The effect of concanavalin A on the rat electro-olfactogram

Differential inhibition of odorant response

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When the rat olfactory mucosa is treated with concanavalin A, it subsequently shows diminished sensitivity towards 60% of the 112 odorants tested (as judged by the amplitude of the electro-olfactogram response). Odorants containing four to six carbon atoms tend to show the largest (absolute) diminutions, suggesting a receptor for this kind of odorant, although the structural specificity is weak. The receptor seems to be of particular importance in the detection of thiols, carboxylic acids and hydrocarbons of the above size, since these compounds lose the highest proportion of their original signal. The concanavalin A appears to be binding to the glycan of one or more cell-surface proteins. The binding may be at, or close to, at least one odorant receptor.

INTRODUCTION

Much of the information about the characteristics of the vertebrate olfactory receptors comes from electrophysiological studies (e.g. Holley, 1974; Revial *et al.*, 1983). There have been biochemical attempts to characterize the receptors (Cagan & Kare, 1981), and several studies based on a variety of chemical-modification techniques (Menevse *et al.*, 1977, 1978; Persaud *et al.*, 1980; Shirley *et al.*, 1981, 1983a; Wood & Dodd, 1982).

The currently held consensus of opinion seems to be that the receptor cells have a fairly wide response spectrum; the typical cell is capable of responding to several odorants. The cells are 'chaotic' in the sense that their response spectra do not fall into well-defined groups (Holley, 1974), although more recent research (Revial *et al.*, 1983) suggests that there may be some pattern.

Of the receptor molecules, much less is known. It has been suggested (Polak, 1973) that a typical odorant interacts with several different receptors, and that the relative degrees of stimulation are important in odour recognition.

Lectins, and in particular Con A, have been widely used as probes for the surfaces of membranes (Bittiger & Schnebli, 1976), and specific inactivation of receptors has been achieved in some instances. We have found that Con A superfused over the olfactory mucosa of a rat preparation *in vitro* affects responses to some odours (Polak *et al.*, 1982; Shirley *et al.*, 1983b). [The EOG is an electrical signal deriving from the generator potential of the primary receptor cells; its underlying mechanisms have been reviewed (Gesteland, 1975).] These small-scale studies suggested that small aliphatic carboxylic acids were particularly affected.

In the present paper we report on the selectivity of the Con A effect, measured on 112 odorants (each at a single concentration), and on its interpretation. The odorants were selected to include several homologous series, so that structure-activity relationships could be perceived. The effects are not confined to carboxylic acids and seem to reflect the hydrocarbon moiety of the molecules.

MATERIALS AND METHODS

Chemicals

Con A (type IV) and α -methyl mannoside were obtained from Sigma. The suppliers and purities of the odorants are noted in Table 1. It was considered unnecessary to purify most of the odorants, as the EOG seems rather insensitive to trace impurities. Those compounds found to contain highly volatile impurities were redistilled. All other reagents were of analytical quality.

Animals

Male Wistar rats weighing 200–250 g were used throughout.

Apparatus

The odour-delivery system has been described previously (Shirley, 1987). EOGs were recorded with a glass micro-pipette filled with Ringer solution (gelled with agarose), silver/silver chloride electrodes and an amplifier of 10 M Ω input impedance. EOGs were displayed on a chart recorder and measured manually, or the signals were digitized (to 12-bit resolution) and amplitudes determined by computer. There were no differences noted between the two methods.

Experimental protocol

The animals was stunned and decapitated. The head was cut in sagittal section and the septum removed to expose the olfactory turbinates (Fig. 1). The head was clamped into a holder and the olfactory area superfused with Ringer solution (154 mM-NaCl/5.6 mM-KCl/2.2 mM-CaCl₂/2.7 mM-NaHCO₃/11 mM-glucose) for 5 min and gassed with CO₂/O₂ (1:19). The flow rate was 2 ml/min. The liquid was removed by aspiration and the tissue was allowed to stabilize under clear air for 10 min (or longer if necessary).

The tissue was then stimulated with odour pulses and the EOGs recorded. The recording locations are shown in Fig. 1. Each pulse was of 1 s duration and the interval

Table 1. Effect of Con A on the EOG response of odorants

Tabulated are the means and 95% confidence intervals (CI) for R :

$$R = \frac{\text{EOG amplitude after Con A}}{\text{EOG amplitude before Con A}} \times \frac{\text{reference EOG before Con A}}{\text{reference EOG after Con A}}$$

Most of the compounds were tested on five control and five Con A-treated animals (95% confidence interval = 2×2.776 s.e.m.). P is the probability that test and control treatments, produced the same result (Student's t test). (n.s. = not significant). A is the mean initial signal divided by the initial amyl acetate signal. Odorant concentration is expressed as a percentage of saturated vapour concentration at 15 °C. The isoamyl acetate concentration was 0.4%. Recording locations 1 and 3 are shown in Fig. 1. The odorant purity is that claimed by the manufacturer, except where an asterisk occurs, in which case it was determined by g.l.c. The suppliers of the odorants are: Fi, Fisons; A, Aldrich; FL, Fluka; Si, Sigma; B, BDH; K, Koch-Light; Al, Alferetta; E, Elf Aquitaine; P, PFW; I, Institut Français du Pétrol.

