

## Role of Stress-Related Brain-Derived Neurotrophic Factor (BDNF) in the Rat Submandibular Gland

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The nerve growth factor (NGF) family comprises NGF, brain-derived neurotrophic factor (BDNF) and neurotrophins (NTs)-3, -4/5, -6 and -7, all of which are collectively referred to as neurotrophins. However, the expression of neurotrophins other than NGF in the salivary gland has not been described in detail. Through interaction with the TrkB receptor, BDNF plays an important role in long-term potentiation. We found that BDNF expression increased within submandibular gland tissue in response to stress, suggesting that the salivary glands are sensitive to stress. In addition, stress caused increases in plasma BDNF derived from the submandibular gland and in TrkB receptor mRNA in the adrenal medulla. Plasma BDNF might activate TrkB receptors in the adrenal medulla during acute stress. The salivary glands are likely to influence not only oral health, but also systemic organs. This review addressed the relationship between hormone-like effects and stress-related BDNF expression in the rat submandibular gland.

**Key words:** brain-derived neurotrophic factor (BDNF), plasma, salivary gland, stress

### I. Introduction

The exocrine salivary glands consist of the parotid, submandibular and sublingual glands as well as numerous minor salivary glands scattered in the oral cavity [39]. The salivary glands secrete saliva to digest food, promote mastication and fight bacteria [28]. However, the salivary glands might also play other important roles because they produce many substances [38]. Salivary products are associated with the maintenance of oral health, which is associated with systemic health, including that of the respiratory tract [43]. Not only the volume, but also the quality of saliva is considered important for good oral health (Fig. 1, arrow A).

Saliva includes many components that are derived from blood, because acinar cells produce saliva from blood

plasma. The identification of salivary products might reflect the status of systemic health or disease. Therefore, dentistry and other medical disciplines have started to search for biomarkers in saliva and to develop relevant laboratory tests (Fig. 1, arrow B).

Although the hormone-like effects of salivary gland products such as cell growth factors were investigated during the late 1980s and early 1990s [43], little is understood about the influence of the salivary gland on the entire body, and the functions *in vivo* of growth factors produced by the salivary gland have not yet been determined. However, since components produced by salivary glands are likely to enter the bloodstream by reabsorption from the duct and sublingual mucosa [3], salivary glands might contribute to both oral and total health. Thus, the potential hormone-like effects of such components on the whole body should be re-examined (Fig. 1, arrow C). The potential clinical roles of factors produced by the salivary gland should be investigated in detail.

We propose “salivary glands and health medicine” as a novel field of study. This concept includes elucidating

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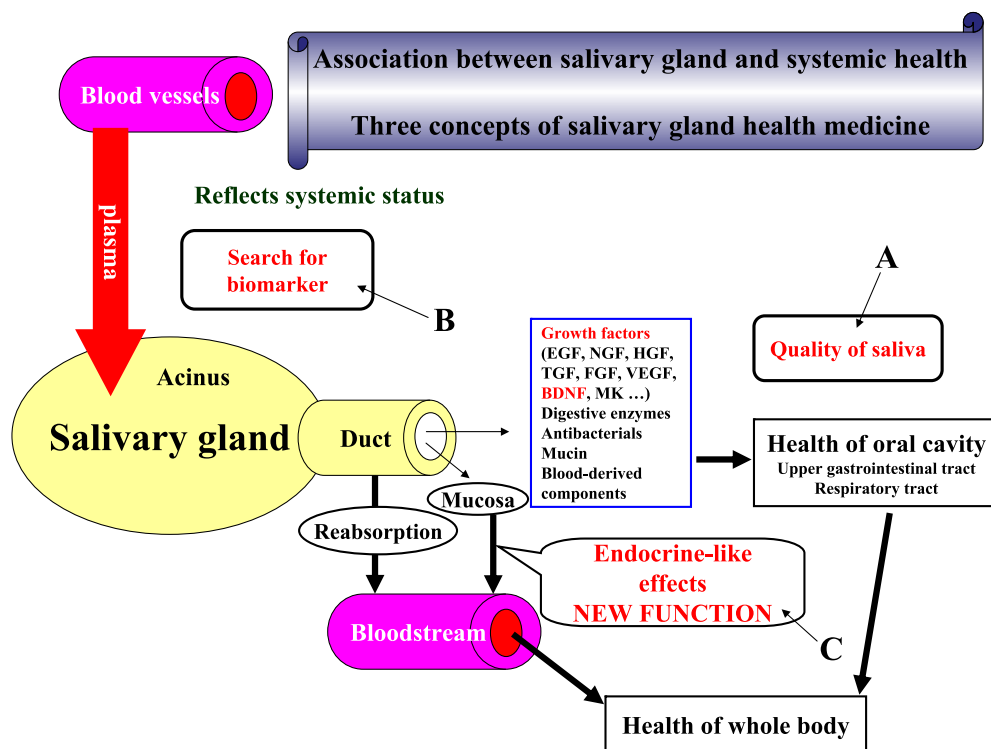


