Biochem. J. (1989) 262, 475–478 (Printed in Great Britain)

Concanavalin A reveals olfactory receptors which discriminate between alkane odorants on the basis of size

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For certain odorants, the amplitude of the rat electro-olfactogram is reduced if the olfactory epithelium is treated with the lectin concanavalin A. When normal and cycloalkanes of one to ten carbon atoms are used as odorants at equimolar concentration, the maximum reduction in amplitude is found to correlate with the size of the stimulus molecule. This observation is consistent with the notion that concanavalin A disables an olfactory receptor molecule which normally responds to the alkyl moiety of odorants in a particular size range. That moiety may thus represent a 'primary' quality-determining component in odour discrimination.

INTRODUCTION

The initial event in olfaction consists of interactions between an odorant and olfactory receptor neurons. These interactions are expected to be non-covalent, to be sensed by an array of receptor sites and to be transduced into a neural excitation pattern characteristic of each odour.

A key objective in understanding the qualitative aspect of odour discrimination is the determination of the molecular properties which are sensed by the putative receptors. Structure–activity relationships based on perceived odour qualities (e.g. woody, musky, fatty, etc.) have not provided adequate insight, presumably because of overlap between receptor response spectra.

To identify receptor classes, we have been examining structure–activity relationships using as activity the electro-olfactogram [EOG; an indicator of the summated generator potential of the receptor cells (Ottoson, 1956)]. We assume that there are receptor sites which respond to particular classes of odorants and that these sites can be selectively inhibited by reagents. The molecular features shared by odorants whose EOG responses are inhibited might be those sensed as primary quality components (Shirley *et al.*, 1983).

Previously, we observed that superfusion of the olfactory mucosa with the lectin concanavalin A (Con A) causes a reduction in the amplitude of the EOGs of certain odorants (Shirley *et al.*, 1987*a*). Responses to aliphatic acids, aldehydes, thiols and hydrocarbons were particularly affected. Maximum inhibition occurred for C_4–C_6 homologues in the n-alkyl series of each of these functional groups. However, no effort had been made to match stimulus concentrations, and the EOG recordings were not all from the same mucosal position.

The Con A effect depends not only on the odorant used, but also on its concentration (Shirley *et al.*, 1987*b*). In the present paper we describe the effect of Con A treatment on the EOGs produced by equimolar concentrations of a series of aliphatic hydrocarbons. (The concentrations in the olfactory mucus are estimated to be equimolar.) The use of hydrocarbons as stimuli minimized the effect of electrostatic interactions and maximized hydrophobic ones (Dodd, 1976). In addition, the low water/air partition coefficients enabled an accurate estimate of concentrations in the olfactory mucus to be made.

METHODS AND MATERIALS

Chemicals

The alkanes, their suppliers, purities and physical properties are listed in Table 1. Con A (type IV) was from Sigma and all other reagents were of analytical grade.

Animals

Male Wistar rats weighing 200–250 g were used.

Olfactometry

The olfactometer has been described previously (Shirley, 1987).

The lower-boiling hydrocarbons (C_1–C_4), suitably diluted with air, were stored over water. Between 2 and 8 ml of each was transferred/min via a peristaltic pump to a chamber where it was mixed with water-saturated air (250 ml/min). Final concentrations were adjusted by means of the initial dilution and pump speed.

The higher-boiling-point compounds were placed in glass evaporators and dry air (0.1–20 ml/min) was passed over the surface. These odorized airstreams were each then mixed with 250 ml of water-saturated air/min. Final concentrations were adjusted by varying the evaporator temperature (from −80 to +15 °C) and dry-air flow rate.

Decane was used as saturated vapour produced by passing 250 ml of water-saturated air/min over decane-soaked filter-paper.