Odorant	M_r	R (mean $\pm$ 95% CI)		P	A	Concn. (%)	Position	Purity (%)	Supplier
		ConA	Control						
Acids									
1 Acetic acid	60	0.69 $\pm$ 0.30	1.07 $\pm$ 0.41	n.s.	0.25	0.20	1	99.7	Fluka
2 Propionic acid	74	0.81 $\pm$ 0.19	0.90 $\pm$ 0.15	n.s.	0.19	0.20	1	99	Aldrich
3 Methacrylic acid	86	0.35 $\pm$ 0.16	0.96 $\pm$ 0.27	0.001	0.35	0.25	1	98.5	Aldrich
4 n-Butyric acid	88	0.39 $\pm$ 0.19	1.01 $\pm$ 0.20	0.001	0.17	0.70	1	99	Aldrich
5 Isobutyric acid	88	0.66 $\pm$ 0.11	0.91 $\pm$ 0.14	0.001	0.40	0.70	1	99	Aldrich
6 4-Pentynoic acid	98	0.55 $\pm$ 0.17	0.81 $\pm$ 0.21	0.02	0.34	11	3	97	Fluka
7 Cyclobutanecarboxylic acid	100	0.39 $\pm$ 0.24	0.90 $\pm$ 0.11	0.001	0.49	2.5	1	98	Aldrich
8 2-Methyl-2-butenic acid	100	0.19 $\pm$ 0.10	0.91 $\pm$ 0.36	0.001	0.15	11	3	97	Fluka
9 2-Methyl-3-butenic acid	100	0.36 $\pm$ 0.08	0.96 $\pm$ 0.13	0.001	0.98	2.5	3	95	Fluka
10 n-Valeric acid	102	0.48 $\pm$ 0.06	0.82 $\pm$ 0.08	0.001	0.30	2.0	1	99	Aldrich
		0.41 $\pm$ 0.23	0.70 $\pm$ 0.12	0.02	0.44	9.0	3		
11 Isovaleric acid	102	0.35 $\pm$ 0.16	0.93 $\pm$ 0.08	0.001	0.42	2.0	1	98	Fluka
12 2-Methylbutyric acid	102	0.51 $\pm$ 0.16	1.04 $\pm$ 0.12	0.001	0.60	2.0	1	98	Aldrich
		0.12 $\pm$ 0.09	0.88 $\pm$ 0.27	0.001	0.70	4.5	3		
13 Trimethylacetic acid	102	0.64 $\pm$ 0.22	0.97 $\pm$ 0.12	0.005	0.40	0.60	1	99	Aldrich
14 Cyclopentanecarboxylic acid	114	0.37 $\pm$ 0.16	0.89 $\pm$ 0.09	0.001	0.37	2.5	1	99	Aldrich
15 n-Hexanoic acid	116	0.55 $\pm$ 0.10	0.94 $\pm$ 0.18	0.001	0.58	12	1	99	Aldrich
16 3-Methylvaleric acid	116	0.60 $\pm$ 0.12	1.01 $\pm$ 0.11	0.001	1.01	4.0	1	97	Aldrich
17 t-Butylacetic acid	116	0.51 $\pm$ 0.19	1.06 $\pm$ 0.10	0.001	0.31	6.0	1	98	Aldrich
18 Cyclohexanecarboxylic acid	128	0.50 $\pm$ 0.09	0.91 $\pm$ 0.10	0.001	0.33	2.4	3	99	Sigma
19 n-Heptanoic acid	130	0.78 $\pm$ 0.12	1.08 $\pm$ 0.16	0.005	0.52	6.0	1	96	Aldrich
20 Cycloheptanecarboxylic acid	142	0.41 $\pm$ 0.07	0.91 $\pm$ 0.16	0.001	0.55	2.1	3	99	Aldrich
21 n-Octanoic acid	144	0.74 $\pm$ 0.27	1.19 $\pm$ 0.26	0.02	0.29	12	1	99.5	Aldrich
Aldehydes									
22 n-Butyraldehyde	72	0.73 $\pm$ 0.16	1.05 $\pm$ 0.23	0.02	0.47	0.04	1	99	Aldrich
23 Isobutyraldehyde	72	0.67 $\pm$ 0.14	0.98 $\pm$ 0.16	0.005	0.53	0.04	1	99	Aldrich
24 trans-2-Methylbut-2-enal	84	0.55 $\pm$ 0.13	0.94 $\pm$ 0.06	0.001	0.74	0.006	3	97	Fluka
25 n-Valeraldehyde	86	0.87 $\pm$ 0.11	0.91 $\pm$ 0.08	n.s.	0.81	0.07	1	99	Aldrich
26 Isovaleraldehyde	86	0.60 $\pm$ 0.21	0.94 $\pm$ 0.07	0.005	0.43	0.02	1	98	Fluka
27 2-Methylbutyraldehyde	86	0.74 $\pm$ 0.20	0.99 $\pm$ 0.20	0.05	0.79	0.07	1	98	Aldrich
		0.36 $\pm$ 0.13	0.96 $\pm$ 0.08	0.001	0.89	0.006	3		
28 Trimethylacetaldehyde	86	0.67 $\pm$ 0.13	0.95 $\pm$ 0.09	0.001	0.48	0.15	1	99	Fluka
29 n-Hexaldehyde	100	0.93 $\pm$ 0.07	1.00 $\pm$ 0.03	0.05	1.36	0.07	1	99	Aldrich
30 n-Heptaldehyde	114	0.96 $\pm$ 0.03	1.02 $\pm$ 0.04	0.01	1.16	0.12	1	95	Aldrich
31 n-Decylaldehyde	156	0.81 $\pm$ 0.15	1.04 $\pm$ 0.15	0.02	0.66	0.30	1	98	Fluka
Alcohols									
32 Propan-1-ol	60	0.93 $\pm$ 0.18	0.96 $\pm$ 0.09	n.s.	0.91	0.06	1	99.5	Bioscience Resource
33 Butan-1-ol	74	0.93 $\pm$ 0.17	1.03 $\pm$ 0.15	n.s.	1.03	0.12	1	99.5	Bioscience Resource
34 Isobutanol	74	0.99 $\pm$ 0.21	0.98 $\pm$ 0.14	n.s.	0.96	0.15	1	99	Aldrich
35 t-Butanol	74	0.85 $\pm$ 0.09	0.97 $\pm$ 0.07	0.02	1.21	0.2	1	99.5	Aldrich
36 Butan-2-ol	74	0.79 $\pm$ 0.12	0.92 $\pm$ 0.05	0.02	0.69	0.03	1	99	Bioscience Resource
37 Cyclopentanol	86	0.85 $\pm$ 0.05	0.92 $\pm$ 0.06	0.05	0.69	0.06	1	99	Aldrich
38 3-Methylbut-2-en-1-ol	86	0.92 $\pm$ 0.16	0.87 $\pm$ 0.30	n.s.	0.67	0.011	3	99	Aldrich
39 3-Methylbut-3-en-1-ol	86	0.91 $\pm$ 0.21	0.78 $\pm$ 0.33	n.s.	0.78	0.013	3	97	Aldrich
40 n-Pentanol	88	0.96 $\pm$ 0.09	0.84 $\pm$ 0.13	n.s.	0.87	0.04	1	99	Aldrich
41 S-(−)-2-Methylbutan-1-ol	88	0.57 $\pm$ 0.13	0.86 $\pm$ 0.19	0.01	0.54	0.0017	3	99.5	Fluka
42 Isopentanol	88	0.71 $\pm$ 0.23	0.97 $\pm$ 0.11	0.05	1.08	0.1	1	99	Sigma
		0.70 $\pm$ 0.21	0.79 $\pm$ 0.23	n.s.	0.43	0.0015	3		
43 Pentan-2-ol	88	0.73 $\pm$ 0.18	0.97 $\pm$ 0.18	0.05	0.72	0.03	1	98	Aldrich
44 Pentan-3-ol	88	0.84 $\pm$ 0.13	0.91 $\pm$ 0.07	n.s.	1.06	0.03	1	99	Aldrich
45 Hexan-1-ol	102	1.02 $\pm$ 0.22	1.00 $\pm$ 0.18	n.s.	1.20	4	1	98	Aldrich
46 Hexan-2-ol	102	0.94 $\pm$ 0.07	1.02 $\pm$ 0.08	n.s.	1.29	0.12	1	99	Aldrich
47 Hexan-3-ol	102	0.95 $\pm$ 0.10	0.97 $\pm$ 0.14	n.s.	1.39	0.4	1	97	Aldrich
Hydrocarbons									
48 n-Butane	58	0.40 $\pm$ 0.18	1.00 $\pm$ 0.11	0.001	0.59	0.14	3	99	Bioscience Resource
49 n-Pentane	72	0.41 $\pm$ 0.13	0.95 $\pm$ 0.12	0.001	0.51	0.13	3	99.9	*I

Table 1. (Cont.)

