LPV I, on the other hand, indicates transformation into smaller components within the oocytes during vitellogenesis. It should be recalled that by the time of egg-laying the heaviest elements had completely disappeared. It is unlikely that the ovary participates in the synthesis of all or part of the LPVs, as it has been proposed in some decapods[8]. In fact, LPV labelling happens very late, only starting to appear in vivo 2–2.5 h after injection of ^{3}H-leucine, that is, much later than VTG labelling (about 45 min)[5]. It is highly probable that the labelling of LPVs is the consequence of the entrance of labelled VTG into oocytes.

Finally, it is notable that these rearrangements are intramolecular: they do not affect the mol.wt of LPV I, which remains stable throughout vitellogenesis (350,000) and hardly differs from that of VTG (400,000)[3]. The physiological significance of this process remains unknown.

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Cytoprotection – organoprotection by somatostatin: gastric and hepatic lesions*

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Summary. In rats, the hemorrhagic gastric erosions produced by ethanol, and the fatal hemorrhagic hepatic necrosis induced by phalloidin, were significantly reduced by regular somatostatin, but not by derivatives devoid of -SH containing cysteines. These effects of the hormone were abolished in animals which received, in addition, the sulfhydryl blocker n-ethylmaleimide before the toxic chemicals. Thus, somatostatin exhibits organoprotection dependent on endogenous sulfhydryls.

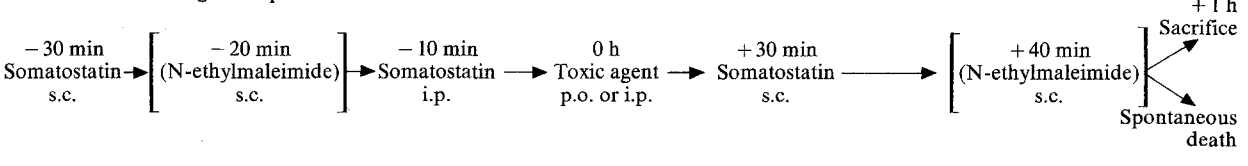
Prostaglandins (PG) were recently shown to exhibit gastric cytoprotection, that is to prevent various gastric lesions such as those induced by ethanol, aspirin, acids or alkali[1,2]. The cytoprotective effect (i.e., inhibition of superficial, hemorrhagic mucosal erosions) of PG differs from the well known anti-ulcerogenic action (i.e., directed against well-formed gastric and duodenal ulcers) of these compounds in that only the latter effect is associated with inhibition of gastric acid secretion[1,2]. The mechanisms of the cytoprotective effect of PG are unknown[3–5]. Local cytoprotection is also offered by other drugs, e.g., cimetidine, probanthine[6] and sulfhydryl-containing chemicals[7].

Somatostatin, besides exhibiting an anti-ulcerogenic effect[8–11], also reduces intoxications involving other organs, e.g., pancreas[12–14], liver[15,16], adrenals and lungs[17,18]. We reported that endogenous sulfhydryls (e.g., glutathione) might mediate the cytoprotective effect of PG[7], and have now recognized that -SH groups are also present in somatostatin, which exerts potent local gastric cytoprotection. We hereby present data and a hypothesis that somatostatin, due to its inherent structure, may provide a systemic or generalized cytoprotection (i.e., histoprotection, organoprotection).

Materials and methods. In the present studies Sprague-Dawley rats with an initial b.wt of 200 g had free access to Purina lab chow and tap water. Each control and experimental group consisted of 3–5 animals; every experiment was performed at least twice and the results were pooled. Somatostatin (Serono) was either dissolved in the original, commercially provided (Serono) protamine sulfate and $ZnCl_2$ for s.c. injection to delay absorption and to prolong the biological half-life of the hormone, or solubilized in distilled water for i.p. administration and for rapid, short-time elevation of blood somatostatin levels. The general design of experiments is presented in table 1.

In the 1st study following an overnight fast, rats recieved s.c. somatostatin, 10 µg/100 g as a 0.2 ml of protamine sulfate and $ZnCl_2$ suspension, 30 min before 1 ml of 100% ethanol p.o. The sulfhydryl blocker N-ethylmaleimide (Sigma) was injected s.c. at the dose of 5 mg/100 g, 10 min after somatostatin (or 20 min before ethanol). A derivative of somatostatin (bis-S-Acm Somatostatin, L361, 728, Merck or di-S-tBu-Somatostatin, Serono) in which the -SH groups (cystein) were replaced, was also injected at 10 µg/100 g s.c., 30 min before ethanol. The animals were killed 1 h after ethanol administration and the lesions in the stomach

Table 1. General design of experiments



were graded on a scale of 0–3 (0 = normal, 1 = 1–4 small petechial hemorrhages in a diameter of about 1 mm; 2 = 5 or more petechiae and hemorrhagic streaks of 1–4 mm; 3 = erosions 5 mm or longer and/or confluent bleeding areas). Seven standardized sections of stomachs (2 from junction of forestomach and glandular stomach, 2 from glandular area, 2 from glandular stomach and antrum and 1 from pylorus including antrum and duodenum) were fixed in 10% buffered aqueous formaldehyde and processed for light microscopic examination.

