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Molecular Cloning and Expression of a Human Secretin Receptor

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SUMMARY

Secretin is a 27-amino acid neuroendocrine peptide that stimulates fluid and electrolyte secretion in the gastrointestinal tract, activates tyrosine hydroxylase activity in the central nervous system, and affects cardiac and renal function. Specific receptors for secretin have been previously characterized on neuroblastoma cells, pancreatic acini, gastric glands, and liver cholangiocytes. We report here the isolation of a 1616-base pair cDNA from human lung tissue that encodes a 440-amino acid, 50-kDa, G protein-coupled human secretin receptor (HSR), with homology of 80% with the rat secretin receptor and 37% with the human type I vasoactive intestinal peptide receptor. Northern blot analysis of human tissue mRNA revealed that the relative intensity for expression of a 2.1-kilobase HSR transcript was pancreas > kidney > small intestine > lung > liver, with trace levels in brain, heart, and ovary. Stable transfectants of HSR in human embryonic kidney 293 cells, termed 293S12, expressed 10^5 binding sites/cell for ^{125}I-secretin, with an apparent K_d of 3.2 nM. Vasoactive intestinal peptide, pituitary adenylyl cyclase-activating peptide-38, and glucagon were less potent (by 3 orders of magnitude) than secretin in competitively inhibiting ^{125}I-secretin binding to 293S12 cells. Secretin evoked concurrent dose-dependent increases in intracellular cAMP and calcium levels in 293S12 cells and stimulated a 4-fold increase in phosphatidylinositol hydrolysis. Thus, the HSR expressed by stable transfectants can couple to two distinct intracellular signaling pathways.

Secretin is a 27-amino acid neuroendocrine peptide that is a member of the VIP, PACAP, and glucagon peptide family and is distributed primarily in the gastrointestinal tract and central nervous system (1, 2). The first hormone to be discovered (3), secretin stimulates biliary, pancreatic, and small intestinal fluid and electrolyte secretion in many mammalian species (1) and tyrosine hydroxylase activity in rat superior cervical ganglion neurons and PC-12 pheochromocytoma cells (4, 5). Secretin also inhibits the release of gastrin and gastric acid (1) and affects cardiac and renal function (6, 7). Immunoreactive secretin was found in the small intestine, primarily in S cells of the duodenum (1), and less abundantly in the central nervous system (2). RT-PCR amplification detected expression of rat secretin precursor mRNA in the small intestine, heart, lung, kidney, brain, and testis (8, 9). For rat brain, RT-PCR amplification revealed distinct expression of the secretin precursor transcript in the hypothalamus, pituitary, medula oblangata, pons, cortex, and brainstem (8, 10).

Distinct receptors for secretin that were coupled to the elevation of $[cAMP]_i$ through a G_s protein were characterized in guinea pig pancreatic acini (11), rat gastric glands (12), rat liver cholangiocytes (13), mouse N18TG2 neuroblastoma cells (14), and mouse-rat NG108–15 neuroblastoma-glioma hybrid cells (15). A RSR cDNA isolated from NG108–15 cells (16) was the first cloned member of a distinct new family of G protein-coupled receptors that now includes receptors for VIP (17–19), PACAP (20–22), glucagon (23, 24), parathyroid hormone (25), and calcitonin (26). Although Northern blot analysis of rat tissue mRNA detected RSR expression in the heart, stomach, and pancreas, no signal was observed for the lung, liver, kidney, or brain (16).

We previously described the cloning and characterization of a HVR1 cDNA from HT-29 intestinal epithelial cells and lung tissue (17). Here we report the cloning, functional expression, and tissue distribution of a cDNA encoding a HSR that was generated from lung tissue mRNA and is also ex-

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ABBREVIATIONS: VIP, vasoactive intestinal peptide; PACAP, pituitary adenylyl cyclase-activating peptide; HSR, human secretin receptor(s); RSR, rat secretin receptor(s); HVR1, human type I vasoactive intestinal peptide/type II pituitary adenylyl cyclase-activating peptide receptor(s); $[cAMP]_i$, concentration of intracellular cAMP; $[Ca^{2+}]_i$, concentration of intracellular calcium; PI, phosphatidylinositol; RT, reverse transcription; PCR, polymerase chain reaction; RACE, rapid amplification of cDNA ends; HEK293, human embryonic kidney 293; SSC, standard saline citrate; SDS, sodium dodecyl sulfate; CMV, cytomegalovirus; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; kb, kilobase(s); HBSS, Hanks' balanced salt solution.

pressed in the pancreas, kidney, small intestine, liver, brain, heart, and ovary.

Materials and Methods

Isolation of cDNA clones. A human lung cDNA library in λgt11 (HL 1004b; Clontech Laboratories, Palo Alto, CA) was screened under low stringency conditions of 6× SSC (1× SSC is 0.15 M NaCl, 0.015 M sodium citrate), 5× Denhardt's solution, 0.25% SDS, 0.01 M sodium phosphate, pH 6.8, 0.001 M EDTA, 100 μg/ml sonicated salmon sperm DNA, and 1 mg/ml nonfat dried milk, at 45° for 18 hr, with a 0.3-kb ^{32}P-labeled *Bam*HI restriction fragment derived from the HVR1 cDNA (17). This fragment encoded a region spanning transmembrane segments II–IV of the HVR1 protein. Filters were washed in 2× SSC/0.05% SDS for 30 min at 22°, followed by 1× SSC/0.05% SDS for 30 min at 45°. A 0.6-kb ^{32}P-labeled insert from a resulting positive cDNA clone, termed HL14.5, was used to rescreen the library under higher stringency conditions of 5× SSC, 5× Denhardt's solution, 0.2% SDS, 50% deionized formamide, and 100 μg/ml sonicated salmon sperm DNA, at 42° for 18 hr. Washing conditions were 1× SSC/0.1% SDS for 30 min at 22°, followed by 0.1× SSC/0.1% SDS for 30 min at 42–55°, which resulted in a positive clone termed HL12.1A.

