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Identification of lymphoid cell lines bearing receptors for somatostatin

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SUMMARY

The MT-2, derived from an adult T-cell leukaemia (ATL) cell, the Molt-4F, a human T-cell line, and the Isk, an EB virus-transformed B-cell line, were found to have high-affinity receptors for somatostatin, a cyclic tetradecapeptide that inhibits the release of substances such as growth hormone, TSH, glucagon, insulin, secretin, gastrin and cholecystokinin. The quantity of radioactivity bound varied linearly with the number of cells, and was displaced by non-radioactive somatostatin in a concentration-dependent manner. Specific binding of ^{125}I-somatostatin was time- and temperature-dependent and at 22° reached equilibrium within 120 min. Scatchard analysis demonstrated one class of specific-binding sites on MT-2 cells, Isk cells and Molt-4F cells that had respective densities and dissociation constants of 109 pM and 0·64 nM, 102 pM and 1·1 nM, and 5·8 pM and 0·22 nM.

INTRODUCTION

Many attempts have been made to determine the bidirectional effects of neuropeptides on the modulation of immunological responses (Bhathena *et al.*, 1981; O'Dorisio *et al.*, 1981; Mascards, Baiton & Sherline, 1984; Ottaway, 1984; Payan *et al.*, 1984b; Payan, Brewster & Goetzl, 1984a; Payan, Hess & Goetzl, 1984c; Payan & Goetzl, 1985; Goetzl *et al.*, 1985). The presence of receptors for substance P (Payan *et al.*, 1984b) and vasoactive intestinal peptide (VIP) (Payan *et al.*, 1984a) on lymphocytes has been reported. Both these compounds stimulate lymphocytes and enhance adenylate cyclase activity.

The tetradecapeptide, somatostatin, was found to exert an anti-proliferative action on activated rat thymocytes (Mascards *et al.*, 1984) and on the human T-cell line, Molt-4 (Payan *et al.*, 1984c). Somatostatin inhibits growth hormone and TSH secretion, and exerts inhibitory effects on cell secretion in the pancreas and gut. Somatostatin is present in the hypothalamus, cortex, midbrain, brain stem, spinal cord, sensory ganglia, delta cells of the islets of Langerhans and epithelium of stomach and intestine. These widespread distributions of somatostatin suggest a diversity of physical functions of this peptide, including immune regulation.

We report here evidence for the presence of certain lymphoid cell lines bearing receptors for somatostatin, and discuss the importance of immuno–neuroendocrine interactions, as related to systemic immunity.

Abbreviations: ATL, adult T-cell leukaemia; B max, maximum binding sites; Isk, EB virus-transformed B-cell line; Kd, dissociation constant; Molt-4F, human T-cell line; MT-2, adult T-cell leukaemia cell line; PMSF, phenylmethylsulphonyl fluoride.

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MATERIALS AND METHODS

Peptides

Synthetic somatostatin was purchased from Sigma Chemical Company (St Louis, MO). Synthetic somatostatin analogue, SMS 201-995, was kindly provided by Dr W. Doepfner, Sandoz Ltd, Basel, Switzerland (Bauer *et al.*, 1982). Insulin and glucagon were obtained from Wako Pure Chemical Industries Ltd, Tokyo, Japan. Somatostatin 1-tyrosine ^{125}I-monoiodinated (^{125}I-somatostatin), with a specific activity of 1200 μCi/μg, was obtained from New England Nuclear, Boston, MA.

Cell lines

The human T-cell lymphotropic/leukaemia virus (HTLV-1)-producer adult T-cell leukaemia (ATL) cell line, MT-2, was provided by Professor I. Miyhoshi, Kochi Medical School, Nangoku, Japan (Miyoshi *et al.*, 1981). The human T-cell leukaemia/lymphoma cell lines Molt-4F, CCRF-HSB-2, Jurkat, the EB-virus transformed human B-cell lines, Isk, ER, EBV-Sh, EBV-Ky and L-KT9 were provided by Professor M. Aizawa, Hokkaido University School of Medicine, Sapporo, Japan (Kasahara *et al.*, 1983). The Fc-ε receptor-positive human

B-cell line, RPMI-8866, was provided by Professor T. Kishimoto, Osaka University School of Medicine, Suita, Japan. All cells were cultured in RPMI-1640 medium (Gibco, Grand Island, NY) with L-glutamine (Gibco) and 10% fetal bovine serum (Gibco) at 37° in a humidified atmosphere of 5% CO_2 in air. The cells had divided by 48–72 hr and were used for studies within the second subdivision.

