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Real-Time Evaluation of Human Secretin Receptor Activity Using Cytosensor Microphysiometry

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Abstract Human secretin receptor is a G protein-coupled receptor that is functionally linked to the cAMP second messenger system by stimulation of adenylate cyclase. To functionally characterize the receptor and evaluate its signal transduction pathway, the full-length human secretin receptor cDNA was subcloned into the mammalian expression vector pRc/CMV and expressed in cultured CHO cells. Intracellular cAMP accumulation of the stably transfected cells was measured by a radioimmunoassay (RIA), while the extracellular acidification rate was measured by the Cytosensor microphysiometer. Human secretin and biotinylated human secretin were equipotent in both assays in a dose-dependent manner. The EC_{50} values of stimulating the intracellular cAMP accumulation and the extracellular acidification rate were 0.2–0.5 nM and 0.1 nM, respectively, indicating that microphysiometry is more sensitive than the cAMP assay in monitoring ligand stimulation of the human secretin receptor. The secretin-stimulated response could be mimicked by forskolin and augmented by the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine, indicating that the extracellular acidification response is positively correlated with intracellular cAMP level. The response could be abolished by the protein kinase A inhibitor H-89, suggesting that protein kinase A plays an essential role in the intracellular signaling of the receptor. Upon repeated stimulation by the ligand, the peak acidification responses did not change significantly at both physiological (0.03 nM and 3 nM) and pharmacological (0.3 μM) concentrations of human secretin, suggesting that the human secretin receptor did not exhibit robust homologous desensitization. J. Cell. Biochem. 72:517–527, 1999.

Key words: human secretin receptor; cAMP; Cytosensor microphysiometry

Secretin, discovered by Bayliss and Starling in 1902, is a 27-amino acid peptide hormone. It is synthesized and released from the endocrine S cells located in the mucosa of the upper small intestine in numerous mammalian species [Straus and Yalow, 1978]. The principal function of secretin is to stimulate the secretion of bicarbonate, water, enzymes, and electrolytes from the exocrine pancreas. The released bicarbonate is important for neutralizing the acidic chyme from the stomach, thus providing an optimum pH for the normal functioning of various digestive enzymes in the small intestine. Secretin mRNA transcripts have been reported to be widely distributed in various regions of the rat brain, suggesting that it may also function as a neurotransmitter or neuromodulator [Fremeau et al., 1983; Kopin et al., 1990; Itoh et al., 1991; McKeon and Zigmond, 1993]. Other physiological actions of secretin include inhibition of gastrin secretion, inhibition of gastric acid release, inhibition of gastric emptying and stimulation of bile secretion [Valenzuela and Defilippi, 1981; Kleibeuker et al., 1984; Walsh, 1987; You and Chey, 1987; Jin et al., 1994].

The human secretin receptor cDNA has recently been cloned and characterized [Chow, 1995; Jiang and Ulrich, 1995; Patel et al., 1995]. Human secretin receptor is a 440-amino acid polypeptide with a predicted molecular weight of 50 kD [Chow, 1995]. It is a member of the glucagon-VIP-secretin receptor family with

Abbreviations used: BSA, bovine serum albumin; CHO, Chinese hamster ovary; FBS, fetal bovine serum; FITC, fluorescein isothiocyanate; GHRH, growth hormone releasing hormone; GLP-1, glucagon-like peptide 1; IBMX, 3-isobutyl-1-methylxanthine; MEM, minimum essential medium; PACAP, pituitary adenylate cyclase activating peptide; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; PHI, peptide histidine isoleucine; RIA, radioimmunoassay; RT, reverse transcription; VIP, vasoactive intestinal peptide.

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seven transmembrane domains characteristic of G protein-coupled receptors. Northern blot analysis of various human tissues indicated that the receptor is expressed in the pancreas, small intestine, kidney, colon, and lung [Chow, 1995; Patel et al., 1995]. Activation of the human secretin receptor is functionally coupled to the cAMP second messenger system via the stimulation of adenylate cyclase. Other intracellular second messengers such as Ca^{2+} and inositol-1,4,5-trisphosphate have also been reported [Patel et al., 1995].

To functionally characterize the receptor and evaluate its signal transduction pathway, the full-length human secretin receptor cDNA was subcloned into the mammalian expression vector pRc/CMV and expressed in cultured Chinese hamster ovary (CHO) cells. The extracellular acidification rates of the stably transfected cells were measured by the Cytosensor microphysiometer system. The system specifically measures the extracellular acidification rate of cells by means of a silicon-based sensor chip device [Parce et al., 1989; McConnell et al., 1992]. During cellular metabolism, ATP is generated to meet the energy demand. At the same time, the cells will continually excrete metabolic wastes, primarily in the form of CO_2 and lactic acid. Since both CO_2 and lactic acid are acidic, the cells will acidify their immediate environment at a rate directly proportional to their metabolic rates. Therefore, any event which increases the cellular ATP usage, such as receptor activation and initiation of signal transduction, will increase the metabolic rate of the cells and hence the rate of CO_2 and lactic acid excretion. As a result, the extracellular acidification rate will increase which can be readily detected by the silicon sensor chip within the sensor chambers of the Cytosensor microphysiometer.

In this report, we describe the functional expression of the human secretin receptor in CHO cells and the subsequent characterization of receptor activation by extracellular acidification measurement and intracellular cAMP accumulation. This is the first report of a real-time monitoring of human secretin receptor activity by means of microphysiometry. This receptor assay tool also represents a good alternative for traditional assay systems and is useful in quantifying human secretin receptor activation and in dissecting the signal transduction pathway of the receptor.

