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SOMATOSTATIN (SSTR2) RECEPTORS MEDIATE PHOSPHOLIPASE C-INDEPENDENT Ca^{2+} MOBILIZATION IN RAT AR42J PANCREAS CELLS

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Rat AR42J pancreas cells, which express somatostatin-SSTR2 type receptors, responded to SSTR2-selective somatostatin (SRIF) agonist ligands with a dose-dependent increase in intracellular Ca^{2+}. In addition to SRIF-14 and SRIF-28, the most potent SRIF peptides were the cyclic octapeptides, BIM-23014C, BIM-23023, SMS 201-995, and the cyclic hexapeptides, MK-678 and BIM-23027. The SSTR3 and SSTR5-selective ligands, BIM-23056 and BIM-23052, were inactive and weakly active, respectively. None of the SRIF peptides stimulated inositol phosphate turnover, indicating that Ca^{2+} mobilization was independent of phospholipase C activation. Incubation in calcium-free medium abolished the increase in intracellular Ca^{2+}. These results indicate that activation of SSTR2 receptors in AR42J cells opens cell-surface calcium channels.

Somatostatin (SRIF) peptides have been shown to be coupled to a variety of G-protein-coupled second messenger systems in native and receptor-transfected cell types. The reported actions include adenylate cyclase inhibition (1-8), antagonism of voltage-dependent calcium channels (9-12) and the Na^+-H^+ antiport (13), K^+ channel activation (9), and tyrosine phosphatase stimulation (14). Subsequent studies have also demonstrated phospholipase C activation coupled to intracellular calcium mobilization (15-17), and phospholipase A2 stimulation (18,19). The present report uniquely demonstrates that activation of native SSTR2 receptors on cultured rat AR42J pancreas cells results in a mobilization of intracellular Ca^{2+} which is phospholipase C-independent, and dependent on extracellular Ca^{2+}.

METHODS

In Vitro Calcium Mobilization Assay: Rat AR42J cells were cultured in DMEM medium containing 10% fetal bovine serum in an atmosphere of 5% CO_2 and 95% air at 37 ° C. After 5 days of culture, the cells were harvested by incubating in a 0.3% EDTA/phosphate buffered saline solution (25° C) and washed twice by centrifugation. The washed cells were resuspended in Hank's - buffered saline solution (HBSS) for loading of the fluorescent Ca^{2+} indicator Fura-2AM. Cell suspensions of approximately 10^6 cells/ml were incubated with 2 µM Fura-2AM for 30 min at 25 ° C. Unloaded Fura-2AM was removed by centrifugation (twice) in HBSS, and final cell suspensions were transferred to a spectrofluorometer (Hitachi F-2000) equipped with a magnetic stirring mechanism and a temperature-regulated cuvette holder. After equilibration to 37 ° C, the SRIF peptides were added for measurement of Ca^{2+} mobilization. The excitation and emission wavelengths were 340 and 510 nm, respectively.

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Phosphoinositide Turnover: AR42J cells were harvested and resuspended in a phosphate-buffered saline solution contained 25 mM glucose and 75 mM sucrose (PBS+GS) and pre-incubated with 25 μCi/ml myo-[^{3}H]inositol for 60 min/37 °C. The cells were washed, resuspended in PBS+GS, and incubated with LiCl (100 mM) and SRIF peptides in a final volume of 0.30 ml. The reaction was terminated by the addition of chloroform/methanol (1:2), and the total [^{3}H]inositol phosphates were isolated as described (20).