Binding studies

To measure somatostatin binding, ^{125}I-somatostatin and 0·5–2×10^6 cells were incubated in 0·2 ml phosphate-buffered saline (PBS; pH 7·4) containing 10 mM $MgCl_2$, 50 μg/ml Thimerosal and 1% bovine serum albumin (BSA). After incubation for 120 min at 22°, the bound radioactivity was separated from the free tracer by centrifugation. The cells were washed twice with the buffer and the radioactivity was counted in a scintillation counter (LKB Industries Inc., Rockville, MD). Specific binding of ^{125}I-somatostatin was calculated by subtracting the non-specific binding (in the presence of 10^{-4}M unlabelled somatostatin) from the total binding.

Determination of specific binding of radioligand

The specificity of ^{125}I-somatostatin binding was determined by competitive inhibition, under the conditions described above. A mixture of 100 μl of the competitors (somatostatin, synthetic somatostatin analogue, insulin and glucagon) at varying dilutions, and ^{125}I-somatostatin were incubated with 2×10^6 cells for 120 min at 22°. After washing, the bound radioactivity was counted. Specific binding of somatostatin to the cell surface was determined by subtracting the binding in the presence of the competitor from the total binding. The dissociation constant (Kd) and number of binding sites (B max) for each cell line were determined by Scatchard plots.

RESULTS

Figure 1 shows the relationship between the cell number and total and non-specific binding of ^{125}I-somatostatin to MT-2 cells. It is apparent that the specific binding to MT-2 cells increased linearly with the MT-2 cell number. In contrast, no specific binding of ^{125}I-somatostatin to normal human peripheral blood lymphocytes was observed (data not shown).

As shown in Fig. 2, the kinetics of ^{125}I-somatostatin binding to MT-2 cells was time- and temperature-dependent. When ^{125}I-somatostatin binding was measured at 15°, 22° and 37°, the specific binding of ^{125}I-somatostatin to MT-2 cells increased to a maximum at 22° within 120 min and remained at a plateau until 300 min. At 15°, a maximum binding was also observed after 180-min incubation. When cells were incubated at 37°, the ^{125}I-somatostatin binding reached a maximum within 30 min, then declined.

Next, competitive inhibition studies were done to confirm the specificity of ^{125}I-somatostatin binding to MT-2 cells, using insulin, glucagon, somatostatin and a synthetic analogue of somatostatin (SMS 201-995) as competitors. Representative inhibition curves are illustrated in Fig. 3. The ^{125}I-somatostatin binding to MT-2 cells was inhibited by somatostatin and a somatostatin analogue. In contrast, concentrations of insulin and glucagon up to 10^{-4} M were without effect on the ^{125}I-somatostatin binding. When the data were plotted using Scatchard analysis a single class of binding sites was revealed,

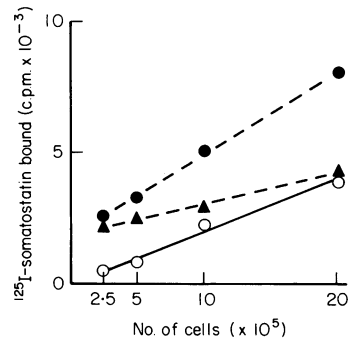

Figure 1. Binding of ^{125}I-somatostatin to MT-2 cells. The abscissa represents the number of cells incubated for 2 hr at 22° in the presence and absence of 10^{-4}M unlabelled somatostatin. The ordinate represents c.p.m. bound. Specific binding of ^{125}I-somatostatin (○) was calculated by subtracting the non-specific binding (▲) from the total binding (●). Note that specificity binding increased linearly with the concentration of MT-2 cell number.

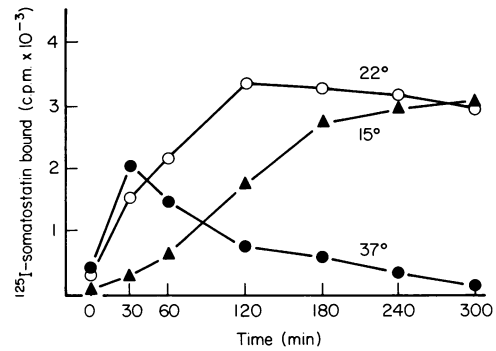

Figure 2. Time- and temperature-dependence of the specific binding of ^{125}I-somatostatin to MT-2 cells. At each temperature, 2×10^6 cells were incubated with ^{125}I-somatostatin for the times indicated at 15° (▲), 22° (○) and 37° (●). Points are the mean of triplicate incubations in one of three similar experiments.