5′-RACE. Poly(A)$^+$ RNA was prepared from human lung tissue with the FastTrack mRNA isolation system (Invitrogen, San Diego, CA). First-strand cDNA was synthesized with Superscript II reverse transcriptase (GIBCO-BRL, Gaithersburg, MD) using the HSB3 gene-specific amplimer spanning the region 5′ to putative transmembrane domain I, 5′-d(CAGCTTCAGCAGCTAGGAGTGCCGC), followed by 3′-d(A)-tailing with terminal deoxynucleotide transferase (Promega, Madison, WI) as described (17). Second-strand cDNA was synthesized with a 5′-RACE adaptor/dT amplimer, 5′-d(GGAATTCTGCAGGATCCCGGGTAAG(T)$_{18}$, and PCR amplification was performed using the 5′-RACE adaptor/amplimer and a nested gene-specific amplimer (HSA3), 5′-d(CTGCTGGTGAGCATCCGGAGGAATC), under the conditions described (17). The primary PCR products were electrophoresed on a 2% agarose gel, and DNA fragments of approximately 0.3 kb in size were isolated (Elu-Quik; Schleicher & Schuell, Keene, NH) and subcloned into the pCRII vector (Invitrogen). Transformants were screened under the low stringency conditions described previously, with a second nested ^{32}P-labeled gene-specific oligonucleotide (HSD3), 5′-d(CACAGAAGAGGGCCAGCAGCTTATG), as a probe. Independently positive clones were sequenced in their entirety, and no PCR errors were found.

Construction of the cDNA encoding the full length lung HSR. The full length cDNA encoding HSR was obtained by PCR amplification of oligo(dT)-primed, reverse transcribed, human lung tissue, poly(A)$^+$ RNA with a 5′-amplimer (HSA) that spans the translation start codon (underlined), 5′-d(GCACCATGCGTCCCCACCTG), and a 3′-amplimer (HSB) that spans a region 3′ to the translation stop codon, 5′-d(CTCTCTTGGTCTCTGTCCG), using *Pyrococcus furiosus* polymerase (Stratagene, La Jolla, CA) under the conditions of 1 min at 94°, 2 min at 55°, and 4 min at 74° for 35 cycles. The 1.4-kb fragment obtained was subcloned into the G418 sulfate antibiotic-selectable pRc/CMV mammalian expression vector (Invitrogen), and the resulting HSR/CMV construct was verified by sequence analysis.

Northern blot analysis of tissue expression of HSR. Northern blot membranes containing poly(A)$^+$ RNA from multiple human tissues (Clontech) were hybridized to a ^{32}P-labeled HL12.1A cDNA probe in Rapid-Hyb buffer (Amersham) at 65° for 2 hr, in an Autoblot microhybridization oven (Bellco, Vineland, NJ). Membranes were washed in 2× SSC/0.1% SDS for 30 min at room temperature, followed by 0.1× SSC/0.1% SDS for 30 min at 65°, and were autoradiographed.

Transfection and selection of stable cell lines. Subconfluent HEK293 cells were grown in 100-mm culture plates and transfected with 10 μg of HSR/CMV using the Lipofectamine transfection reagent (GIBCO-BRL), according to the manufacturer's instructions. Transfected cells were grown for 1 week in medium containing 0.5 mg/ml geneticin (GIBCO-BRL) and were subcloned under limiting dilution. Individual colonies were isolated using sterile cloning cylinders and expanded, and cloned cells were screened for HSR expression initially by specific binding of ^{125}I-secretin (986 Ci/mmol; Peninsula Laboratories, Belmont, CA). Replicate aliquots of 10^6 cells were incubated for 30 min at 22° with ^{125}I-secretin (50,000–100,000 cpm) in 0.3 ml of Dulbecco's modified Eagle medium containing 10 mg/ml hen ovalbumin (ICN, Costa Mesa, CA), pH 7.3, in the presence or absence of 1 μM unlabeled human secretin (American Peptide Co., Sunnyvale, CA). Cell-associated and unbound radiolabel were separated by rapid centrifugation through 100 μl of a mixture of *n*-butyl phthalate and dioctyl phthalate oils (3:2, v/v; VWR Scientific) in 0.4-ml polypropylene tubes. The tips of the tubes containing the cell pellets were cut off, and cell-associated and unbound radioactivity was determined in a γ counter. An HSR-expressing clonal cell line, termed 293S12, was selected from a range of stable transfectants for further analysis.

Competitive displacement of bound ^{125}I-secretin. Replicate aliquots of 293S12 cells (3.3×10^6/ml) were incubated with ^{125}I-secretin in the presence of 0–10 μM unlabeled peptide for 30 min at 22°, as described previously. K_i and B_{max} values were analyzed with the LUNDON-2 competitive Scatchard analysis program (Lundon Software, Cleveland, OH).

