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Release of somatostatin-like immunoreactivity from enriched enteric nerve varicosities of rat ileum

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Abstract

Synaptosomes were isolated from rat ileum by various steps of differential centrifugation. The peptide content for somatostatin-like immunoreactivity was used as marker for neuronal membranes. The enriched synaptosomal fraction (P2) showed a good enrichment of somatostatin content (4-fold) in comparison to the post-nuclear supernatant. The basal release of somatostatin-like immunoreactivity was 26 ± 3 pg/mg tissue protein. KCl-evoked depolarization (65 mM) caused a significant increase of somatostatin-like immunoreactivity release (72 ± 11 pg/mg, $n = 12$, $P < 0.001$) compared to basal release. In Ca^{2+}-free medium the evoked release of somatostatin-like immunoreactivity was abolished. A substantial increase of somatostatin-like immunoreactivity release (52 ± 7 pg/mg, $n = 12$, $P < 0.05$) was also observed in the presence of the Ca^{2+} ionophore A-23187. The cholinergic agonist carbachol elicited a dose-dependent release of somatostatin-like immunoreactivity (10^{-7} M: 54 ± 8 pg/mg, 10^{-6} M: 63 ± 6 pg/mg, 10^{-5} M: 53 ± 5 pg/mg, $n = 12$, $P < 0.001$), which was blocked by atropine (10^{-6} M: 35 ± 6 pg/mg, $n = 12$, $P < 0.001$), but not by hexamethonium. Other presynaptic modulating substances such as serotonin, the selective neurokinin-B agonist [βAsp^4,MePhe^7]neurokinin B-(4–10), neurotensin, cholecystokinin-8, caerulein and pentagastrin had no stimulatory effect on release of somatostatin-like immunoreactivity. In summary, somatostatin-like immunoreactivity can be released from enteric synaptosomes by both depolarization with KCl and cholinergic stimulation via a muscarinic mechanism. The synaptosomes of intrinsic nerves offer an approach to study release of neuronal somatostatin on the subcellular level.

Keywords: Somatostatin; Synaptosome; Ileum, rat; Acetylcholine; Presynaptic mechanism

1. Introduction

Somatostatin is localized in endocrine cells as well as in nerves and therefore it may have a dual functional role as hormone or paracrine mediator and as putative neurotransmitter. The presence of somatostatin-like immunoreactivity has been demonstrated in D cells of the stomach, small intestine and pancreas (Arimura et al., 1975; Hökfelt et al., 1975a; Penman et al., 1983), in intrinsic enteric neurons (Costa et al., 1980; Darvodelsky et al., 1988; Keast et al., 1984; Schultzberg et al., 1980) and extrinsic efferent or afferent neurons supplying the gut (Costa and Furness, 1984; Hökfelt et al., 1975a,b). In the rat and guinea pig small intestine somatostatin-containing nerves were found in both the submucous and the myenteric plexus. In the gastrointestinal tract release of somatostatin-like immunoreactivity has been studied in various experimental models as isolated perfused pancreas, isolated perfused stomach (Madaus et al., 1990; Schusdziarra et al., 1986), isolated antrum and fundus (Schubert et al., 1988), isolated perfused ileum (Hermansen, 1985; Schmidt et al., 1993) and isolated D-cells (Chang and Soll, 1988). Somatostatin released in these studies is mainly due to release from endocrine cells and it is not possible to clearly distinguish between endocrine and neuronal release. In order to study somatostatin release from neuronal origin several techniques, such as release from muscle strips (Allescher et al., 1991; Grider, 1989), release from isolated enteric ganglia (Grider, 1989) and release from cultured enteric submucosal neurons (Buchan et al., 1989) or cultured submucous ganglia (Accili et al., 1993) have been employed. Preparations of intact muscle with adherent myenteric plexus or isolated ganglia leave the synaptic

