Biochemical studies of olfaction: Binding specificity of radioactively labeled stimuli to an isolated olfactory preparation from rainbow trout (*Salmo gairdneri*)

(binding sites/olfactory receptors/olfactory specificity/amino acids)

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ABSTRACT   The extent of binding of 10 radioactively labeled odorant amino acids to a sedimentable fraction (fraction P2) derived from the olfactory rosettes of the rainbow trout *Salmo gairdneri* corresponded closely with their reported relative stimulatory effectiveness measured electrophysiologically. L isomers were bound to a greater extent than their respective D isomers. Binding of L-alanine was strongly and irreversibly inhibited by mercurials but was not affected by sulfhydryl-blocking reagents. Binding was saturable and reversible. Scatchard analyses gave evidence of two types of binding sites for most of the amino acids studied. The K_d values of the higher-affinity binding sites were similar among the amino acids, being in the range of 10^{-6} M; differences occurred in the relative numbers of sites, n. These results, coupled with those from competition experiments, lead to the postulate that a multiplicity of types of olfactory binding sites exist in the trout: site TSA, which binds L-threonine, L-serine, and L-alanine; site L, which binds L-lysine; and site A_B which binds β-alanine. Tentative assignments are: site V, which binds L-valine; site H, which binds L-histidine; and site A_D, which binds D-alanine. Site A_D may be a lower affinity site for L-alanine. Binding of olfactory stimulus molecules appears to be an initial discrimination step in olfaction.

The olfactory system is remarkable in its responsiveness to a wide range of molecules. Despite a florid history of hypotheses (1, 2) as to the underlying mechanisms, virtually nothing is known about the biochemical basis of the specificity of interaction between an olfactory stimulus molecule and an olfactory receptor. Research on olfactory perception in human subjects frequently has used structure–activity correlations (2) and cross-adaptation approaches (3) in attempts to infer the molecular features of the receptor sites. Electrophysiological measurements in animals, especially the electro-olfactogram (4), record the changes in electrical parameters of the olfactory epithelium in response to stimulation with chemicals. An attempt to study the initial interaction in olfaction biochemically was reported by Ash (5), who measured changes in difference spectra that were later shown to arise from oxidation of ascorbic acid (6) and were noted to be brought about by only 7 of 28 chemicals used as representative odorants (7).

The paucity of information on the initial interaction of olfactory stimuli with receptor sites is referable largely to the unavailability, up to now, of a suitable biochemical assay for measurement of initial interactions of odorant molecules with olfactory receptor preparations. In addition, there has not been a convenient experimental animal to serve as a biochemical model for vertebrate olfaction. It is well established that salmonid fishes use olfaction, in addition to vision, in order to migrate to their home streams to mate (8). The salmonid fishes were shown (9, 10), by use of electrophysiological techniques,

to be responsive to various amino acids as olfactory stimuli. The olfactory rosettes, which contain the olfactory receptor cells, are readily accessible on the snout of the fish. It has been shown (11) by using radioactively labeled taste stimuli with a catfish taste preparation, that binding is a measure of an initial interaction in taste. That assay procedure seemed eminently suitable for studying similar events in olfaction. We have reported (12) that the trout olfactory system is a useful model for experimental biochemical studies of olfaction, and in this paper we describe the details of the binding of several odorant amino acids to a sedimentable preparation derived from rainbow trout (*Salmo gairdneri*) olfactory epithelium.

