Reduction in the sweaty smell of 3-methyl-2-hexenoic acid by cross-adaptation using its pleasant-smelling methyl esters

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Synopsis

Magnitude estimates for a 10:1 mixture of (<u>E</u>)- and (<u>Z</u>)-3-methyl-2-hexenoic acid (3M2H), a principal component of human underarm odor, decreased following adaptation to a mixture of methyl esters of 3M2H (ME3M2H), which possess a pleasant, fruity odor. These results provide further demonstration that structurally similar, yet perceptually distinct, odorants may cross-adapt and suggest that the extent of cross-adaptation may be affected by the degree of structural, as well as perceptual, similarity.

INTRODUCTION

The extent of olfactory cross-adaptation, the decrease in sensitivity to one odorant following exposure to a different odorant, may represent the degree to which odors share common sensory channels (1–4). Thus, by documenting the extent of cross-adaptation among different odorants, inferences about structure–activity relationships and coding in the olfactory system can be drawn. Yet, surprisingly little is known about the determinants of olfactory self- and cross-adaptation. The present report continues our long-term study of the structural features of odorous compounds as they affect cross-adaptation between sweaty-smelling 3-methyl-2-hexenoic acid and its fruity-smelling esters.

It is now well established that the occurrence of cross-adaptation typically entails a

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degree of similarity, either perceptual or structural, between the tested odorants. There is a good deal of evidence demonstrating that perceptual similarity affects cross-adaptation since perceptually similar odorants, irrespective of structural similarity, cross-adapt (1-5). Conversely, structurally similar odorants, irrespective of perceptual similarity, can cross-adapt as well (6-10).

One system we have used to study structural similarity and cross-adaptation (10) is an isomeric mixture of (<u>E</u>)- and (<u>Z</u>)-3-methyl-2-hexenoic acid (3M2H). Both isomers are present in axillary sweat of males and females, albeit in different ratios (11,12). The E-isomer of 3M2H is a major component (analytically) in male underarm sweat (11,13). A synthetic mixture of the two isomers was formulated in the 10:1 E:Z ratio found in the male secretions (11,13) and tested for cross-adaptation with three homologous ethyl esters: the ethyl esters of 3-methyl-2-hexenoic acid (EE3M2H), 3-methyl-2-octenoic acid (EE3M2O), and 3-methyl-2-pentenoic acid (EE3M2P). The esters, in contrast to the volatile sweaty-smelling organic acids, are pleasant and fruity-smelling.

Significant cross-adaptation was observed between 3M2H and its ethyl esters (10) since exposure to EE3M2H resulted in significantly reduced magnitude estimates for the intensity of 3M2H. Cross-adaptation was asymmetric; adaptation to 3M2H did not significantly affect the perceived intensity of EE3M2H. Further, there was no significant cross-adaptation between 3M2H and the ethyl esters of the lower (EE3M2P) and higher (EE3M2O) molecular weight homologues (10). Similarity ratings between the ethyl esters and 3M2H revealed no significant differences among the ethyl esters in their mean similarity rating to 3M2H; i.e., each was rated equally dissimilar to 3M2H (10). Thus, it is unlikely that the differences among the ethyl esters in their efficacy in crossadapting 3M2H were attributable to perceptual differences. A more likely explanation for the pattern of cross-adaptation observed is the greater structural similarity between EE3M2H and 3M2H as revealed by molecular modeling studies (10). We have recently also established that the E-isomer is singularly more effective in cross-adapting 3M2H than is the Z-isomer (9).

The methyl esters of 3M2H (ME3M2H) have a pleasant, fruity odor; however, the odor is much weaker than that possessed by the ethyl esters (9,10). In the present work, we sought to determine whether the ME3M2H would be as effective in cross-adapting 3M2H as the ethyl esters. We assessed cross-adaptation between a mixture of methyl esters of 3M2H and a 10:1 E:Z mixture of 3M2H.

EXPERIMENTAL

SUBJECTS

Twelve paid subjects (five males and seven females; mean age of 25.3 years) were recruited from among colleagues and the surrounding university community. All subjects were first screened for sensitivity to the odorants used in the experiment and paid to participate.