The final concentrations in air of the hydrocarbons were measured before and after EOG recording with a flame-ionization detector at the outlet of the olfactometer. The interior of the detector was maintained at 20 kPa (150 mmHg) below atmospheric pressure, causing

Abbreviations used: Con A, concanavalin A; EOG, electro-olfactogram.

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Table 1. Physical properties of the ordorants

The codes used to designate suppliers are: a, Phase Separations Ltd. (g.l.c.-standard gasses diluted in air); b, Aldrich; c, Fluka; d, Elf-Aquitaine; e, Merck. The values in parentheses are apparent purities by g.l.c. The solubility data are taken (or extrapolated) from McAuliffe (1966), except that for cyclopropane, which is from Inga & McKetta (1961). For the substances which are liquid at room temperature, the tabulated value is the concentration in an aqueous solution which is in equilibrium with the liquid hydrocarbon and hence with vapour at the saturated vapour pressure. For the gases, the value is the concentration in an aqueous solution which is in equilibrium with the gas at a pressure of 101 325 Pa (1 atm). The corresponding data for the gas phase have been interpolated from tables (Weast, 1976/1977) or calculated from the boiling point. All data refer to a temperature of 25 °C. The partition coefficient is the ratio of the molar concentrations of the compound in water and air. The concentration of the reference odorant (isopentyl acetate) was 10 μM in the gas phase (corresponding to 710 μM in water).

Compound	Source	Saturated vapour concn. (nM)	Solubility in water (mM)	Partition coefficient (water/air)
Methane	a	40.9	1.53	0.037
Ethane	a	40.9	2.01	0.049
Propane	a	40.9	1.45	0.034
Butane	a	40.9	1.06	0.026
Pentane	b,c (99.8)	28.7	0.534	0.019
Hexane	c (99.7)	7.80	0.110	0.014
Heptane	b (100)	2.30	0.0292	0.013
Octane	d (98.9)	0.705	0.0058	0.0082
Nonane	d (99.4)	0.252	0.0017	0.0067
Decane	d (99.7)	0.095	0.00031	0.0033
Cyclopropane	b (99)	40.9	12.0	0.239
Cyclopentane	c (99.5)	17.1	2.22	0.130
Cyclohexane	e (100)	5.18	0.654	0.126
Cycloheptane	d (100)	1.09	0.306	0.281
Cyclo-octane	b (99)	0.296	0.0704	0.238
Cyclodecane	c (99.4)	0.025	0.01	0.4

the device to draw in an air sample at 5 ml/min via a stainless-steel capillary.

The gas-phase concentrations were chosen to produce equal (300 nM) concentrations in the olfactory mucus for each odorant. This value was chosen because it is near the maximum attainable with decane. As mucus/air partition coefficients are, in general, unknown, we have used water/air partition coefficients. Mucus may be a better solvent than water; Hornung *et al.* (1980) showed that twice as much n-octane was absorbed by frog olfactory mucus than by water. Differences of this order are of no significance in this work. The physical data for the compounds is collected in Table 1.

Experimental protocol

The head of a freshly decapitated rat was cut in sagittal section and the septum removed to gain access to the ethmoturbinates. The half-head was clamped in a holder (about 18 °C) and the olfactory area superfused with Ringer's solution (154 mM-NaCl/5.6 mM-KCl/2.2 mM-$CaCl_2$/2.7 mM-$NaHCO_3$/11 mM-glucose, gassed with CO_2/O_2 (1:19) for 5 min at a flow rate of about 2 ml/min. The liquid was removed by aspiration and the tissue allowed to stabilize under clean air for 10 min (or longer if necessary). The tissue was then stimulated with a train of odour pulses and the resulting EOGs recorded. Each pulse was of 1 s duration and the interpulse interval was 1 min. The recording was via a glass capillary (approx. 40 μm tip diameter, filled with Ringer solution gelled with 0.3% agarose) and Ag/AgCl electrodes connected to an amplifier of 10 MΩ input impedance. The signals were digitized to 12-bit resolution, and peak amplitudes were determined by computer. After this recording period the microcapillary was raised and the tissue superfused with Con A (0.5 mg/ml) in Ringer solution for 5 min and rinsed with Ringer solution for 10 min. Control animals were treated with Ringer solution alone for 15 min. Liquid was then aspirated away, the tissue allowed to stabilize, and stimulation and recording were repeated.

