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ISOPRENE AND SLEEP

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Summary

Isoprene is one of the main constituents of endogeneous origin in exhaled human breath. The concentration of isoprene seems to vary with states of sleep and wakefulness,increasing during sleep and decreasing sharply just after awakening. Thus,isoprene may be involved in in sleep upholding.

The analysis by gas chromatography of organic compounds in human breath has clearly demonstrated the presence of isoprene (2-methyl-1,3-butadiene) of endogeneous origin (Jansson and Larsson (1), Conkle et al. (2), De Master and Nagasawa (3), Gelmont et al. (4)). Conkle et al. (2) suggested that the isoprene in the breath might be associated with psychological stress whereas De Master and Nagasawa (3) observed a circadian variation in isoprene concentration with a maximum during the night. According to Gelmont et al. (4) the amount of isoprene does not depend on age, sex, diet,fasting or non-fasting states. From a biochemical point of view, Deneris et al.(5-6) showed that isoprene could result from mevalonate metabolism via isopentenyl pyrophosphate and dimethylallyl pyrophosphate.

Our studies of the breath of healthy volunteers by gas chromatography and mass spectrometry demonstrate that the concentration of isoprene depends on sleep and wakefulness rather than on primitive circadian rhythms.

Methods

Breath samples were collected in 1 l Teflon bags : each volunteer was instructed to inhale moderately and then exhale as much breath as possible into the bag. Fifty volunteers, 30 women and 20 men, aged 15 to 60 years, provided samples of air exhaled at various times of the day. Twenty-five of them also collected samples at different moments of the night, either while entirely awake or just after being awakened and allowed to fall asleep again. For practical reasons, samples obtained immediately after spontaneous or induced awakening were considered to be identical to those that might have been collected during sleep.

Isoprene and acetone were determined according to a method previously described (7). In brief, 50 ml of expired air were taken with a syringe from the bag and injected into a refrigerated Tenax trap. Then the trap was rapidly heated to 280° C and the adsorbed substances were flushed into two capillary columns (RSL 160 Alltech) of a gas chromatograph (Varian model 6000).One

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capillary column was connected to a flame ionisation detector and the other to a mass spectrometer ion trap detector (ITD 800 Finnigan) to confirm the identification of isoprene.

Results

The concentration of isoprene in the breath taken at different moments of the daytime period, between 8 and 23 hours, from 50 healthy volunteers while fully awake was 14.6 +/- 6.4 nmol/L. This result, in agreement with the values obtained by De Master and Nagasawa (3) in similar conditions, shows that the elimination of isoprene does not appreciably change during the diurnal period in individuals who stay awake.

Figure 1 shows the concentration of isoprene in the breath of healthy volunteers asleep or awake during the night. No significant difference was found between the two groups at 23 hours when all subjects were awake. Remarkably enough, the isoprene concentration at 2, 4, 6 hours in the 13 subjects who were allowed to sleep was significantly higher than in the 9 subjects who stayed awake. The isoprene concentration in individuals awake during the night (17.7 +/- 7.0 nmoles/L) was similar to that in individuals awake during the day (14. 6 +/- 6.4 nmoles/L). Thus, in the absence of sleep during the night, the concentration of isoprene in the breath did not increase. On awakening in the morning or in the middle of the night, isoprene concentration was observed to fall sharply in less than 20 minutes. At 6 hours the isoprene concentration decreased from 45.3 +/- 16.5 to 23.3 +/- 7.7 nmoles/L (n = 14) and at 2 hours from 42.4 +/- 13.5 to 18.2 +/- 4.7 nmoles/L (n = 6).

