The Integration of Salivary Immunoglobulin A by the Repetitive Stressful Task

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Abstract

Recent psychophysiological studies have revealed that the human endocrine and immune secretion changes its level sensitively according to participants' subjective stress and relaxation. In this study, one of the major secretory immune substances, salivary Immunoglobulin A (IgA), was assessed to investigate the human secretion response induced by a short-term stress. In the experiments, subjects were required to conduct a short period of a calculation task repeatedly with intervals. The result indicated that the IgA changed clearly along with the calculation/interval schedule. Thus the IgA could be assumed to reflect the participants' subjective stress level. Besides, the integrative change of IgA was also observed depending on the duration of the stressful task. The relatively slower and long lasting modulation feature of the secretion response could result in such an integrative change. This, in turn, shows the possibility of estimating the intermittent workload stress in our daily life by tracing IgA.

Keywords: Immunoglobulin A; stress marker; acute stress; Psychoneuroendocrinology; Psychoneuroimmunology

Introduction

This study is targeted on the estimation of human mental stress by introducing one of the human secretory substances, salivary immunoglobulin A (IgA). This intriguing new field of study has been started by the findings in 70's that the human secretory substances changes its volume depending on his/her mental states. That field of study has then drastically developed for the last decade accompanying with the development of molecular analysis techniques, and nowadays, it forms an interdisciplinary scientific field called as *Psychoneuroendocrinology (PNE)* and/or *Psycho-neuroimmunology (PNI)* (Ader, Felten & Cohen, 2001)[1]. In the numerous past psychophysiological studies, stress estimation has been frequently investigated introducing central and autonomic nervous system indices, such as brain wave, heart rate, blood pressure, and respiration. PNEI studies, by contrast, focus on the change of human secretion inside the body by assessing the variety of hormones and immune substances. Those substances are considered to interact both central and autonomic nervous system, thus PNEI is expected to reveal the underlying mechanism of how the stressor recognized in a brain brings forth the physiological change inside human body.

However, even though past PNEI studies successfully observed physiological changes in the human body depending on subjects' mental states (see the review e.g., Bosh, Ring, de Geus, Veerman & Amerongen, 2002), the number of studies is still small and some studies have reported controversial results. Besides, there are some technical and methodological issues need to be improved. For example, few studies have been investigated precise change of secretory substances in time series. Most of the past studies assessed human secretion, such as saliva, urine, and blood, just before and after 20-30 minutes of psychological intervention but covering all the experimental period. Some reasons can be thought for that small numbers of assessing human secretory substances; acceptability of subjects for repetitive sampling that is especially for blood and urine, expense of quantitative determination of secretory substances. However, the change of secretory substance in the human body are recently turned out to be much more sensitive in terms of reaction speed ever thought before. It thus is possible that the small time difference of secretion collection could result in big difference of the amount of secretory substances, and that would lead to the controversial results against the same psychological interventions (Valdimarsdottir & Stone, 1997). In addition, such physiological responses are also turned out not to be a merely reflection for the given interventions but higher cognitive processes including individual personality can mediate those responses (Valdimarsdottir & Stone, 1997; Ohira, 2001; Bosh, Ring, de Geus et al., 2002). Therefore, for further investigation, it is quite important to design an experiment more systematic, well controlled, and reproducible.

[1] In this paper, we use the term *PNEI* (Psychoneuroendocrine-immunology) for indicating both PNE and PNI.

In this study, we investigated the precise change of salivary immunoglobulin A (IgA) induced by a short-term stressful task paying attention to the methodological issues mentioned above. IgA is one of the immune substances secreted in saliva, and it is known to increase by mental stress and frequently introduced in past PNEI studies. But no study has investigated the precise change of IgA in time series. We thus designed an experiment aiming to obtain a basic and useful knowledge for future PNEI study.