SSTR2 Receptor Binding: Membranes for the SSTR2 receptor binding assay were obtained by homogenizing the cultured AR42J cells (Polytron, setting 6, 15 sec) in ice-cold 50 mM Tris-HCl and centrifuging twice at 39,000 x g (10 min), with an intermediate resuspension in fresh buffer. The final pellets were resuspended in 10 mM Tris-HCl for assay. For assay, aliquots of the membrane preparation were incubated for 90 min at 25 ° C with approximately 0.05 nM [^{125}I]MK-678 in 50 mM HEPES (pH 7.4) containing bovine serum albumin (10 mg/ml; fraction V, Sigma Chem.), $MgCl_2$ (5 mM), Trasylol (200 KIU/ml), bacitracin (0.02 mg/ml), and phenylmethylsulphonyl fluoride (0.02 mg/ml). The final assay volume was 0.3 ml. The incubations were terminated by rapid filtration through GF/C filters (pre-soaked in 0.3% polyethylenimine) using a Brandel filtration manifold. Each tube and filter were then washed three times with 5 ml aliquots of ice-cold buffer. Specific binding was defined as the total [^{125}I]MK-678 bound minus that bound in the presence of 200 nM MK-678.

RESULTS

An initial screen of rat AR42J acinar pancreas cells for neuropeptide (10 nM) stimulation of intracellular Ca^{2+} mobilization resulted in stimulation for bombesin (524 %), PACAP-38 (1000 %), for CCK-8 (1060 %), and substance P (540 %). In addition, somatostatin-14 (SRIF-14), which has been shown to both mobilize intracellular Ca^{2+} through a phospholipase C-dependent pathway in receptor-transfected cell lines (15-17), and function as a Ca^{2+} channel antagonist in other cellular systems (9-12), increased intracellular Ca^{2+} (280 %) in the AR42J cells. Since AR42J cells were known to be highly enriched in SSTR2 receptors (21-23), SRIF ligands of known selectivity were also examined for their effects. Figure 1 shows the dose response curves for SRIF-14, SRIF-28, and other SRIF peptides having specificity for SSTR2, SSTR3, and SSTR5, and Table I lists the calculated EC_{50} values in comparison with SSTR2 binding inhibition constants (Ki). The rank order of potency for Ca^{2+} mobilization for the SRIF analogues was similar to that observed for the *in vitro* inhibition of [^{125}I]MK-678 binding and highly-indicative of SSTR2 specificity (21, 24): BIM-23023 = MK-678 > SMS 201-995 > BIM-23014C. BIM-23052 and BIM-23056, previously shown to have some selectivity for the rat SSTR5 and SSTR3 receptors (24-25), respectively, were inactive or weakly active, up to a concentration of 100 nM. Interestingly, the maximum response for the cyclic octapeptide and hexapeptide SRIF ligands was consistently approximately 80% of that obtained with the natural ligands, and the linear peptides, BIM-23052 and BIM-23056, exhibited very weak responses in spite of moderate binding affinity.

In view of other studies demonstrating that host cells, when transfected with the human SSTR's 1-5, couple to the activation of phospholipase-C (PLC) and Ca^{2+} mobilization (15-17), the AR42J cells we examined PLC activation by various SRIF peptides. In these experiments SRIF peptides were inactive (data not shown) in the range of 1 - 1000 nM as activators of phosphoinositide turnover, whereas control peptides

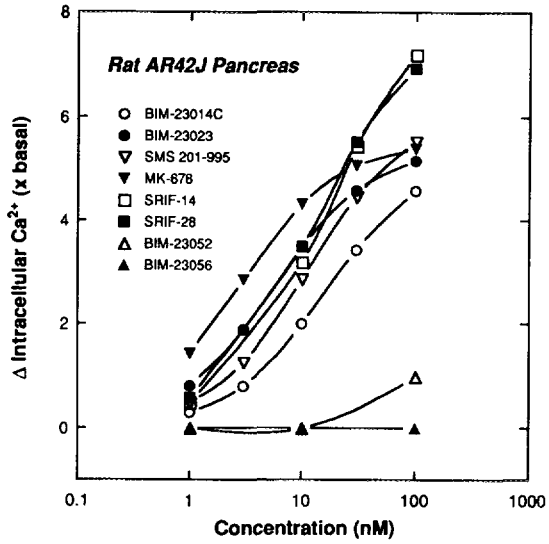

Fig. 1. Dose-response curves for the mobilization of intracellular Ca^{2+} in rat AR42J pancreas cells. Curves are the mean of four to eight independent determinations.