MATERIALS AND METHODS

Cell Culture and Transfection

CHO-K1 (ATCC, Rockville, MD) cells were cultured in minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS) and antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin). Two kinds of plasmid vectors were used in the transfection studies, the mammalian expression vector pRc/CMV (Invitrogen, Leek, The Netherlands) and the constructed pRc/CMV-HSR containing the full-length human secretin receptor cDNA (Fig. 1). The cDNA insert contains 109 bp of the 5′-untranslated region, 1320 bp of the entire coding region of the human secretin receptor, and 288 bp of the 3′-untranslated region [Chow, 1995]. Transfection of CHO cells was performed using Lipofectin reagent (Gibco-BRL, Gaithersburg, MD) according to the recommended procedure of the manufacturer. A stable cell line expressing the human secretin receptor (CHO-HSR) was established by selection in the presence of 500 µg/ml G418 (Gibco-BRL) in the growth medium for 2–3 weeks. Another batch of CHO cells was also transfected with the pRc/CMV vector alone. G418-resistant cells from

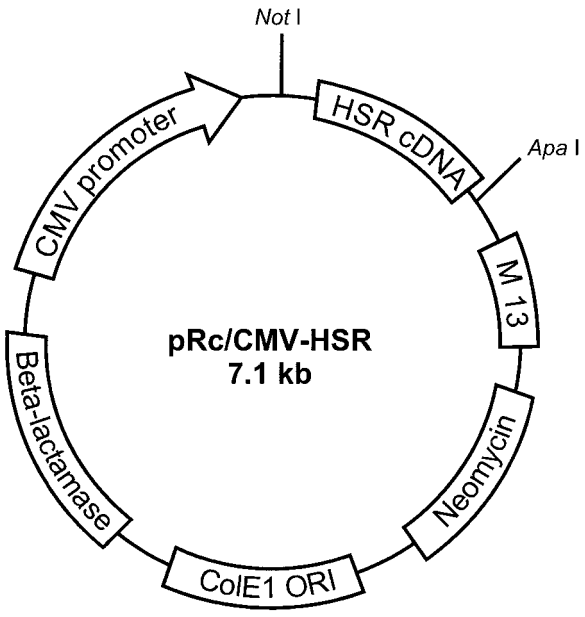

Fig. 1. pRc/CMV-HSR, the 1.7-kb full-length human secretin receptor (HSR) cDNA was subcloned into the *Not*I and *Apa*I sites of the mammalian expression vector.

this transfection were maintained as a control cell line.

Reverse Transcription-Polymerase Chain Reaction

Total RNA was isolated from the transfected CHO cells by the guanidinium thiocyanate/phenol extraction method [Chomczynski and Sacchi, 1987], followed by treatment with DNase I (Gibco-BRL) to eliminate residual genomic DNA. Poly(A)$^+$ mRNA was extracted from the total RNA by PolyATtract mRNA isolation system (Promega, Madison, WI). First-strand cDNA was synthesized using MMLV reverse transcriptase (Gibco-BRL) and random hexamer as primer. PCR was carried out using gene-specific primers designed according to the published cDNA sequence [Chow, 1995]. The sequences of the primers are HSR5′ (CAGGATCCATGCGTCCCCACCTGTCGCCGCCG) and HSR3′ (CAGAATTCTTACAGCTTCAGCAGGTAGGAGTG). Each 50-µl reaction contained 50 pmole of each primer, 0.2 mM dNTPs, and 2.5 U *Taq* polymerase (Gibco-BRL). The reaction was performed with an initial denaturation of 5 min at 94°C, followed by 30 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C. The PCR product was fractionated on a 0.8% ethidium bromide stained agarose gel and visualized by an ultraviolet (UV) transilluminator. The expected size of the human secretin receptor PCR product is 445 bp. RT-PCR of β-actin was also carried out in parallel as a control.

Measurement of Intracellular cAMP Production

A total of 200,000 CHO-HSR cells were seeded onto each well of 6-well plates (Costar, Cambridge, MA) 2 days before the cAMP assays. The cells were washed once with MEM containing bovine serum albumin (BSA) at 1 mg/ml. The washed cells were incubated with the same medium containing 0.2 mM 3-isobutyl-l-methylxanthine (IBMX) (RBI, Natick, MA) for 30 min at 37°C and then exposed to different concentrations of human secretin (Bachem, Bubendorf, Switzerland) or biotinylated human secretin (Neosystem, Strasbourg, France) for 45 min at 37°C. After incubation, the medium was removed, and the cells were lysed by the addition of 1 ml ice-cold ethanol. The cell debris was pelleted by centrifugation at 14,000 rpm for 10 min, and the supernatant was dried on a vacuum concentrator. The cAMP levels were measured by a radioimmunoassay (RIA) kit (Amersham, Buckinghamshire, UK) according to the procedure as specified by the manufacturer.

Measurement of the Extracellular Acidification Rate

Extracellular acidification rates were measured using the Cytosensor microphysiometer (Molecular Devices, Sunnyvale, CA). CHO-HSR cells were seeded into sterile 12-mm capsule cups (Molecular Devices) at 6×10^5 cells/capsule in MEM supplemented with 10% FBS and antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin). The cells were incubated at 37°C in 5% CO_2 for 18 h. A spacer and a capsule insert (Molecular Devices) were placed in each capsule cup to define the internal size of the capsule at 50 µM high and 6 mm in diameter and to trap the cells between two microporus polycarbonate membranes. The assembled capsule cups were installed into the sensor chambers of the Cytosensor. Bicarbonate free MEM containing 1 mg/ml BSA was used as a running medium in the Cytosensor. The running medium was pumped across the cells at a rate of 100 µl/min, and the extracellular pH was continuously monitored during each 90-s pump interval. To measure the acidification rate, the pumps were momentarily stopped for 20 s, and the rate of excretion of acidic metabolites from the cells (extracellular acidification rate) was measured by the instrument. After the establishment of a stable basal acidification rate, diluted peptides or drugs in the running medium were delivered to the sensor chambers via a second fluid path. Peak acidification response was achieved at approximately 10 min. The basal acidification rates from different sensor chambers were normalized to 100%, and the peak acidification responses were expressed as a percentage of the basal value before stimulation.