Salivary immunoglobulin A (IgA)

A glycoprotein called immunoglobulin A (IgA) is one of the most important substances for human immune system. IgA exists within serum and other various secretory fluids, such as saliva, breast milk, and nasal, gastrointestinal, bronchial, and urogenital secretions. IgA containing in those secretory fluid (IgA) are normally in the dimeric form combined with other glycoprotein named J chain and secretory component (SC) which stabilizes the IgA molecular and protects it from degradation in those fluid (Tsujita & Morimoto, 1999). The salivary IgA antibodies work nonspecifically and, therefore, play a very important role for our health, e.g., for preventing bacteria from forming colonies, neutralizing toxins and enzymes produced by bacteria, and inhibiting pathogenic viruses to penetrate into the epithelial cell. It is thus considered that the salivary IgA works as the first line of defense from influenza or other upper respiratory tract infection (URTI) illnesses. Actually, clinical studies suggested that the correlation between relatively higher levels of salivary IgA and a lower incidence of an acute URTI (Jemmott III, & McClelland, 1989). It was also reported that the relation of salivary IgA level with caries and periodontitis (Gregory, Kim, Kindle, Hobbs, & Lloyd, 1992).

On the other hand, by the 70's behavioral-immune studies, it was found that salivary IgA changes its level accompanying with various types of psychosocial factors (see review, e.g., Valdimarsdottir & Stone, 1997; Bosh, Ring, de Geus et al., 2002), such as desirable or undesirable daily events (Stone, Neale, Cox, Napoli, Valdimarsdottir & Kennedy-Moore, 1994), daily hassles (Martin & Dobbin, 1988), negative or positive mood (Martin, Guthrie & Pitts, 1993), academic stress such as an examination (Jemmott III, Borysenko, Borysenko, McClelland, Chapman, Meyer & Benson, 1983) and presentation (Evans, Bristow, Hucklebridge, Clow & Pang, 1994), a short-term stress (Jemmott III & McClelland, 1989) and relaxation (e.g., Green & Green, 1987; Knight & Rickard, 2001). A review article indicated there are distinguishable two types of stress effect on IgA: 1) increasing IgA secretion immediately after a shot-term stress, named "*immediate stress effect*", and 2) decreasing IgA secretion several days after stress, named "*delayed stress effect*" (Tsujita & Morimoto, 1999). Because the delayed stress effect could be easily masked by the immediate stress effect, it is concluded that the IgA can be a useful stress maker for a short-term stress.

However, the precise IgA modulation against a short-term stress is still unknown. We then conducted an experiment investigating the changes of IgA concentration in time series induced by a short-term stressful task with repetitive saliva collection.

Method

Subjects

Subjects recruited for this study were ten healthy male students, ages ranged from 21 to 31 years old. They were well informed about the experiment and the purpose of our study before the experiment. They all had no problem about physical condition and did not have any infectious illness when they experienced the experiment. Subjects were indicated not to eat and drink without water for one hour prior to the experiment because pH of saliva could affect quantitative determination of IgA.

A Short-Term Stressful Task

Subjects were instructed to conduct a simple calculation task as a short-term stressful experience. The calculation task was a simple addition of two double-digit integers, and it was repeatedly presented on the laptop monitor (INSPIRON 700m with 12.1 inch thin film transistor-liquid crystal display, DELL Inc., USA) every 3.0 seconds with changing the figures. Subjects were instructed to conduct the calculation task as fast and correct as possible.

Such a simple calculation task is quite similar to so-called Kraepelin psychodiagnostic test, which is frequently introduced for the researches investigating the effects of the mental stress on physiological indices. The result of our experiment thus expects to compare with broad research fields of study.

Experiments

Two types of experiments differing the duration of calculation task were prepared for this study to compare the IgA changes in time series. In experiment A, subjects were instructed to conduct two sets of 18 minutes of the calculation tasks and 9 minutes of breaks after that. Thus the duration of the calculation and break was 36 and 18 minutes in total. In experiment B, subjects were instructed to conduct six sets of 6 minutes of the calculation tasks and 3 minutes of breaks after that. The total duration of the calculation tasks and breaks was the same as the experiment A. Each subject daily experienced one of those types of experimental procedures.

Saliva samples were taken by small cotton every three minutes during the calculation tasks and breaks. Saliva was also taken 3, 10 and 20 minutes after the last set of calculation/break. Subjects were instructed to place the cotton under his tongue, not to chew, for three minutes. Those cottons were centrifuged at 1500 rpm for 10 minutes to remove mucin, and stored in freezing chamber at –20 Celsius before quantitative analysis.

Each experiment A and B was conducted in the afternoon for reducing the influence of the diurnal change of IgA. The order of those experiments was counterbalanced among the subjects. All the experimental procedure was conducted in a dark and soundproof room one by one.

