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Effects of Phytoncides on Spontaneous Activities and Sympathetic Stress Responses in Wistar Kyoto and Stroke-Prone Spontaneously Hypertensive Rats

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Phytoncides are volatile substances mainly released from trees that have been reported to reduce the cardiovascular response to restraint stress in stroke-prone spontaneously hypertensive rats (SHRSP). To clarify the more general effects, we examined the effects of phytoncides on spontaneous activities without stress and on sympathetic responses to cold and restraint stress in SHRSP and Wistar-Kyoto rats (WKY). When exposed to phytoncides, spontaneous activity was decreased both in WKY and SHRSP. The cold stress-induced increase in urine adrenaline and noradrenaline amounts was not affected by phytoncides. Under restraint stress, both in WKY and SHRSP, heart rate and blood pressure tended to be lower, and the plasma concentration of cathecholamines was lower in the phytoncides group than in the control. These results suggest that phytoncides decrease spontaneous activity as well as cardiovascular responses to restraint stress but not to cold stress in both WKY and SHRSP.

Key words: phytoncides, restraint stress, spontaneous activity, stroke-prone spontaneously hypertensive rat, Wistar-Kyoto rat

INTRODUCTION

Phytoncides are volatile substances released mainly from trees. "Phytoncides" is a compound word coined by Tokin in about 1930, with the first part "phyto," meaning plants, and the second part, "cide," meaning to kill (1). Phytoncides per se or forest bathing has been reported to have both relaxing effects and anti-bacterial effects (2,3,4,5). Phytoncides was also reported to enhance human natural killer activity (6). The organic compounds terpenoids, which are the major ingredients of phytoncides, are reported to reduce blood pressure and heartbeats, as well as to calm down cerebral frontal lobe activity in humans under a non-stress condition (7). We also reported that phytoncides, whose composition is the same as those in the present study, reduced the cardiovascular response to restraint stress in stroke-prone spontaneously hypertensive rats (SHRSP) (8). Under the assumption that phytoncides will be used as a forest bathing substitute, not only hypertensive people or those under stress but also normotensive people and those without apparent stress will be exposed to phytoncides.

In this study, therefore, we aimed to prove the more general effects of phytoncides with the same composition as those in our previous study (8). We investigated the effects of phytoncides on spontaneous activity under a non-stress condition as well as different types of physiological stress in not only SHRSP, but also in Wistar-Kyoto rats (WKY). WKY/Izm which was used in this study is the original strain of SHRSP but without the apparent disease trait or hypersensitivity to stress characteristic to the SHRSP.

MATERIALS AND METHODS

Animals

A total of 78 male SHRSP/Izm (SLC Inc. Japan) and 58 male WKY/Izm (SLC Inc. Japan) 8 to 20 weeks old were used. This study was approved by the Ethical Committee for Animal Research of Shimane University, and all the experimental procedures were performed according to the institutional guidelines. The animals were reared in the Department of Experimental Animals, Center for Integral Research in Science, Shimane University, at a room temperature

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of 23±2℃, humidity 55±10%, ventilation 10-13 times/hour, and under artificial lighting (light term: 7:00-19:00, dark term:19:00-7:00), and given commercial chow (MF, Oriental Yeast, Tokyo, Japan) and drinking tap water ad libitum. For each of the following experiments, SHRSP and WKY were divided into two groups with equivalent distributions of age, blood pressure, and body weight, and assigned to the phytoncide group (exposed to atomized phytoncides: P group) or control group (distilled-water: C group). Okamoto *et al.* succeeded in successive selective breeding of SHR with cerebrovascular lesions from substrain families and they called these SHR offspring stroke-prone SHR (SHRSP) in 1974 (9). SHRSP shows blood pressures higher than SHR, symptoms of apoplexy, which causes death at younger ages (9), and abnormal catecholamine metabolism (10).

Phytoncides

The phytoncides used in this study were the same as those used in our previous study (PT-150, Phyton Tao 118 Inc., Osaka, Japan) (8). They were extracted from a mixed homogenate of 118 different kinds of plants. The same standardized batch was used in all the experiments of this study. The compositions were as follows: D-limonene (18.7%), α-terpineol (15.2%), methyl salicylate (11.1%), 3-phenyl propenal (10.5%), α-pinene (7.3%), ß-linalool (5.6%), bornyl acetate (4.5%), N-amyl acetate (3.8%), eugenol (3.4%), and others (15.6%).

