Histol Histopathol (2010) 25: 1317-1330

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Review

The role of neurotrophins related to stress in saliva and salivary glands

Juri Saruta[1,3,4], Sadao Sato[1] and Keiichi Tsukinoki[2,3,4]

[1]Department of Craniofacial Growth and Development Dentistry, Division of Orthodontics, Kanagawa Dental College, Kanagawa, Japan, [2]Department of Maxillofacial Diagnostic Science, Division of Pathology, Kanagawa Dental College, Kanagawa, Japan, [3]Research Institute of Salivary Gland and Health Medicine, Kanagawa Dental College, Kanagawa, Japan and [4]Department of Oral Pathology, Kanagawa Dental College, Postgraduate School of Dentistry, Kanagawa, Japan

Summary. Nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) are well-studied neurotrophins involved in neurogenesis, differentiation, growth, and maintenance of selected peripheral and central populations of neuronal cells during development and adulthood. Neurotrophins, in concert with the hypothalamic-pituitary-adrenal (HPA) axis, play key roles in modulating brain plasticity and behavioral coping, especially during ontogenetic critical periods, when the developing brain is particularly sensitive to external stimuli. Early life events, such as psychophysical stress, affect NGF and BDNF levels and induce dysregulation of the HPA axis, thereby affecting brain development and contributing to inter-individual differences in vulnerability to stress or psychiatric disorders. Immobilization stress modifies BDNF mRNA expression in some organs. We studied the effect of immobilization stress on BDNF and its receptor tyrosine receptor kinase B (TrkB) in rat submandibular glands, and found increased BDNF expression in duct cells under immobilization stress. Upon further investigation on the influence of salivary glands on plasma BDNF using an acute immobilization stress model, we found that acute immobilization stress lasting 60 min significantly increases the plasma BDNF level. However, plasma BDNF elevation is markedly suppressed in bilaterally sialoadenectomized rats. This suggests that salivary glands may be the primary source of plasma BDNF under acute immobilization stress. This report reviews the structure of salivary glands, the role of neurotrophins in salivary glands, and the significance of BDNF in saliva and salivary glands, followed by a summary of the evidence that indicates the relationship between immobilization stress and BDNF expression within salivary glands.

Key words: Brain-derived neurotrophic factor (BDNF), Neurotrophins, Saliva, Salivary gland, Stress

Offprint requests to: Prof. Keiichi Tsukinoki, DDS, Ph.D., Department of Maxillofacial Diagnostic Science, Division of Pathology, Kanagawa Dental College, 82 Inaoka-cho, Yokosuka, Kanagawa 238-8580, Japan. e-mail: ktsukino@kdcnet.ac.jp

Introduction

The salivary glands consist of the major salivary glands, including the parotid, submandibular, and sublingual glands, and numerous minor salivary glands scattered throughout the oral cavity (Mese and Matsuo, 2007). The main role of the salivary glands is to secrete saliva to assist in food digestion, as well as to promote mastication and antimicrobial activities (Pedersen et al., 2002; Doel et al., 2004). They are predicted to have other important roles, as the salivary glands produce a variety of substances.

Salivary glands produce several cell growth factors and play an important role in human health (Tsukinoki et al., 2005). Cell growth factors such as epidermal growth factor (EGF) and nerve growth factor (NGF), in particular, are found in the rat submandibular gland, leading to the acknowledgment of new functions of the salivary glands (Cohen, 1960, 1962). Mouse salivary gland tissue expresses a high level of NGF (Aloe et al., 1986). The NGF family consists of NGF, brain-derived neurotrophic factor (BDNF), and neurotrophins (NT)-3, -4/5, -6, and -7, all of which are collectively referred to as neurotrophins (Lewin and Barde, 1996). However, few reports have described the expression of neurotrophins other than NGF in the salivary gland.

Neurotrophins interact with tyrosine receptor kinase

(Trk) family high-affinity protein kinase receptors. Specifically, BDNF interacts with the TrkB receptor (Lewin and Barde, 1996). This BDNF-TrkB interaction promotes the survival and differentiation of neurons, and is involved in modification of neurotransmission and synaptic plasticity of the central and peripheral nervous systems (Leibrock et al., 1989). BDNF is predominantly found in the hippocampus and is associated with episodic memory (Egan et al., 2003). Immobilization stress reduces mRNA levels of neurotrophins such as NGF, BDNF, and NT-3 in the rat brain, especially in the hippocampus (Ueyama et al., 1997). In contrast, NGF expression is increased in response to stress in the mouse salivary gland (Aloe et al., 1986). The production of various cell growth factors is often increased during episodes of stress to maintain homeostasis in the salivary gland (Aloe et al., 1986; Konturek et al., 1991).

In this review, we describe the structure of the salivary glands, explain the role of neurotrophins in the salivary glands, and elaborate on the significance of BDNF in saliva and salivary glands. We also summarize evidence that indicates the relationship between immobilization stress and BDNF expression within the salivary gland. The effect of immobilization stress on BDNF and TrkB expression in male rat submandibular glands is reported.

Structure of the salivary glands

The human salivary glands basically consist of a secretory portion and a ductal system (Hand, 1980) (Fig. 1). In the secretory portion, there are serous cells and mucous cells, and depending on the composition of these cells, the glands can be classified into serous, mucous, or mixed glands (Hand, 1980). The ductal system consists of ductal epithelia and is classified into intercalated, striated, and interlobular ducts according to morphology. Intercalated ducts have been considered to be the site of origin of many salivary gland tumors (Dardick et al., 1990). Functionally and histologically, the striated ducts resemble the distal renal tubules of the kidneys (Bradley, 1995). The ductal system plays the role of a discharge route for saliva produced in the acinus, as well as contributing to the nature of saliva (Bradley, 1995). Myoepithelial cells stack in the peripheries of acinar cells and intercalated ducts (Hand, 1980). In rats and mice, on the other hand, granular ducts are found between the intercalated and striated ducts (Gresik, 1994). Granular ducts are not found in human salivary gland tissue, but are remarkably well developed in rodent males (Chretien, 1977; Gresik, 1994). The granular ducts are androgen-dependent tissues and the salivary glands in rodents are regarded distinctly as a sex hormone-dependent organ differing from humans (Amano and Iseki, 1998). Human salivary glands are regulated by the autonomic nervous system, rather than hormonal factors. Anatomically, the major salivary glands are located differently in rodents and humans. Therefore, we emphasize the need to consider the differences from humans when analyzing salivary glands of rodents.

Production of saliva

Saliva is a complex secretion: about 90% by volume is secreted by the major salivary glands and the remaining about 10% by the minor glands. These glands are located in every region of the mouth except for the gums and the anterior part of the hard palate. Saliva is sterile when it leaves the salivary glands but ceases to be so as soon as it mixes with the crevicular fluid, remains of food, microorganisms, desquamated oral mucous cells, etc. (Sreebny, 1987). Saliva is first produced by acinar cells, which are largely divided into two types: serous and mucous cells. The serous acinar cells of the parotid gland produce a largely serous secretion. While this gland synthesizes most of the alpha-amylase, it produces less calcium than the submandibular gland. The mucins are mainly produced by the submandibular and sublingual glands, and proline-rich proteins (PRPs) and histatins by the parotid and submandibular glands. The minor salivary glands are made essentially of mucous cells (Pedersen et al., 2002; Mese and Matsuo, 2007). In healthy individuals, the daily production and swallowing of saliva normally ranges from 0.5 to 1.5 L. At rest, secretion ranges from 0.25 to 0.35 ml/min and is

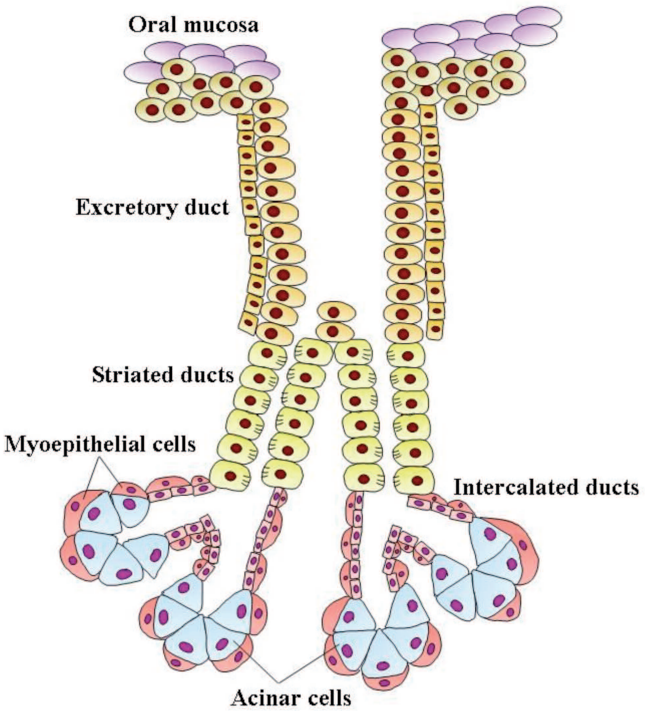

Fig. 1. Basic structure of human salivary gland. Multiple secretory endpieces are connected to the oral cavity through a system of branching ducts consisting of intercalated ducts, striated ducts, and a major excretory duct that merges with the oral mucosa.

mostly produced by the submandibular and sublingual glands. Sensory, electrical, or mechanical stimuli can raise the secretion rate to 1.5 ml/min. The greatest volume of saliva is produced before, during, and after meals, reaching a maximum peak at around 3 p.m. and falling considerably at night while sleeping. Water comprises 99% of saliva and the remaining 1% is composed of organic and inorganic molecules. Saliva is a good indicator of plasma levels of various substances such as hormones and drugs and can therefore be used as a non-invasive method for monitoring plasma concentrations of medicines or other substances (Hofman, 2001; Llena-Puy, 2006).

