SUMMARY

Structural, biochemical, and pharmacological studies have provided numerous insights into the role of rodent submandibular glands in a variety of physiopathological functions. In this review we briefly highlight past and present findings published by our group and others regarding the role of rodent submandibular glands and nerve growth factor in inflammatory events. Accordingly, the role of mouse salivary glands and nerve growth factor in neuroinflammatory responses, body temperature and parasitic infection are discussed, and potential future lines of studies aimed at elucidating their physiopathological roles are suggested.

INTRODUCTION

The submandibular glands (SMG) of rodents are divided in two different functional and anatomical parts, the acinar and ductal regions. The acinar region contains the secretory cells which produce and release their products in the saliva. The acinar cells are structurally connected to each other by ducts linked to the granulated convoluted tubules, and then to the striated excretory ducts which store the wide variety of biologically active polypeptides.

SMG are innervated by sympathetic, parasympathetic and peptidergic nerves, and each set of terminal fields contributes to the secretory regulation (1-4). Sympathetic activation has been shown to induce high protein secretion, and parasympathetic activation lead to more liquid saliva production (2-4). The SMG of mice contain small intrinsic cholinergic/peptidergic ganglia whose function is not clearly known. Structurally, mouse SMG are formed by highly differentiated epithelial cells capable of secreting more than 25 different biologically active polypeptides (2,5-8). They contribute, with significant overlapping, to several functional activities involved in the regulation of homeostasis and digestion (4,9). There is considerable evidence indicating that growth factors released by SMG can contribute to reestablishing normal tissue function by promoting cell growth and proliferation, and by regulating inflammatory cell processes (9). Among these proteins, nerve growth factor (NGF) is the first polypeptide identified in salivary glands owing to its property of promoting growth and differentiation of peripheral sympathetic and embryonic sensory neurons (10). In the last few years, a number of studies have provided evidence that NGF plays a functional role in forebrain cholinergic neurons, and is also involved in inflammatory events (11-14) inside and outside the nervous system.

The secretion of molecules stored in the SMG cells is regulated by stimuli arising from adrenergic, cholinergic and peptidergic neurons (15,16). Postganglionic sympathetic axons originating in the superior cervical ganglion (SCO) are located in a typical adrenergic ground plexus closely surrounding the SMG acini, while the ducts seem to be completely devoid of adrenergic innervation (17-20). Structural and biochemical studies have shown that adrenergic stimulation results in a depletion of secretion granules containing growth factors, including NGF (17,21). Removal of SCG decreased catecholamine fluorescence and thyroxine hydroxylase immunoreactivity in SCG (22,23), suggesting that, through adrenergic innervation, SCG neurons receive trophic support from the glands. Both α- and (3-adrener-
ergic agents trigger the release of growth factors produced and stored in the glands, although degradation of cells is more strongly induced by α- than by (3-adrenergic stimuli. Exogenous administration of cyclopyrine, a drug stimulating α-adrenergic receptors, produced drastic depletion of NGF from the granular convoluted tubules and increased NGF level in the blood (24). Decentralization of the SCG by surgical lesion of the preganglionic innervation did not significantly affect the SCG neurons (16) and/or the amount of NGF stored in the gland (unpublished observations). Reduction of adrenergic innervation occurs with age, an effect most probably associated with a low availability of NGF in the SMG. Administration of the cholinergic drug pilocarpine to adult rodents induced exocytosis of secretion granules in granular convoluted tubule cells (25). Decentralization or superior cervical ganglionectomy did not significantly affect the amount of substance P (SP) present in the SMG, which implies that this neuropeptide is not regulated by preganglionic or postganglionic sympathetic neurons projecting to the SMG (16). This observation suggests that SP is not associated with the SMG sympathetic innervation, but is anatomically and functionally related to the chorda tympani nerve and possibly involved in the parasympathetic innervation of the gland. Accordingly, sectioning of the chorda tympani nerve results in a significant decrease in SP content of the salivary glands.

The SMG also possess rather small intrinsic ganglia located between the lingual nerve, and the submandibular and sublingual gland ducts (26,27). The role of these intrinsic neurons in the activity of the SMG is not fully known; they are supposed to contribute to the secretory activity of the gland.

**SALIVARY NGF AMD THE ENDOCRINE SYSTEM**

- Although displaying mainly exocrine activity, the SMG also possess an endocrine function. One of the first indications that these glands displayed endocrine activities was reported by Ogata (28) whereas other studies provided evidence that the SMG are able to secrete various proteins (21). They are released by the secretory ducts into the bloodstream, most probably through the fenestrated capillaries underlying the ducts (1). Several studies have shown that NGF is produced, stored and released by the granular convoluted tubules of the SMG, and that its NGF content is higher in male than in female mice (29). The synthesis of growth factors in SMG is hormonally regulated (30). Indeed, testosterone enhances the levels of growth factors, while removal of testis and thyroid gland decreases them (31). Moreover, other studies have revealed that removal of the pituitary gland in rats induces atrophy of the SMG and decreases some immunosuppressive capabilities (32), while SMG extirpation is quickly followed by atrophy of the thymus associated with depletion of the thymus-dependent lymphocytes in the spleen and lymph nodes (33). This effect seems to be independent of age and sex.