Measurements of [cAMP]$_i$. 293S12 cells (5×10^6/ml) were preincubated with 0.6 mM 3-isobutyl-1-methylxanthine (Calbiochem, San Diego, CA) and 0.02 mM Ro 20–1724 (GIBCO-BRL) for 30 min at 22°. Replicate aliquots of 10^5 cells were treated with picomolar to micromolar levels of peptide for 30 min at 37°, in 0.2 ml of Dulbecco's modified Eagle medium containing 1 mg/ml hen ovalbumin. The reactions were terminated with 70% ethanol, and cell supernates were lyophilized and reconstituted in 0.05 M sodium acetate buffer, pH 6.2. [cAMP]$_i$ was analyzed by cAMP enzyme-linked immunosorbent assay (PerSeptive Diagnostics, Cambridge, MA) according to the manufacturer's protocol.

Measurements of [Ca^{2+}]$_i$. 293S12 cells (4×10^6/ml) were incubated in the dark with 2.5 μM fura-2/acetoxymethyl ester (Molecular Probes, Eugene, OR), in HBSS containing 25 mM HEPES, pH 7.4, and 1 mg/ml hen ovalbumin (HBSS1), for 30 min at 37°. After three washes with phosphate-buffered saline, the cells were resuspended at a density of 10^6/ml of HBSS1 and stored in the dark at 4° until use. Fura-2-loaded cells (2×10^6) were analyzed for [Ca^{2+}]$_i$ changes induced by 0.1–100 nM peptide with a Perkin Elmer LS 50B fluorescence spectrometer (340/380-nm excitation, 510-nm emission, 10-nm slit width). Calibration of fura-2 dye taken up by the cells was performed by lysing the cells in 0.2% (v/v) Triton X-100, 1.2 mM $CaCl_2$ (R_{max}), followed by removal of all calcium with 10 mM EGTA (R_{min}). [Ca^{2+}]$_i$ values were calculated with the assistance of the Fluorescence Data Manager program (Perkin Elmer, Buckinghamshire, UK).

Measurements of PI hydrolysis. 293S12 cells (2×10^6/ml) were incubated with 5 μCi of *myo*-[^{3}H]inositol (Amersham), in HBSS containing 16 mM HEPES, pH 7.4, and 0.1 mg/ml bovine serum albumin (HBSS2), for 2 hr at 37°, with gentle shaking. After three washes with HBSS2, the cells were resuspended in HBSS2 containing 10 mM LiCl and 1 mM unlabeled *myo*-inositol (Sigma) and were incubated for an additional 10 min at 37°, with gentle shaking. Analysis of the time course of PI hydrolysis was undertaken by treating replicate 0.5-ml aliquots of 10^6 cells with 1 μM secretin, or with buffer control, for the indicated times at 37°. Dose-dependent induction of PI hydrolysis by secretin was determined by treating replicate 0.5-ml aliquots of 10^6 cells with 0–1 μM secretin for 30 min at 37°. Reactions were terminated with the addition of 0.1 volume of 50% (w/v) trichloroacetic acid, followed by centrifugation for 1 min at 15,000 rpm. Supernates were extracted three times with 1 ml of water-saturated ethyl ether, neutralized with 0.1 volume of 0.2 mM

Tris·HCl, pH 8.0, and chromatographed on AG 1-X8 (Bio-Rad, Hercules, CA) columns to resolve [^{3}H]inositol phosphates, according to the method of Berridge *et al.* (27). Radioactive eluates were quantified in a Beckmann liquid scintillation counter.

Results

Cloning of lung HSR. Low stringency screening of a human lung cDNA library with a probe derived from the HVR1 cDNA resulted in several clones bearing HVR1 sequences and one clone containing a distinctive 0.6-kb cDNA insert, termed HL14.5 (Fig. 1). Sequence analysis of HL14.5 revealed the presence of a 196-amino acid open reading frame, which contained part of the amino terminus and transmembrane domains I–IV of a putative G protein-coupled receptor that had 84% identity with RSR and 37% identity with HVR1. Rescreening of the human lung cDNA library under higher stringency conditions with the HL14.5 cDNA insert as a probe yielded two positive clones, the longer of which contained a 1.4-kb insert, termed HL12.1A (Fig. 1).