IgA determination

Salivary IgA concentration was determined by enzyme-linked immunosorbent assay (ELISA) (Salivary Secretory IgA Indirect Enzyme Immunoassay Kit, Salimetrics, LLC., USA). ELISA is nowadays the major molecular determination technique. ELISA is much easier in treatment and cheaper in running cost than other molecular determination techniques, such as the radioimmunoassay (RIA) that needs to be assessed with radioactive substance and the high performance liquid chromatography (HPLC) that costs higher than ELISA for samples in small number. The principle of ELISA is based on the antigen-antibody reaction for capturing a target substance and the enzyme reaction for detecting the mass via optical density of reaction produced color. The ELISA Kit we use is employing competitive method. Brief description of the analysis procedure is as follows; first, each saliva sample (or standard human IgA solution) is mixed with a constant amount of the anti-human IgA conjugated to horseradish peroxidase (HRP). Next, the unbind IgA antibody is added to human IgA-coated 96-well micro-plate. Because the bottom of the micro-plate is coated with human IgA, the unbind IgA antibody conjugated to HRP is captured. The amount of the bind enzyme conjugate can be detected as optimal color strength (near 450nm) cased by enzyme reaction. Therefore, that optimal density is inversely proportion to the concentration of IgA containing in each saliva sample. Finally, IgA concentration of each sample is determined by referencing the optimal density of the standard samples. All the analysis procedure takes roughly 8 hours for one micro-plate.

Results

Behavioral profiles

The averaged score of the calculation task during the experiment A and B indicated no significant difference. On the other hand, according to the results of five points' scaled questionnaire, five out of ten subjects replied the experiment B was relatively stressful than the experiment A.

IgA Changes in Experiment A and B

As for an averaged profile, salivary IgA concentration has changed along with the calculation/break schedule in experiment A and B, as in Figure 1 and 2. It shows our experimental design was adequate for investigating the effects of a short-term stress on human secretory substance.

In the experiment A, IgA got increased immediately after the calculation task and decreased when the task removed.

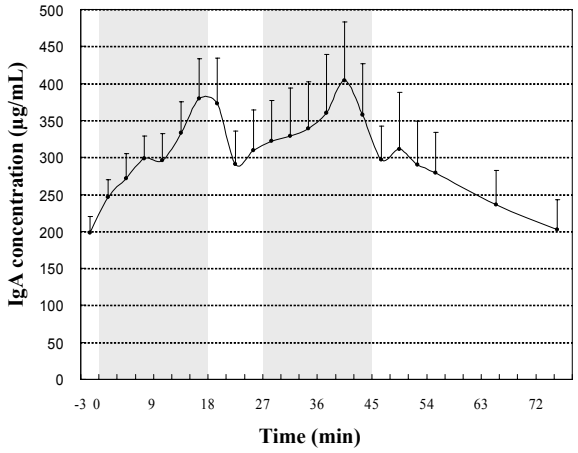

Figure 1: IgA concentration in the experiment A. Error bar indicates standard error. The period indicated as in gray band represents the calculation task.

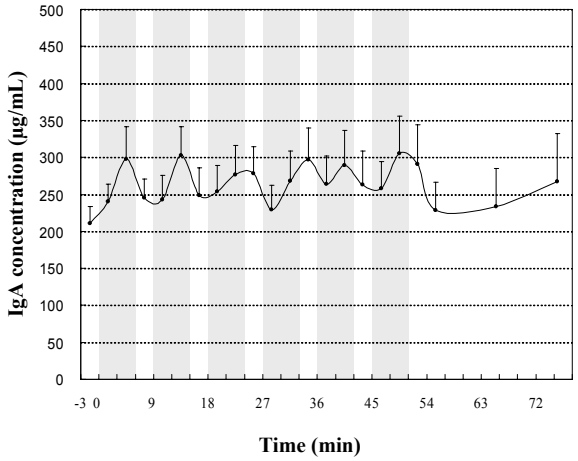

Figure 2: IgA concentration in the experiment B. Error bar indicates standard error. The period indicated as in gray band represents the calculation task.