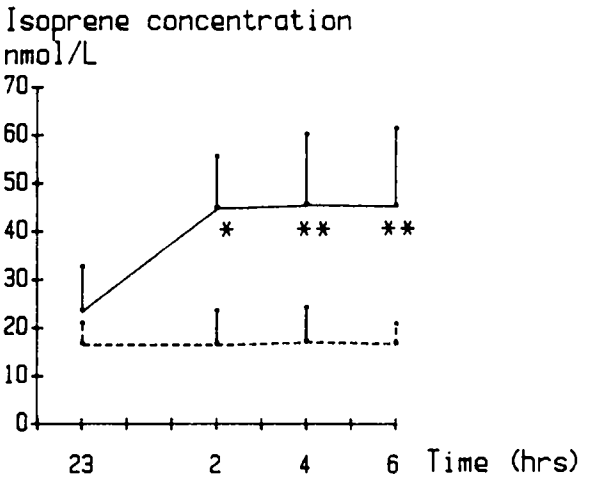

Fig. 1
Isoprene concentration (m +/- SD) during the night in adult breath of 13 subjects allowed to sleep after 23 H (●———●) and of 9 subjects prevented from sleeping (●- - -●). Comparisons between the 2 groups at the same hour were performed by Student's test.
* $p < 0.001$ ** $p < 0.005$.

Nine of the sleeping individuals from whom samples of breath for isoprene determination were taken at 2, 4 and 6 hours were continuously monitored. High levels of isoprene were observed during stages I, II, III, IV and REM (Rapid Eye Movement). But, no particular relationship was found between levels of isoprene and any of the specific sleep stages. Other experiments showed that the position of the individuals : standing or lying, the illumination of the room :light or dark, did not noticeably change the concentration of isoprene. The increase of isoprene during sleep and the sharp decrease after awakening as well as the absence of variation with position or illumination suggest that the increase in isoprene concentration depends on sleep.

However, well-characterized sleep stages may be observed unaccompanied by any increase of breath isoprene. In two healthy individuals, frequently disturbed by complete awakening during the night, once or more often each hour,periods of sleep with no concomittant increase of breath isoprene were recorded. When the night sleep of these two individuals was only interrupted by short awakenings at 2, 4, 6 hours, there was a significant increase of isoprene concentration.

Discussion

Our observations suggest that the variation of isoprene concentration in human breath depends on states of sleep and wakefulness. We do not know if the increase of isoprene in breath is a consequence of sleep or a factor involved in sleep upholding. The increase of isoprene concentration in breath could be, at least partially, a physiological consequence of the decrease of pulmonary ventilation during sleep. But, according to recent data (8-9) the decrease of ventilation during sleep in healthy subjects is slight, from 5 % to 15 %, and not sufficient to explain the increase of isoprene in breath. Whereas isoprene concentration in breath decreased by about 50 % in less than 20 minutes after awakening, the concentration of acetone simultaneously measured in the same samples, did not decrease : 18.7 +/- 7.6 nmoles/L at awakening and 18.8 +/-7.8 nmoles/l 20 minutes latter, values in agreement with recent data (10-11). Since acetone is an endogenous substance like isoprene with a fairly low boiling point, it might have been expected that variations of ventilation would lead to similar variations of the concentration of isoprene and acetone, although acetone is very water soluble in opposition to isoprene.

Moreover, even if there is a passive component in the increase of isoprene during sleep, isoprene could play a role in sleep upholding. According to Gotsinkii (12) isoprene, given by inhalation to mice, is a hypnotic and as some experiments on rats and mice in our laboratory confirm this result, we favour the hypothesis that isoprene plays a role in sleep upholding. Isoprene, highly volatile with a boiling point at 34 °C, can be rapidly eliminated through the lungs and is thus compatible with quick changes in the degree of vigilance such as are produced when passing from sleep to wakefulness.

The body organs producing isoprene found in the breath have not yet been identified. Although it appears difficult to demonstrate in vivo that the human brain produces isoprene, the probability that it does so is high because brain tissue contains cholesterol as well as dolichols (13) the synthesis of which requires isopentenyl pyrophosphate and dimethylallyl pyrophosphate. The synthesis of both these molecules depends on the enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase which may thus play a role in the production of isoprene. This enzyme which has a multivalent feedback regulation (14-15) could additionally be under the control of hormones such as norepinephrine (16-17).

Our findings suggest that the concentration of isoprene in human breath depends on states of sleep or wakefulness. To further this investigation, we are now studying patients with well-defined sleep disorders.

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