Confocal Microscopy of CHO-HSR Cells

Cell surface receptors, after binding with the biotinylated ligand, could be visualized by fluorescein isothiocyanate (FITC)-conjugated streptavidin [Skulstad et al., 1995]. A total of 200,000 CHO-HSR cells and vector transfected control CHO cells were seeded separately into P35G-01 capsules (MatTet, Ashland, MA) 2 days before the experiment. The cells were washed once with MEM containing 1 mg/ml BSA. The washed cells were incubated with the same

medium containing 50 nM biotinylated human secretin for 1 h at 4°C. After incubation, the medium was removed and the cells were washed twice with 2 ml phosphate-buffered saline (PBS). The washed cells were fixed with 2% paraformaldehyde in PBS for 10 min. The cells were washed twice with PBS and incubated with PBS containing 1 mg/ml BSA for 1 h at room temperature and then exposed to a 1:40 diluted FITC-conjugated streptavidin reagent (Pierce, Rockford, IL). The cells were observed under a confocal laser microscope MRC 600 (BioRad, Richmond, CA) at an excitation wavelength of 488 nm. Pictures were taken with a 35 mm camera using Kodak 35 mm T-MAX film.

RESULTS AND DISCUSSION

Expression of the Human Secretin Receptor in CHO Cells

To functionally characterize the human secretin receptor, a stable cell line expressing the human secretin receptor was established. This was done by transfecting CHO cells with the expression vector pRc/CMV carrying the full-length human secretin receptor cDNA (Fig. 1). CHO cells were chosen because they have been widely used as a heterologous expression system for studying G protein-coupled receptors by means of Cytosensor microphysiometry, including adrenergic [Owicki et al., 1990], dopaminergic [Chio et al., 1994], and muscarinic [Baxter et al., 1994] receptors. Expression of the human secretin receptor in the transfected CHO cells was confirmed by RT-PCR (Fig. 2). A prominent band of 445 bp representing the RT-PCR product of the human secretin receptor was detected in CHO-HSR cells, but not in the control CHO cells. RT-PCR of β-actin was also performed in parallel and positive signals were detected in both cell lines. The results indicate that the human secretin receptor is expressed in CHO-HSR cells, but not in the control cells.

Secretin Stimulates cAMP Accumulation in CHO-HSR Cells

In order to demonstrate that the expressed human secretin receptor in CHO cells could initiate signal transduction, intracellular cAMP levels in response to increasing concentrations

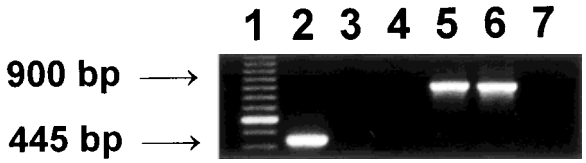

Fig. 2. Reverse transcription-polymerase chain reaction (RT-PCR) analysis of human secretin receptor expression in the stably transfected Chinese hamster ovary (CHO) cells. RT-PCR was performed as described under Materials and Methods. **Lane 1**, 100-bp DNA size marker; **lane 2**, RT-PCR performed on CHO-HSR cells; **lane 3**, RT-PCR performed on the control CHO cells transfected with pRc/CMV vector alone; **lane 4**, negative control with no template; **lanes 5–7**, are the RT-PCR of β-actin on CHO-HSR cells, control CHO cells and no template negative control, respectively. The 445-bp fragment represents the human secretin receptor PCR product and the 900-bp fragment represents the β-actin PCR product.

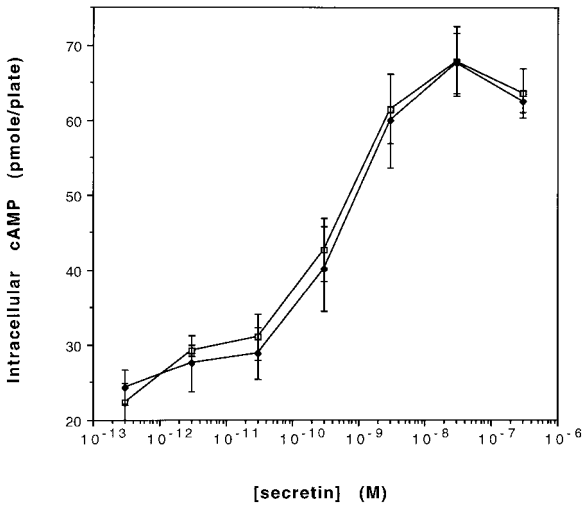

Fig. 3. Ligand stimulation of intracellular cAMP accumulation in Chinese hamster ovary (CHO)-HSR cells. CHO-HSR cells were incubated with various concentrations of human secretin (open symbol) and biotinylated human secretin (solid symbol). The assays were done in triplicates, and each point is the mean ±SD.

of human secretin and biotinylated human secretin were measured by RIA (Fig. 3). Both secretin and biotinylated secretin could stimulate intracellular accumulation of cAMP in CHO-HSR cells in a dose-dependent manner. The response peaked at 30 nM, and the EC_{50} was around 0.2–0.5 nM. These results indicate that the expressed human secretin receptor in CHO cells is functional and is coupled to the cAMP second messenger pathway. The results are similar to the cAMP assays performed on transfected COS-7 cells [Chow, 1995; Jiang and Ulrich, 1995].

Visualization of Expressed Human Secretin Receptors in CHO-HSR Cells

The biotinylated secretin used in this study has a biotin group linked to the C-terminus of the secretin molecule. Our results show that the biotinylated secretin is equipotent to secretin in stimulating cAMP production in CHO-HSR cells and is therefore a biologically active secretin analog. This secretin analogue is a very useful reagent for probing the human secretin receptor. We have demonstrated that the biotinylated secretin bound on the expressed human secretin receptors in CHO-HSR cells could be coupled to FITC-conjugated streptavidin and observed under a confocal laser microscope (Fig. 4). This microscopic study confirms that the human secretin receptor is expressed and correctly addressed at the cell surface of CHO-HSR cells. As a matter of fact, through coupling to streptavidin-linked antibodies or enzymes, the biotinylated secretin could be potentially useful for studying receptor–ligand interaction or for the histocytochemical investigation of the human secretin receptor.