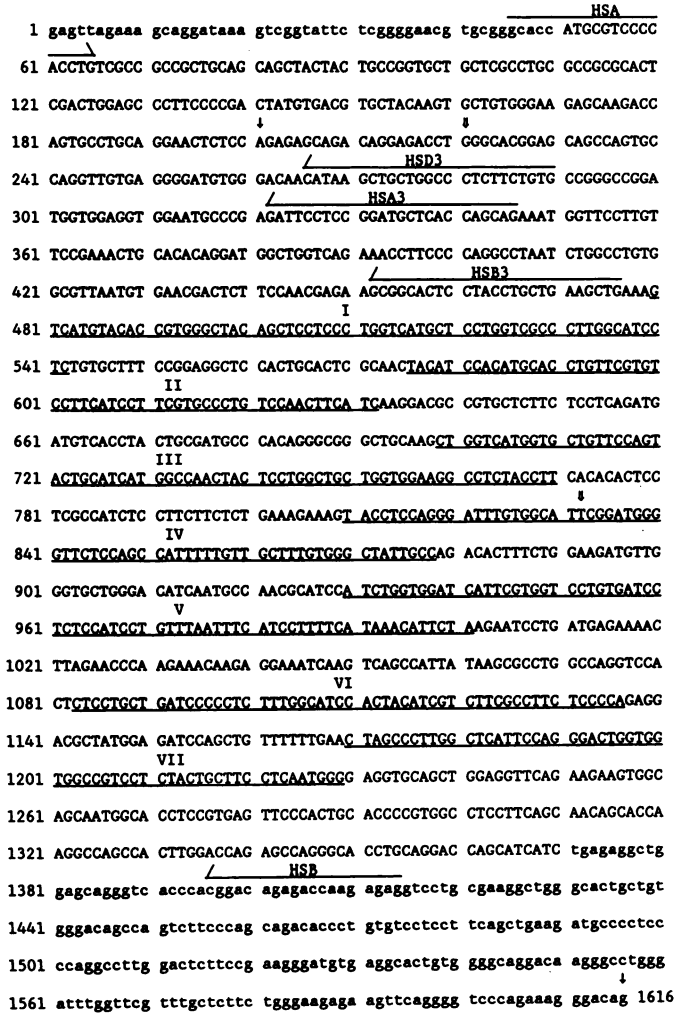

Fig. 1. Nucleic acid sequence for lung HSR cDNA. *Uppercase letters*, region encoding the receptor protein; *vertical arrows*, limits of cDNA inserts HL14.5 (⇓) and HL12.1A (↓); *horizontal arrows*, orientation and location of gene-specific amplimers described in the text; *underlining*, positions of transmembrane segments I–VII. The sequence for the HSR cDNA has been deposited in the GenBank database, with accession number U13989.

Sequence analysis indicated that the HL14.5 insert was contained within HL12.1A, which showed an overall nucleic acid identity of 79% with RSR. The HL12.1A-encoded protein contained seven membrane-spanning hydrophobic domains and a carboxyl terminus terminating in a stop codon, with 243 base pairs of 3′ untranslated sequence that lacked a poly(A)$^+$ tail. Alignment of HL12.1A and RSR sequences revealed, however, that about 0.2 kb of the 5′ region that encoded the amino terminus, including the translation start codon, was absent in HL12.1A (Fig. 1).

Repeated screenings of the human lung library did not yield additional 5′ sequence information; therefore, the 5′-RACE technique (28) was applied to human lung tissue poly(A)$^+$ RNA, using nested HL12.1A-specific primers. This resulted in the amplification of a 0.3-kb RACE fragment containing 0.2 kb of 5′ sequence beyond that obtained for HL12.1A and encoding a putative translation start codon followed by an open reading frame that was homologous to the amino-terminal region of RSR (Fig. 1).

Structure of HSR. The 1616-base pair cDNA had an overall nucleic acid identity of 76% with RSR and encoded a 440-amino acid HSR protein with a predicted molecular mass of 50 kDa. The HSR protein shared an amino acid identity of 80% with RSR and 37% with HVR1. Based on nucleotide homology and a match of the first three amino acid residues for HSR, RSR, and HVR1, the initial ATG start codon for HSR was aligned with the second of the two in-frame ATG codons reported for the 5′ region of RSR (Fig. 2). The amino acid homology between HSR and RSR was greatest within the transmembrane domains; however, HSR differed from RSR in having four additional residues in the amino-terminal region and nine fewer residues in the carboxyl-terminal tail segment (Fig. 2). The amino-terminal region of HSR contained eight cysteine residues, seven of which were simi-

Fig. 2. Alignment of the primary amino acid sequences for HSR, RSR, and HVR1. *Uppercase letters*, residues common to two or more receptors. Extracellular amino-terminal cysteine residues (*), potential *N*-glycosylation (|) and serine/threonine phosphorylation (+) sites, and putative transmembrane segments I–VII (*overlined*) are indicated for HSR. Gaps have been introduced to maximize the alignment.

larly located for RSR, and the extracellular portions of HSR carried five conserved potential *N*-glycosylation sites (Fig. 2). Several serine and threonine residues that could serve as potential regulatory phosphorylation sites were located in the intracellular portions of the receptor (Fig. 2). The entire HSR coding insert was independently obtained through RT-PCR amplification of human lung tissue poly(A)$^+$ RNA, using the gene-specific amplimers HSA and HSB.

Tissue expression of HSR. Northern blot analysis of multiple human tissue poly(A)$^+$ RNAs hybridized to an HL12.1A probe detected the presence of a prominent 2.1-kb transcript in several of the tissues characterized (Fig. 3). The size of the observed transcript indicated that the HSR cDNA isolated and reported in this study was missing about 0.5 kb of sequence. The relative intensity for HSR mRNA tissue expression was pancreas > kidney > small intestine > lung > liver, with trace levels of expression in the brain, heart, and ovary (Fig. 3).

Binding of ^{125}I-secretin by stable transfectants of HSR. Five independent, stably transfected clones of the HSR/CMV mammalian expression construct in HEK293 cells were obtained, and they bound ^{125}I-secretin with similar high levels of specificity. One representative clone, termed 293S12, was subjected to a detailed analysis of binding characteristics and signal transduction properties.