Odorant	M_r	R (mean $\pm$ 95% CI)		P	A	Concn. (%)	Position	Purity (%)	Supplier
		ConA	Control						
50 Hex-1-yne	82	0.54 $\pm$ 0.22	0.92 $\pm$ 0.17	0.01	0.60	0.02	3	99	I
51 Hex-1-ene	84	0.60 $\pm$ 0.12	0.90 $\pm$ 0.17	0.01	0.84	0.34	3	99.9	I
52 n-Hexane	86	0.71 $\pm$ 0.21	0.94 $\pm$ 0.14	0.05	0.76	4.5	3	99.7	I
		0.48 $\pm$ 0.08	0.89 $\pm$ 0.08	0.001	0.46	0.56	3	94	*Fi
53 n-Heptane	100	0.69 $\pm$ 0.08	0.92 $\pm$ 0.18	0.02	0.45	1.8	3	99.9	*I
54 n-Octane	114	0.65 $\pm$ 0.08	0.97 $\pm$ 0.09	0.001	0.56	5.7	3	88	*I
55 n-Nonane	128	0.54 $\pm$ 0.13	0.93 $\pm$ 0.11	0.001	0.42	6.6	3	99.9	*I
56 Naphthalene	128	0.92 $\pm$ 0.02	1.03 $\pm$ 0.02	0.001*	0.98	9	1	99	*B
57 n-Decane	142	0.76 $\pm$ 0.24	0.96 $\pm$ 0.11	0.01	0.17	10	3	95.8	*I
Thiols									
58 Ethanethiol	62	0.75 $\pm$ 0.15	1.06 $\pm$ 0.14	0.01	1.18	0.06	3	97	FI
59 Propan-1-thiol	76	0.65 $\pm$ 0.24	0.97 $\pm$ 0.14	0.02	1.25	0.04	3	99	*FI
60 Propan-2-thiol	76	0.87 $\pm$ 0.09	1.03 $\pm$ 0.12	0.02	1.65	0.03	3	98	FI
61 Butan-1-thiol	90	0.56 $\pm$ 0.20	0.86 $\pm$ 0.31	n.s.	1.00	0.03	3	98	*FI
62 Butan-2-thiol	90	0.46 $\pm$ 0.14	0.92 $\pm$ 0.20	0.001	1.14	0.03	3	85	*FI
63 2-Methylpropan-1-thiol	90	0.51 $\pm$ 0.12	1.10 $\pm$ 0.33	0.01	1.10	0.04	3	98	*FI
64 t-Butanethiol	90	0.55 $\pm$ 0.09	0.94 $\pm$ 0.22	0.002	0.63	0.05	1	89	*FI
65 Pentan-1-thiol	104	0.80 $\pm$ 0.13	0.90 $\pm$ 0.04	n.s.	0.89	0.04	3	99	*FI
66 2-Methylbutan-1-thiol	104	0.61 $\pm$ 0.17	0.96 $\pm$ 0.06	0.01	0.98	0.03	3	98	*A
67 3-Methylbutan-1-thiol	104	0.71 $\pm$ 0.14	0.84 $\pm$ 0.12	n.s.	0.82	0.005	3	96	*A
68 2-Methylbutan-2-thiol	104	0.50 $\pm$ 0.35	0.87 $\pm$ 0.09	0.05	1.05	0.05	3	85	*A
69 2-Furylmethanethiol	114	0.83 $\pm$ 0.10	0.93 $\pm$ 0.11	n.s.	1.53	0.05	3	98.2	*A
70 Thiophenol	110	0.78 $\pm$ 0.11	0.95 $\pm$ 0.19	n.s.	0.74	0.07	3	99	*A
71 Cyclohexanethiol	116	0.68 $\pm$ 0.07	1.03 $\pm$ 0.09	0.001	1.27	0.1	3	94	*A
72 n-Hexanethiol	118	0.80 $\pm$ 0.05	1.00 $\pm$ 0.05	0.001	1.27	0.1	3	96.4	*A
73 Benzyl mercaptan	124	0.98 $\pm$ 0.06	0.93 $\pm$ 0.10	n.s.	1.14	0.13	3	99	*A
74 n-Heptanethiol	132	0.79 $\pm$ 0.14	0.96 $\pm$ 0.08	0.02	1.51	0.14	3	95	K
75 2-Phenylethanethiol	138	1.00 $\pm$ 0.05	1.02 $\pm$ 0.09	n.s.	1.09	0.4	3	99	*A
76 n-Octanethiol	146	0.68 $\pm$ 0.15	0.98 $\pm$ 0.17	0.01	0.65	0.12	1	95.5	*FI
		0.90 $\pm$ 0.10	0.89 $\pm$ 0.06	n.s.	0.84	1.0	3		
77 3-Phenylpropanethiol	152	0.88 $\pm$ 0.06	1.05 $\pm$ 0.04	0.01	1.36	0.7	3	99.6	*A
78 n-Decanethiol	174	0.57 $\pm$ 0.15	0.78 $\pm$ 0.28	n.s.	0.64	14	3	99.6	*FI
Sulphides									
79 Dimethyl sulphide	62	0.89 $\pm$ 0.15	1.05 $\pm$ 0.13	n.s.	1.31	0.05	3	97	A
80 Tetrahydrothiophene	88	0.82 $\pm$ 0.16	1.10 $\pm$ 0.24	0.05	1.19	0.003	3	98	*A
81 Dimethyl disulphide	94	0.45 $\pm$ 0.18	0.93 $\pm$ 0.13	0.001	1.16	0.04	3	98	*A
82 Dimethyl trisulphide	126	0.89 $\pm$ 0.11	1.08 $\pm$ 0.12	0.02	1.42	0.04	3	98.6	E
83 Diethyl sulphide	90	0.83 $\pm$ 0.17	1.03 $\pm$ 0.18	n.s.	1.48	0.06	3	97.4	E
84 Methylpropyl sulphide	90	0.85 $\pm$ 0.27	1.08 $\pm$ 0.16	n.s.	1.87	0.04	3	99.4	E
85 Diethyl disulphide	122	0.86 $\pm$ 0.20	1.08 $\pm$ 0.24	n.s.	1.09	0.1	3	98.2	E
86 2,4,5-Trimethylthiazole	118	0.75 $\pm$ 0.08	0.98 $\pm$ 0.08	0.002	1.31	0.1	3	†	*P
87 Methyl phenyl sulphide	124	0.84 $\pm$ 0.05	1.07 $\pm$ 0.05	0.001	1.78	1.0	3	99	*K
88 Di- <i>n</i> -butyl sulphide	146	0.68 $\pm$ 0.06	0.90 $\pm$ 0.11	0.01	1.62	1.0	3	93	*FI
Miscellaneous odorants									
89 Isobutylnitrile	69	0.83 $\pm$ 0.07	0.91 $\pm$ 0.14	n.s.	0.63	0.07	1	99	A
90 γ -Butyrolactone	86	0.61 $\pm$ 0.07	0.81 $\pm$ 0.20	0.05	0.37	1.9	3	99.5	FI
91 Nitrobutane	103	0.83 $\pm$ 0.10	1.06 $\pm$ 0.10	0.01	1.05	0.012	3	99	FI
92 1-Bromo-2-methylpropane	137	0.80 $\pm$ 0.14	1.01 $\pm$ 0.13	0.02	0.65	0.3	1	97	A
93 n-Valeronitrile	83	0.94 $\pm$ 0.12	1.00 $\pm$ 0.08	n.s.	1.29	0.2	1	97	FI
94 n-Butyl isocyanide	83	0.97 $\pm$ 0.09	0.91 $\pm$ 0.04	n.s.	0.66	0.015	1	97	A
95 Cyclopentyl ketone	84	0.93 $\pm$ 0.09	0.99 $\pm$ 0.09	n.s.	0.83	0.04	1	99	A
96 Isoamylamine	87	1.04 $\pm$ 0.35	1.19 $\pm$ 0.27	n.s.	0.81	0.07	1	98	FI
97 t-Butyl methyl ether	88	0.79 $\pm$ 0.21	1.01 $\pm$ 0.11	0.05	0.64	0.06	1	97	A
98 Butyl isocyanate	99	0.72 $\pm$ 0.12	0.96 $\pm$ 0.26	n.s.	0.37	0.023	3	98	FI
99 Methyl isobutyrate	102	0.94 $\pm$ 0.09	0.95 $\pm$ 0.08	n.s.	0.82	0.03	1	99	A
100 Isobutyl formate	102	0.73 $\pm$ 0.13	0.94 $\pm$ 0.06	0.005	0.91	0.03	1	98	A
101 Butyl isothiocyante	115	0.93 $\pm$ 0.05	0.97 $\pm$ 0.15	n.s.	1.17	0.19	3	98	FI
102 Isoamyl nitrite	117	0.68 $\pm$ 0.08	0.93 $\pm$ 0.10	0.001	0.48	0.04	3	97	A
103 n-Butyl methyl ketone	100	0.93 $\pm$ 0.04	0.99 $\pm$ 0.04	0.05	1.25	0.03	1	99	A
104 Isobutyl methyl ketone	100	0.88 $\pm$ 0.10	0.98 $\pm$ 0.06	n.s.	0.88	0.05	1	99.5	A
105 <i>m</i> -Cresol	108	1.00 $\pm$ 0.23	0.84 $\pm$ 0.18	n.s.	0.69	4	1	99	A
106 Acetophenone	120	0.88 $\pm$ 0.08	1.02 $\pm$ 0.10	0.05	1.26	0.07	1	99	A
107 Dimethylethylpyrazine	136	0.84 $\pm$ 0.12	0.91 $\pm$ 0.08	n.s.	1.06	0.04	1	†	Al
108 Isobutylphenone	148	0.92 $\pm$ 0.12	0.95 $\pm$ 0.11	n.s.	1.12	0.2	1	97	A
109 L-Carvone	150	0.90 $\pm$ 0.05	0.97 $\pm$ 0.04	0.05	1.13	0.12	1	98	A
110 1,8-Cineole	154	0.75 $\pm$ 0.17	1.01 $\pm$ 0.10	0.01	0.90	0.15	1	99	A
111 Linalyl acetate	196	0.92 $\pm$ 0.14	0.99 $\pm$ 0.09	n.s.	0.47	0.6	1	95	FI

