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Identification and Distribution of mRNA Encoding the Y1, Y2, Y4, and Y5 Receptors for Peptides of the PP-fold Family in the Rat Intestine and Colon

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Peptide YY (PYY), neuropeptide Y (NPY) and pancreatic polypeptide (PP) are structurally related peptides which have potent antisecretory effects in small and/or large intestines. Receptors mediating these effects are still unknown with the exception of a PYY-preferring receptor expressed in small intestinal crypts. In the present study, expression of recently cloned Y1, Y2, and Y5 receptors which have similar affinity for PYY and NPY and Y4 receptors which have a high affinity for PP was investigated in gut by RT-PCR analysis. The data show that all Y receptors are expressed in small intestine and/or colon but with specific distributions. Y1 receptors are only expressed in nonepithelial colonic tissue, whereas Y2 and Y4 receptors are present in both epithelial and nonepithelial tissues of the small or large intestine. In contrast, Y5 receptor expression appears to be restricted to epithelial crypts of the small intestine and nonepithelial tissue of colon. Sequencing of PCR products showed 100% identity with the corresponding sequences of the cloned Y1, Y4, or Y5 receptors. The PCR product obtained with Y2 primers from rat crypt cells showed 84% identity with the cloned human Y2 receptor. These data indicate a wide distribution of Y receptors in small intestine and colon. They also suggest that Y1, Y2, Y4, and Y5 receptors may be responsible for still unexplained effects of PYY, NPY, or PP on secretion in small and large intestines.

Peptide YY (PYY), neuropeptide Y (NPY) and pancreatic polypeptide (PP) belong to a family of structurally related 36-amino acid peptides that have functions in neural and endocrine signalling (1). PYY and NPY are very potent inhibitors of small intestinal fluid and electrolyte secretion (2). Receptors for PYY were discovered in the rat small intestine epithelium (3) and were defined as "PYY-preferring" because they display a slightly higher affinity for PYY than for NPY. In contrast they have a very low affinity for PP. They are exclusively present in proliferative crypt cells and appear to be quenched when epithelial cells migrate onto the villi, stop to divide and differentiate into mature enterocytes (4). They are not expressed by colonic epithelium (3). PYY or NPY binding to intestinal epithelial PYY receptors results in a potent inhibition of cAMP production (5).

Altogether these observations supported that cryptic PYY-preferring receptors could be responsible for the antisecretory actions of PYY and NPY in small intestine (2). However an increasing number of observations do not fit with this simple view: (i) PYY or NPY inhibitory effects on small intestinal secretion are partially blocked by tetrodotoxin (6) supporting the existence of a neurally-mediated mechanism; (ii) PYY or NPY inhibits fluid secretion in colon (7) despite the absence of expression of PYY receptors in colon epithelium (3); (iii) NPY and PYY stimulate intestinal absorption (8), an event which probably occurs in intestinal villi which do not express PYY receptors; (iv) PP or some analogs of PYY or NPY that have a very low affinity for cryptic PYY receptors are nonetheless potent inhibitors of intestinal secretion (9).

Several receptor subtypes that bind NPY, PYY and/or PP have been cloned and belong to the so-called Y family of heptahelical G protein-coupled receptors (see refs. 10, 11, 12 for reviews). They include Y1, Y2 and Y5 receptors, which have similar affinity for NPY and PYY, Y4 receptors, which have a high affinity for PP, and Y6 receptors, the pharmacological profile of which is still under debate (11).

In this context, the present work has been designed to determine the expression of Y1, Y2, Y4 and Y5 receptors in the rat small intestine and colon. For that purpose we have developed highly specific RT-PCR for each

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receptor mRNA and looked after PCR products in the epithelium (isolated small intestinal crypt cells or villus cells and isolated colonic cells) and nonepithelial layers of gut. Our data provide evidence for a wide distribution of Y receptors in intestine with differences in their cellular or tissular expression.

MATERIALS AND METHODS

Biological materials. Three-month-old male Wistar rats (280-300g) fed *ad libitum* were used in this study. Samples from gut (jejunum and colon), hypothalamus, lung and testis were removed after decapitation. Colon epithelial cells were prepared on ice as described (3). Jejunal crypt cells were separated from villus cells by shaking the everted jejunum for successive periods in a dispersing solution containing EDTA as previously described in detail (13). Nonepithelial tissues (jejunum or colon) were obtained after complete removal of epithelial cells.Three-month-old male Wistar rats (280-300g) fed *ad libitum* were used in this study. Samples from gut (jejunum and colon), hypothalamus, lung and testis were removed after decapitation. Colon epithelial cells were prepared on ice as described (3). Jejunal crypt cells were separated from villus cells by shaking the everted jejunum for successive periods in a dispersing solution containing EDTA as previously described in detail (13). Nonepithelial tissues (jejunum or colon) were obtained after complete removal of epithelial cells.

