# Inhibition by somatostatin of the vasopressin-stimulated adenylate cyclase in a kidney-derived line of cells grown in defined medium

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LLC-$PK_{1L}$ cells, a kidney-derived cell line grown in defined medium, possess a vasopressin-sensitive adenylate cyclase. Somatostatin was able to inhibit the vasopressin-induced increase in adenylate cyclase activity, without affecting the basal enzyme activity. This inhibition was competitive. No effect of somatostatin could be detected on [$^3$H]vasopressin binding suggesting an interaction of somatostatin with the vasopressin-sensitive system distal to the hormone–receptor interaction. At variance with *N*6-L-2-phenylisopropyladenosine (PIA), GTP did not potentiate the inhibition by somatostatin. The inhibition of the vasopressin stimulation by somatostatin and that by PIA were additive. Changing the composition of the cell growth medium increased the number of vasopressin receptors per cell. Cells with a high number of vasopressin receptors were less sensitive to inhibition by somatostatin. Such results suggested that somatostatin and vasopressin receptors and/or the inhibitory (Ni) and stimulatory (Ns) regulatory transducing components are regulated by different mechanisms.

*Somatostatin* *Vasopressin* *Cell culture* *Defined medium* *Adenylate cyclase*

## 1. INTRODUCTION

Somatostatin has been reported to exert a wide variety of physiological actions. Among these effects, several transport processes have been reported to be inhibited by somatostatin [1–3]. In addition somatostatin has been found to have a physiological action only on those tissues which contain somatostatin [4,5]. Despite the antagonizing effect of somatostatin on antidiuretic hormone action on the mammalian kidney [6,7], somatostatin has not been detected in this tissue. This is likely to be due to the high cellular heterogeneity within the kidney. Authors in [8] detected immunoreactive somatostatin in the toad urinary bladder, a useful established model for studying the mechanisms of antidiuretic hormone action in vivo. Addition of exogenous somatostatin inhibited reversibly the vasopressin-stimulated osmotic water flow without affecting the increase in the short-circuit current elicited by vasopressin. However, although the results suggest a direct effect of somatostatin on adenylate cyclase activity, this has not been formally proven.

The cell culture technique has also been extensively proven to be a valid tool for studying some aspects of renal functions [9–12]. Using the LLC-$PK_{1L}$ cell line, derived from the kidney, we demonstrated a somatostatin-induced inhibition of the increase in adenylate cyclase elicited by vasopressin.

## 2. METHODS

### *2.1. Adenylate cyclase assay*

The incubation medium was composed of 50 mM Tris–HCl (pH 7.4), 100 $\mu$M [$\alpha$-$^{32}$P]ATP (0.5 $\mu$Ci/assay), 5 mM $MgCl_2$, 100 $\mu$M cyclic [$^3$H]AMP (10 000 cpm/assay), 5 units/ml adenosine deaminase, 50 $\mu$M papaverine, 120 mM NaCl, 0.5 mg/ml

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creatine kinase, 2.5 mg/ml creatine phosphate and 0.3–0.5 mg/ml particulate enzyme (final volume 50–100 $\mu$l). Incubation time was 20 min at 30°C. The reaction was stopped as in [19] and cyclic [$^{32}$P]AMP searated according to [14]. Sources of all materials were as in [15]. Somatostatin was from Peninsula.

### 2.2. Cell culture

LLC-PK$_{1L}$ cells were a generous gift from Dr J.S. Handler (NIH, Bethesda). They were grown as in [16] except for the following: (i) penicillin and streptomycin concentrations were half those reported, (ii) the splitting ratio was 1:3, (iii) the trypsinisation procedure was stopped by means of soybean trypsin inhibitor and (iv) the cells were used 2 weeks after seeding. The minimum cell culture medium was that of [17]. When cells were induced for an increased vasopressin receptor number, an enriched medium was used. Its composition will be described in another paper (in preparation).

### 2.3. Membrane preparation

Petri dishes were washed 3 times with 10 ml $Ca^{2+}$- and $Mg^{2+}$-free phosphate-buffered saline. The cells were then disrupted with a tight-fitting Dounce homogenizer (15 strokes) in 5 mM Tris–HCl (pH 7.4) and 1 mM EDTA. The homogenate from one Petri dish was diluted to 10 ml and spun at 10000 × $g$ for 10 min. This procedure was repeated once and the final pellet used as a source of enzyme immediately after enzyme preparation. Proteins were determined as in [18].

