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Attenuated Cortisol Response to Psychological Stress but not to CRH or Ergometry in Young Habitual Smokers

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KIRSCHBAUM, C., C. J. STRASBURGER AND J. LANGKRÄR. *Attenuated cortisol response to psychological stress but not to CRH or ergometry in young habitual smokers.* PHARMACOL BIOCHEM BEHAV 44(3) 527–531, 1993.— Salivary cortisol and heart rate responses to a) psychological stress (public speaking and mental arithmetic), b) human corticotropin-releasing hormone (hCRH), and c) bicycle ergometry until exhaustion were investigated in 10 smokers and 10 nonsmokers. Compared to d), an injection of physiological saline, psychological stress as well as hCRH resulted in significant elevations of salivary cortisol levels in the total group. Ergometry workload induced only moderately enhanced cortisol concentrations. Profound changes in heart rates were observed following bicycle ergometry [+83 beats per minure (bpm)] and after the psychological stress (+29 bpm). hCRH injection increased heart rate by 5 bpm while heart rates dropped after saline administration (−2 bpm). Smokers showed an attenuated cortisol response to the psychological stressor. Mean cortisol increases reached only one third in smokers compared to nonsmokers. Similarly, cortisol levels in smokers tended to be lower after hCRH injection; however, this difference was not statistically significant. Cortisol responses to ergometry did not differ between the two groups. Likewise, heart rates did not reveal different profiles in any of the three stimulations in smokers compared to nonsmokers.

Cortisol Saliva Heart rate Psychological stress Ergometry Humans Smoking

ACUTE nicotine administration has been shown to elicit endocrine responses in several hormones including a hypersecretion of corticotropin (ACTH), β-endorphin, growth hormone, vasopressin, luteinizing hormone (LH), and glucocorticoid steroids (1,3,15). In addition to acute elevation following cigarette smoking, we recently observed enhanced cortisol levels in habitual smokers compared to nonsmokers studied over a 12-h period (11). The difference in overall cortisol levels did not reflect profoundly elevated baseline levels in smokers but rather an increase due to repeated cigarette smoking. Moreover, there was a tendency toward larger meal-induced cortisol secretion in the same subjects, probably as a result of additive or synergistic action of insulin and nicotine in smokers.

While the stimulatory effect of nicotine on the hypothalamic-pituitary-adrenal (HPA) axis is well established (6,7, 11,14,18,24,25), there are only few data available to date on possible differences in endocrine responsiveness between smokers and nonsmokers besides different reactions to nicotine exposure. Sellini and coworkers (17) recently described attenuated cortisol responses to lysin-8-vasopressin in smokers compared to nonsmokers. However, they did not observe a similar difference between both groups after insulin-induced hypoglycemia. Similarly, a recent study by Tersman et al. (21) could not reveal different cortisol levels between smokers and nonsmokers after a mental stressor; however, they reported differences in cardiovascular responses.

In view of the hypothesis that a robust response of glucocorticoid hormones to stressors may counterbalance some of the potentially detrimental reactions of the body (12,20), the impact of habitual smoking on cortisol responses to different stimuli deserves further investigation. In the present study, we therefore measured cortisol responses to psychological stress, to an injection of human corticotropin-releasing hormone (hCRH), and following bicycle ergometry in healthy subjects. We hypothesized that due to repeated stimulation of adrenocortical cells by nicotine habitual smokers show attenuated cortisol responses to stimulation of the HPA.

METHOD

Subjects

Ten male habitual smokers and 10 male nonsmokers were paid for participation. Their mean age was 24.7 ± 3.3 years (mean ± SD) and mean height was 180.5 ± 5.8 cm. Accord-

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ing to information provided by subjects, smokers consumed a mean of 19.9 ± 1.95 cigarettes per day with a nicotine content of 0.97 ± 0.09 mg per cigarette. One smoker and one nonsmoker were assigned to a pair matched for age, body weight, and physical fitness. Written consent was obtained from each subject before entering the first test. They had to pass a brief medical examination to ensure absence of illness and health risk factors (e.g., hypertension). All subjects were medication free.

General study outline

Both individuals of one pair attended all experimental sessions on the same day starting between 10:00 a.m. and 11:00 a.m. Subjects were asked to get up at 8:00 a.m. before each experimental session and refrain from smoking and intense physical exercise at least 1 h prior to the start of the experiments. After entering the laboratory, subjects were equipped with an electrode belt for wireless transduction of heart rates. Thereafter, an indwelling catheter was inserted into the antecubital vein and kept open by physiological saline. After an initial rest period of 30 min and subsequent stimulation, subjects were monitored for 90 min relative to stimulation onset. Thus, the total duration of each experiment was 2 h. Subjects had no information about the sequence of tests, which was randomized across the 10 pairs of subjects studied. Further, they were blinded with respect to the nature of the substance injected (either saline or hCRH; see below).