The PKSV-PCT cell line was derived from microdissected proximal convoluted tubules from kidney of transgenic mice (14). A clone of PKSV-PCT cells expressing PYY-preferring receptors (referred to as Cl. 10) was used in this study and was cultured as described (15, 16).

Extraction of RNA. For extraction of RNA, solid tissues including nonepithelial intestine or colon were homogenized (Ultra-Turrax) in a RNAxel (Eurobio, Les Ulis, France) solution (1 ml for 100 mg of tissue). Isolated cells or cultured cells were homogenized in RNAxel solution by 10 passages through a needle (1.2 mm diameter). The extraction of RNA was then performed by the method of Chomczynski and Sacchi (17). All RNA preparations were treated with RNAse free-DNAse (Promega, Charbonnières, France) for 30 min at 37°C (10 units of DNAse for 10 μg of total RNA).For extraction of RNA, solid tissues including nonepithelial intestine or colon were homogenized (Ultra-Turrax) in a RNAxel (Eurobio, Les Ulis, France) solution (1 ml for 100 mg of tissue). Isolated cells or cultured cells were homogenized in RNAxel solution by 10 passages through a needle (1.2 mm diameter). The extraction of RNA was then performed by the method of Chomczynski and Sacchi (17). All RNA preparations were treated with RNAse free-DNAse (Promega, Charbonnières, France) for 30 min at 37°C (10 units of DNAse for 10 μg of total RNA).

RT-PCR experiments. Ten micrograms of total RNA was reverse-transcribed at 37°C for 1 hour using oligo$(dT)_{15}$ (Promega, Charbonnieres, France) and Moloney murine leukemia virus (M-MLV, Life Technologies, Cergy, France) reverse transcriptase (100 U). Five μl of the resulting cDNA mixtures were then submitted to PCR using specific primers for glyceraldehyde-3-phosphate-dehydrogenase (GAPDH), taken as a control for a house-keeping protein, and Y1, Y2, Y4 and Y5 receptors (Table 1). For every Y receptors, sense and antisense primers (see Table 1) were used for 35 cycles (denaturated at 92°C, one minute; annealed at 64°C, one minute; extended at 72°C, one minute). For GAPDH, sense and antisense primers (see Table 1) were used as described for receptors but annealing was carried out at 55°C. The PCR products were then analyzed by electrophoresis on 1% agarose gels and transferred to Hybond membranes (Amersham, Les Ulis, France) which were subsequently submitted to Southern hybridization with [^{32}P]-labelled cDNA probes, i.e., full-length rat Y1 (18), human Y2 (19) and rat Y4 (20) receptor cDNA, and rat Y5 receptor cDNA fragment 834-1357.

PCR product cloning and sequencing. PCR products were cloned using the LigATor kit (R&D Systems, Abingdon, UK) as specified by the manufacturer and then sequenced with a DNA sequencing kit (Amersham, Les Ulis, France).

RESULTS

RT-PCR analysis of Y1, Y2, Y4 and Y5 receptors in rat intestine and colon, the Cl.10 mouse kidney cell clone and control tissues (hypothalamus, testis and/or lung) was developed by using specific oligonucleotide primers (see Table 1). It was verified that: (i) the expected PCR product for GAPDH (983 bp), taken as a control, was observed in all biological preparations (Figure 1); (ii) no PCR product could be observed for GAPDH or receptors (Y1, Y2, Y4 or Y5) when the retrotranscription step was omitted (not shown), assessing for the absence of contaminating DNA in RNA preparations; (iii) no PCR product could be observed when water was added (Figure 1) instead of cDNA preparations, assessing for the absence of contamination during the RT-PCR process. The following data were obtained for receptors:

Y1 receptor. A PCR product with the expected size (546 bp) was observed in hypothalamus (Figure 1) from which this receptor was initially cloned (18). In contrast, no PCR product was observed in the Cl.10 cell line and most small and large intestinal tissues with the notable exception of nonepithelial colonic tissue. DNA sequencing of the PCR product (position 499-770) obtained from this tissue (not shown) revealed 100% identity with the corresponding sequence in the cloned rat Y1 receptor cDNA (18).

