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ScienceDirect
Neuroscience Letters 416 (2007) 241–246
Neuroscience Letters
www.elsevier.com/locate/neulet

Mechanism of changes induced in plasma glycerol by scent stimulation with grapefruit and lavender essential oils

Jiao Shen [a,*], Akira Niijima [b], Mamoru Tanida [a], Yuko Horii [a], Takuo Nakamura [a], Katsuya Nagai [a]

[a] *Institute for Protein Research, Osaka University, 3-2 Yamada-Oka, Suita, Osaka 565-0871, Japan*
[b] *Niigata University School of Medicine, Niigata, Niigata 951-8122, Japan*

Received 29 November 2006; received in revised form 27 December 2006; accepted 28 December 2006

Abstract

In a previous study, we found that stimulation with scent of grapefruit oil (SGFO) elevated plasma glycerol levels in rats. However, stimulation with scent of lavender oil (SLVO) triggered a negative effect. To identify the mechanism of these changes during lipolysis, we examined the role of autonomic blockers and bilateral lesions of the hypothalamic suprachiasmatic nucleus (SCN) in the modification of plasma glycerol in rats exposed to SGFO and SLVO. We found that intraperitoneal injection of propranolol hydrochloride and atropine sulfate eliminated the changes in plasma glycerol levels induced by SGFO and SLVO, respectively. Bilateral lesions of the SCN completely abolished the effects of SGFO and SLVO on lipolysis. In addition, we investigated tyrosine phosphorylation of the transmembrane glycoprotein BIT (a brain immunoglobulin-like molecule with tyrosine-based activation motifs, a member of the signal-regulator protein family), which was found to be involved in the activation of renal sympathetic nerves and increase in body temperature on cold exposure. SGFO was found to enhance the immunoreactivity of BIT to the 4G10 anti-phosphotyrosine antibody in the SCN, whereas SLVO decreased the immunoreactivity. The changes in BIT phosphorylation resulting from the exposure to SGFO and SLVO were eliminated by the corresponding histamine receptor antagonists, which eliminated the changes in plasma glycerol concentration. The results suggest that SGFO and SLVO affect the autonomic neurotransmission and lipolysis. The SCN and histamine neurons are involved in the lipolytic responses to SGFO and SLVO, and tyrosine phosphorylation of BIT is implicated in the relevant signaling pathways.

Keywords: Grapefruit; Lavender; Glycerol; BIT; Histamine; SCN

In aromatherapy, grapefruit is used to treat depression and induce euphoria, whereas lavender is beneficial in reducing stress and relaxing the mind. This raises the possibility that stimulation with grapefruit or lavender may influence the activity of sympathetic nerves. Previously, we reported that stimulation with scent of grapefruit oil (SGFO) excited the sympathetic nerves, innervating the white adipose tissues (WAT), and elevated the plasma glycerol levels in rats [24]. However, stimulation with scent of lavender oil (SLVO) triggered a negative effect [25]. In this study, we conducted further tests to understand the mechanism of changes during lipolysis induced by SGFO and SLVO.

Experiments in rats have clearly shown the functional, sympathetic and parasympathetic innervation of WAT, where the autonomic nerves play an important role in the regulation of lipid metabolism [1,11,10]. Generally, activation of the sympathetic nerves lead to hydrolysis of triglycerides stored within the adipose tissues to form glycerol and free fatty acids (FFA). Moreover, glycerol release should be accompanied by a concomitant increase in the plasma FFA levels [21]. However, in certain conditions, stimulation of lipolysis results in an increase of only glycerol, without a proportional release of FFA [31]. Therefore, we examined whether the effects of SGFO and SLVO on lipolysis were induced through the autonomic nerves by measuring plasma glycerol and FFA concentrations.

It has been documented that the suprachiasmatic nucleus (SCN) controls the daily changes in the activity of the autonomic nervous system and the balance between sympathetic and parasympathetic output to peripheral organs [10,6,5]. Therefore,

* Corresponding author at: YMP-i, ANBAS Project, 4th Floor, Nakajima Building 4-12-17, Toyosaki, Kita-Ku, Osaka 531-0072, Japan.
Tel.: +81 6 6486 1080; fax: +81 6 6486 1081.
E-mail address: jshen@ymp-i.co.jp (J. Shen).

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doi:10.1016/j.neulet.2006.12.063

we also examined the effects of autonomic blockers and bilateral lesions of the SCN on the changes in plasma glycerol, resulting from the exposure to SGFO and SLVO in conscious rats.

Moreover, tyrosine phosphorylation of BIT (a brain immunoglobulin-like molecule with tyrosine-based activation motifs, a member of the signal-regulator protein family), in the SCN has been reported to be involved in the activation of renal sympathetic nerves and increase in body temperature in rats on cold exposure [13,23,28,29]. Therefore, we examined tyrosine phosphorylation of BIT in the SCN using immunoblotting to elucidate its function in the signal transduction pathways on stimulation with SGFO and SLVO.

