ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1999 Jan; 20 (1): 59 - 64
http://www.simm.ac.cn; www.chinainfo.gov.cn/periodical

Binding characteristics of pancreatic polypeptide receptors on rat hepatic membranes[1]

ZHANG Xin-Tang, YAO Shi-Yin, XU Ying-Gao, ZHU Shang-Quan[2]
(*Shanghai Institute of Biochemistry, Chinese Academy of Sciences, Shanghai 200031, China*)

KEY WORDS pancreatic polypeptide; pancreatic hormones; peptide receptors; competitive binding; binding sites; liver; cell membrane

ABSTRACT

AIM: To study the binding characteristics of pancreatic polypeptide (PP) receptors on rat hepatic membranes. **METHODS**: ^{125}I-PP suitable to study interaction between ligand and receptors were prepared. ^{125}I-porcine PP and ^{125}I-duck PP were used to study PP receptor binding in the controlled conditions. **RESULTS**: The binding of ^{125}I-porcine PP to receptors on rat hepatic membranes was time- and temperature-dependent. The specific binding of ^{125}I-porcine PP was inhibited by unlabeled porcine PP in a concentration-dependent manner, whereas duck PP was only partially inhibited in the high concentration (> 500 nmol $\cdot$ L^{-1}). Scatchard analysis produced a curvilinear plot, suggesting multiple affinity binding sites, ie, high-affinity and low-affinity with dissociation constants (K_d) 5.4 and 158 nmol $\cdot$ L^{-1}, respectively. **CONCLUSION**: Rat hepatic membranes possessed specific PP receptors and porcine PP binding activity was much higher than that of duck PP.

INTRODUCTION

Pancreatic polypeptide (PP) is a single chain peptide hormone with 36 amino acids and amidation at the carboxyl terminus without disulfide bond. PP was initially isolated and characterized from avian[1] and mammalian pancreas[2]. PP inhibited exocrine pancreatic secretion[3] and gallbladder contraction[4] and augmented insulin inhibition of hepatic glucose production[5]. In our laboratory, we have isolated and purified duck PP and determined its amino acid sequence[6].

PP, like other peptide hormones, elicited its biological action through interactions with specific cell-surface receptors. The PP receptors were expressed in small intestine and brain[7,8]. Though the PP binding protein on rat liver membranes was identified using bovine PP as ligand[9], the binding characteristics of PP receptors are still not well studied. In the present study, the methods to prepare ^{125}I-porcine PP and ^{125}I-duck PP, and the characterization of the interaction between PP and its receptors on rat hepatic membranes were studied.

MATERIALS AND METHODS

Materials Porcine PP and duck PP prepared in our laboratory using the method described by XU *et al*[6] were homogenous in HPLC and on polyacrylamide gel electrophoresis. Bovine serum albumin (BSA fraction V) and glucagon were purchased from Sigma Chemical Co. Bacitracin was from Serva, [^{125}I]NaI from Amersham Corp. All other chemicals used were of analytical reagent grade. Male Sprague-Dawley rats (Grade II, Certificate № 005) were

[1] Project supported by Chinese 863 High Technology Foundation, № 863-103-13-01-01.
[2] Correspondence to Prof ZHU Shang-Quan.
Phn 86-21-6437-4430, ext 283. Fax 86-21-6433-8357.
E-mail zhusq@sunm.shcnc.ac.cn
Received 1997-10-08 Accepted 1998-08-27

ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1999 Jan; 20 (1)
E-mail aps@server.shcnc.ac.cn Phn/Fax 86-21-6474-2629

obtained from Shanghai Experimental Animal Center, Chinese Academy of Sciences.