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connection of neuronal elements intact and they provide a good model for the study of integrative regulation and interaction of peptide release. On the other hand, these models are less suited to the study of presynaptic cellular and subcellular mechanisms of peptide release. Preparation of synaptosomes, an established method to study neurotransmitter release in the central nervous system, offers the unique opportunity to examine intracellular and subcellular mechanisms of neurotransmitter release without interference of the neuronal network present in vivo or in the intact isolated organ. Furthermore presynaptic modulation of a functionally distinct subpopulation of terminals can be studied. Release of acetylcholine (Briggs and Cooper, 1982; Christofi and Cook, 1986; Reese and Cooper, 1982), serotonin (Jonakait et al., 1979), purines (White, 1977), bombesin (Kurjak et al., 1994) and neuropeptide Y (Cheng et al., 1987) from enteric synaptosomes has been shown; however, the release of somatostatin has not yet been investigated. In contrast to the enteric nervous system the neuronal release of somatostatin has been extensively studied in the central nervous system. The aim of this study was to examine the basic release mechanisms of somatostatin-like immunoreactivity from enriched synaptosomes isolated from the rat ileum and to characterize the action of cholinergic agonists and other substances which have been shown to release neuronal somatostatin in the central nervous system.

2. Materials and methods

2.1. Tissue handling and isolation of synaptosomes

Isolation of synaptosomes was performed as described previously (Kurjak et al., 1994). Briefly, male Wistar rats were killed by cervical dislocation and the small intestine was quickly removed and suspended in ice-cold isolation buffer (25 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS), 10 mM $MgCl_2$, 8% w/v sucrose, pH 7.4). All further preparative steps were carried out at 0–4°C. The ileum was dissected, cleaned of mesenteric arcade and fat, and opened along the mesenteric attachment line. The mucosal layer was removed. For preparation of synaptosomes the tissue was resuspended in isolation buffer, minced with scissors and homogenized with a Polytron PT20 homogenizer at approximately 1500 rpm setting for 15 s (3 × 5 s).

The tissue homogenate was centrifuged in two steps of $800 \times g$ for 10 min. The supernatant was collected (post-nuclear supernatant) and re-centrifuged at $3500 \times g$ for 10 min to obtain the crude synaptosomal fraction (P1). The supernatant after this spin was centrifuged at $120000 \times g$ for 60 min. The pellet from this spin (microsomal 1) was re-suspended and centrifuged again at $10000 \times g$ for 10 min. The resulting pellet and the supernatant were then further referred to as enriched synaptosomal fraction (P2) and microsomal fraction 2, respectively.

2.2. Enzyme and protein assays

Protein was measured by the Folin phenol method of Lowry as described elsewhere (Allescher et al., 1989). The specific activity of 5′-nucleotidase (EC 3.1.3.5) was determined by incubation of the membrane fraction (20–50 μg of protein) for 30 min at 37°C in 50 mM imidazole-buffer pH 7.0 containing 5 mM AMP-sodium salt and 5 mM $MgCl_2$ to a final volume of 1 ml. The reaction was stopped by adding 1 ml of ice-cold trichloroacetic acid. The precipitated protein was sedimented by centrifugation and the liberated phosphate was determined.

2.3. Peptide release

Peptide release studies were carried out as described previously (Kurjak et al., 1994). Briefly, 1050 μl of Krebs-Ringer bicarbonate solution (NaCl 115.5 mM, $MgSO_4$ 1.16 mM, NaH_2PO_4 1.16 mM, glucose 11.1 mM, $NaHCO_3$ 21.9 mM, $CaCl_2$ 2.5 mM, KCl 4.16 mM, gassed with 95% O_2 and 5% CO_2) and 150 μl of drugs or Krebs-Ringer solution serving as blank were incubated in separate test tubes at 37°C using a gently shaking water bath. The reaction was started by adding 300 μl of synaptosomes (300 μg of protein) to each tube at timed intervals and they were incubated for 5 min. Preliminary experiments with an incubation of a given amount of exogenous somatostatin demonstrated that the loss of somatostatin-like immunoreactivity due to proteolytic breakdown is less than 5%, when the incubation period was reduced to 5 min, compared to a loss of somatostatin-like immunoreactivity of more than 10%, when the incubation period was 30 min.

2.4. Radioimmunoassay

Somatostatin-like immunoreactivity was determined as described elsewhere (Harris et al., 1978) with an antibody raised against [^{125}I][Tyr^1]somatostatin. Antiserum 80c for measurements of somatostatin-like immunoreactivity was generously provided by Dr. R.H. Unger (Dallas, TX, USA). [^{125}I][Tyr^1]Somatostatin and synthetic somatostatin as standard were generously supplied by Dr. Jean Rivier (Salk Institute, La Jolla, USA). After incubation of the assay probe (100 μl) with the tracer (2000–3000 cpm/100 μl) for 72 h at 4°C the mixture was chromatographed on a Sephadex G-25 column, equilibrated with 0.1 M acetic acid containing 0.1% w/v gelatin. The sensitivity of the assay

was 12.5 pg/ml. No cross-reactivity was observed with cholezystokinin, pentagastrin or caerulein.