MATERIALS AND METHODS

Reagents. L-[3-³H]Alanine (5.6, 6.8, 7.0, and 13.2 Ci/mmol), L-[4-³H]proline (20 Ci/mmol), L-[2,3-³H]valine (6.0 Ci/mmol), and unlabeled L-alanine were obtained from Schwarz/Mann. β-[N-3-³H]Alanine (37.5 Ci/mmol), L-[N-3-³H]arginine (10.4 Ci/mmol), L-[3-³H]histidine (10.2 and 8.4 Ci/mmol), D-[1-¹⁴C]leucine (24.6 mCi/mmol), L-[1-¹⁴C]leucine (28.7 mCi/mmol), L-[G-³H]lysine (2.51 Ci/mmol), L-[G-³H]phenylalanine (5.32 Ci/mmol), L-[G-³H]serine (3.38 Ci/mmol), L-[G-³H]-threonine (2.09 Ci/mmol), D-[1-¹⁴C]alanine (17.9 mCi/mmol), and L-[1-¹⁴C]alanine (58.2 mCi/mmol) were obtained from New England Nuclear. The other unlabeled amino acids, N-ethylmaleimide, p-hydroxymercuribenzoate (sodium salt), and N-(2-acetamido)-2-aminoethanesulfonic acid (Aces) were purchased from Sigma. Other chemicals were of the highest purity available from commercial sources. Filters (Type HAWP, 0.45 μm) for the binding assays were obtained from the Millipore Corp.

Tissue. Trout were decapitated and the heads were transported on ice to the laboratory. The fish were approximately 24–29 cm long. Storage at −65° for up to 2 weeks was without discernible effect on the gross integrity of the tissue. The trout heads were stored frozen (−65°) for 2–6 days in most cases and sometimes for up to 2 weeks for practical reasons. Trout heads stored at −65° for various periods of time were used to prepare fraction P2 (see below), which was then assayed with L-[³H]-alanine, L-[³H]serine, L-[³H]lysine, or L-[³H]valine as the ligand. The binding activity of fraction P2 from trout heads stored for the above time periods was stable. In contrast, the binding activity of the isolated fraction P2 was rather labile, decreasing by 40% when it was stored at −65° for 7 days and by 66% if it was quick-frozen in liquid N_2 prior to storage at −65°. Binding activity decreased by 77% when fraction P2 was stored overnight at 0°.

Fractionation of Olfactory Tissue. The procedure was essentially that used with catfish taste tissue (11) as described

Abbreviation: Aces, N-(2-acetamido)-2-aminoethanesulfonic acid.
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(13–15). In a typical experiment, 100 olfactory rosettes from 50 trout heads were removed by dissection, carefully trimmed of excess tissue, and placed in a chilled beaker. The pooled tissue was washed twice with 5 ml of 0.3 M sucrose/1 mM $CaCl_2$ and once with 5 ml of the homogenizing solution (1 mM $CaCl_2$ adjusted to pH 7.5 with $NaHCO_3$). The wet weight of the tissue (lightly blotted) was then determined. Olfactory rosettes from 50 animals yielded about 600–900 mg of tissue.

The tissue was minced with a small scissors for 5 min in 2–3 ml of homogenizing medium. Then the tissue was homogenized and fractionated as described (11), except that the centrifugations were at 1100 × g for 15 min to obtain fraction P1 and supernatant S1 and at 7300 × g for 60 min to obtain fraction P2, and that the filtration through the gauze was carried out on the supernatant resulting from the centrifugation at 1100 × g. The original homogenate (H1) was ultimately fractionated into pellet P1, pellet P2, and supernatant S2. Between 2 and 3 mg of protein in fraction P2 was generally obtained from 50 animals. Pellet P2 was suspended in 0.05 M Aces buffer, pH 7.0. Protein was determined by the Lowry method (16) with human serum albumin as a standard.

Binding of Amino Acids. The approach was essentially that used by Krueger and Cagan (11) to measure binding of taste stimuli. Binding is operationally defined. For total binding, each vessel contained 2.0 ml of fraction P2 in 0.05 M Aces buffer (pH 7.0) plus 0.15 ml of amino acid. The ligand was 6 μM L-[3H]-alanine where not specified otherwise. Nonspecific entrapment was measured on additional samples by using the same mixture except that excess unlabeled amino acid was included. After incubation on ice for 1 hr, replicate 1-ml samples were filtered through Millipore filters and rinsed with 10 ml of Aces buffer. The radioactivity was determined (17) by using a Packard Tri-Carb liquid scintillation counter (model 3375) (37% efficiency for 3H and 72% for 14C). "Specific" binding was calculated from the difference between total counts bound and nonspecific counts bound. Binding of L-[3H]alanine was linear with protein concentration (at least over the range 20–100 μg/ml), showed a pH optimum between 6.9 and 7.2, and was reversible upon addition of excess unlabeled L-alanine.