STIMULI SYNTHESIS AND PURIFICATION

The stimuli used are presented in Figure 1. The 3M2H was synthesized and purified in

a manner described previously (11,14). The methyl esters of 3M2H were subsequently made as follows: A mixture of approximately 3:1 E- and Z-3-methyl-2-hexenoic acids (1.0 gm, 7.8×10^{-3} mol) was dissolved in methanol (25 ml) under N₂. Concentrated sulfuric acid (0.5 ml, 9.4×10^{-3} mol) was added, and the reaction mixture was brought



Methyl esters of 3-methyl-2-hexenoic acid (ME3M2H)



Methyl esters of 3-methyl-3-hexenoic acid



exo-3-methylidine-methyl-hexenoate

Figure 1. Chemical stimuli used in the present study. On the left are the (E)-isomers, on the right, the (Z)-isomers.

Purchased for the exclusive use of nofirst nolast (unknown) From: SCC Media Library & Resource Center (library.scconline.org) to reflux for 1.5 h. Reaction progress was monitored by aliquot workup and gas chromatography (Perkin-Elmer 990 GC; 30-m \times 0.52-mm (i.d.) column with 1.0- μ coating of Stabilwax [Restek, Bellefonte, PA] held at 100°C for 17 minutes and programmed at 6°C per minute to 200°C).

The reaction mixture was poured into chilled water. Dichloromethane was added, and the aqueous phase was basified to pH 8 with sodium bicarbonate. The organic components were extracted into dichloromethane (2×25 ml) and distilled. Separation of the acids from the esters was accomplished by column chromatography through silica gel with dichloromethane doped with 1% NH₄OH as eluent.

Analysis of the acid-free ester mixture was performed by combined gas chromatography/ mass spectrometry (GC/MS; Finnigan/MAT 4510) utilizing the same chromatographic conditions described above. This analysis indicated that in addition to the desired methyl esters, a small amount of three other products derived from double-bond migration to the 3- and exo-position were present. These data indicated that the following percentages of methyl esters were present: E (56.7%)-and Z (17.6%)-3-methyl-2hexenoates and the remaining 25.7% consisting of the E, Z-3-methyl-3-hexenoates (16.3%) and exo-3-methylidine-methyl-hexenoate (9.4%). We did not attempt to further separate the isomer mixture and used it as the cross-adapting stimulus.

STIMULI PRESENTATION

Odorants were diluted in odorless, light, white, mineral oil and presented in 270-ml polypropylene squeeze-bottles with plastic, flip-top caps. Each bottle contained 10 ml of the odorant/mineral oil solution.

A twelve-step binary dilution series was prepared for each odorant. Initially, 20 mg of each odorant was diluted in 20 ml of odorless, light, white, mineral oil to yield a 1 mg/ml (0.1% w/v) solution with molar concentrations of 7.81 mM for 3M2H and 7.04 mM for ME3M2H. The dilution scheme for each odorant was the same, ranging from 1.0×10^{-1} w/v (step 12) to 4.88×10^{-5} % w/v (step 1).

Subjects were tested in two 30-minute sessions using a procedure described previously (3,9,10). Briefly, a forced-choice, staircase procedure was used at the beginning of each session to equate intensities of the test stimuli for each subject. A two-minute rest was imposed following perceptual matching. Subjects then rated, using magnitude estimation, the intensities of step 10 of the adapting stimulus and the intensity-matched concentration of the other test odorant. Subjects assigned numerical ratings to each of these two stimuli twice. If the means of the magnitude estimates for each odor were dissimilar (greater than 20% discrepancy), the matching procedure was repeated. In this manner, initial magnitude estimates ensured that the two stimuli were perceptually equivalent for that subject. Thus, in a given session, the test odorant judged to be most similar in intensity by the individual subject; the stimulus used for adaptation was a fourfold higher concentration (i.e., step 12) than the test stimulus.

After making the initial magnitude estimates, subjects began to sniff repeatedly the adapting stimulus. Every 15 seconds during this adaptation period, subjects sniffed and rated a test stimulus between sniffs of the adapting stimulus. The test stimulus, either 3M2H or ME3M2H, alternated on sequential trials so that subjects made a total of 20

ratings (ten 3M2H, ten ME3M2H) during the five-minute adaptation period. Following these ratings, the adapting stimulus was removed and subjects continued to rate test stimuli every 15 seconds during a five-minute post-adaptation period. Subjects thus made a total of 20 ratings during this recovery period.

