Abstract

Male Sprague-Dawley rats were exposed to conditioned emotional stimuli in a communication box, which is much more psychologically conditioned stress than the commonly used restraint and water immersion, to investigate the induction of oxidative DNA damage by psychological stress. Significantly higher levels of 8-hydroxy-2′-deoxyguanosine in rat liver nuclear DNA than in the controls (1.46 ± 0.19 SD) 8-hydroxy-2′-deoxyguanosine/10^5 deoxyguanosine were detected immediately after the second (1.90 ± 0.27, P < 0.01), third (3.10 ± 0.94, P < 0.01), and fourth exposure (2.95 ± 1.17, P < 0.01) to conditioned emotional stimuli. This is the first evidence that oxidative damage to nuclear DNA is induced by psychological stress.

Introduction

The relationship between psychological stress and disease has become a very important issue ever since the stress theory was proposed by Selye (1). It is well known that circulatory diseases and ulceration become a very important issue ever since the stress theory was proposed. The observation of suppression of immunity by stress in experimental animals has been cited as evidence of such a relationship (3–5).

However, there is no evidence that stress resulting in DNA damage can lead to cancer. Male Sprague-Dawley rats (6 weeks old) were exposed to CES in a communication box (6) to investigate DNA damage by psychological stress. The index of DNA damage used was the level of OH^*dGuo in nuclear DNA. OH^*dGuo is known as a DNA lesion produced spontaneously by endogenous hydroxyl radicals and induced artificially by chemical carcinogens in their target organs and by ionizing radiation (7–9).

Materials and Methods

Stress Exposure. As shown in Fig. 1, the communication box has 30 chambers, 15 for senders, which are electrically shocked intermittently, and 15 for responders, which receive CES such as visual, auditory, and olfactory sensations from the senders. Male Sprague-Dawley rats (Slc, Japan; 6 weeks old) were exposed to CES for 5 h a day from 10 a.m. to 3 p.m., except for one experiment in which exposure lasted for 10 h (8 a.m. to 6 p.m.). Four exposures to CES in 4 days was the longest condition. Control rats were left in their cages without food and water for 5 h before sacrifice. Sham CES rats were sacrificed immediately after the completion of 4 days of treatment as the responder rats in the chamber except for the electrical shock to sender rats. Responder rats were sacrificed immediately after the completion of CES and 1, 3, 6, and 18 h after the third CES. Immediately prior to the fourth CES, 2-nitropropane was injected i.p. (100 mg/kg body weight) into responder rats which had been exposed to CES 3 times. Plasma adrenalin and noradrenalin concentrations were determined by high-performance liquid chromatography in all rats. After CES, rats appeared almost perfectly well and no signs of dehydration were observed at autopsy.

Nucleotide Extraction and Hydrolysis. The procedure for OH^*dGuo analysis was described in our previous paper (10), and is based on the method of Kasai et al. (11). All procedures, except the incubations with enzymes, were performed at 4°C. Nuclear fractions were obtained by centrifugation of 0.5 g of liver tissue at 1000 x g after gentle homogenization in 10 ml of 250 mM mannitol-70 mM sucrose-5 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid buffer, pH 7.4 (saturated with argon). The nuclear fractions were homogenized in 3.0 ml of 1.0% sodium dodecyl sulfate containing 1 mM EDTA (saturated with argon) and incubated at 37°C for 30 min with proteinase K (Sigma; 500 mg/ml). The lysed nuclear fractions were shaken for 5 min with 3 ml of phenol (saturated with 50 mM Tris buffer, pH 7.4, containing 0.05% tert-butylhydroxytoluene). The supernatant recovered by centrifugation at 2000 x g for 5 min was shaken for 3 min with 1.5 ml of phenol and 1.5 ml of chloroform:isoamyl alcohol (24:1, v/v, containing 0.05% tert-butylhydroxytoluene). The supernatant recovered by centrifugation at 2000 x g for 3 min was shaken again with 3 ml of chloroform:isoamyl alcohol (described above). After the addition of 0.3 ml of saturated NaCl solution to 3 ml of supernatant recovered by centrifugation at 2000 x g for 3 min, DNA and RNA were precipitated by the addition of 7 ml cooled (~20°C) ethanol. After the precipitate was dissolved in 3 ml of Milli-Q water (saturated with argon; Millipore), it was heated at 95°C for 3 min and then cooled in ice. Nucleic acids (0.5 ml) were hydrolyzed to nucleosides by nuclease P1 (Yamasita, Japan; 8 units) at 45°C for 30 min in 20 mM acetate buffer, pH 4.8, and 0.1 mM ZnCl2 and then alkaline phosphatase (Sigma, 1.0 unit) at 37°C for 60 min in 50 mM Tris buffer, pH 7.4.