Stimulation procedures

Psychological stress. The psychological stressor consisted of a largely standardized public speaking task and mental arithmetic in front of an audience as recently described (10). Briefly, subjects had to prepare a short talk in which they had to take over the role of an individual who applied for a job (10 min). This talk (5 min) was given in front of an audience of three subjects with a videocamera and tape recorder installed. Immediately following was the second task, in which subjects had to serially subtract 13 as quickly and accurately as possible starting at 1,022. It should be noted that none of the subjects had prior experience with public speaking other than oral presentations as part of their university education.

CRH and placebo tests. A standard CRH test was performed using a bolus injection of 100 μg synthetic hCRH (Bissendorf Peptide, Wedemark, Germany). In the placebo test, subjects received a bolus injection of 1 ml physiological saline. With the exception of the nature of the injected substance, both experiments were identical. The latter test was chosen as a control for possible stress effects due to the procedure of venipuncture, injection, etc.

Ergometry. At time 0 min, subjects started to work on a bicycle ergometer at an initial workload of 100 W. Workload was increased by 50 W every minute until exhaustion. The experiment was also stopped when the individual's heart rate exceeded 190 beats per minute (bpm).

Sampling and biochemical assay of cortisol

Cortisol levels were determined from saliva samples because they represent the unbound, that is, biologically active hormone fraction. Saliva cortisol is highly correlated with serum free and total cortisol levels and has been shown to be independent of saliva flow rate. The noninvasive sampling makes saliva steroid measurement the method of choice for investigations of stress effects on cortisol levels [for reviews, see (9,16,22)].

Saliva samples were obtained by subjects at −10, 0, 10, 20, 30, 40, 60, and 90 min with respect to stimulation onset. For easy and hygienic sampling of saliva, the Salivette sampling device (Sarstedt, Rommelsdorf, Germany) was employed as described elsewhere (4). Samples were stored at −20°C until being assayed. For cortisol analysis, a time-resolved fluorescence immunoassay was used (2). Intra- and interassay precision was less than 6 and 8%, respectively.

Heart rate

Heart rate was monitored in 15-s intervals by wireless signal transduction (Sport-Profi, Polar Instruments, Eschborn, Germany). A total of 480 heart rates per subject were sampled over the 2-h period of each experiment. For statistical analysis, heart rates were averaged over 5-min intervals for the period −5 min to +90 min. Due to technical problems, heart rates could be obtained from only 19 subjects in the CRH and NaCl experiments and from only 17 subjects under psychological stress.

Statistical analysis

Analyses of variance (ANOVAs) for repeated measurement were performed to reveal possible time and/or group effects with respect to cortisol levels and heart rates measured in each subject. The individual baselines were introduced as covariates to control for possible differences in initial values. Two-tailed unpaired Student's *t*-tests were computed for comparison of baseline levels; paired *t*-tests were computed for comparison of peak heart rates. Results with a probability of $\alpha \leq 5\%$ were considered statistically significant.

RESULTS

In the total group, cortisol levels were significantly higher after psychological stress, CRH administration, and ergometry when compared to saline injection ($F = 9.9$, 15.4, and 8.3, respectively; all $p < 0.001$; Fig. 1A). From similar baseline levels in all four tests, cortisol decreased consistently after NaCl injection (time effect, $F = 8.7$, $p < 0.001$). However, cortisol levels were increased almost twofold after psychological stress and threefold after CRH, respectively. Although ergometry workload did not lead to significant elevations compared to baseline levels, cortisol levels did not decrease up to 60 min after start of exercise, either. Mean heart rates increased by 83 bpm under ergometry ($t = 12.7$, $p < 0.001$), 29 bpm following psychological stress ($t = 6.1$, $p < 0.001$), and 5 bpm after CRH injection ($t = 3.1$, $p < 0.01$). Under saline administration, heart rates dropped by 2 bpm ($t = 1.8$, $p = 0.08$; Fig. 1B). While baseline heart rates and heart rate responses were not significantly different between smokers and nonsmokers in any of the four experiments (all F values < 1), cortisol levels revealed different profiles under psychological stress. Although a similar time course could be observed in both groups, nonsmokers had a mean cortisol response that was almost threefold higher than the increase in the smokers (9.1 vs. 3.2 nM/l; $F = 2.1$, $p < 0.05$; Fig. 2). There was no significant group effect ($F = 3.3$, $p > 0.05$).