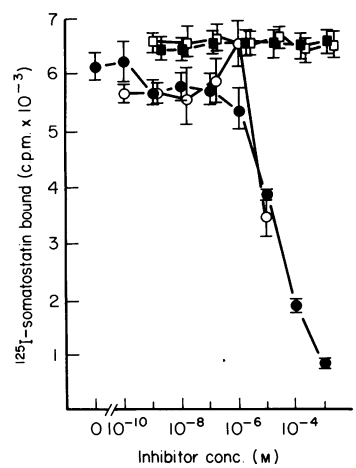

Figure 3. Specific binding of ^{125}I-somatostatin to MT-2 cells. MT-2 cells (2×10^6 cells) were incubated with ^{125}I-somatostatin in the presence indicated concentration of somatostatin (●), somatostatin analogue (○), insulin (■) and glucagon (□) for 120 min at 22°.

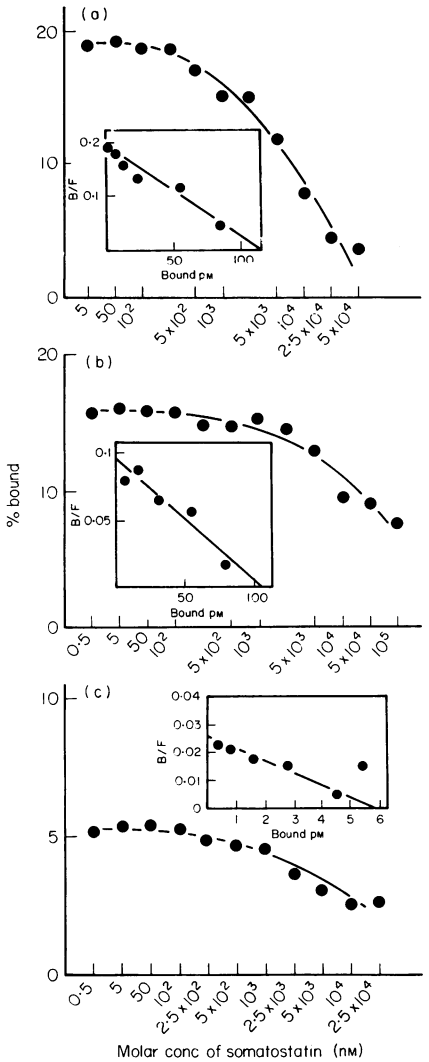

Figure 4. Inhibition of the specific binding of ^{125}I-somatostatin to MT-2 cells (a), Isk cells (b) and Molt-4F cells (c) in the presence of increasing concentrations of unlabelled somatostatin. Each point indicates the mean of triplicate representative experiments. Insert, corresponding Scatchard plot.

with a Kd of 0·64 nM. The B max for somatostatin were calculated to 109 pM (Fig. 4a). The same analytical studies of somatostatin receptor on several human lymphoid cell lines were done using ^{125}I-somatostatin and competitor. Isk cells, as well as MT-2 cells, revealed high-affinity binding, with a Kd of 1·1 nM and a B max of 102 pM (Fig. 4b). Less amount of binding sites (Kd, 0·22 nM; B max, 5·8 pM) was observed on Molt-4F cells (human T cell line; Fig. 4c). No detectable binding sites for somatostatin were found on other types of human lymphoid cell lines, as listed in Table 1.

DISCUSSION

We obtained evidence that some lymphoid cell lines bear receptors for somatostatin, as determined by a radiobinding assay with ^{125}I-somatostatin. MT-2 and Isk cells had a large amount of receptors for somatostatin, while other lymphoid cell lines possessed less amounts.

Table 1. Somatostatin receptors on human lymphoid cell lines

Cell line	Origin	Somatostatin receptor
MT-2	T	++
Molt-4F	T	+
CCRF-HSB-2	T	−
Jurkat	T	−
Isk	B	++
ER	B	+
RPMI-8866	B	+
EBV-Sh	B	−
L-KT9	B	−
EBV-Ky	B	−

The presence of receptors for somatostatin on pituitary membranes and other endocrine organs has been well characterized, and the binding specificity of the receptors represented features similar to those described in recent papers (Enjalbert *et al.*, 1982; Esteve *et al.*, 1984; Srikant & Patel, 1985a,b).