Calculation of results

The amplitude of each hydrocarbon EOG was divided by the amplitude of the reference (isopentyl acetate) EOG interpolated from neighbouring pulses. The mean of this quantity is called the 'normalized amplitude' (A). Changes in the normalized amplitude are termed 'ΔA':

$$A = \frac{\text{amplitude of odorant EOG}}{\text{amplitude of reference EOG}}$$

$$\Delta A = A_{\text{initial}} - A_{\text{final}}$$

RESULTS AND DISCUSSION

The values of normalized EOG amplitudes and the Con A-induced changes are given in Table 2. The alkanes containing five or more carbon atoms are fairly potent stimuli for the rat. For instance, nonane at 300 nM elicits an EOG which has half the amplitude of that produced by 700 μM-isopentyl acetate.

In the series of the normal hydrocarbons, the maximum response is shown with nonane, whereas the maximum Con A-induced reduction in response occurs with pentane. The effect of Con A is clearly odorant-dependent.

With a large variety of odorants, we observed (Shirley *et al.*, 1987*a*) that large Con A-induced reductions in

Table 2. Normalized amplitudes, Con A-induced changes and molecular parameters

The values for the normalized amplitude (A) are means and 95% confidence intervals, based on reading from at least 20 animals (C_5–C_{10} compounds) or at least ten animals (C_1–C_4). The values for ΔA are means and 95% confidence intervals and are based on at least ten animals (C_5–C_{10}) or at least five animals (C_1–C_4). The mean initial reference signal (isopentyl acetate) was 9.80 mV and its mean survival was 84% (Ringer treatment) and 74% (con A treatment). Molecular lengths are measured from Dreiding models fitted with hydrogen and are the maximum dimension of the conformation considered most stable for the cyclohydrocarbons (Clark & McKervey, 1979). For the normal hydrocarbons, the all-staggered extended-chain conformation has been used. The values in the surface area column refer to aqueous cavity surface areas. For methane, ethane and propane the values have been taken from the data of Harris *et al.* (1973). Otherwise they have been calculated from the water solubilities of the hydrocarbons (Reynolds *et al.*, 1974) using a constant factor of 8780 J/nm² and, where comparative data are available, are in good agreement with the values given by Harris *et al.* (1973). The value for cyclopropane should be regarded with caution, as considerable extrapolation of the solubility data is required.

Odorant	Normalized amplitude (A)	Change (ΔA)	Length (nm)	Surface area (nm²)	Molar volume (ml/mol)	M_r
Methane	0.03±0.03	0.02±0.06	0.377	1.52	53.3	16.0
Ethane	0.04±0.03	0.04±0.06	0.503	1.97	84.6	30.1
Propane	0.04±0.03	−0.02±0.04	0.626	2.35	86.8	44.1
Butane	0.06±0.04	0.05±0.09	0.752	2.84	99.5	58.1
Pentane	0.29±0.04	0.22±0.07	0.876	3.26	114.4	72.2
Hexane	0.31±0.05	0.13±0.05	1.001	3.71	129.8	86.2
Heptane	0.24±0.03	0.05±0.03	1.123	4.08	145.6	100.2
Octane	0.41±0.05	0.07±0.05	1.250	4.54	161.5	114.2
Nonane	0.54±0.04	0.10±0.06	1.375	4.89	177.7	128.3
Decane	0.30±0.03	0.07±0.03	1.500	5.37	193.9	142.3
Cyclopropane	0.04±0.04	0.04±0.12	0.500	1.91	58.5	42.1
Cyclopentane	0.28±0.05	0.22±0.08	0.608	2.86	93.5	70.1
Cyclohexane	0.39±0.06	0.22±0.05	0.692	3.21	107.5	84.2
Cycloheptane	0.33±0.05	0.20±0.05	0.680	3.44	121.1	98.2
Cyclo-octane	0.35±0.04	0.24±0.07	0.708	3.84	134.5	112.2
Cyclodecane	0.32±0.03	0.15±0.05	0.880	4.39	163.1	140.3