A similar picture emerged in the CRH condition. Again, smokers tended to respond with less pronounced cortisol increases; however, the difference between both groups (11.0 vs. 15.6 nM/l) did not reach statistical significance and no main effect was found ($F < 1$). Likewise, no significant differences could be observed after ergometry ($F < 1$). As indicated above, significant time effects were observed under all

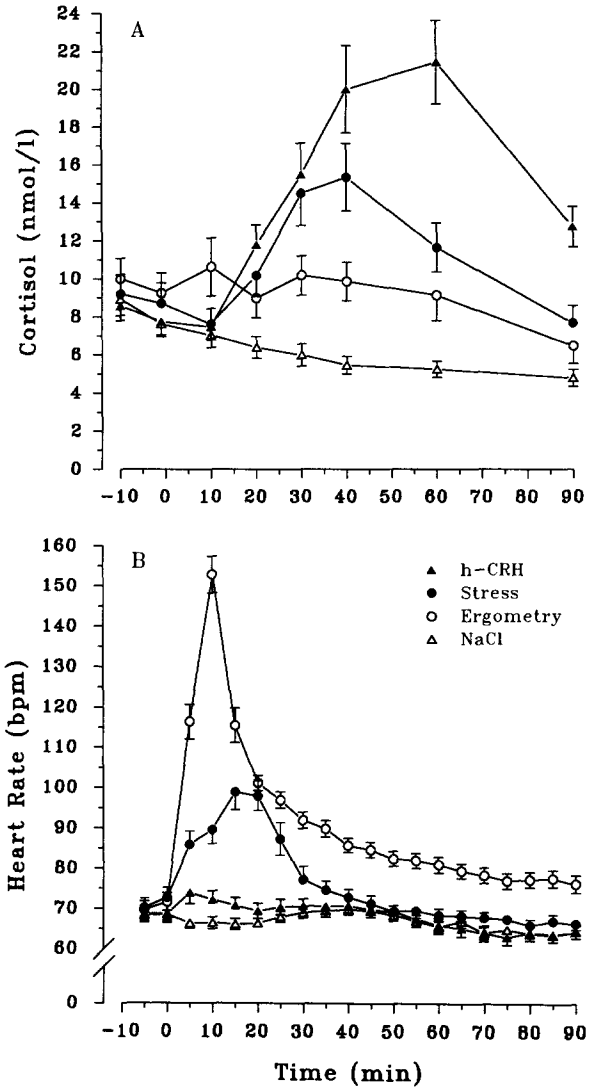

FIG. 1. Cortisol levels in saliva (A) and heart rates (B) following corticotropin-releasing hormore (CRH) injection, psychological stress, bicycle ergometry until exhaustion, and placebo administration in 20 male volunteers (means ± SE).

three stimulations (F values 20.8, 25.9, and 3.8, respectively; all $p < 0.01$). It should be noted that baseline cortisol levels were not statistically different between smokers and nonsmokers in any test (t-values 1.6, 0.4, 0.5, and 0.4, respectively; all $p > 0.05$).

DISCUSSION

The present article provides preliminary evidence for attenuated cortisol responses in habitual smokers compared to age- and weight-matched nonsmokers. While a trend toward smaller responses was observed following CRH administration, cortisol levels following psychological stress were significantly lower in smokers than in nonsmokers. Recently, Tersman and coworkers (21) investigated—among other measures—cortisol levels in 15 smokers and 15 nonsmokers following mental arithmetic as a psychological stressor. In contrast to our findings, they did not observe different cortisol levels in these groups. However, they only obtained one post-stress sample at +15 min without a baseline measure. Thus, they may have missed the peak cortisol responses in their subjects because we observed the highest mean cortisol levels 30 min after stimulation onset. Moreover, Tersman et al. studied male and female subjects simultaneously and did not take into account the possible interaction of smoking and gender with respect to cortisol levels after psychological stress. In fact, in a series of studies we found consistently lower cortisol stress responses in females compared to males (8b).