Using an atomizing device (Fine Mist Master, Phyton Tao 118 Inc., Osaka, Japan), PT-150 was atomized and the P group animals were exposed to the mist at 0.1-0.2 ppm. Distilled water was similarly atomized and exposed to the control animals (C group).

Effects of PT-150 on spontaneous activities

Using an activity monitoring device (Toyo-Sangyo Co. Ltd, Okayama, Japan, 48 X 56 X 30 cm), home-cage spontaneous ambulatory actions were analyzed. After measurement of body weight, amount of drinking water and chow, rectal temperature, and blood pressure, the WKY (n=7 for each group) and SHRSP (n=11) were individually housed, and after two days of habituation, exposure to PT-150 was started. The exposure time was set from 9:00 a.m. to 1:00 a.m. the next day. By the near infrared sensor, small movements such as grooming were detected at a minimum of a 12 mm distance and were designated as Move 1. A square of 3 times larger than the animal body size was set, and when the animal moved out of the square the movement was counted as a large motion (Move 2). In addition, the number of rearings were counted (Rearing). Data for these three movements were obtained for two days, and the daily average was calculated.

Effects of PT-150 on sympathetic stress reaction

Cold stress

WKY (n=5) and SHRSP (n=7) 11-13 week old rats were used. After oral administration of distilled water 1 ml/100 g body weight, each animal was put into a metal metabolic cage and atomization was started. The cage was then put in the room at either 23±2℃ or 4℃ for 4 hours. Urine was collected in the cup with 1 ml of 1N HCl as an antiseptic. The urine concentration of catecholamine (adrenaline and noradrenaline) was measured, and the amount of catecholamine excretion per 4 hours was calculated. To prevent catecholamine reaction to the metal, dimethyldichlorosilane was applied to the metabolic cage two days before the experiment.

Restraint stress

WKY (n=9) and SHRSP (n=16) 11-13 week old rats were used. For blood sampling after the restricted stress experiment, a cannula was introduced in the jugular vein under ether anesthesia, and after two days recovery, the rats were placed in holders used for the tail-cuff blood-pressure measurement for 5 hours. Heart rate and systolic blood pressure were monitored every 1 hour by the tail-cuff method (Softron Inc, Tokyo, Japan). Atomizing was started after the first blood-pressure measurement and continued to the end of the restraint experiment. Immediately after the experiment, 0.3 - 0.5 ml blood was collected from the cannula, and the plasma catecholamine concentration was measured using high-performance liquid chromatography.

Statistics

Data are expressed as the mean ± standard deviation. We used analysis of variance (ANOVA) and Fisher's Post-Hoc test for statistical analyses. $P < 0.05$ was accepted as statistically significant.

RESULTS

PT-150 reduced spontaneous action

SHRSP generally tended to show larger amounts of activities than WKY (Fig. 1a,b for WKY vs. Fig. 1c,d for SHRSP). In both WKY and SHRSP, the P group generally showed lower mean values in all three types of activities and in both the light and dark periods. Rearing in the light period of WKY and Move 1 in the dark period of SHRSP as well as in the light and dark periods of SHRSP were significantly lower in the P group than in the C group (Fig. 1).

PT-150 suppressed sympathetic stress reactions to restraint stress but not to cold stress

At a low temperature, both the WKY and SHRSP showed significant increases in the amount of urine catecholamine excretion compared with at room temperature (Fig. 2a,b). In WKY, adrenaline was markedly increased, while noradrenaline was more apparently increased than adrenaline in SHRSP, and thus the strain difference was noticed in the reaction pattern against low-temperature stress. However, exposure to PT-150 did not cause significant effects on the cold-induced increase in urine catecholamine excretion both in WKY and SHRSP (Fig. 2a,b).