Salivary secretion related to stress

Salivary secretion is exclusively controlled by the sympathetic and parasympathetic autonomic nervous systems (Hand, 1980). Hormones do not usually initiate salivary secretion (Mese et al., 2007). The parasympathetic nerve is mainly responsible for the secretion of water and electrolytes, while the sympathetic nerve is mainly responsible for the secretion of proteins accompanied by exocytosis in acinar cells (Garrett et al., 1991).

Stimulation of the sympathetic nerve, or ß-adrenergic receptors, causes exocytosis but less fluid secretion (Hand, 1980). Activation of ß-adrenoceptors increases the intracellular level of adenosine 3',5'-cyclic monophosphate (cAMP), the primary second messenger for amylase secretion (Hand, 1980). cAMP is thought to activate protein kinase, also known as PKA. Although the target proteins phosphorylated by PKA have not yet been identified, they may regulate the process by which cells release the contents of their secretion granules. This involves the fusion of the granule membrane with the luminal plasma membrane of the secretory cell followed by rupture of the fused membranes (Turner and Sugiya, 2002). The released content of granules comprises a wide variety of proteins which are unique to saliva and show biological functions of particular importance to oral health. For example, amylase secreted by the parotid gland is a digestive enzyme for starch and glycogen; lipase secreted by the von Ebner's gland may be responsible for fat digestion; lysozyme and peroxidase are representative proteins that have anti-bacterial and/or anti-viral properties; histatins have a potent anti-candidal effect; PRPs, which comprise about 70% of the total protein content of parotid saliva, have roles in lubrication and the acquired pellicle on the surface of teeth together with mucins; and mucins, the major organic component of submandibular and sublingual saliva, are multifunctional glycoproteins involved in mechanical protection and prevention of dehydration of the oral tissues, as well as in lubrication of solid food and trapping of microorganisms (Slowey et al., 1968; Tenovuo and Knuuttila, 1977; Kousvelari et al., 1980; Field et al., 1987; Troxler et al., 1990; Kurahashi and Inomata, 1999).

Two primary neuroendocrine systems have been of specific interest in the study of human stress: the hypothalamus-pituitary-adrenocortical (HPA) system, with the secretion of cortisol, and the sysmpathetic adrenomedullary (SAM) system, with the secretion of catecholamine (Brown and Fisher, 1984; Streeten et al., 1984). In the HPA system, cortisol secretion is regulated by the adrenocorticotropic hormone (ACTH) from the pituitary gland (Brown and Fisher, 1984; Streeten et al., 1984). Salivary cortisol levels are closely correlated to blood cortisol levels and therefore reliably reflect HPA activity (Kirschbaum and Hellhammer, 1994). Many reports have shown that various kinds of psychological stress activate the HPA system and consequently induce significant increases in salivary cortisol levels (Toda et al., 2005). In the SAM system, direct measurements of salivary catecholamine do not reflect SAM activity (Schwab et al., 1992). Recent reports have elucidated various saliva stress markers, such as alpha-amylase, immunoglobulin A (IgA), brain-derived neurotrophic factor (BDNF), and chromogranin A (CgA) (Tsukinoki et al., 2006; Lucas et al., 2007; Okamura et al., 2008; Toda and Morimoto, 2008; van Stegeren et al., 2008). In a previous study, we demonstrated that CgA is produced in human submandibular glands (Saruta et al., 2005). Using immunoelectron microscopy, we showed that immunoreactivity for CgA is localized to secretory granules as well as the saliva matrix of ductal cavities and that CgA is produced predominantly by serous cells and then secreted into saliva.

Currently, it is believed that measurement of these salivary proteins is a useful tool for evaluating the activation of the SAM system (Takai et al., 2004; Kanamaru et al., 2006; van Stegeren et al., 2006; Grillon et al., 2007; Tsukinoki et al., 2007).

Neurotrophins found in the salivary glands

Neurotrophic factors (NTs) are growth factors that act directly on neurons to support their growth, differentiation, and survival. NTs belong to several families of structurally and functionally related molecules, including the NGF superfamily, the glial cell line-derived neurotrophic factor (GDNF) family, and the neurokine or neuropoietin superfamily (Barde, 1990). The NGF superfamily includes NGF, BDNF, NT-3, NT-4/5, and NT-6 (Lewin and Barde, 1996). NGF was discovered in the 1950s as a key player in target-mediated regulation of peripheral innervation (Levi-Montalcini, 1987).

Biochemical, pharmacological, and morphological studies have revealed that NGF is synthesized within the granular convoluted tubules of the submandibular gland soon after puberty and that the female gland produces less NGF than the male gland (Levi-Montalcini and Angeletti, 1968; Levi-Montalcini, 1987). The functional significance of the large amounts of NGF observed in the mouse salivary gland is not fully understood, although past studies have demonstrated that the NGF

produced in the salivary gland is released into the bloodstream following intraspecific aggressive behavior acting on nerve cells, chromaffin cells, mast cells, and lymphocytes (Aloe et al., 1986, 1990, 1994). Moreover, although the salivary gland has been regarded as a major source of NGF and high concentrations of NGF in the submandibular gland have been reported in animal studies (Watson et al., 1985; Mathison et al., 1994), there are currently very few reference values for measuring the expression and localization of NGF in human salivary glands (De Vincente et al., 1998). However, there are many reference values for NGF levels in

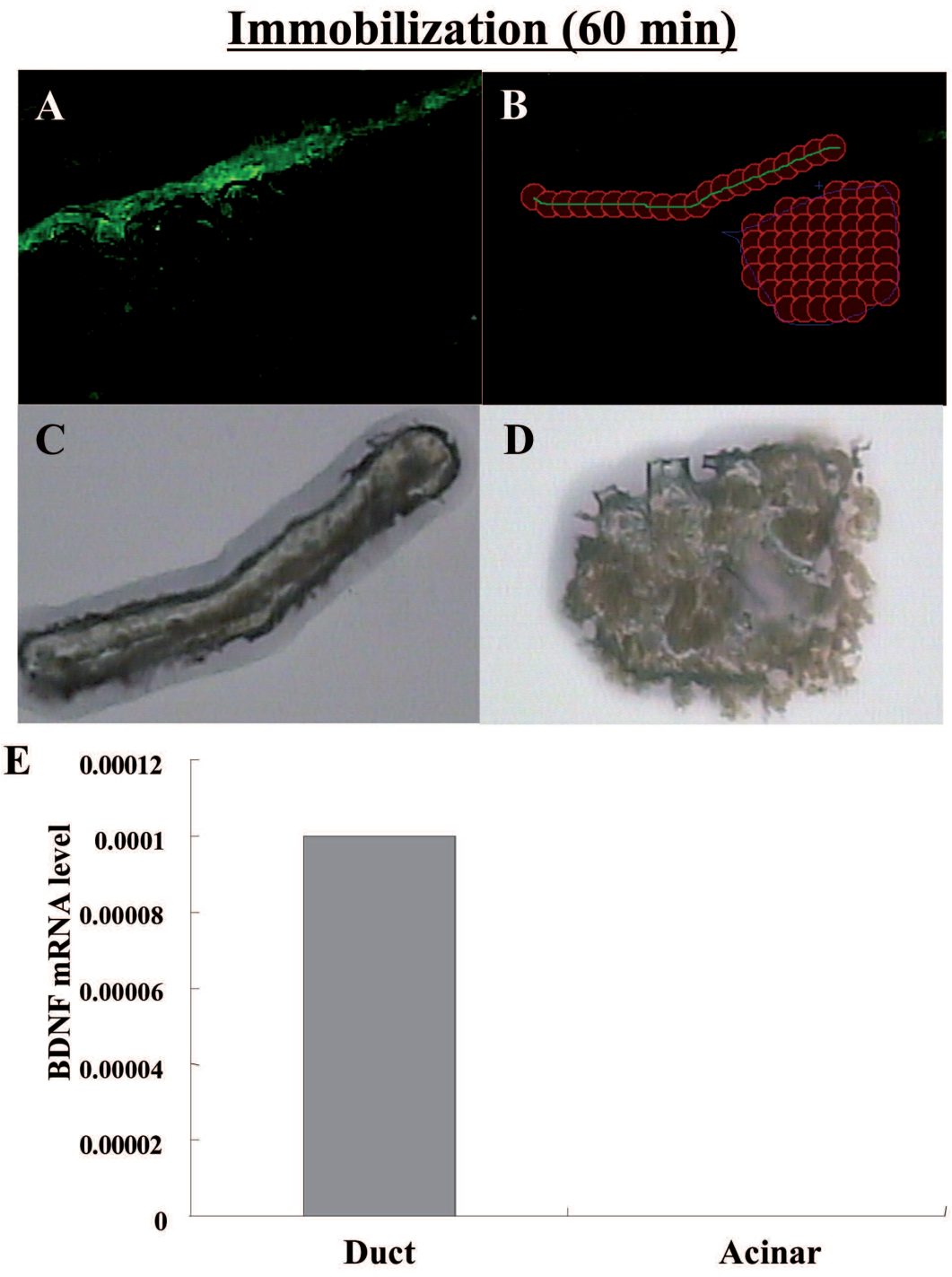

Fig. 2. Microdissection analysis of BDNF mRNA and protein expression in rats following immobilization stress for 60 min. **A.** BDNF reactivity was found within duct cells, but not acinar cells, by immunofluorescence staining (original magnification, x 200). **B.** Duct cells and acinar cells dissected from tissue sections (original magnification, x 200). **C.** Cap image showing dissected duct cells (original magnification, x 400). **D.** Cap image showing dissected acinar cells (original magnification, x 400). **E.** Graph showing BDNF/ß-actin ratios of the dissected samples. BDNF mRNA level was 0.0001 for duct cells, and 0 for acinar cells. Duct cells showed the presence of BDNF mRNA, but acinar cells did not. (modified from Tsukinoki et al., 2006).

human saliva (Glantz et al., 1989; Fischer et al., 1998; Ruhl et al., 2004; Nam et al., 2007). In a previous study, De Vicente et al. (1998) reported the expression and localization of neurotrophin proteins in human and mouse salivary glands using immunohistochemistry (IHC). They investigated 14 human (4 parotid, 6 submandibular, and 4 sublingual glands) and 5 mouse salivary glands. No neurotrophins were detected in human salivary glands. The only neurotrophin found in the mouse salivary glands was NGF (submandibular gland). Although salivary gland localization of NGF in mice has been demonstrated, the expression of NGF is poorly understood in humans.