* Naphthalene was tested on 65 test and 65 control animals.

† Mixed isomers (3,5-dimethyl- and 3,6-dimethyl-2-ethylpyrazine).

‡ 99.6%, mixture of *cis*- and *trans*- Δ^3 .

Fig. 1. A section through a rat head showing the areas ('positions 1 and 3') from which EOGs were recorded

between pulses was 1 min. Isopentyl acetate was chosen as a reference odorant and was used on every preparation. In most experiments, seven other odours were also used with each preparation. Every fourth or fifth pulse was of the reference material. At location 1, recording continued until each odour had been applied at least three times and the resulting EOG amplitudes

were stable. At location 3, each odour was applied twice.

The tissue was then superfused with Ringer solution containing 0.5 mg of Con A/ml for 5 min, and then with Ringer solution for 10 min. The liquid was aspirated away and the odour stimuli and recording were repeated. In the control experiments, this superfusion was with Ringer alone for 15 min. Parts of typical traces are shown in Fig. 2.

Acetic acid and propionic acid and the butyraldehydes proved too adaptive for the above procedure, and an alternative protocol was adopted. After the initial wash, the tissue was stimulated with isopentyl acetate and naphthalene until EOGs of constant amplitude were obtained. The tissue then received a single stimulus of the 'difficult' odour. The tissue was then washed with Ringer solution and the process repeated. After three or four such cycles the tissue was treated with Con A as described above and the recording procedure repeated.

In total, each odour was applied to five control and five Con A-treated animals, with the exceptions of isopentyl acetate (applied to all preparations) and naphthalene, which was used with 65 control and 65 Con A-treated animals. Naphthalene was used on many preparations to detect any excessive animal-to-animal variation.

Odorant concentrations

The concentrations of the odorants are given in Table 1. But it is necessary to make a few comments about concentration. Our olfactometer delivers 250 ml of

Fig. 2. Parts of EOG recordings from position 3 (see Fig. 1)

These traces have been reconstituted from digitized data and show a series of EOGs before (i) and after (ii) Con A treatment (a) and before (i) and after (ii) control (Ringer solution) treatment (b).

Table 2. Inhibition of Con A-induced EOG diminutions by methyl mannoside

The experiment was carried out as described in the Materials and methods section, except for the inclusion of 20 mM- α -methyl mannoside where indicated.

Odorant	Ringers solution . . . Con A . . . Methyl mannoside . . .	R value (mean $\pm$ 95% CI)			
		+	+	+	+
Cyclopentanecarboxylic acid		0.89 $\pm$ 0.09	0.37 $\pm$ 0.16	0.78 $\pm$ 0.19	0.79 $\pm$ 0.14
Isovaleric acid		0.93 $\pm$ 0.08	0.35 $\pm$ 0.19	1.04 $\pm$ 0.25	0.92 $\pm$ 0.11
Isovaleraldehyde		0.94 $\pm$ 0.07	0.60 $\pm$ 0.21	0.90 $\pm$ 0.14	0.88 $\pm$ 0.14
Isoamyl alcohol		0.97 $\pm$ 0.11	0.71 $\pm$ 0.23	0.99 $\pm$ 0.23	0.92 $\pm$ 0.21
Octan-1-thiol		0.98 $\pm$ 0.17	0.68 $\pm$ 0.15	0.97 $\pm$ 0.09	0.94 $\pm$ 0.14
1,8-Cineole		1.01 $\pm$ 0.10	0.75 $\pm$ 0.17	0.98 $\pm$ 0.08	0.99 $\pm$ 0.12
Naphthalene		1.03 $\pm$ 0.02	0.92 $\pm$ 0.02	1.00 $\pm$ 0.15	0.99 $\pm$ 0.04

air/min, and this impinges on an area of olfactory surface of about 3×5 mm. In the rat the thickness of the olfactory mucus is at least $5 \mu\text{m}$, and so the odorized area has a volume of at least $0.075 \mu\text{l}$. Therefore in 1 s a maximum of 50000 vol. of air pass over 1 vol. of mucus.

