Specific Somatostatin-binding to Cytosol of Bovine Gallbladder Mucosa

E. Arilla, B. Colás, and J. C. Prieto

Received January 23, 1986

KEY WORDS: somatostatin; gallbladder; mucosa

The binding of somatostatin was studied in the cytosolic fraction of bovine gallbladder mucosa. The binding reaction depended on time, temperature and pH, and was reversible, saturable and specific. Stoichiometric data suggested the presence of two classes of binding sites: a class with high affinity ($K_d = 23.6$ nM) and low capacity (3.7 pmol somatostatin/mg protein) and a class with low affinity ($K_d = 284.6$ nM) and high capacity (85.0 pmol somatostatin/mg protein) at $37^\circ C$ and pH 7.4. The binding sites were highly specific for somatostatin since peptides such as [Leu]enkephalin, neurotensin, vasoactive intestinal peptide and substance P showed practically no effect upon somatostatin binding. The presence of somatostatin-binding sites in the cytosolic fraction of gallbladder mucosa, together with the known occurrence of somatostatin nerve endings in the gallbladder strongly suggests that this peptide may be involved in the physiology and physiopathology of gallbladder mucosa.

INTRODUCTION

Somatostatin, a tetradecapeptide initially isolated from ovine hypothalamus (Brazeau et al., 1973), has been identified by immunologic techniques in a variety of neuronal and non-neuronal cells (Elde et al., 1978; Reichlin, 1983). Several findings indicate that the peptide acts at the vicinity of target cells in a local manner by interaction with specific binding sites (Larsson et al., 1981; Forssman et al., 1978).

Nerves containing immunoreactive somatostatin have recently been described in the lamina propria underneath the columnar epithelium of the gallbladder (Cai et al., 1983). Furthermore, it has been demonstrated that somatostatin, at a high concentration, will inhibit the fluid transport response to vasoactive intestinal peptide...
(VIP) and secretin in the gallbladder in vitro (Wood et al., 1982). In another context, patients with somatostatinoma, presenting high circulating levels of somatostatin, usually have gallstones (Krejs et al., 1979).

The purpose of the present study was to investigate the occurrence of specific somatostatin binding sites in a cytosolic fraction isolated from bovine gallbladder mucosa. This characterization of the first step of somatostatin action is of particular interest since it would further support a role of somatostatin as a neuromediator influencing the behaviour of gallbladder epithelium in physiological and physiopathological conditions.

**MATERIALS AND METHODS**

Synthetic Tyr$^1$-somatostatin was purchased from Universal Biologicals Ltd. (Cambridge, UK); somatostatin from Cambridge Research Biochemicals (Cambridgeshire, UK); neuropeptide, substance P and VIP from Peninsula Laboratories (Belmont, CA, USA); [Leu]enkephalin, trypsin inhibitor and bovine serum albumin (fraction V) from Sigma (St Louis, MO, USA); Iodogen (1,3,4,6-tetrachloro-3,6-diphenylglycoluril) from Pierce Chemicals Co. (Rockford, IL, USA); and carrier-free Na$^{125}$I (IMS 30, 100 mCi/ml) from the Radiochemical Centre (Amersham, UK). Synthetic Tyr$^1$-somatostatin was radioiodinated (as in Fraker and Speck, 1978); the specific radioactivity of tracer being about 360 Ci/g. All other chemicals were of reagent grade.

Bovine gallbladders were removed within ten minutes of killing the animal at the slaughter-house, immediately placed on ice, opened and the bile carefully removed. The cytosolic fraction of the gallbladder mucosa was isolated (as in Reyl-Desmars and Lewin, 1982). Protein was estimated by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

Under standard conditions, the cytosolic fraction of the bovine gallbladder (0.16 mg protein/ml) was incubated in 0.5 ml of a medium of the following composition: 0.5 mM Na$_2$HPO$_4$; 80 mM NaCl; 4 mM KCl; 1 mM CaCl$_2$; 1.5 mM MgCl$_2$; 50 mM HEPES (pH 7.4); 11 mM glucose; 0.1% bovine serum albumin; 0.1 mg/ml trypsin inhibitor and 35 pM $^{125}$I-Tyr$^1$-somatostatin either alone or together with increasing concentrations of unlabelled somatostatin (up to 2 pM) or of other peptides.

