Electrophysiological Evidence for Acidic, Basic, and Neutral Amino Acid Olfactory Receptor Sites in the Catfish

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ABSTRACT  Electrophysiological experiments indicate that olfactory receptors of the channel catfish, *Ictalurus punctatus*, contain different receptor sites for the acidic (A), basic (B), and neutral amino acids; further, at least two partially interacting neutral sites exist, one for the hydrophilic neutral amino acids containing short side chains (SCN), and the second for the hydrophobic amino acids containing long side chains (LCN). The extent of cross-adaptation was determined by comparing the electro-olfactogram (EOG) responses to 20 "test" amino acids during continuous bathing of the olfactory mucosa with water only (control) to those during each of the eight "adapting" amino acid regimes. Both the adapting and test amino acids were adjusted in concentrations to provide approximately equal response magnitudes in the unadapted state. Under all eight adapting regimes, the test EOG responses were reduced from those obtained in the unadapted state, but substantial quantitative differences resulted, depending upon the molecular structure of the adapting stimulus. Analyses of the patterns of EOG responses to the test stimuli identified and characterized the respective "transduction processes," a term used to describe membrane events initiated by a particular subset of amino acid stimuli that are intricately linked to the origin of the olfactory receptor potential. Only when the stimulus compounds interact with different transduction processes are the stimuli assumed to bind to different membrane "sites." Four relatively independent L-α-amino acid transduction processes (and thus at least four binding sites) identified in this report include: (a) the A process for aspartic and glutamic acids; (b) the B process for arginine and lysine; (c) the SCN process for glycine, alanine, serine, glutamine, and possibly cysteine; (d) the LCN process for methionine, ethionine, valine, norvaline, leucine, norleucine, glutamic acid-γ-methyl ester, histidine, phenylalanine, and also possibly cysteine. The specificities of these olfactory transduction processes in the catfish are similar to those for the biochemically determined receptor sites for amino acids in other species of fishes and to amino acid transport specificities in tissues of a variety of organisms.

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INTRODUCTION

Electrophysiological studies on the olfactory system of fishes ranked amino acids in terms of their relative stimulatory effectiveness at a single test concentration. Rank order lists of amino acids obtained from olfactory receptor responses (electro-olfactogram [EOG] and/or integrated multiunit activity) were reported for the Atlantic salmon (Sutterlin and Sutterlin, 1971), white catfish (Suzuki and Tucker, 1971; Caprio, 1980), channel catfish (Caprio, 1978; Erickson and Caprio, 1984), Atlantic stingray (Silver, 1979), spotted bullhead and sea catfish (Caprio, 1980), and the American eel (Silver, 1982). Similarly, lists of stimulatory amino acids were compiled from data obtained by olfactory bulbar EEG recordings in salmonids (Hara et al., 1973; Belghaug and Døving, 1977), red sea bream and mullet (Goh and Tamura, 1980), and channel catfish (Byrd and Caprio, 1982). Although the previous studies established conclusively that the olfactory receptors of fishes detect with high sensitivity a number of different amino acids, electrophysiological information was lacking concerning possible amino acid receptor site "types" present on the olfactory receptor cells. Although species-specific differences in the previous data are evident, further analyses revealed a considerable similarity for the highly stimulatory amino acids (Goh et al., 1979; Caprio, 1984). In general, L-α-amino acids with unbranched and uncharged side chains were the more potent stimuli, as determined electrophysiologically. This similar spectrum of highly effective amino acids suggests the presence of similar proportions and/or binding affinities of receptor sites for odorant amino acids across different species of fishes.

Single unit recordings from olfactory receptor cells of fishes would lend some insight into the types of olfactory receptor sites present on individual neurons. However, with the exception of Suzuki's (1978) work, olfactory receptor unit data in fishes are lacking. This is due to the small size of the primary receptor neurons (cell body, 5–8 μm; axons, 0.2–0.3 μm diam; Easton, 1971; Kreutzberg and Gross, 1977; Caprio and Raderman-Little, 1978), their high packing density, and, in the majority of the fishes tested, the instability of the delicate lamellae of the olfactory organ, which tend to float in the water bathing the capsule. Multiunit olfactory receptor recordings utilizing cross-adaptation, however, can provide evidence for the possible different types of olfactory receptor "transduction processes" present within the olfactory receptor mucosa, but their distribution per cell (i.e., the chemospecificity of individual olfactory neurons) still requires unit analysis. The term "transduction process" is used here to include the stimulus binding site and all related events subsequent to stimulus binding that result in the production of the generator current within the olfactory neuron. For example, two amino acids might share a transduction process by simply binding to the same membrane site, or they may bind to separate, independent membrane sites that are intricately linked through later events occurring within the membrane that lead to the initiation of ionic transmembrane currents. Only when stimulus compounds are shown to interact with different transduction processes is that considered appropriate experimental evidence in the present report for the existence of different stimulus binding sites.