RESULTS

Fractionation of Olfactory Tissue. The specific binding activity of each fraction was determined using L-[3H]alanine as a ligand. Fraction P2 consistently showed the highest specific binding activity (Table 1), with binding being enriched 4- to 12-fold over that of homogenate H1. In addition, the major portion (40–70%) of the binding activity was recovered in fraction P2. This distribution of binding activity is similar to that obtained with catfish taste tissue (11). Under the fractionation conditions used here, plasma membranes are expected (13–15, 18) to sediment in fraction P2. In preliminary experiments with brook trout (*Salvelinus fontinalis*), we also found similar specific binding of L-[3H]alanine to fraction P2.

As a control, a nonolfactory fraction P2 was prepared from the brain of rainbow trout. Its specific binding activity for L-[3H]alanine was only 16% of that in fraction P2 from the olfactory tissue. We also found that heating olfactory fraction P2 at 100° for 10 min abolished specific binding of L-[3H]ala-nine.

Correspondence between Stimulus Binding and Olfactory Responses. Electrophysiological measurements from peripheral and central olfactory tissue of the salmon *S. salar* (9) and from the olfactory bulb of the rainbow trout *S. gairdneri* (10) showed that these salmonid fishes are responsive to a number of amino acids as olfactory stimuli. The summated electrical activity from the surface of the rainbow trout olfactory bulb was recorded

Table 1. Distribution of binding activity toward L-[3H]alanine after fractionation of homogenate of trout olfactory epithelium

Exp.	Fraction*	Protein, mg†	Amount bound, pmol†	Specific binding, pmol/mg
1	H1	51.0 (46.6)	1018 (501)	19.9
	P1	31.3	362	11.6
	P2	2.8	364	128
	S2	11.5	187	16.3
	Recovery	98%	182%	
2	H1	22.7 (16.8)	607 (569)	26.7
	P1	9.9	185	18.6
	P2	3.4	362	106
	S2	3.2	114	36.1
	Recovery	98%	116%	
3	H1	35.5 (29.3)	481 (712)	13.6
	P1	17.6	46	2.6
	P2	3.0	276	92.5
	S2	8.2	32	3.9
	Recovery	98%	50%	
4	H1	34.4 (28.7)	695 (1024)	20.2
	P1	16.0	217	13.5
	P2	3.0	742	246
	S2	9.5	295	30.9
	Recovery	99%	122%	

* The olfactory tissue from 50 rainbow trout (*Salmo gairdneri*) (100 olfactory rosettes) was fractionated. The fractions resulting are: H1, whole homogenate; P1, initial pellet; P2, higher-speed pellet; and S2, final supernatant.

† In parentheses are shown the values for the sum of P1 + S1 (representing the initial division of the whole homogenate) upon which the recoveries are based, because of the variability encountered upon sampling the whole homogenate itself.

by Hara (10) in response to solutions of amino acids (0.1 mM) that were flowed over the olfactory rosette *in situ*. We therefore measured the binding of 10 of these amino acids, representing stimuli of high, medium, and low electrophysiological effectiveness, in order to establish the relevance of the binding measurements to an independent measure of olfactory stimulation. The amounts of amino acids bound and their relative stimulatory effectiveness measured electrophysiologically corresponded remarkably well (Fig. 1), with a correlation coefficient of +0.795 (*P* < 0.01).