BDNF, purified from pig brain, is more abundantly expressed and widely distributed than NGF in the CNS, acting as a trophic factor for dopaminergic neurons of the substantia nigra/ventral mesencephalon, in addition to cholinergic ones (Barde et al., 1982; Knusel et al., 1991). In addition to being retrogradely transported, BDNF is also anterogradely transported in the CNS and acts as both a target-derived neurotrophic factor and an autocrine/paracrine modulator (Altar and DiStefano, 1998). At the synapse, BDNF has been shown to play an important role in long-term potentiation (Kafitz et al., 1999; Lu and Chow, 1999; Pang et al., 2004). In the hippocampus in particular, the expression of BDNF varies depending on stress (Givalois et al., 2004), stress + biting behavior (Lee et al., 2008), exercise (Adlard and Cotman, 2004), and learning (Egan et al., 2003), and BDNF plays an important role in facilitating the formation of neural networks. BDNF is also found elsewhere, such as in the lachrymal glands (Ghinelli et al., 2003), lymphocytes (Sobue et al., 1998), and vascular endothelial cells (Nakahashi et al., 2000). As an activity-dependent NT, with receptors densely distributed throughout the CNS, including the limbic system and midbrain, BDNF clearly has emerged as a major regulator of synaptic plasticity (Altar et al., 1994; Ghosh et al., 1994; Duman et al., 1997; Castren, 2004).

Using multiple techniques, we demonstrated increased expression of BDNF mRNA and protein in rat submandibular gland tissue following stress (Tsukinoki et al., 2006). Localization of BDNF protein and mRNA to ductal epithelium was observed in samples of rat submandibular gland tissue in a novel detection system, which combined microdissection of BDNF immunofluorescent-positive cells and quantitative RT-PCR (Fig. 2). Ernfors et al. (1990) were the first to describe BDNF

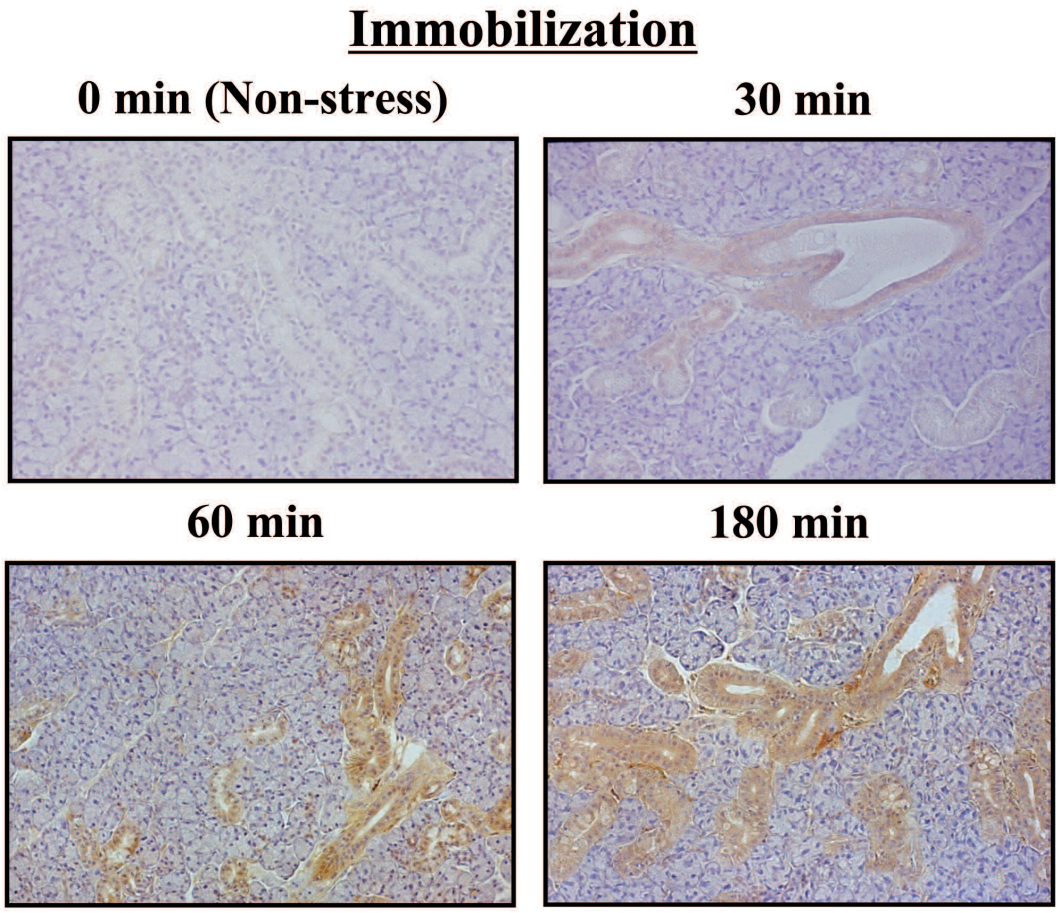

Fig. 3. BDNF protein levels following immobilization stress. Photomicrographs showing the immunohistochemical localization of BDNF protein in paraffin-embedded tissues from rats immobilization-stressed with the anti-BDNF monoclonal antibody (n=6). BDNF protein expression was observed in duct cells (original magnification, x 100). (from Tsukinoki et al., 2006).

expression in rat submandibular gland tissue in the absence of stress, but BDNF mRNA was not detected by in situ hybridization (ISH) with an oligonucleotide probe (Ernfors et al., 1990). Our findings were consistent with these results in the non-stress condition (Fig. 3). In general, a high level of BDNF expression has been observed in the central and peripheral nervous systems, since BDNF mediates cell survival and differentiation in neurons (Lewin and Brade, 1996). However, BDNF has also been reported to be present in non-neural tissues of rats such as the heart (Timmusk et al., 1993), lung (Timmusk et al., 1993), platelets (Radka et al., 1996), lymphocytes (Sobue et al., 1998), and lacrimal glands (Ghinelli et al., 2003). Our studies identified the rat submandibular gland as a BDNF-expressing organ in immobilization stress (Fig. 3).

Single or repeated immobilization stress markedly reduces BDNF mRNA and protein expression in the rat hippocampus (Ghinelli et al., 2003). However, increased BDNF mRNA and protein levels occur in the pituitary glands of rats stressed for 60 min, while decreased levels occur following stress for 180 or 300 min (Givalois et al., 2001). In our study, significant increases in BDNF mRNA and protein in the submandibular gland were observed in immobilization-stressed, compared with non-stressed, rats (Figs. 4, 5). Immobilization stress was performed according to a well-established protocol (Hori et al., 2004, 2005). This protocol is known to rapidly induce ACTH and corticosterone (Ghinelli et al., 2003). A sustained elevation of BDNF expression was observed in immobilization-stressed, compared with non-stressed, rats. Of note, a marked increase in BDNF mRNA was observed in rats following immobilization stress for 30 min (Fig. 4). Moreover, BDNF levels were decreased after 180 min of post-immobilization stress, compared with levels in non-stressed rats (Fig. 4). These findings suggest that the salivary gland is sensitive to stress; specifically, BDNF expression increases within submandibular gland tissue in response to stress. An earlier study showed that BDNF expression is not observed in human or murine submandibular gland tissue (De Vincente et al., 1998). Although not observed in submandibular gland tissue in non-stress conditions (De Vincente et al., 1998), an alteration of BDNF expression may be induced in stress conditions. We recently reported that BDNF expression in the submandibular gland is up-regulated by a chronic stressor (Saruta et al., 2009), and increased BDNF mRNA and protein expressions were observed in salivary duct cells as a result of immobilization stress and biting behavior (Saruta et al., in press). However, the expression of BDNF is poorly understood in humans, although salivary gland localization of BDNF in rats has been demonstrated (Tsukinoki et al., 2006). Therefore, in our study, we investigated the expression and localization of BDNF in the human submandibular gland (HSG) using various methods. BDNF was consistently localized in serous and ductal cells in HSG, as detected by IHC and ISH (data not shown). Reactivity was stronger in serous cells than in ductal cells. Western blotting gave one significant immunoreactive band at 14 kDa in the HSG and saliva (data not shown). BDNF was also detected in secretory granules of serous and ductal cells by immunoelectron microscopy (data not shown). Thus, BDNF in humans is produced by HSG and secreted into saliva.

Interestingly, in non-stress and time-course stress treatments, TrkB mRNA was not detected in submandibular gland tissue or oral or esophageal mucosa by RT-PCR, despite the fact that increased levels of BDNF mRNA and protein were observed (Fig. 6). In the absence of stress, previous reports failed to demonstrate TrkB expression in the human salivary gland (De Vincente et al., 1998) or esophageal mucosa (Shibayama and Koizumi, 1996). BDNF derived from the submandibular gland might act at distant sites following secretion into the bloodstream. NGF is

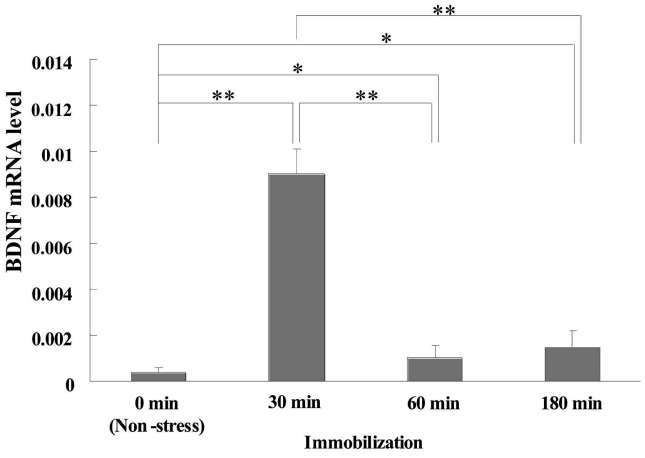

Fig. 4. BDNF mRNA quantification in rat submandibular gland tissue. Data are BDNF/ß-actin mRNA ratios. Graph showing BDNF mRNA of immobilization-stressed rats. There were significant differences between non-stress and 30, 60, or 180 min. Values are means ± S.E.M.; n=6 rats in each group. *: p<0.05, **: p<0.01, ANOVA/Tukey's. (modified from Tsukinoki et al., 2006).