Secretin Stimulates Extracellular Acidification of CHO-HSR Cells

The effects of human secretin and biotinylated human secretin on CHO-HSR cells were measured by the Cytosensor microphysiometer. Secretin could bring about an increase in the extracellular acidification rate in a dose-dependent manner (Fig. 5). Again, human secretin and biotinylated human secretin were equipotent in this respect. The response peaked at 3 nM, and the EC_{50} was found to be 0.1 nM. That

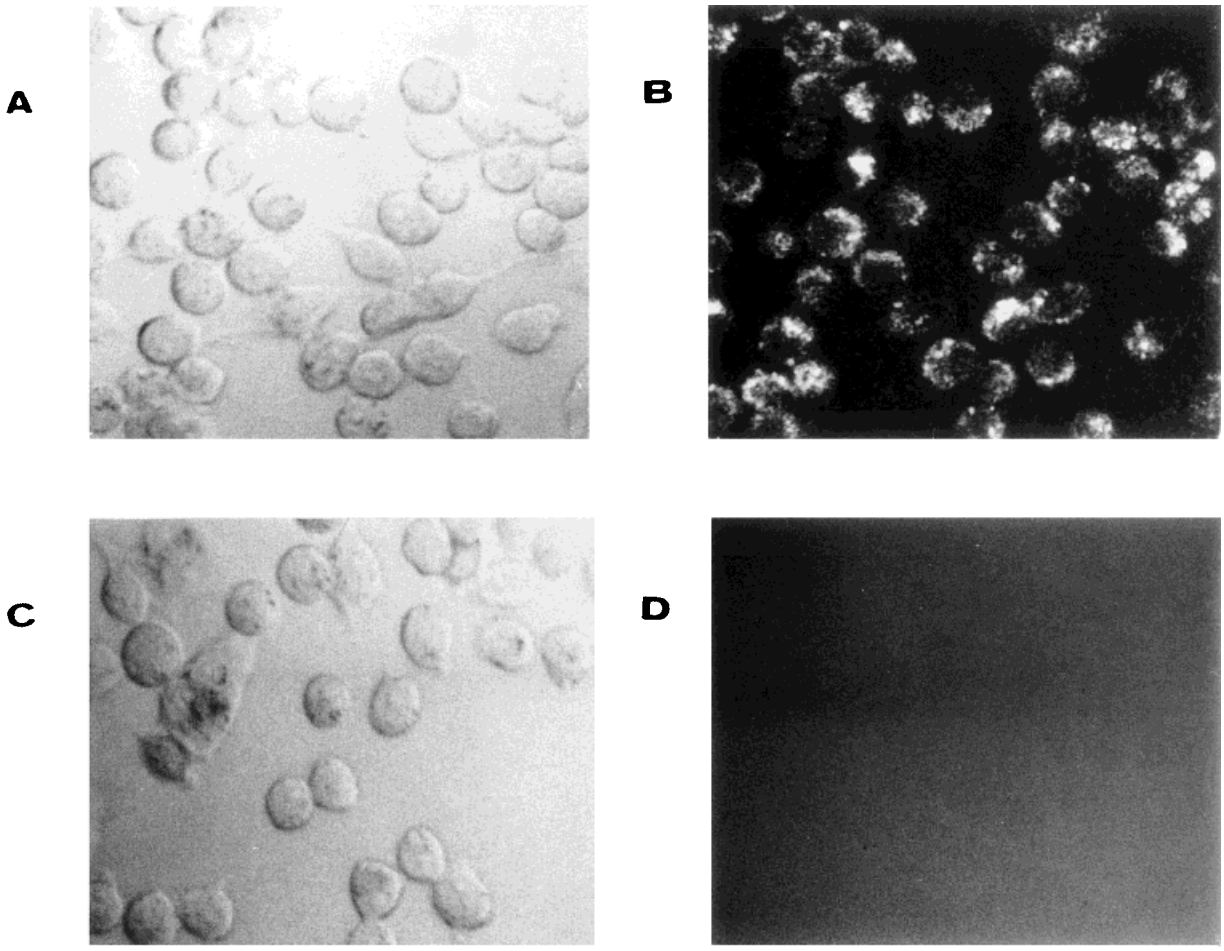

Fig. 4. Detection of human secretin receptor in Chinese hamster ovary (CHO)-HSR cells. The bound biotinylated secretin was coupled to FITC-conjugated streptavidin as described under Materials and Methods. **A,B:** CHO-HSR cells observed under a light microscope and a confocal laser microscope, respectively. **C,D:** Vector-transfected control cells observed under a light microscope and a confocal laser microscope, respectively.

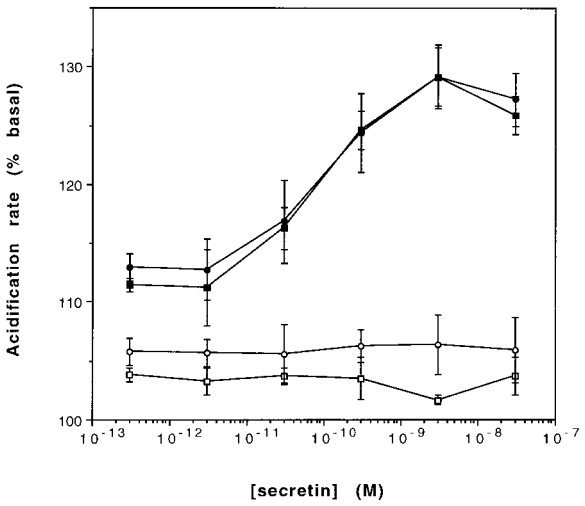

Fig. 5. Ligand stimulation of extracellular acidification in Chinese hamster ovary (CHO)-HSR cells. CHO-HSR cells (solid symbols) and control CHO cells (open symbols) were exposed to human secretin (square) and biotinylated human secretin (circle) at different concentrations as described under Materials and Methods. The peak response at each ligand concentration was expressed as a percentage of the basal acidification rate. The values are the means ±SD of the measured acidification rates from three sensor chambers.