## RESULTS AND DISCUSSION

Somatostatin induced a dose-dependent inhibition of vasopressin-sensitive adenylate cyclase from LLC-PK$_{1L}$ cells (fig.1). The relative magnitude of inhibition increased as the (8-lysine) vasopressin (LVP) concentration was lowered. In addition the threshold dose of somatostatin eliciting inhibition was lower at low LVP concentrations than at higher ones. No effect of somatostatin could be detected on basal adenylate cyclase activity. The plot of the data according to Eadie showed that somatostatin behaved as a competitive inhibitor of the increase in activity due to vasopressin. As the somatostatin concentration increased, the apparent affinity for adenylate cyclase activation by LVP was decreased. Using maximum LVP concentrations, similar maximum velocities were obtained.

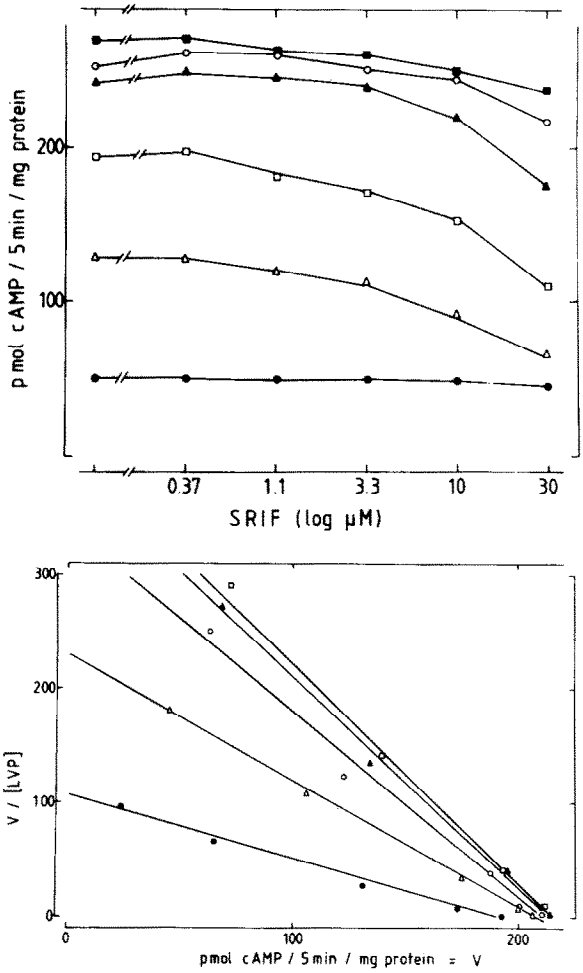


Fig.1. Inhibition of vasopressin-stimulated adenylate cyclase as a function of somatostatin concentration. (Upper) Adenylate cyclase activity was measured at different LVP concentrations as a function of the somatostatin (SRIF) concentration. Cells grown in enriched medium were used for enzyme preparations to increase the vasopressin response. The incubation time was 20 min at 30°C. (●, no LVP; △, 0.2 nM LVP; □, 1 nM LVP; ▲, 5 nM LVP; 0.25 nM LVP and ■, 20 $\mu$M LVP). (Lower) Data presented in the upper panel were plotted according to Eadie. The increase in adenylate cyclase ($V$) due to LVP was plotted as a function of $V$/LVP concentration at various somatostatin concentrations (□, no SRIF; ▲, 1.1 $\mu$M SRIF; ○, 3.3 $\mu$M SRIF; △, 10 $\mu$M SRIF and ●, 30 $\mu$M SRIF).

