Expression of receptors for gut peptides in pancreata of BOP-treated and control hamsters

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The growth of pancreatic cancers may be influenced by certain gut peptides. However, the alteration of gut peptide receptors in the progress of pancreatic carcinogenesis is largely unknown. With storage phosphor autoradiography, this study visualized and characterized receptors for cholecystokinin (CCK), somatostatin (SST), bombesin (BBS), secretin and vasoactive intestinal peptide (VIP) in pancreata of control hamsters (\(n = 7\)) and pancreatic neoplastic lesions (\(n = 10\)) or adenocarcinomas (\(n = 10\)) of \(N\)-nitrosobis(2-oxopropyl)amine (BOP)-treated hamsters. The specific CCK-A and secretin receptors expressed in normal pancreata were markedly reduced in pancreatic neoplastic lesions and absent in adenocarcinomas. In the development of pancreatic tumours, the subgroup of SST receptors did not change, but both the affinity and binding capacity declined. In comparison with the binding of VIP to normal pancreata, specific VIP binding was significantly lower in neoplastic lesions and almost absent in pancreatic adenocarcinomas. No specificity binding for BBS was detected in normal pancreas or (pre)neoplastic lesions of hamster pancreas. The reduction or absence of receptors for CCK, secretin, SST and VIP in hamster pancreas with the progress of carcinogenesis suggests that in BOP-treated hamsters, pancreatic adenocarcinomas have, to a large extent, lost the hormone-dependent characteristics of the original tissue.

Introduction

Cancer of the exocrine pancreas has a very poor prognosis. The low resectability and ineffective radiotherapy and chemotherapy make it imperative to seek new therapeutic approaches. Various experimental studies suggest that growth of exocrine pancreatic carcinomas may be influenced by gastrointestinal hormones (1–2). Attempts are being made to develop hormonal therapies for pancreatic cancers by using synthetic peptide antagonists or analogues of various hormones, such as cholecystokinin (CCK*), somatostatin (SST) and bombesin (BBS)/gastrin releasing peptide, singly or in combination, to counteract these growth stimulatory effects (3–5).

To develop hormonal therapies for pancreatic cancers, an understanding of the biology of this tumour is of primary importance. Evaluation of factors involved in the development and growth of this tumour is also essential. In recent years, animal models of neoplasm of the pancreas have provided information regarding histogenesis and molecular changes associated with the development of this tumour. Interestingly, in rats treated with azaserine acinar cell tumours develop, whereas in Syrian golden hamsters ductal cell neoplasms of pancreas can be induced. A few acinar cell carcinomas or mixed acinar/ductal carcinomas have been reported in carcinogen-treated hamsters (6). The \(N\)-nitrosobis(2-oxopropyl)amine (BOP)-treated hamster is the best characterized and generally used model for studying the development of ductal adenocarcinomas of the pancreas (7).

The role of gut peptides and the status of their corresponding receptors in human pancreatic cancer are still controversial and unclear (1,8–9). In a previous study we have presented part of the spectrum of gut peptide receptors in pancreata of azaserine-treated rats (10). However, by far the greatest number (89–95%) of human adenocarcinomas of the pancreas are of ductal type and only a small percentage (1–4%) shows acinar cell differentiation (11). Because of the similarity of the induced tumours to those occurring in humans, the BOP-hamster model is considered to provide a unique model to study pancreatic carcinogenesis.

Though both consistent and inconsistent effects of gut peptides on growth of pancreas or of pancreatic tumours in hamsters have been reported (12–19), characterization of peptide receptors in hamster pancreas and in pancreatic tumours has not been performed. To elucidate whether pancreatic tumours induced in hamsters by BOP will retain some of the hormone-dependent characteristics of the original pancreas, this study visualized and characterized the receptors for CCK, SST, BBS, secretin and vasoactive intestinal peptide (VIP) in pancreata of saline control and BOP-treated hamsters by storage phosphor autoradiography.

Materials and methods

CCK-8, (Leu\(^8\),p-Tyr\(^{22}\),Tyr\(^{23}\))SST-28, SST-14, Tyr-B-Ala-secretin and gastrin-17 were purchased from Sigma Chemical Co. (St Louis, MO). Lorglumide was a gift from Rota Research Laboratories (Milan, Italy). L365,260 and MK 329 (L364,718) were obtained from Merck Sharp & Dohme Research Laboratory (Merk & Co. Inc., Rahway, NJ). Tyr\(^2\)-Octreotide was supplied by Sandoz Pharma Ltd (Basel, Switzerland). Tyr\(^3\)-BBS was purchased from Peninsula Laboratories Europe Ltd. Porcine VIP-28 was obtained from Saxon Biochemicals GmbH (Hannover, Germany). \(^{125}\)I-Tyr\(^2\)-BBS were purchased from New England Nuclear (Boston, MA). The labelled peptide had a specific activity of 2200 Ci/mmol.

