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Salivary alpha-amylase response to acute psychosocial stress: The impact of age

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ABSTRACT

The impact of stress on health varies across the different stages of human life. Aging is associated with psychobiological changes that could limit our ability to cope with stressors. Therefore, it is crucial to clarify the physiological mechanisms that underlie the stress response and the changes that occur in them as we age. Our aim was to investigate age differences in the salivary alpha amylase (sAA) response to stress, and its relationship with other typical stress biomarkers such as cortisol and heart rate (HR). Sixty-two participants divided into two age groups (younger group: $N = 31$, age range: 18–35 years; older group: $N = 31$, age range: 54–71 years) were exposed to the Trier Social Stress Test and a control condition in a crossover design. No age differences were found in the sAA or HR responses to stress. However, the sAA global output was higher in older than younger adults. Additionally, in the stress condition, the total amount of cortisol released was positively related to the total sAA released, while the HR increase was positively related to the sAA increase. Our results do not support the existence of an attenuated autonomic nervous system response to stress in older adults, but rather a heightened sympathetic tone. Furthermore, we found further evidence of the coordination between the hypothalamus–pituitary–adrenal system and the autonomic nervous system in their response to acute psychosocial stress.

1. Introduction

Lifetime exposure to stress can have important consequences for health. Stress has been related to a large number of pathologies that have a higher incidence in old age, such as cardiovascular disease, atherosclerosis, cancer or Type 2 diabetes (Chrousos and Kino, 2007; Steptoe, 1991). Aging is associated with several psychobiological changes, such as increased vulnerability to oxidative stress, imbalances in central neurotransmitter pathways, and changes in emotional regulation (Alameida et al., 2011; Ferrari and Magri, 2008; Salmon et al., 2010), which could limit our ability to cope with stressors (Pardon, 2007). Therefore, it is important to clarify the physiological mechanisms that underlie the stress response, as well as the changes that occur in them as we age.

Two main body systems are involved in the stress response, the autonomic nervous system (ANS) and the hypothalamus–pituitary–adrenal axis (HPA-axis). Recently, salivary alpha amylase (sAA), an oral cavity enzyme, has been identified as a possible biomarker of ANS reactivity to stress (for reviews see: Nater and Rohleder, 2009; Rohleder and Nater, 2009). This enzyme increases rapidly in response to physiological and psychosocial stress conditions, such as exercise and written examinations (Chatterton et al., 1996), the cold pressor stress test (van Stegeren et al., 2008), and the Trier Social Stress Test (Nater et al., 2005, 2006; Rohleder et al., 2004). Several studies have been performed to profile the sAA response to stress mainly in children (Granger et al., 2006; Räikkönen et al., 2010; Spinrad et al., 2009), adolescents (Gordis et al., 2006; Sumter et al., 2010; Susman et al., 2010) and young adults (Nater et al., 2005, 2006; Rohleder et al., 2006; Schoofs et al., 2008). However, data available on older people are very sparse and the results are mixed. Previous studies have suggested that old age has no effect on basal sAA levels (Aguirre et al., 1987; Pajukoski et al., 1997; Salvolini et al., 1999), although a more recent study has shown that older adults have higher overall sAA output throughout the day (Strahler et al., 2010a). To our knowledge, only one published study investigated the effect of old age on the acute sAA response to stress, finding an attenuated response in older adults (59–61 years) compared to young adults (20–31 years) (Strahler et al., 2010b).

More studies have been performed to examine the impact of aging on other ANS biomarker responses to stress, such as heart rate, heart rate variability or plasma epinephrine and norepinephrine. According to an exhaustive review by Seals and Dinenno (2004), it appears that the primary effect of aging on

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the human ANS is an elevation in the tonic sympathetic activity. However, the influence of aging on the ANS response to stress remains controversial. For example, several studies have reported no changes (Esler et al., 1995; Wood et al., 2002), a decreased response (Kudielka et al., 2004a; Strahler et al., 2010b), or even an enhanced response to stress with older age (Pascualy et al., 1999; Uchino et al., 1999).

In contrast to sAA, the impact of aging on the end product of HPA-axis activation, cortisol, has been investigated more extensively (for reviews and a meta-analysis see: Kudielka et al., 2009; Otte et al., 2005; Seeman and Robbins, 1994). Studies using pharmacological stimulation of the HPA-axis have consistently shown that older adults have an elevated HPA-axis response compared to young adults (e.g. Born et al., 1995; Heuser et al., 1994; Kudielka et al., 1999; Luisi et al., 1998). Nevertheless, results are mixed when studying the effects of aging on the cortisol response to different kinds of stressors (Kudielka et al., 2009). While several studies did not find any effect of aging on the cortisol response to psychosocial stressors (Kudielka et al., 1999, 2000; Nicolson et al., 1997; Rohleder et al., 2002), others found a higher cortisol response with increasing age (Gotthardt et al., 1995; Kudielka et al., 2004b; Seeman et al., 2001; Strahler et al., 2010b; Traustadóttir et al., 2005).

In the current study, we subjected a group of young and older participants to both a psychosocial stressor (TSST, Kirschbaum et al., 1993) and a control situation in a crossover design. Although no sex differences have been found in basal sAA levels (Nater et al., 2007; Rantonen and Meurman, 2000), or in the acute sAA response to stressors (Kivlighan and Granger, 2006; Takai et al., 2007), the HPA-axis shows sexual dimorphism. In fact, the cortisol response to stress is up to twice as high in men as it is in women, regardless of age (Kudielka et al., 2009). Furthermore, this response is dependent on the phase of women's menstrual cycle (Kirschbaum et al., 1999). For these reasons, the current study included men and women in equal numbers in each age group. Additionally, as it has been shown that taking oral contraceptives does not alter basal sAA levels or sAA responses to stress (Laine et al., 1991; Schoofs et al., 2008), and in order to avoid the effect of menstrual cycle phase on cortisol concentrations, we decided to select only young women taking oral contraceptives. Before, during and after the stress task, we measured cortisol and sAA concentrations and, as a complementary measure of the ANS, heart rate (HR). Following the only study that has assessed the effect of aging on the sAA response to stress (Strahler et al., 2010b), we expected attenuated sAA and HR responses to stress in the older group. Furthermore, due to the mixed results regarding the effect of older age on basal sAA levels and cortisol reactivity to stress, we investigated whether the sAA overall output and the stress-induced cortisol response were different between age groups.

