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The effect of concanavalin A on the rat electro-olfactogram at various odorant concentrations

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We have studied the effect of concanavalin A (Con A) on the rat electro-olfactogram response to several odorants. Each odorant was applied over a range of concentrations. For hydrophobic odorants whose response was affected by Con A, the diminution in response was maximal at odorant concentrations of about 1 μM in the olfactory mucus. The (odour) concentration-dependence of the change is compatible with the idea that Con A inactivates one or more types of olfactory receptor that normally bind odorants with dissociation constants of the order of 100 nM. With hydrophilic odorants we had to apply concentrations very much higher than this to elicit any response from the system. At these high concentrations we could observe Con A-induced diminutions in response.

INTRODUCTION

A knowledge of receptor–ligand affinity is important in the study of any receptor system. In mammalian olfaction, however, there are complicating factors. Since there are many possible ligands (odorants) and an unknown number of receptors, there is undoubtedly a large number of different affinities. Secondly, odours are usually delivered in air to the olfactory system, and it is necessary to know the concentration of odour in the mucus in order to calculate affinities.

Nevertheless, there have been attempts to estimate affinities. It has been suggested (Tucker, 1963; Poynder, 1974*a*,*b*) that the concentration-dependence of the amplitude of the electro-olfactogram reflects odorant–receptor interactions. This idea has been developed by Senf *et al.* (1980). These authors concluded that, at high odour concentrations, the shape of the EOG-amplitude-versus-concentration curve could be explained by assuming a single dissociation constant for any one of their odours (a series of alcohols). The logarithms of these dissociation constants were in the range 0 to -5 and varied smoothly over the series of compounds, supporting the notion that the interaction of odour and receptor was primarily hydrophobic.

The olfactory system responds to odorant concentrations much lower than those used in that study. At low concentrations the shape of the EOG-amplitude-versus-concentration curve cannot be explained by a single dissociation constant. The amplitude of response is proportional to a power (between 0.3 and 0.5) of the stimulus concentration (Ottoson, 1956, 1970).

We have suggested [the accompanying paper (Shirley *et al.*, 1987)] that (i) the EOG can, approximately, be regarded as the sum of components each of which originates with a different type of receptor molecule, and (ii) that one or more of these types is susceptible to the lectin Con A. If so, the Con A-induced change in the shape of the amplitude-versus-concentration curve might be explained by a single dissociation constant. We would then have a means of measuring at least some of the dissociation constants.

METHODS AND MATERIALS

Chemicals

(*S*)-(−)-Nicotine was obtained from Sigma and redistilled under reduced pressure to 99.9% purity as determined by capillary gas chromatography. n-Butyl cyanide (98%) and isopentanoic acid (98%) were supplied by Fluka. The other odorants: methyl disulphide (99%), isobutyl mercaptan (97%), isobutyraldehyde (99%), hexan-1-ol (98%) and isopentyl acetate (97%) were supplied by Aldrich. Con A (type IV) was obtained from Sigma. All other reagents were of analytical quality.

Animals

Male Wistar rats weighing 200–250 g were used.

Olfactometer

The olfactometer has been described previously (Shirley, 1987). This experiment involved the application of odours at very low concentrations. We paid great attention to the cleaning of the olfactometer. The machine was disassembled and the components washed in chloroform and baked *in vacuo* at 120 °C. This process was repeated until the rat head preparation described below (the most sensitive available detector) stimulated with nominally clean air gave responses much less than the smallest odour-induced response.

Experimental protocol

All recordings were obtained from location 3, defined in the accompanying paper (Shirley *et al.*, 1987) and, except for the following, the experimental protocol was as described in that paper. The concentrations of the reference odorant (isopentyl acetate) was 7.9×10^{-8} M in

Abbreviations used: Con A, concanavalin A; EOG, electro-olfactogram [a tissue surface potential caused by the generator current of the olfactory primary cells (Ottoson, 1956)]; nicotine, 3-(1-methyl-2-pyrrolidinyl)pyridine; *A*, amplitude (*not* absorbance in this paper).

the gas phase. Each preparation was stimulated with the reference odorant and with different concentrations of one or two other odorants. All amplitudes were determined by computer from the digitized record and a low-pass (2.5 Hz cut-off) numerical filter (phase-sensitive Fourier series) was used to enhance the signal/noise ratio for the lower amplitude EOGs.