Twenty Syrian golden hamsters (obtained at 3 weeks of age from Charles River Wiga, Sulzfeld, Germany) were injected s.c. once weekly with 20 mg BOP/kg body wt at 6, 7 and 8 weeks of age according to an injection protocol as previously described (20). BOP (Ash Stevens Inc., Detroit, MI) was dissolved freshly in 0.9% NaCl solution. The control group of seven hamster was conducted 19 weeks after the first injection of BOP. All animals were anaesthetized with ether, exsanguinated by cannulating the abdominal aorta.
and then examined for gross pathological changes. The pancreata were removed and rapidly frozen at -80°C. Parts of the pancreata were processed for light microscopy by cryostat sectioning at 5 μm and stained with haematoxylin and eosin. The different types of BOP-induced putative (pre)neoplastic ductal lesions were evaluated as described previously (20). Tubular, cystic and intermediate ductal complexes as well as intraductal hyperplasia exhibiting the characteristics suggestive of a neoplastic change were classified as preneoplastic or borderline lesions. Ductal adenocarcinoma is a lesion of ductal cells with a degree of anaplasia.

CCK-33 was acetylated with 125I-labelled Bolton–Hunter reagent as described by Jansen and Lamers (22). The sp. act. was ~2200 Ci/mmol. (Leu<sup>9</sup>,D-Trp<sup>2</sup>,Ty<sup>3</sup>) SST-28, Tyr-B-Ala-secretin and VIP-28 were iodinated using the Chloramine-T oxidation method. Iodinated peptides were separated from unincorporated 125I by gel filtration on a Sephadex G25 F<sub>2</sub> or G50-SF column pre-equilibrated with 0.1 N acetic acid and 0.1% gelatin or 0.25 M ammonium hydrogen carbonate (23,24). The specific activities of (125I)Leu<sup>9</sup>,D-Trp<sup>2</sup>,Ty<sup>3</sup>) SST-28 and (125I)Tyr-B-Ala-secretin were ~2200 Ci/mmol and that of (125I)VIP-28 ~4000 Ci/mmol.

For radioligand binding assay, cryostat tissue sections (10 μm) were cut at -20°C, mounted on gelatin-coated slides and dried overnight at -80°C. Binding of various radioligands to pancreatic tissue sections was according to Tang et al. (10). In brief, sections mounted on slides were air dried for 30 min and pre-incubated in 50 mM Tris buffer, pH 6.5, containing 5 g/l bovine serum albumin at 22°C for 20 min. Binding of (125I)BH-CCK-8, (125I)Tyr-B-BBS, (125I)Tyr-B-Ala-secretin and (125I)VIP-28 to pancreatic tissue sections was performed by incubating the sections at 22°C in 50 mM Tris, 0.25 g/l bacitracin, 4 mg/l leupeptin, 2 mg/l chymostatin, 130 μM NaCl, 7.7 mM KCl, 5 mM MgCl<sub>2</sub>, 1 mM EGTA, and 100 μM each radioligand at pH 6.0, 6.5, 7.0 and 7.4 separately for 180 min. The SST receptor binding assay was performed by incubating the sections at 22°C in 25 mM Tris, 5 mM MgCl<sub>2</sub>, 0.5 g/l bacitracin, 250 000 U/l kallikrein inactivator trasylol, 0.5 g/l phenylmethylsulfonyl fluoride and 100 μM (125I)SST-28 at pH 7.4 for 60 min. Alternate slides were incubated with addition of 1 mM of the corresponding non-radioactive peptide to determine the extent of non-specific binding. The dried tissue sections and one slide with two dried drops of 10 μl each labelled peptide were placed in a storage phosphor cassette for 24 h at 22°C. The latent image stored in the storage phosphor cassette was converted to digital images using a PhosphorImager<sup>®</sup> and the data were processed with ImageQuant<sup>®</sup> software (Molecular Dynamics, Sunnyvale, CA).