Under restraint stress, heart rates decreased gradually in the control WKY group, but were not significantly changed in the control SHRSP group (Fig. 3a,c). By the exposure to PT-150, heart rate was significantly decreased at 2 and 3 hours in SHRSP, but was not significantly changed in WKY (Fig. 3a,c). Systolic blood pressure increased slightly in WKY under restraint stress and PT-150 exposure significantly decreased systolic blood pressure at all time points from 1 to 5 hours (Fig. 3b). On the other hand, systolic blood pressure increased more apparently in SHRSP, and PT-150 exposure tended to

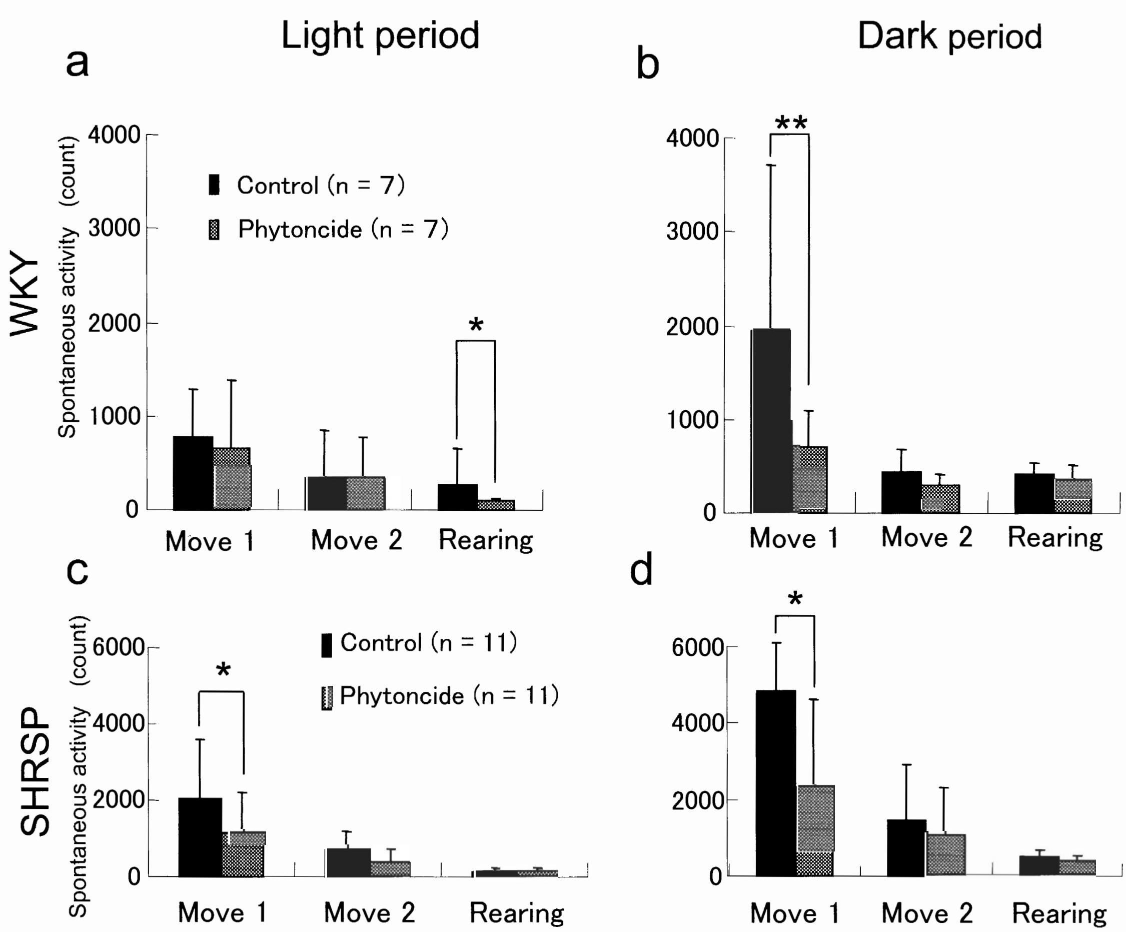

Fig. 1. Spontaneous activities with or without exposure to phytoncides PT-150 in WKY (a,b) and SHRSP (c,d) during light (a,c) and dark (b,d) periods. Move 1 and Move 2 mean small and large movements, respectively (see text for details). Counts are per day. Note that counts are generally higher in SHRSP (c,d) than in WKY (a,b). * : $P<0.01$; ** : $P<0.001$

lower blood pressure from 3 hours onward, although a statistically significant difference was not noticed (Fig. 3d). The exposure to PT-150 significantly decreased the concentrations of both adrenaline and noradrenaline after restraint stress both in WKY and SHRSP (Fig. 4a,b), while concentrations of catecholamines are generally higher in SHRSP than in WKY (Fig. 4a,b).