Evidence is accumulating that the immune and neuroendocrine systems communicate by virtue of signal molecules and receptors common to both systems (Bhathena *et al.*, 1981; Mascards *et al.*, 1984; Payan *et al.*, 1984a, b and c; Payan & Goetzl, 1985). The finding that nerve fibers were distributed in lymphoid tissues may support the concept mentioned above (Felten *et al.*, 1985). O'Dorisio *et al.* (1981) and Ottaway (1984) showed the presence of receptors for vasoactive intestinal peptide (VIP), a 28-amino acid peptide that acts as a neurotransmitter and stimulates both intestinal secretion of water and electrolytes and pancreatic acinar cell secretion of electrolytes and enzymes on immune effector cells. They postulated that VIP modulates lymphocyte functions by binding to the specific VIP receptors on T lymphocytes that leads to the activation of adenylate cyclase and cyclic AMP-dependent protein kinase to induce phosphorylation of specific lymphocyte proteins (O'Dorisio, Wood & O'Dorisio, 1985). Payan *et al.* (1984b) demonstrated that 21% of human blood T lymphocytes were reactive with substance P using a fluorescence-activated cell sorter and radiobinding assay. They also found cultured human IM-9 lymphoblastoid cells had receptors for substance P (Payan *et al.*, 1984a).

Somatostatin is a cyclic tetradecapeptide that inhibits the release of certain biologically active substances, such as growth hormone, TSH, glucagon, insulin, secretin, gastrin and cholecystokinin. This inhibition results from the depression of intracellular cyclic AMP levels. It is also known that somatostatin reduces membrane permeability to calcium. There are reports that somatostatin may play a regulatory role in lymphocyte-mediated immune responses (Bhathena *et al.*, 1981; Mascards *et al.*, 1984; Payan *et al.*, 1984c). Mascards *et al.* (1984) noted that somatostatin exerted an anti-proliferative action on Con A-activated rat thymocytes. Payan *et al.* (1984c) has shown that somatostatin is a potent inhibitor of the proliferation of human blood lymphocytes and of lymphoblasts of the human T cell line, Molt-4. When taken together, these observations led to the question of whether lymphocytes, especially lymphoblastoid cells, possessed receptors for soma-

tostatin. We found that receptors for somatostatin were present on lymphoblastoid cells, high-affinity receptors on MT-2 and Isk cell lines and fewer of receptors on Molt-4F and ER cell lines, using a radiolabelled ligand. The specificity of somatostatin binding to the receptors was confirmed by the competitive inhibition radiobinding assay using insulin, glucagon, somatostatin and a somatostatin analogue as competitors.

We used a MT-2 cell line derived from an adult T-cell leukaemia cell that produced a novel human type C retrovirus (Miyoshi *et al.*, 1981). We also used an EB virus-transformed B-cell line, Isk, established from normal human PBL (Kasahara *et al.*, 1983). We anticipated that the expression of somatostatin receptors on these cell lines might be referable to the viral infection. However, so far examined, mitogen-activated lymphocytes from normal individuals and most cells from patients with acute and chronic lymphocytic leukaemia were also found to express the high- and low-affinity receptors for somatostatin on their surface (T. Koike, H. Nakamura, K. Hiruma, T. Sato, H. Tomioka and S. Yoshida, manuscript in preparation). Therefore, it is likely that the expression of somatostatin receptors may not result from the neoplastic transformation by the viral infection but from acquisition during the activation process of lymphocytes similar to those of 4F2 antigen (Haynes *et al.*, 1981), Ia antigen (Reinherz *et al.*, 1979), transferrin receptor (T9 antigen) (Terhorst *et al.*, 1981) and IL-2 receptor (Tac antigen) (Wano *et al.*, 1984).

In conclusion, we identified receptors of somatostatin on certain lymphoid cell lines. Although the role of these receptors on lymphoblastoid cells in the immune regulation or dysregulation remains obscure, these cell lines may be useful tools to elucidate precise characteristics and functions of the somatostatin receptors, from the standpoint of immune–neuroendocrine interactions.

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