Given that smokers show less psychoendocrine reactions to psychological stress, one could argue that this merely reflects differences in personality traits and/or coping capacities rather than differences in physiological reactivity. In fact, data from several studies appear to support this view because personality differences between smokers and nonsmokers have been reported (19,23,26). However, employing the same

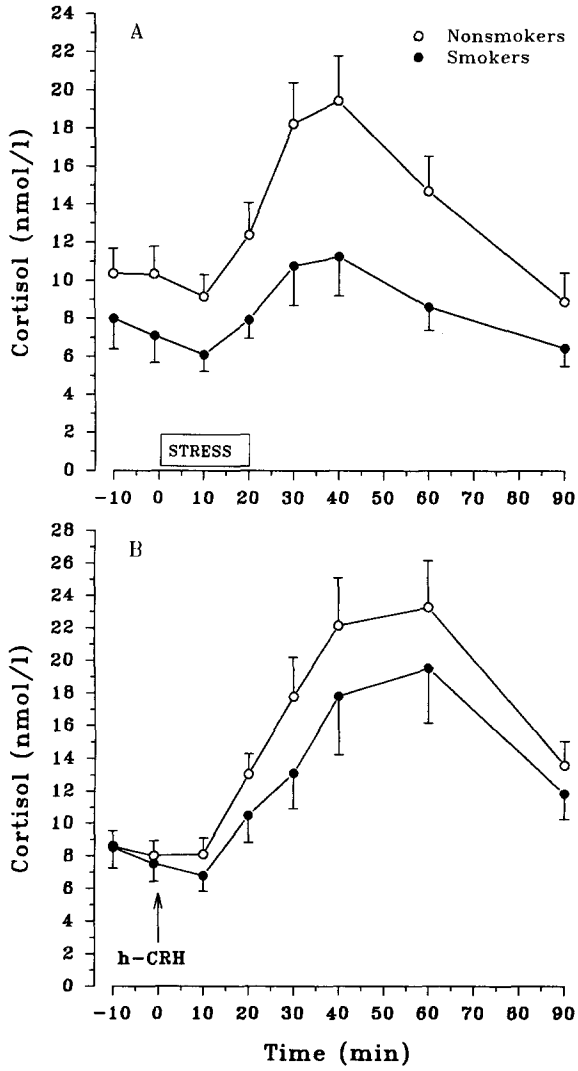

FIG. 2. Salivary cortisol levels following psychological stress (A) and after corticotropin-releasing hormone (CRH) administration (B) in smokers (n = 10) and nonsmokers (n = 10; means ± SE).

stress model as described in the present article we were unable to detect significant correlations between cortisol levels and personality traits in two independent studies (8a).

The small sample investigated in the present study does not allow to infer from our data that frequent intermittent nicotine consumption may lead to a lower responsiveness of the HPA system as measured by free cortisol concentration in saliva. Although we observed smaller increases after pharmacological stimulation in smokers, the variability of responses to CRH was too high to reveal statistically significant differences. However, both the direction and magnitude of response differences observed in this study support findings from Sellini and coworkers, who reported on attenuated cortisol responses in habitual smokers after lysin-8-vasopressin stimulation (17). If nicotine exposure is able to alter the responsiveness of the adrenal cortex, the duration of smoking becomes an important variable to control. The individuals investigated in the present study were rather young and it can be speculated that in subjects who already smoked for decades the profiles of cortisol responses might diverge more profoundly between smokers and nonsmokers. The mechanisms mediating this effect need to be elucidated. Changes in the glucocorticoid receptor level could be responsible for differences in release and feedback action of cortisol following stimulation. As a consequence, smokers may show differences in hypothalamic CRH, vasopressin, and subsequent ACTH release compared to nonsmokers. Results from a recent study by Heuser et al. (5) are in agreement with our hypothesis that a frequent and prolonged stimulation of the HPA axis can lead to an altered responsiveness of the system. However, in contrast to the present findings Heuser and coworkers observed enhanced cortisol secretion following a combined dexamethasone/CRH test in elderly endurance runners. The reason for the contradictory findings is unknown. It may be suspected that different endocrine and/or neural mediators are involved in long-term alteration of HPA functioning after repeated physical stress and habitual smoking, respectively. Regardless of the mechanisms mediating nicotine-induced alteration of HPA responsiveness, our data suggest that habitual smoking should be considered an intervening variable for cortisol responses to psychological stress. In concert with other variables, smoking may account for some of the interindividual variability in adrenocortical activity observed in humans. Besides elucidating possible mechanisms leading to lowered HPA responsiveness, future research should also investigate the impact of cortisol on nicotine sensitivity. Recent experiments in rodents suggest that corticosteroids released in response to nicotine exposure may prevent the organism from potential physiological and behavioral consequences of nicotine (13).

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