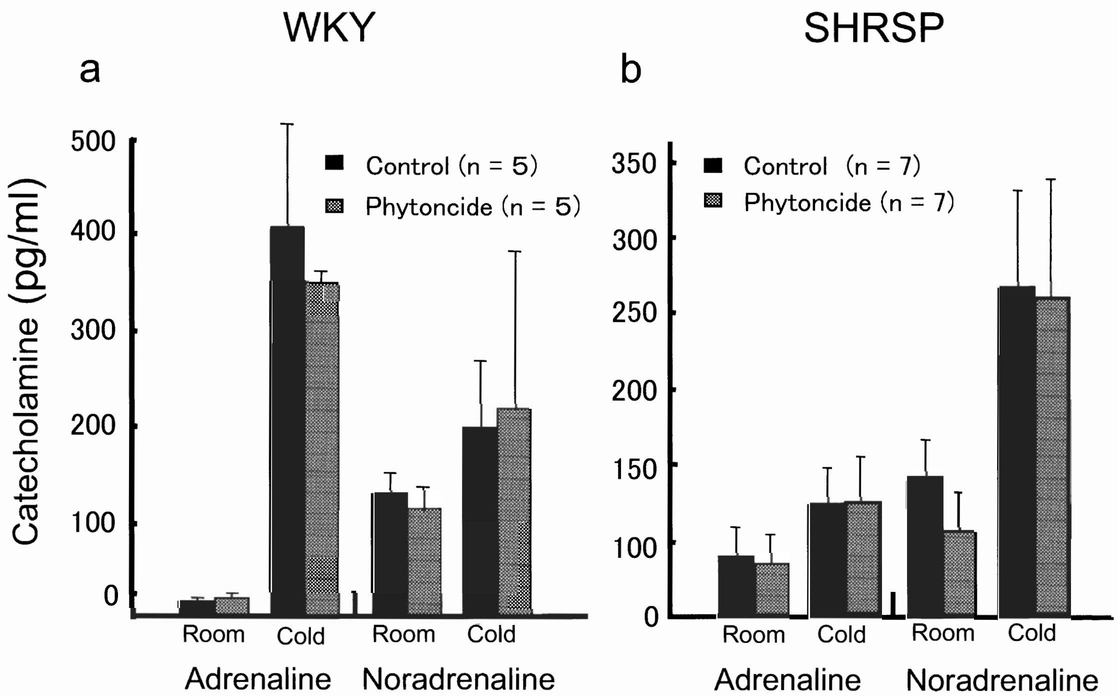

Fig. 2. Amounts of urine excretion of adrenaline and noradrenaline at either 23±2℃ (Room) or 4℃ (Cold) for 4 hours with or without exposure to phytoncides PT-150 in WKY (a) and SHRSP (b).