When the air is odorized, the concentration of odour in the mucus starts to rise. If there were perfect transfer of odour between air and mucus then equilibrium would be approached with an exponential time constant of $K/50000$ s, where K is the mucus/air partition coefficient of the odour. In practice, the rise time will be somewhat longer. The carboxylic acids used in the present study have water/air partition coefficients of the order of 10000000 (at neutral pH). The mucus concentrations of these substances will therefore be very much less than the equilibrium value during a 1 s stimulation. The profile of the stimulus concentration for the acids will be a ramp rather than a rectangular pulse.

Most of the other odorants used in the present study have partition coefficients very much less than 10000, so the mucus concentration of these substances should be of the order of the equilibrium value, and the stimulus shape should approximate to a pulse. The alcohols have partition coefficients of 1000–5000 and should show intermediate behaviour.

These intrinsic differences in pulse shape arising from the physical chemistry of the odorants should be kept in mind when discussing the physiological response to the various classes of odorants. Odour concentration in the mucus and pulse-shape distortion are discussed elsewhere (Shirley, 1987).

Definition of symbols

I is used to denote the mean amplitude of the EOG (in mV); subscripts i and f refer to the initial (pre-Con A) and final (post-Con A) recording periods respectively. Further subscripts refer to a particular odour.

$L (= I_f/I_i)$ is used to denote the survival of the signal; subscripts (where used) refer to odorant and reagent.

The absolute amplitude of the EOG to any given stimulus is variable between rats; it can also vary in an individual animal. Variations in the shape and size of the turbinates and, particularly, variations in mucus thickness, all affect the electrical resistance and the pattern of current flow and hence the size of the observed potential. Consequences of these effects can in large part be

removed by stimulating the tissue with a reference odour (in this case isopentyl acetate). Dividing the amplitude of the odour signal by that of the pentyl acetate signal yields a quantity (A) which is more nearly constant between animals.

Changes induced by the Con A are expressed in two ways:

$$\Delta A = A_i - A_f$$

$$R = A_f/A_i$$

These symbols have been used to maintain consistency with a previous, and the accompanying, paper (Shirley *et al.*, 1983a, 1987). In the present study, the survival of the isopentyl acetate signal was high, so R can be simply interpreted as a direct measure of EOG survival.

RESULTS

Stability of preparations

The rat olfactory preparation *in vitro* is sufficiently stable for biochemical studies (Shirley *et al.*, 1983a). Life-times of 3–5 h are typical. The experiments described here are much shorter than this, typically 1.5 h.

At location 1, after treatment with Con A, about 40% of preparations showed elevated EOG amplitudes, which then declined with time. This decline seemed general, affecting all odours equally and so leaving R unaffected. For the few preparations which showed a signal loss of 30% or more over the recording period, the results were discarded. However, Con A seems to have a general destabilizing effect on the preparation.

At location 3 the instabilities were more severe. We also observed other differences between the locations.

Firstly, at position 3, the survival of the isopentyl acetate signal was lower and more variable, namely 0.80 ± 0.28 and 0.94 ± 0.35 (mean $\pm$ s.d.) for the Con A and control treatments respectively. This compares with values of 1.00 ± 0.20 and 1.01 ± 0.20 for position 1. Between locations the differences in variance are significant ($P < 0.05$ for Con A and $P < 0.01$ for the controls). For Con A treatment, the mean value at position 3 is significantly lower than that at position 1 ($P < 0.001$).

Secondly, the value of R for any odorant during the control treatment should be 1.00. The mean of this

Table 3. Con A-induced diminution of EOG amplitude

The means and 95% confidence intervals of the Con A-induced reduction are shown. Each entry represents replicate determinations on five animals, except for naphthalene (65 animals). Students *t* test was used to generate the *P* values (n/s, not significant).

