Chem. Senses 30 (suppl 1): i262–i263, 2005 doi:10.1093/chemse/bjh215

Green Odor Reduces Pain Sensation and Fatigue-like Responses Without Affecting Sensorimotor Function

Shuji Aou[1], Masaharu Mizuno[1], Yuko Matsunaga[2], Kazuhiko Kubo[2], Xue-Liang Li[2] and Akikazu Hatanaka[3]

[1]Department of Brain Science and Engineering, Kyushu Institute of Technology, Kitakyushu 808-0196, Japan, [2]Department of Integrative Physiology, Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582, Japan and [3]Department of Applied Biological Chemistry, Yamaguchi University, Yamaguchi 753-8511, Japan

Correspondence to be sent to: Shuji Aou, e-mail: aou@brain.kyutech.ac.jp

Key words: fatigue, forced swimming, green odors, nociception, open-field test

Introduction

Green leaves produce a specific odor, the so called 'green odor' which is composed of carbon-6 alcohols and aldehydes (Hatanaka, 1996). These chemicals were synthesized from α-linolenic and linoleic acids through lipoxygenase/peroxidase-dependent cascades, a synthesizing pathway similar to those of lipid mediators of animals such as leukotriene and prostanoids. (3*Z*)-hexenol and (2*E*)-hexenal are the main components of green odor and they have been reported to act as biological signals inducing bioprotective responses in plants. It is used as pheromone-like substance and regulates feeding, reproduction and communication in insects. Recently, it has been shown that green odor affects stress responses in rats (Akutsu *et al.*, 2002; Nakashima *et al.*, 2004) and electeroencephalograms in humans (Sano *et al.*, 2002). Here we show that green odor attenuates a fatigue-like response and nociception without affecting normal sensorimotor function and emotion in rats and human.

Materials and methods

Reaction time was evaluated to measure the latency of lever press to visual cue during exposure to green odor or vehicle. In the measurements of simple reaction time, 20 pictures were randomly presented to the subjects who were asked to press the lever as soon as possible after the presentation of the visual cue. Selective reaction time was evaluated to measure the latency of lever press to two categories of pictures, 20 male pictures for one side, and 20 female ones for the other side. The threshold of mechanical pain was measued using a plastic cone which was gradually lowered onto the center area of the second finger. The score when subjects started to feel uncomfortable or pain was used as the pain threshold.

In the fatigue test, each rat was put into the swimming pool for 15 min, then put in the cage for 5 min. Rats were then tested in the open field for 10 min. The total distance that the animal moved, the number of instances of rearing behavior (rats that stood up on their hind legs) and time spent in the center area were recorded with a video camera and analyzed automatically with a computer (Ethovision v 1.96; Noldus Info. Tech.). The anxiety levels of the rats were evaluated using the elevated plus-maze test. The plus maze consisted of two open arms, 50 × 10 cm and two enclosed arms, 50 × 10 × 40 cm, with an open roof and with the two open arms opposite to each other. The maze was elevated to a height of 50 cm. A video tracking motion analysis and behavior recognition system was used to measure locomotion activity and the number of times entered into the arms and the durtion of time that they stayed at each arm. Every rat was tested for 5 min. All measurements were entered directly into a computer.

All experiments were performed in accordance with the Declaration of Helsinki and the *Guiding Principles in the Care and Use of Animals* (DHEW Publication, NIH 86-23). All results are presented as the mean (±SEM). Unpaired Student's *t*-tests were used for comparisons of the results of the passive avoidance, hot plate and the elevated plus maze tests. Differences were considered to be significant if $P < 0.05$.

Results

To examine the effects of green odor on sensorimotor function and nociception, we measured the reaction time and pain threshold in humans (Table 1). Neither simple reaction time to visual cues nor selective reaction time in the visual discrimination task were affected by exposure to green odor. In contrast, the threshold of pain sensation significantly increased during exposure to green odor, but not to isoamyl acetate or woody odor used as controls. Green odor did not affect scores of the State-Trait Anxiety Inventory (STAI) and a face scale test (Lorish and Maisiak, 1986). The anxiety score estimated by STAI1 and STAI2 did not show any difference between the treated groups and the control group. In the face scale test, green odor did not affect the emotional state subjectively scored according to happiness–sadness feeling. There was no difference in the mean values of face scales between the treated groups and the controls. Analgesic action was not caused by its effect on sensorimotor function.

Locomotor activity and exploratory behaviour in the open field in rats were greatly reduced after swimming for 15 min (Figure 1). Exposure to green odor for 5 min after swimming attenuated swimming-induced depression of motor activity. Exposed rats

Table 1 Effect of green odor on reaction time, nociception and emotion in human

	Green odor (+)	Green odor (–)
Simple reaction time (s)	351.5 ± 3.1 (14)	342.0 ± 14.0 (16)
Selected reaction time (s)		
Category A	587.4 ± 16.1 (14)	588.8 ± 14.1 (17)
Category B	584.8 ± 19.7 (14)	574.8 ± 15.7 (17)
Pain threshold	10.8 ± 0.6* (56)	10.1 ± 0.5 (56)
STAI 1	46.5 ± 1.1 (48)	43.4 ± 1.5 (42)
STAI 2	50.1 ± 1.2 (48)	47.6 ± 1.3 (42)
Face scale	9.6 ± 0.5 (48)	9.8 ± 0.5 (41)
Heart rate	68.5 ± 1.4 (48)	67.7 ± 2.4 (15)

Mean ± SEM (*n*); *$P < 0.05$.