is to say, both the concentrations of the ligand to reach maximal response and half-maximal response are smaller than that determined by the cAMP assays on transfected COS-7 cells [Chow, 1995; Jiang and Ulrich, 1995] as well as on CHO-HSR cells (Fig. 3). By contrast, no significant increase in acidification rate was detected in the control CHO cells challenged with human secretin or biotinylated human secretin. This is the first report that functionally expressed human secretin receptor in CHO cells could respond to ligand stimulation in increasing the rate of extracellular acidification, rendering the receptor amenable to real-time noninvasive monitoring by Cytosensor microphysiometry.

In order to show that the expressed human secretin receptor could in fact specifically interact with secretin, CHO-HSR cells were challenged with various secretin-related peptides including vasoactive intestinal peptide (VIP), pituitary adenylate cyclase activating peptide (PACAP), growth hormone-releasing hormone (GHRH), peptide histidine isoleucine (PHI), glucagon, and glucagon-like peptide 1 (GLP-1) (All peptides were from Bachem, Bubendorf, Switzerland). At 3 nM, only secretin could increase the extracellular acidification rate of CHO-HSR cells whereas no significant response was detected for all the other peptides, indicating that the expressed human secretin receptor could specifically interact with secretin at a physiological concentration (Fig. 6a). At a pharmacological concentration (0.3 μM), however, VIP, PACAP, and PHI could also stimulate an increase in acidification rate (Fig. 6b). The results may be explained by the fact that VIP, PACAP and PHI are structurally more similar to secretin, particularly at the N-terminal region [Chow, 1995]. Therefore, these peptides

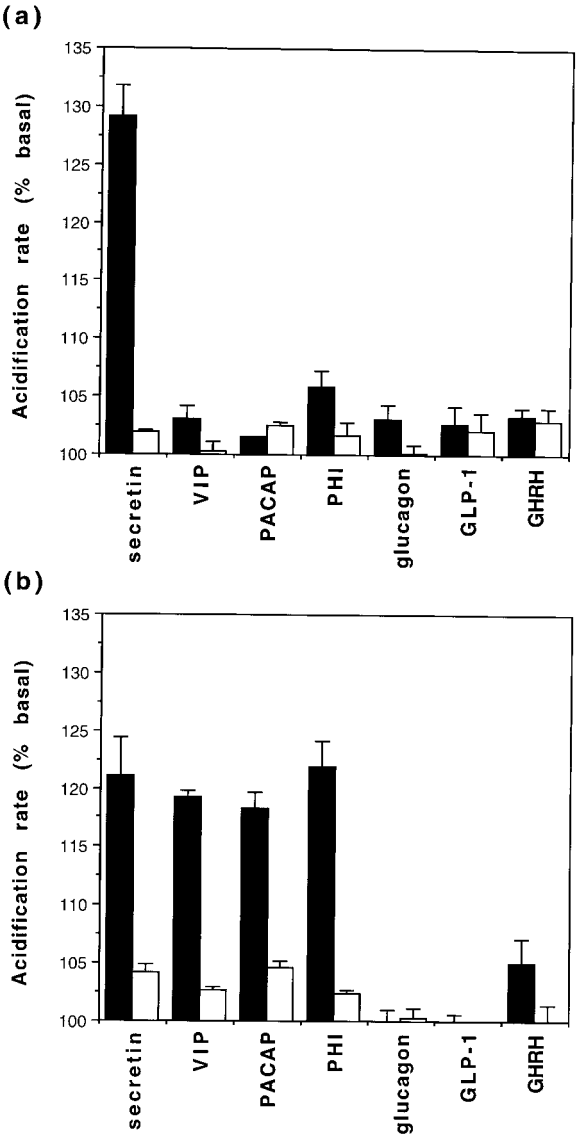

Fig. 6. Stimulation of extracellular acidification in Chinese hamster ovary (CHO)-HSR cells by peptide hormones. CHO-HSR cells (solid bars) and control CHO cells (open bars) were stimulated by **(a)** 3 nM and **(b)** 0.3 μM of the various human peptide hormones as shown. Values are the means ±SD from four sensor chambers.

may cross-react with the secretin receptor at pharmacological concentrations. On the other hand, the control CHO cells did not respond to the peptides (Fig. 6).

Characterization of the Human Secretin Receptor cAMP Second Messenger Pathway by the Cytosensor

To demonstrate that the activation of the cAMP second messenger pathway is positively correlated with the increase in the extracellular acidification rate of CHO-HSR cells, the cells were exposed to membrane-permeable drugs that increase the intracellular cAMP levels, including forskolin (Calbiochem, La Jolla, CA) [Laurenza et al., 1989] and IBMX [Turner et al., 1993]. As a cAMP mimetic, forskolin increased the acidification rate of CHO-HSR cells in a dose-dependent manner (Fig. 7a). IBMX, being a phosphodiesterase inhibitor, could also increase the acidification rate dose-dependently (Fig. 7b). CHO-HSR cells were also exposed to various concentrations of secretin in the presence of 10 μM IBMX (Fig. 8). IBMX was found to potentiate the effect of secretin, suggesting that an increase in the intracellular cAMP level could directly augment the secretin-stimulated acidification response. The potentiating effect of IBMX was more pronounced at a low concentration (0.03 nM) of secretin. At higher concentrations (0.3 nM and 3 nM) of secretin, the potentiating effect of IBMX was less pronounced because the cells were almost maximally stimulated. These results therefore provide evidence that the secretin-stimulated acidification response of CHO-HSR cells is mediated through the activation of the cAMP second messenger pathway.