The correspondence is even more notable when the large differences in the types of preparation are considered. In our
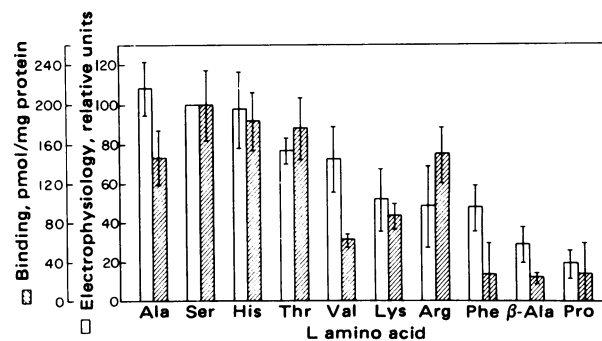

FIG. 1. Comparison of binding and electrophysiological responsiveness for olfactory stimulus amino acids in the trout. Binding of the 3H-labeled amino acids to fraction P2 from rainbow trout (*S. gairdneri*) olfactory tissue was measured with the ligand concentration at 6 μM throughout (pH 7.0). The binding data represent four experiments, in each of which binding of every amino acid was measured. The values were corrected for nonspecific binding, which was measured for each amino acid in all experiments. The electrophysiological data are from the report by Hara (10); the relative effectiveness is compared with serine as 100. All data are expressed as the mean ± SD.

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experiments the pH was maintained at 7.0 by using a buffered system in order to compare binding under a uniform set of conditions, whereas Hara (10) apparently added no additional buffering capacity to the dechlorinated tap water used as the medium, the pH of which was unspecified. The higher stimulus concentration used by Hara (10) was not used in our experiments because of the increased possibility of nonspecific binding at the higher ligand concentration. When the many differences in experimental procedure are considered, the agreement between our two sets of data is remarkably good and strongly supports the conclusion that binding measured with the present system is a relevant measure of an early event in olfaction.

The ability of the olfactory system to discriminate many stereoisomers is well known (2, 19). With salmonid fishes, the L-amino acids are more effective electrophysiological stimuli than the D isomers, with the stimulation elicited by the D isomer varying between 15 and 70% of that with the L form (9, 10). We observed a generally similar degree of stereospecific selectivity in the binding interaction. For example, binding of L-[^{14}C]-leucine and of D-[^{14}C]leucine were compared (at 6 μM): 233 pmol/mg versus 101 pmol/mg for the L and D isomers, respectively, with one preparation of fraction P2 and 175 and 84 pmol/mg with a second preparation. The binding activity of fraction P2 with L-[^{14}C]alanine was higher than with D-[^{14}C]alanine throughout the saturation curve, although significant binding of D-alanine was observed. Therefore, although the L isomer is more effective electrophysiologically and binds to a greater extent, appreciable electrophysiological activity and binding can also occur with the D form. These findings indicate that the stereospecific discrimination for the amino acids that occurs at the peripheral receptor level in this species is not absolute.

Binding Characteristics of Olfactory Stimulus Amino Acids. The close correspondence of the binding and the electrophysiological data shown in Fig. 1 encouraged us to investigate the binding properties of several odorants in greater detail. Accordingly, saturation curves were determined for several amino acids and the data were subjected to Scatchard analysis (20). In all cases, the binding curves (Fig. 2) showed saturation, although it was not complete for most compounds over the concentration range used. One striking aspect of the results (Table 2) is that the major differences among the amino acids appeared to be in the relative numbers of binding sites (n) rather than in the measure of the affinities (K_d). All but one of
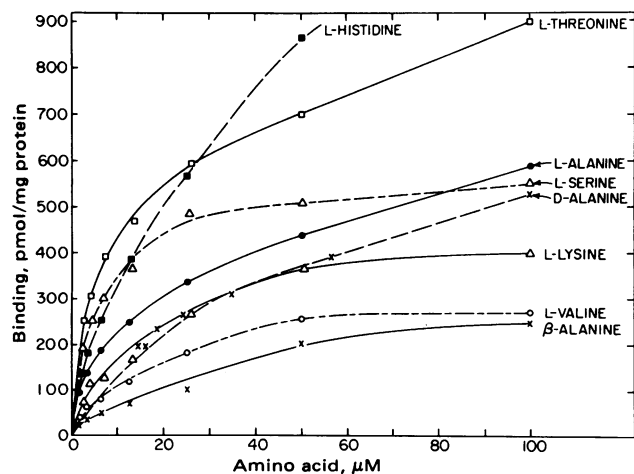
FIG. 2. Binding curves for amino acid olfactory stimuli to fraction P2 from the rainbow trout. The data for each amino acid are the mean values from two separate preparations. The binding value for L-histidine at 0.1 mM, 1217 pmol/mg, is omitted from the curve to save space.