In addition, interestingly, the average IgA concentration during the second set of the calculation task in the experiment A was higher than that of the first set (a significant main effect in period; $F(21,189)=2.74$, $p<.01$ and post hoc comparisons between two sets; $p<.05$ by paired t-test), as in Table 1. That could happen because the duration of the first break was not enough to recover the IgA to the baseline.In the experiment B, IgA got slight increase and decrease repeatedly along with the six sets of the calculation/break. The IgA integration effect during the experiment B was not observed unlike the experiment A, i.e., no significant main effect in period has found.

Comparing the experiment A and B, the average baseline of IgA had no significant difference, but the average IgA concentration during all through the experiment and during through 36 minutes of the calculation task in the experiment

A was significantly higher than that of B (p<.01 by paired t-test respectively), as in Table 2.

When one considers those differences in IgA changes, it could assume that the IgA increase by a short-term stress would be in the form of exponential. If IgA increased and decreased linearly, the integration effects would be found, or not found, for both experiments because the rate of the duration of the calculation/break was the same (2:1) in both experiments. Thus, if a short-term stress does not last long, IgA would decreases to the baseline during a short break, and vice versa.

Saliva flow

The total amount of sampled saliva volume in the experiment A was significantly smaller than that of in the experiment B (p<.01 by paired t-test). However there was no correlation between saliva volume and IgA in both experiments (by Pearson's correlation; r = -0.25, p>.05 and r = -0.23, p>.05 for the experiment A and B).

Table 1: Average IgA concentration (standard deviation) during the first and second set of task.

First task period	Second task period	*t-test*
304.1(123.1)	351.8(210.8)	p<.05

Unit of IgA concentration is μg/mL.

Table 2: Average IgA concentration (standard deviation).

Period	Experiment A	Experiment B	*t-test*
Base	198.5(68.3)	210.4(74.3)	Ns
Task	327.9(173.5)	269.0(119.8)	p<.01
Break	278.8(166.0)	256.3(133.9)	Ns
Total	305.6(171.5)	263.2(126.3)	p<.01

Unit of IgA concentration is μg/mL.

Discussion

IgA as a psychophysiological index

Our experimental results that the average IgA concentration changed according to a short-term stress and break schedule is quite interesting. Such an IgA change has been expected when one reviews the numbers of past IgA studies (e.g., Valdimarsdottir & Stone, 1997). However almost all those studies have assessed IgA just before and after a stressor, thus no study has shown such a detail IgA change in time series. Especially, in the experiment B, such an IgA change observed even by 6 minutes of a stressful tasks and 3 minutes of breaks, while it was indicated as an averaged profile. It implies that the IgA change induced by a short-term stress can occur much more sensitive and rapid than ever thought before.

On the other hand, IgA concentration had no correlation with salivary flow rate. Therefore our experimental results can bring basic knowledge about human IgA secretion against mental stress, such as IgA secretion speed and acceleration. Actually, the IgA integrative change observed in the experiment A can reflect the difference in IgA increasing speed by a stressor and decreasing by break. If one assumes that IgA increase by a stressor would be in the form of exponential against the duration of the stressor, it is accountable that 1) the recovering by break in the experiment A was not enough unlike in the experiment B, 2) averaged IgA concentration during all through the experiment A was higher than that of B, and 3) it took much time to recover the IgA to the baseline in the experiment A than B.

As a whole, our experimental result could imply the possibility of IgA as a psychophysiological index in two aspects. One is as a transient short-term stress marker, and another is as a long lasting or recursive stress marker.

Application of IgA for stress management

Our experimental result was quite unique and remarkable in the point that subjects' subjective change of mental state could be explained by a single physiological index of IgA. Besides, stress estimation by salivary IgA has several methodological advantages, for instance, it is easy for sampling, non-invasive, less stress and repeatable sampling unlike blood, conservable for a long time, and so on. That means IgA can be applicable for a broad stress management field, while we need to integrate much more studies on IgA or other secretory substances. For example, the simple calculation task introduced in our experiment possesses the same characteristics of a typical visual display terminal work (VDT work) in terms of its repetitive, boring and endless feature. When one compares the behavioral profiles in our experiments, there was no difference in performance significantly. However the physiological change, that is the IgA change, induced by such tasks was significantly different; total amount of IgA secretion was higher in the experiment A and an integration effect was observed in the experiment A. Certainly, one might not engage such a calculation task in actual daily VDT work, but point is the fact that the same performance does not necessary result in the same effects inside the human body. Thus it leads to the idea of the stress management for the intermittent type of office work. We have already proposed the simple mathematical model, which could successfully mimic the IgA change during intermittent calculation task in our experiment (Nomura, 2006). The model is designed as the nonlinear ordinary differential equation consisting of the first order of exponential increasing term and the second order of nonlinear decreasing term. We suggested that the stress level caused deskwork could be predictable by such a simple model and derive an optimal work/break schedule. This idea might be applicable for other intermittent type of work situations like as long distance driving.