As an initial step to understand the signal transduction pathway of the human secretin receptor, CHO-HSR cells were challenged with various concentrations of secretin in the presence of 25 μM H-89 (Calbiochem, La Jolla, CA), a pharmacological agent that blocks the cAMP pathway by inhibiting protein kinase A [Chijiwa et al., 1990]. It was found that H-89 dramatically reduced the secretin-stimulated acidification response of CHO-HSR cells (Fig. 9). Therefore, protein kinase A of the cAMP signal transduction cascade is believed to be an essential component of the acidification response in CHO-HSR cells. Since the response of the cells can be continuously and quantitatively monitored in a noninvasive manner, the present system opens up the potential of further dissecting the intracellular signaling mechanisms of the human secretin receptor by the judicious deployment of suitable pharmacological agents.

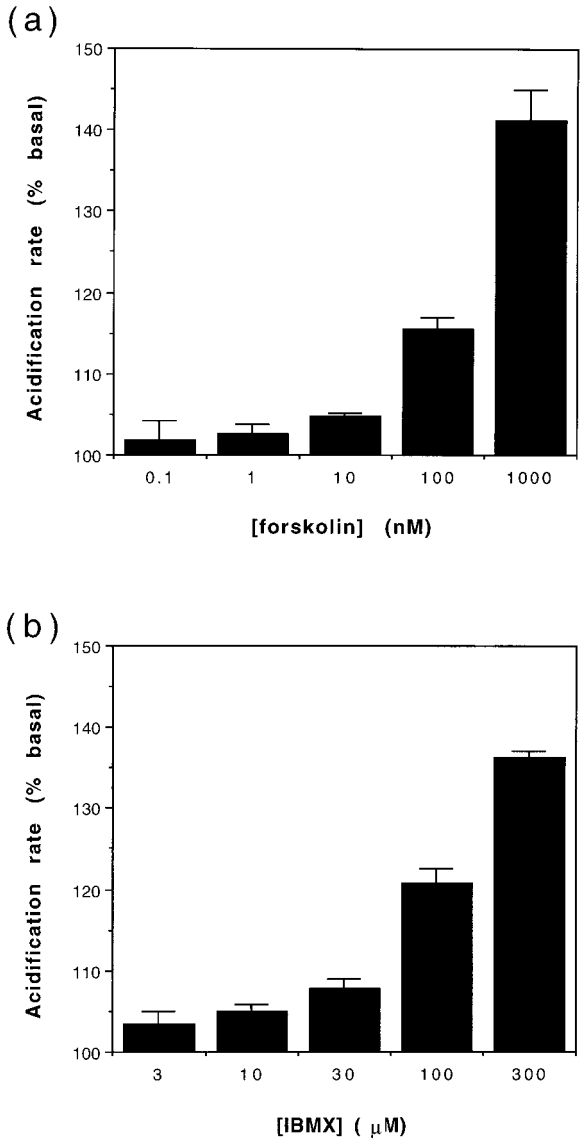

Fig. 7. Stimulation of extracellular acidification in Chinese hamster ovary (CHO)-HSR cells by **(a)** forskolin and **(b)** IBMX. Various concentrations of the drugs were used and the peak responses at 10 min were taken. Values shown are the means ±SD from four sensor chambers.

Desensitization Studies on the Human Secretin Receptor

The interaction of cell surface receptors with ligands could be accompanied by a brief or prolonged refractory period during which the receptors are not responsive to extracellular

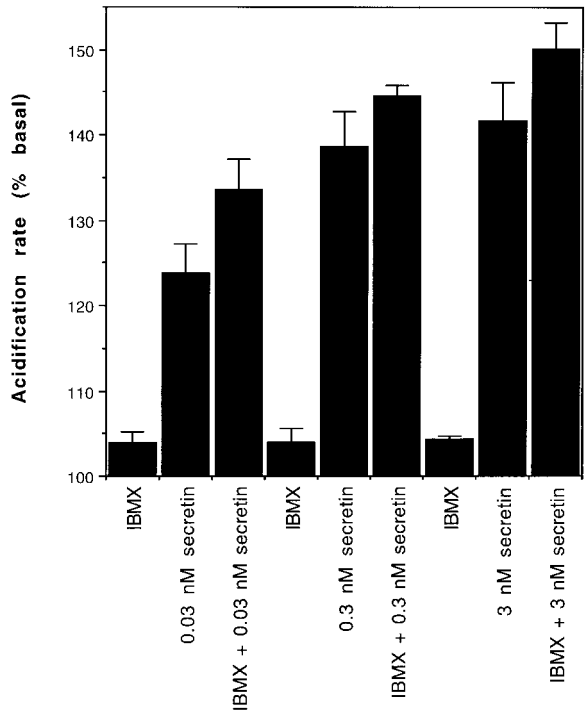

Fig. 8. IBMX potentiation of secretin-stimulated extracellular acidification in Chinese hamster ovary (CHO)-HSR cells. Three different concentrations (0.03, 0.3, and 3 nM) of human secretin were used. IBMX, when used alone or in conjunction with secretin, was at 10 μM. Values are the means ±SD from four sensor chambers.

