Vol. 171, No. 1, 1990 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 31, 1990 Pages 330-335

IDENTIFICATION OF A NOVEL 65-kDa CELL SURFACE RECEPTOR COMMON TO PANCREATIC POLYPEPTIDE, NEUROPEPTIDE Y AND PEPTIDE YY

Koji Nata, Hideto Yonekura, Hiroshi Yamamoto and Hiroshi Okamoto*

Department of Biochemistry, Tohoku University School of Medicine
Sendai 980, Miyagi, Japan

Received July 19, 1990

SUMMARY: By affinity cross-linking and sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis, we identified a novel cell surface receptor on intact rat cells, which bound, with similar dissociation constants, pancreatic polypeptide (PP), neuropeptide Y (NPY) and peptide YY (PYY), the members of the PP family. The receptor was detected on pancreatic islet and acinar cells, hepatocytes and epithelial cells of the stomach, duodenum and small intestine. Its molecular weight was estimated to be 65,000, and the cross-linking of [^{125}I]labeled ligands was inhibited by an excess of unlabeled PP, NPY or PYY. The results suggest that the 65-kDa molecule is a common receptor for PP family peptides.

Pancreatic polypeptide (PP), neuropeptide Y (NPY), and peptide YY (PYY) are homologous 36-amino acid peptides with a carboxyl-terminal tyrosylamide (1, 2). The three peptides constitute a family of regulatory peptides, characterized by a common distinct tertiary structural feature, the PP-fold (3). PP, a hormone synthesized in the islets of Langerhans (2,4,5), has been related to gastrointestinal and pancreatic functions (6). NPY is a neuropeptide present in both the central and peripheral nervous systems (7). PYY is a gut hormone found in the lower intestine (8). Three classes of receptors for PP family peptides have so far been reported; one for PP (9, 10), one for NPY (11), and the other for PYY and NPY (9, 12-14).

In the present study, by affinity cross-linking between intact rat cells and radiolabeled ligands and subsequent SDS-PAGE, we have identified a novel 65-kDa cell surface receptor that binds all three PP family peptides with similar affinities.

* To whom correspondence should be addressed.

Abbreviations: PP, pancreatic polypeptide; NPY, neuropeptide Y; PYY, peptide YY; DSS, disuccinimidyl suberate; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

0006-291X/90 $1.50

MATERIALS AND METHODS

Materials: Synthetic rat PP and PYY were purchased from Peninsula Laboratories (Belmont, California) and synthetic rat NPY from Peptide Institute, Inc. (Osaka, Japan). Na[^{125}I] was from Amersham International (Amersham, UK), Enzymobead from Bio-Rad (Richmond, California), and disuccinimidyl suberate (DSS) from Pierce Chemical Co. (Rockford, Illinois). Male Wistar rats weighing 200-300 g were used for all experiments. Pancreatic islets of Langerhans and pancreatic acinar cells were isolated by the collagenase digestion method (15). Parenchymal hepatocytes were isolated as described before (16). Gastric, duodenal, and small intestinal mucosae were stripped by slide glasses and dispersed by incubation with 0.05% collagenase (Wako Pure Chemical Industries Co., Osaka, Japan). 3Y1, a rat fibroblast cell line (17), was a gift from Dr. K. Hamanaka (Hiroshima University). H-4-II-E, a rat hepatoma cell line, was purchased from American Type Culture Collection (Rockville, Maryland).

Peptide iodination: The synthetic peptides were radioiodinated by a solid phase glucose oxidase-lactoperoxidase procedure (18) and purified by chromatography on Sephadex G-10.

Binding and cross-linking: Cells (3 x 10^5 cells or 200 islets) were incubated at 4°C for 15 h with [^{125}I]labeled peptides in 1.0 ml of Dulbecco's modified Eagle's medium containing 0.2% bovine serum albumin. The receptor-bound radioligand was pelleted by centrifugation, washed, and resuspended in 0.5 ml of phosphate-buffered saline containing 0.5 mM $MgCl_2$ and 0.5 mM $CaCl_2$. DSS was added to a final concentration of 1 mM, and the cross-linking reaction was carried out for 30 min at 4°C. The reaction was then quenched with 0.8 ml of ice-cold 25 mM Tris/HCl, pH 7.4, containing 0.14 M NaCl and 1 mM EDTA. Cross-linked materials were pelleted by centrifugation and solubilized in 62.5 mM Tris/HCl, pH 6.8, containing 2% sodium dodecyl sulfate (SDS) and 10% glycerol with or without 5% 2-mercaptoethanol.