Whereas rigorous amino acid cross-adaptation and binding experiments are
currently rare in studies of olfaction and taste in fishes, numerous examples exist of
competition studies with amino acids in the transport systems of organisms
from bacteria to mammals. Odorant detection by olfactory receptors is thought
to involve, at least initially, only the physical adsorption (and desorption) of
stimulus molecules to membrane receptor sites without the subsequent translo-
cation of the amino acid molecule into the receptor cell (Tucker, 1963). Such
binding interactions of amino acids have been demonstrated directly with olfac-
tory preparations from rainbow trout (Cagan and Zeiger, 1978; Rhein and
Cagan, 1980, 1983; Brown and Hara, 1981) and channel catfish (Cancalon,
1978). The specificities for amino acid transport systems found in tissues of
widely differing organisms are similar, although not identical, to the respective
specificities of the olfactory transduction processes for amino acids in the catfish.
Thus, the initial interaction of amino acids with transport receptor sites is thought
to be somewhat analogous to amino acid binding to olfactory receptor sites, and
information obtained from either type of experiment may lead to a better
understanding of both phenomena.

This study was initiated to determine the possible classes and chemospecificities
of transduction processes for amino acids present in the olfactory receptor
epithelium of the channel catfish and to compare these chemospecificities with
the chemical affinities for amino acids determined from olfactory receptor
binding experiments and from a variety of amino acid transport systems found
in vertebrates and invertebrates.

**METHODS**

**Animal Immobilization and EOG Recording Technique**

Juvenile (100–200 g) channel catfish, *Ictalurus punctatus*, obtained from a local catfish
farm, were transported to the laboratory in oxygenated pond water. The catfish were
equilibrated to 25°C water temperature before being transferred to charcoal-filtered well
water in 75-liter aquaria, which were maintained on a 12:12 light-dark regime. The
catfish, immobilized with Flaxedil (gallamine triethiodide; 0.1 mg/100 g body weight;
Davis & Geck Dept., American Cyanamid, Pearl River, NY) injected into the flank
musculature, were wrapped in damp tissue paper and placed in a plexiglass and metal
holding device before dissection. Supplemental doses of Flaxedil were delivered to the
fish as necessary. The gills were perfused throughout the experiment with aerated,
charcoal-filtered well water.

The olfactory aemellae were exposed by removing the skin, connective tissue, and
cartilage dorsal to the nasal capsule. The underwater EOG was recorded as described by
Silver et al. (1976). The amplified DC signal was displayed on an oscilloscope and recorded
permanently on chart paper. The absolute magnitude (in millivolts) of the EOG response
was determined by comparison to a known calibration signal. A level of at least 3 mV
EOG response magnitude to receptor stimulation with 10^{-4} M L-alanine was used as an
indicator of a “good” physiological preparation. Generally, 70% of the catfish tested
reached this criterion. All data reported in these experiments were obtained from a total
of 11 animals.

**Stimuli and Application Scheme**

Stock solutions of commercially obtained amino acids were prepared weekly at 10^{-3} M
and stored at 4°C; dilutions (in charcoal-filtered well water) were made prior to each
experiment. The pH of all amino acid solutions at the concentrations tested was ~8, the pH of the well water solvent. Amino acid "test" solutions (0.5 ml) at room temperature were hand-injected uniformly from disposable Pasteur pipettes into the flow (14 ml/min) of either well water alone or into the adapting solution that continuously bathed the olfactory mucosa. These stimuli were diluted, and the maximal concentration delivered to the receptors was 42% of the concentration injected as determined by photodensitometry of the dye solutions (values in the text are the undiluted concentration). The rise time of the dye was much faster than its decline and the pulse duration was ~5 s measured at 37% of the peak response. The extended time course of the EOG responses measured near baseline (Fig. 1) results from a slight recirculation of the test stimulus, prior to its complete clearance, within the olfactory chamber (Caprio, 1980). Cross-contamination of the stimulus injection port was kept to a minimum by continuously flushing it with the same solvent as that bathing the olfactory mucosa (i.e., well water in the unadapted condition and the adapting stimulus during the respective adapting regime).