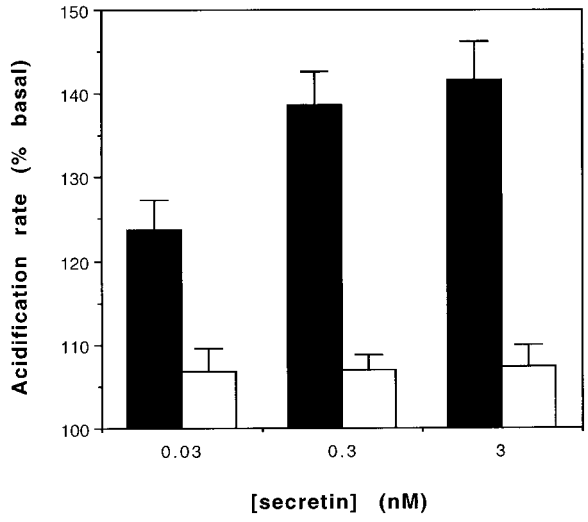

Fig. 9. H-89 blockade of secretin-stimulated extracellular acidification in Chinese hamster ovary (CHO)-HSR cells. CHO-HSR cells were pretreated with 25 μM H-89 for 45 min (open bars). Controls without H-89 were performed in parallel (solid bars). After the establishment of a steady basal acidification rate, the cells were challenged with different concentrations of human secretin shown. Values are the means ±SD from four sensor chambers.

stimuli, a phenomenon known as receptor desensitization. In previous desensitization studies on the rat secretin receptor, it was found that after pretreating CHO cells bearing the wild-type rat secretin receptor with 10 nM secretin for 5 min or 12 h before washing, restimulation with secretin failed to elicit any cAMP response, an observation that was explained by receptor internalization [Holtmann et al., 1996]. However, a recent report demonstrated that coexpression of G protein-coupled receptor kinases in human embryonic kidney cells expressing the rat secretin receptor could lead to a 40% decrease in the maximal cAMP response to secretin, suggesting that receptor phosphorylation was the major desensitization mechanism [Shetzline et al., 1998]. Although secretin receptor desensitization has been studied in rat, there is still no information on whether the same occurs for the human secretin receptor. The present study attempts to address this issue by means of Cytosensor microphysiometry. The advantage of the system is that the cellular response to ligand stimulation can be promptly registered and continuously monitored in real-time. To demonstrate whether homologous desensitization exists for the human secretin receptor, CHO-HSR cells were continuously exposed to 0.1 mM secretin for 30 min. The acidification responses of the cells increased dramatically at first and then leveled off after about 20 min (Fig. 10). This profile is similar to the time-dependent cAMP accumulation profile reported in previous studies on the rat secretin receptor upon continuous stimulation by the ligand [Holtmann et al., 1996; Shetzline et al., 1998], further substantiating the conclusion that the acidification response is dependent on intracellular cAMP level. Shetzline et al. [1998] interpreted this profile as a rapid desensitization process in that the rate of the cAMP accumulation decreased with time and no further increases in cAMP accumulation occurred after 20 min. In this respect, our data might also suggest the possible occurrence of acute receptor desensitization that acts to limit the maximal acidification response of the cells.

However, when CHO-HSR cells were challenged by successive exposures to human secretin for 9 min at three different concentrations, viz. 0.03, 3, and 300 nM, the peak responses of the cells did not change significantly from the first to the third exposure, suggesting that the

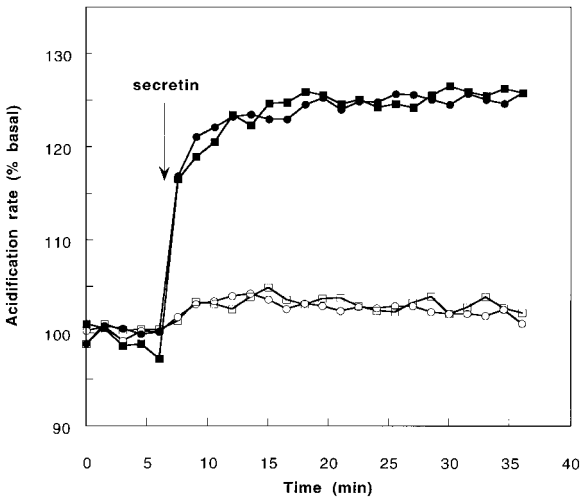

Fig. 10. Continuous ligand stimulation of extracellular acidification in Chinese hamster ovary (CHO)-HSR cells. CHO-HSR cells (solid symbols) and control CHO cells (open symbols) were continuously exposed to 0.1 μM human secretin for 30 min. Arrow, time of switching the fluid path to the secretin-containing medium. Tracings from four independent sensor chambers are shown.

human secretin receptor may not exhibit robust homologous desensitization (Fig. 11). It appears that the desensitization event, if present, could be reversed by washing off the ligand since restimulation with successive doses of secretin could still elicit acidification responses of similar magnitude and kinetics. We postulate that the possible desensitization of the human secretin receptor may be followed by a rapid resensitization process. During the washing periods, it can be observed that the acidification rate of the cells gradually returned to basal, suggesting that the cells may have recovered from the effect of ligand stimulation and become resensitized.

Our observation is at variance with a previous report on the rat secretin receptor [Holtmann et al., 1996] in that the receptor was unable to respond to subsequent restimulation by the ligand in terms of cAMP accumulation. Whether this apparent discrepancy is due to an inherent difference between human and rat secretin receptors remains to be investigated. It could also be argued that the discrepancy may be attributed to the fact that rather different aspects of post-receptor events were measured in these studies. Intracellular cAMP accumulation is an early event in the signaling cascade. On the other hand, extracellular acidification rate reflects the summed effect of various signaling pathways or metabolic events that are stimulated by the ligand. The Cytosensor therefore monitors a downstream effect of the ligand. However, our data indicate that the Cytosensor response of the human secretin receptor is tightly coupled to the cAMP pathway in that any pharmacological manipulation to alter the cell's cAMP status or its downstream signaling would result in corresponding changes in the extracellular acidification rate (see Figs. 7–9). Thus, the acidification responses shown in Figures 10 and 11 should indicate the extent of cAMP accumulation and the effect of desensitization, if any, that occurred during secretin stimulation. In this regard, our data would seem to lend support to the notion of a lack of robust homologous desensitization for the human secretin receptor.