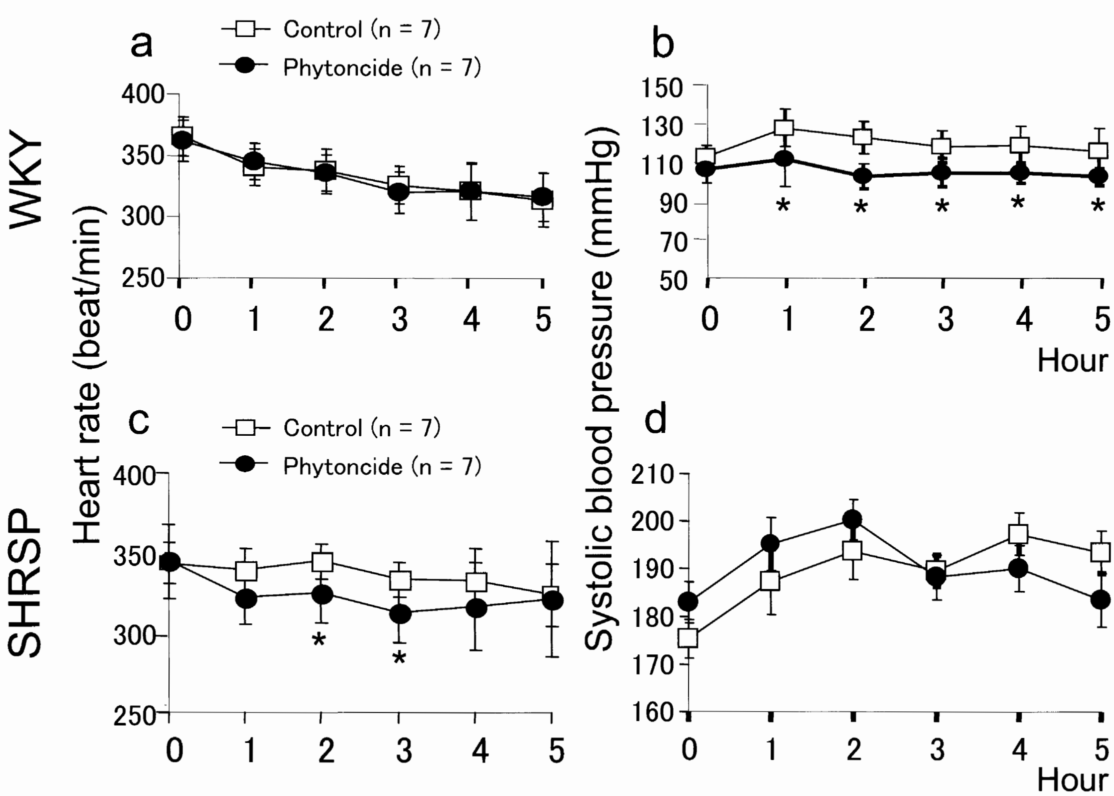

Fig. 3. Heart rate (a,c) and systolic blood pressure (b.d) in WKY (a,b) and SHRSP (c,d) under restraint stress for 5 hours with or without exposure to phytoncides PT-150. Animal numbers are 7 in all groups. * : P<0.05