SDS-PAGE analysis and autoradiography: SDS-PAGE was performed according to Laemmli (19) using 7.5% polyacrylamide slab gel. After electrophoresis, gels were dried and exposed to Fuji (Minamiashigara, Japan) RX films at -80°C. Autoradiograms were scanned using a Model 620 Video Densitometer (Bio-Rad).

RESULTS

Incubation of [^{125}I]PP with rat pancreatic islet cells and hepatocytes followed by DSS cross-linking gave rise to a single band on SDS-PAGE (Fig. 1, lanes 1, 5). The band was eliminated by the addition of a molar excess of non-radioactive PP during the incubation with [^{125}I]PP (Fig. 1, lanes 2, 6). The apparent molecular weight of the cross-linked ligand-receptor complex was 69,000, yielding a receptor size of 65,000 by subtraction of the molecular weight of ligands (4,000), assuming that one ligand molecule was cross-linked per receptor molecule. The migration pattern of the cross-linked complex was not altered in non-reducing conditions (Fig. 1, lane 9).

We next examined the effect of NPY and PYY on the formation of the 69-kDa complex. The addition of an excess of either

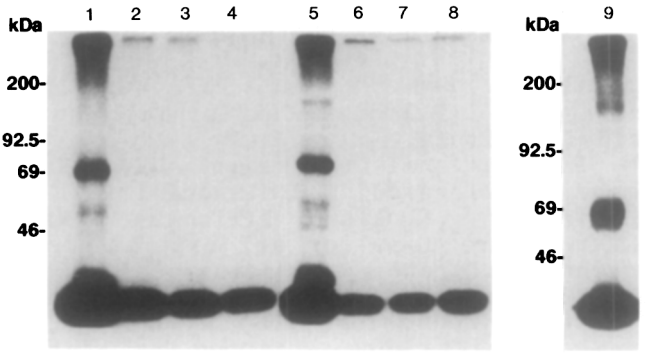


Fig. 1. SDS-PAGE analysis of [^{125}I]PP-receptor complex after covalent cross-linking with bifunctional reagent. Pancreatic islet cells (lanes 1-4) or hepatocytes (lanes 5-9) were incubated with [^{125}I]PP (400 nM in lanes 1-4 and 200 nM in lanes 5-9) in the absence (lanes 1, 5 and 9) and in the presence of 40 μM unlabeled PP (lanes 2 and 6), NPY (lanes 3 and 7), or PYY (lanes 4 and 8). The peptide-bound cells were then treated with DSS, solubilized and electrophoresed under reducing (lanes 1-8) or non-reducing (lane 9) conditions. Positions of size markers are presented on the left of lanes 1 and 9.

unlabeled NPY or PYY was found to inhibit the [^{125}I]PP binding (Fig. 1, lanes 3,4,7,8) as did unlabeled PP. When [^{125}I]NPY or [^{125}I]PYY was cross-linked to hepatocytes, only one band was detected at 69 kDa, the same position to which the [^{125}I]PP-labeled complex migrated, and could be eliminated by unlabeled PP as well as by NPY or PYY (Fig. 2). Similar results were obtained with pancreatic islet cells (data not shown).

To determine the relative affinity of the 65-kDa receptor for PP, NPY, and PYY, the characteristics of binding to hepatocytes

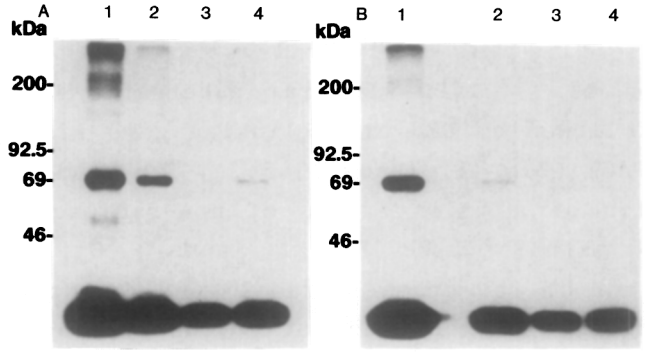
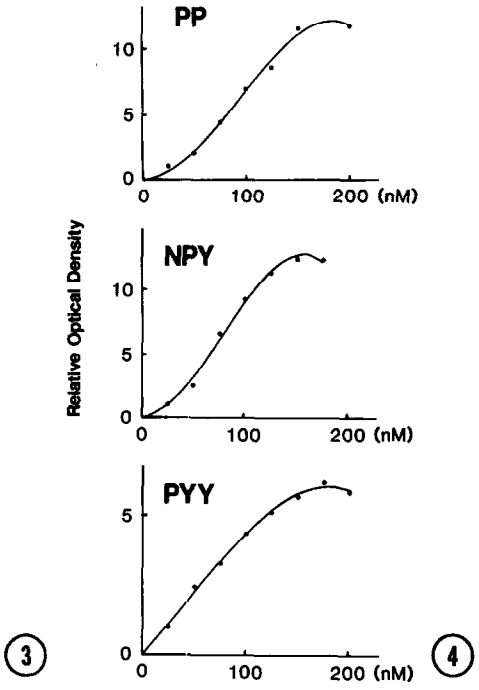