(a) Values of ΔA recorded at position 1			
Compound	ΔA		<i>P</i>
	Con A	Control	
3-Methylvaleric acid	0.39 ± 0.15	-0.01 ± 0.12	0.001
3-Methylbutyric acid	0.31 ± 0.07	0.03 ± 0.03	0.001
3-Methylbutanol	0.30 ± 0.21	0.04 ± 0.12	0.02
Cyclobutanecarboxylic acid	0.30 ± 0.07	0.04 ± 0.05	0.001
t-Butanethiol	0.29 ± 0.09	0.05 ± 0.12	0.002
Hexanoic acid	0.27 ± 0.06	0.03 ± 0.11	0.001
2-Methylbutyric acid	0.27 ± 0.05	-0.03 ± 0.08	0.001
1,8-Cineole	0.25 ± 0.19	0.00 ± 0.09	0.02
Methacrylic acid	0.24 ± 0.12	-0.01 ± 0.13	0.01
Pentan-2-ol	0.24 ± 0.11	0.06 ± 0.16	0.05
Isobutyl formate	0.23 ± 0.10	0.05 ± 0.06	0.01
2-Methylbutanal	0.21 ± 0.18	0.03 ± 0.15	n/s
Cyclopentanecarboxylic acid	0.20 ± 0.08	0.05 ± 0.05	0.01
t-Butanol	0.19 ± 0.11	0.03 ± 0.09	0.02
Isobutanol	0.19 ± 0.07	0.01 ± 0.07	0.002
Octanethiol	0.18 ± 0.10	0.04 ± 0.12	0.05
Pivalic aldehyde	0.17 ± 0.08	0.03 ± 0.06	0.01
t-Butylacetic acid	0.17 ± 0.09	-0.01 ± 0.03	0.001
Dimethylethylpyrazine	0.16 ± 0.06	0.10 ± 0.10	n/s
Acetophenone	0.16 ± 0.12	0.00 ± 0.11	0.02
Pentanoic acid	0.15 ± 0.06	0.06 ± 0.05	0.01
Isobutyric acid	0.15 ± 0.08	-0.05 ± 0.04	0.001
Pentan-3-ol	0.15 ± 0.12	0.12 ± 0.10	n/s
3-Methylbutanal	0.14 ± 0.06	0.03 ± 0.03	0.002
Butanal	0.14 ± 0.09	0.00 ± 0.07	0.01
Decanal	0.14 ± 0.12	-0.02 ± 0.09	0.02
2-Methylbromopropane	0.13 ± 0.10	-0.01 ± 0.07	0.02
t-Butyl methyl ether	0.13 ± 0.14	-0.02 ± 0.07	0.05
Pivalic acid	0.13 ± 0.10	0.01 ± 0.05	0.01
Hexan-3-ol	0.12 ± 0.15	0.03 ± 0.19	n/s
Butyric acid	0.12 ± 0.07	0.00 ± 0.02	0.01
Pentanal	0.12 ± 0.10	0.07 ± 0.07	n/s
Heptanoic acid	0.11 ± 0.09	-0.04 ± 0.07	0.01
Butan-2-ol	0.11 ± 0.05	0.06 ± 0.03	0.05
L-Carvone	0.11 ± 0.06	0.03 ± 0.06	0.05
Isobutylnitrile	0.11 ± 0.08	0.07 ± 0.11	n/s
Isobutylphenone	0.11 ± 0.14	0.05 ± 0.11	n/s
Isobutyl methyl ketone	0.11 ± 0.08	0.02 ± 0.06	0.05
Butanol	0.10 ± 0.16	-0.01 ± 0.12	n/s
Hexanal	0.10 ± 0.11	0.00 ± 0.04	0.05
Acetic acid	0.09 ± 0.08	-0.02 ± 0.09	0.05
Butyl methyl ketone	0.09 ± 0.06	0.02 ± 0.05	0.05
Cyclopentanol	0.09 ± 0.05	0.06 ± 0.06	n/s
Valeronitrile	0.09 ± 0.14	-0.01 ± 0.10	n/s
Naphthalene	0.09 ± 0.02	-0.02 ± 0.02	0.001
Hexan-2-ol	0.08 ± 0.10	-0.02 ± 0.11	n/s
Propanol	0.08 ± 0.19	0.04 ± 0.09	n/s
Octanoic acid	0.07 ± 0.05	-0.04 ± 0.06	0.01
Methyl isobutyrate	0.05 ± 0.07	0.05 ± 0.06	n/s
Heptanal	0.05 ± 0.03	-0.03 ± 0.05	0.01
Pentanol	0.05 ± 0.10	0.13 ± 0.11	n/s
Cyclopentyl ketone	0.05 ± 0.06	0.02 ± 0.08	n/s
Propionic acid	0.04 ± 0.04	0.02 ± 0.03	n/s
Linalyl acetate	0.04 ± 0.07	0.00 ± 0.03	n/s
Isobutanol	0.04 ± 0.15	0.04 ± 0.15	n/s
Butyl isocyanide	0.02 ± 0.06	0.05 ± 0.02	n/s
Isoamylamine	0.01 ± 0.24	-0.09 ± 0.07	n/s
m-Cresol	0.01 ± 0.12	0.12 ± 0.15	n/s
Hexanol	-0.02 ± 0.25	0.01 ± 0.21	n/s

Table 3. (Cont.)

(b) Values of ΔA recorded at position 3			
Compound	ΔA		<i>P</i>
	Con A	Control	
2-Methylbut-3-enoic acid	0.67 ± 0.24	0.04 ± 0.12	0.001
2-Methylbutyric acid	0.66 ± 0.19	0.07 ± 0.18	0.001
Isobutanethiol	0.63 ± 0.20	0.05 ± 0.15	0.001
Butane-2-thiol	0.62 ± 0.16	0.07 ± 0.21	0.001
Dimethyl disulphide	0.60 ± 0.21	0.11 ± 0.17	0.002
2-Methylbutanal	0.57 ± 0.18	0.04 ± 0.07	0.001
t-Butyl sulphide	0.53 ± 0.15	0.12 ± 0.24	0.01
2-Methylbutane-2-thiol	0.48 ± 0.34	0.14 ± 0.09	0.05
Butanethiol	0.44 ± 0.21	0.12 ± 0.29	0.05
Cyclohexanethiol	0.44 ± 0.08	−0.04 ± 0.10	0.001
Propanethiol	0.44 ± 0.29	0.05 ± 0.16	0.02
Butane	0.39 ± 0.19	0.00 ± 0.07	0.001
2-Methylbutane-1-thiol	0.38 ± 0.23	0.04 ± 0.06	0.01
trans-2-Methylbuten-2-al	0.33 ± 0.16	0.05 ± 0.06	0.002
Cycloheptanecarboxylic acid	0.33 ± 0.04	0.04 ± 0.08	0.001
Hex-1-ene	0.33 ± 0.14	0.09 ± 0.15	0.02
Trimethylthiazoline	0.32 ± 0.10	0.03 ± 0.12	0.001
Decanethiol	0.30 ± 0.08	0.20 ± 0.21	n/s
Pentanoic acid	0.30 ± 0.19	0.13 ± 0.13	n/s
Furfuryl mercaptan	0.29 ± 0.17	0.05 ± 0.15	0.02
Thioanisole	0.29 ± 0.12	−0.12 ± 0.10	0.001
Hex-1-yne	0.28 ± 0.19	0.04 ± 0.11	0.02
Hexane	0.28 ± 0.17	0.05 ± 0.07	0.01
2-Methylbutan-1-ol	0.27 ± 0.23	0.16 ± 0.18	n/s
Hexanethiol	0.26 ± 0.08	0.00 ± 0.06	0.001
Pentane	0.26 ± 0.12	0.04 ± 0.08	0.005
3-Methylbutane-1-thiol	0.24 ± 0.17	0.13 ± 0.10	n/s
Thiophane	0.24 ± 0.24	−0.06 ± 0.21	0.05
Cyclohexanecarboxylic acid	0.23 ± 0.15	0.02 ± 0.02	0.01
Nitrobutane	0.20 ± 0.12	−0.06 ± 0.10	0.002
Octane	0.19 ± 0.07	0.02 ± 0.06	0.001
Pentanethiol	0.19 ± 0.16	0.09 ± 0.05	n/s
Thiophenol	0.18 ± 0.09	0.03 ± 0.04	0.05
Nonane	0.17 ± 0.09	0.05 ± 0.06	0.02
3-Methylbutanol	0.17 ± 0.19	0.18 ± 0.20	n/s
3-Methylbutyl nitrite	0.17 ± 0.06	0.04 ± 0.05	0.002
Pent-4-ynoic acid	0.16 ± 0.18	0.06 ± 0.05	n/s
3-Phenylpropanethiol	0.16 ± 0.09	−0.06 ± 0.13	0.01
γ-Butyrolactone	0.15 ± 0.03	0.06 ± 0.07	0.01
2-Methylbut-2-enoic acid	0.14 ± 0.06	0.02 ± 0.04	0.001
Heptane	0.13 ± 0.06	0.04 ± 0.12	n/s
Butyl isocyanate	0.12 ± 0.06	0.01 ± 0.07	0.01
3-Methylbut-3-enoic acid	0.12 ± 0.27	0.21 ± 0.30	n/s
Benzyl mercaptan	0.11 ± 0.09	0.08 ± 0.12	n/s
Butyl isothiocyanate	0.07 ± 0.06	0.07 ± 0.26	n/s
3-Methylbut-2-en-1-ol	0.06 ± 0.13	0.15 ± 0.32	n/s
Decane	0.03 ± 0.05	0.01 ± 0.02	n/s
2-Phenylethanethiol	0.00 ± 0.05	−0.02 ± 0.10	n/s

variable at position 1 was 0.97 ± 0.07 (S.D.); at position 3 it was significantly lower, namely 0.94 ± 0.08 ($P < 0.05$).