2.5. Drugs

All reagents were purchased from the indicated sources: veratridine, tetrodotoxin, neurotensin, capsaicin, carbachol, 5-hydroxytryptamine, Ca^{2+} ionophore A-23187, EGTA (ethylene glycol-bis[β-aminoethylether]-*N,N,N',N'*-tetraacetic acid), 1,1-dimethyl-4-phenyl-piperazinium iodide (DMPP), atropine sulphate (Sigma, Munich, Germany), (4-hydroxy-2-butynyl)-1-trimethylammonium-*m*-chlorocarbanylate chloride (McN-A-343) (Research Biochemical, Heidelberg, Germany), pentagastrin, CCK-8 (cholecystokinin-8), caerulein (Peninsula, Heidelberg, Germany), pirenzepine, 11-2[[2-(diethylamino)methyl]-1-piperidinyl]acetyl-5,11-dihydro-6*H*-pyrido-[2,3-*d*][1,4]-benzodiazepine-6-one (AFDX 116) (Dr. K. Thomae, Biberach, Germany), [βAsp^4,MePhe^7]neurokinin B-(4–10) (gift from Drs. G. Drapeau and D. Regoli, University of Sherbrooke, Canada). Adequate controls were performed with the vehicles used for solubilizing each reagent.

2.6. Statistics

Data are given as means ± S.E.M., n indicates the number of independent observations in separate experiments from separate preparations. For each value of a given drug of a single preparation the release study was carried out in duplicate. Analysis of variance test and t-test for paired samples were used to compare the mean values and values of $P < 0.05$ or less were considered significant.

3. Results

3.1. Isolation of synaptosomes

Differential centrifugation led to a substantial enrichment (4-fold) of content of somatostatin-like immunoreactivity (8.4 ± 0.7 ng/mg) in the enriched synaptosomal fraction (P2), when compared to postnuclear supernatant (2.2 ± 0.3 ng/mg). The P2 fraction was used for further release experiments.

3.2. Neuropeptide release

Basal peptide release

The basal release of somatostatin-like immunoreactivity in the supernatant of the Krebs-Ringer solution after an incubation time of 5 min was 26 ± 3 pg/mg protein. The basal level of somatostatin-like immunoreactivity was equivalent to about 0.35% (range 0.31–0.39%) of the total amount of somatostatin-like immunoreactivity present in the synaptosomes. The stimulated peptide release relative to basal release within a certain preparation was stable and consistent. This basal value of somatostatin-like immunoreactivity was not due to a Ca^{2+}-dependent release of somatostatin-like immunoreactivity, as the basal level of somatostatin-like immunoreactivity release was not significantly changed when the incubation was carried out in Ca^{2+}-free medium containing 0.25 mM EGTA (23 ± 8 pg/mg, not significant).

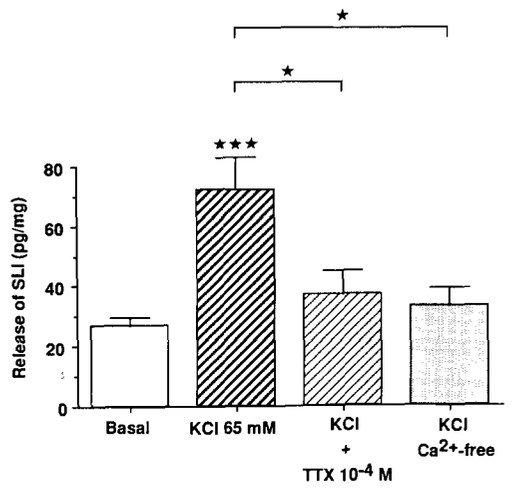

Fig. 1. Release of somatostatin-like immunoreactivity (SLI) from enriched synaptosomes in response to depolarization with KCl (65 mM, $n = 12$). In the presence of tetrodotoxin (TTX, 10^{-4} M) the release was reduced; Ca^{2+}-free medium blocked the evoked release completely. Values are expressed as means ± S.E.M. of $n = 12$ independent experiments, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Depolarization-induced release of somatostatin-like immunoreactivity