Preparation of rat hepatic membranes The rat hepatic membranes were prepared according to the method of Cuatrecasas[10] with some modifications. All procedures were carried out at 0 – 4 ℃. Fresh rat liver (from 2 rats) was washed 3 times with ice-cold saline containing $NaHCO_3$ 1 mmol·L^{-1} and minced in ice-cold sucrose 0.25 mol·L^{-1} containing $NaHCO_3$ 1 mmol·L^{-1}, then homogenized with glass homogenizer. After centrifugation at 1000 × g for 5 min, the supernatant was centrifuged at 12 000 × g for 10 min. The supernatant was centrifuged again at 64 000 × g for 30 min. The pellet was suspended in 200 mL of phosphate buffer 10 mmol·L^{-1} (pH 7.4) containing NaCl 130 mmol·L^{-1}, homogenized and centrifuged. The resuspension and centrifugation steps were repeated twice. Finally the pellet was resuspended in 20 mL phosphate buffer 10 mmol·L^{-1} containing NaCl 130 mmol·L^{-1}, homogenized gently in glass homogenizer and stored frozen at – 80 ℃ after aliquot.

Protein concentration The protein concentration of the membrane preparation was determined by the Hartree[11] modification of the Lowry method using BSA as standard.

Preparation of ^{125}I-PPs Porcine PP was iodinated using Iodogen method[12]. Twenty μL of phosphate buffer 0.5 mol·L^{-1} (pH 7.2), 20 μL (20 μg) of porcine PP solution in HCl 5 mmol·L^{-1}, 10 μL of [^{125}I]NaI (37 MBq) was added to 1.5 mL Eppendorf tube coated with 10 μg of Iodogen, respectively. The reaction was carried out for 10 min at (25 ± 3) ℃ with shaking. The iodination mixture was fractionated and purified by discontinuous polyacrylamide gel electrophoresis using a modification of the method of Linde and Hansen[13]. After electrophoresis, the running gel was cut in 2 mm slips. The gel slips were put in 1 mL phosphate buffer 50 mmol·L^{-1} (pH 7.4) containing BSA 1 % and the protein fractions with high radioactivity were collected and stored frozen at – 20 ℃. The duck PP (4.2 μg) was also ^{125}I-labeled using the same method, however the Sephadex G-50 gel filtration was used to purify ^{125}I-duck PP.

Receptor binding assay Receptor binding assay was performed in 4 mL polystyrene tubes (γ-counter tube). The membrane preparations containing 150 μg protein/100 μL of binding buffer (phosphate buffer 50 mmol·L^{-1}, pH 6.5, containing BSA 1 % and bacitracin 1 g·L^{-1}) were incubated with 50 μL of ^{125}I-porcine PP or ^{125}I-duck PP (approximately 2.5 kBq) in the presence or absence of unlabeled hormones in a final volume of 0.2 mL at 4 ℃ for 1 h. The reaction was terminated by dilution with 3 mL of ice-cold washing buffer (phosphate buffer 50 mmol·L^{-1}, pH 6.5 containing BSA 0.1 %). The tubes were centrifuged at 3000 × g at 4 ℃ for 30 min. The supernatant was decanted and the pellet was washed twice with ice-cold washing buffer. Finally, radioactivity of the pellet was measured in a gamma counter (65 % counting efficiency). Nonspecific binding was determined in the presence of an excess of unlabeled porcine PP or duck PP (4.23 μg/tube). Specific binding was calculated by subtracting the nonspecific binding from the total binding. All assays were performed in triplicates.

RESULTS

^{125}I-labeled ligands To avoid the possibility of oxidation of methionine in porcine PP molecule, porcine and duck PPs were labeled and separated, respectively, as described above. The labeling yield was 73.5 % for porcine PP and 77.1 % for duck PP. After separation by electrophoresis mono-^{125}I-porcine PP was obtained. The radioactivity of ^{125}I-duck PP was calculated to be 6808 GBq·g^{-1}.

^{125}I-porcine PP and ^{125}I-duck PP binding

to rat hepatic membranes In the presence of a constant of ^{125}I-porcine PP, the specific binding of ^{125}I-porcine PP to rat hepatic membranes markedly increased with membrane concentration, although the nonspecific binding was only slightly altered (Fig 1), indicating that specific ^{125}I-porcine PP binding was directly dependent on the membrane concentration in the incubation medium.