On the other hand, autonomic nervous system related indices such as heart rate and blood pressure might also seem to be useful markers for evaluation of intermittent workload stress. But those indices are under strong and rapid homeostatic control, so it would not be suitable for

evaluation of long lasting, from an order of minutes to hours, workload stress. Actually, the heart rate showed On/Off like change in our experiments, i.e., rose right after the task started and fell immediately after finishing the task, but did not show any integrative change. Respect to this point, human secretion related indices would take an advantage as for its slow and integrative change.

Possible mediators and other stress markers

We have to admit there must be uncountable potential mediator for our experiment, e.g., personality, chronic stresses, sex, age. For example, the subjects categorized in Type A behavioral pattern showed higher baseline of IgA and lower reactivity against acute stressor, as in Ohira, Watanabe, Kobayashi and Kawai (1999). Ohira also suggested that the idea of controllability against an acute stress unconsciously determined the salivary IgA (2001). Those studies thus suggested that the higher cognitive process could mediate the IgA secretion within human body. But studies targeting on those potential mediators are still small in number, it is necessary to investigate in future works.

In addition, there are some possible stress markers within human saliva besides IgA, such as cortisol (Vining & McGinley, 1987; Kirschbaum & Hellhammer, 1994; Ocknfels, Porter, Smyth, Kirschbaum, Hellhammer & Stone, 1995; King & Hegadoren, 2002), amylase (e.g., Yamaguchi, Kanemori, Kanemaru, Takai, Mizuno & Yoshida, 2004; they argue that salivary alpha-amylase responds only for distress but for eustress.), dehydroepiandrosterone (DHEA), free-3-mehoxy-4-hydroxyphenylglycol (free-MHPG) (e.g., Buchsbaum, Muscettola & Goodwin, 1981), and Chromogranin (Nakane, Asami, Yamada & Ohira, 2002). Especially, cortisol has been frequently introduced to the PNEI study investigating the effect of mental stress on human secretory circulation because it is considered that the cortisol in saliva and/or serum directory reflects the activation of the hypothalamic-pituitary-adrenal axis (HPA axis), which is thought to be the major biological pathway against mental stress. In addition, like as IgA, there are some studies reporting possible mediators of cortisol change, such as diurnal change, personality like as anxiety trait (Schlotz, Schulz, Hellhammer, Stoen & Hellhammer, 2005), reactivity for stressor (Nagamine, Murota, & Shimizu, 2002), and hypertensive (Nyklicek, Bosh, Nieuw Amerongen, 2005). On the other hand, the integrative PNEI researches assessing variety of physiological indices in blood, such as active natural killer (NK) cell level, varieties of T lymphocyte, dopamine, norepinephrine, and epinephrine, are recently undergoing, and showed rapid change of composition of those substances (Isowa, Ohira & Murashima, 2004; Kimura, Isowa, Ohira, Murashima, 2005). But far more research would need for discussing those results.

PNEI study is rapidly developing as mentioned above, but there are still few studies investigating the detail change of those substances against a short-term stress like this study. Therefore, far more systematic and integrative researches on the effects of a short-term stress on changes of human salivary and/or serum substances must be necessary for better understanding of underlying mechanism of stress driven psychophysiological interaction. More numbers of such researches will also promises to make a reasonable criterion for human mental stress toward the application of this fields, while it is not necessary to be thought that the modulation of human mental state is continuous in time.

Conclusion

In this paper, we illustrated the IgA as a useful transient stress marker and as a long lasting stress marker. In contrast, there are also some studies focusing on the effects of the positive change of human mental state on IgA and other secretory fluid. For example, the music listening helps to recover stress-induced increased IgA to the baseline (Nomura, Tanaka & Nagashima, 2006). Such a positive change of human mental states might be mediated strongly by higher cognitive process than the stress. Therefore, the PNEI studies would possibly reveal new research methods for cognitive science.

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