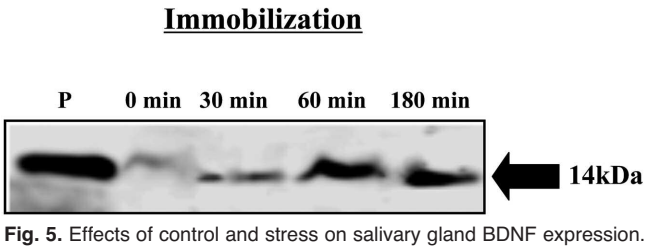

Fig. 5. Effects of control and stress on salivary gland BDNF expression. Western blot analysis of BDNF expression with equal amounts of protein from submandibular glands of control and stress rats. Western blotting demonstrated bands of 14 kDa in brain tissue and submandibular gland tissues of the immobilization-stressed group (n=6). P, Positive control (brain tissue). (from Tsukinoki et al., 2006).

released from salivary glands into the bloodstream following stress induced by fighting (Aloe et al., 1986). There is a positive correlation between serum and brain BDNF protein levels (Karege et al., 2002). However, serum BDNF is unlikely to have an effect on the central nervous system, since serum BDNF is derived from platelets (Yamamoto and Gurney, 1990). Low levels of free BDNF exist in rat plasma (Radka et al., 1996). Since BDNF is able to cross the blood-brain barrier (Pan et al., 1998), levels of free BDNF in plasma might play a more significant role than serum levels of BDNF with regard to effects on the central nervous system. Although it is generally thought that trauma-induced alterations in neurotrophins and their receptors within the central nervous system might protect against neuronal damage (Givalois et al., 2004), free BDNF in plasma might contribute to recovery against a decrease of BDNF. However, the source and role of plasma BDNF remain poorly understood. The results of our study indicate that the rat submandibular gland may be an important source of plasma BDNF.

Neurotrophins and plasma

In a previous animal study, Radka et al. (1996) reported that ELISA analysis of human serum resulted in BDNF values exceeding 2.5 ng/ml, and plasma levels were approximately 50 pg/mL. Rat serum had mean BDNF values of approximately 1 ng/ml, while mean plasma levels were approximately 150 pg/mL. These small plasma values for rats compared to humans are probably due to the greater difficulty involved in obtaining plasma with minimal platelet lysis from rats versus humans. The surprising finding was the lack of a BDNF ELISA signal in either the serum or plasma of mice (Radka et al., 1996). The very low levels of BDNF in rat or human plasma suggest that BDNF is not normally present in circulation in the absence of platelet lysis. This finding is consistent with the suggestion by Yamamoto and Gurney (1990) that BDNF plays a role specifically during tissue trauma, nerve injury, or hemorrhage when platelets are activated and when their contents are released into circulation (Yamamoto and Gurney, 1990).

Stress is an important onset factor for mental disorders in humans, but few studies have measured the levels of plasma BDNF in conditions of acute immobilization stress. Plasma is useful for investigating the role of BDNF as a hormone or humoral factor. Hence, in our study, we determined whether plasma BDNF is altered by acute immobilization stress, and whether plasma BDNF is affected by the submandibular gland.

When rats were exposed to stress for 30, 60, or 180 min, plasma BDNF levels in the 60- and 180-min groups were significantly higher than those in the control and 30-min groups (Fig. 7). Thus, we confirmed that acute immobilization stress markedly increases plasma BDNF levels. It has been reported that elevated plasma BDNF protects against neural damage from methamphetamine (Kim et al., 2005). However, because a decrease in plasma BDNF level is correlated with the severity of schizophrenia accompanied by tardive dyskinesia, tardive dyskinesia may be induced by the reduction of neural cell protection provided by BDNF (Tan et al., 2005). In addition, BDNF is able to pass through the blood-brain barrier (Pan et al., 1998). Free BDNF entering the plasma (endogenous BDNF) is likely to

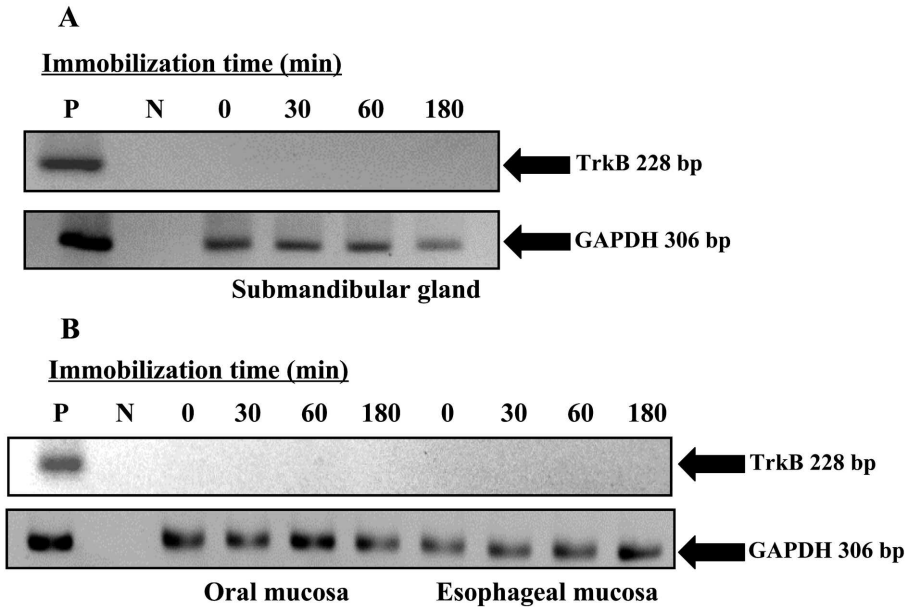

Fig. 6. Effects of immobilization stress on TrkB mRNA expression in rats. **A.** Submandibular gland tissue. **B.** Oral and esophageal mucosa. TrkB mRNA was not detected in any sample, except for brain tissue. P, Positive control (brain tissue); N, Negative control; 0, no stress; 30, immobilization stress for 30 min; 60, immobilization stress for 60 min; 180, immobilization stress for 180 min. (from Tsukinoki et al., 2006).

protect neural cells and maintain neural cell function. Therefore, in the early stages, an increase in plasma BDNF may contribute to the protection of neural cells against damage caused by acute stress conditions. Furthermore, accumulating evidence from clinical, preclinical, and animal studies indicate a key role of neurotrophins in the pathophysiology of psychiatric diseases. Indeed, low plasma levels of NGF were found in schizophrenic patients (Bersani et al., 1999; Parikh et al., 2003), while low serum BDNF levels have been reported in both schizophrenic patients and patients with depressive disorders (Karege et al., 2002; Toyooka et al., 2002). A recent study performed on patients affected by major depression reported that low plasma BDNF is associated with suicidal behavior and that plasma BDNF levels may be a biological marker of suicidal depression (Kim et al., 2007). Moreover, Bersani et al. (2004) have clearly shown that NGF plasma levels in healthy subjects follow an ultradian rhythm and that this same rhythm is disrupted in schizophrenia (Bersani et al., 2004). Another study also demonstrated the presence of a diurnal rhythm of BDNF in humans: BDNF levels were found to show a circadian rhythm, being significant higher in the morning than at night, and being positively correlated with cortisol (Begliuomini et al., 2008). The correlation between BDNF and the cortisol circadian trend suggests that these two factors may be physiologically co-regulated in order to maintain the homeostasis of integrated cerebral activities. Indeed, the diurnal rhythm of corticosterone regulates the stimulating action of nitric oxide inhibitors on BDNF, as well as on neurogenesis in the dentate gyrus (Pinnock et al., 2007).

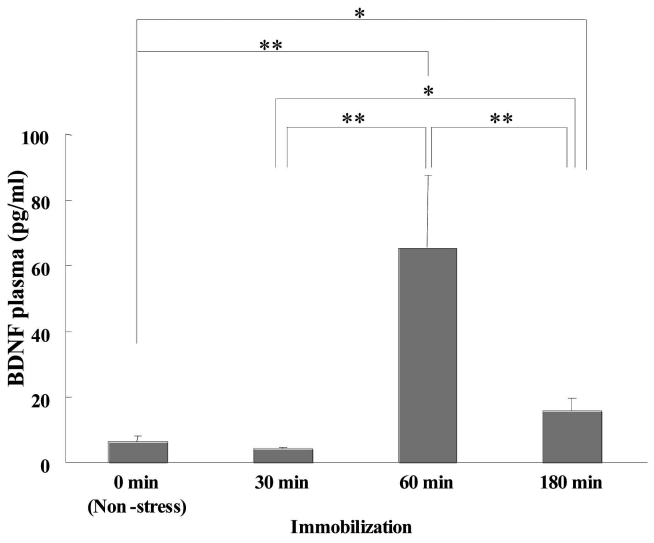

Fig. 7. BDNF protein levels following immobilization stress. Plasma BDNF level was 6.15±1.64 pg/mL for non-stressed rats. For stressed rats, plasma BDNF levels were 4.31±0.33 pg/mL at 30 min, 65.5±19.7 pg/mL at 60 min, and 15.64±3.32 pg/mL at 180 min. Significant differences were found in plasma BDNF levels between non-stressed rats and those that experienced 60 min or 180 min of acute immobilization stress. Significant differences were noted between rats that experienced 30 min and those that experienced 60 or 180 min of acute immobilization stress, and also between rats that experienced 60 min and 180 min of stress. Acute immobilization stress for 60 min significantly increased the level of plasma BDNF. Values are means ± S.E.M.; n=6 rats in each group. *: $p<0.05$, **: $p<0.001$, ANOVA/Tukey's. (modified from Tsukinoki et al., 2007).