In the 2nd study 250 µg of somatostatin in 1 ml of protamine sulfate and $ZnCl_2$ suspension were injected s.c., 30 min before and 30 min after the administration of phalloidin (kindly supplied by Dr H. Faulstich, Max Planck Institute, Heidelberg, FRG) 0.12 mg/100 g i.p. In addition, 10 min before the toxin, another dose of 250 µg of somatostatin in 1 ml of distilled water was given i.p. Other groups of rats also received the sulfhydryl blocker N-ethylmaleimide (alone or in combination with somatostatin), 2 mg/100 g s.c., 20 min before and 40 min after phalloidin. The -SH free derivative of somatostatin (Serono) was also used instead of the regular somatostatin in the same dose and time. Groups of rats were either sacrificed 1 h after intoxication, or were observed for mortality (which invariably occurred within 2–4 h) and survivors were killed in 24 h. The hemorrhagic liver lesions were evaluated grossly and microscopically on a scale of 0–3, where 0 = normal, 1 = multifocal (1–5 mm) hemorrhages, 2 = large hemorrhagic areas entirely involving one (usually the left and/or medium) lobe, 3 = enlargement and uniform hemorrhage involving the whole liver.

The results of gastric and hepatic lesions are expressed as mean ± SEM and they were evaluated for statistical significance by the 2-tailed Student's t-test, with correction for multiple comparisons.

Results. Results of the 1st study (table 2) revealed that the regular somatostatin significantly reduced the intensity of the gastric mucosal lesions. On the other hand, injection of somatostatins with their -SH groups replaced, or the sulfhydryl blocker N-ethylmaleimide alone or with regular somatostatin had no cytoprotective effect in the ethanol model.

Table 2. Effect of somatostatin and N-ethylmaleimide on the gastric lesions produced by ethanol

Group	Pretreatment (before ethanol)	Gastric erosions (Scale: 0–3)
1	None	2.7 ± 0.2
2	Somatostatin	1.5 ± 0.2*
3	N-Ethylmaleimide (N-EM)	3.0 ± 0.1
4	Somatostatin + N-EM	2.9 ± 0.2
5	bis-S-Acm-somatostatin	2.6 ± 0.1
6	di-S-tBu-somatostatin	2.5 ± 0.2

Each group consisted of 6–10 rats. Values are expressed as mean ± SEM. * = $p < 0.05$, compared to group 1.

Table 3. Effect of somatostatin and N-ethylmaleimide on the toxicity of phalloidin

Group	Pretreatment (before phalloidin)	Hepatic lesions (Scale: 0–3)	Mortality (%)
1	None	2.9 ± 0.1	100
2	Somatostatin	0.7 ± 0.2*	20*
3	N-Ethylmaleimide (N-EM)	2.3 ± 0.2	90
4	Somatostatin + N-EM	2.1 ± 0.3	86
5	di-S-tBu-somatostatin	2.7 ± 0.2	92

Each group contained 6–10 rats. Values for hepatic lesions are presented as mean ± SEM. * = $p < 0.05$, compared to group 1.

The 2nd study (table 3) showed the well known focal or generalized hemorrhagic enlargement and necrosis of the liver found in rats given phalloidin alone as described previously[16,19]. These lesions as well as the mortality were significantly diminished by somatostatin treatment. In contrast to this, N-ethylmaleimide counteracted the protective action of ordinary somatostatin, and the sulfhydryl-free derivatives of somatostatin were without effect.

Discussion. These experiments suggest that somatostatin reduces certain organ lesions (e.g., gastric erosions, hepatic necrosis) caused by chemicals. Previous results from our laboratories and from the literature also showed a protective effect against damage to the pancreas[12–14], adrenals and lungs[17,18]. The action of somatostatin, like that of PG, on the stomach is both antiulcerogenic in experimental animals and man, and cytoprotective. The systemic cytoprotective (or organoprotection) might not actually be limited to specific organs, but may involve a widespread tissue like the cardiovascular system and vascular endothelium which could be the substrate for the 'anti-shock' effect[13,17,18] of somatostatin (e.g., pleural effusion, ascites, edema and hemorrhage in the liver, pancreas, lungs and adrenals).

Since these local (e.g., gastric protective) actions of somatostatin and PG are similar, it is conceivable that one of them is the endogenous, ultimate mediator of the other. A recent report[20] suggests a PG-sensitive release of somatostatin from the gastric mucosa. We have reported that sulfhydryl compounds like cysteamine which exerted gastric cytoprotection[7] also released somatostatin from gastric and duodenal mucosa[21,22]. Hence, it is possible that some of these actions of PG or sulfhydryl drugs might be mediated and/or modulated by somatostatin. It remains to be seen whether these pharmacologic actions of somatostatin may have physiologic roles as well, especially concerning the separation of endocrine and organoprotective effects of the hormone. Further research is also needed to measure the endogenous levels of protein and nonprotein sulfhydryls to assess whether tissue thiols and/or sulfhydryls in somatostatin are involved in somatostatin-induced organoprotection. Additional studies with somatostatin antibodies should also provide information about whether endogenous somatostatin is important in resistance against certain chemically-induced gastric and hepatic lesions.

