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SUBMANDIBULAR GLAND PEPTIOES AND THE MODULATION OF ANAPHYLACTIC AND ENDOTOXIC REACTIONS

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SUMMARY

• *Sitbmandibular glandpeptide-T (SGP-T), a hepta-peptide with the sequence ofthreonine, aspartate, isoleucine, phenylalanine, glutamate, glycine, gfycine (TDIFEGG), was isolated from the Sitbmandibular glands of rats based on the ability of extracts of these glands to reduce the hypotension induced by bacterial lipopolysaccharide. SGP-T was also found to decrease the severity of the cardiovascular shock provoked by antigen administration to ovalbumin-sensitized rats. An analysis of the structure-activity relationship re vealed that three amino acids, phenylalanine, gluta mate,glycine (PEG), located in the carboxy terminal of SGP-T were sufficient to inhibit intestinal anaphylaxis* in vitro. *Inter estingly, the D-isomeric form of PEG (feG) did not inhibit anaphylaxis in the* in vitro *assay. However, both tripeptides, FEG and feG, significantly reduced anaphylactic hypotension and intestinal anaphylaxis* in vivo. *SGP-T may be a prototype of a family of small peplides that modulate the immune and smooth muscle reactions to severe inflammatory stress. SGP- T preferentially inhibits cardiovascular anaphylaxis, whereas feG exhibits a high degree of selectivity for inhibiting intesti nal anaphylaxis* in vivo. ***(Biomed Rev 1998; 9: 101-106)***

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INTRODUCTION

• Even though endocrine secretion from the salivary glands was described over 40 years ago, these glands are still primarily considered as accessory digestive organs that also have a primary role in the maintenance of oral health. Although this latter role is of utmost importance, as reduced exocrine salivary secretions may cause important and debilitating distur bances to oral homeostasis (1,2), a more subtle but nonetheless consequential ro le for the salivary glands in the regulation of the *milieu interiew* is now appreciated and accepted. A variety of studies, some of which are reviewed in this volume, attest to the importance of salivary endocrine secretions in modulating a variety of morphological, immunological and physiological functions that have an important impact on systemic health.

Salivary endocrine secretions are involved in maintaining the structure and function of the digestive tract (3-5), mammary glands (6), liver (7-9), reproductive tract (10,11), and the immune system. Through the secretion of a variety of growth factors, salivary glands exert modulatory effects on lymphocyte (12,13), mast cell (14,15), neutrophil (16,17), and macrophage (18) functions. The submandibular glands (SMG) also regulate inflammatory reactions associated with the late-phase pulmonary inflammation induced by allergen in sensitized rats (19,20). Closely associated with the regulatory actions of salivary gland endocrine secretion and modulation of immune system function is the modulation of physiological functions by the salivary glands. By far the most intensely studied is the role that the salivary glands play in esophageal (21) and gastric ulcers (3,22) whereas more recent studies have shown that these glands participate

in the regulation of cardiovascular responses to endotoxic (23) and anaphylactic (24) shock.

The purpose of this short review is to illustrate the importance of the salivary glands, and in particular some small peptides isolated from them, in the regulation of acute pathophysiologi-cal sequelae provoked by endotoxic and allergic reactions.

REDUCTION OF ENDOTOXIC HYPOTENSION BY SALIVARY GLAND PEPTIDES

• Several years ago, we found that removal of the SMG exacerbates the severity of the hypotension provoked by the intravenous administration of a bacterial endotoxin, lipopolysaccharide (LPS), to rats (25). After this observation we initiated a series of experiments to isolate and identify the antiendotoxic shock factors, and much to our surprise we were not dealing with any of the growth factors (molecular weight over 6 kD), but rather with material that had a molecular weight of less than 2 kD. Several SMG peptides were identified thatreduced the severity of endotoxic hypotension (23): the pentapeptide SGP-S, sequence of Ser-Gly-Glu-Gly-Val (SGEGV) and theheptapeptide SGP-T, sequenceofThr-Asp-Ile-Phe-Glu-Gly-Gly(TDIFEGG). Although the parent protein for these peptides was not isolated or identified, a Genbank search (26) identified these two sequences in a preprohormone found exclusively in the rat SMG. SGP-S is found closer to the amino terminal of this protein, submandibular rat 1 protein(SMRl)(27,28)whereasSGP-Tislocatedveryclose to the carboxy terminal. SMR1 isapreprohormonethatprovides several maturation peptides, an undeca-, a hexa- and a penta peptide, that are under multifactorial endocrine control and released both locally into the oral cavity and systemically (29,30).