Definition of symbols

The amplitude of each EOG was divided by the amplitude of the reference EOG (interpolated from the neighbouring reference pulses) and the mean of this quantity is termed 'A' and referred to as 'normalized amplitude'. Changes in A caused by Con A treatment are referred to as 'ΔA'.

Solubilities

Where odorant solubilities were not available from the literature, they were determined. Increasing amounts of the material were added to vials, each containing 20 ml of pure water, and the vials were shaken periodically for 30 min. The solubility was taken to be the highest concentration which did not show turbidity under side illumination. The concentration steps were a factor of 1.5.

RESULTS

The vapour pressure and solubility data are collected in Table 1. The odorant concentrations, the normalized EOG amplitudes and the changes induced by Con A are shown in Table 2 and Fig. 1.

The variation of EOG amplitude with odour concentration can be described fairly well by a power law with an exponent between 0.3 and 0.5; this is in line with the results obtained previously (Ottoson, 1956; von Sydow, 1968).

The EOG for one odour, butyl cyanide, was not affected by the Con A treatment. For three odours whose mucus concentration can be estimated with some certainty the Con A-induced change in EOG shows a maximum at a (mucus) concentration of about 1 μM. Hexanol, the fourth odorant in this class, shows an increasing change with increasing concentration, but the concentration range was rather limited. There is evidence that the EOG for isopentyl acetate, the reference substance, was decreased to a small extent.

Nicotine and isopentanoic acid both have high water/air partition coefficients, which makes estimates of the mucus concentration rather unreliable. The Con A-induced changes in EOG for these compounds apparently occur at very much higher odorant concentrations (at least four orders of magnitude).