Male Wistar rats (8 weeks old, weighting 280–300 mg) were used. They were allowed free access to food and water, and housed in a room illuminated daily from 07:00 to 19:00 (a 12-h light/12-h dark cycle). All studies were conducted in accordance with the guidelines of Institutional Animal Care and Use Committee of Osaka University.

A gauze was fully soaked with a suspension of either grapefruit or lavender essential oil and water and placed on top of the cover of the animal cage. The cage was wrapped with a nylon sheet for retaining the scent during the test. Stimulation with the scents continued until the end of sampling. Grapefruit essential oil (*Citrus paradisi*, Pranarom International, Belgium) and lavender essential oil (*Lavandula angustifolia*, Pranarom International) were suspended in 10,000-fold and 100,000-fold water, respectively. The stronger scent excited the animals and they almost jumped out of the cage.

Blood samples were collected from a chronically indwelling silicone catheter implanted in the right external jugular vein, with its end at a point just outside the atrium. Surgical catheterizations were performed under anaesthesia with intraperitoneal (ip) Nembutal 3 days before the experiment. The plasma was separated immediately for the assay of glycerol and FFA. Glycerol was detected according to the protocol of the F-kit glycerol (Glycerol UV-method, Cat.# 10148270035; R-Biopharm AG, Darmstadt), and FFA was assayed using the acyl CoA synthetase-acyl CoA oxidase method with the NEFA-C-test kit (Wako Pure Chemical Industries, Ltd. Cat.# 279-75401).

As described previously, bilateral electrolytic lesions of the SCN were performed on some rats 2–3 weeks before the scent stimulation experiments. In brief, under pentobarbital anaesthesia (35 mg/kg, ip), a stainless steel electrode was inserted into the SCN using the following coordinates: A–P, 0.85–0.9 mm posterior to the bregma; L, 0 mm from the midline; V, 8.5 mm from the skull surface, as per the atlas of Paxisons and Watson [19]. A 1.0 mA anodal direct current was then passed through the electrode for 20 s. After operation, the animals were housed individually for 1 week and then locomotive activity was measured with an Animex (MK-Animex, Muromachi Kikai Co., Tokyo). Only rats with complete loss of the circadian rhythm of activity with SCN lesion were used, since SCN lesion is known to eliminate the circadian rhythm of locomotive activity [15]. At the end of the experiment, the brain was removed and histologically examined using cresyl violet staining, to verify placement of the bilateral lesions in the SCN. Only rats ($n = 10$) in which the lesions were accurately placed were included in the final data set.

Following scent stimulation for 5 or 15 min, the rats were sacrificed. The brain was obtained as soon as possible and immediately frozen with dry ice. The SCN were punched out with a needle using the dissection procedure described by Palkovits and Brownstein [18] as a general guide. The punches were homogenized in radioimmunoprecipitation assay (RIPA) buffer. Supernatants containing 40 μg protein were then incubated overnight with Concanavalin A (Con A)-Sepharose beads at 4 °C. After immunoblotting with an anti-phosphotyrosine antibody 4G10, the same blot was probed with anti-BIT antibody. The levels of tyrosine phosphorylation of BIT were quantified by scanning densitometry using the National Institutes of Health (NIH) Image program.

Data are expressed as mean ± S.E.M. Statistical analyses were performed using the Mann–Whitney *U* test and analysis of variance (ANOVA) with repeated measures. In all the cases, $p < 0.05$ was considered statistically significant.

As shown in Fig. 1, the levels of plasma glycerol and FFA increased significantly in response to SGFO (a and b) and decreased markedly in response to SLVO (e and f), as compared with the water-treated group (expressed as a percentage of the value at 0 min). Basal glycerol levels were not significantly different between the groups (Tables 1 and 2).

The plasma FFA levels changed proportionally to the glycerol levels. This suggests that the changes induced by SGFO and SLVO in lipolysis may be caused by stimulation of the autonomic nerves [21]. To clarify the relationships between the

Table 1
Basal levels of plasma glycerol in experimental groups

Groups	Number of rats	Plasma glycerol (μM) (means ± S.E.M.)
Experiment 1		
Grapefruit	5	102.7 ± 24.4
Water	5	87.7 ± 23.5
Experiment 2		
Water + saline	4	74.1 ± 12.2
Grapefruit + saline	4	72.6 ± 9.5
Grapefruit + propranolol	3	85.5 ± 21.9
Grapefruit + atropine	4	64.1 ± 16 7
Experiment 3		
Grapefruit-SCN-lesioned	6	188.7 ± 43.9
Grapefruit-sham-operated	6	119.6 ± 18.1
Experiment 4		
Lavender	5	136.4 ± 13.2
Water	5	90.1 ± 13.7
Experiment 5		
Water + saline	5	92.2 ± 15.6
Lavender + saline	4	132.1 ± 22.8
Lavender + propranolol	4	88.8 ± 4.2
Lavender + atropine	3	121.6 ± 7.3
Experiment 6		
Lavender-SCN-lesioned	4	126.3 ± 5.5
Lavender-sham-operated	4	199.4 ± 11

Differences in respective basal values were not statistically significant (Mann–Whitney *U* test).