Depolarization with KCl (65 mM) increased release of somatostatin-like immunoreactivity significantly (72 ± 11 pg/mg, $n = 12$, $P < 0.001$) compared to the referring basal value (28 ± 3 pg/mg). When the stimulation with KCl was carried out in Ca^{2+}-free medium the release of somatostatin-like immunoreactivity in the presence of 65 mM KCl was not significantly different from basal release (Fig. 1), indicating that the release induced by KCl is Ca^{2+}-dependent. In the presence of tetrodotoxin the KCl-induced release was reduced but not abolished (37 ± 8 pg/mg, $n = 7$, $P < 0.05$).

The depolarizing agent veratridine, an alkaloide which serves to open sodium channels, thus depolarizing the cell membrane, had a significant effect on the somatostatin-like immunoreactivity levels (basal 29 ± 3 pg/mg, veratridine 10^{-6} M: 47.3 ± 5 pg/mg, $n = 5$, $P < 0.05$). In the presence of tetrodotoxin the release of somatostatin-like immunoreactivity was blocked (Fig. 2).

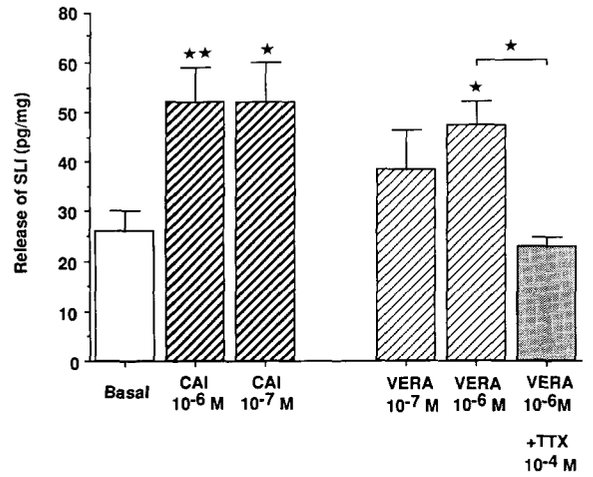

Fig. 2. Release of somatostatin-like immunoreactivity (SLI) in response to veratridine (VER 10^{-6} M, 10^{-5} M, $n = 7$), in the absence and presence of tetrodotoxin (TTX, 10^{-4} M) and to the Ca^{2+} ionophore A-23187 (CAI 10^{-6} M, 10^{-5} M, $n = 13$). The values are given as means ± S.E.M., * $P < 0.05$, ** $P < 0.01$.

The Ca^{2+} ionophore A-23187, a pharmacological tool for raising intracellular Ca^{2+} levels, also caused a potent release of somatostatin-like immunoreactivity (10^{-6} M: 52 ± 7 pg/mg, $P < 0.01$; 10^{-5} M: 52 ± 8 pg/mg, $P < 0.05$, $n = 13$) as compared to basal release (26 ± 4 pg/mg) (Fig. 2).

Effect of cholinergic mechanisms on release of somatostatin-like immunoreactivity

Acetylcholine is one of the major enteric neurotransmitters and accordingly the role of cholinergic mechanisms was studied using the muscarinic agonist carbachol, the nicotinic agonist 1,1-dimethyl-4-phenylpiperazinium (DMPP) and the selective muscarinic M_1 receptor agonist McN-A-343. Carbachol (10^{-8}–10^{-5} M) stimulated release of somatostatin in a dose-dependent manner (Fig. 3A) (basal: 21 ± 3 pg/mg, carbachol 10^{-8} M: 38 ± 11 pg/mg, not significant; carbachol 10^{-7} M: 54 ± 8 pg/mg, $P < 0.01$; carbachol 10^{-6} M: 63 ± 6 pg/mg, $P < 0.001$; carbachol 10^{-5} M: 53 ± 6 pg/mg, $P < 0.01$, $n = 12$). In the presence of atropine the stimulatory effect of carbachol (10^{-6} M) was significantly inhibited (carbachol 10^{-6} M + atropine 10^{-6} M: 35 ± 6 pg/mg, $n = 12$, $P < 0.001$) (Fig. 3B). The muscarinic M_2 receptor antagonist AFDX 116 (10^{-6} M) attenuated the carbachol-induced release of somatostatin-like immunoreactivity (carbachol 10^{-6} M + AFDX 116 10^{-6} M: 41 ± 11 pg/mg, $n = 5$, not significant). The muscarinic M_1 receptor antagonist pirenzepine did not influence the carbachol-induced release of somatostatin-like immunoreactivity (carbachol 10^{-6} M + pirenzepine 10^{-7} M: 59 ± 13 pg/mg, $n = 8$) (Fig. 3B).