Thirdly, there is poor agreement between *R* values recorded for the same odorant at the two different locations. Five compounds were tested at both locations (although not necessarily at the same concentration), and the correlation coefficient between the resulting *R* values is 0.54, which is non-significant.

It is possible that there may be a real difference between the two locations. In addition, the change in

experimental protocol which accompanied the change in recording location may be partially responsible. The two sets of data are not, therefore, directly comparable.

Con A-induced changes

It can be seen from Table 1 that many odorants gave a significantly lower *R* value after Con A than after the corresponding Ringer-solution treatment. In all, 19 out of 21 acids, 9 out of 10 aldehydes, 6 out of 16 alcohols, 8 out of 10 hydrocarbons, 13 out of 21 thiols, 6 of the 10 sulphides and 8 of the 20 miscellaneous compounds

showed this behaviour. The same breadth of effect can be seen in Table 3, which lists the values of ΔA .

There seems to be a pattern in the data which relates to the hydrocarbon portion of the molecule (see Fig. 5 below and the Discussion section).

Inhibition of effects by α -methyl mannoside

α -Methyl mannoside can exhibit, and sometimes reverse, the binding of Con A via its sugar-specific site (Goldstein & Hayes, 1978). The diminution in the EOGs caused by Con A can be inhibited by including 20 mM- α -methyl mannoside in the superfusion medium (see Table 2). This concentration of mannoside, in the absence of Con A, does not affect the EOGs.

However, once the EOGs had been inhibited by Con A, we were unable to reverse the effect. After Con A treatment, the application of methyl mannoside in Ringer solution (20–100 mM; 5–30 min) caused only small and unreproducible changes in R .

DISCUSSION

General considerations

The number of different types of receptor molecules in the mammalian olfactory system is unknown. Various authors have postulated different numbers, ranging from seven (Amoore, 1970) upwards. Electrophysiological recordings from individual olfactory receptor cells have been interpreted to suggest that each cell carries many different kinds of receptor molecule (Holley, 1974). If this is true, then obtaining structure-activity relationships from chemical-modification data may not be straightforward.

It is necessary to discuss briefly the general problem of the interpretation of chemical-modification data ob-

tained from the olfactory system, as no previous authors seem to have addressed this point.

If a reagent acts on a receptor molecule as an antagonist, then changes in the observed sensitivity of the tissue should have their origin in the odour-response spectrum(a) of the affected type(s) of molecule. However, if the reagent has an agonistic effect on the receptor molecule, then the receptor cell will be permanently stimulated and show diminished (or abolished) response to odorants binding at other receptor sites on the same cell (the effect would be analogous to cross-adaptation). In this case the changes in sensitivity of the tissue have their origin in the response spectra of the affected cells. It is also possible that a reagent acting remotely from the receptor molecules could produce either of these effects.

We have no independent lines of evidence concerning the mode of action of Con A. However, examination of the data presented here suggests that we are observing events at the molecular level. Of course, if each olfactory cell carries only one kind of receptor molecule, then we are observing at both levels of the system.

There is a second problem in interpreting chemical-modification data in a multi-receptor system. We must assume that the EOG can be regarded as a signal which is composed of components each of which originate from a single type of receptor molecule. In a complex and non-linear system, this assumption is almost certainly untrue. However, it may serve as a workable approximation.

Fig. 3 shows that there may be some merit in the idea. Odorants with Con A-resistant EOGs tend to have EOGs which are particularly susceptible to the thiol reagent mersalyl. If the number of receptor-molecule types is fairly small and no receptor is attached by both Con A and mersalyl, then the phenomenon is explained. Before the tissue is treated, components of the EOG will arise from the Con A-sensitive receptors, from the mersalyl-sensitive receptors and from the receptors sensitive to neither reagent. An odorant which favours the first group will survive Con A treatment poorly and mersalyl treatment well; one which favours the second will show the opposite behaviour. The explanation of the phenomenon becomes very much harder if we accept a model that does not allow the association of a certain component of the EOG with a certain class of receptor.

We are now in a position to assign meaning to the parameters measured in this experiment. The following five conditions describe the simplest possible case: (1) there is no non-specific damage to the olfactory mucosa; (2) the reagent is antagonistic; (3) the reagent disables only one type of receptor molecule; (4) the reagent disables the type completely; (5) components of the EOG arise from each type of receptor separately and are additive.

ΔA can then be identified with the component of the EOG which was produced by the affected receptor. It will vary with the following factors: (1) the density of the receptor sites in the vicinity of the electrode; (2) the concentration of the odorant; (3) the chemical nature of the odorant.

Ideally, all odorants should be presented so that their concentrations in the mucus are the same. Since this condition was not applied (and for some odorants could not be applied), we hope that the structure-activity relationships are strong enough not to be masked by this experimental imperfection.

Fig. 3. Plot of the mean survival (L) of the EOGs of various odorants after treatment with Con A as against the mean survival after treatment with mersalyl

The Con A values are taken from the present paper, whereas the mersalyl values are taken from a previous study (Shirley *et al.*, 1983a). The odorants are: 1, isopentyl acetate; 2, m-cresol; 3, L-carvone; 4, 1,8-cineole; 5, naphthalene; 6, dimethylethylpyrazine; 7, decanal; 8, octanethiol; and 9, isopentanoic acid. All data were obtained from recording position 1 (see Fig. 1).

On the other hand, R (strictly $1 - R$) indicates what fraction of the original signal came from the affected receptor.

Essentially ΔA should indicate how good a stimulus an odorant is for the receptor and R should indicate the relative importance of the receptor in the recognition of the odour. These are different concepts. An odorant which is primary (in the sense used by Amoore, 1970) stimulates one and only one receptor. However, the primary nature of an odorant does *not* imply tight binding to, or strong stimulation of, the receptor.

Concanavalin A effects

The first feature of interest in the data (Tables 1 and 3) is the broad specificity of the receptor(s) interrogated. Con A treatment significantly diminished the signal from 37 of the 59 odorants in the first group; 33 of the 48 odorants in the second group were also affected.

Since we expected ΔA to vary with the density of the receptor sites near the electrode, it is not surprising that the absolute values obtained at location 1 and location 3 differ (Table 3).

