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Somatostatin Acts Via a Pertussis Toxin-Sensitive Mechanism on Calcitonin Secretion in C-Cells

Angela Zink,* Hans Scherubl,† Friedhelm Raue,* and Reinhard Ziegler*

The effect of the somatostatin analog octreotide on cAMP-mediated calcitonin (CT) secretion and cAMP accumulation in C-cells was investigated. Glucagon stimulated cAMP accumulation and CT secretion with a maximal effect at a concentration of 10^{-5} M. The cAMP antagonist RpcAMPs blocked the glucagon-induced CT secretion down to control levels. Therefore, no other second messengers seem to be involved in glucagon-stimulated CT secretion. Octreotide in increasing doses (10^{-6} to 10^{-5} M) inhibited cAMP accumulation and CT secretion with a maximal effect at a concentration of 10^{-7} (40% and 29% of control values, respectively). Pretreatment of the cells with 100 ng/mL pertussis toxin for 24 hours abolished the inhibitory effect of octreotide on cAMP accumulation and CT secretion (82% and 58% of control values, respectively). Similar results were obtained under the influence of the phosphodiesterase inhibitor IBMX. Therefore, we conclude that somatostatin modulates adenylate cyclase-coupled CT secretion in C-cells via a pertussis toxin-sensitive G-protein possibly in an autocrine/paracrine way. (Henry Ford Hosp Med J 1992;40:289-92)

Somatostatin is a physiologically important inhibitor of growth hormone release and is also known to inhibit hormone secretion in a variety of extrapituitary tissues including brain, thyroid, pancreas, and gut (1,2). Concerning C-cells in vivo, somatostatin inhibits calcitonin (CT) secretion (3,4), but the physiological role of somatostatin and the mechanism of action in CT-secreting cells is still unclear.

Hormone secretion by C-cells is mainly regulated through changes in the extracellular calcium concentration via voltage-dependent calcium channels, but various peptides such as glucagon or growth hormone-releasing peptide influence CT secretion in C-cells via the adenylate cyclase pathway (5). It has been shown that in pituitary cells somatostatin acts independently on both second messenger systems, the cAMP-mediated and the calcium-mediated pathway (6). In C-cells, the effect of somatostatin on adenylate cyclase-mediated CT secretion is still unclear. Therefore, we studied the effect of somatostatin on the glucagon-stimulated CT secretion in an established rat medullary carcinoma cell line (rMTC 6-23) (7) in order to elucidate the role of somatostatin on cAMP-mediated CT secretion and to study the possible involvement of a pertussis toxin-sensitive mechanism in cAMP-mediated CT secretion.

Methods

Cell culture
rMTC 6-23 cells were purchased from the American Type Culture Collection and grown as monolayers in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 15% horse serum in a humidified atmosphere with 5% CO₂ and 95% air.

Determination of cAMP
Confluent cells on 35 mm dishes were washed twice with PBS-buffer and further incubated with medium containing test agents or vehicle alone at 37 °C. After 15 minutes, medium was removed, cells were washed twice with PBS-buffer, and denaturated with ice-cold ethanol (100% pH 3). After 2 hours at 4 °C, the supernatant was evaporated at 37 °C under N₂, and the resulting pellet was resuspended in the cAMP-assay buffer. cAMP was determined by competitive protein binding assay as reported previously (8). Total cell protein was determined by the method of Bradford (9).

Secretion experiments
To determine CT secretion, confluent cells on replicate 35 mm dishes were preincubated with serum-free DMEM for 2 hours. Subsequently, cells were washed twice with PBS-buffer and further incubated with medium containing test agents or vehicle alone. After 2 hours, medium from each dish was collected and stored at −20 °C until assayed. CT was measured by radioimmunoassay. Total cell protein was determined after the method of Bradford (9). Viability as tested by trypan blue exclusion was > 90% in each experiment.