The selective muscarinic M_1 receptor agonist McN-A-343 had no stimulatory effect on somatostatin release (McN-A-343 10^{-6} M: 49 ± 19 pg/mg, $n = 3$, not significant).

Hexamethonium slightly reduced the carbachol-induced somatostatin release (carbachol 10^{-6} M: 63 ± 6 pg/mg; carbachol 10^{-6} M + hexamethonium 10^{-4} M: 46 ± 5, $n = 12$, not significant). 1,1-Dimethyl-4-phenylpiperazinium (DMPP) also caused only a small increase of somatostatin levels which did not reach statistical significance (basal: 33 ± 5 pg/mg; 10^{-4} M: 46 ± 11 pg/mg, $n = 4$).

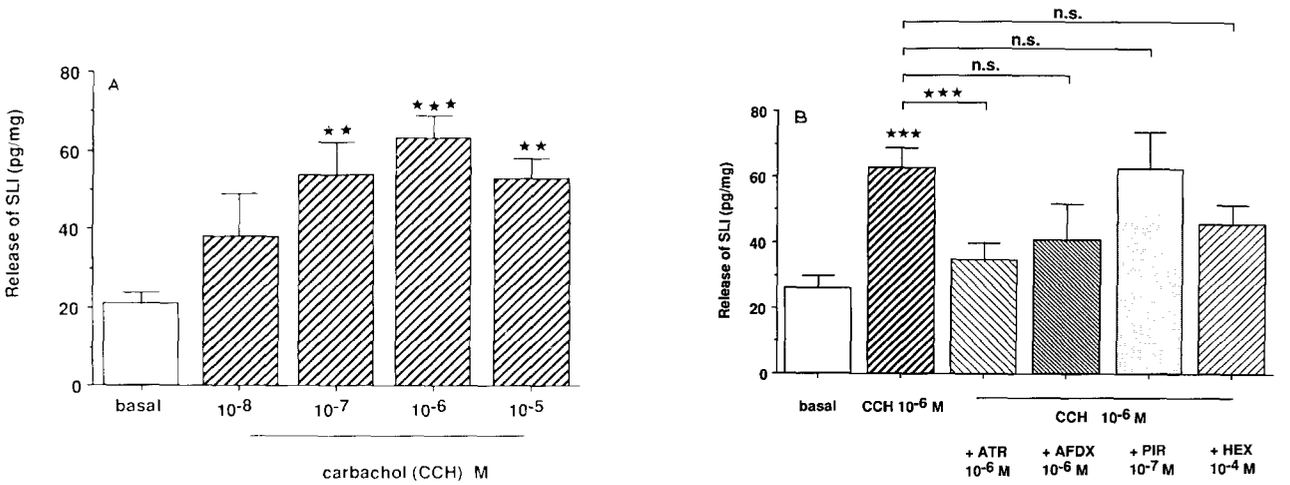

Fig. 3. (A) Dose-dependent effect of carbachol (CCH) on the release of somatostatin-like immunoreactivity (SLI) from enriched synaptosomes of the rat ileum. The histogram gives means ± S.E.M. of $n = 12$ independent experiments, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. (B) Effect of atropine (ATR 10^{-6} M, $n = 12$), pirenzepine (PIR 10^{-7} M, $n = 5$), AFDX 116 (AFDX 10^{-6} M, $n = 4$) and hexamethonium (HEX 10^{-4} M, $n = 12$) on release of somatostatin-like immunoreactivity (SLI) in the presence of carbachol (CCH 10^{-6} M, $n = 12$). The values are given as means ± S.E.M. Statistical comparison was performed using analysis of variance or Student's t-test for paired samples (n.s.: not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Table 1
Effect of neurotensin (NT, $n = 12$), neurokinin B (NKB, $n = 6$), 5-hydroxytryptamine (5-HT, $n = 6$), cholecystokinin-8 (CCK-8, $n = 5$), caerulein (CAE, $n = 5$) and pentagastrin (PENT, $n = 5$) at the doses indicated on the release of somatostatin-like immunoreactivity from enriched enteric synaptosomes