The mechanism of action of somatostatin and PG is not completely understood, although our present and recent[7,21,22] results indicate that endogenous sulfhydryls may mediate or modulate these effects. The vascular endothelium might be a possible target organ for the systemic protective action of somatostatin. The presence of sulfhydryls in somatostatin itself seems to be important (e.g., for optimal configuration, reactivity or stability of the hormone and for receptor interactions). Since somatostatin derivatives which are devoid of -SH lack organoprotective properties, a further search is required for somatostatin analogs which may exert only minimal or no endocrine actions while providing short or long lasting histoprotection or organocytoprotection.

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Calcium causes the biphasic dose-response curve for pancreatic amylase secretion

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Summary. High concentrations of bethanechol (10^{-4} to 10^{-3} M) were effective stimulants of amylase secretion from the mouse pancreas if incubations are performed in low [Ca^{2+}] (0.1 mM) solutions but not if normal Krebs solution (2.56 mM Ca^{2+}) was used. This inhibitory effect of Ca^{2+} at high secretagogue concentrations did not appear to be mediated through the microtubules or microfilaments.

Agonists which act on muscarinic or cholecystokinin receptors of pancreatic acinar cells stimulate amylase secretion when their concentration is low, but are not such effective secretagogues when they are used at higher concentrations[1-3]. This decrease in secretion with high concentration of agonists is not due to tachyphylaxis or some other event in receptor activation, but is a post-receptor phenomenon[1,3]. Savion and Selinger showed that damage occurs to the apical area of the acinar cells when they are exposed to high concentration of secretagogues and they suggest that this leads to an impairment of secretion[1]. Williams[3] suggested that Ca^{2+} is the 2nd messenger responsible for inhibition of secretion, but it is possible that a Ca^{2+}-independent phenomenon, such as membrane changes resulting from secretagogue-induced phosphatidylinositol hydrolysis[4], may explain the impaired secretory response to high concentrations of agonists. The experiments described in this paper were undertaken in an attempt to elucidate the mechanisms underlying this biphasic dose-response curve.

Methods. Male mice were starved for 16 h (with water ad libitum) and then killed by cervical dislocation. Pancreata were removed and cross cut (0.6 mm) with a McIlwain tissue chopper. Tissue pieces were washed in Krebs solution for 2 × 10 min (extended to 3 × 10 min in experiments using colchicine), and incubated for 30 min at 37 °C in 5 ml of Krebs which contained bethanechol at appropriate concentrations. A sample of the incubating medium was

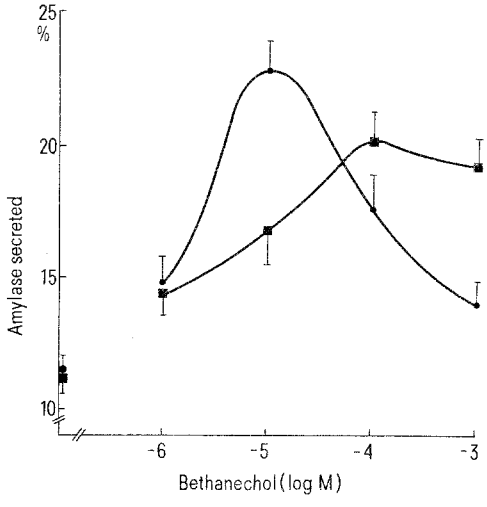

Figure 1. Effect of extracellular calcium concentration on amylase secretion by fragments of mouse pancreas. Tissues were incubated in Krebs solution containing either 0.1 mM $CaCl_2$ (■) or 2.56 mM $CaCl_2$ (●). Each point represents the mean ± SE for 8 or 9 samples. Amylase secretion is expressed as a percentage of total amylase present in the tissue.

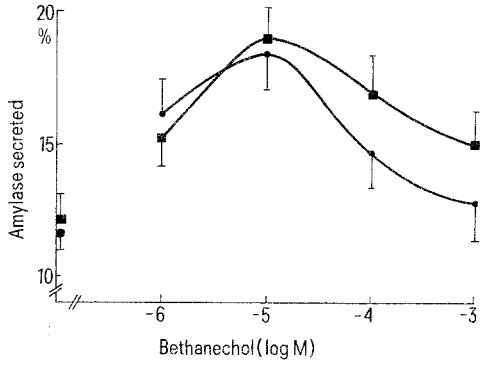

Figure 2. Effect of cytochalasin B and colchicine on amylase secretion by the mouse pancreas. Tissues were pre-washed and incubated in the presence of either 1.1×10^{-6} M cytochalosin B (■) or 10^{-4} M colchicine (●). Each point represents the mean ± SE for 8 or 9 samples.