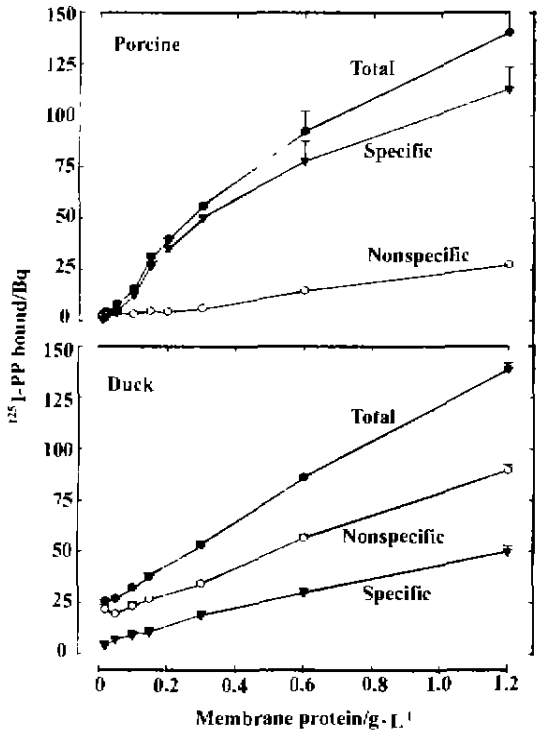

Fig 1. ^{125}I-porcine PP and ^{125}I-duck PP binding to rat hepatic membrane as a function of membrane concentration. n = 3 experiments from 6 rats. $\bar{x} \pm s$.

However, under the same conditions the specific binding of ^{125}I-duck PP to rat hepatic membranes was much lower than that of ^{125}I-porcine PP due to that the nonspecific binding of ^{125}I-duck PP rapidly increased with membrane concentration (Fig 1). When membrane concentration was constant, the specific binding of ^{125}I-porcine PP to membranes appeared as steady state in the presence of excess of ^{125}I-porcine PP.

Effect of time and temperature on specific binding of ^{125}I-porcine PP to rat hepatic membranes The binding of ^{125}I-porcine PP to rat hepatic membranes was clearly dependent upon conditions of time and temperature. The specific binding increased rapidly at 24 ℃ and reached a maximum of 6 % at 8 min and then decreased gradually to 3 % at 120 min (Fig 2).

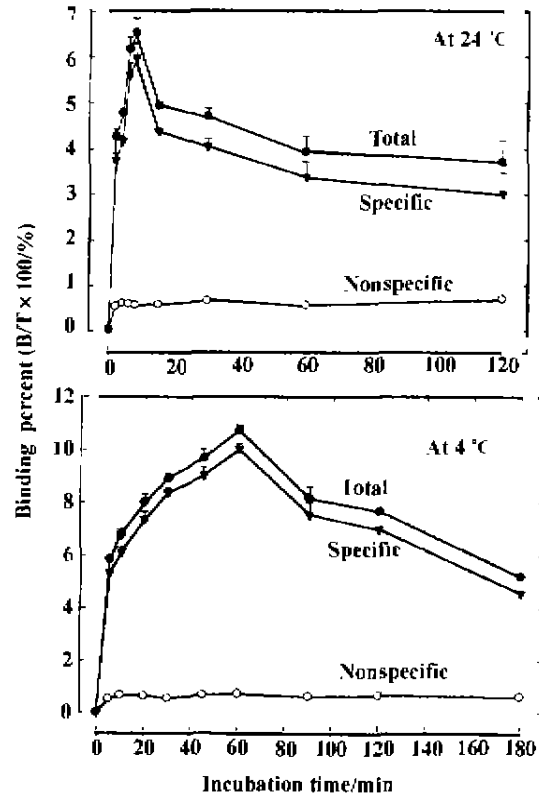

Fig 2. Effects of time and temperature on ^{125}I-porcine PP binding to rat hepatic membrane. n = 3 experiments from 6 rats. $\bar{x} \pm s$.