Fig. 1. Schema of the role of the salivary gland in health medicine. Important research concepts regarding salivary glands are shown.

the effects of the body on the salivary glands and the reciprocal effects of the salivary glands on the body, as well as the clinical applicability of laboratory tests of saliva (Fig. 1, arrow A–C). Especially, we reported regarding hormone-like effects of salivary gland in workshop of the 52nd annual meeting of the Japan Society of Histochemistry and Cytochemistry.

Salivary glands produce various cell growth factors, such as epidermal growth factor (EGF), nerve growth factor (NGF) and hepatocyte growth factor [39, 40]. In fact, EGF and NGF purified from the rat submandibular gland led to the acknowledgment of new salivary gland functions [8, 9]. Mouse salivary gland tissues express high levels of NGF [3]. The NGF family consists of NGF, brain-derived neurotrophic factor (BDNF), and neurotrophins (NTs)-3, -4/5, -6, and -7, all of which are collectively referred to as neurotrophins [6, 23, 32]. However, the expression of neurotrophins other than NGF in the salivary gland has not been analyzed. Immobilization stress reduces mRNA levels of neurotrophins such as NGF, BDNF, and NT-3 in the rat brain, especially in the hippocampus [44]. In contrast, NGF expression increases in response to stress in the mouse salivary gland [3]. The production of various cell growth factors often increases during episodes of stress to maintain homeostasis in the salivary gland [3]. We describe the relationship between hormone-like effects and stress-related BDNF expression of the rat submandibular gland in this review.

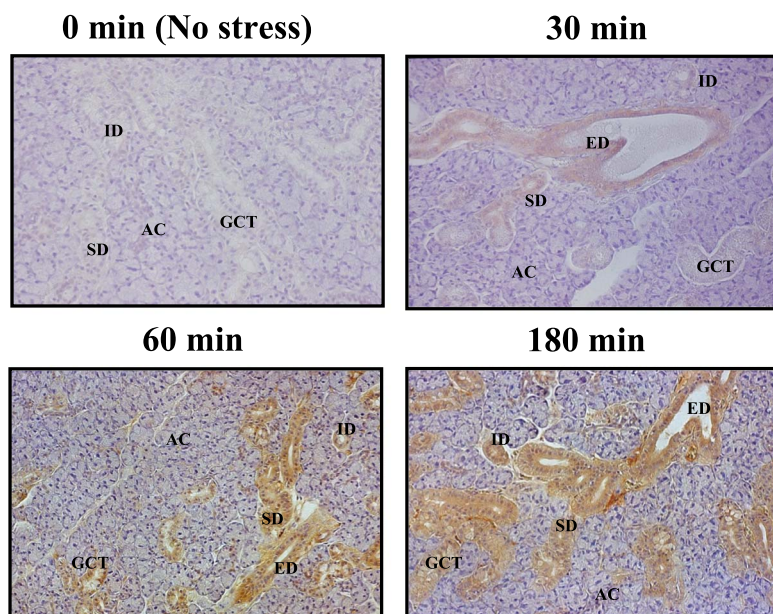
## II. What is BDNF?