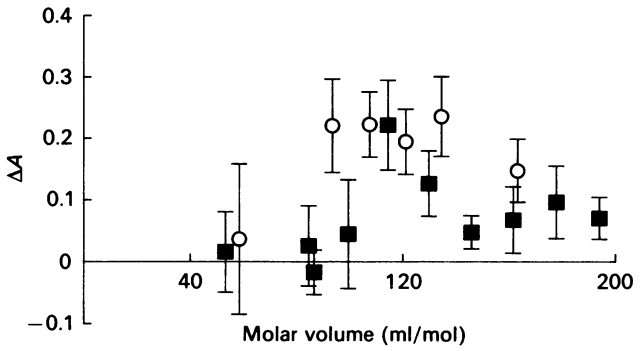

Fig. 1. Relationship between the Con A-sensitive component of the EOG and the molar volume of the odorant

The error bars represent 95% confidence intervals. The normal alkanes (■) are methane to n-decane and the cyclo compounds (○) are cyclopropane, cyclopentane to cyclo-octane and cyclodecane.

response are most common among substances with a molar volume in the range 80–130 ml/mol, suggesting a size-based hydrophobic interaction of the kind described by Wishnia & Pinder (1966), Robillard & Wishnia (1972) and Reynolds *et al.* (1968) for soluble proteins. This pattern is confirmed by the present observations (Fig. 1). Hydrophobic interactions can easily discriminate between compounds, as demonstrated by hydrophobic chromatography (reviewed by Shaltiel, 1984). Since the aqueous concentrations of the hydrocarbons were calculated to be equimolar, we can have some confidence that this pattern is intrinsic and not the result of arbitrary choices of concentrations.

Correlation of the Con A-induced reductions with M_r or length produce less regular patterns than that obtained with molar volume. However, the data correlate well with molecular surface area (Fig. 2*a*). The component of the EOG which survives Con A treatment also seems to depend on surface area (Fig. 2*b*).

We have previously suggested (Shirley *et al.*, 1987*a*,*b*) that the EOG can be regarded as the sum of a series of components, each of which arises from a different type of receptor molecule; and that Con A disables (at least) one receptor, removing one of the components. The Con A-induced reduction in EOG may thus give the response spectrum of a single olfactory receptor.

On this basis, the EOGs evoked by the hydrocarbons would involve predominantly two receptors: one Con A-sensitive and capable of accepting alkanes of about 3.5 nm²; the other Con A-insensitive and responding to hydrocarbons of about 5 nm². That the receptors should discriminate between alkanes on the basis of area is not surprising, as hydrophobicity is the strongest interaction which could operate with these molecules, and the hydrophobic force is area-dependent.

It is also of interest that we have previously implicated the existence of a receptor capable of responding to C_8–C_{10} aliphatics (i.e. molecules of about 5 nm²) (Shirley *et al.*, 1983), this receptor being susceptible to enzymic iodination.