Table 2. Values of K_d and n for the binding of olfactory stimulus amino acids to fraction P2*

Amino acid	K_{d_1}, M $\times 10^6$	n_1, mol/mg $\times 10^{10}$	K_{d_2}, M $\times 10^5$	n_2, mol/mg $\times 10^{10}$
L-Threonine	3.7	5.9	1.7	10
L-Serine	4.6	5.5	—	—
L-Alanine	5.6	4.4	3.7	8
L-Histidine	3.9	3.9	5.6	19
L-Lysine	4.2	2.0	3.1	6
L-Valine	4.8	1.4	3.1	4
β-Alanine	3.9	0.8	14	8
D-Alanine	—	—	4.7	8

* The values are calculated from Scatchard analysis of the binding data presented in Fig. 2; the lines were fit by least-squares calculations. Although a saturation curve was carried out with L-phenylalanine, the high degree of nonspecific binding of this compound to the Millipore filters made the data considerably less reliable for evaluation of K_d and n, and they are therefore not included.

the L-amino acids showed evidence of multiple sites, and only L-serine and D-alanine gave straight-line Scatchard plots without a breakpoint. The other plots were indicative of two types of binding sites, which are referred to here as the "high-affinity site" (K_{d_1} and n_1) and the "low-affinity site" (K_{d_2} and n_2). The binding of L-histidine may possibly be more complex, because the Scatchard plot for L-histidine was different from that for the other amino acids in not having as clear a breakpoint.

In order to assess the relative specificity of binding sites for amino acids in fraction P2, a series of competition experiments was carried out with four of the amino acids as ligands—L-[^{3}H]threonine, L-[^{3}H]serine, L[^{3}H]alanine and L-[^{3}H]valine. Seven different amino acids were added individually as potential competitors of binding of the labeled ligand. The results shown in Table 3 reveal several interesting points. A similar extent of mutual inhibition of binding among three amino acids—L-threonine, L-serine, and L-alanine—was clearly demonstrated. Inhibition of L-[^{3}H]valine binding also occurred with these three amino acids as competitors, but the extent of inhibition was less. L-Valine competed to some extent with binding of each of these three ^{3}H-labeled ligands. Partial competition effects were observed also among some of the other amino acids. It was striking that addition of L-lysine had no effect on binding of any of the four ^{3}H-labeled ligands used.

Effects of Metal Ions and Sulfhydryl Reagents. Addition of 1 mM Hg^{2+} (as HgCl$_2$) inhibited binding of L-[^{3}H]alanine by 97%, and washing twice by successive centrifugation and

Table 3. Competition among various amino acids for binding to fraction P2*

Competing amino acid	Binding of [^{3}H]ligand, %			
	L-Thr	L-Ser	L-Ala	L-Val
L-Threonine	—	26	32	42
L-Serine	18	—	22	48
L-Alanine	18	22	—	47
L-Valine	38	50	57	—
L-Histidine	48	61	71	43
β-Alanine	84	80	68	92
D-Alanine	75	68	52	83
L-Lysine	94	98	104	98

* The binding assays contained the competing amino acid (60 μM) in the assay mixture with the radioactive ligand at 6 μM. The data are from four separate experiments; in each experiment, a single radioactive ligand was tested with each of the competing amino acids.
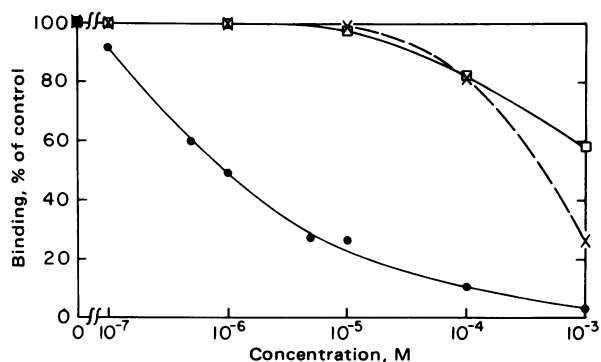
FIG. 3. Effects of Hg^{2+} (●), Cu^{2+} (□), and Cd^{2+} (✕) on binding of L-[³H]alanine to fraction P2. Hg^{2+} and Cd^{2+} were added as the chlorides and Cu^{2+}, as the sulfate. The data for Hg^{2+} and Cu^{2+} are means of two experiments, and those for Cd^{2+} are from a single experiment.