Y2 receptor. A PCR product with the expected size (442 bp) was observed in hypothalamus (Figure 1) from which the human receptor was initially cloned. PCR products were observed in the Cl. 10 cell line and all small and large intestinal tissues but nonepithelial colonic tissue in which no band could be detected. DNA sequencing of the PCR product obtained from jejunal crypt cells (corresponding to position 561-791 in the human Y2 receptor cDNA) showed 84% identity with the human sequence (Fig. 2).

Y4 receptor. PCR products of the expected size (492 bp) were observed in testis, lung and hypothalamus (Figure 1) which represented control tissues in which the Y4 receptor mRNA had been previously shown to be expressed (20, 21). PCR products were also obtained in all small intestinal and colonic tissues (Figure 1). In contrast, no PCR product could be observed in the mouse kidney cell line Cl. 10 (Figure 1). DNA sequencing of the PCR product (position 776-1089) obtained from jejunal crypt cells (not shown) revealed 100% identity with the corresponding sequence in the cloned rat Y4 receptor cDNA (20).

Y5 receptor. A PCR product with the expected size (524 bp) was observed in hypothalamus (Figure 1) from

TABLE 1

Sequence of Primers Used in RT-PCR

Receptor subtype	Organism	Primers position	Sequence (5′ → 3′)[b]	PCR product's (bp)
Y1	Rat/Mouse[a]	Rat: 499/1044	GCT TCT TCT CTG CCC TTY GTG RGT CTC GTA GTC RTC GTC TCG	546
Y2	Human	349/790	AAA TGG GTC CTG TCC TGT GCC TGC CTT CGC TGA TGG TAG TGG	442
Y4	Rat/Mouse	Rat: 623/1115	GAC TTG CTA CCC ATC CTC ATM ATC ACC ACC GYC TCA TCT AYA	492
Y5	Rat	843/1357	CCA GGC AAA AAC CCC CAG CAC GGC AGT GGA TAA GGG CTC TCA	524
GAPDH	Human	11/992	TGA AGG TCG GAG TCA ACG GAT TTG GT CAT GTG GGC CAT GAG GTC ACA CAC	983

[a] Degenerated oligonucleotides from rat and mouse sequences.
[b] Y = C + T; R = A + G; M = A + C.

which the receptor was initially cloned (22). DNA sequencing of the PCR product (position 834-1068) obtained from this tissue (not shown) revealed 100% identity with the corresponding sequence in the cloned rat Y5 receptor cDNA (22). PCR products were observed in jejunal epithelial crypt cells and nonepithelial colonic tissue but not in other intestinal tissue preparations. A band was also obtained in the mouse kidney cell line Cl. 10 (Figure 1).

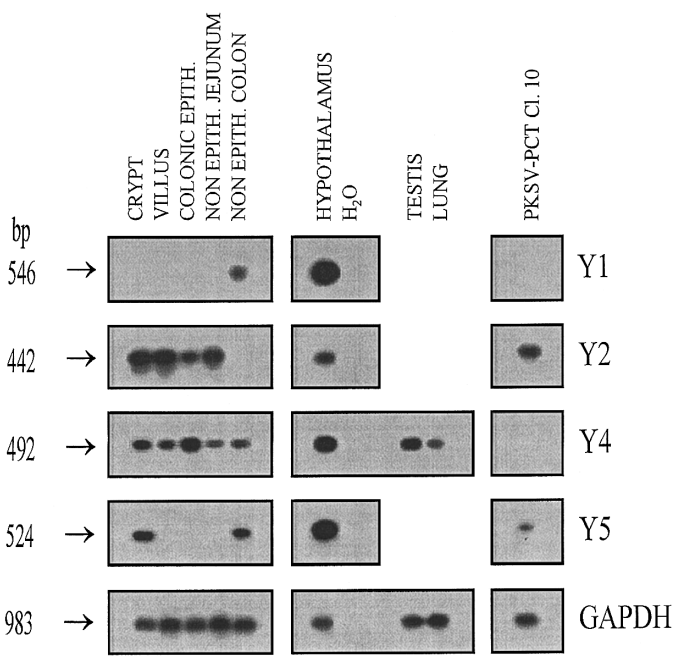

FIG. 1. PCR-based detection of mRNA expression of Y1, Y2, Y4, and Y5 receptors in intestine, colon, PKSV-PCT Cl. 10 cells and control tissues. cDNAs from indicated tissues were used as templates for PCR reactions using specific primers (see Table 1). Southern hybridization with specific [^{32}P]-labelled probes (see Materials and Methods) are shown. Abbreviations: Colonic epith., Colonic epithelium; Non epith. jejunum, Non epithelial jejunum; Non epith. colon, Non epithelial colon.