More BDNF (purified from pig brain) is expressed and it is more widely distributed than NGF in the central nervous system (CNS), where it acts as a trophic factor for dopaminergic and cholinergic neurons of the substantia nigra/ventral mesencephalon [6, 23]. In addition to being retrogradely transported, BDNF that is anterogradely transported in the CNS acts as both a target-derived neurotrophic factor and an autocrine/paracrine modulator [4]. Furthermore, BDNF plays an important role in long-term synapse potentiation [18]. The amount of BDNF expressed in the hippocampus varies depending on the amount of stress [13], stress plus biting behavior [22], exercise [1] and learning [11], and it plays an important role in facilitating the formation of neural networks. On the other hand, the lacrimal glands [13], lymphocytes [36], vascular endothelial cells [24] and salivary glands of rats [30, 31] and humans [33] express BDNF. BDNF may play important roles in the various organs except for CNS.

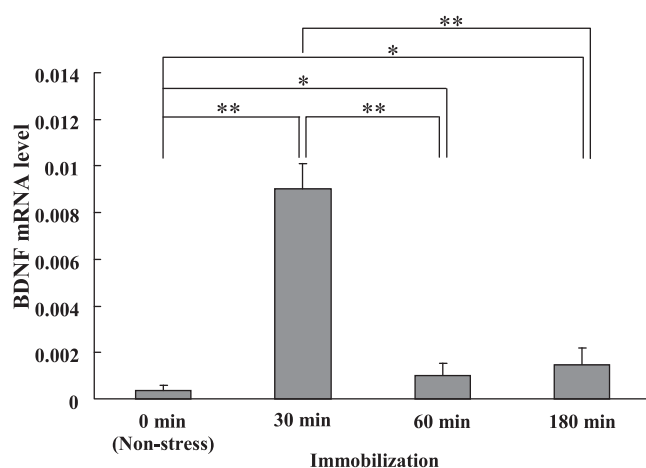
## III. Salivary BDNF Expression Increases under Acute Stress

Male Sprague-Dawley rats aged 7–9 weeks (Japan SLC, Shizuoka, Japan) were immobilized using a stress model according to an established protocol [17] that rapidly induces ACTH and corticosterone [13]. We previously demonstrated increased BDNF mRNA and protein expres-

## Immobilization



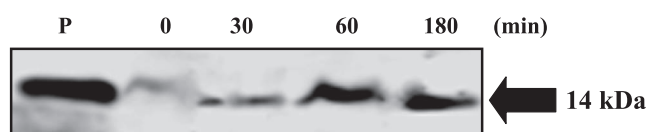
**Fig. 2.** Immunohistochemical localization of BDNF in male rat submandibular gland following immobilization stress. Immunohistochemical localization of BDNF protein in paraffin-embedded tissues from stressed rats using anti-BDNF monoclonal antibody (n=6). Duct cells express BDNF protein (original magnification,  $\times 100$ ). AC, acinar cells; ED, excretory ducts; GCT, granular convoluted tubule; ID, intercalated ducts; SD, striated ducts (modified from Tsukinoki *et al.*, 2006).



**Fig. 3.** Effect of immobilization stress on BDNF mRNA levels in rat submandibular gland. Levels of BDNF mRNA determined in rat submandibular gland using quantitative RT-PCR. Data are BDNF/ $\beta$ -actin mRNA ratios. Graph of BDNF mRNA in stressed rats shows significant differences between non-stress and at 30, 60 or 180 min of stress. Values are means $\pm$ SEM; n=6 rats per group. \* $p < 0.05$ , \*\* $p < 0.01$ , ANOVA/Tukey's test (modified from Tsukinoki *et al.*, 2006).

sion in rat submandibular gland tissue using this model [41]. Ernfors *et al.* [12] originally described BDNF 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 [12]. Our findings under non-stressed conditions were consistent with these (Fig. 2). The imposition of acute immobilization stress resulted in BDNF protein becoming localized to the rat ductal epithelium including stratified and granular duct