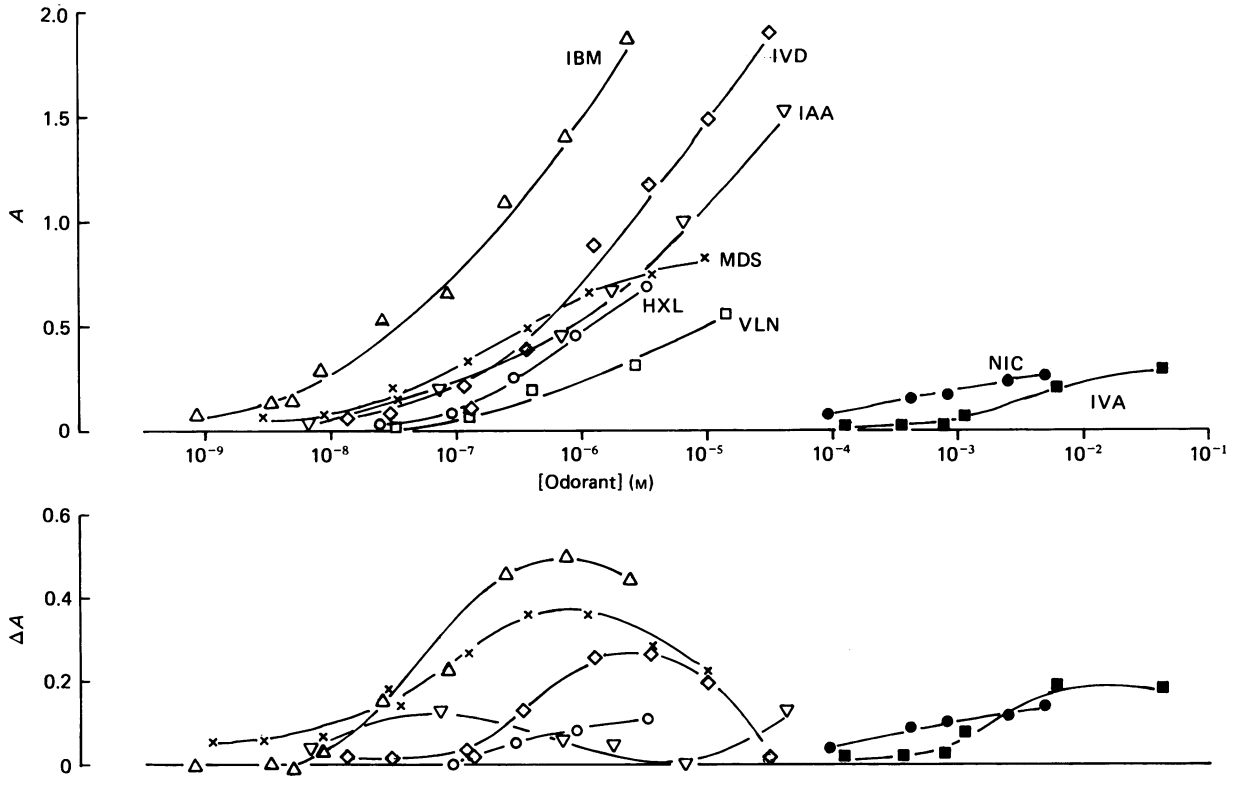

Fig. 1. Mean normalized EOG amplitude (A) and its change upon Con A treatment (ΔA) plotted against the logarithm of the concentration of the odorants in the mucus

For the insoluble odorants this concentration has been estimated by multiplying the water/air partition coefficient by the vapour-phase concentration. For the high-partition-coefficient odorants, nicotine and isopentanoic acid, this method breaks down. The concentration of these odorants is estimated by multiplying the vapour-phase concentration by 50000 (an instrumental factor) and the resulting estimate is almost certainly somewhat high. Abbreviations: IAA, isopentyl acetate; HXL, hexan-1-ol; IBM, isobutyl mercaptan; IVD, isopentylaldehyde; MDS, methyl disulphide; VLN, butyl cyanide; NIC, nicotine; IVA, isopentanoic acid. For clarity, zero values at low concentrations have been omitted, as has the ΔA curve for butyl cyanide, which was not affected by Con A at any concentration. The symbols in the lower panel correspond to those identified in the upper panel.

Table 1. Physical data for the compounds

The vapour pressures (15 °C) have been interpolated from standard tables (Weast, 1978–1979; Dreisbach, 1955–1961), except for isopentylaldehyde and isobutyl mercaptan, which have been calculated from the boiling point. The solubilities where marked * were measured; the others were taken from Stephen & Stephen (1963). For the compounds marked † the tabulated partition coefficient is between all forms of the compound in solution in water at pH 7. The isopentanoate ion is the dominant species in the case of isopentanoic acid. The partition coefficient for free isopentanoic acid is about 22000. Abbreviation used: Misc., miscible in all proportions.

	Saturated vapour			Saturated soln.		
	Vapour pressure			Concn.		
Compound	mmHg	Pa	Concn. (mol/l of air)	(g/100 ml)	(M)	Partition coefficient
Isopentyl acetate	2.9	390	1.4×10^{-4}	0.17	0.013	81
Methyl disulphide	16	2100	8.7×10^{-4}	0.11	0.011*	13
Isopentylaldehyde	62	8300	3.5×10^{-3}	0.28	0.033*	9
Isobutyl mercaptan	53	7100	2.9×10^{-3}	0.067	0.0075*	2.5
Butyl cyanide	4.1	550	2.3×10^{-4}	0.71	0.086	370
Hexan-1-ol	0.53	71	3.0×10^{-5}	0.59	0.58	2000
Isopentanoic acid	0.25	33	1.4×10^{-5}	3.6	0.35	4000000†
Nicotine	0.05	7	2.7×10^{-6}	Misc.	6.2	230000†

There was no significant effect of the control treatment on the EOG for any odorant.

The Con A treatment caused some diminution in the reference EOG. The survival was 0.80 ± 0.20 (S.D., $n = 45$). Since this 20% decrease reflects both specific (receptor) and non-specific damage, we have some confidence that the specific component is itself small, and, although it may cause some distortion of the results, it should not obscure the main findings.

DISCUSSION

Odorant concentration

To estimate the dissociation constant of the receptor–odorant complex, we need to know the odorant concentration in the mucus. We cannot measure this concentration in our preparation and so must rely on estimates. There is a review on odorant access to the receptors (Getchell *et al.*, 1984). For present purposes we make some simplifying assumptions. We neglect diffusion; on the time scale of the observations (about 1 s) in an animal with a thin layer of mucus (about 5 μm; Menco, 1980) odorants should diffuse almost to equilibrium. The method of calculating the mucus concentration of an odorant is outlined in the legend to Fig. 1 and covered in detail elsewhere (Shirley, 1987).