DISCUSSION

The present study demonstrates the presence of mRNA encoding Y receptors for peptides of the PP-fold family in the rat small and large intestines. This includes Y1, Y2 and Y5 receptors, the natural ligands of which are both NPY and PYY, and also Y4 receptors which are selective for PP. Most of these Y receptors are widely expressed in epithelial and nonepithelial tissues of the gut but each of them has a specific distribution.

Although the use of RT-PCR for analyzing the expression of Y receptors provides nonquantitative information on mRNA and not on functional proteins, the very high specificity of the method is a clue for understanding the co-expression of Y receptors in tissues. Indeed the use of classical ligand binding assays (10, 11, 12) would have been very uneasy or even impossible since there is still substantial confusion regarding the pharmacological profile of Y receptors (11) due to the fact that i) the different Y receptors exhibit numerous cross-reactivities of their natural ligands; ii) few specific agonists and/or antagonists of Y receptors are available (10). In this context, it is worth pointing out that the present work unambiguously demonstrates for the first time the expression of Y1, Y2, Y4 and/or Y5 receptor mRNA since all PCR products were hybridized by Southern blots using specific probes and were also sequenced (see Figures 1 and 2).

Our data on the expression of Y receptor mRNA in the rat intestine and colon can be tentatively interpreted in the light of the pharmacological actions of PYY, NPY, PP and their analogs: (i) the expression of Y1 receptors in nonepithelial colonic tissue may be related to the fact that the Y1 agonist Leu^{31},Pro^{34} NPY (23)

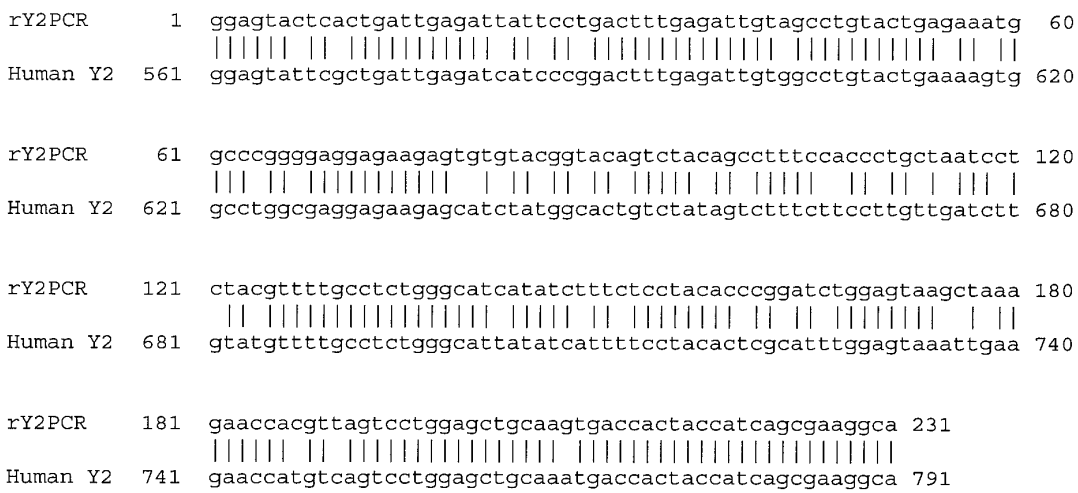

FIG. 2. Nucleotide sequence alignment of the rat Y2-PCR product (rY2PCR) [Genbank: AF054870] obtained from jejunal crypt cells and the human Y2 receptor [Genbank: U36269].