Eight amino acids with a wide range of side chain structural variations were chosen as the adapting stimuli. The concentrations of the 20 test amino acids were selected to the nearest whole log concentration that elicited similar EOG response magnitudes as that of the standard, $10^{-5}$ M L-alanine (Fig. 2); this reduced the variation in response caused by differences in stimulatory effectiveness. Responses were first recorded with only a flow of charcoal-filtered well water bathing the olfactory mucosa. The adapting amino acid was then presented continuously to the olfactory mucosa for 6–10 min before the phasic responses to the test amino acids were recorded. The adapting stimulus bathed the olfactory capsule both during the presentation of the test stimuli and throughout the 4-min interstimulus intervals. This method of keeping the olfactory receptors in a constant background of the adapting stimulus during the relatively long interstimulus intervals ensured a continuous level of adaptation; however, during the brief application of the test stimuli, the concentration of the adapting stimulus was maximally diluted to 58% of its interstimulus concentration. Finally, the preparation was returned to well water alone and allowed to recover for 30–45 min before the test amino acids were again presented in a background flow of each test amino acid. Standards were regularly tested to check the reproducibility of the preparation.

**Data Analyses**

Representative amino acids of each structural group were used as adapting stimuli: acidic, basic, short side chain neutral (SCN), and long chain neutral (LCN) amino acids. The data were analyzed by first subtracting the (small) control responses from the unadapted and adapted peak phasic responses to the amino acids and then standardized as a percentage of the unadapted responses to the respective stimuli:

$$\frac{\text{adapted response} - \text{control A}}{\text{unadapted response} - \text{control W}} \times 100 = \%,$$

where control A is the peak phasic response to a 0.5-ml aliquot of the respective adapting amino acid during each of the eight adapting regimes, and control W is the response to a 0.5-ml aliquot of well water presented during the well water flow bathing the olfactory capsule in the unadapted state.

The significance of the main effects (adapting and test amino acids) was tested with a two-level factorial ANOVA with randomized blocks (Freund and Littell, 1981). Unequal subclasses were used to generate least-squared means, which were used in the Pearson Product Moment Correlations of the eight adapting regimes. A principal-components analysis (Cooley and Lohnes, 1971) was conducted to determine the relationships among...
all adapting regimes and test stimuli. This multivariate statistical technique (a) determined similarities among the adapting regimes based on the EOG responses to the test stimuli (Fig. 3, A and B) and (b) related each test compound to all others based on the similarities of the EOG responses to the test stimuli across the different adapting regimes (Fig. 4). The programs of Bloom et al. (1977) calculated and plotted these relationships on a coordinate axis system.

RESULTS

Cross-Adaptation: Relationship of Adapting Stimuli

A modified cross-adaptation technique was employed to determine the possible types of olfactory transduction processes that exist for amino acids in the olfactory receptor pathway of the channel catfish. The differing patterns of depression of the EOG responses to the 20 test amino acids by the 8 adapting amino acids were used as indicators of the different types and specificities of the olfactory transduction processes. A similar pattern of reduction of EOG responses to the test stimuli across the different adapting regimes indicated the existence of a particular transduction process; further, the amino acid side chain structures of the test and adapting amino acids defined in part the molecular specificity of the transduction process.

The receptor (EOG) response to amino acid stimulation is characterized by an initial phasic component followed by a rapid decline to a steady state tonic level that continues throughout the stimulus duration. Upon termination of the amino acid stimulus and return to well water alone, the EOG response returns quickly to baseline. Thus, during the competition experiments, a tonic level of activity is maintained to the constant presentation of the “adapting” stimulus. Also, the phasic response to the addition of a “test” stimulus is depressed to varying degrees, and sometimes eliminated, depending on the molecular characteristics of the adapting and test stimuli used (Fig. 1). It is this depression in the phasic EOG response, compared with its unadapted value, that is used as a measure of the degree of cross-reactivity among the stimulus compounds. Those compounds that interact with the same transduction process would strongly cross-adapt, whereas competition among compounds would not be significant when they interact with independent transduction processes (i.e., independent receptor sites). The results of an extensive series of competition experiments are summarized in Fig. 2.