Fig. 2. Specificity of binding of [^{125}I]NPY and [^{125}I]PYY. (A) Hepatocytes were incubated with 100 nM [^{125}I]NPY in the absence (lane 1) or presence of a 100-fold excess of unlabeled PP (lane 2), NPY (lane 3), and PYY (lane 4). (B) Hepatocytes were incubated with 100 nM [^{125}I]PYY in the absence (lane 1) or presence of a 100-fold excess of unlabeled PP (lane 2), NPY (lane 3), and PYY (lane 4). Positions of size markers are presented on the left.



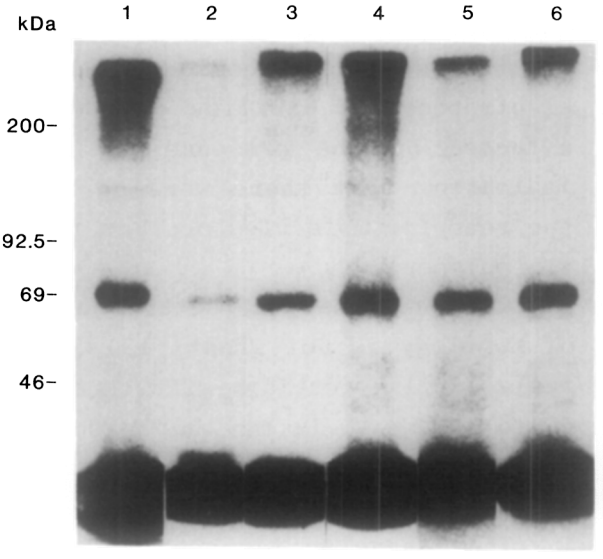


Fig. 3. Saturation binding kinetics of PP, NPY, and PYY. Hepatocytes were incubated with an increasing amount of the labeled ligands. Absorbance of the 69-kDa band was determined by densitometry and plotted versus the ligand concentration.

Fig. 4. [^{125}I]PP binding in various rat tissues. Lane 1, hepatocytes; lane 2, pancreatic islet cells; lane 3, pancreatic acinar cells; lane 4, gastric epithelial cells; lane 5, duodenal epithelial cells; lane 6, small intestinal epithelial cells. [^{125}I]PP was used at 200 nM. The cross-linking of labeled PP was blocked by adding an excess of unlabeled PP (data not shown). Positions of size markers are presented on the left.

were examined using increasing amounts of labeled ligands to reach saturation. Densitometric scanning of autoradiographs and integration of the peaks indicated that the labeling of the 69-kDa component was half-maximally saturated at 92 nM [^{125}I]PP, 78 nM [^{125}I]NPY, and 67 nM [^{125}I]PYY (Fig. 3).

To see the distribution of the 65-kDa receptor, a variety of rat tissues were examined using [^{125}I]PP as ligand. As shown in Fig. 4, the formation of the 69-kDa complex was observed in rat pancreatic acinar cells and epithelial cells of the stomach, duodenum and small intestine in addition to hepatocytes and pancreatic islet cells. On the other hand, in a rat fibroblast cell line, 3Y1, and a rat hepatoma cell line, H-4-II-E, the 65-kDa receptor could not be detected (data not shown).

DISCUSSION

In the present study, we have identified a cell surface molecule, which binds PP, NPY and PYY with similar dissociation constants, on various rat tissues. The cell surface molecule can thus serve as a common receptor for PP family peptides. The electrophoretic mobility of the receptor-ligand complex was not affected by the presence or absence of the reducing agent, indicating that there were no intersubunit disulfide bridges in the receptor molecules or that the receptor was a single monomeric polypeptide.