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Glucagon stimulated cAMP accumulation and CT secretion in rMTC 6-23 cells in a dose-dependent manner as shown in Fig 1. Maximal stimulation occurred at a concentration of $10^{-8}$ M and reached levels 300% and 150% over control levels for cAMP and CT secretion, respectively. The cAMP antagonist RpcAMPs blocked the glucagon-induced CT secretion down to control values (Table). Increasing doses of octreotide ($10^{-9}$ to $10^{-6}$ M) inhibited the glucagon-induced cAMP accumulation and CT secretion maximally to 40% and 29%, respectively, by a concentration of $10^{-7}$ M octreotide (Fig 2). Similar results were seen under the influence of the phosphodiesterase inhibitor IBMX ($10^{-4}$ M, data not shown). Fig 3 shows the effect of pretreatment of the cells for 24 hours with pertussis toxin. This pretreatment partially blocked the inhibitory effect of octreotide on cAMP accumulation and CT secretion to 82% and 58% of control values, respectively (Fig 3). A maximal effect of pertussis toxin was reached by a concentration of 100 ng/mL. Similar results were obtained under the influence of IBMX ($10^{-4}$ M, data not shown).

**Discussion**

The glucagon-induced CT secretion in our experiments paralleled the glucagon-induced cAMP accumulation, which is consistent with previous findings (5). Generation of other second messengers such as IP3, which influences intracellular calcium by glucagon, is unlikely in C-cells, as it has been shown earlier that glucagon had no effect on intracellular calcium concentration in C-cells measured with fura-2-loaded cells (10). Furthermore, addition of the cAMP antagonist RpcAMPs completely suppressed glucagon-stimulated CT secretion. Therefore, we considered activation of adenylate cyclase the sole signal-transducing pathway mediating the glucagon-induced CT secretion in our cell system and used it as a model to study the effect of somatostatin on cAMP-mediated CT secretion.

Somatostatin is able to inhibit cAMP accumulation as well as cAMP-mediated CT-secretion in C-cells. This is consistent with findings in pituitary or adrenal glomerulosa cells, where somatostatin inhibits cAMP-mediated hormone secretion in a similar way (11,12). In the rMTC 6-23 cells as well as in parafollicular and GH-pituitary cells, the inhibitory effect of somatostatin was not complete (13-15). The reason for this is unclear. Binding studies with different somatostatin analogs revealed similar or even higher binding affinities or biological effects of the analogs in pituitary cells (16). Thus structural differences are not likely to be the reason for this partial inhibitory effect. As C-
cells have been shown to secrete somatostatin (17,18), an auto-
crine or paracrine desensitization of the cells might explain the
incomplete effect of somatostatin. A similar desensitization by
somatostatin has been described in pituitary cells (19).
The octreotide-induced inhibition of cAMP accumulation as
well as the inhibition of CT secretion could be prevented by pre-
treatment of the cells with pertussis toxin. This indicates that a
pertussis toxin-sensitive mechanism is involved in the cAMP-
mediated CT secretion in C-cells. As the effect of pertussis toxin
occurred with or without IBMX, it is not due to changes in deg-
radation of cAMP. More likely, octreotide acts via somatostatin
receptors which are coupled to adenylate cyclase via inhibitory
pertussis toxin-sensitive G-proteins, as reported in other cell
systems (20,21).

The physiological role of the somatostatin effect on C-cells
remains unclear. In pituitary cells an effect of somatostatin on
Ca++- and voltage-gated K+-channels has been described (22),
and there is evidence that somatostatin may influence secretory
processes via more distal events (23). In C-cells, somatostatin
has been shown to inhibit calcium-induced CT secretion via G-
proteins directly coupled to the voltage-gated calcium channels
(24) and chronic exposure of C-cells to somatostatin inhibits
proliferation of the cells (Mekonnen Y, et al, personal communi-
cation, 1991). Together with the finding that somatostatin itself
is secreted by C-cells, a modulatory role of somatostatin on pro-
liferation and CT secretion in an autocrine or paracrine way
seems to be evident.

Fig 2—Effect of increasing doses of octreotide on glucagon-
stimulated cAMP accumulation (upper panel) and CT secretion
(lower panel) in rMTC cells. Cells were grown and experiments
performed as described in the text. Points show mean ± SEM of
four representative experiments.

Fig 3—Effect of different doses of pertussis toxin on the inhibi-
tory effect of octreotide on glucagon-stimulated cAMP accu-
mulation and CT secretion in rMTC cells. Cells were pretreated
with pertussis toxin for 24 hours before stimulation was per-
formed. For details, see the text. Points show mean ± SEM of
four representative experiments.

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