suspension in buffer failed to reverse the inhibition. At 0.1 mM, p-hydroxymercuribenzoate was also strongly inhibitory (80% inhibition). Addition of 10 mM dithioerythritol to the preparation prior to 1 mM Hg^{2+} decreased the inhibition to only 25%, whereas addition of the dithioerythritol after the Hg^{2+} was less effective in decreasing the inhibition (75% inhibition). The concentration dependence of the inhibition by Hg^{2+}, along with the smaller effects of Cu^{2+} and Cd^{2+} on binding, are illustrated in Fig. 3. Strong inhibition by Hg^{2+} was also observed with an olfactory fraction P2 prepared from the brook trout (Salvelinus fontinalis). In addition, certain other metal ions were tested with rainbow trout fraction P2 at 1 mM in single experiments. The ions and extents of inhibition were as follows: Ag^+, 93%; Pb^{2+}, 42%; and Zn^{2+}, 18%. The first two were added as the nitrates and the third as the sulfate.

The inhibition of odorant binding by mercurials led us to consider the possibility of an —SH group being involved. It had been suggested that —SH groups might be involved at olfactory receptor sites in salmon (9) and frog (21). Upon further study, we observed that neither 1 mM iodoacetic acid nor 0.1 mM N-ethylmaleimide inhibited binding, although a 10-fold higher concentration of the latter compound did show some inhibition. We therefore suggest that the inhibitory effect of the mercurials may not be due to specific blocking of an —SH group at the olfactory binding site for L-alanine.

DISCUSSION

The nature of the initial interaction of a stimulus molecule with an olfactory receptor and the nature of the olfactory receptor macromolecules have been the subjects of considerable speculation but relatively little direct biochemical study. The correspondence between the binding measurements and the electrophysiological responses shown in Fig. 1 is striking and lead us to conclude that binding (operationally defined) is a physiologically relevant measure of an initial step in olfactory selectivity. To emphasize further that the order of binding observed here is not fortuitous, we note that binding of some of the same amino acids was studied with a similar preparation from catfish taste tissue (11), but the relative amounts bound differed substantially from the situation with the rainbow trout.

The results of the Scatchard analyses (Table 2) and those from relatively limited series of competition experiments (Table 3) are considered together in assessing the types of binding sites present in fraction P2 isolated from the trout olfactory tissue. Perhaps the most striking result of the Scatchard analyses is the absence of marked differences among the values of K_d for the

various amino acids. The values for the high-affinity sites, which are in the range of 10^{-6} M, are within experimental error. Similarly, the values for the low-affinity sites (except that for β-alanine) are also within experimental error among themselves. At this level of analysis, however, differences emerge for the relative numbers of binding sites, n. The relative importance, in olfactory sensing, of the high-affinity sites compared with the low-affinity sites cannot yet be inferred, and these are not distinguished in our discussion. The results do indicate, however, that olfactory discrimination does not occur by means of different affinities of a single type of binding site for all of the olfactory stimulus compounds; consequently, this implies the existence of a multiplicity of types of sites, a point that is addressed directly in the following analysis of our experimental findings that leads us to postulate the existence of several different types of binding sites.