At 4 ℃, the specific binding also increased quickly in the first 10 min and then progressively increased to reach a maximum of 10 % at 60 min. When the incubation was continued, the specific binding was also gradually down to below 7 % at 120 min (Fig 2). At 4 ℃ the nonspecific binding was slightly lower and total

ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1999 Jan; 20 (1)
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binding was higher than that at 24 ℃. Therefore, the optimal conditions to study the interaction between porcine PP and its receptors seem to be at 4 ℃ for 1 h. These conditions were used in other experiments.

Competition for binding of ^{125}I-porcine PP to rat hepatic membranes The specific binding of ^{125}I-porcine PP to rat hepatic membranes was inhibited by unlabeled porcine PP in a concentration-dependent manner. The IC_{50} was found to be 40 nmol·L^{-1} (Fig 3).

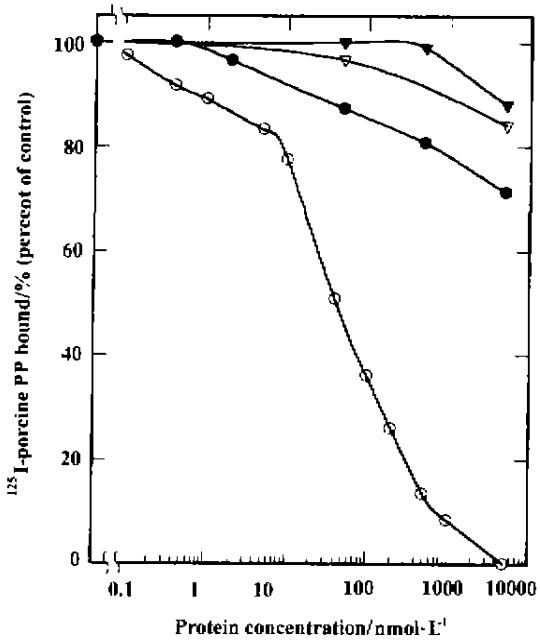

Fig 3. Competitation for specific binding of ^{125}I-porcine PP to rat hepatic membranes by increasing concentration of unlabeled porcine PP (○), duck (●), glucagon (△), and insulin (▲).

Other pancreatic peptide hormones, glucagon and insulin, did not affect the binding of ^{125}I-porcine PP to its receptors. Duck PP was much less active than that of porcine PP. Only about 20 % of bound ^{125}I-porcine PP were replaced in the presence of duck PP 500 nmol·L^{-1}.

Based on Fig 3, when the ratio of bound to free ^{125}I-porcine PP was plotted as a function of bound porcine PP concentration using Scatchard analysis[14], a curvilinear plot was observed (Fig 4).

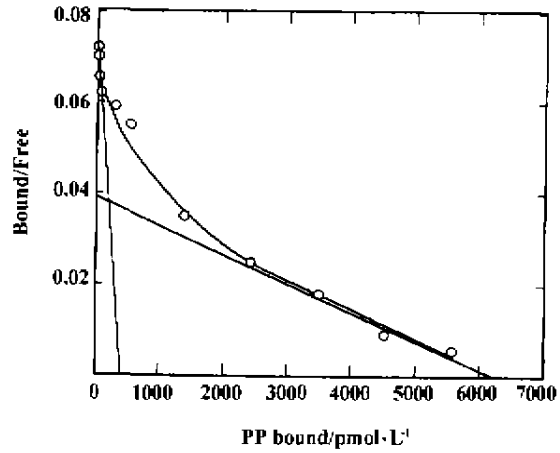

Fig 4. Scatchard plot of specific binding of ^{125}I-porcine PP to rat liver membrane.

That meant 2 classes of porcine PP binding sites in rat hepatic membranes. The high affinity site displayed a K_d of 5.4 nmol·L^{-1} and the binding capacity (B_{max}) was 541 pmol·g^{-1} (protein). The low affinity site displayed a K_d of 158 nmol·L^{-1}, and the B_{max} was 8.29 nmol·g^{-1} (protein).