has proabsorptive effect and inhibits VIP-stimulated secretion (24) in rat colon. The absence of Y1 receptor mRNA in colonic epithelium and its presence in nonepithelial colonic tissue is also in line with the fact that tetrodotoxin blocks PYY effect on VIP-stimulated short-circuit current in rat colon (24). However, the potent antisecretory action of Leu^{31},Pro^{34} NPY in rat jejunum in vivo (9) contrasts markedly with the absence of Y1 receptor mRNA in rat jejunum; (ii) the widespread expression of Y2 receptor mRNA in all gut preparations but nonepithelial colonic tissue is in line with the potent antisecretory effects of PYY, NPY (9, 25, 26) and Y2-selective C-terminal fragments of these peptides (9) in rat jejunum in vivo. The distribution of Y2 receptor mRNA in both epithelial and nonepithelial tissues of rat jejunum is also consistent with the fact that the inhibition of short-circuit current by NPY in rat jejunum involves both tetrodotoxin-dependent (27) and tetrodotoxin–independent (26, 27) components. The expression of Y2 receptor mRNA in colonic epithelium may be related to the postsynaptic site of action of PYY for inhibition of electrogenic Cl^- secretion in rat colon (28). It is now clear that the expression of Y2 receptors is not restricted to the central nervous system as it was initially claimed (29) but includes peripheral organs. Beyond the expression of Y2 receptors in gut, the presence of Y2 receptor mRNA in the mouse kidney cell line Cl.10 further argues for the peripheral expression of Y2 receptors in kidney (30); (iii) Y4 receptor mRNA are present in all gut preparations. Since the Y4 receptor exhibit a selectivity for PP, Y4 receptor in rat jejunum may be responsible for the inhibition by PP of VIP-induced fluid secretion in rat jejunum (9). The presence of Y4 receptor mRNA in rat colon suggests that PP may be active at this site, a view in consonance with the fact that PP inhibits VIP-stimulated increases in short-circuit current in colon (31); (iv) finally, the expression of Y5 receptor mRNA appears to be restricted to jejunal epithelial crypt cells and nonepithelial colonic tissue. Since Y5 receptors recognize PYY and NPY with similar affinities, such expression may contribute to many of the above-described actions of PYY and NPY in rat jejunum and colon. However due to the current unavailability of highly selective Y5 receptor agonist or antagonist, nothing is known regarding the role of Y5 receptors in the control of secretion in small and large intestines.

The present data clearly demonstrate that the distribution of Y1, Y2 and Y4 receptor mRNA is quite different from the distribution of functional PYY-preferring receptors negatively coupled to cAMP production in rat small intestine and colon (2,4). This provides a further argument supporting the fact that PYY-preferring receptors and Y2 receptors are distinct though they share a common pharmacological profile more especially with regard to the high affinity for C-terminal fragments of PYY or NPY (10). In contrast, the epithelial distribution of functional PYY-preferring receptors (2,3) and Y5 receptors is very similar: presence in jejunal crypt cells but not in villus cells and absence in colonic epithelium. Furthermore, both PYY-preferring receptors (15, 16) and Y5 receptor mRNA can be detected in the mouse proximal kidney cell clone Cl.10. Although PYY-preferring receptors discriminate between PYY and NPY whereas Y5 receptors do not, these observations indicate that further studies are certainly needed to compare the pharmacological profiles of PYY-preferring receptors and Y5 receptors. Such studies are currently in progress in our laboratory.