Changes in plasma levels of neurotrophins in psychiatric disorders may reflect the altered activity of the biological clock, thus representing early markers of vulnerability. In addition, NGF and BDNF have the potential for use as indices of therapeutic efficacy, following pharmacological or light-therapy treatment (Benedetti et al., 2007).

In our study, levels of plasma BDNF were significantly higher in the 60-min stress group than in all other groups (Fig. 7). Sialoadenectomy (removal of the salivary glands) suppressed the increase of BDNF in plasma when 60-min stress was experienced (Fig. 8). However, because suppression was not complete in sialoadenectomized rats, expression of BDNF was investigated in organs relating to peripheral BDNF: heart, lung (Timmusk et al., 1993), liver (Cassiman et al., 2001), pancreas (Hanyu et al., 2003), and spleen (Schuhman et al., 2005). Our results showed that expression of BDNF mRNA in these organs did not significantly increase with immobilization (data not shown). In a previous report, BDNF mRNA and protein in the pituitary gland were found to increase in rats exposed to acute immobilization stress for 60 min (Givalois et al., 2004). Because the pituitary gland produces various hormones, pituitary BDNF may be released into the bloodstream. This suggests the possibility that not only the salivary gland, but also the pituitary gland, influence plasma BDNF in acute immobilization stress. However, we hold that the submandibular glands are the primary source of plasma BDNF in acute immobilization stress, because the increase in plasma BDNF concentration was greater in non-sialoadenectomized rats than in sialoadenectomized rats. In addition, in response to acute immobilization stress, salivary BDNF may enter the bloodstream from the submandibular gland.

In recent years, many studies have reported that BDNF is produced by various organs outside of the nervous system. BDNF is found in blood cells such as lymphocytes (Sobue et al., 1998), macrophages (Rost et al., 2005), and eosinophils (Raap et al., 1999). BDNF may be involved in the protection of neural cells in inflamed tissue. In allergic diseases, such as atopic dermatitis, BDNF released from eosinophils raises plasma BDNF levels (Raap et al., 1999). In asthma, enhanced local BDNF production in the lung contributes to neural hyperreactivity and pathological broncho-constriction (Braun et al., 2004). Alterations in plasma BDNF levels are also observed in acute coronary syndrome (Manni et al., 2005) and during the menstrual cycle (Lommatzsch et al., 2005). At the cellular level, vascular endothelial cells are considered to be an

important source of BDNF (Nakahashi et al., 2000). Plasma BDNF may play different roles in various conditions and processes, such as inflammation, allergy, heart disease, and menstruation. Therefore, acute stress not only causes neural cell injury, but also damages the gastrointestinal tract (e.g., acute gastric ulcer). Rapid increases in plasma BDNF may provide protection for the gastrointestinal organs.

Neurotrophins in saliva

Human saliva contains growth factors, including insulin-like growth factor (IGF)-I, IGF-II (Costigan et al., 1988), hepatocyte growth factor (HGF) (Tsukinoki et al., 2003), transforming growth factor-α (TGF-α) (Nak et al., 1995), vascular endothelial growth factor (VEGF) (Taichman et al., 1998), EGF (Dagogo-Jack et al., 1985), NGF (Lipps, 2000), BDNF (Mandel et al., 2009), and fibroblast growth factor-2 (FGF-2) (Ishizaki et al., 2000). In a previous immunohistochemical study using antibodies against mouse submandibular NGF, sections of flash-frozen human whole saliva were examined by both light and transmission electron microscopy (Glantz et al., 1989). NGF antibody-treated sections heavily stained for bacteria-like particles were frequently observed. Moreover, Nam et al. (2007) reported measurable concentrations of NGF in all three sources of saliva; the concentration was affected by the source of the stimulated parotid and submandibular saliva, the age of stimulated submandibular saliva, and gender differences for resting whole saliva and stimulated parotid saliva (Nam et al., 2007). The mean concentrations of NGF were 901.4±75.6 pg ml^{-1} in resting whole saliva, 885.9±79.9 pg ml^{-1} in stimulated parotid saliva, and 1066.1±88.1 pg ml^{-1} in stimulated submandibular/sublingual saliva. The stimulated submandibular saliva showed lower NGF concentrations with increasing age. The NGF concentrations of resting whole saliva and stimulated parotid saliva were significantly higher in women than men. The NGF concentration of stimulated submandibular saliva was significantly higher than stimulated parotid saliva and significantly correlated with stimulated parotid saliva NGF levels.

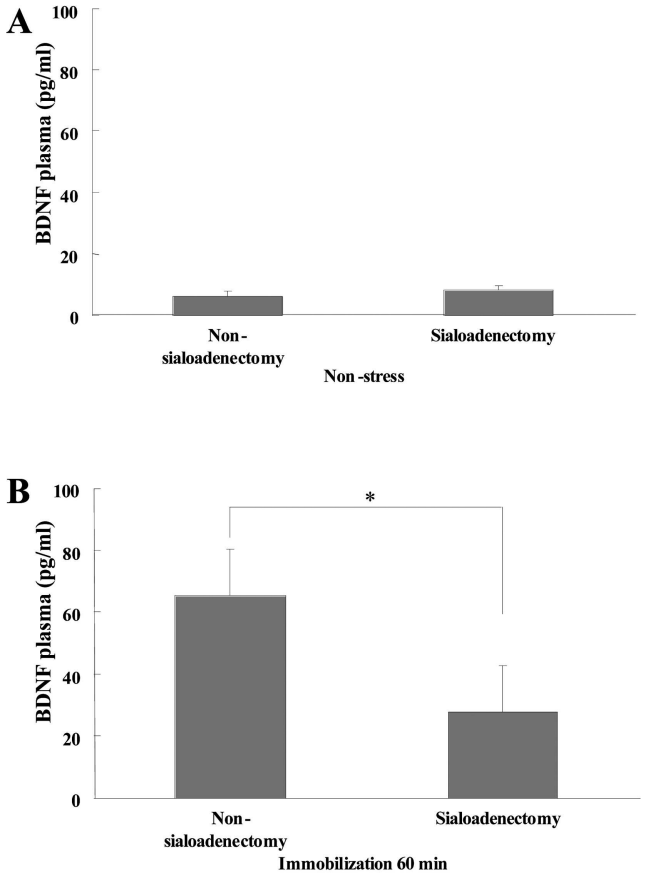

Fig. 8. BDNF protein levels in sialoadenectomized rats (removal of submandibular glands before acute immobilization stress). **A.** Plasma BDNF levels in non-stressed rats. Plasma BDNF levels were 6.15±1.64 pg/mL in normal rats and 8.01±5.07 pg/mL in sialoadenectomized rats. **B.** Plasma BDNF levels in stressed rats. Plasma BDNF levels were 65.5±19.7 pg/mL in normal rats and 27.9±14.8 pg/mL in sialoadenectomized rats. There were significant differences in plasma BDNF levels between non-sialoadenectomized and sialoadenectomized rats. Values are means ± S.E.M.; n=6 rats in each group. *: $p<0.05$, Student's t-test. (modified from Tsukinoki et al., 2007).

Mandel et al. (2009) demonstrated through immunoblotting and enzyme digestions that the mature and pro-forms of BDNF are present in human whole saliva (Mandel et al., 2009). There were considerable individual differences in the expression and relative concentrations of each form of the protein; not all participants expressed every form. In that study, the immunoreactive specificity of the anti-BDNF antibody was confirmed by peptide neutralization and by lack of crossreactivity with the other neurotrophins, verifying that the antibody truly binds BDNF. Although BDNF mRNA expression has been observed in rodent submandibular glands (Tirassa et al., 2000; Tsukinoki et al., 2006), we did not investigate the presence of BDNF in the animals' saliva. A recent salivary proteome analysis in humans failed to identify growth factors such as NGF and BDNF (Denny et al., 2008). To our knowledge, this was the first report of BDNF in salivary secretions, whether in humans or other species.

With regard to the roles of these growth factors in saliva, it has been reported that the act of licking a wound accelerates wound healing in rodents (Hutson et al., 1979; Bodner, 1991). We measured the concentration of HGF in saliva and blood before and after an operation for a salivary gland tumor using an ELISA system and found a significant increase in HGF levels after surgery (Tsukinoki et al., 2003). Thus, it is highly possible that growth factors in saliva accelerate wound healing. On the other hand, the blood concentration of NGF decreases in mice in which the submandibular glands have been resected (Alleva and Francia, 2009; Cirulli

and Alleva, 2009a; Cirulli et al., 2009b). Although the details of the blood distribution route of growth factors produced in the salivary glands are unknown, reabsorption from the sublingual area is considered a likely route. The sublingual area is the place where nitroglycerin tablets are administered because of the thin mucous membrane and the abundance of blood vessels. The openings for the submandibular glands and sublingual glands are present here and it is reasonable to consider that growth factors in saliva are reabsorbed from the sublingual area. Although NGF is not detected in human salivary glands, other unknown neurotrophic factors could be produced in the salivary glands and reabsorbed from the sublingual area affecting the cerebrum. We consider that the quality of saliva may be assessed as an indicator of the amount of its production, assuming that growth factors are one of the factors that benefit a living body.

Conclusions

Under conditions of acute immobilization stress, rat submandibular glands increased BDNF production, thereby contributing to the elevation of plasma BDNF levels. The increase in plasma BDNF concentration is significantly greater in non-sialoadenectomized than sialoadenectomized rats. Rat submandibular glands are therefore confirmed to be the major source of plasma BDNF under conditions of acute immobilization stress. Although the physiological mechanisms responsible for the increase in salivary BDNF under acute immobilization stress conditions are not understood, upregulation of salivary tissue BDNF may directly affect the salivary glands via acute immobilization stress.