In the primary structures of PP family peptides, the degree of homology is not great (13), but these peptides display strikingly similar tertiary structures, which have become known as the PP-fold (3). It may be reasonable to assume that it is this common three-dimensional structure that is recognized by the 65-kDa receptor.

There have so far been several reports concerning receptors or binding activities for the members of the PP family, including for PP (9, 10), for NPY (11), and for PYY and NPY (9, 12-14). The 65-kDa receptor characterized in this study appears to be distinct in several respects. Most importantly, it binds equally all the members of the PP family, whereas the previously reported species are specific for PP, for NPY, or for PYY and NPY. The size of the receptor is 65 kDa, whereas the sizes of NPY-specific receptors are 52-59 kDa and 37-39 kDa (11) and those of PYY/NPY-specific receptors are 70 kDa and 50 kDa (12, 14). In addition, this receptor has a relatively lower affinity to the ligands than the others (9,10,12,14). We failed to detect the previously reported species in the present study using intact cells. This may be due to the difference in the tissues examined, the sample preparation (e.g. membrane fractions versus intact cells), or low abundance of these receptor species.

REFERENCES

1. Tatemoto, K. (1989) In Neuropeptide Y (V. Mutt, K. Fuxe, T. Hökfelt, and J.M. Lundberg, Eds.), pp. 13-21, Raven Press, New York.
2. Yamamoto, H., Yonekura, H., and Nata, K. (1990) In Molecular Biology of the Islets of Langerhans (H. Okamoto, Ed.), pp. 107-124, Cambridge University Press, Cambridge.
3. Glover, I.D., Barlow, D.J., Pitts, J.E., Wood, S.P., Tickle, I.J., Blundell, T.L., Tatemoto, K., Kimmel, J.R., Wollmer, A., Strassburger, W., and Zhang, Y.-S. (1985) Eur. J. Biochem. 142, 379-385.

4. Yamamoto, H., Nata, K., and Okamoto, H. (1986) J. Biol. Chem. 261, 6156-6159.
5. Yonekura, H., Nata, K., Watanabe, T., Kurashina, Y., Yamamoto, H., and Okamoto, H. (1988) J. Biol. Chem. 263, 2990-2997.
6. Hazelwood, R.L. (1981) In The Islets of Langerhans (S.J. Cooperstein, and D. Watkins, Eds.), pp. 275-318, Academic Press, New York.
7. Mutt, V., Fuxe, K., Hökfelt, T., and Lundberg, J.M. (1989) Neuropeptide Y, Raven Press, New York.
8. Tatemoto, K. (1982) Proc. Natl. Acad. Sci. USA 79, 2514-2518.
9. Schwartz, T.W., Sheikh, S.P., and O'Hare, M.M.T. (1987) FEBS Lett. 225, 209-214.
10. Gilbert, W.R., Frank, B.H., Gavin III, J.R., and Gingerich, R.L. (1988) Proc. Natl. Acad. Sci. USA 85, 4745-4749.
11. Nguyen, T.D., Heintz, G.G., Kaiser, L.M. Staley, C.A., and Taylor, I.L. (1990) J. Biol. Chem. 265, 6416-6422.
12. Inui, A., Okita, M., Inoue, T., Sakatani, N., Oya, M., Morioka, H., Shii, K., Yokono, K., Mizuno, N., and Baba, S. (1989) Endocrinology 124, 402-409.
13. Schwartz, T.W., Fuhlendorff, J., Langeland, N., Thøgersen, H., Jørgensen, J.C., and Sheikh, S.P. (1989) In Neuropeptide Y (V. Mutt, K. Fuxe, T. Hökfelt, and J.M. Lundberg, Eds.), pp. 143-151, Raven Press, New York.
14. Sheikh, S.P., and Williams, J.A. (1990) J. Biol. Chem. 265, 8304-8310.
15. Okamoto, H. (1981) Mol. Cell. Biochem. 37, 43-61.
16. Inoue, C., Yamamoto, H., Nakamura, T., Ichihara, A., and Okamoto, H. (1989) J. Biol. Chem. 264, 4747-4750.
17. Kimura, G., Itagaki, A., and Summers, J. (1975) Int. J. Cancer 15, 694-706.
18. Habener, J.F., Rosenblatt, M., Dee, P.C., and Potts, J.T. Jr. (1979) J. Biol. Chem. 254, 10596-10599.
19. Laemmli, U.K. (1970) Nature 227, 680-685.