An involvement of SMG in endocrine mechanisms is also suggested by behavioral studies. Aggressive behavior causes massive degradation in SMG and release of biologically active compounds such as epidermal growth factor, renin, and NGF (34). As hypothesized some years ago by Levi-Montalcini (10), the mouse salivary NGF seems to be implicated in the regulation of offensive and defensive behaviors. In fact, the increase of blood NGF in fighting mice was found to be associated with the number of agonistic episodes, and is more pronounced in submissive mice than in dominant mice (34-36). Thus the circulating NGF level in submissive mice was increased two-fold compared with dominant animals (3 5). This study also indicates a relationship between the circulatingNGF levels in aggressive mice and the number of fighting episodes, though this relationship is valid only for dominant attacking mice when plasma NGF is analysed in both dominant and submissive animals. The amount of NGF released into the circulation of mice without SMG decreased drastically suggesting that during aggressive behavior, the main source of circulatingNGF is the SMG (3 5). It was also observed that chronic aggressive behavior in mice, which induces a massive release of salivary NGF into the bloodstream, can lead to the production of NGF autoantibodies, thus causing a significant decrease in peripheral sympathetic innervation(37).

Recent studies suggest that adrenergic innervation of SMG participates to some extent in the regulation of NGF release into the circulation. For example, fighting mice, immunosympathectomized with NGF antibody since birth or chemically sympathectomized with 6-hydroxydopamine, still release high levels of NGF into the c irculation (35). Adrenalectomy failed to blockNGF release in fight ing mice, and injections of ACTH or crude extract of adrenal gland or hypophysis in isolated adult male mice did not result in NGF release into the bloodstream, suggesting that mediators released by these glands do not exert a primary role in submandibular NGF release (35,38). A correlation between NGF and endocrine functions is suggested by other findings, indicating that NGF is able to stimulate the pituitary-adrenocortical axis releasing ACTH and glucocorticoids (39), and that adrenal gland hormones alter the synthesis of NGF (38,40). Furthermore, administration of NGF antibodies into rat fetuses, which inhibits the availability and activity of endogenous NGF, induced a significant loss in body growth and a marked neuroendocrine deficit in the offspring (41). Likewise, deleterious neuroendocrine effects of NGF have been found in rabbits and guinea pigs after maternal exposure toNGF antibodies (42). Moreover, reproductive organs store and release significant physiological amounts ofNGF, suggesting that this molecule is implicated in the functional activity of these tissues (43), while changes in NGF concentration were found during late pregnancy or delivery in both plasma and central nervous system (44,45).
NEUROINFLAMMATORY RESPONSES AND NGF

- The first evidence showing that NGF is associated with inflammation was reported by Levi-Montalcini who found that experimentally induced granulomas are characterized by altered levels of NGF (46). Subsequent studies demonstrated that NGF promotes proliferation and degranulation of mast cells, and it was found that a large number of inflammatory diseases is characterized by high local NGF levels (reviewed in 44,45). Salivary NGF enhances vascular permeability (47), promotes differentiation of granulocytes (48), and induces lymphocyte proliferation (49) and mast cell degranulation (50). The evidence that NGF promotes wound healing (51) further suggests that this molecule is involved in the modulation of different inflammatory responses (9). Though the tissues of the immune system are known to be innervated by the sympathetic nervous system (52), the mechanisms regulating the innervation of specific tissues are at present not fully understood. Sympathetic nerve terminals in immune tissues are found to surround blood vessels and T cells areas within lymphoid organs, such as spleen, lymph nodes, and gut-associated lymphoid tissues (53). NGF is also involved in peripheral inflammatory responses (54), playing a crucial role in neuropathologies associated with sensory deficits (55). Our laboratory studies indicate that NGF increases in several neuroinflammatory diseases (44). A high NGF level has been found in patients with rheumatoid arthritis (56), systemic scleroderma (57), lupus erythematosus (58,59), Kawasaki disease (59a), multiple sclerosis (60) as well as in rodents affected by experimental allergic encephalomyelitis (61,62), an animal inflationary disorder model considered to closely resemble the human diseases multiple sclerosis and rheumatoid arthritis. We have also shown that pretreatment with NGF antibody reduces or prevents the development of arthritis induced by carrageenan, suggesting a functional role of NGF in this type of peripheral inflammation (63). Similar results were obtained with arthritic transgenic mice expressing high levels of TNF-cc in knee joints (64).

It has been reported that certain neuroinflammatory diseases may also be characterized by low levels or absence of circulating NGF and, surprisingly, by a concomitant increase in NGF antibodies (64a). This may due to the generation of autoantibodies against NGF by chronic exposure to supranormal NGF amounts in the bloodstream. In fact, evidence supporting this hypothesis was obtained in our laboratory in chronically stressed mice (37). Furthermore, it was recently reported that the NGF level is decreased, whereas the p75 NGF receptor is overexpressed in human atherosclerotic coronary arteries (65).