In the second session, the adapting odorant, either 3M2H or ME3M2H, was reversed and the procedure repeated. The adapting odorant used in a particular session was counterbalanced across sessions for all subjects.

Each magnitude estimate was converted to a percentage of the initial magnitude estimate for that odorant; the resulting percentages are presented in Figure 2. These data were analyzed by a series of repeated, single-factor ANOVAs; a separate analysis was performed for each comparison. The ANOVAs were calculated after each estimate was first subtracted from 100, allowing an assessment of whether estimates were significantly different from the initial estimates (100%).

RESULTS

Both 3M2H and ME3M2H showed significant self-adaptation (Table I). The pattern of self-adaptation observed was consistent with a pattern we have observed previously (3,10); strong self-adaptation occurred quickly and continued for the duration of the adaptation period. Following removal of the adapting odorant, each self-adapted odorant displayed a pattern of recovery to baseline levels (Table I).

Significant cross-adaptation between 3M2H and ME3M2H was observed asymmetrically. Exposure to ME3M2H significantly reduced the perception of 3M2H via cross-adaptation, but there was no effect of 3M2H exposure on the perception of ME3M2H (Table I; Figure 2).

DISCUSSION

These results suggest that the cross-adaptation relationship previously observed between 3M2H and its ethyl esters (10) may be a general one, as the methyl esters displayed a strikingly similar pattern of effectiveness in reducing the perception of 3M2H intensity. Thus, the initial reduction in perception following 15 seconds of exposure (35.0% following exposure to EE3M2H vs 36.5% following ME3M2H exposure), the shape of the adaptation curve, and the overall reduction in perceived odor intensity (mean reduction of 35.1% following EE3M2H exposure; 34.3% following ME3M2H) are consistent between the ethyl and methyl ester exposures. In addition, this similarity was seen even though the methyl ester mixture contained three minor components whose homologues were not present in the ethyl ester mixture previously employed (10). Also of note is that a significant reduction in the perception of 3M2H was achieved by the methyl ester mixture; this level of reduction was not seen either with the EE3M2P or EE3M20, which are stronger smelling, fruity homologues of EE3M2H (10). Subsequent studies will include a purified mixture of only the E-Z-methyl ester isomers and/or the E- and Z-isomers alone, as per our previous studies (9).

These results, suggesting a possible receptor interaction for an acid and its esters, have a neurophysiological parallel. Sato *et al.* (15) examined tuning specificities in mouse



Figure 2. Mean magnitude estimates (with standard errors) as a percentage of the initial estimates for 3M2H and ME3M2H following adaptation to each compound.

olfactory receptor cells and found that sensitivity depended upon both carbon chain length and the terminal functional group (carboxyl, hydroxyl, or amino), with some cells responding specifically to an acid and its esters.

Overall, these results further demonstrate that structurally similar, yet perceptually

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Odorant	Adaptation		Recovery	
	<u>X</u> (S.E.)	<u>F</u>	<u>X</u> (S.E.)	F
	Exposure to 3M2H			
3M2H	48.1% (7.07)	53.95***	79.9% (9.12)	4.87*
ME3M2H	79.5% (8.59)	5.69*	86.0% (9.08)	2.37
	Exposure to ME3M2H			
3 M 2H	65.7% (12.26)	7.80**	86.3% (13.04)	1.10
ME3M2H	32.2% (6.75)	100.95***	83.0% (6.15)	7.66**

 Table I

 Mean Magnitude Estimates as a Percentage of Initial Estimates and Associated F-Values for Comparisons of Mean Magnitude Estimates for 3M2H and ME3M2H During and Following Adaptation to Each of the Adapting Odorants

* p < .05; **p < .02; ***p < .001.

Note: The values in the table represent the mean magnitude estimate for an odorant expressed as a percentage of the initial estimate. Each F-test compares these mean magnitude estimate with the initial magnitude estimates for that odorant. Degrees of freedom for all F-tests = (1,11).

distinct, odorants may cross-adapt, and support the idea that the extent of crossadaptation may be affected by the degree of structural similarity among odorants. Practically, the easy-to-prepare methyl and ethyl esters of 3M2H may be incorporated into a variety of consumer products used to reduce the perception of malodors that contain organic acids.

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