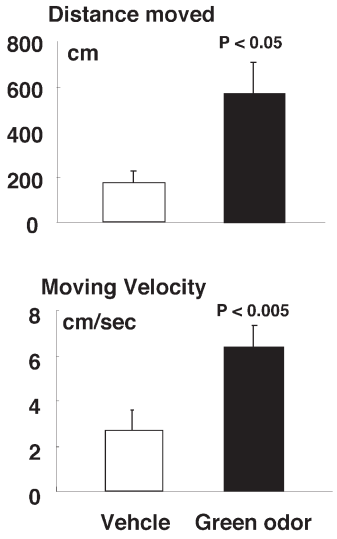

Figure 1 Effects of green odor on forced-swim-induced fatigue in rats. Five minutes exposure to green odor just after forced swimming for 15 min increased locomotor activity (upper) and moving velocity (lower) in the open-field test.

showed longer distance moved, more frequent rearing and entering the center area than control rats. In the usual open-field test without swimming, however, green odor did not affect any activities.

Discussion

The present study has demonstrated that green odor reduces nociception and fatigue-like response, while not affecting normal sensory, motor and emotional function under non-stress conditions. The mechanism that produces the fatigue-reducing and analgesic effects of green odor is currently unknown. Several plant-derived fragrances including green odors have been shown to have beneficial effects on humans and animals in a wide variety of stress conditions (Fujiwara *et al.*, 1998; Akutsu *et al.*, 2002; Nakashima *et al.*, 2004) and the cingulate cortex is suggested to be one of the specific targets of green odor (Sasabe *et al.*, 2003). We found that plant-derived odors, such as orange, borneol and isoamyl acetate induced changes in the activity of glucose-sensitive neurons in the lateral hypothalamic area (Karadi *et al.*, 1989; Oomura *et al.*, 1994). Glucose-sensitive neurons have been shown to produce orexin and orexin facilitates locomotor activity (Nakamura *et al.*, 2000) and suppresses nociception (Bingham *et al.*, 2001). Therefore some of the effects observed in the present experiment may be elicited by activation of glucose-sensitive neurons in the lateral hypothalamic area.

Acknowledgements

This work was partly supported by the Japanese Ministry of Education, Culture, Sports, Science and Technology via a Special Coordination Fund for Promoting Science and Technology.

References

Akutsu, H., Kikusui, T., Takeuchi, Y., Sano, K., Hatanaka, A. and Mori, Y. (2002) *Alleviating effects of plant-derived fragrances on stress-induced hyperthermia in rats.* Physiol. Behav., 75, 355–360.

Bingham, S., Davey, P.T., Babbs, A.J., Irving, E.A., Sammons, M.J., Wyles, M., Jeffrey, P., Cutler, L., Riba, I., Johns, A., Porter, R.A., Upton, N., Hunter, A.J. and Parsons, A.A. (2001) *Orexin-A, an hypothalamic peptide with analgesic properties.* Pain, 92, 81–90.

Fujiwara, R., Komori, T., Noda, Y., Kuraoka, T., Shibata, H., Shizuya, K., Miyahara, S., Ohmori, M., Nomura, J. and Yokoyama, M.M. (1998) *Effects of a long-term inhalation of fragrances on the stress-induced immunosuppression in mice.* Neuroimmunomodulation, 5, 318–22.

Hatanaka, A. (1996) *The fresh green odor emitted by plants.* Food Rev. Int., 12, 303–350.

Karadi, Z., Oomura, Y., Nishino, H. and Aou, S. (1989) *Olfactory coding in the monkey lateral hypothalamus: behavioral and neurochemical properties of odor-responding neurons*. Physiol. Behav., 00, 1249–1257.

Lorish, C.D. and Maisiak, R. (1986) *The Face Scale: a brief, nonverbal method for assessing patient mood*. Arthritis Rheum., 29, 906–909.

Nakamura, T., Uramura, K., Nambu, T., Yada, T., Goto, K., Yanagisawa, M. and Sakurai, T. (2000) *Orexin-induced hyperlocomotion and stereotypy are mediated by the dopaminergic system.* Brain Res., 873, 181–187.

Nakashima, T., Akamatsu, M., Hatanaka, A. and Kiyohara, T. (2004) *Attenuation of stress-induced elevations in plasma ACTH level and body temperature in rats by green odor*. Physiol. Behav., 80, 481–488.

Oomura, Y., Karadi, Z., Nishino, H., Aou, S. and Scott, T.R. (1994) *Functional correlation between feeding-related neurons and chemical senses*, In Kurihara, K., Suzuki, N. and Ogawa, H. (eds), Olfaction and Taste XI. Springer, Berlin, pp. 502–506.

Sano, K., Tsuda, Y., Sugano, H., Aou, S. and Hatanaka, A. (2002) *Concentration effects of green odor on event-related potential (P300) and pleasantness.* Chem. Senses, 27, 225–230.

Sasabe, T., Kobayashi, M., Kondo, Y., Onoe, H., Matsubara, S., Yamamoto, S., Tsukada, H., Onoe, K., Watabe, H., Iida, H., Kogo, M., Sano, K., Hatanaka, A., Sawada, T. and Watanabe, Y. (2003) *Activation of the anterior cingulate gyrus by 'green odor': a positron emission tomography study in the monkey.* Chem. Senses, 28, 565–572.