## Immobilization



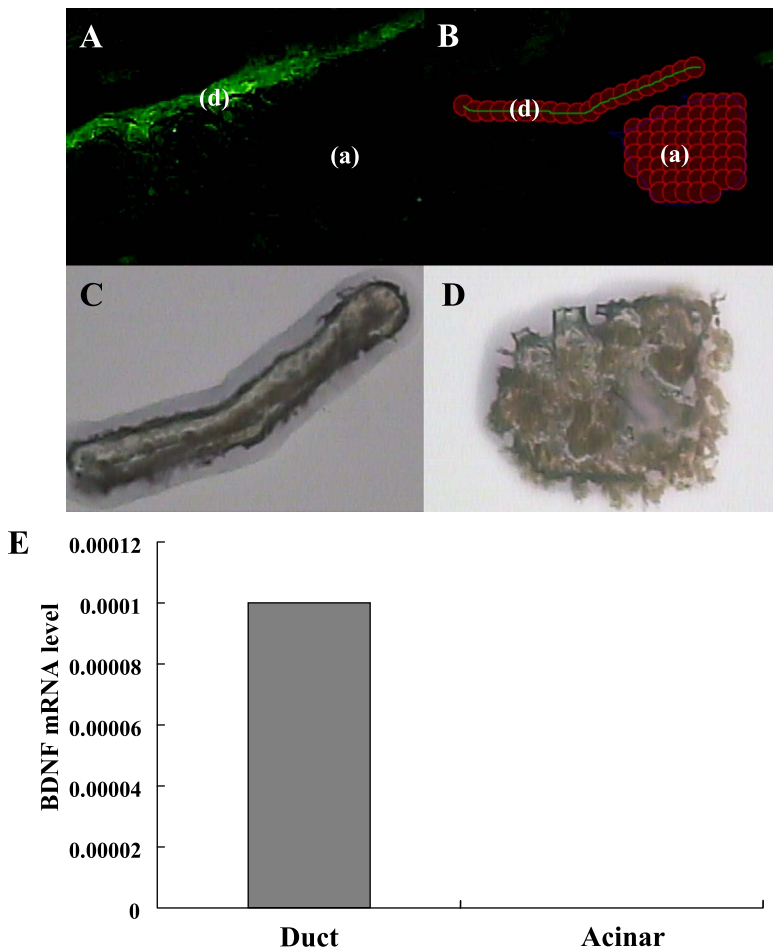
**Fig. 4.** Effects of immobilization stress on BDNF protein levels in rat submandibular gland. Western blots of BDNF expression in equal amounts of total protein from submandibular glands of control and stressed rats show 14 kDa bands in brain and submandibular gland of stressed rats (n=6). P, Positive control (brain tissue) (from Tsukinoki *et al.*, 2006).

cells (Fig. 2). Levels of BDNF mRNA (Fig. 3) and protein (Fig. 4) in the submandibular gland significantly increased in stressed rats compared with non-stressed rats [41]. Levels of BDNF mRNA notably increased in rats that were immobilized for 30 min (Fig. 3). The microdissection of cells that were BDNF-immunofluorescence positive combined with quantitative RT-PCR revealed BDNF protein and mRNA localization in the ductal epithelium of rat submandibular glands (Fig. 5). These findings indicated associations between morphological findings and BDNF mRNA expression, and suggested that the salivary gland is sensitive to stress. That is, the submandibular gland responds to stress by expressing more BDNF.

## IV. Expression of TrkB in the Submandibular Gland under Acute Stress

BDNF interact with high-affinity protein kinase receptors for the tyrosine receptor kinase (Trk) family [5, 36],

### Immobilization (60 min)



**Fig. 5.** Microdissection analysis of BDNF mRNA and protein expression in rats after immobilization stress for 60 min. (A) Immunofluorescence staining identified BDNF reactivity within duct, but not acinar cells (original magnification,  $\times 200$ ). (a) Acinar cells. (d) Duct cells. (B) Duct and acinar cells dissected from tissue sections (original magnification,  $\times 200$ ). (a) Acinar cells. (d) Duct cells. (C) Cap image shows dissected duct cells (original magnification,  $\times 400$ ). (D) Cap image shows dissected acinar cells (original magnification,  $\times 400$ ). (E) Levels of BDNF mRNA in rat submandibular gland measured using quantitative RT-PCR. Graph shows BDNF/ $\beta$ -actin ratios of dissected samples. Levels of BDNF mRNA in duct and acinar cells are 0.0001 and 0, respectively. Duct, but not acinar cells express BDNF mRNA (modified from Tsukinoki *et al.*, 2006).

particularly the TrkB receptor [5]. Notably, RT-PCR did not detect TrkB mRNA in the submandibular gland, in the oral or esophageal mucosa in non-stressed rats, and in time-course stress studies despite increased BDNF mRNA and protein expression (Fig. 6A and B) [41]. Others have also shown that TrkB is not expressed in human salivary glands [10] or in esophageal mucosa [35] in the absence of stress. Thus, BDNF derived from the submandibular gland might act at distant sites after release into the bloodstream.