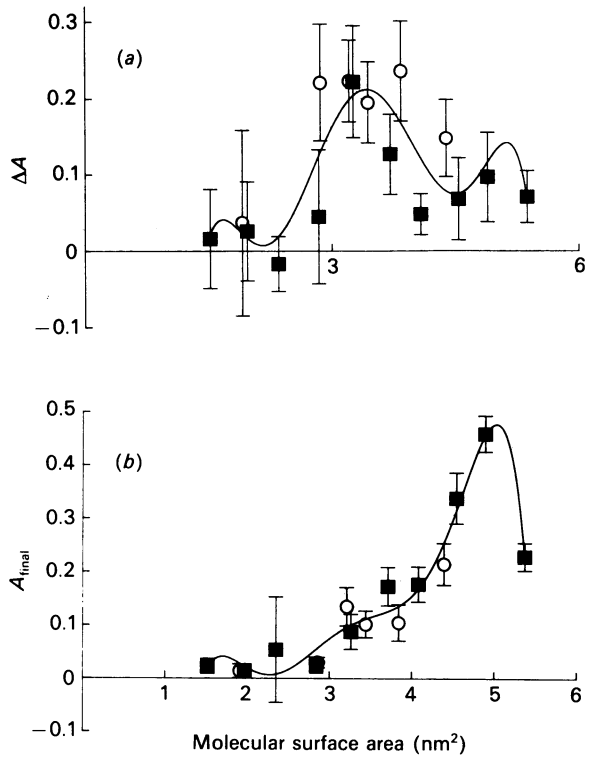

Fig. 2. Relationship between the Con A-sensitive component of the EOG (*a*) and the Con A-insensitive component (*b*) and the surface area of the stimulating hydrocarbon

The method of calculation of area is given in the legend to Table 2. All stimuli were applied at 300 nM in the olfactory mucus. The error bars represent 95% confidence intervals, and the lines are unweighted sixth-order polynomials fitted to the data for both normal alkanes (■) and cycloalkanes (○).

The five compounds whose EOGs are most affected by Con A share (within experimental error) the same value of ΔA: 0.22, 0.22, 0.22, 0.20 and 0.24 for n-pentane and cyclopentane to cyclo-octane respectively, although this by no means represents a constant proportion of the initial signal (76, 79, 56, 65 and 69% respectively). By using the interpretation given above, the equality of the ΔA values is explicable: those hydrocarbons which best fit the receptor are, at equimolar concentration, about equally affective at stimulating (possibly driving it near saturation). On other models (e.g. those with very highly specific receptors or those with transduction mediated by the lipids of the membrane) this equality would be more difficult to explain.

There seems to be a tendency for the cycloalkanes to return higher values of ΔA than do the normal alkanes. This is at least partially explained by there being more cyclo- than normal alkanes within the critical size range.

Thus each receptor site seems to respond to a range of alkanes, discriminating on the basis of some size-related parameter, such as surface area. A full explanation of the response spectra will undoubtedly have to involve consideration of shape factors as well. A concrete example of this kind of shape/size discrimination can perhaps be found in the binding of aliphatic C_2–C_6 isonitriles to haemoglobin (Reisberg & Olsen, 1980).

Compounds for which Con A-induced reductions in response are found tend to have C_4–C_5 skeletons (Shirley *et al.*, 1987*a*). But the possession of such a skeleton by an odorant does not guarantee that a reduction will be observed. For a Con A-induced reduction of response to be detectable, it is necessary that the Con A-sensitive receptor be a major contributor to the system's total response to the odorant. The addition of a polar functional group to a suitable skeleton may permit interactions with other receptors and increase the overall response to the point where the effect of Con A is masked. Such a substituent might also weaken the hydrophobic interaction.

The perceived quality of an odorant would arise from the comparison of the responses of a number of different types of receptor molecule, each sensing a different profile of the odour molecule (Polak, 1973). An odorant with an alkyl group of acceptable size and shape (possibly coupled to a not-unsuitable polar group) would trigger the Con A-sensitive receptor. Such an alkyl group would therefore be a requirement for one of the 'primary' quality-determining responses.

We thank Elf-Aquitaine for supplying several of the hydrocarbons, RAM Ltd. for financial support, and Michael Jenkins for his technical assistance in part of this project.

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Received 17 November 1988/28 March 1989; accepted 31 March 1989