Stereospecificity of the sensory irritation receptor for nonreactive chemicals illustrated by pinene enantiomers

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Abstract To clarify the existence of a receptor protein for sensory irritants in trigeminal nerve endings, D- [i.e. (+)] and L- [i.e. (−)] enantiomers of α- and β-pinene as models of nonreactive chemicals were evaluated for their potency in outbred OF1 and NIH/S mice using ASTM E981-84 bioassay. All pinenes possess sensory irritation properties and also induced sedation and signs of anaesthesia but had no pulmonary irritation effects. According to the ratio of RD50 (i.e. concentration which causes a 50% decrease in respiratory rate, f) and vapour pressure (P°), all pinenes are nonreactive chemicals. For nonreactive chemicals, P° and olive oil-gas partition (LOil) can be used to estimate their potency as sensory irritant. Thus, for enantiomers with identical physico-chemical properties, the estimated RD50 values are the same. In addition, although α- and β-pinene do not have identical P° and LOil values, their estimated potencies are quite close. However, the experimental results showed that D-enantiomers of pinenes were the most potent as sensory irritants and a difference in potency also exists between α- and β-pinene. RD50 for D-enantiomers of α- and β-pinene were almost equal, 1053 ppm and 1279 ppm in OF1 strain and 1107 ppm and 1419 ppm in NIH/S strain, respectively. Values differed by a factor of 4 to 5 from L-β-pinene for which the RD50 was 4663 ppm in OF1 and 5811 ppm in NIH/S mice. RD50 could not be determined for L-α-pinene; this pinene was almost inactive. D-α-pinene seems to best fit the receptor because its experimental RD50 was one-half of the estimated value while for D-β-pinene those values were equal. On the contrary, L-β-pinene was about 3 to 4 times less potent than estimated. L-α-pinene was only slightly active although it was estimated to be as potent as D-α-pinene. The remarkable difference in potency between L-enantiometers is most likely due to a structural difference between α- and β-pinene: the more flexible β-pinene can bend to fit into the receptor better than the rigid α-pinene. The results showed that the commonly used physicochemical descriptors cannot fully explain the potency of these chemicals; their three-dimensional structure should also be considered. Because of the stereospecificity of pinenes, a target site for nonreactive sensory irritants is most likely a receptor protein containing a chiral lipophilic pocket.

Key words Sensory irritation · Stereospecificity · Enantiomers · α-Pinene, β-pinene · Chirality

Introduction

The mouse bioassay (Alarie 1996) has been used to evaluate the sensory irritation potency of airborne compounds by measuring a decrease in breathing rate due to stimulation of the trigeminal nerve endings in the nasal mucosa. From the concentration-response relationship, the RD50, i.e. the concentration which causes a 50% decrease in respiratory frequency, has been determined for c. 300 chemicals or mixtures (Schaper 1993). Such a database offers an excellent opportunity to study the mechanisms of sensory irritation. For nonreactive chemicals, a ratio RD50/saturated vapour pressure was observed to exceed 0.1 and to be approximately constant (Nielsen and Alarie 1982; Abraham et al. 1994). Thus, following Ferguson’s principle (1939), a physical rather than chemical interaction with receptors at trigeminal
nerve endings has been suggested for nonreactive chemicals (Nielsen and Alarie 1982; Abraham et al. 1994). For these chemicals, vapour pressure can be used to estimate RD50 values (Alarie and Luo 1986; Abraham et al. 1994; Alarie et al. 1995, 1996, 1998) as well as the nasal pungency in humans (Cometto-Muñiz and Cain 1994; Abraham et al. 1996). Recently, Abraham et al. (1990, 1994, 1996) and Alarie et al. (1995, 1996, 1998) have shown that lipophilicity of nonreactive chemicals plays a major role in sensory irritation both in mice and in humans. Also, the solute dipolarity and hydrogen bond acidity are significant descriptors (Abraham et al. 1990, 1996; Alarie et al. 1995, 1998). Thus, for nonreactive sensory irritants moderately dipolar, fairly hydrogen bond basic and highly lipophilic receptor characteristics are expected (Alarie et al. 1995).

Sensory irritation for nonreactive compounds is assumed to be a direct, reversible interaction between a chemical and a finite and amphiphilic pocket of a receptor (Nielsen 1991; Abraham et al. 1994). The 'cut-off' phenomenon and stereospecific sensory irritation effects have been noted (Alarie 1990; Abraham et al. 1994; Alarie et al. 1995), suggesting that the receptor has defined dimensions and shape. Previously, Alarie (1990) has shown that the cis-isomer of 4-cyclohexyl methylecyclohexylamide was a potent sensory irritant while the trans-isomer was inactive. Another form of the stereoisomerism occurs when chemicals differ from each other by their three-dimensional structure, being non-superimposable mirror images i.e. enantiomers. Their physical and chemical properties are identical in a non-chiral environment, but the ability to turn plane-polarized light and to interact with other stereospecific compounds, such as proteins, are different (Fessenden and Fessenden 1990). Thus, enantiomers might offer good probes to determine stereospecific properties of the sensory irritant receptor.