The competition data (Table 3) and the binding parameters (Table 2) are consistent with a common site (called site TSA) that accommodates L-threonine, L-serine, and L-alanine. Some binding of L-valine and L-histidine may occur at site TSA. The binding properties of D-alanine (Table 2) are similar to those of the low-affinity site for L-alanine, and D-alanine competes to some extent with L-[³H]alanine (Table 3). We tentatively postulate site A_D, which binds D-alanine but which may be a low-affinity site for L-alanine. We also postulate site A_B for binding β-alanine. The binding parameters for L-lysine (Table 2) indicate an affinity similar to that of site TSA but with fewer sites. These data, coupled with the complete absence of inhibition of binding by L-lysine of the ³H-labeled amino acids tested (Table 3), lead us to postulate the existence of site L, which binds L-lysine. Although the data (Tables 2 and 3) suggest that L-valine can bind to site TSA, we tentatively postulate an additional binding site for L-valine, site V. Other interpretations are possible, however. Our postulate of site H, which binds L-histidine, is also regarded as tentative.

The major question that has emerged is What types of olfactory sites exist? A number of authors have suggested that there are multiple types of olfactory receptor sites (e.g., refs. 2, 19, and 21). Binding sites are defined in terms of the particular set of stimuli chosen for study, and therefore those that we postulate represent a minimum. The data presented here, although far from complete in terms of a full definition of this problem in olfaction, do begin to establish on a biochemical basis an important principle: a multiplicity of olfactory binding sites exists, with different but not necessarily exclusive specificities for various olfactory stimuli.

Our interpretation is in sharp contrast to that of Hara (22), who postulated a single hypothetical olfactory receptor site in the trout, based upon structure–activity correlations with electrophysiological recordings from the olfactory bulb of the brain. Our present results also suggest that a conclusion regarding general involvement of —SH groups in the binding interaction at olfactory receptor sites is premature. The inhibition of odorant binding by Hg^{2+} does, however, explain the blocking by Hg^{2+} of the electrophysiological responsiveness of the salmon olfactory apparatus (9). Our evidence points to the olfactory receptors being a possible locus of action of environmental toxicants. In a species that relies upon olfaction for its reproductive behavior, the conventional assessments of "toxicity" would not necessarily reveal this type of toxic effect on the individual, whereas the effect could prove detrimental to the population by means of interfering with the olfactory sense. The assay method we describe could be a useful screening tool for other potential toxicants, because it enables examination of their effects on odorant binding.

Current understanding of the biochemical mechanisms of olfaction is rudimentary and has been hampered by the lack

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of suitable experimental systems. Our direct binding method which uses radioactively labeled ligands and a sedimentable preparation from the trout olfactory epithelium (12) is one approach. Recently, a binding study was reported (23) which used a whole homogenate of sow olfactory tissue. As ligand, ^{3}H-labeled 5α-androst-16-en-3-one, a sexually active pheromone from boars, was used. Although the authors noted that a significant amount of the steroid bound nonspecifically, they also presented evidence for specific binding. In each of these two biochemical systems, the choice of the biological model is a species with a well-developed, behaviorally functional, olfactory sense and known to use olfaction in its natural habitat. In each case also, relevant olfactory stimulus compounds were used as ligands rather than chemicals selected randomly or by uncritical extrapolation from human olfactory perception.

The binding sites for amino acids in the trout are localized in a sedimentable fraction. Analytical criteria have previously (11) shown a similar P2 preparation from taste tissue to be enriched in plasma membranes (18). The localization of the binding sites for the steroid was not reported, and it will be interesting to determine whether they occur on the plasma membrane or within the cytoplasm of the cell where other steroid-binding receptor proteins are found (24).

The ability to clearly define each type of binding site when using other than highly purified receptor macromolecules is fraught with problems of interpretation. Nevertheless, guidelines can be established for studies on more highly purified preparations. The present studies offer biochemical evidence indicating a multiplicity of olfactory binding sites, some of which are able to accommodate more than one type of odorant compound. These are facts which investigators performing structure–activity correlations by measuring responses at higher neural levels will now need to consider. Olfactory receptor molecules have been assumed to be proteins. The data presented here show that the receptor sites are part of the sedimentable membrane-containing fraction of the cell. Whether the receptor molecules are able to maintain the proper conformation to show specific binding activity when released from the membrane matrix is a question yet to be answered. The ability of the binding sites to effect reasonably high degrees of selectivity suggests that several different proteins may function as olfactory receptor molecules in the receptor membranes.

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