The data presented in fig.2 show that the inhibi-

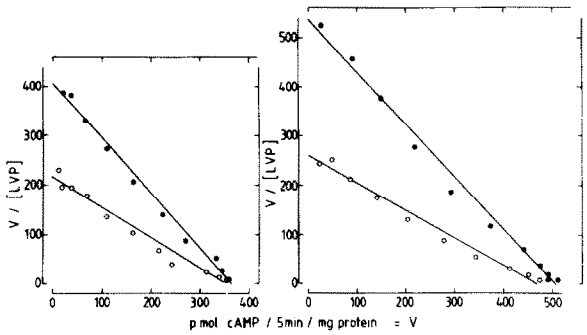


Fig.2. Eadie plot of vasopressin-dependent adenylate activity: effects of somatostatin and GTP. Cells grown in modified culture were used. Adenylate cyclase activity was measured as a function of the LVP concentration with (○) or without (●) 10 $\mu$M somatostatin (SRIF). The data were plotted as in fig.1, lower panel. The basal adenylate cyclase activities in the absence of GTP were equal to 34 (no SRIF) and 33 (+SRIF) pmol cAMP/5 min per mg protein and to 46 (no SRIF) and 43 (+SRIF) pmol cAMP/5 min per mg protein in the presence of 0.25 $\mu$M GTP. (Left) With (and without) somatostatin the $K_{app}$ for enzyme activation and maximum velocities were respectively equal to 1.60 nM and (0.89 nM), 344 and (359) pmol cAMP/5 min per mg protein. (Right) Maximum velocities and $K_{app}$ for enzyme activation were equal to 503 pmol cAMP/5 min per mg protein and 0.94 nM (without somatostatin) and 464 pmol cAMP/5 min per mg protein and 1.80 nM (with somatostatin).

tory effects of somatostatin on vasopressin stimulation were neither potentiated nor abolished upon addition of 0.25 or 10 $\mu$M (not shown) GTP to the adenylate cyclase assay medium. Provided high enough LVP concentrations were used, the same maximum velocity was reached. In addition GTP did not affect the affinity of the adenylate cyclase system for somatostatin since the ratio of the apparent affinities for LVP measured with and without somatostatin were identical irrespective of the presence of GTP. These data confirmed the competitive action of somatostatin on the LVP-induced response.

Such a competition could be explained by a direct interaction of somatostatin with the LVP binding to the antidiuretic hormone receptor. Therefore a Scatchard analysis of [$^3$H]LVP binding data was undertaken (not shown). The $K_d$ values (±SD) for hormonal binding were found to be equal to 5.6 ± 2.3, 8.9 ± 3.1 and 7.6 ± 1.7 nM in the control condition or with 10 $\mu$M GTP or 10 $\mu$M somatostatin, respectively. For the same experimental conditions, the respective maximum binding capacities were found to be equal to 253 ± 17, 320 ± 20 and 252 ± 10 fmol receptor/mg protein (±SD). From these data it was therefore concluded that somatostatin was not competing for [$^3$H]LVP binding. Both hormones must therefore interact with each other at a step beyond their receptors to antagonize the LVP-induced adenylate cyclase activation.

*N*6-L-2-Phenylisopropyladenosine (PIA) is able to lower basal and to a greater extent hormonally stimulated adenylate cyclase activities (in preparation). However PIA inhibition of the adenylate cyclase activity was potentiated by GTP in contrast to that with somatostatin described above. In addition, PIA inhibition could not be relieved by in-

Table 1

Interactions between PIA and somatostatin on the increase in adenylate cyclase activity due to LVP

| PIA ($\mu$M) | GTP ($\mu$M) | Increase in activity due to 1 nM LVP (pmol/5 min per mg protein) | % decrease in activity due to 10 nM somatostatin | % of response to 1 $\mu$M LVP | |
|---|---|---|---|---|---|
| | | | | −SRIF | +SRIF |
| 0 | 0 | 110 | 25 | 43 | 33 |
| 30 | 0 | 94 | 31 | 40 | 30 |
| 0 | 10 | 118 | 37 | 46 | 32 |
| 30 | 10 | 71 | 37 | 41 | 71 |

The basal adenylate cyclase activities were equal to: 70 (0 PIA, 0 GTP); 181 (+ PIA, 0 GTP); 117 (0 PIA, + GTP) and 231 (+ PIA, + GTP) pmol cAMP/5 min per mg protein. Results are means of triplicate determinations

