multifactorial and/or different for each hormone. Also, SSH interact differently with rats with atypical acinar cells than with hamsters with ductal cell preneoplastic lesions.

Clinical trials for cancer of the pancreas with hormonal therapy have been performed based on the evidence mentioned here; however, the data are not conclusive for hormone manipulation. Nonetheless, this approach in combination with other drugs seems to exert some beneficial effect and should be evaluated in view of the poor results achieved with current therapies [43].

T = testosterone

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Pay no attention to what the critics say. No statue has ever been put up to a critic

Jean Sibelius, Finnish composer (1865–1975)