It should be pointed out at this juncture that Cytosensor microphysiometry has been successfully applied to desensitization studies of a large variety of different receptor types. Among these studies, some receptors showed homologous desensitization, e.g. kappa opioid receptor [Ling et al., 1998], opioid receptor-like receptor 1 [Pei et al., 1997], neurotensin receptors and purinoceptors [Richards et al., 1997], and parathyroid hormone receptor [Barret et al., 1997]. After washing off the ligand, these receptors gave much diminished Cytosensor responses upon subsequent exposure to the same ligand. On the other hand, other receptors did not show such desensitization, e.g. $GABA_A$ receptor [Brown et al., 1997] and CCK_B receptor [Denyer et al., 1994]. For these receptors, subsequent repetitive exposure after washing off the ligand in between did not give rise to reduced responses. In our hands, the behavior of the human secretin receptor is similar to those receptors that exhibited no desensitization. It appears that homologous desensitization does not occur in the human secretin receptor, or if it does at all, it is reversible upon removal of the ligand.

In conclusion, the human secretin receptor has been functionally expressed in CHO cells and characterized by cAMP assay and Cytosensor microphysiometry. The expressed receptor in CHO cells could specifically interact with secretin, leading to a dose-dependent increase in extracellular acidification. This cellular response was found to be mediated via the cAMP signal transduction cascade. Upon repeated stimulation by the ligand, the human secretin receptor did not exhibit homologous desensitiza-

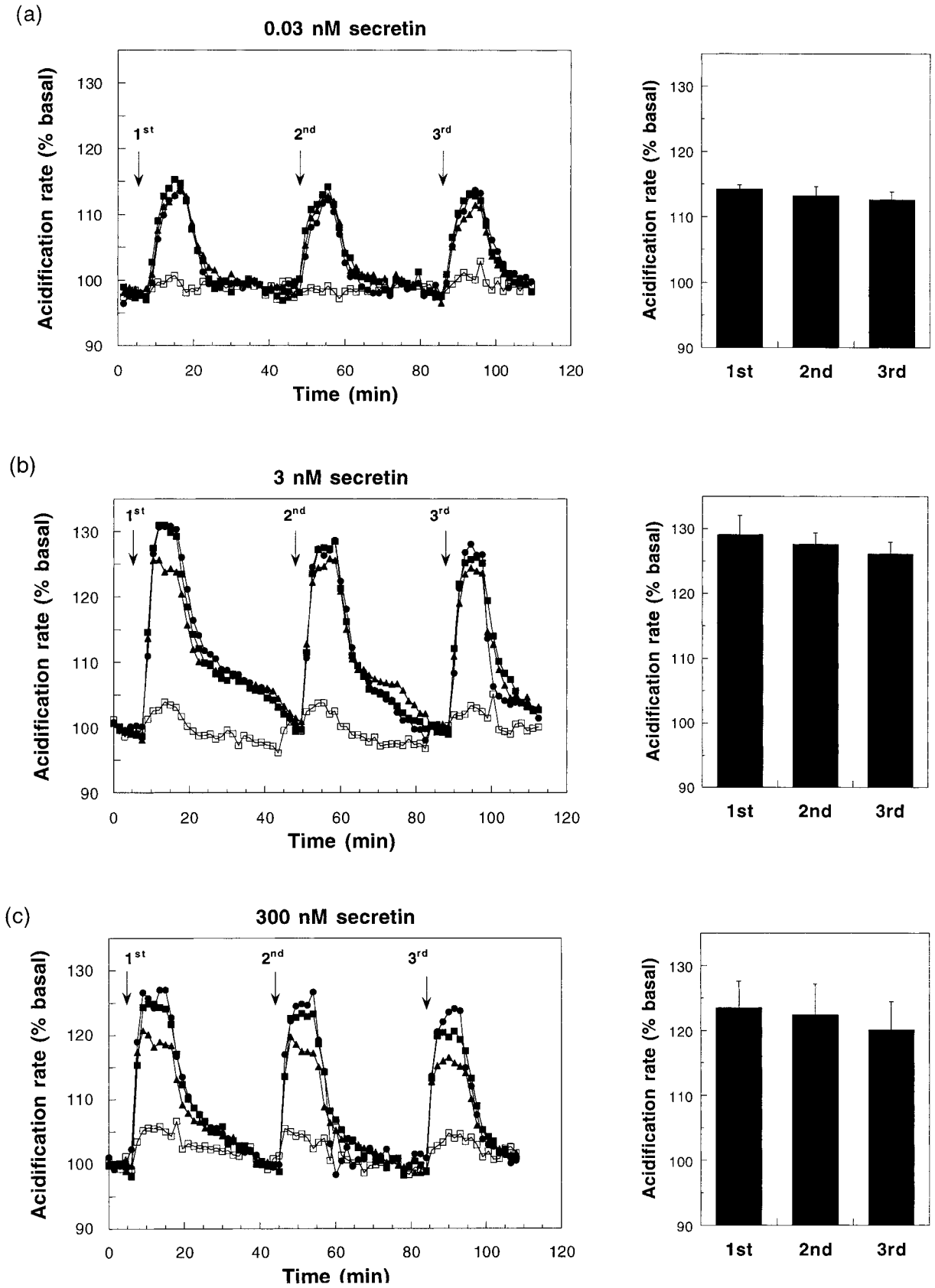

Fig. 11. Repeated ligand stimulation of extracellular acidification in Chinese hamster ovary (CHO)-HSR cells. CHO-HSR cells (solid symbols) and CHO cells (open symbol) were stimulated by three successive exposures to human secretin for 9 min at **(a)** 0.03 nM, **(b)** 3 nM, and **(c)** 300 nM, respectively. Between successive exposures to the ligand, the cells were washed with the running medium without secretin for 30 min. Restimulation with secretin was instituted only when the acidification rates returned to basal. Left, real-time signals from four independent sensor chambers. Arrows, time of switching the fluid path to the secretin-containing medium and the adjacent bar charts on the right show the peak response of the first, second, and the third exposure to secretin. Values are the means ±SD from three sensor chambers.

tion. Compared with the cAMP assay, our results suggest that microphysiometry is a more sensitive method with which to detect the ligand-stimulated response of the human secretin receptor and is a rapid *in vitro* bioassay to evaluate human secretin receptor physiology and its signal transduction pathway.

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