Computerized Scatchard analysis of the competitive displacement by unlabeled human secretin of ^{125}I-secretin bound to 293S12 cells revealed the presence of about 10^5 binding sites/cell, with an apparent K_d of 3.2 nM (Fig. 4). This binding affinity for secretin observed with 293S12 cells was in concordance with apparent K_d values of 1–10 nM reported for transiently expressed RSR in COS cells (16) and native secretin receptors of guinea pig pancreatic acini (11), mouse N18TG2 neuroblastoma cells (14), and rat-mouse NG108–15 hybridoma cells (15). Human PACAP-38 (American Peptide Co.), VIP, and glucagon (Sigma) weakly displaced ^{125}I-secretin bound to 293S12 cells (Fig. 3). PACAP-38 (IC_{50} ~ 4 μM) was slightly more effective than VIP (IC_{50} ~ 8 μM), whereas glucagon was markedly less potent (IC_{50} ~ 30 μM).

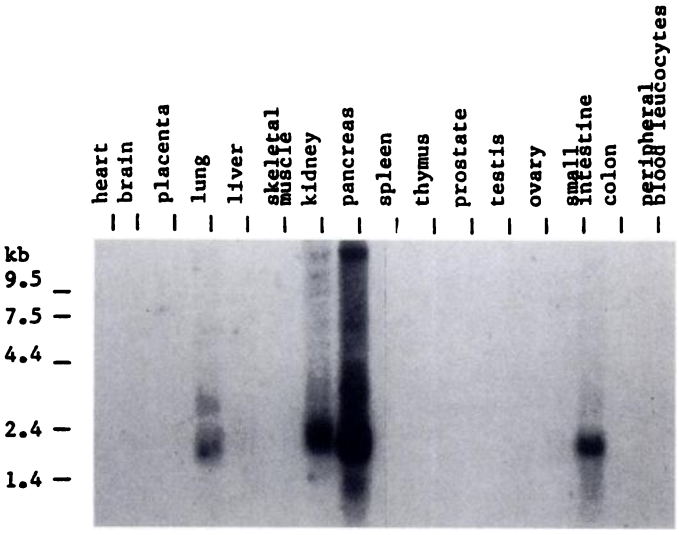

Fig. 3. Tissue expression of HSR. In this Northern blot, membranes containing poly(A)$^+$ RNA from the indicated human tissues were hybridized to the ^{32}P-labeled HL12.1A cDNA insert and autoradiographed. *Numbers to the left*, sizes and locations of RNA molecular mass markers (GIBCO-BRL).

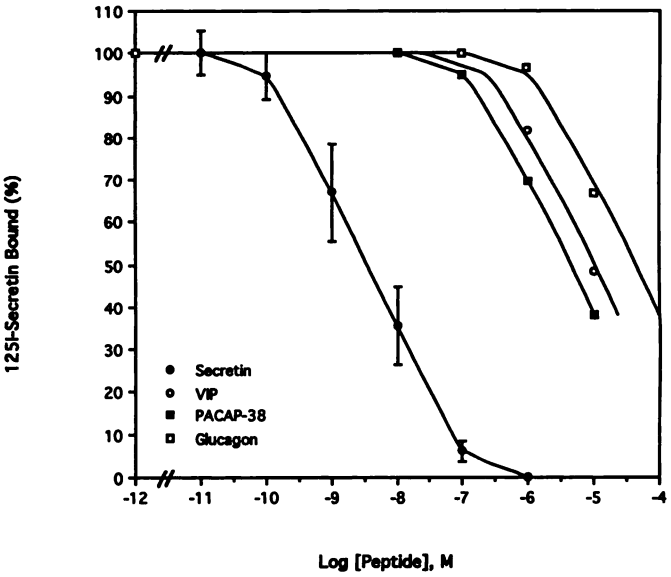

Fig. 4. Competitive displacement of ^{125}I-secretin bound to 293S12 cells. The binding of ^{125}I-secretin to stable transfectants of HSR in 293 cells was determined in the presence of 0–10 μM secretin, VIP, PACAP-38, or glucagon. Results are the mean (± standard error for secretin) of three separate experiments performed in duplicate and are expressed as a percentage of the maximal response, after subtraction of the nonspecifically bound counts. Nonspecific binding, measured in the presence of 10 μM secretin, was 1260 ± 105 cpm, and maximal total ^{125}I-secretin bound was 13,307 ± 680 cpm.

Increase in $[cAMP]_i$ induced in stable transfectants of HSR. Secretin dose-dependently induced an increase in $[cAMP]_i$ in 293S12 cells, with a half-maximally effective concentration of 2 nM (Fig. 5), which was similar to values of 1–6 nM reported for recombinant RSR transiently expressed in COS cells that overexpress the α-subunit of Gs (16) and native secretin receptors of rat liver cholangiocytes (13) and guinea pig pancreatic acini (11). VIP (EC_{50} ~ 0.2 μM) and PACAP-38 (EC_{50} ~ 0.1 μM) also increased $[cAMP]_i$ in 293S12 cells in a dose-dependent manner, although 10 μM glucagon had no discernible effect (Fig. 5).