Unless otherwise indicated, incubations were performed at 37°C for 90 min. $^{125}$I–Tyr$^1$-somatostatin associated with cytosolic protein was determined after removal of unbound tracer by the addition of 0.25% activated charcoal, 0.5% bovine serum albumin and 0.025% T70 dextran (Ogawa et al., 1978; Reyl-Desmars and Lewin, 1982). “Specific” binding was estimated as the difference between “total” binding (i.e., in the presence of tracer alone) and “nonspecific” binding as measured in the presence of 4 pM unlabelled somatostatin. This nonspecific component represented about 50% of the binding observed in the absence of unlabelled somatostatin. Each individual experiment was performed in triplicate. The integrity of bound $^{125}$I–Tyr$^{11}$-somatostatin was assessed by talc absorption as previously described (Arilla et al., 1984).
RESULTS

The specific binding of $^{125}$I–Tyr$^{11}$-somatostatin to the cytosolic fraction of gallbladder mucosa was dependent on time and temperature (Fig. 1). At 37°C, it was maximal at 90 min and did not change significantly from 90 to 180 min. Reducing the incubation temperature to 25 or 15°C decreased the specific binding. In subsequent experiments, incubations of the cytosolic fractions were then performed at 37°C for 90 min, i.e., when optimal equilibrium conditions are observed.

![Graph showing specific binding of $^{125}$I–Tyr$^{11}$-somatostatin](image)

Fig. 1. Specific binding of $^{125}$I–Tyr$^{11}$-somatostatin to the cytosolic fraction of bovine gallbladder mucosa as a function of time and temperature. $^{125}$I–Tyr$^{11}$-somatostatin ($^{125}$I–Tyr$^{11}$-SS, 35 pM) was incubated at 15°C (▲), 25°C (○), and 37°C (●) for various time intervals with the cytosolic fraction (0.16 mg protein/ml). Each point is the mean of triplicate determinations in a single experiment.

The dissociation of the tracer-cytosolic fraction complex was studied by the addition of 4 μM somatostatin to the incubation mixture at steady-state (Fig. 2). The time-course of dissociation followed multi-order kinetics that could represent two classes of binding sites with different affinities.

When increasing amounts of cytosolic fraction were added to the incubation medium, the specific binding of $^{125}$I–Tyr$^{11}$-somatostatin increased linearly with protein concentration (Fig. 3) up to 0.2 mg protein/ml. Higher cytosolic concentrations resulted in binding values lower than expected. This finding could be explained, at least in part, by the dependence of degradation of $^{125}$I–Tyr$^{11}$-somatostatin on cytosolic fraction concentration (Fig. 3). Consequently the characteristics of binding sites were studied at a protein concentration of 0.16 mg protein/ml, i.e., when only about 20% of $^{125}$I–Tyr$^{11}$-somatostatin was degraded.

The dependence of binding and degradation of labelled peptide on pH was studied in the 6.5–8.5 range (Fig. 4). Maximal binding was observed at pH 7.1–7.4. Degradation
Arilla, Colás, and Prieto

Fig. 2. Dissociation of $^{125}\text{I}$-Tyr$^{11}$-somatostatin binding from cytosolic fraction of bovine gallbladder mucosa. $^{125}\text{I}$-Tyr$^{11}$-somatostatin ($^{125}\text{I}$-Tyr$^{11}$-SS, 35 pM) was preincubated at 37°C for 90 min with the cytosolic fraction (0.16 mg protein/ml). Unlabelled somatostatin (4 μM) was then added and incubation continued for the indicated time intervals. Results are expressed as percentage of specific binding at the end of the preincubation period. Each point is the mean of triplicate determinations in a single experiment.

was also maximal at pH 7.1–7.4, suggesting that binding and inactivation of $^{125}\text{I}$–Tyr$^{11}$-somatostatin occurred, at least in part, by related processes.

In competition studies (Fig. 5), the specific binding of $^{125}\text{I}$–Tyr$^{11}$-somatostatin was inhibited by increasing concentrations of native somatostatin. When interpreted by the method of Scatchard (1949) (Fig. 5, inset), the data were compatible with the existence of two classes of binding sites: a high-affinity site with a $K_d$ of $23.6 \pm 3.5\text{nM}$ and a binding capacity of $3.7 \pm 0.2\text{ pmol somatostatin/mg protein}$ and a low affinity site with a $K_d$ of $284.6 \pm 10.9\text{ nM}$ and a binding capacity of $85.0 \pm \text{pmol somatostatin/mg protein}$.

The binding sites appeared to be highly specific for somatostatin since high concentrations (1 μM) of other peptides including [Leu]enkephalin, neurotensin, substance P and VIP showed practically no degree of competition (Fig. 6).
Fig. 3. Dependence of binding and degradation of $^{125}$I-Tyr$^{11}$-somatostatin by cytosolic fraction of gallbladder on cytosol concentration. Increasing cytosol concentrations were incubated with 35 pM labelled peptide ($^{125}$I-Tyr$^{11}$-SS) at 37°C for 90 min. The specific binding (●) and the integrity (○) of $^{125}$I-Tyr$^{11}$-somatostatin were determined and plotted as a function of cytosolic protein concentration. Each point is the mean of triplicate determinations in a single experiment.