Reference odour

The purpose of the reference odorant is explained in the accompanying paper (Shirley *et al.*, 1987). However, it is possible that the receptors involved in the transduction of the reference signal are themselves affected by the reagent. Such effects are fairly small (see the Results section), but would cause an underestimate of ΔA, worsening at high values of A.

Effect of Con A

If Con A disables one or more of the olfactory receptors and ΔA is the affected component of the EOG (Shirley *et al.*, 1987), then we would expect the ΔA-versus-concentration curve to approximate to that of an ideal receptor:

$$\Delta A = m \times [c/(c + K_d)]$$

where c is the odorant concentration and m is a constant (or possibly to several such curves superposed). The comparisons are shown in Fig. 2. The approximation is good despite the errors inherent in this experiment and the main deviations occur at high concentrations. One reason for this has been mentioned above (Con A can affect the receptors for the reference odour). A second possibility is self-shunting of the EOG (Poynder, 1974*b*).

If ΔA is the response of the Con A-affected receptors, we must conclude that the dissociation constants for the insoluble odorants are of the order of 100 nM. For the soluble odorants they are higher.

Senf *et al.* (1980) estimated much weaker binding for a series of alcohols. This is not at variance with our present finding since, at high odorant concentrations, most of our initial signal is unaffected by Con A, so there must be lower-affinity sites.

For four of the compounds the data suggests a single Con A-sensitive receptor (see Fig. 2). For three of the others the data are not extensive enough to reach a conclusion, and the remaining compound was unaffected.

The question remains: is this a single receptor, responding to all four compounds, or four separate receptors responding to one compound each? The maximum responses (as described by ΔA) are different for the four compounds, but it would be rather coincidental for three receptors, each sensitive to Con A, to show such similar dissociation constants toward three such disparate compounds as methyl disulphide, isobutyl mercaptan and isovaleraldehyde. On the basis of these data the question must remain open.

Polak (1973) has suggested that an odorant interacts with more than one receptor and is recognized by the relative degree of binding. Since we have examples of a single receptor providing between 30 and 50% of the entire signal, it seems that the total number of receptor

Table 2. Variation of normalized EOG amplitude and Con A-induced change of normalized EOG amplitude with vapour-phase odorant concentration

Fitting the data to the equation:

$$\log A = \text{constant} + (m \cdot \log c)$$

yields the following: r, the correlation coefficient (obtained by fitting each data block to the linear equation); m, the slope; S_v ($= -\text{constant}/m$), the logarithm of the vapour-phase concentration (M) at which $A = 1$ (i.e. the concentration at which the odorant would produce the same amplitude of response as the reference stimulus); S_1 (related to S_v via the partition coefficient), the logarithm of the corresponding liquid-phase (mucus) concentration. The P values, which pertain to ΔA, were calculated by Student's t test. Abbreviations: n/s not significant; CI, confidence interval.