	NT	NKB	5-HT	CCK-8	CAE	PENT
Basal	37± 8	38± 7	38± 7	35±12	35±12	35±12
10^{-10} M	n.d.	n.d.	n.d.	34±13	n.d.	n.d.
10^{-9} M	33± 8	36±10	32± 9	n.d.	31± 9	n.d.
10^{-8} M	38±13	31± 9	36±11	34±12	31±13	n.d.
10^{-7} M	36±12	n.d.	38±11	n.d.	35±12	33±11
10^{-6} M	37±11	34± 8	36±10	35± 7	31±12	33±13
10^{-5} M	n.d.	n.d.	33± 9	n.d.	n.d.	n.d.

Values are expressed as pg/mg tissue, means ± S.E.M. n.d. = effect at that particular dose not determined.

Effect of capsaicin

In the presence of capsaicin the level of somatostatin-like immunoreactivity did not differ significantely from the respective basal value (basal: 36 ± 5 mg/pg; capsaicin 10^{-6} M: 43 ± 12 pg/mg, $n = 10$).

Effect of various presynaptic modulating substances

Changes of somatostatin release were investigated in the presence of increasing concentrations of the neurokinin 3 receptor agonist [β-Asp^4,MePhe^7]neurokinin B-(4–10), 5-hydroxytryptamine, neurotensin, cholecystokinin-8, caerulein and pentagastrin, as these transmitters were either reported to stimulate somatostatin in other systems or to be involved in synaptic events. None of these putative presynaptic agonists had a stimulatory effect on somatostatin release at the particular dose depicted in Table 1.

4. Discussion

This study describes for the first time the release of somatostatin-like immunoreactivity from synaptosomes isolated from the enteric nervous system. The synaptosomal fraction used for release studies showed substantial enrichment of somatostatin content. The synaptosomes are capable of releasing somatostatin in response to membrane depolarization using KCl or veratridine, in response to Ca^{2+} ionophore A-23187 and in response to cholinergic stimulation with carbachol. There was no significant effect of hexamethonium on the carbachol-induced release of somatostatin-like immunoreactivity and also no effect of the nicotinic receptor agonist 1,1-dimethyl-4-phenylpiperazinium (DMPP) on the basal somatostatin release suggesting that nicotinic mechanisms are probably not involved in the synaptic regulation of somatostatin release.

The mechanisms of somatostatin release from neuronal elements of the gut have mainly been studied in in vivo or in vitro models with an intact neuronal network of the myenteric plexus, in cultured myenteric neurons or in isolated myenteric ganglia. To date experimental data on the Ca^{2+} requirements of somatostatin release and depolarization-induced release of somatostatin from enteric neurons are not available. In the central nervous system somatostatin release can be stimulated by K^+ depolarization from in vitro preparations (Berelowitz et al., 1978; Parel, 1978; Robbins et al., 1982a) and in synaptosomes (Bennett et al., 1979). A dose-dependent release of somatostatin by depolarizing agents such as KCl, veratridine, oubain and the Ca^{2+} ionophore A-23187 was demonstrated in cell dispersions of mouse hypothalamic cells (Richardson and Twente, 1985). The release was diminished or abolished by omission of extracellular Ca^{2+}, chelation of extracellular Ca^{2+} using EGTA or in the presence of the Ca^{2+} channel blocker verapamil. The depolarization or A-23187-induced somatostatin release was reduced in the presence of the calmodulin inhibitors trifluperazine, or the naphthalene sulfonamide calmodulin inhibitor W7 suggesting a role of calmodulin in the release process (Richardson and Twente, 1985). Considering our data there is no reason to suspect the enteric neuronal somatostatin-like immunoreactivity is released any differently from its counterpart in the brain.