To date, there has been limited investigation of changes in plasma BDNF using experimental models which are characterized by physical stress (Alleva and Francia, 2009). In clinical studies, blood BDNF levels have been found to be lower in individuals with schizophrenia (Tan et al., 2005) or depression (Karege et al., 2005). Because plasma BDNF levels increase with the use of psychotropic agents (Shimizu et al., 2003), the BDNF content in serum and plasma may reflect the clinical stage of such mental disorders. Interestingly, elevation of BDNF levels by exogenous BDNF contributes to the protection of neural cells in the rat hippocampus (Radecki et al., 2005). Since BDNF is able to pass through the blood-brain barrier (Pan et al., 1998) and utero-placental barrier (Kodomari et al., 2009), circulating BDNF is likely to contribute to protecting neural cells and maintaining their function. Hence an increase in plasma BDNF levels may be an important neuroprotective response under acute immobilization stress conditions. The salivary glands are likely to affect not only oral health, but also systemic organs. We believe that the salivary glands can influence the health of distant organs. Future studies should investigate the mechanisms of this connection to general health.

Acknowledgements. This research was supported in part by a Grant-in Aid for Young Scientists (Start-up, #1989023), Scientific Research (B, #20390467), Research Institute on Occlusive Medicine of Kanagawa Dental College, and Open Research from the Japanese Ministry of Education, Culture, Sports, Science, and Technology.

References

Adlard P.A. and Cotman C.W. (2004). Voluntary exercise protects against stress-induced decreases in brain-derived neurotrophic factor protein expression. Neuroscience 124, 985-992.

Alleva E. and Francia N. (2009). Psychiatric vulnerability: suggestions from animal models and role of neurotrophins. Neurosci. Biobehav. Rev. 33, 525-536.

Aloe L., Alleva E., Bohm A. and Levi-Montalcini R. (1986). Aggressive behavior induces release of nerve growth factor from mouse salivary gland into the bloodstream. Proc. Natl. Acad. Sci. USA 83, 6184-6187.

Aloe L., Alleva E., Bohm A. and De Simone R. (1990). Change of NGF level in mouse hypothalamus following intermale aggressive behaviour: biological and immunohistochemical evidence. Behav. Brain. Res. 39, 53-61.

Aloe L., Bracci-Laudiero L., Alleva E., Lambiase A., Micera A. and Tirassa P. (1994). Emotional stress induced by parachute jumping enhances blood nerve growth factor levels and the distribution of nerve growth factor receptors in lymphocytes. Proc. Natl. Acad. Sci. USA 91, 10440-10444.

Altar C.A. and DiStefano P.S. (1998). Neurotrophin trafficking by anterograde transport. Trends. Neurosci. 21, 433-437.

Altar C.A., Siuciak J.P., Wright P., Ip N.Y., Lindsay R.M. and Wiegand S.J. (1994). In situ hybridization of trkB and trkC receptor mRNA in rat forebrain and association with high-affinity binding of [125I]BDNF, [125I]NT-4/5 and [125I]NT-3. Eur. J. Neurosci. 6, 1389-1405.

Amano O. and Iseki S. (1998). Occurrence and nuclear localization of cAMP response element-binding protein in the postnatal development of the rat submandibular gland. Histochem. J. 30, 591-601.

Barde Y.A. (1990). The nerve growth factor family. Prog. Growth. Factor. Res. 2, 237-248.

Barde Y.A., Edgar D. and Thoenen H. (1982). Purification of a new neurotrophic factor from mammalian brain. EMBO J. 1, 549-553.

Begliuomini S., Lenzi E., Ninni F., Casarosa E., Merlini S., Pluchino N., Valentino V., Luisi M., Luisi M. and Genazzani A.R. (2008). Plasma brain-derived neurotrophic factor daily variations in men: correlation with cortisol circadian rhythm. J. Endocrinol. 197, 429-435.

Benedetti F., Barbini B., Colombo C. and Smeraldi E. (2007). Chronotherapeutics in a psychiatric ward. Sleep Med. Rev. 11, 509-522.

Bersani G., Iannitelli A., Maselli P., Pancheri P., Aloe L., Angelucci F. and Alleva E. (1999). Low nerve growth factor plasma levels in schizophrenic patients: a preliminary study. Schizophr. Res. 37, 201-203.

Bersani G., Iannitelli A., Massoni E., Garavini A., Grilli A., Di Giannantonio M., Conti C.M. and Pancheri P. (2004). Ultradian variation of nerve growth factor plasma levels in healthy and

schizophrenic subjects. Int. J. Immunopathol. Pharmacol. 17, 362-372.

Bodner L. (1991). Effect of parotid submandibular and sublingual saliva on wound healing in rats. Comp. Biochem. Physiol. A. Comp. Physiol. 100, 887-890.

Bradley R.M. (1995). Salivary secretion. In: Essentials of oral physiology. Mosby. St. Louis. pp 161-186.

Braun A., Lommatzsch M., Neuhaus-Steinmatz U., Quarcoo D., Glaab T., McGregor G.P., Fischer A. and Renz H. (2004). Brain-derived neurotrophic factor (BDNF) contributes to neuronal dysfunction in a model of allergic airway inflammation. Br. J. Pharmacol. 141, 431-440.

Brown M.R. and Fisher L.A. (1984). Brain peptide regulation of adrenal epinephrine secretion. Am. J. Physiol. 247, E41-E46.

Cassiman D., Denef C., Desmet V.J. and Roskams T. (2001). Human and rat hepatic stellate cells express neurotrophins and neurotrophin receptors. Hepatology 33, 148-158.

Castren E. (2004). Neurotrophins as mediators of drug effects on mood, addiction, and neuroprotection. Mol. Neurobiol. 29, 289-302.

Chretien M. (1977). Action of testosterone on the differentiation and secretary activity of a target origin: The submandibular gland of the mouse. Int. Rev. Cytol. 50, 333-396.

Cirulli F. and Alleva E. (2009a). The NGF saga: from animal models of psychosocial stress to stress-related psychopathology. Front. Neuroendocrinol. 30, 379-395.

Cirulli F., Francia N., Berry A., Aloe L., Alleva E. and Suomi S.J. (2009b). Early life stress as a risk factor for mental health: role of neurotrophins from rodents to non-human primates. Neurosci. Biobehav. Rev. 33, 573-585.

Cohen S. (1960). Purification of nerve-growth promoting protein from the mouse salivary gland and its neuro-cytotoxic antiserum. Proc. Natl. Acad. Sci. USA 46, 302-311.

Cohen S. (1962). Isolation of a mouse submaxillary gland protein accelerating incisor eruption and eyelid opening in the newly born animal. J. Biol. Chem. 237, 1555-1562.

Costigen D.C., Guyda H.J. and Posner B.I. (1988). Free insulin-like growth factor I (IGF-I) and IGF-II in human saliva. J. Clin. Endocrinol. Metab. 66, 1014-1018.

Dagogo-Jack S., Atkinson S. and Kendall-Taylor P. (1985). Homologous radioimmunoassay for epidermal growth factor in human saliva. J. Immunoassay 6, 125-136.

Dardick I., Byard R.W. and Carnegie J.A. (1990). A review of the proliferative capacity of major salivary glands and the relationship to current concepts of neoplasia in salivary glands. Oral Surg. Oral Med. Oral Pathol. 69, 53-67.

Denny P., Hagen F.K., Hardt M., Liao L., Yan W., Arellanno M., Bassilian S., Bedi G.S., Boontheung P., Cociorva D., Delahunty C.M., Denny T., Dunsmore J., Faull K.F., Gilligan J., Gonzalez-Begne M., Halgand F., Hall S.C., Han X., Henson B., Hewel J., Hu S., Jeffrey S., Jiang J., Loo J.A., Ogorzalek Loo R.R., Malamud D., Melvin J.E., Miroshnychenko O., Navazesh M., Niles R., Park S.K., Prakobphol A., Ramachandran P., Richert M., Robirison S., Sondej M., Souda P., Sullivan M.A., Takashima J., Than S., Wang J., Whitelegge J.P., Witkowska H.E., Wolinsky L., Xie Y., Xu T., Yu W., Ytterberg J., Wong D.T., Yates J.R. 3rd and Fisher S.J. (2008). The proteomes of human parotid and submandibular/sublingual gland salivas collected as the ductal secretions. J. Proteome Res. 7, 1994-2006.

De Vincente J.C., Garcia-Suárez O., Esteban I., Santamaria J. and Vega J.A. (1998). Immunohistochemical localization of neurotrophins and neurotrophin receptors in human and mouse salivary glands. Ann. Anat. 180, 157-163.

Doel J.J., Hector M.P., Amirtham C.V., Al-Anzam L.A., Benjamin N. and Allaker R.P. (2004). Protective effect of salivary nitrate and microbial nitrate reductase activity against caries. Eur. J. Oral. Sci. 112, 424-428.

Duman R.S., Heninger G.R. and Nestler E.J. (1997). A molecular and cellular theory of depression. Arch. Gen. Psychiatry 54, 597-606.

Egan M.F., Kojima M., Callicott J.H., Goldberg T.E., Kolachana B.S., Bertolino A., Zaitsev E., Gold B., Goldman D., Dean M., Lu B. and Weinberger D.R. (2003). The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell 112, 257-269.

Ernfors P., Wetmore C., Olson L. and Persson H. (1990). Identification of cells in rat brain and peripheral tissues expressing mRNA for members of the nerve growth factor family. Neuron 5, 511-526.

Field R.B., Dromy R. and Hand A.R. (1987). Regulation of secretion of enzymes from von Ebner's gland of rat tongue. J. Dent. Res. 66, 586-587.

Fischer H.P., Eich W. and Russell I.J. (1998). Possible role for saliva as a diagnostic fluid in patients with chronic pain. Semin. Arthritis Rheum. 27, 348-359.