Storage phosphor autoradiography requires no chemical development and can be erased and re-used. When compared with film autoradiography, this new technique is faster to localize and quantify peptide receptors in tissue sections and easier to perform, due to its higher sensitivity and wider linear dynamic range (25-26).

The abilities of various CCK receptor agonists and antagonists to affect binding of (125I)BH-CCK-33 to CCK receptors in pancreata of both groups of hamsters were further studied. (125I)SST-28 binding in pancreata of both groups of hamsters was competed by SST-28, SST-14 and Tyr<sup>3</sup>-octreotide respectively to distinguish the subgroup of identified SST receptors. Binding parameters (K<sub>d</sub> (dissociation constant) and B<sub:max</sub> (maximum binding capacity)) were determined for each binding site using a non-linear least squares curve fitting program (LIGAND; 27). Significance of differences was analyzed using Student’s t-test.

**Results**

This study has shown that the binding of the gut peptides, CCK, secretin, SST and VIP to the hamster pancreas decreased with the progress of BOP-induced carcinogenesis.

Storage phosphor autoradiographs revealed specific CCK and secretin receptors in pancreata of the control group and normal pancreas of BOP-treated hamsters (Figure 1). Prenecrotic lesions induced by BOP presented very faint specific binding for CCK and marked reduction of (125I)SST-28 in pancreata of both groups of hamsters was competed by SST-28, SST-14 and Tyr<sup>3</sup>-octreotide respectively to distinguish the subgroup of identified SST receptors. Binding parameters (K<sub>d</sub> (dissociation constant) and B<sub:max</sub> (maximum binding capacity)) were determined for each binding site using a non-linear least squares curve fitting program (LIGAND; 27). Significance of differences was analyzed using Student’s t-test.

Dose–inhibition curves of normal pancreata as well as preneoplastic lesions displayed potent inhibition of (125I)BH-CCK-33 binding by CCK-8 and the CCK-A receptor antagonists MK329 and lorglumide. Little competitive binding of (125I)BH-CCK-33 was found with gastrin and the CCK-B antagonist L365,260 (Figure 3). Analysis of a non-linear curve fitting suggested the presence of a single class of binding site of CCK-A receptors. Similarly, one receptor binding site for secretin was revealed in normal pancreata and preneoplastic lesions.

In a comparison of the SST and VIP receptors in normal pancreata, the specific SST and VIP receptors decreased significantly in preneoplastic lesions and almost disappeared in pancreatic adenocarcinomas, due to reduction of both affinity and binding capacity (Figures 1 and 2 and Table I). In the radioligand binding study, binding of (125I)SST-28 in pancreata of the control group could be replaced by unlabelled SST-28 and SST-14, but it could not be inhibited by unlabelled Tyr<sup>3</sup>-octreotide (Figure 4). Similar inhibition patterns of SST-28, SST-14 and Tyr<sup>3</sup>-octreotide were present in preneoplastic lesions and adenocarcinomas of BOP-treated hamsters. The dose–inhibition curves were best fitted by a one site model for SST-28 and SST-14.

The normal pancreata of control hamsters did not express BBS receptors. In the progress of BOP-induced carcinogenesis no specific binding of BBS was detected in either preneoplastic lesions or adenocarcinoma (Figure 1). The failure to find specific binding of labelled BBS to the hamster pancreas is not due to the radioactive ligand employed, since this labelled BBS does bind to the pancreas of rat and to the brain of hamster.

**Discussion**

In the present study, several aspects of the spectrum of gut peptide receptors in normal pancreata and pancreatic (pre)neoplastic lesions of BOP-treated hamster have been reported for the first time.

In rat pancreatic acini CCK-A receptors with two binding sites have been revealed previously (28–29). However, little information on the characteristics of CCK receptors in hamster pancreas is available. Unlike rat pancreas, this study showed CCK-A receptors with one binding site in the normal pancreas of control and BOP-treated hamsters.

Several studies have focused on the role of CCK in pancreatic carcinogenesis. The trophic effect of CCK on putative preneoplastic lesions in azaserine-treated rats has been attributed to the interaction of CCK with its specific cellular receptors (30). In addition, previous studies by our and other groups have demonstrated an increased binding capacity of high affinity CCK receptors in putative pancreatic preneoplastic lesions and carcinomas induced by azaserine (10,29).