Furthermore the results of our study suggest the existence of an excitatory muscarinic presynaptic receptor as the carbachol-induced release of somatostatin-like immunoreactivity was reduced by atropine. In a recent paper Grider (1989) described somatostatin release induced by the nicotinic receptor agonist 1,1-dimethyl-4-phenylpiperazinium (DMPP) in isolated ganglia of the guinea pig small intestine and from muscle strips. These results are not in contrast to the negative findings of our study using DMPP, as in synaptosomes the neuronal circuitry is disrupted. In the muscle strips and the isolated ganglia, the synaptic connections are intact and thus the stimulatory effect could be due to receptors on the cell soma of the somatostatin containing neurons but could also be due to nicotinic stimulation of an excitatory interneuron. Furthermore interspecies difference may play a role.

The experiments with muscarinic M_1 and M_2 receptor antagonists as well as the specific M_1 receptor agonist McN-A-343 in our preparation suggest that the stimulatory effect could be due to an activation of a muscarinic M_2 receptor subtype. So far data on excitatory presynaptic muscarinic receptors regarding peptide release in the enteric nervous system are rather sparse. Presynaptic receptors identified either by muscle strip experiments or by electrophysiological studies were mainly regarded as inhibitory mediating a short loop inhibition (Kilbinger, 1984; Kilbinger and Nafziger, 1985). There is, however, electrophysiological evidence

for the presence of excitatory muscarinic receptors which are present on about 25% of all intrinsic neurons of the guinea pig ileum (North and Tokimasa, 1982) and there is also functional evidence from release studies of other transmitters such as acetylcholine of the existence of presynaptic muscarinic receptors which facilitate neurotransmitter release (Kilbinger, 1984; Kilbinger and Nafziger, 1985). From our data it could be speculated that part of the presynaptic muscarinic inhibitory effects observed in in vitro experiments (Richardson and Twente, 1985; Richardson et al., 1980) are due to the release of an inhibitory mediator such as somatostatin. This could partly explain the controversial data concerning the release of somatostatin in the central nervous system in response to cholinergic stimuli. Acetylcholine stimulated somatostatin release from cultured cortical cells and this effect was atropine sensitive (Robbins et al., 1982b), whereas in the hypothalamus stimulation with acetylcholine showed no effect (Maeda and Frohmann, 1980) or a inhibition (Richardson et al., 1980) of somatostatin release.

In the central nervous system the effects of various neuropeptides on somatostatin release have been investigated. Serotonin (10 pM–1 μM) had no influence on somatostatin release from cultured cortical cells (Robbins et al., 1982b) and from hypothalamic tissue (Chihara et al., 1979; Maeda and Frohmann, 1980). Neurotensin showed a consistent stimulatory effect on somatostatin release in the central nervous system (Maeda and Frohmann, 1980; Sheppard et al., 1979). In our model serotonin was also ineffective stimulating release of somatostatin-like immunoreactivity, in contrast neurotensin did not stimulate somatostatin release from enteric synaptosomes. This suggests a different local regulation between central and enteric nervous system. Further investigation in this topic is needed. Other substances known to presynaptically modulate neurotransmitter release as cholecystokinin, caerulein or the tachykinin NK_3 receptor agonist [β-Asp^4,MePhe^7]neurokinin B-(4–10) did also not stimulate somatostatin release from enriched enteric synaptosomes.

Nerve endings from extrinsic afferent sensory neurons terminating in the gut wall contain somatostatin (Hökfelt et al., 1975b). These nerves are capsaicin-sensitive, whereas intrinsic neurons are resistant to capsaicin (Holzer, 1988). In the present study there was no consistent effect of capsaicin on somatostatin release, thus the somatostatin-like immunoreactivity released from the enriched synaptosomes is supposed to be confined to intrinsic nerves. In this context, it is noteworthy that in our experimental system synaptosomes of both the submucosal and the myenteric plexus are present, and therefore different properties of somatostatin release cannot be ruled out. Further studies are necessary to clearly identify release patterns of submucosal and myenteric neurons. Furthermore two molecular forms of somatostatin with 28 and 14 amino acids are present in the gastrointestinal tract. In the present study the molecular form of somatostatin was not further characterized. However, it has been demonstrated that the somatostatin released from small intestine and from human enteric neurons in culture was identical with somatostatin-14 (Accili et al., 1993; Schmidt et al., 1993) and similar findings were reported in cortical synaptosomes (Sheppard et al., 1982).

In conclusion, the present study demonstrates the isolation of enriched enteric synaptosomes from rat small intestine and the release mechanisms of somatostatin-like immunoreactivity in response to depolarization and cholinergic stimuli.

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