Sialadenectomized rats consistently exhibited a more severe hypotensive response to LPS than unoperated rats (25). With the unoperated rats, SGP-T at a dose of 100lag/kg effectively prevented the characteristic LPS-induced drop in mean arterial blood pressure when given 90 or 30 min prior to LPS, as well as when administered 10 min after LPS injection. Compared with pre-LPS values, overall SGP-T reduced by 60% the decrease and the percent decrease in mean arterial blood pressure induced by LPS. With sialadenectomized rats, SGP-T inhibited endotoxin induced hypotension at doses as low as 1 tig/kg (18). This result implies that a sensitization of the SGP-T receptor occurs upon removal of the SMG.

SGP-S, on the other hand, regardless of the treatment schedule, had no effect on the shock induced by endotoxin in unoperated rats. However, with sialadenectomized rats. SGP-S effectively reduced the severity of endotoxic hypotension by 3 8% relative to the untreated controls. Subsequent studies have concentrated exclusively on SGP-T, and SGP-S remains to be examined in other biological assays.

INHIBITION OF IMMEDIATE HYPERSEMSITIVITY REACTIONS

» Since salivary glands participate in the severity of pulmonary anaphylactic reactions (31). we decided to examine the effects of SGP-T on anaphylactic reactions. Several different anaphylactic models have been explored, and the results of these studies are briefly reviewed.

• **Intestinal anaphylaxis *in vitro***

Using a classical anaphylactic model, the Schultz-Dale reaction, we found that SGP-T *in vitro,* at doses of 6.8 X 10^{-6}M, inhibited by approximately 50% allergen-induced contractions of the jejunal segments obtained from ovalbumin-sensitized rats (Table 1). Subsequent structure activity relationship studies revealed that the tripeptide, PEG, located at the carboxy terminal ofSGP-Talso inhibited, toasimilarextent, intestinal anaphylaxis in the organ bath (32). In contrast, the D-isomeric form of PEG, the tripeptide feG, was inactive.

• **Intestinal anaphylaxis //; *vivo***

SGP-T and the tripeptides were then evaluated for their ability to inhibit intestinal anaphylaxis *in vivo (24,33).* The intragastric administration of allergen to fasted, ovalbumin-sensitized Hooded Lister rats results in a disruption of the fasting pattern of gastrointestinal motility which is characterized by repeated oral-to-aboral propagation of smooth muscle contractions (migrating myoelectric complexes, MMC) that exhibit a periodicity of ~10min(34). Allergen disrupted this rhythmic pattern of myoelectricity to replace it with a continuous apparently unorganized pattern of smooth muscle contractions, and invariably provoked diarrhea, a clinical sign of intestinal anaphylaxis. The disruption of MMC lasted for 30 to 60 min before the normal fasting MMC periodicity was reestablished. Intravenous SGP-T, FEG, and feG at doses of 100lig/kg significantly reduced the allergen-provoked disruption of myoelectric activity and fewer rats developed diarrhea. Furthermore, the oral administration of feG at a dose of 3 50ug/kg totally blocked the anaphylactic reaction.

• **Anaphylactic hypotension**

Using ovalbumin-sensitized rats, SGP-T was administered intravenously at doses ranging from 35 to350ug/kg 10 min prior to intravenous injection of the antigen. In saline-treated rats, the anaphylaxis provoked-drop in blood pressure was reduced significantly, 60% to 70%, by intravenous SGP-T at doses of 35 and 100l.ig/kg. Intravenous FEG and feG, at doses of 1 OOug/kg given 1 Omin prior to challenging the animal with antigen, reduced the anaphylactic hypotension by approximately 50% and 30%, respectively. A dose of 350ug/kg feG, given by gavage one hour before antigen challenge, reduced anaphylactic hypotension by more than 50% (32,33) (Table 1).