The data (Figs. 1 and 2) show clearly that the EOG phasic response to each of the 20 test amino acids was subject to some reduction (by at least 24% of its unadapted value) in the standardized EOG amplitude by all 8 of the adapting compounds, but there were substantial quantitative differences. The more pronounced reductions in the EOG responses were to those α-amino acids most closely related by side chain structure and charge to the adapting stimulus. Thus, at least three general patterns of response across the eight different cross-adapting regimes resulted and are indicated in Fig. 2. During adaptation with basic amino acids (L-arginine, L-lysine; Fig. 2, A and B), the EOG responses to acidic and neutral amino acids were not greatly affected. Similarly, during adaptation with an acidic amino acid (L-glutamic acid; Fig. 2C), the EOG
FIGURE 1. Representative EOG records obtained from a single catfish to 14 "test" α-amino acids presented in either a background of well water (A and D), $10^{-4}$ M L-arginine (B), or $10^{-5}$ M L-methionine (C). The phasic EOG responses in A and D rise from baseline activity, whereas in B and C the EOG records arise from an ongoing tonic level of activity to the respective adapting stimulus. The amino acids are arranged in the order of acidic (1, L-aspartic acid; 2, L-glutamic acid), basic (3, L-arginine; 4, L-lysine), and neutral compounds with short side chains (SCN) (5, glycine; 6, L-alanine; 7, D-alanine; 8, L-serine; 9, L-cysteine), and neutral compounds with long side chains (LCN) (10, L-methionine; 11, L-ethionine; 12, L-leucine; 13, L-norleucine; 14, L-glutamic acid-γ-methyl ester). Ct indicates the control response to well water injected into either a continuous flow of well water (A and D; i.e., control W in Eq. 1), or into the respective adapting solutions (B and C) bathing the olfactory mucosa. The depression of Ct seen in B and C indicates a decline in the ongoing tonic response levels caused by water blanks. Responses 3B and 10C served as controls (i.e., control A in Eq. 1) during the respective adapting regimes. Note in B that an arginine adapting solution selectively eliminated the phasic EOG response to itself (3B, adaptation) and L-lysine (4A, cross-adaptation) (see also Fig. 2A). Note in C that a methionine adapting solution reduced the responses to the LCN test amino acids (10-14C) to a greater degree than those to the other amino acid groups, leaving evident the responses to the acidic (1, 2) and basic (3, 4) amino acids (see also Fig. 2D). Breaks in the records indicate 4-min interstimulus intervals.

responses to basic and neutral amino acids were least affected. Finally, during adaptation with the L-isomers of neutral amino acids (methionine, norleucine, glutamic acid-γ-methyl ester [GME], alanine, and cysteine; Fig. 2, D–H), the responses to acidic and basic amino acids were clearly reduced the least. As a qualitative (arbitrary) aid in examining Fig. 2, a value of >50% unadapted response indicated that minimal cross-adaptation between the adapting and test compounds had occurred; if the response was 20–50%, moderate cross-adaptation had resulted, and if the response was <20%, strong cross-adaptation had occurred.

FIGURE 2. (opposite) Mean ± SE phasic EOG responses to 20 "test" amino acids during the continuous application of 8 "adapting" amino acids individually presented (listed at top of histograms, A–H) to the olfactory mucosa. Test amino acids are listed in the order of acidic, basic, neutral (SCN), neutral (LCN), and imino groupings. N = 5 fish tested per adapting regime unless otherwise noted. See text for details.
Fig. 2D–F
For the basic amino acid transduction process, the adapting effects of arginine and lysine were significantly correlated ($P < 0.001$, $r = 0.84$; Table I). L-Arginine completely adapted the receptor responses to L-lysine (0%), while the response to the acidic amino acid L-aspartate was little affected (57%) (Fig. 2A). The receptor responses to the neutral amino acids, with the exceptions of L-alanine (20%) and L-proline (9%), were moderately adapted by L-arginine. The adapting stimulus L-lysine provided a pattern of receptor responses (Fig. 2B) to the test amino acids similar to that for arginine (Table I), although lysine at $10^{-5}$ M was a slightly less potent adapting stimulus than $10^{-4}$ M L-arginine. L-Lysine effectively adapted the responses to L-arginine, reducing the EOG response to 21% of the unadapted response, and accordingly the responses to L-aspartic and L-glutamic acids were minimally affected (63 and 56%, respectively). Also, like arginine, lysine moderately affected the EOG responses to the neutral amino acids. The acidic transduction process, which was characterized with L-glutamic acid, did not strongly cross-adapt any of the test amino acids (Table I), although the response to aspartic (28%) acid was more affected than any other of the patterns resulting from the adapting regimes (Fig. 2C).