Fig. 4. Effect of pH on binding (●) and degradation (○) of $^{125}$I-Tyr$^{11}$-somatostatin by the cytosolic fraction of gallbladder mucosa. The cytosolic fraction (0.16 mg protein/ml) was incubated with 35 pM tracer ($^{125}$I-Tyr$^{11}$-SS) for 90 min at 37°C. The incubation medium was that reported in Methods, adjusted to the indicated pH values. The integrity of the labelled peptide was determined and plotted as a percentage of the maximal value. Results are the means of triplicate determinations in a single experiment.
DISCUSSION

The present study demonstrates for the first time the existence of specific binding sites for somatostatin in the cytosolic fraction of bovine gallbladder mucosa. The use of $^{125}$I-Tyr$^{11}$-somatostatin appears justified for such studies since previous reports (Schonbrunn et al., 1983) have indicated that this derivative exhibits biological and binding activities very similar to those of the native peptide.

The somatostatin binding sites exhibited properties such as dependence on time, temperature and pH, reversibility, saturability and specificity in the interaction with the peptide. These are common features in other somatostatin binding systems such as gastric cells (Rey al et al., 1979) pituitary membranes (Leitner et al., 1979), ghosts of blood cells (Bathena and Recant, 1980), cytosolic fractions of pancreas (Diel et al., 1981) and gastrointestinal mucosa (Arilla et al., 1984; Gonzalez-Guijarro et al., 1985).
Interpretation of the stoichiometric data by the method of Scatchard (1949) disclosed two classes of somatostatin binding sites with different affinities, dissociation experiments further supporting this possibility. Two classes of somatostatin binding sites have also been described in many other systems (Reyl et al., 1979; Enjalbert et al., 1982) with the exception of pituitary (Enjalbert et al., 1982), brain (Czernik and Petrack, 1983; Epelbaum et al., 1982) and adrenal glomerulosa zone (Aguilera et al., 1982) membranes where a unique class of sites was described. The affinities of the two classes of binding sites are higher than those described for the binding of somatostatin to cytosolic fraction of rat small intestinal epithelial cells (Arilla et al., 1984). However, somatostatin bound with a higher affinity to other cytosolic fraction (Reyl-Desmars and Lewin, 1982) and membrane (Enjalbert et al., 1982; Aguilera et al., 1982) systems. Present results on somatostatin binding to the cytosolic fraction of bovine gallbladder mucosa are not compatible with the low circulating levels of the peptide; however, the presence of somatostatin in nerve endings at the gallbladder mucosa (Cai et al., 1983) should be borne in mind. This neuroparacrine condition allows local concentrations of somatostatin high enough to interact with the reported binding sites. In this context,
present \( K_d \) values are even lower than those observed in the study of somatostatin action on fluid transport in the gallbladder (Wood et al., 1982).

The cytosolic condition of somatostatin binding sites in gallbladder mucosa is in accordance with previous reports on somatostatin binding to cytosolic fraction of pancreas (Diel et al., 1981; Draznin et al., 1982) and gastric mucosa (Reyl-Desmars and Lewin, 1982; Arilla et al., 1984). In contrast, somatostatin binding sites appear to be located on the plasma membranes in systems such as brain (Epelbaum et al., 1982) and pituitary (Leitner et al., 1979). Obviously, the present results imply that the peptide can cross the plasma membrane. The process by which such a penetration would occur is so far unknown but it may be tentatively suggested to involve internalization of surface receptors, as proposed for insulin (Schlessinger et al., 1978).

The physiological significance of somatostatin binding sites in the gallbladder mucosa remains to be understood. However, it has been demonstrated that somatostatin interferes with the regulation of the gallbladder concentrating function (Bjorck and Svanvik, 1984). Moreover, it is probable that somatostatin reaches its specific binding sites after local release by somatostatin-containing nerves. Such an hypothesis is supported by the finding that somatostatin-containing nerves are present in the lamina propria just beneath the gallbladder epithelium (Cai et al., 1983). These previous findings together with present results, suggest that somatostatin may exert a physiological role in the regulation of gallbladder epithelial functions acting in a local manner.

ACKNOWLEDGEMENTS

The authors express their gratitude to Drs. J. Fernández, E. González, and J. Serrano (slaughter-house of Alcalá de Henares) for valuable assistance. This work was supported by grants from the Fondo de Investigaciones Sanitarias de la Seguridad Social (85/1213) and the Comisión Asesora de Investigación Científica y Técnica (699/82).

REFERENCES