Garrett J.R., Suleiman A.M., Anderson L.C. and Proctor G.B. (1991). Secretory responses in granular ducts and acini of submandibular glands in vivo to parasympathetic or sympathetic nerve stimulation in rats. Cell Tissue Res. 264, 117-126.

Ghinelli E., Johansson J., Rios J.D., Chen L.L., Zoukhrin D., Hodges R.R. and Dartt D.A. (2003). Presence and localization of neurotrophins and neurotrophin receptors in rat lacrimal gland. Invest. Opthalmol. Vis. Sci. 44, 3352-3357.

Ghosh A., Carnahan J. and Greenberg M.E. (1994). Requirement for BDNF in activity-dependent survival of cortical neurons. Science 263, 1618-1623.

Givalois L., Arancibia S., Alonso G. and Tapia-Arancibia L. (2004). Expression of brain-derived neurotrophic factor and its receptors in the median eminence cells with sensitivity to stress. Endocrinology 145, 4737-4747.

Givalois L., Marmigère F., Rage F., Ixart G., Arancibia S. and Tapia-Arancibia L. (2001). Immobilization stress rapidly and differentially modulates BDNF and TrkB mRNA expression in the pituitary gland of adult male rats. Neuroendocrinology 74, 148-159.

Glantz P.O., Meenaghan M.A., Hyun K.H. and Wirth S.M. (1989). On the presence and localization of epidermal and nerve growth factors in human whole saliva. Acta. Odontol. Scand. 47, 287-292.

Gresik E.W. (1994). The granular convoluted tubule (GCT) cell of rodent submandibular glands. Micros. Res. Technol. 27, 1-24.

Grillon C., Duncko R., Covington M.F., Kopperman L. and Kling M.A. (2007). Acute stress potentiates anxiety in humans. Biol. Psychiatry 62, 1183-1186.

Hand A.R. (1980). Salivary glands. In: Bhaskar SN, editor. Orban's oral histology and embryology. Mosby. St. Louis. MO. pp 336-370.

Hanyu O., Yamatani K., Ikarashi T., Soda S., Maruyama S., Kamimura T., Kaneko S., Hirayama S., Suzuki K., Nakagawa O., Nawa H. and Aizawa Y. (2003). Brain-derived neurotrophic factor modulates glucagons secretion from pancreatic alpha cells: Its contribution to glucose metabolism. Diabetes Obes. Metab. 5, 27-37.

Hatton M.N., Loomis R.E., Levine M.J. and Tabak L.A. (1985). Masticatory lubrication. The role of carbohydrate in the lubricating

property of a salivary glycoprotein-albumin complex. Biochem. J. 230, 817-820.

Hofman L.F. (2001). Human saliva as a diagnostic specimen. J. Nutr. 131, 1621S-1625S.

Hori N., Yuyama N. and Tamura K. (2004). Biting suppresses stress-induced expression of corticotrophin-releasing factor (CRF) in the rat hypothalamus. J. Dent. Res. 83, 124-128.

Hori N., Lee M.C., Sasaguri K., Ishii H., Kamei M., Kimoto K., Toyoda M. and Sato S. (2005). Suppression of stress-induced nNOS expression in the rat hypothalamus by biting. J. Dent. Res. 84, 624-628.

Hutson J.M., Niall M., Evans D. and Fowler R. (1979). Effect of salivary glands on wound contraction in mice. Nature 279, 793-795.

Ishizaki H., Westermark A., van Setten G. and Pyykkö I. (2000). Basic fibroblast growth factor (bFGF) in saliva-physiological and clinical implications. Acta. Otolaryngol. Suppl. 543, 193-195.

Kafitz K.W., Rose C.R., Thoenen H. and Konnerth A. (1999). Neurotrophin-evoked rapid excitation through TrkB receptors. Nature 401, 918-921.

Kanamaru Y., Kikukawa A. and Shimamura K. (2006). Salivary chromogranin-A as a marker of psychological stress during a cognitive test battery in humans. Stress 9, 127-131.

Karege F., Bondolfi G., Gervasoni N., Schwald M., Aubry J.M. and Bertschy G. (2005). Low brain-derived neurotrophic factor (BDNF) levels in serum of depressed patients probably results from lowered platelet BDNF release unrelated to platelet reactivity. Biol. Psychiatry 57, 1068-1072.

Karege F., Perret G., Bondolfi G., Schwald M., Bertschy G. and Aubry J.M. (2002). Decreased serum brain-derived neurotrophic factor levels in major depressed patients. Psychiatry Res. 109, 143-148.

Karege F., Schwald M. and Cisse M. (2002). Postnatal developmental profile of brain-derived neurotrophic factor in rat brain and platelets. Neurosci. Lett. 328, 261-264.

Kim D.J., Roh S., Kim Y., Yoon S.J., Lee H.J., Han C.S. and Kim Y.K. (2005). High concentrations of plasma brain-derived neurotrophic factor in methamphetamine users. Neurosci. Lett. 388, 112-115.

Kim Y.K., Lee H.Y., Won S.D., Park E.Y., Lee H.Y., Lee B.H., Lee S.W., Yoon D., Han C., Kim D.J. and Choi S.H. (2007). Low plasma BDNF is associated with suicidal behavior in major depression. Prog. Neuropsychopharmacol. Biol. Psychiatry 31, 78-85.

Kirschbaum C. and Hellhammer D.H. (1994). Salivary cortisol in psychoneuroendocrine research: recent development and application. Psychoneuroendocrinology 19, 313-333.

Knusel B., Winslow J.W., Rosenthal A., Burton L.E., Seid D.P., Nikolics K. and Hefti F. (1991). Promotion of central cholinergic and dopaminergic neuron differentiation by brain-derived neurotrophic factor but not neurotrophin 3. Proc. Natl. Acad. Sci. USA 88, 961-965.

Kodomari I., Wada E., Nakamura S. and Wada K. (2009). Maternal supply of BDNF to mouse fetal brain through the placenta. Neurochem. Int. 54, 95-98.

Konturek S.J., Brzozowski T., Konturek P.K., Majka J. and Dembinski A. (1991). Role of salivary glands and epidermal growth factor (EGF) in gastric secretion and mucosal integrity in rats exposed to stress. Regul. Pept. 32, 203-215.

Kousvelari E.E., Baratz R.S., Burke B. and Oppenheim F.G. (1980). Immunochemical identification and determination of proline-rich proteins in salivary secretions, enamel pellicle, and glandular tissue specimens. J. Dent. Res. 59, 1430-1438.

Kurahashi M. and Inomata K. (1999). Relationship between parotid amylase secretion and osmolality in the gastric contents of rats fed a pelleted or liquid diet. Jpn. J. Physiol. 49, 507-512.

Lee T., Saruta J., Sasaguri K., Sato S. and Tsukinoki K. (2008). Allowing animals to bite reverses the effects of immobilization stress on hippocampal neurotrophin expression. Brain Res. 1195, 43-49.

Leibrock J., Lottspeich F., Hohn A., Hofer M., Hengerer B., Masiakowski P., Thoenen H. and Barde Y.A. (1989). Molecular cloning and expression of brain-derived neurotrophic factor. Nature 341, 149-152.

Levi-Montalcini R. (1987). The nerve growth factor 35 years later. Science 237, 1154-1162.

Levi-Montalcini R. and Angeletti P.U. (1968). Nerve growth factor. Physiol. Rev. 48, 534-569.

Lewin G.R. and Barde Y.A. (1996). Physiology of the neurotrophins. Annu. Rev. Neurosci. 19, 289-317.

Lipps B.V. (2000). Isolation of nerve growth factor (NGF) from human body fluids; saliva, serum and urine: comparison between cobra venom and cobra serum NGF. J. Nat. Toxins 9, 349-356.

Llena-Puy C. (2006). The role of saliva in maintaining oral health and as an aid to diagnosis. Med. Oral. Pathol. Oral. Cir. Bucal. 11, E499-E455.

Lommatzsch M., Zingler D., Schuhbaeck K., Scholetcke K., Zingler C., Schuff-Werner P. and Virchow J.C. (2005). The major of age, weight, and gender on BDNF levels in human platelets and plasma. Neurobiol. Aging 26, 115-123.

Lu B. and Chow A. (1999). Neurotrophins and hippocampal synaptic transmission and plasticity. J. Neurosci. Res. 58, 76-87.

Lucas R.M., Ponsonby A.L. and Dear K. (2007). Mid-life stress is associated with both up- and down-regulation of markers of humoral and cellular immunity. Stress 10, 351-361.

Mandel A.L., Ozdener H. and Utermohlen V. (2009). Identification of pro- and mature brain-derived neurotrophic factor in human saliva. Arch. Oral. Biol. 54, 689-695.

Manni L., Nikolova V., Vyagova D., Chaldakov G.N. and Aloe L. (2005). Reduced plasma levels of NGF and BDNF inpatients with acute coronary syndromes. Int. J. Cardiol. 102, 169-171.

Mathison R., Davison J.S. and Befus A.D. (1994). Neuroendocrine regulation of inflammation and tissue repair by submandibular glands factors. Immunol. Today 15, 527-532.

Mese O. and Matsuo R. (2007). Salivary secretion, taste and hyposalivation. J. Oral. Rehabil. 34: 711-723.

Mogi M., Inagaki H., Kojima M., Minami M. and Harada M. (1995). Transforming growth factor alpha in human submandibular gland and saliva. J. Immunoassay 16, 379-394.

Nakahashi T., Fujimura H., Altar C.A., Li J., Kambayashi J., Tandon N.N. and Sun B. (2000). Vascular endothelial cells synthesize and secrete brain-derived neurotrophic factor. FEBS Lett. 470, 113-117.

Nam J.W., Chung J.W., Kho H.S., Chung S.C. and Kim Y.K. (2007). Nerve growth factor concentration in human saliva. Oral. Dis. 13, 187-192.

Okamura M., Yoshida A., Saruta J., Tsukinoki K., Sasaguri K. and Sato S. (2008). Effect of bruxism-like activity on the salivary chromogranin A as a stress marker. Stomatologie 105, 33-39.