Odorant	[Odorant] in vapour phase (M)	Mean normalized EOG amplitude (A + 95% CI)	Mean Con A-induced change (ΔA + 95% CI)	P
Methyl disulphide	8.4×10^{-11}	0.15±0.05	0.06±0.09	n/s
($r = 0.988$; $m = 0.32$;	2.3×10^{-10}	0.14±0.04	0.06±0.7	0.1
$S_v = -7.1$; $S_1 = 6.0$)	6.7×10^{-10}	0.19±0.04	0.07±0.08	0.1
	2.6×10^{-9}	0.29±0.05	0.14±0.10	0.02
	2.3×10^{-9}	0.33±0.08	0.18±0.09	0.01
	9.6×10^{-9}	0.55±0.15	0.27±0.11	0.01
	3.0×10^{-8}	0.84±0.19	0.36±0.11	0.001
	8.6×10^{-8}	1.24±0.21	0.36±0.11	0.001
	2.9×10^{-7}	1.64±0.24	0.28±0.12	0.01
	7.7×10^{-7}	1.83±0.25	0.23±0.14	0.02
Isopentylaldehyde	1.3×10^{-9}	0.07±0.04	0.02±0.03	n/s
($r = 0.969$; $m = 0.47$;	3.0×10^{-9}	0.08±0.05	0.01±0.05	n/s
$S_v = -6.4$; $S_1 = -5.4$)	1.3×10^{-8}	0.11±0.05	0.02±0.03	n/s
	1.2×10^{-8}	0.23±0.04	0.03±0.02	0.02
	3.5×10^{-8}	0.40±0.08	0.13±0.07	0.01
	1.3×10^{-7}	0.89±0.10	0.26±0.05	0.001
	3.5×10^{-7}	1.19±0.14	0.27±0.06	0.001
	1.1×10^{-6}	1.49±0.19	0.20±0.12	0.01
	3.3×10^{-6}	1.91±0.23	0.02±0.40	n/s
Isobutyl mercaptan	3.2×10^{-10}	0.08±0.05	−0.01±0.02	n/s
($r = 0.980$; $m = 0.42$;	1.3×10^{-9}	0.14±0.05	0.00±0.06	n/s
$S_v = -7.0$; $S_1 = -6.6$)	1.8×10^{-9}	0.14±0.04	−0.01±0.04	n/s
	3.2×10^{-9}	0.30±0.08	0.03±0.10	n/s
	9.6×10^{-9}	0.53±0.10	0.15±0.12	0.05
	3.2×10^{-8}	0.66±0.11	0.23±0.10	0.01
	9.3×10^{-8}	1.11±0.15	0.46±0.23	0.01
	2.8×10^{-7}	1.41±0.16	0.50±0.24	0.01
	8.6×10^{-7}	1.88±0.16	0.44±0.20	0.01
Isopentyl acetate	8.3×10^{-11}	0.06±0.01	0.04±0.02	0.01
($r = 0.989$; $m = 0.38$	8.7×10^{-10}	0.20±0.05	0.12±0.06	0.01
$S_v = -7.1$; $S_1 = -5.2$)	8.7×10^{-9}	0.47±0.06	0.06±0.11	n/s
	2.1×10^{-8}	0.70±0.04	0.05±0.04	0.05
	7.9×10^{-8}	1	0	–
	4.9×10^{-7}	1.53±0.05	0.13±0.11	0.05
Hexan-1-ol	1.3×10^{-12}	0.03±0.01	0.01±0.01	0.05
($r = 0.968$; $m = 0.49$;	1.3×10^{-11}	0.04±0.02	0.00±0.05	n/s
$S_v = -8.5$; $S_1 = -5.2$)	4.6×10^{-11}	0.09±0.03	0.00±0.02	n/s
	1.5×10^{-10}	0.26±0.07	0.05±0.05	0.05
	4.4×10^{-10}	0.47±0.08	0.08±0.07	0.05
	1.7×10^{-9}	0.70±0.10	0.11±0.11	0.05
Isopentanoic acid	2.4×10^{-9}	0.03±0.01	0.02±0.01	0.01
($r = 0.956$; $m = 0.46$;	7.1×10^{-9}	0.03±0.01	0.02±0.01	0.01
$S_v = -5.1$; $S_1 = -0.3$)	1.5×10^{-8}	0.04±0.02	0.03±0.02	0.01
	2.2×10^{-8}	0.08±0.03	0.08±0.04	0.01
	1.2×10^{-7}	0.21±0.04	0.19±0.06	0.001
	8.4×10^{-7}	0.30±0.05	0.19±0.18	0.05
n-Butyl cyanide	1.1×10^{-11}	0.03±0.01	0.00±0.02	n/s
($r = 0.997$; $m = 0.37$;	8.1×10^{-11}	0.06±0.01	0.01±0.01	0.05
$s_v = -6.8$; $S_1 = -4.3$)	3.3×10^{-10}	0.10±0.02	0.02±0.01	0.01
	1.0×10^{-9}	0.19±0.03	0.03±0.03	0.05
	7.2×10^{-9}	0.32±0.05	−0.02±0.05	n/s
	3.6×10^{-8}	0.58±0.07	0.01±0.03	n/s
Nicotine	1.9×10^{-9}	0.08±0.001	0.04±0.03	0.05
($r = 0.984$; $m = 0.29$;	8.2×10^{-9}	0.15±0.04	0.08±0.06	0.05
$S_v = -5.1$; $S_1 = -0.4$)	1.6×10^{-8}	0.17±0.05	0.10±0.05	0.01
	4.9×10^{-8}	0.23±0.06	0.12±0.08	0.02
	9.8×10^{-8}	0.26±0.09	0.14±0.39	n/s