In the BOP–hamster model, however, the effects of CCK on pancreatic carcinogenesis are rather inconsistent (12–14). Enhancing as well as inhibitory effects and also an absence of any effect of CCK on the development of ductal pancreatic tumours induced in hamsters by nitrosamine derivatives have been reported (12–14). The results of the present study show that specific CCK-A receptors were expressed with high affinity in normal pancreata of hamsters, but with very low affinity in pancreatic preneoplastic lesions, while they were absent in adenocarcinomas. Thus, the effect of CCK on the normal pancreas of hamsters, if present, is likely to be mediated by CCK-A receptors in the pancreas. Since these receptors were found to gradually disappear with progressive malignant changes, it is likely that the direct effect of CCK on ductal pancreatic carcinogenesis would also disappear. This observation may explain our previous finding that CCK did not...
Peptide receptors in BOP-treated hamster pancreas

CCK  SST  VIP  Secretin  BBS

Normal pancreas

Preneoplastic lesion

Adenocarcinoma

Fig. 1. Autoradiographs of total and non-specific binding of $^{[125]}$BH-CCK-33, $^{[125]}$SST-28, $^{[125]}$secretin, $^{[125]}$VIP-28 and $^{[125]}$Tyr4-BBS in pancreata of control hamsters and putative pancreatic preneoplastic lesions and adenocarcinomas. Row T represents total binding and row N shows non-specific binding. In the images of row T of the preneoplastic lesions, N denotes normal pancreas, while P denotes preneoplastic lesions. Magnification ~1-3X.

enhance the development of ductal lesions in BOP-treated hamsters (14).

SST analogues, such as octreotide (sandostatin) or octastatin (RC-160) have shown a marked inhibition of tumour growth in hamsters with BOP-induced preneoplastic ductal lesions or pancreatic cancer (15,31). SST receptors have been demonstrated in BOP-induced pancreatic cancer of hamsters (32). Although specific binding of SST was also identified in BOP-induced pancreatic cancers of hamsters in the current study, the decline in SST receptors during pancreatic carcinogenesis may be very important. It is, however, unclear to what extent suppression of tumour growth was mediated by the low number of SST receptors in pancreatic cancers of this model.

Five different SST receptor subtypes have been identified so far (33). Sequence comparisons of the SST receptor subtypes clearly revealed two subgroups of receptors in the SST receptor (SSTR) gene family. SSTR1 and SSTR4 share common characteristics that differ from those of the subgroup containing the subtypes SSTR2, SSTR3 and SSTR5. This separation into two subgroups is also reflected in pharmacological characteristics. Short peptide analogues of SST, such as octreotide or octastatin, demonstrate specific binding only for the SSTR2, SSTR3 and SSTR5 subtype. While all five SSTR subtypes bind SST-28 and SST-14 with high affinity (33). The present study has demonstrated that binding of $^{[125]}$SST-28 in normal pancreas of hamster could be inhibited by SST-28 and SST-14, but not selectively blocked by Tyr4-octreotide. This finding indicates that the SST receptors in normal pancreas of hamsters appear to belong to the subgroup containing the subtypes SSTR1 and SSTR4. In the development of pancreatic carcinogenesis, the subgroup of SST receptor did not change, but the affinity and binding capacity declined gradually. The present findings suggest that the inhibitory effect of the SST analogues octreotide and octastatin on tumour growth in the BOP-hamster
other growth factors, rather than a direct inhibitory action on SST receptors, in the BBS-preneoplastic lesion group (31) does not give any explanation for the contradictory findings mentioned above. Absence of BBS receptors in normal pancreas of hamsters, preneoplastic pancreatic lesions and pancreatic adenocarcinoma in the BBS-hamster model in this study suggests that the effects of BBS and its receptor antagonist on normal pancreas and pancreatic tumours of hamsters are not mediated directly by BBS receptors in the pancreas. In this respect it is interesting to note that the inhibitory effects of BBS and RC-3095 on hamster pancreatic tumours induced by BOP were invariably linked to a down-regulation of epidermal growth factor receptors (31, 34). The lack of BBS receptors in hamster pancreatic tumours in the present study is in contrast to the description of BBS receptors in a human pancreatic cancer cell line, CFPAC-1 (35). No further data on BBS receptors in human pancreatic cancers have been reported. Although BOP-induced pancreatic cancer in the hamster shows similarities to those occurring in man in terms of morphological characteristics, the different species may result in inconsistent expression of peptide receptors. It is difficult to understand the conflicting findings that in BOP-induced pancreatic cancer of hamsters BBS receptors could be identified in the cell membranes (31), but not in tissue sections in the present study. Interestingly, a similar discrepancy has also been described in the detection of CCK receptors in a human pancreatic cancer cell line (36, 37).