Pang P.T., Teng H.K., Zaitsev E., Woo N.T., Sakata K., Zher S., Teng K.K., Yung W.H., Hempstead B.L. and Lu B. (2004). Cleavage of proBDNF by tPA/plasmin is essential for long-term hippocampal plasticity. Science 306, 487-491.

Pan W., Banks W.A., Fasold M.B., Bluth J. and Kastin A.J. (1998).

Transport of brain-derived neurotrophic factor across the blood-brain barrier. Neuropharmacology 37, 1553-1561.

Parikh V., Evans D.R., Khan M.M. and Mahadik S.P. (2003). Nerve growth factor in nerve-medicated first-episode psychotic and medicated chronic schizophrenic patients: possible implications for treatment outcome. Schizophr. Res. 60, 117-123.

Pedersen A.M., Bardow A., Jensen S.B. and Nauntofte B. (2002). Saliva and gastrointestinal functions of taste, mastication, swallowing and digestion. Oral. Dis. 8, 117-129.

Pinnock S.B., Balendra R., Chan M., Hunt L.T., Turner-Stokes T. and Herbert J. (2007). Interactions between nitric oxide and corticosterone in the regulation of progenitor cell proliferation in the dentate gyrus of the adult rat. Neuropsychopharmacology 32, 493-504.

Raap U., Goltz C., Deneka N., Bruder M., Renz H., Kapp A. and Wedi B. (1999). Brain-derived neurotrophic factor is increased in atophic dermatitis and modulates eosinophil functions compared with that seen in nonatopic subjects. J. Allergy Clin. Immunol. 115, 1268-1275.

Radecki D.T., Brown L.M., Martinez J. and Teyler T.J. (2005). BDNF protects against stress-induced impairments in spatial learning and memory and LTP. Hippocampus 15, 246-253.

Radka S.F., Holst P.A., Fritsche M. and Altar C.A. (1996). Presence of brain-derived neurotrophic factor in brain and human and rat but not mouse serum detected by a sensitive and specific immunoassay. Brain. Res. 709, 122-130.

Rost B., Hanf G., Ohnemus U., Otto-Knapp R., Groneberg D.A., Kunkel G. and Noga O. (2005). Monocytes of allergics and non-allergics produce, store, and release the neurotrophins NGF, BDNF, and NT-3. Regul. Pept. 124, 19-25.

Ruhl S., Hamberger S., Betz R., Sukkar T., Schmalz G., Seymour R.A., Hiller K.A. and Thomason J.M. (2004). Salivary proteins and cytokines in drug-induced gingival overgrowth. J. Dent. Res. 83, 322-326.

Saruta J., Tsukinoki K., Sasaguri K., Ishii H., Yasuda M., Osamura Y.O., Wtanabe Y. and Sato S. (2005). Expression and localization of Chromogranin A gene and protein in human submandibular. Cells Tissues Organs 180, 237-244.

Saruta J., Lee T., Shirasu M., Takahashi T., Sato C., Sato S. and Tsukinoki K. (2010). Chronic stress affects the expression of BDNF in rat submandibular glands. Stress 13, 53-60.

Saruta J., Kondo Y., Sato C., Shiiki N., Tsukinoki K. and Sato S. (2010). Salivary glands as the source of plasma BDNF in stressed rats engaged in biting behavior. Stress (in press).

Schuhman B., Dietrich A., Sel S., Hahn C., Klingenspor M., Lommatzsch M., Gudermann T., Braun A., Renz H. and Nockher W.A. (2005). A role brain-derived neurotrophic factor in B cell development. J. Neuroimmunol. 163, 15-23.

Schwab K.O., Heubel G. and Bartels H. (1992). Free epinephrine, norepinephrine and dopamine in saliva and plasma of healthy adults. Eur. J. Clin. Chem. Clin. Biochem. 30, 541-544.

Shibayama E. and Koizumi H. (1996). Cellular localization of the Trk neurotrophin receptor family in human non-neuronal tissues. Am. J. Pathol. 148, 1807-1818.

Shimizu E., Hashimoto K., Okamura N., Koike K., Komatsu N., Kumakiri C., Nakazato M., Watanabe H., Shinoda N., Okada S. and Iyo M. (2003). Alterations of serum levels of brain-derived neurotrophic factor (BDNF) in depressed patients with or without antidepressants. Biol. Psychiatry 54, 70-75.

Slowey P.R., Eidelman S. and Klebanoff S.J. (1968). Antibacterial activity of the purified peroxidase from human parotid saliva. J. Bacteriol. 96, 575-579.

Sobue G., Yamamoto M., Doyu M., Li M., Yasuda T. and Mitsuma T. (1998). Expression of mRNAs for neurotrophins (NGF, BDNF, and NT-3) and their receptors (p75 NGFR, trk, trkB, and trkC) in human peripheral neuropathies. Neurochem. Res. 23, 821-829.

Sreebny L.M. (1987). The salivary system. In: Salivary electrolytes and fluid protection in health and disease. CRC Press. Boca Raton, Florida. pp 95-122.

Streeten D.H., Anderson G.H., Dalakos T.G., Seeley D., Mallov J.S., Eusebio R., Sunderlin F.S., Badawy S.Z. and King R.B. (1984). Normal and abnormal function of the hypothalamic-pituitary-adrenocortical system in man. Endocr. Rev. 5, 371-394.

Taichman N.S., Cruchley A.T., Fletcher L.M., Hagi-Pavli E.P., Paleolog E.M., Abrams W.R., Booth V., Edwards R.M. and Malamud D. (1998). Vascular endothelial growth factor in normal human salivary glands and saliva: a possible role in the maintenance of mucosal homeostasis. Lab. Invest. 78, 869-875.

Takai N., Yamaguchi M., Aragaki T., Eto K., Uchihashi K. and Nishikawa Y. (2004). Effect of psychological stress on the salivary cortisol and amylase levels in healthy young adult. Arch. Oral. Biol. 49, 963-968.

Tan Y.L., Zhou D.F. and Zhang X.Y. (2005). Decreased plasma brain-derived neurotrophic factor levels in schizophrenic patients with tardive dyskinesia: association with dyskinetic movements. Schizoph. Res. 74, 263-270.

Tenovuo J. and Knuuttila M.L. (1977). Antibacterial effect of salivary peroxidases on a cariogenic strain of streptococcus mutans. J. Dent. Res. 56, 1608-1613.

Timmusk T., Palm K., Metsis M., Reintam T., Paalme V., Saarma M. and Persson H. (1993). Multiple promoters direct tissue-specific expression of the rat BDNF gene. Neuron 10, 475-489.

Tirassa P., Manni L., Stenfors C., Lundeberg T. and Aloe L. (2000). RT-PCR ELISA method for the analysis of neurotrophin mRNA expression in brain and peripheral tissues. J. Biotechnol. 84, 259-272.

Toda M. and Morimoto K. (2008). Effect of lavender aroma on salivary endocrinological stress marker. Arch. Oral. Biol. 53, 964-968.

Toda M., Den R., Nagasawa S., Kitamura K. and Morimoto K. (2005). Relationship between lifestyle scores and salivary stress markers cortisol and chromogranin A. Arch. Environ. Occup. Health. 60, 266-269.

Toyooka K., Asama K., Watanabe Y., Muratake T., Takahashi M., Someya T. and Nawa H. (2002). Decreased levels of brain-derived neurotrophic factor in serum of chronic schizophrenic patients. Psychiatry Res. 110, 249-257.

Troxler R.F., Offner G.D., Xu T., Vanderspek J.C. and Oppenheim F.G. (1990). Structural relationship between human salivary histatins. J. Dent. Res. 69, 2-6.

Tsukinoki K., Yasuda M., Asano S., Karakida K., Ota Y., Osamura R.Y. and Watanabe Y. (2003). Association of hepatocyte growth factor expression with salivary gland tumor differentiation. Pathol. Int. 53, 815-822.

Tsukinoki K., Yasuda M., Miyoshi Y., Mori Y., Ootsuru M., Saruta J., Sato S., Kaneko A., Watanabe Y. and Osamura Y. (2005). Role of hepatocyte growth factor and c-Met receptor in neoplastic conditions of salivary glands. Acta Histochem. Cytochem. 38, 25-30.

Tsukinoki K., Saruta J., Sasaguri K., Miyoshi Y., Jinbu Y., Kusama M., Sato S. and Watanabe Y. (2006). Immobilization stress induces

BDNF in rat submandibular glands. J. Dent. Res. 85, 844-848.

Tsukinoki K., Saruta J., Muto N., Sasaguri K., Sato S., Tan-Ishii N. and Watanabe Y. (2007). Submandibular glands contribute to increases in plasma BDNF levels. J. Dent. Res. 86, 260-264.

Turner R.J. and Sugiya H. (2002). Understanding salivary fluid and protein secretion. Oral. Dis. 8, 3-11.

Ueyama T., Kawai Y., Nemoto K., Sekimoto M., Tone S. and Senba E. (1997). Immobilization stress reduced the expression of neurotrophins and their receptors in the rat brain. Neurosci. Res. 28, 103-110.

van Stegeren A., Rohleder N., Everaerd W. and Wolf O.T. (2006). Salivary alpha-amylase as marker for adrenergic activity during stress: Effect of betablockade. Psychoneuroendocrinology 31, 137-141.

van Stegeren A., Wolf O.T. and Kindt M. (2008). Salivary alpha amylase and cortisol responses to different stress tasks: Impack of sex. Int. J. Psychophysiol. 69, 33-40.

Watson A.Y., Anderson J.K., Siminoski K., Mole J.E. and Murphy R.A. (1985). Cellular and subcellular colocalization of nerve growth factor and epidermal growth factor in mouse submandibular glands. Anat. Rec. 213, 365-376.

Yamamoto H. and Gurney M.E. (1990). Human platelets contain brain-derived neurotrophic factor. J. Neurosci. 10, 3469-3478.

Accepted March 26, 2010