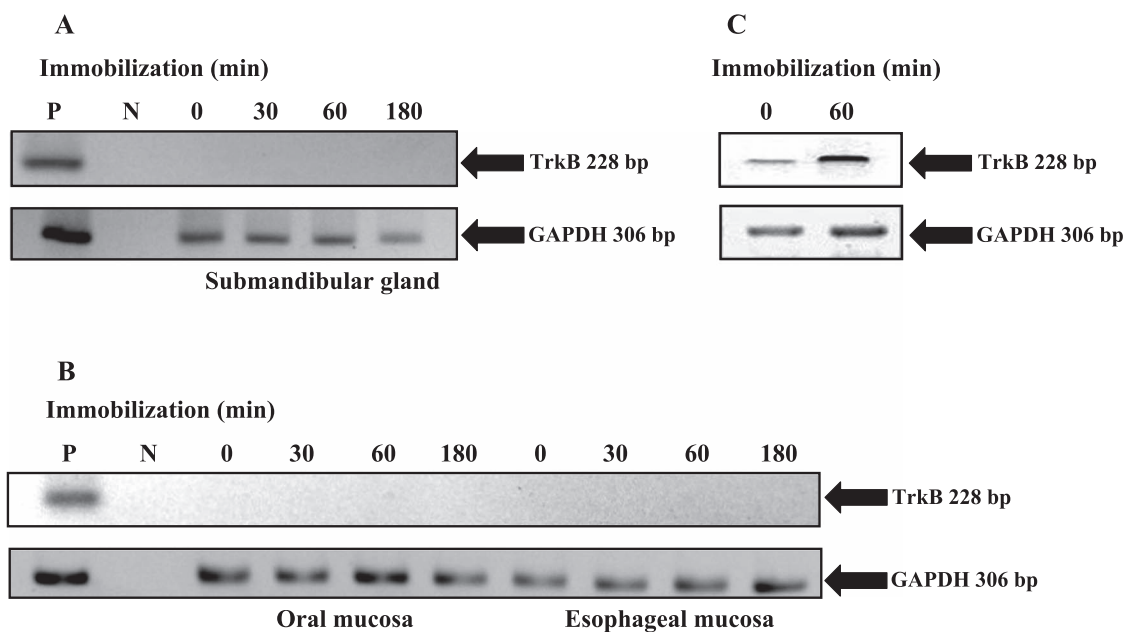
### V. Association of Plasma BDNF and Salivary Glands

The salivary glands release NGF into the bloodstream after stress induced by fighting [2, 3], and serum and brain BDNF protein levels positively correlate [19]. However, serum BDNF is unlikely to affect the CNS, since serum BDNF is derived from intact platelets [45]. Rat plasma contains low levels of free BDNF [27]. Since BDNF can cross the blood-brain barrier [26], the effects of free plasma BDNF on the central nervous system might be more significant than those of serum of BDNF. Although trauma-induced changes in neurotrophins and their receptors within

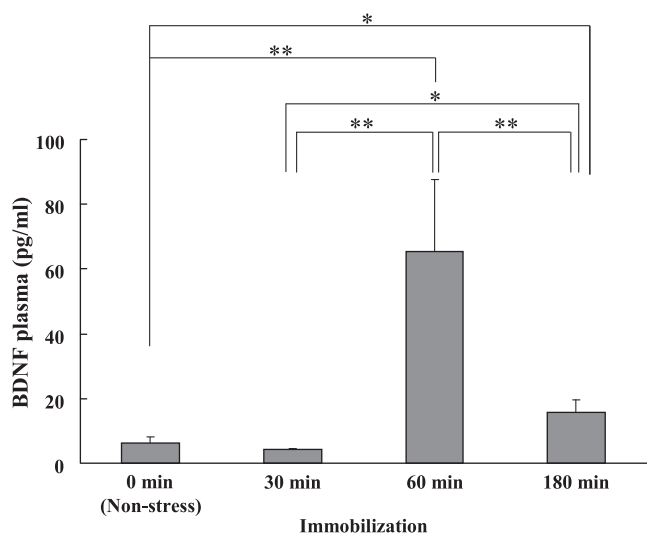
the central nervous system might protect against neuronal damage [14], free plasma BDNF might contribute to recovery from a decrease of BDNF. However, the source and roles of plasma BDNF are poorly understood under the physiological conditions.