Increase in $[Ca^{2+}]_i$ induced in stable transfectants of HSR. Although secretin receptors were previously reported to couple only to increases in $[cAMP]_i$, presumably through G_s proteins, other members of the secretin receptor subfamily, including HVR1, were found to concurrently couple to an increase in $[Ca^{2+}]_i$ (29, 30). We therefore examined 293S12 cells for the effects of secretin on $[Ca^{2+}]_i$. Fura-2-loaded 293S12 cells showed a dose-dependent increase in $[Ca^{2+}]_i$ upon treatment with 1–100 nM secretin (Fig. 6). Increases in $[Ca^{2+}]_i$ above the base-line were, however, difficult to resolve at concentrations of secretin below 0.1 nM, due to noise in the detection system. Stimulation by secretin resulted in a rapid transient increase in $[Ca^{2+}]_i$ that peaked by 20 sec and fell to a sustained level above the basal level (Figs. 6 and 7). The late sustained elevation in $[Ca^{2+}]_i$ depended on extracellular calcium and rapidly fell to base-line levels after chelation of extracellular free calcium with 2 mM EGTA (Fig. 7). Pretreatment with 2 mM EGTA before challenge with secretin resulted in a smaller transient increase in $[Ca^{2+}]_i$, which, however, returned rapidly to basal values (Fig. 7). These results indicate that the rapid early increase in $[Ca^{2+}]_i$ was probably due to release from intracellular calcium stores, with the

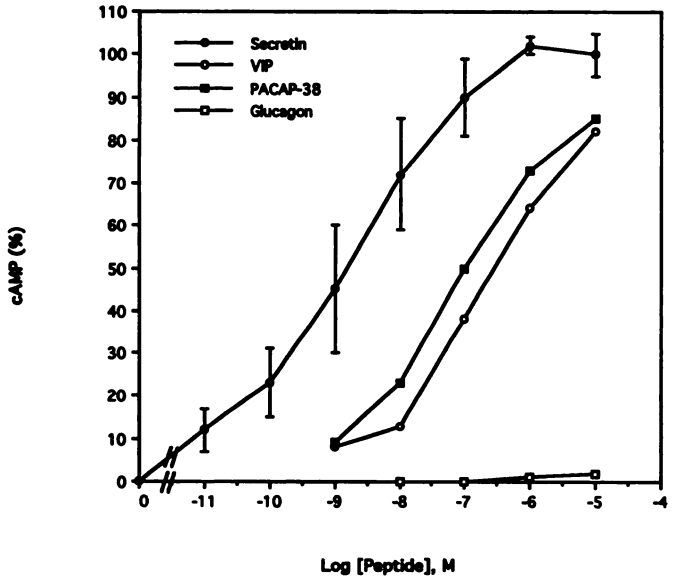

Fig. 5. Increase in $[cAMP]_i$ induced in 293S12 cells. Cells were incubated with the indicated concentrations of secretin, VIP, PACAP-38, or glucagon, and the cAMP content of cell lysates was evaluated by enzyme-linked immunosorbent assay. The values reported are the mean (± standard error for secretin) of three separate experiments carried out in duplicate and are expressed (per 10^5 cells) as a percentage of the maximal response, with the basal level subtracted. Uninduced basal $[cAMP]_i$ was 6 ± 2 pmol/10^5 cells and maximal $[cAMP]_i$ induced by 10 μM secretin was 126 ± 6 pmol/10^5 cells.

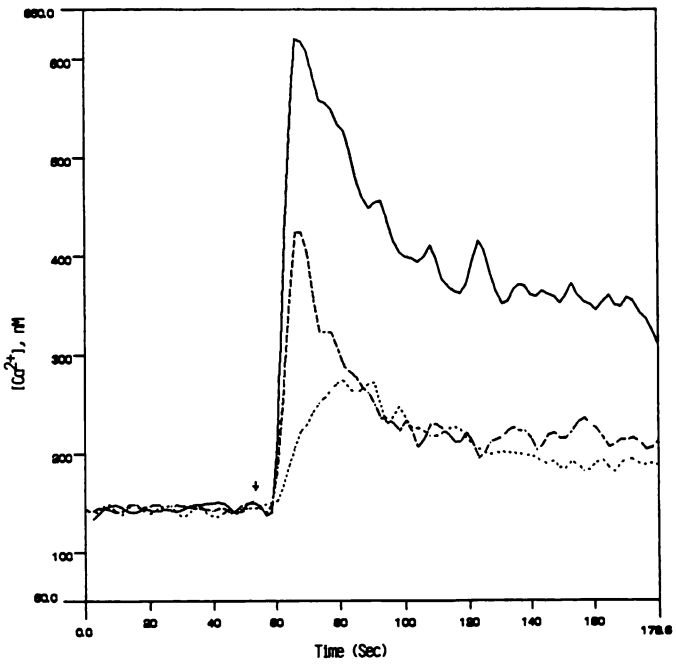

Fig. 6. Dose-dependent increase in $[Ca^{2+}]_i$ evoked in 293S12 cells. Fura-2-loaded cells were stimulated with 1 nM (· · · · ·), 10 nM (– – –), or 100 nM (——) secretin at the time indicated (↓) and changes in $[Ca^{2+}]_i$ with respect to time were monitored. Each analysis was carried out with cells not previously exposed to peptide, because of rapid desensitization of the secretin-induced response.

influx of extracellular calcium enhancing the peak maximum. Further confirmation was obtained when secretin failed to elicit an increase in $[Ca^{2+}]_i$ in 293S12 cells that had been pretreated with 20 nM thapsigargin (Sigma) (Fig. 7), which depletes intracellular calcium stores by inhibiting endoplasmic reticulum Ca^{2+}-ATPases (31).