(CCK, 1750 %; bombesin, 850 %; substance P, 190 %; PACAP-38, 320 %) demonstrated a marked stimulation at a concentration of 10 nM.

In order to further characterize the mechanism for Ca^{2+} mobilization, the cells were incubated in Ca^{2+} - free buffer. As observed in Table II, the absence of extracelluar Ca^{2+}

Table I. Somatostatin Peptide Stimulation of Intracellular Calcium Mobilization in Rat AR42J Pancreas Cells

SRIF Peptide	EC_{50} (nM)[1]	SSTR2 (Ki, nM)[2]
SRIF-14	21.3 ± 5.8	0.16 ± 0.05
SRIF-28	27.1 ± 5.5	0.22 ± 0.05
BIM-23023 *D-Phe-cyclo[Cys-Tyr-D-Trp-Lys-Abu-Cys]-Thr-NH_2*	4.3 ± 0.8	0.15 ± 0.02
MK-678 *cyclo[D-Trp-Lys-Val-Phe-meAla-Tyr]-NH_2*	4.8 ± 2.3	0.16 ± 0.07
SMS 201-995 *D-Phe-cyclo[Cys-Phe-D-Trp-Lys-Thr-Cys]-Thr-ol*	12.2 ± 4.0	0.28 ± 0.02
BIM-23014C *D-Nal-cyclo[Cys-Tyr-D-Trp-Lys-Val-Cys]-Thr-NH_2*	19.0 ± 4.6	0.35 ± 0.09
BIM-23052 *D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH_2*	>1000	7.5 ± 3.2
BIM-23056 *D-Phe-Phe-Tyr-D-Trp-Lys-Val-Phe-D-Nal-NH_2*	>1000	251 ± 53

1 Mean (± SEM) of four to eight independent determinations.

2 *In vitro* inhibition of [^{125}I]MK-678 binding: Mean (± SEM) of five to 10 independent determinations.

Table II. Somatostatin Peptide Stimulation of Intracellular Calcium Mobilization in Rat AR42J Pancreas Cells: Extracellular Calcium Dependency

SRIF Peptide	Δ Calcium (nM)	
	(+) Calcium-Buffer	(-) Calcium Buffer
BIM-23014C		
1.0 nM	10	0
10 nM	74	0
30 nM	219	0
SMS 201-995		
1.0 nM	9	0
10 nM	67	0
30 nM	321	0

For the (-) calcium assay, AR42J cells were incubated as described in the Methods with HBSS, which was modified to contain 0 mM Ca^{2+}, 2.0 mM $MgCl_2$, and 1.0 mM EGTA.

in the incubation media completely blocked the mobilization action by the cyclic octapeptides, BIM-23014C and SMS 201-995.

DISCUSSION

In summary, this study demonstrates for the first time that SRIF peptides mobilize intracellular Ca^{2+} in cells expressing the native SSTR2 type. Several previous studies employing cloned SRIF receptors have indicated that SRIF peptides can mobilize Ca^{2+} intracellular stores through activation of PLC (15-17). However, the data with AR42J pancreas cells clearly show that SRIF peptide-mediated Ca^{2+} mobilization is PLC-independent, and unique, as demonstrated by a dependence on extracellular Ca^{2+} .

Although the AR42J cells express SSTR1 mRNA (22), these cells are extraordinarily enriched in SSTR2 mRNA and SSTR2 binding (21-23). It was not surprising, therefore, that the rank order of potency for Ca^{2+} mobilization was precisely that expected for an SSTR2-mediated response (21,24-25). Whether a similar activity is present in other tissues expressing the endogenous SSTR2 receptor remains to be established.

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