Analysis of OH^*dGuo. The hydrolysate was filtered through Ultrafree C3TK (Millipore) to remove enzymes and other macromolecules. High-performance liquid chromatography analysis (pump, Shimazu LC9A; degasser, Shimazu DGU3A; column oven, Shimazu CTO6A, 40°C; column, Beckman Ultraspherc ODS 4.6 x 250 mm; eluent, 5% methanol aqueous 10 mM NaH2PO4; flow rate, 1.0 ml/min) was performed with a UV detector (Shimazu SPD6AV; 290 nm) for dGuo and an electrochemical detector (ESA Coulomet 5100A; guard cell, +0.4 V; detector 1, +0.2 V; detector 2, +0.35 V) for OH^*dGuo. Peaks were monitored, and their areas were calculated by Chromatopac CR3A and CR6A (Shimazu). The OH^*dGuo standard was synthesized by the method of Kasai et al. (12) and purified by preparative high-performance liquid chromatography (Wako Pure Chemicals, Inc., Osaka, Japan).

Results and Discussion

The results are shown in Fig. 2. After a single exposure to CES, no increases in OH^*dGuo were detected in rat liver nuclear DNA in the 5-h exposure group (1.46 ± 0.19 OH^*dGuo/10^5 dGuo) or the 10-h exposure group (1.33 ± 0.47) in comparison with the control rats (1.46 ± 0.19) and sham CES rats (1.85 ± 0.40). However, OH^*dGuo levels have been induced to significantly higher levels than in the controls immediately after the second (1.90 ± 0.27, P < 0.01), third (3.10 ± 0.94, P < 0.01), and fourth exposures (2.95 ± 1.17, P < 0.01). The induced OH^*dGuo had decreased to its spontaneous level in the kidney or lung, except when excised immediately after the
OXIDATIVE DNA DAMAGE INDUCED BY PSYCHOLOGICAL STRESS

Fig. 1. Communication box (made of polyvinyl chloride; width, 1000 mm; depth, 800 mm; height, 250 mm) was separated into 30 chambers. The chambers for responders and senders were arranged as above. The floor of the box was metallic bars (electrodes for stimulus) at intervals of 10 mm. The floor of the responder’s chamber was covered by a plate of polyvinyl chloride. Conditions of electric stimulus were 2.0 mA for 10- and 110-s interval for 5 or 10 h of CES.

Fig. 2. Mean OH^*dGuo levels nuclear DNA of rat livers after CES. Bars, SD. *, Significantly different with control (P < 0.05); **, Significantly different with control (P < 0.01) in Student’s t test. Numbers in parentheses, number of rats examined. 2NP, 2-nitropropane.
mice exposed to CES for 2 h.\(^3\) We also observed that the number of lung tumors in urethane (ethyl carbamate)-injected mice after 4 months of CES on alternate days was higher than in mice exposed to urethane alone.\(^3\) This result might be considered as a promoting effect of CES on lung tumor development.

In conclusion, repetition of CES causes oxidative damage to liver nuclear DNA, which is repaired rapidly after the termination of CES. This suggests that intermittent and long-term stress in humans may cause point mutations in DNA when repair has failed. There are many problems in evaluating the relationship between psychological stress and cancer, e.g., classifying personalities and grading the severity of psychological stress in humans. Nevertheless, further studies, not only experimental, but epidemiological, appear necessary to draw any conclusions regarding which cancer sites are related to psychological stress.

Finally, we hope readers will understand that CES in the communication box was the only method for studying the biological effects of stress and was not intended to cause the rats any suffering.

References

Oxidative Damage of Nuclear DNA in Liver of Rats Exposed to Psychological Stress

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