2. Methods

2.1. Participants

The sample was composed of sixty-two participants divided into two age groups: older adults ($N = 31$; 16 men and 15 women; age range: 54–71 years) and young adults ($N = 31$; 16 men and 15 women; age range: 18–35 years). Within both age groups, there were no sex differences in age, body mass index (BMI), subjective socioeconomic status (subjective SES scale: Adler et al., 2000) or education level (for all $p > 0.11$) (see Table 1).

Most of the young participants (90%) were university students from a wide range of college studies, such as Psychology, Medicine, History, and unemployed (90%). Most of the older participants were retired (90%) and belonged to a study program at the University of Valencia for people over 50 years of age (84%). For subject recruitment, announcements were posted and informative talks were held in the various departments of the University campus. Volunteers were interviewed by trained psychologists and completed an extensive questionnaire to check whether they met the study prerequisites. Ninety-two volunteers were excluded from participation. The criteria for exclusion were: alcohol or other drug abuse, dental, visual or hearing problems, presence of cardiovascular, endocrine, neurological or psychiatric disease, and the presence of a stressful life event during the last year. Participants were excluded if they were using any medication directly related to emotional or cognitive function, or one that was able to influence hormonal and sAA levels, such as glucocorticoids, β-blockers, antidepressants, benzodiazepines, asthma medication, thyroid therapies, and psychotropic substances. Vitamins, sporadic use of painkillers, and natural therapies were allowed. All the older women were postmenopausal, having had their last menstrual period at least four years before, and none of them were receiving estrogen replacement therapy. All the young women were taking oral contraceptives (monophasic formulas). The use of contraceptives is widespread in Western society; therefore, women using this medication constitute an interesting research group in itself. None of the participants were habitual smokers, although in each age group two participants reported sporadic smoking (less than 10 cigarettes a week).

Participants meeting the criteria were contacted by telephone and asked to attend two sessions that took place in a laboratory at the Faculty of Psychology. Before each session, participants were asked to maintain their general habits, sleep as long as usual, refrain from heavy physical activity the day before the session, and not consume alcohol since the night before the session. Additionally, they were instructed to drink only water, not eat, smoke or take any stimulants, such as coffee, cola, caffeine, tea or chocolate, 2 h prior to the session, and not brush their teeth at least 1 h prior to the session. The study was conducted in accordance with the Declaration of Helsinki, and the protocol and conduct were approved by the Ethics Research Committee of the University of Valencia. All the participants received verbal and written information about the study and signed an informed consent form.

2.2. Procedure

This study employed a within-subject design with two completely randomized and counterbalanced conditions in two separate sessions: a stress condition and a control condition, with about two weeks between sessions. The sessions consisted of several phases of equal duration for both conditions. Each session took 1 h and 15 min to complete, and they were always held between 16.00 and 20.00 h. Each participant started his or her two sessions at the same hour. Upon arrival at the laboratory, the weight and height of the participants were measured, and the experimenter checked to see whether they had followed the instructions given previously (see Section 2.1).

2.2.1. Stress condition

To produce stress, we subjected the participants to the Trier Social Stress Test (TSST). The stress task consisted of 5 min of free speech (job interview) and a 5 min arithmetic task, performed in front of a committee composed of a man and a woman. The participants remained standing at a distance of 1.5 m from the committee. Additionally, a video camera and a microphone were clearly visible. Both the speech and arithmetic tasks were filmed.

The protocol started with a habituation phase of 15 min to allow the participants to adapt to the laboratory setting. During this phase, the participants remained seated. Five minutes after the start of this phase, they completed the mood questionnaire (PANAS Pre-task). After the habituation phase, the introduction phase started (duration 5 min). In this phase the participants were informed about the procedure for the stress task. They received the instructions in front of the committee in the same room where the task took place. Next, the participants had 10 min to prepare for the speech at hand. Following the preparation phase, the stress task was car-

Table 1
Descriptive statistics (mean ± SEM) of younger ($N = 31$) and older groups ($N = 31$).

	Younger group			Older group		
	Total	Men	Women	Total	Men	Women
Age (years)	22.4 (0.8)	22.1(1.4)	22.7(0.8)	61.8 (0.8)	60.5 (1.2)	63.1 (1.0)
BMI (kg/m^2)	23.4 (0.5)	24.1 (0.8)	22.7 (0.5)	26.6 (0.5)	27.0 (0.5)	26.1 (1.0)
SES[a]	6.5 (0.2)	6.4 (0.2)	6.6 (0.2)	6.1 (0.2)	6.1 (0.3)	6.1 (0.3)
Education level[b]	2.5 (0.1)	2.4 (0.2)	2.5 (0.2)	2.9 (0.2)	2.7 (0.3)	3.0 (0.3)

[a] SES: Subjective Socio-Economic Status scale, ranging from 1 (lowest SES) to 10 (highest SES) (Adler et al., 2000).
[b] Range: 0 = no studies, 1 = primary school, 2 = secondary education, 3 = university and higher education, 4 = postgraduate (Master, PhD). Values are means (±SEM).