REFERENCES

1. Laburthe, A., Kitabgi, P., Couvineau, A., and Amiranoff, B. (1993) *Handbook Exp. Phamacol.* **106,** 133–176.

2. Laburthe, M. (1990) *Trends Endocr. Metab.* **1,** 168–174.
3. Laburthe, M., Chenut, B., Rouyer-Fessard, C., Tatemoto, K., Couvineau, A., Servin, A., and Amiranoff, B. (1986) *Endocrinology* **118,** 1910–1917.
4. Voisin, T., Rouyer-Fessard, C., and Laburthe, M. (1990) *Am. J. Physiol.* **258,** G753–G759.
5. Servin, A., Rouyer-Fessard, C., Balasubramaniam, A., St-Pierre, S., and Laburthe, M. (1989) *Endocrinology* **124,** 692–700.
6. Souli, A., Chariot, J., Presset, O., Tsocas, A., and Rozé, C. (1997) *Eur. J. Pharmacol.* **333,** 87–92.
7. Strabel, D., and Diener, M. (1995) *Br. J. Pharmacol.* **115,** 1071–1079.
8. MacFadyen, R. J., Allen, J. M., and Bloom, S. R. (1986) *Neuropeptides* **7,** 219–227.
9. Souli, A., Chariot, J., Voisin, T., Presset, O., Tsocas, A., Balasubramanian, A., Laburthe, M., and Rozé, C. (1997) *Peptides* **18,** 551–557.
10. Laburthe, M., Couvineau, A., Amiranoff, B., and Voisin, T., (1994) *Baillieres Clin. Endocrinol. Metab.* **8,** 77–110.
11. Blomqvist, A. G., and Herzog, H. (1997) *Trends Neurosci.* **20,** 294–298.
12. Larhammar, D. (1996) *Regul. Pept.* **65,** 165–174.
13. Laburthe, M., Rouyer-Fessard, C., and Gammeltoft, S. (1988) *Am. J. Physiol.* **254,** G457–G462.
14. Cartier, N., Lacave, R., Vallet, V., Hagege, J., Hellio, R., Robine, S., Pringault, E., Cluzeaud, F., Briand, P., Kahn, A., and Vandewalle, A. (1993) *J. Cell Sci.* **104,** 695–704.
15. Voisin, T., Bens, M., Cluzeaud, E., Vandewalle, A., and Laburthe, M. (1993) *J. Biol. Chem.* **268,** 20547–20554.
16. Voisin, T., Lorinet, A. M., Maoret, J. J., Couvineau, A., and Laburthe, M. (1996) *J. Biol. Chem.* **271,** 574–580.
17. Chomczynski, P., and Sacchi, N. (1987) *Anal. Biochem.* **162,** 156–159.
18. Eva, C., Keinanen, K., Monyer, H., Seeburg, P., and Sprengel, P. (1990) *FEBS Letter* **271,** 81–84.
19. Gerald, C., Walker, M. W., Vaysse, P. J. J., He, C., Branchek, T. A., and Weinshank, R. L. (1995*) J. Biol. Chem.* **270,** 26758–26761.
20. Lundell, I., Statnick, M. A., Johnson, D., Schober, D. A., Startback, P., Gehlert, D. R., and Larhammar, D. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 5111–5115.
21. Whitcomb, D. C., Taylor, I. L., and Vigna, S. R. (1990) *Am. J. Physiol.* **259,** G687–G691.
22. Gerald, C., Walker, M. W., Criscione, L., Gustafson, E. L., Batzl-Hartmann, C., Smith, K. E., Vaysse, P., Durkin, M. M., Laz, T. M., Linemeyer, D. L., Schaffhauser, A. O., Whitebread, S., Hofbauer, K. G., Taber, R. I., Branchek, T. A., and Weinshank, R. L. (1996) *Nature* **382,** 168–171.
23. Fuhlendorff, J., Gether, U., Aakerlund, L., Langeland-Johansen, N., Thorgersen, H., Melberg, S. G., Olsen, U. B., Thastrup, O., and Schwartz, T. W. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 182–186.
24. Whang, E. E., Hines, O. J., Reeve, J. R., Grandt, D., Moser, J. A., Bilchik, A. J., Zinner, M. J., McFadden, D. W., and Ashley, S. W. (1997) *Dig. Dis. Sci.* **42,** 1121–1127.
25. Saria, A., and Beubler, E. (1985) *Eur. J. Pharmacol.* **119,** 47–52.
26. Cox, H. M., Cuthbert, A. M., Hakanson, R., and Wahlestedt, C. (1988) *J. Physiol. (London)* **398,** 65–80.
27. McKay, D. M., Berin, M. C., Fondacaro, J. D., and Perdue, M. H. (1996) *Am. J. Physiol.* **271,** G987–G992.
28. Nakanishi, T., Kanayama, S., Kiyohara, T., Okuno, M., Shinomura, Y., and Matsuzawa, Y. (1996) *Regul. Pept.* **61,** 149–154.
29. Rose, P. M., Fernandez, P., Lynch, J. S., Frazier, S. T., Fisher, S. M., Kodukula, K., Kienzle, B., and Seethala, R. (1995) *J. Biol. Chem.* **270,** 22661–22664.
30. Sheik, S. P., Hansen, A. P., and Williams, J. A. (1991) *J. Biol. Chem.* **266,** 23959–23966.
31. Ballantyne, G. H., Goldenring, J. R., Fleming, F. X., Steven, R. J., Scott, F., Fielding, L. P., Binder, H. J., and Modlin, I. M. (1993) *Am. J. Physiol.* **264,** G849–G854.