Plasma BDNF levels were significantly higher after stress for 60 and 180 min than in controls and after 30 min of stress (Fig. 7) [42]. Thus, we confirmed that acute immobilization stress increases plasma BDNF levels. Elevated plasma BDNF protects against neural damage by methamphetamine [20]. However, a decrease in plasma BDNF correlates with the severity of schizophrenia accompanied by tardive dyskinesia, indicating that a reduction in neural cell protection elicited by BDNF is responsible for the tardive dyskinesia [15, 29]. In addition, since BDNF can pass through the blood-brain barrier [26], free BDNF entering the plasma (endogenous BDNF) might protect neural cells and maintain their functions. Therefore, an increase in plasma BDNF might help to protect cells against damage caused by acute stress at the early stages.

Removing the salivary glands (sialoadenectomy) suppressed the increase in plasma BDNF at 60-min of stress (Fig. 8) [42]. However, since the suppression was partial in

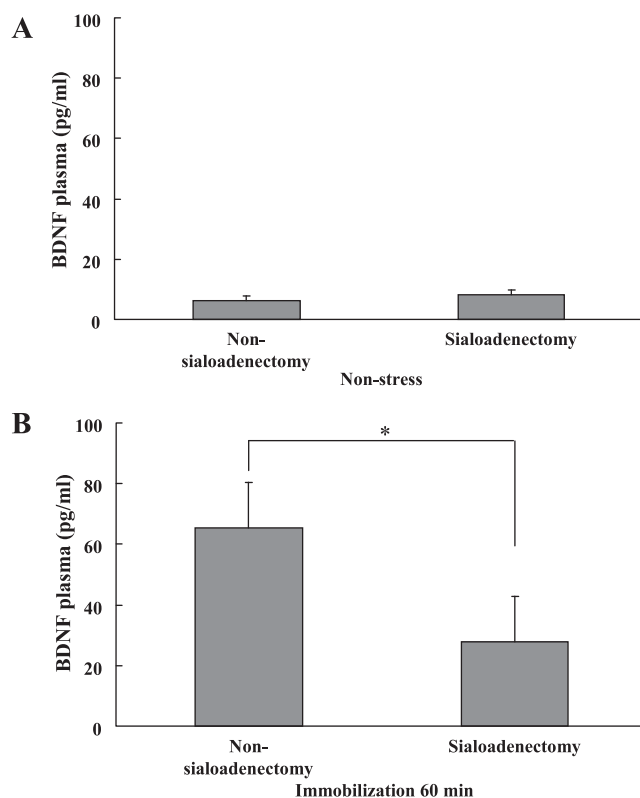


**Fig. 6.** Effects of immobilization stress on TrkB mRNA expression in rats. Levels of TrkB mRNA in rat tissues were investigated using conventional RT-PCR. (A) Submandibular gland tissue. (B) Oral and esophageal mucosa. TrkB mRNA was detectable only brain tissue. (C) Adrenal gland tissue. P, Positive control (brain tissue); N, Negative control (DNase/RNase-free deionized, distilled water); 0, no stress; 30, 60, 180, immobilization stress for 30, 60, and 180 min, respectively (modified from Tsukinoki *et al.*, 2006).