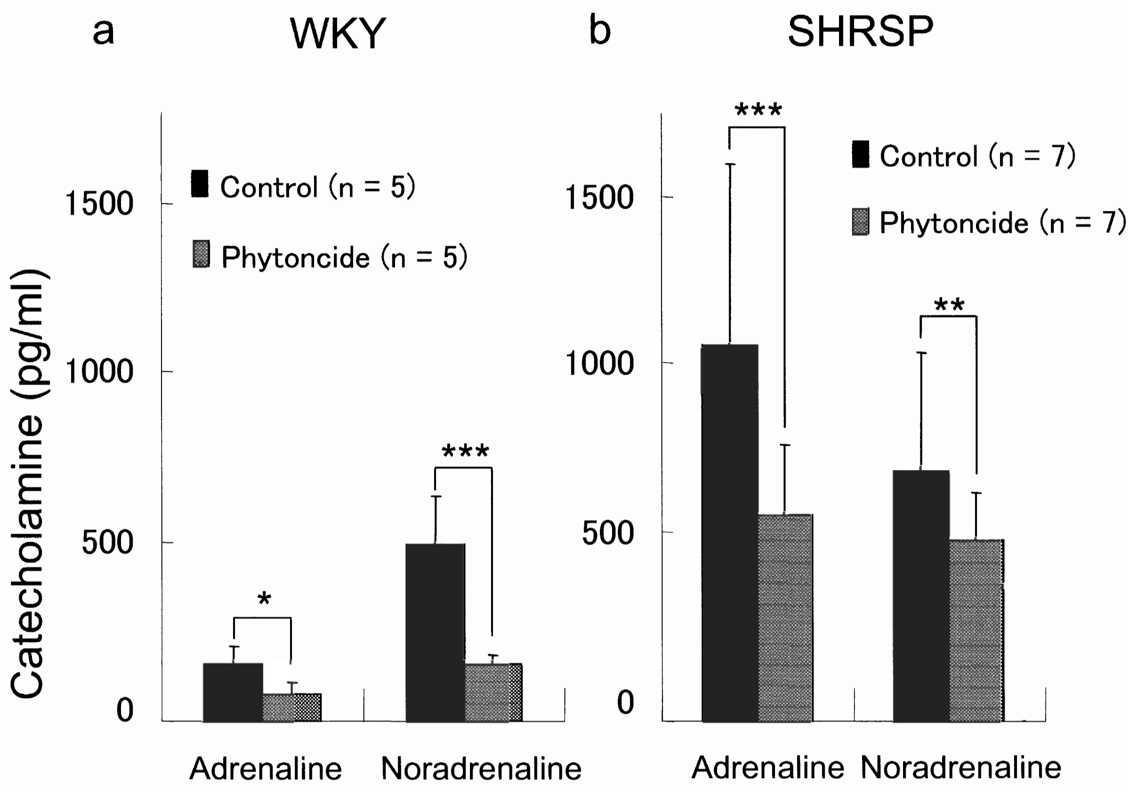

Fig. 4. Plasma catecholamine concentration immediately after the 5-hour restraint stress with or without exposure to phytoncides PT-150 in WKY (a) and SHRSP (b). Note that both adrenaline and noradrenaline concentrations are significantly decreased by PT-150 exposure in both WKY and SHRSP. * : $P<0.05$; ** : $P<0.01$; *** : $P<0.001$

DISCUSSION

Forest bathing and phytoncides as its major effective factors have been implicated to have relaxing effects (2,3,4,5), and the fragrances are expected to have beneficial effects on diseases (11,12,13). In our previous study, we showed that the phytoncides PT-150 lowered systolic blood pressure as well as the plasma concentration of catecholamines in response to restraint stress in SHRSP (8). In the present study, to clarify the more general effects under a stress or non-stress environment as well as in disease-prone and hypersensitive animals or normal animals, we examined the effects of the same PT-150 on spontaneous actions and on sympathetic responses to different types of stress not only in SHRSP, but also in WKY. The present SHRSP showed not only higher blood pressure and plasma catecholamine concentration than the WKY but also higher spontaneous activities of especially small movements (Move 1). It is suggested that higher blood pressure may underlie the higher activity (14). Exposure to PT-150 significantly reduced the amount of Move 1 activity not only in SHRSP but also in WKY. This suggests the primary relaxing effects of phytoncides and/or the secondary effect of decreased blood pressure. Under restraint stress, PT-150 exposure not only significantly decreased the heart rate in SHRSP but also significantly prevented the stress-induced rise of systolic blood pressure in WKY. In our previous report (8), PT-150 suppressed the restraint stress-induced rise of systolic blood pressure in SHRSP, and similar effects were observed also in the present study. It has not been proven whether mental and emotional effects through the olfactory stimulation (15,16) and/or the pharmacological action, possibly through ionotropic γ-aminobutylic acid ($GABA_A$) receptors (2), of components. However, the present results, including the significant decrease in catecholamines by exposure to PT-150 in both WKY and SHRSP, suggested that PT-150 effects are not specific to disease-prone and hypersensitive animals, but are more general in normal animals. The present results also suggest that the effects occur, at least in part, via the suppression of the sympathetic response to restraint stress. On the other hand, PT-150 exposure did not significantly affect the cold stress-induced increase in the urine excretion of catecholamines in both WKY and SHRSP in this study. Although in cold-stress experiments the urine excretion, but not the plasma concentration, of catecholamines were examined and thus a co-relative analysis may be difficult, the results may suggest that the effect of PT-150 differs depending on the severity and/or nature of the stress.

In conclusion, the present study indicated that phytoncides decrease spontaneous activity under a non-stress condition and suppress cardiovascular responses to restraint stress but not to cold stress. These effects were observed not only in disease-prone SHRSP, but also in normal WKY, suggesting that phytoncides have more general relaxing effect than previously reported.

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