Early investigations described how secretin stimulated the growth of transplanted hamster pancreatic cancers (17) and boosted the in vitro proliferation of a pancreatic ductal tumour cell line (WD PaCa) which was originally induced by BOP in hamsters (18). The normal pancreas of hamsters in the present study expressed specific receptors for secretin. However, the affinity and binding capacity of secretin receptors dramatically decreased in pancreatic preneoplastic lesions and no specific secretin binding was detected in adenocarcinomas of the pancreas. The molecular basis of stimulation of pancreatic cancer growth by secretin mentioned above could not be

In our previous study, BBS receptors were detected in normal rat pancreas (10). However, with the same method, no expression of BBS receptors in normal hamster pancreas was found in the current study. This discrepancy suggests important differences between the species. Studies performed with the BOP-hamster model have demonstrated that BBS causes an increase in growth of the hamster pancreas, but in contrast to rats, BBS treatment reduced the number of (pre)neoplastic lesions (16). Administration of BBS or gastrin releasing peptide alone did not stimulate growth of pancreatic tumours and, in fact, had a slightly suppressive effect on cancers (34). Surprisingly, a tumour growth inhibitory effect of the BBS receptor antagonist RC-3095 was also shown by the same group (31, 34). The expression of BBS receptors in BOP-induced pancreatic tumours (31) does not give any explanation for the contradictory findings mentioned above. Absence of BBS receptors in normal pancreas of hamsters, preneoplastic pancreatic lesions and pancreatic adenocarcinoma in the BOP-hamster model in this study suggests that the effects of BBS and its receptor antagonist on normal pancreas and pancreatic tumours of hamsters are not mediated directly by BBS receptors in the pancreas. In this respect it is interesting to note that the inhibitory effects of BBS and RC-3095 on hamster pancreatic tumours induced by BOP were invariably linked to a down-regulation of epidermal growth factor receptors (31, 34). The lack of BBS receptors in hamster pancreatic tumours in the present study is in contrast to the description of BBS receptors in a human pancreatic cancer cell line, CFPAC-1 (35). No further data on BBS receptors in human pancreatic cancers have been reported. Although BOP-induced pancreatic cancer in the hamster shows similarities to those occurring in man in terms of morphological characteristics, the different species may result in inconsistent expression of peptide receptors. It is difficult to understand the conflicting findings that in BOP-induced pancreatic cancer of hamsters BBS receptors could be identified in the cell membranes (31), but not in tissue sections in the present study. Interestingly, a similar discrepancy has also been described in the detection of CCK receptors in a human pancreatic cancer cell line (36, 37).

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Table I. The characteristics of peptide receptors in three types of pancreatic tissue of hamsters

<table>
<thead>
<tr>
<th>n</th>
<th>$K_D$ (nM) (mean ± SD)</th>
<th>$B_{max}$ (pmol/mg protein) (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CCK</td>
<td>SST</td>
</tr>
<tr>
<td>Normal pancreas</td>
<td>7</td>
<td>59.3 ± 25</td>
</tr>
<tr>
<td>Preneoplastic lesion</td>
<td>10</td>
<td>637 ± 42</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>10</td>
<td>ND</td>
</tr>
</tbody>
</table>

The data for SST receptor are from the inhibition curves of SST-28. ND, not detectable.

$p < 0.01$ compared with normal pancreas.

$p < 0.01$ compared with preneoplastic lesion.
confirmed by the secretin receptor assay used. These inconsistent results may reflect the different biological features of pancreatic cancer of hamsters under various experimental conditions.

Unlike secretin, VIP, which is structurally related to secretin, suppressed the growth of the transplantable hamster pancreatic adenocarcinoma (19). However, the data in Table I showed a reduced binding capacity of VIP receptors in preneoplastic lesions and a significant decline in affinity of VIP receptors in adenocarcinomas. These data suggest that VIP receptors may mediate pancreatic carcinogenesis in the BOP-hamster model only in the early stages.

The present findings that receptors for CCK, SST, secretin and VIP decreased or disappeared with the progress of BOP-induced pancreatic carcinogenesis suggest that pancreatic tumours induced in the hamster pancreas by BOP have, to a large extent, lost the hormone-dependent characteristics of the original tissue.

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References


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