Table 2

Effects of vasopressin receptor density on the relative inhibition by somatostatin

| Cells grown | GTP (10 $\mu$M) | Changes in activity (pmol cAMP/5 min per mg protein) due to | | % inhibition due to somatostatin (B/A) |
|---|---|---|---|---|
| | | 2 nM LVP (A) | 2 nM LVP + 5 $\mu$M somatostatin (B) | |
| Minimum medium | 0 | 10.1 | 3.7 | 63 |
| Minimum medium | + | 15.7 | 5.9 | 62 |
| Modified medium | 0 | 117 | 96 | 18 |
| Modified medium | + | 150 | 121 | 19 |

The basal adenylate cyclase activities measured with and without GTP were respectively equal to 44 and 87 pmol cAMP/5 min per mg protein for cells grown in the minimum medium and to 42 and 83 pmol cAMP/5 min per mg protein for cells grown in the modified medium. Data are means of triplicate values which agreed within ±2%

creasing the dose of LVP. Since the modalities of inhibition by PIA and somatostatin appeared to be different it was of interest to see whether they share a common mechanism of action (table 1). PIA inhibited the adenylate cyclase response to a greater extent when GTP was present. Somatostatin, irrespective of the presence of PIA and/or GTP, was able to induce additional inhibition of enzyme activity. The relative magnitude of this inhibition was independent of the degree of inhibition already achieved with PIA. Therefore the apparent affinity of somatostatin for vasopressin inhibition of adenylate cyclase was not affected by the presence of PIA (PIA per se did not change the apparent affinity of LVP for the enzyme activation) (not shown).

The LLC-$PK_1$ cells, i.e., the parent cell line of the cells used here, are grown in the presence of 10% foetal bovine serum. These cells have more than 50-times the vasopressin receptor number compared to LLC-$PK_{1L}$ cells and their vasopressin-sensitive adenylate cyclase cannot be altered upon somatostatin addition (not shown). By making appropriate modifications of the cell culture medium, it was possible to increase by 10–20-fold the receptor number in LLC-$PK_{1L}$ cells. The results listed in table 2 show that these cells were still responsive to somatostatin. The absolute decrease in activity due to somatostatin was larger when the enzyme response to vasopressin was the highest. However, the relative inhibition was less. Again the addition of GTP to the adenylate cyclase assay medium did not alter the inhibitory property of somatostatin. Such data, taken together with those obtained on LLC-$PK_1$ cells may suggest the following:

(i) The addition of serum did not allow the existence or expression of a somatostatin receptor;
(ii) Vasopressin and somatostatin receptors have distinct regulatory pathways and can be expressed independently;
(iii) The transduction mechanism of the somatostatin receptor becomes a limiting factor for regulating high levels of stimulation induced by vasopressin.

## 4. CONCLUSIONS

Somatostatin has been shown to be effective in inhibiting the hormone stimulation in many [3,19,20] but not all tissues [21]. To our knowledge, this is the first demonstration of an inhibitory effect of somatostatin on the antidiuretic hormone-stimulated adenlyate cyclase. The stimulated state of adenylate cyclase appeared to be a prerequisite for demonstrating inhibition by somatostatin [22]. However, all stimulated states of adenylate cyclase are not inhibitable by somatostatin as exemplified in the case of enzyme activation by PIA (when no GTP was added). In contrast inhibition by PIA and that by somatostatin were additive (in the presence of GTP).

The adenylate cyclase system is a multicomponent regulatory complex. It is composed of a

receptor (R), a catalytic unit (C) and a transduction mechanism (N). At present two distinct N units have been described. $N_s$ stimulates C and $N_i$ mediates the inhibition of C. The LVP-mediated response is supposed to regulate adenylate cyclase activity through $N_s$, while inhibitory effects of PIA are supposed to be mediated through the $N_i$ unit. Stimulations of C by LVP and PIA are additive. Similarly inhibitions of the LVP response by PIA and somatostatin are also additive (in the presence of GTP). Both somatostatin and PIA can inhibit the LVP response but had no effect on PIA stimulation. This would exclude the possibility that somatostatin has a direct effect on C. However it has been shown to be able to inhibit the forskolin- and cholera toxin-activated state of C [22]. Alternatively somatostatin might act on $N_i$ coupled to C. This would imply that the mechanisms of enzyme activation by PIA and by LVP are different since they do not exhibit the same sensitivity to PIA inhibition.

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