SIALECTOMY, NGF AND THERMOREGULATION

- It was recently demonstrated that salivary NGF is implicated in the regulation of the temperature set point in adult mice (66). Thus, circulating NGF levels are low in hypothermic and high in hyperthermic mice, whereas injection of purified NGF into the tail vein of normal mice induces an increase in body temperature lasting about 6 hours (66). The observation that body temperature returned to normal values after 6 hours, while the concentration of circulating NGF was still high about 48 hours suggests that NGF may be involved in the early response, most probably via the activation of cells of the neuroendocrine axis (39,67). The hypothesis that NGF plays a role in temperature response is also suggested by other observations. For example, supranormal levels of NGF occur in pathophysiological conditions associated with changes in body temperature (reviewed in 68), such as inflammatory responses (69-71). The mechanism leading to NGF increase as a result of body temperature elevation is at present not known. Since NGF synthesis is known to be regulated by cytokines (69,72), the possibility exists that the high levels of NGF are due to the supranormal expression of these molecules during inflammation.

Other studies have shown that SMG are involved in inflammatory responses (9), and that salivary NGF acts on cells of the endocrine and immune systems (52,73). Because the hypothalamic-mes encephalic NGF and NGF receptors (13,35,74-76), another possibility is that hypothalamic NGF-positive neurons might themselves be involved in these events. This hypothesis is supported by evidence demonstrating that a dramatic increase in the expression of NGF occurs in the hypothalamus in response to infection and high body temperature (77). Interestingly, brain ischemia, which alters body temperature (78), is also characterized by an increase in NGF and mRNANGF, while in-tracerebral administration of NGF antibodies during fetal life induces in the offspring physical and behavioral characteristics associated with deficits in thermoregulation (41). Since NGF acts on a variety of cells (10,14,73), the release of NGF during variation of body temperature may well serve to activate NGF responsive cells associated with homeostatic regulation.

SALIVARY GLANDS AMD PARASITIC INFECTION

- To gain further information about the role of salivary NGF in inflammatory responses, we recently earned out studies on mice infected with the trematode Schistosoma mansoni. Schistosomiasis is a disease that affects more than two million people in countries of South America, Africa, and Asia. A key pathogenetic event in this parasitic infection is the formation of granulomas around schistosome eggs trapped in the intestine and liver (79). Immunological, pharmacological, biochemical, and molecular studies have shown that cytokines secreted by macrophages, eosinophils, mast cells, and Th-1 and Th-2 lymphocytes play a crucial role in the formation of these granulomas (79,80). We have shown that NGF occupies a key position in the regulation of parasitic inflammation. Our studies demonstrated that in addition to immunological changes, Schistosoma manso-
inflammatory cells and/or cytokines remains to be verified. The increase of NGF followed by an accumulation of mast cells around the granulomas (77,81). We also found that the exogenous administration of NGF antibodies significantly reduced the presence of these cells and restored the altered thermoregulation induced by schistosomiasis (66). Our findings also indicated a hyperalgesic effect in infected compared with uninfected animals on the hot-plate (82), whereas this nociceptive effect was not observed in infected-sialectomized animals (83). Since NGF administration induces hyperalgesia(54,55), and NGF is known to increase during chronic infection in the paws and in the brain (81), it has been hypothesized that the altered nociceptive expression is related to an increased level of paw NGF in infected mice, while the absence of hyperalgesia in infected-sialectomized mice show similar NGF levels in the plasma compared with uninfected controls (66). However, whether these latter effects are directly induced by NGF or mediated through the effect of NGF on inflammatory cells and/or cytokines remains to be verified.

CONCLUSION

- Since its discovery, the presence of large quantities of NGF and other growth factors secreted by the mouse SMG has aroused considerable interest in their pathophysiological function. Although salivary glands products are found in the bloodstream under conditions of stress, a still unresolved question is whether under normal conditions SMG have to be considered exclusively an exocrine gland or a gland displaying also endocrine activity. As peripheral blood and endocrine cells are receptive to NGF action, it is highly possible that in appropriate physiological states, SMG-derived growth factors can reach the bloodstream regulating the activity of these cells. This hypothesis is supported by findings reported by our group and others showing that mouse SMG release NGF and other growth factors during aggressive encounters. Because NGF has been implicated in autoimmune inflammatory disorders, a potentially important line of research that might be pursued involves the role of SMG and NGF in Sjögren’s syndrome (SS), an autoimmune inflammatory disease associated with systemic lupus erythematosus (85), characterized by periductal infiltration of mononuclear cells leading to severe immunological, neurological, and functional deficits of the SMG (86). Significant, a recent study indicated that the constitutive level of epidermal growth factor, a peptide also produced in the SMG (4), under went significant changes in SS (87). The availability of a mouse strain that spontaneously develops a systemic lupus erythematosus-like syndrome renders the possibility of addressing the se questions more feasible. Other areas of research involve studying the hypothesis that SMG and/or growth factors secreted by these glands are involved in tumor growth (88), muscular dystrophy (89,90), pulmonary inflammation (91,92), and aging (93).

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