DISCUSSION

In the present study, specific PP receptors were identified in rat hepatic membranes. Binding of ^{125}I-porcine PP to these membranes was rapid, specific, and depended on time and temperature. The optimum conditions seemed to be at 4 ℃, for 1 h. After over-incubation, the specific binding was down, this might be due to the degradation of labeled ligand and/or receptors.

The reaction pH was important for the interaction of PP with its receptors and the optimum pH in this study was 6.5 as reported by Whitcomb *et al*[8].

The specific binding of ^{125}I-porcine PP to rat hepatic membranes was inhibited by unlabeled porcine PP in a concentration-dependent manner. Porcine glucagon and insulin, unrelated pancreatic peptide hormones with PP in the structure, did not inhibit ^{125}I-porcine PP binding to rat hepatic membranes. It was noted that duck PP had a very low competitive activity for ^{125}I-porcine PP binding to rat hepatic membranes. The low specific binding of ^{125}I-duck PP to rat hepatic membrane was observed (Fig 1). The difference of amino acid sequences was a possible explanation for the low binding activity of duck PP. The sequence homology among PPs from various species was very low and only 7 amino acids in 36 amino acids residues of PP molecule were absolutely conservative[15]. From the comparison of amino acid sequence of rat PP, porcine PP and duck PP (Tab 1), the homology of rat PP and porcine PP was 75 % and only 44 % homology between rat PP and duck PP.

The Scatchard analysis showed classes of PP receptors on rat hepatic membranes. The similar results were also observed in the binding of bovine PP to PP receptors in dog intestinal basolateral membranes[8].

Although the presence of specific binding sites of porcine PP in rat hepatic membranes was shown, it remained difficult to conclude that these binding sites were functional receptors for PP, because we did not know whether PP appeared to have any metabolism effect on liver or isolated hepatocytes. Therefore, it was necessary to understand the structural characteristics of PP receptors on rat hepatic membrane and post-binding events.

In conclusion, rat hepatic membranes possessed specific PP receptors and porcine PP binding activity was much higher than that of duck PP.

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Tab 1. Comparison of amino acid sequence of rat-PP, porcine PP and duck PP.

	5	10	15	20	25	30	35
Rat-PP	A P L E P M Y P G D Y A T H E Q B A Q Y E T Q L R R Y I N T L T R P R Ya						
Porcine-PP	A P L E P V Y P G D D A T P E Q M A Q Y A A E L R R Y I N M L T R P R Ya						
Duck-PP	G P S Q P T Y P G D D A P V E D L V R F Y N D L Q Q Y L N V V T R H R Ya						

ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1999 Jan; 20 (1)
E-mail aps@server.shcnc.ac.cn Phn/Fax 86-21-6474-2629

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大鼠肝膜上胰多肽受体的结合特征[1]

张新堂，姚矢音，许英镐，朱尚权[2]（中国科学院上海生物化学研究所，上海 200031，中国）

关键词 胰多肽；胰腺激素；肽受体；竞争性结合；结合位点；肝；细胞膜

目的：研究大鼠肝膜胰多肽受体的结合特性。**方法**：在控制条件下，用^{125}I标记的胰多肽进行胰多肽受体的结合特性研究。**结果**：制备了适用于进行配体和受体相互作用研究的^{125}I标记的猪胰多肽和鸭胰多肽。^{125}I-猪胰多肽与大鼠肝膜胰多肽受体结合依赖于时间和温度，而这一专一结合能被未标记的猪胰多肽以剂量关系所抑制。鸭胰多肽只有在高浓度下才显示出部分抑制作用。Scatchard 作图分析表明大鼠肝膜存在两种胰多肽的结合位点，高亲和结合位点和低亲和结合位点，它们的结合解离常数 K_d 分别为 5.4 nmol·L^{-1}和 158 nmol·L^{-1}。**结论**：大鼠肝膜存在胰多肽的专一结合受体，而且猪胰多肽对这些受体的结合活性要比鸭胰多肽高得多。

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