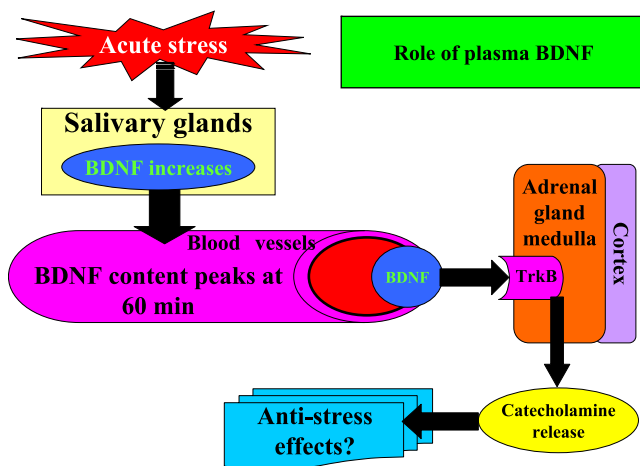
**Fig. 7.** Plasma BDNF levels following immobilization stress. Plasma BDNF concentrations assayed using ELISA kits. Values are means $\pm$ SEM; n=6 rats per group. Significant differences: \* $p$ <0.05, \*\* $p$ <0.001 ANOVA/Tukey's test (modified from Tsukinoki *et al.*, 2007).

sialoadenectomized rats, we investigated BDNF expression in the rat heart, lungs [37], liver [7], pancreas [16], and spleen [34], as they might be associated with peripheral BDNF. The expression of BDNF mRNA in these organs did not significantly increase with immobilization [42]. A previous study found increased BDNF mRNA and protein expression in the pituitary glands of rats exposed to acute



**Fig. 8.** Levels of BDNF protein in sialoadenectomized rats before and after acute immobilization stress. Plasma BDNF levels in non-stressed (A) and stressed (B) rats. Values are means $\pm$ SEM; n=6 rats per group. Significant difference, \* $p$ <0.05 Student's t-test (modified from Tsukinoki *et al.*, 2007).





**Fig. 9.** Possible roles of stress-induced BDNF production by salivary glands. Stress-related salivary BDNF transits from salivary gland to bloodstream. Plasma BDNF derived from salivary gland circulates and binds to TrkB in adrenal medulla where TrkB activation induces catecholamine release.

immobilization stress for 60 min [13]. The pituitary gland produces various hormones and thus might also release BDNF into the bloodstream. If so, then not only the salivary gland but also the pituitary gland influences plasma BDNF during acute immobilization stress. However, we speculate that the submandibular glands are the primary source of plasma BDNF during acute immobilization stress, because plasma BDNF increased more in non-sialoadenectomized rats than in sialoadenectomized rats. In addition, the submandibular gland might release salivary BDNF into the bloodstream in response to acute immobilization stress.

## VI. Role of Plasma BDNF in the Adrenal Gland

We previously described several novel findings regarding the role of BDNF and its receptor TrkB during immobilization stress [21]. Firstly, levels of TrkB mRNA in organs including the cerebral cortex, hippocampus, lung, stomach, liver, pancreas, kidney, pituitary gland and adrenal gland were determined by real-time PCR using a primer set that recognizes the TrkB extracellular domain (pan-TrkB). We found that acute immobilization stress for 60 min did not affect TrkB mRNA expression in the cerebral cortex, hippocampus, lungs, stomach, liver, pancreas and kidneys. In contrast, TrkB mRNA expression in the pituitary and adrenal glands was modified and TrkB receptor expression was maximally increased at 60 min of stress in the adrenal medulla compared with that in the absence of stress (Fig. 6C). Secondly, plasma BDNF might activate TrkB receptors of the adrenal medulla during acute stress. Thirdly, BDNF directly induces the release of catecholamines from PC12 cells [25] derived from chromaffin cells of the rat adrenal medulla by activation of TrkB-T1 receptors. Thus, BDNF-TrkB interactions might modulate

catecholamine release from adrenal chromaffin cells under acute stress (Fig. 9).

## VII. Conclusion

The expression of BDNF was increased in the submandibular glands of rats under acute immobilization stress, and contributed to increased plasma BDNF levels. The increase in the plasma BDNF concentration was significantly greater in non-sialoadenectomized rats than in sialoadenectomized rats. Thus, we demonstrated that the rat submandibular glands are the major source of plasma BDNF under acute immobilization stress. Furthermore, TrkB receptor expression was increased under acute stress and peaked at 60 min of stress in the adrenal medulla compared with levels under non-stressed conditions. Further studies should investigate BDNF-TrkB interactions in organs including the brain, and not simply focus on the adrenal gland that expresses high levels of TrkB.

## VIII. Acknowledgments

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