Induction of PI hydrolysis by secretin in stable transfectants of HSR. Because the release of calcium from intracellular stores is primarily evoked by inositol-1,4,5-trisphosphate, which is produced by phospholipase C_β-mediated hydrolysis of PI (32), 293S12 cells labeled with *myo*-[^{3}H]inositol were examined for the effect of secretin on PI hydrolysis. After 1 hr of incubation at 37°, 1 μM secretin induced a 4-fold increase in the production of total [^{3}H]inositol phosphates, compared with buffer-treated control 293S12 cells (Fig. 8). Inositol monophosphates were the major components of the inositol phosphates produced, although levels of inositol bisphosphates and inositol trisphosphates were also increased by 1 μM secretin, compared with buffer-treated control cells (data not shown). Secretin dose-dependently increased PI hydrolysis in 293S12 cells, with a half-maximally effective concentration of 16 nM after 30 min of incubation at 37° (Fig. 9).

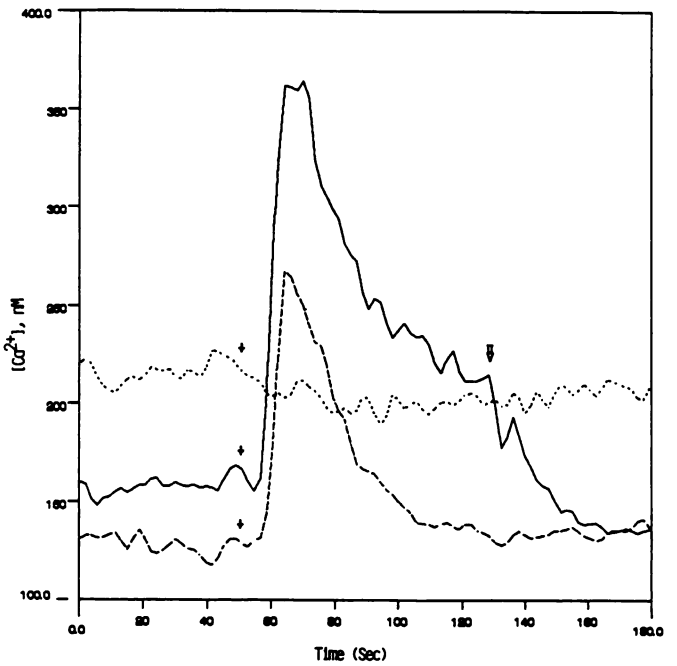

Fig. 7. Source of calcium mobilized by secretin in 293S12 cells. Fura-2-loaded 293S12 cells were pretreated with 2 mM EGTA (– – –), 20 nM thapsigargin (· · · · ·), or buffer control (——) and were challenged with 100 nM secretin at the indicated time point (↓). After stimulation with secretin, 2 mM EGTA was added to the buffer control during the sustained phase of $[Ca^{2+}]_i$ elevation, at the time indicated (↓). Representative tracings of changes in $[Ca^{2+}]_i$ are shown.

Discussion

Neuroendocrine peptide mediators such as secretin, VIP, and PACAP are structurally related peptides that regulate several processes, including those of the central nervous system and gastrointestinal tract, cells and tissues of which have been previously reported to bear receptors for secretin (11–15). Although secretin precursor transcripts were detected in rat lung tissue by RT-PCR analysis (10), there were only a few reports that hinted at the probable presence of secretin receptors in the lung (33, 34) and none describing a role for secretin in lung physiology. The cloning of a secretin

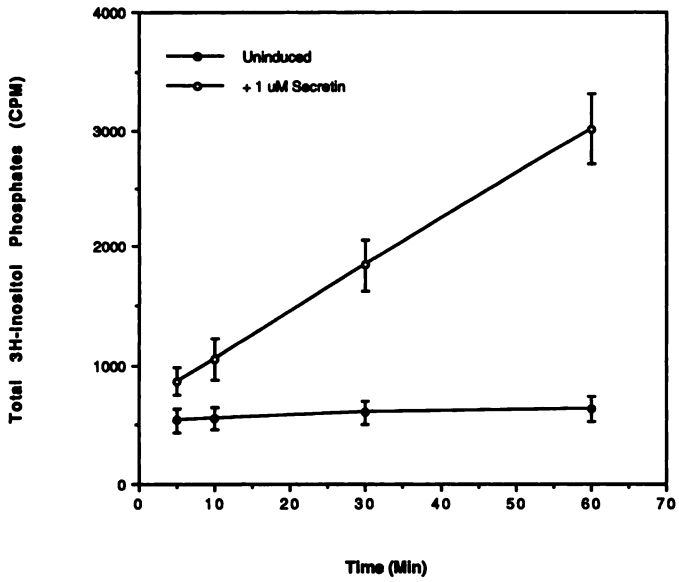

Fig. 8. Time course of stimulation of PI hydrolysis in 293S12 cells. Cells were labeled with *myo*-[^{3}H]inositol and incubated with (○) or without (●) 1 μM secretin for the indicated times, at 37°. Total ^{3}H-labeled inositol phosphates produced were isolated and quantified in a liquid scintillation counter. The values reported are the mean ± standard error of three separate experiments carried out in duplicate.