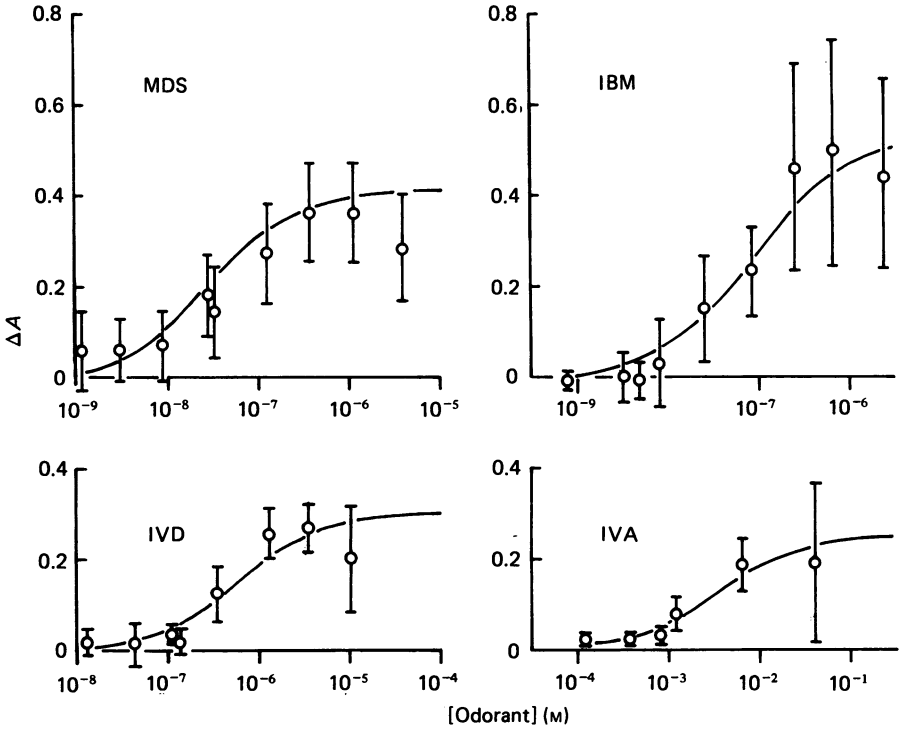

Fig. 2. Effect of odorant concentration in the mucus on the diminution of EOG amplitude (*A*) induced by Con A

The error bars represent 95% confidence intervals (in most cases 2.8 S.E.M. each side of the mean). For four odorants the shape of the curve seems explicable by the assumption of a single ideal receptor, giving the equation:

$$\Delta A = (M \times c)/(c+K)$$

where M and K are constants and c is the odorant concentration. The curves were fitted by non-linear regression to the data shown in the Figure, each mean being weighted inversely with its error. For methyl disulphide (MDS) the parameters were: $M = 0.412$, $K = 3.0 \times 10^{-8}$, and 92% of the variance is explained by the equation. For isobutyl mercaptan (IBM), the corresponding values are 0.52, 8.8×10^{-8} and 96%. For isopentylaldehyde (IVD) they are 0.30, 5.8×10^{-7} and 89%, and for isopentanoic acid (IVA), they are 0.26, 3.4×10^{-3} and 90%.

types responding to any one odorant is fairly small: perhaps only two, three or four.

The final concern is the weak stimulation caused by the soluble odorants. Concentration estimates of these compounds may be poor, but are unlikely to be four orders of magnitude higher. Workers have previously suggested that the hydrophobic force may be the main cause of interaction between ligand and receptor in some chemosensory systems [Ueda & Kobatake, 1977; the accompanying paper (Shirley *et al.*, 1987)]. It is possible that hydrophobic interactions could explain 100 nM dissociation constants. It would then not be surprising that hydrophilic substances tend to interact rather weakly with the receptors.

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