Pinenes are chiral, similar to many naturally occurring compounds, and occur in e.g. coniferous trees and are monoterpenes, which are widely used in industry. Pinenes are bicyclic hydrocarbons with one double-bond and lack polar functional groups, and thus, may be regarded as 'nonreactive'. The focus of this study was to compare the sensory irritation potency of pinene isomers: D-α-pinene, [i.e. (+)-α-pinene], L-α-pinene [i.e. (-)-α-pinene], D-β-pinene, [i.e. (+)-β-pinene] and L-β-pinene, [i.e. (-)-β-pinene] to determine whether or not stereospecificity exists for sensory irritation evoked by nonreactive chemicals.

### Materials and methods

#### Animals

One-hundred and sixty outbred Ico: OF1 (I.O.P.S. Caw) male mice (3 to 4 weeks of age; supplied from Iffa Credo, Domaines des Oncins, Saint-Germain sur l’Arbrelse, France) and 100 outbred KTL (Hsd/Ola):NIH/St(SPF) male mice (3 to 4 weeks of age; supplied from Division of Environmental Health, National Public Health Institute, Kuopio, Finland) were used. Animals were first held and observed for a week in steel cages with dried aspen chips as bedding material. The animals were provided food and tap water ad libitum. The animal rooms were maintained on a 12:12 h light/dark cycle at 20°C and at 40–55% relative humidity of air. Animals used in the experiment were of 21.7–35.6 g body weight and 4 to 6 weeks of age. For each experiment, a new group of four mice was used. After the test, the animals were housed for 1 week (one test group per cage), and 1 week after experiment were weighed to assure reasonable weight gain.

#### Chemicals and test atmospheres

D-α- [i.e. (+)-α-] (>97%), L-α- [i.e. (-)-α-] (~99%), D-β- i.e. (+)-β- (~99%) and L-β- i.e. (-)-β- pinene (~99%) from Fluka (Buchs, Switzerland) were used without further purification. The chemicals were led to evaporate into a Pitt no. 1 glass generator using a constant flow rate controlled by a syringe pump and with compressed air. The output of the generator was mixed with room air at the entrance of the exposure chamber (2.3 l; Vijayaraghavan et al. 1994). The airflow through the chamber was set at 13 to 21 l/min. The nominal concentration was calculated from the amount of the chemical led into the generator and the airflow in the exposure chamber. The concentration of the exposure chamber was continuously monitored by infrared spectroscopy (Miran 1B, Foxboro so. Norwalk, CT., USA). The analyser was calibrated prior to each exposure with known amounts of the chemical. The difference between the measured and nominal concentration was usually <10%.

#### Exposure conditions and measurement of inspiratory and expiratory airflow (V) in mice

For each experiment, each of four animals was placed in a body plethysmograph (glass tubes) attached to the exposure chamber via a glass joint so that only a head protruded into the chamber. Mice were kept in place by a latex collar around their neck and a cork sealing the end of the glass tube allowing incidental head, feet and body movements (Vijayaraghavan et al. 1994). Before recording an airflow, a 10 to 15 min adaptation period in the test apparatus were allowed. All tests lasted for 1 h, consisting of 15 min control period (baseline period) when room air only was introduced, 30 min exposure to the tested chemical or room air (i.e. sham exposure), and 15 min recovery period (room air only).

To measure inspiratory and expiratory airflow a differential pressure transducer (model 8T-2, Gaeltec, Dunvegen, Isle of Skye, Scotland, UK) was attached to a pneumotachograph (model i/a 7319, OEM Medical, Richmond, Va., USA) joined to the top port of each plethysmograph. The continuous, constant airflow (215 ml/min) was passed through each pneumotachograph and plethysmograph by a pump and critical orifice attached to the rear of each plethysmograph (Vijayaraghavan et al. 1994). The analog signal (V) from the differential pressure transducer was amplified and displayed on the Gould WindoGraph recorder and fed to an A/D converter (DAS-16; Keitley/Metrabyte, Taunton, Mass., USA) set at a sampling rate of 300 samples/s. Digitizations were stored on the hard disk of a 486 computer.

#### Data analysis and computer programs for data handling

The computer program (Vijayaraghavan et al. 1994; Boylstein et al. 1995) calculated six variables for each breath of each animal using the digitization of V during inspiration (VI) and expiration (VE). Tidal volume (VT; unit ml) was obtained by integrating V values with time. Hence, airflow during expiration at 0.5 VTE (VD; ml/s), time of pause after expiration (TP; s), time of braking after inspiration (TB; s), duration of inspiration (TI; s) and duration of expiration (TE; s) were calculated. The seventh variable, respiratory frequency (f or BPM; breaths per minute), was calculated for each collection period (15 s). Based on data collected during 15 min