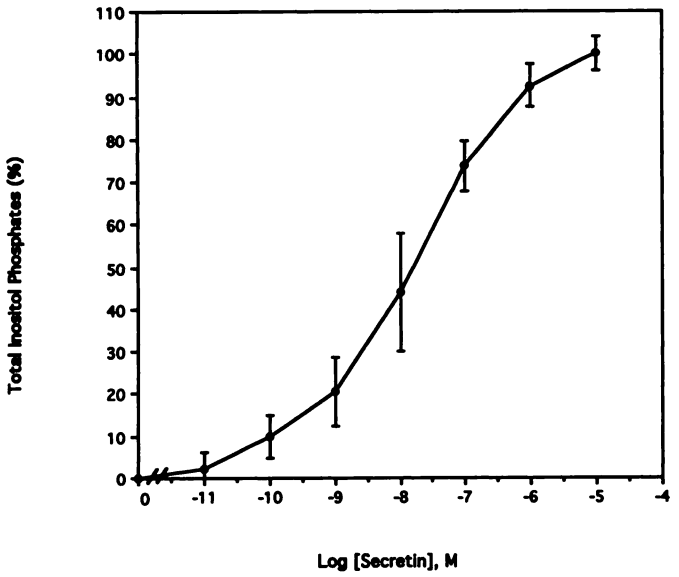

Fig. 9. Dose-dependent activation of PI hydrolysis in 293S12 cells. Cells were labeled with *myo*-[^{3}H]inositol and incubated with 0–1 μM secretin for 30 min at 37°. Total ^{3}H-labeled inositol phosphates produced were isolated and quantified in a liquid scintillation counter. The values reported are the mean ± standard error of three separate experiments carried out in duplicate and are expressed as a percentage of the maximal response, after subtraction of the basal level. The uninduced basal value for ^{3}H-labeled inositol phosphates produced was 592 ± 93 cpm, and the maximal amount of inositol phosphates produced by incubation with 10 μM secretin was 2670 ± 99 cpm.

receptor-encoding cDNA from human lung tissue is, therefore, the first molecular evidence for the presence of specific receptors for secretin in the lung.

HSR belongs to a seven-transmembrane domain, G protein-coupled, receptor subfamily that includes receptors for VIP, PACAP, and glucagon. These homologous receptors have little sequence identity with the catecholamine, opsin, tachykinin, and chemokine G protein-coupled receptor subfamily (30) and possess cysteine-rich extracellular amino termini (Fig. 2), which may be involved in the recognition of the peptide ligand (16). Although there were significant differences in the amino- and carboxyl-terminal regions, HSR and RSR were highly homologous within the region of the seven transmembrane domains (Fig. 2).

Befitting the classical role of secretin in stimulating secretion from the pancreas, small intestine, and liver, the 2.1-kb HSR transcript was detected in these tissues, with the highest levels of expression being found in the pancreas (Fig. 3). In pancreas, lung, and kidney tissues, low levels of a 3-kb transcript were also detected (Fig. 3). This may represent cross-hybridization to a related receptor gene or to alternatively spliced transcripts or RNA-splicing intermediates of HSR. The pattern of tissue expression observed for HSR differed slightly from that reported for RSR, perhaps reflecting species differences in tissue sublocalization of expression. Whereas HSR was well represented in the kidney, moderately expressed in the lung, and weakly expressed in the heart (Fig. 3), RSR was reported to be prominently expressed in rat heart and was not detected in kidney or lung tissues (16).

Stably expressed HSR of 293S12 cells bound ^{125}I-secretin with high affinity and specificity (Fig. 4). In contrast, untransfected HEK293 cells showed no discernible binding affinity for secretin (data not shown). VIP, PACAP-38, and glucagon weakly interacted with HSR, being about 3 orders of magnitude less potent than secretin in displacing ^{125}I-secretin bound by 293S12 cells (Fig. 4), which was similar to the results reported for COS cells transiently expressing RSR (16). Conversely, secretin was 4 orders of magnitude less effective than VIP in displacing ^{125}I-VIP bound to 293 cells stably expressing HVR1 (data not shown), which are of the type I VIP receptor subtype. It has been reported, however, that secretin does not interact with the type II VIP receptor subtype (18), indicating a useful pharmacological distinction between the two VIP receptor subtypes. The availability of cloned HSR and type I VIP receptors will now enable the construction of chimeric receptors to delineate receptor structural elements that discriminate between VIP and secretin.

Activation of HSR on 293S12 cells led to the concurrent production of two intracellular signals, i.e., increases in $[cAMP]_i$ and $[Ca^{2+}]_i$ (Figs. 5–7), possibly mediated through specific G proteins. Multiple signaling pathways were also reported for several members of the secretin receptor subfamily. For example, recombinant HVR1 stably expressed by HEK293 cells, as well as native HVR1 of human colonic adenocarcinoma HT-29 cells, couple to concurrent increases in both $[cAMP]_i$ and $[Ca^{2+}]_i$ (29), as do type I PACAP receptors and receptors for glucagon, parathyroid hormone, and calcitonin (22–26).

Although the EC_{50} for secretin effects on cAMP production was virtually identical to the IC_{50} for secretin effects on ^{125}I-secretin binding, the EC_{50} values for VIP and PACAP-38 effects on cAMP production were 40-fold lower than their respective IC_{50} values for binding. The influx of calcium from intracellular stores elicited by secretin was probably the result of a parallel increase in phospholipase C_β-mediated PI hydrolysis (Figs. 8 and 9). The EC_{50} value for secretin in cAMP production was, however, 1 order of magnitude lower

than the EC_{50} for secretin in inositol phosphate production. It is currently unclear what physiological receptor functions are manifested by these distinct intracellular signals.

In conclusion, we have cloned a high affinity secretin receptor from human lung tissue. Stable transfectants expressing the secretin receptor demonstrate specific binding of ^{125}I-secretin, resulting in increases in $[cAMP]_i$, $[Ca^{2+}]_i$, and PI hydrolysis. Identification of transcripts encoding the HSR in human lung tissue will now open new areas of investigation into the role and function of secretin in human lung physiology.

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