Table 1. *Effects ofSGP-T, PEG, andfeG in several anaphylactic reactions*

Peptide	Percent Decrease in Blood Pressure	% Rats with Disrupted MMC[1]	URE/OA Ratio on Rat Jejunum[2]
Control	-45.7	100	5.7±1.0
SGP-T(iv)[4]	±6.5[3] -	29	13.9±3.6
FEG(iv)	9.8±5.6	39	12.1±2.8
feG(po)[5]	-21.0 ±6.2	0	6.5±3.8

'MMCs, migrating myoelectric complexes; [2]URE/OA, ratio of the contractile response of isolated rat jejunum to urecholine (URE) and ovalbumin (OA); [3]mean ± SEM; [4]iv. peptide administered intravenously; [5]po, peptide administered into the stomach.

- **Multiple receptors for SGP-T and feG?**

The results presented above indicate that SGP-T, FEG, and feG are effective in reducing anaphylactic reactions and endotoxic hypotension. Nonetheless, it is apparent that the peptides were not equally effective in all assays. To highlight their differential effectiveness, the ratio of control to experimental (i.e. in presence of the peptides) response was determined for the various bioassays (Table 2). These calculations revealed that SGP-T was most effective in preventing anaphylactic hypotension and the anaphylaxis disruption of MMC. The tripeptide feG was a very potent inhibitor of intestinal anaphylaxis *in vivo,* and substantially less active in the other assays. The tripeptide FEG was generally less effective than SGP-T and feG when these peptides showed high activity, and FEG did not exhibit specificity for any particular reaction as it was equally effective in all assays. These data also indicate that the isolated jejunal segments are not predictive of biological activity *in vivo,* and the whole animal environment appears to provide an additional component to the actions of the peptides. The nature of these additional elements are presently unknown, but could involve actions on nerves or interactions with other endocrine hormones. SGP-T exerts arelatively rapid action, since in numerous assays the inhibitory effect was observed within 10 minutes of peptide administration, and orally administered feG effectively prevented cardiovascular anaphylaxis when given one hour before antigen challenge. Thus, the peptides may have one or more of the following characteristics: (/) a rapid mechanism of action that probably does not require *tie novo* protein synthesis, (//') prolonged receptor binding or long term deactivation of a cellular activation process or slow release from serum binding proteins, and (///') an absence in the stereospecificity of the receptor. Concerning this latter point, receptors are generally believed to exhibit stereoselective actions for their ligands although this constraint does not seem to apply to some peptides. Several D-isomeric forms of peptides have been shown to have greater activity than their L-isomeric congeners. These include: YYW1GIRK-NH2 in inhibitingneutroph.il infiltration in a thioglycolate-induced mouse peritonitis model (35), and acetyl-RRWWCR-NH2, an inhibitor of interleukin-8 (IL-8) in inducing neutrophil chemotaxis and binding of macrophage-inhibitory protein-2(3 to neutrophils (36).

- **Mechanism of reducing anaphylaxis by the SGP-T andfeG**

To examine the effects of the peptides on mast cells, the mesentery was stained with 1 % safranine solution for 2 min, and then small sections were excised and mounted on slides (37). Fifty mast cells per slide were examined and categorized as degranulated if they had a disperse granular appearance or intact when the stained granules were restricted to a defined ovoid shape (Fig. 1). Unstimulated mast cells are generally ovoid in shape and their granules, stained with safranine, are localized and restricted to the interior of the cell. However, upon stimulation with antigen the mast cells take on a more diffuse appear-

***Table** 2. Summary of biological effects of SGP-T, FEG and feG on anaphylactic reactions*

Assav	SGP-T	FEG	feG
Endotoxic hypotension	2.11	2.09	1.06
Anaphylactic hypotension	4.76	- • 2.40	1.58
Intestinal anaphylaxis and MMC	3.48	2.63	5-100[1]
Intestinal anaphylaxis *(in vitro)*	2.39	2.12	1.14

'The 5-100 ratio depends upon whether feG was administered intravenously (ratio = 5) or orally (ratio = 100).

ance and granules are dispersed into the extracellular space. In rats treated with saline, 100 or 350 ug/kg of feG, 68.6%, 68.6%, and 50% of mast cells showed a degranulated appearance, respectively. There was no significant correlation found between the dose of feG given and the percentage of mast cells that were degranulated after anaphylaxis(p=0.524).