If the odorants in the first set (position 1) are arranged by functional group, the mean values of ΔA are: acids, 0.19 ($n = 16$); aldehydes, 0.14 ($n = 9$); alcohols, 0.12 ($n = 13$); miscellaneous compounds, 0.12 ($n = 21$). Statistically this is just significant ($F = 2.78$, with 3/55 d.f., $P < 0.05$). However, it is not possible to estimate the liquid-phase concentrations of the acids with any certainty (see the Materials and methods section). In view of this and the small difference in ΔA , it is not possible to conclude that an acid-specific receptor exists. The analogous grouping of the odorants in the second set (position 3) yields a non-significant result ($F = 1.33$ with 4/33 d.f.). The means for the groups are: acids, 0.36 (7); thiols, 0.32 (16); alcohols, 0.16 (4); hydrocarbons, 0.23 (9); others, 0.30 (12).

There does seem to be a dependence of ΔA on the hydrocarbon portion of the odorant. We use a simple and crude classification of the odorants which produces three roughly equal groups: (1) compounds of four, five

Fig. 4. Con A-induced reduction of the EOG (ΔA) plotted against molar volume of the odorant

Fig. 5. Variation of R with chain length for various homologous series of straight-chain saturated aliphatic compounds

R is the fraction of an odorant's EOG which survives Con A treatment divided by the fraction of the isopentyl acetate EOG which survives Con A treatment. In the 'Alcohols' panel: $\circ$, 1-ol; $\bullet$, 2-ol.

or six carbon atoms arranged in a single straight aliphatic chain; (2) compounds of four, five or six carbon atoms arranged in a single branched aliphatic chain; (3) all other compounds.

The mean values for ΔA for the first set of odorants are: straight, 0.12 (17); branched, 0.19 (18); others, 0.12 (24). The classification is significant ($F = 5.44$ with 2/56 d.f., $P < 0.01$). Compounds containing the but-2-ene grouping seem to be an exception to the rule. If these are omitted from the second set of data then the classification is still significant, with group means as follows: straight, 0.29 (14); branched, 0.41 (12); others, 0.24 (19) ($F = 3.92$ with 2/42 d.f., $P < 0.05$).

The scatter plot of ΔA against molecular volume (Fig. 4) suggests that molecular size may also be a determinant of the response of the receptor(s). The greatest diminutions in EOG amplitude seem to cluster in the range 80–130 ml/mol, and this could indicate a receptor which responds to odorants of that size.

We can find traces of structure–activity relationships in the data. The pattern is reminiscent of that obtained from the hydrophobic binding of small ligands to proteins (Wishnia & Pinder, 1966; Robillard & Wishnia, 1972; Reynolds *et al.*, 1968). It is unlike the pattern normally generated by a neurotransmitter–receptor interaction or an enzyme–substrate interaction.

Plots of R against carbon number tend to show minima in the region of four to five carbon atoms (Fig. 5). Compounds with the isopentyl skeleton show a lower value of R than do their n-pentyl analogues (paired test; $t = 6.64$, $n = 4$, $P < 0.01$). As far as the carboxylic acids are concerned, this is in accord with the findings of Amoore (1967). Amoore (1967) attributed the phenomenon of specific anosmia (the inability of certain people to detect certain compounds, in particular isovaleric acid) to a missing or altered receptor protein.

The results of the methyl mannoside experiments suggest that Con A is binding via its sugar-specific site to the carbohydrate portion of a glycoprotein. Glycoproteins are prominent in preparations of olfactory membranes (Chen & Lancet, 1984; Chen *et al.*, 1986).

We conclude that Con A inactivates one or more types of receptor molecule (or cell), among which is a receptor particularly important for the detection of C_4 – C_6 alkyl acids, thiols and hydrocarbons. However, the receptor is by no means specific for this class of compound, inasmuch as it responds to most short-chain alkyl compounds.

The results are compatible with the notion that an odorant interacts with several types of receptor molecule (Polak, 1973) and that an odour is recognized by the relative degree of stimulation.

If this is so, the effect of Con A will change with odorant concentration. This is examined in the accompanying paper (Shirley *et al.*, 1987).

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REFERENCES

- Amoore, J. E. (1967) *Nature* (London) **214**, 1095–1098
 Amoore, J. E. (1970) *The Molecular Basis of Odour*, Thomas, Springfield, U.S.A.
- Bittiger, H. & Schnebli, H. (eds.) (1976) *Concanavalin A as a Tool*, John Wiley and Sons, London and New York
 Cagan, R. & Kare, M. (eds.) (1981) *Biochemistry of Taste and Olfaction*, Academic Press, New York
 Chen, Z. & Lancet, D. (1984) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 1859–1863
 Chen, Z., Pace, U., Ronen, D. & Lancet, D. (1986) *J. Biol. Chem.* **261**, 1299–1305
 Gesteland, R. C. (1975) in *Methods in Olfactory Research* (Moulton D. G., Turk, A. & Johnston, Jr., J. W., eds.), pp. 269–323, Academic Press, London, New York and San Francisco
 Goldstein, I. J. & Hayes, C. E. (1978) *Adv. Carbohydr. Chem.* **35**, 127–340
 Holley, A. (1974) in *Transduction Mechanisms in Chemoreception* (Poynder, T. M., ed.), pp. 275–306, IRL Press, London
 Menevse, A., Dodd, G. H., Poynder, T. & Squirrell, D. (1977) *Biochem. Soc. Trans.* **5**, 191–194
 Menevse, A., Dodds, G. H. & Poynder, T. (1978) *Biochem. J.* **176**, 845–854
 Persaud, K., Wodd, P. & Dodd, G. H. (1980) *Olfaction Taste, Proc. Int. Symp.* 7th, p. 98
 Polak, E. (1973) *J. Theor. Biol.* **40**, 469–484
 Polak, E., Shirley, S. G. & Dodd, G. H. (1982) *Eur. Chemorecept. Res. Organ. Congr.* 5th, Regensburg, 59 (abstr.)
 Revial, M. F., Sicard, G., Duchamp, A. & Holley, A. (1983) *Chem. Senses* **8**, 179–194
 Reynolds, J., Herbert, S. & Steinhardt, J. (1968) *Biochemistry* **7**, 1357–1361
 Robillard, K. A., Jr. & Wishnia, A. (1972) *Biochemistry* **11**, 3835–3840
 Shirley, S. G. (1987) *Chem. Senses*, in the press
 Shirley, S. G., Polak, E. & Dodd, G. H. (1981) *Biochem. Soc. Trans.* **9**, 108–109
 Shirley, S. G., Polak, E. & Dodd, G. H. (1983a) *Eur. J. Biochem.* **132**, 485–494
 Shirley, S. G., Polak, E. & Dodd, G. H. (1983b) *Biochem. Soc. Trans.* **11**, 780–781
 Shirley, S. G., Polak, E. H., Edwards, D. A., Wood, M. A. & Dodd, G. H. (1987) *Biochem. J.* **245**, 185–189
 Wishnia, A. & Pinder, T. W., Jr. (1966) *Biochemistry* **5**, 1534–1542
 Wood, P. & Dodd, G. H. (1982) *Assoc. Chemorecept. Sci. Meet.* 4th, Sarasota, 29 (abstr.)

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