The mechanism of action by which SGP-T and feG exert their effects remains unknown, but these peptides neither affect mast cell degranulation nor inhibit the actions of myoactive mediators of anaphylaxis on smooth muscle. An assay of rat mast cell protease II showed that the release of this enzyme from mast cells after antigen challenge was not significantly reduced by SGP-T (24), we were unable at present to find histological evidence that feG acted as a mast cell stabilizer. In regard to inhibition of the mediators of anaphylaxis, SGP-T did not antagonize the myogenic actions of serotonin, histamine or prostaglandin E2 (23), the principle mediators ofjejunal anaphylaxis in the rat (34). Other mechanisms must account for the inhibitory effect of SGP-T and the tripeptides FEG and feG on anaphylaxis. The possibility remains to be considered that these peptides inhibit the synthesized mediators such as platelet activating factor or leukotrienes, whose synthesis is activated when antigen binds to the *IzE* antibodies on mast cells. Nonetheless, other mechanisms may need to be considered. These include actions on the nervous system and modification of cell-cell interactions that participate in hypersensitivity reactions (38).

CONCLUSION

• With the exception of bradykinin, which is generated by kallikreins released from salivary gland and may act locally to modulate blood flow (39), it is generally believed that salivary glands exert their systemic morphological and immunological effects through the release of a variety of polypeptide growth factors (40,41). However, the present and other recent studies (27-30) illustrate that salivary glands are a source of small, mo lecular weight less than IkD, biologically active peptides that can exert effects in the peripheral circulation by very specific mechanisms. These observations will change the perspective by which we examine the role of the salivary glands in regula ting homeostatic responses (42).

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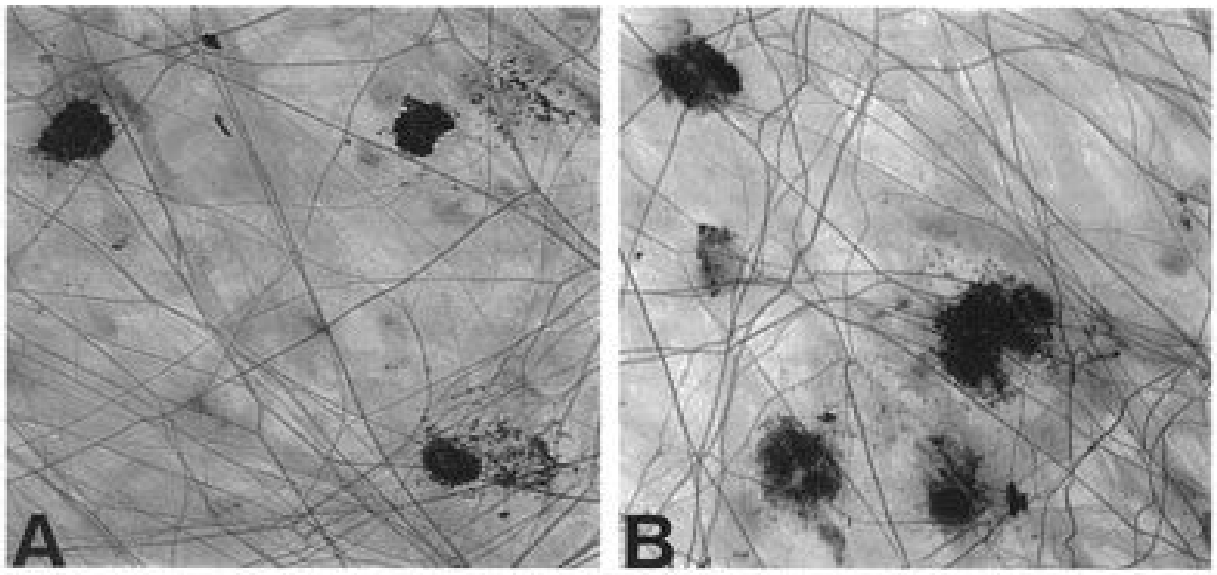

Figure 1. *Mesenleric mast cells stained with 1% safranine 30 min after injection oj antigen to u\'a/biimin-seiisili:ecl rt/ts that were either pretreated with the 0.9% saline vehicle (A) or 350/.ig/kg of the tripeptic/e feG (B). Degranulation of the mast cells is seen in vehicle andpeptide-treated rats,* x *815.*

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