Under all of the neutral amino acid adapting regimes, the EOG responses to both the basic and acidic amino acids were relatively unaffected (Fig. 2, D–H, Table I); however, the lower than expected response to glutamic acid under GME adaptation may possibly have resulted from a slight contamination of the ester by the parent compound, L-glutamic acid, resulting in self-adaptation. The
TABLE I
Product-Moment Correlations Between the Effects of the Eight "Adapting" Amino Acids on the EOG Responses to the "Test" Amino Acids

<table>
<thead>
<tr>
<th></th>
<th>L-Met</th>
<th>L-GME</th>
<th>L-nLeu</th>
<th>L-CysH</th>
<th>L-Ala</th>
<th>L-Glu</th>
<th>L-Arg</th>
<th>L-Lys</th>
</tr>
</thead>
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<tr>
<td>L-Met</td>
<td>—</td>
<td>0.93*</td>
<td>0.91*</td>
<td>0.69*</td>
<td>0.58*</td>
<td>-0.38</td>
<td>-0.09</td>
<td>-0.11</td>
</tr>
<tr>
<td>L-GME</td>
<td>—</td>
<td>0.79*</td>
<td>0.71*</td>
<td>0.75*</td>
<td>-0.08</td>
<td>-0.06</td>
<td>-0.18</td>
<td>—</td>
</tr>
<tr>
<td>L-nLeu</td>
<td>—</td>
<td>0.76*</td>
<td>—</td>
<td>0.45*</td>
<td>-0.41</td>
<td>-0.07</td>
<td>-0.03</td>
<td>—</td>
</tr>
<tr>
<td>L-CysH</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.06*</td>
<td>-0.12</td>
<td>-0.17</td>
<td>-0.21</td>
<td>—</td>
</tr>
<tr>
<td>L-Ala</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.17</td>
<td>0.08</td>
<td>-0.10</td>
<td>—</td>
</tr>
<tr>
<td>L-Glu</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.04</td>
<td>-0.20</td>
<td>—</td>
</tr>
<tr>
<td>L-Arg</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.84*</td>
<td>—</td>
</tr>
<tr>
<td>L-Lys</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
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* P < 0.05, t test.
† P < 0.01.
‡ P < 0.001.

data (Fig. 2, D and E; Table I) for L-methionine and L-norleucine adaptation indicate that the presence of a sulfur atom in the gamma position of methionine is equivalent to a methylene group in norleucine, and that these compounds are recognized by the same transduction process. However, the data also show that the sulfur atom in the adapting stimulus L-methionine could be eliminated, resulting in the compound L-norvaline, which is affected similarly to methionine during the eight adapting regimes (Fig. 2). Further, the similarity in the cross-adapting effectiveness for GME (Fig. 2F; methyl esterification of glutamic acid removes the anionic charge from the side chain and increases its length to four carbons, the same as norleucine), as compared with methionine and norleucine (Fig. 2, D and E; Table I), indicates that the interaction between the side chain of an amino acid and its receptor site(s) is mainly hydrophobic in nature, and that a number of amino acids containing differing hydrophobic side chains may interact with the same transduction process and possibly the same binding site.

Whereas Table I lists pairwise correlations for the effects of the different adapting compounds on the test stimuli, Fig. 3 presents a principal-components ordination that relates all adapting regimes to each other on the same coordinate axis system. Fig. 3A is a visual representation of the data based on the similarity in the cross-adapting effects on the 20 test compounds by the 8 adapting stimuli. The three vectors in Fig. 3A account for 87% of the data variance, and three distinct groups located in different quadrants result: one for the basic, another for the acidic, and a grouping for the neutral amino acids. A simpler way of viewing this is seen in a two-dimensional graph (Fig. 3B) in which two vectors account for 79% of the data variance. Again, the basic adapting compounds are closely correlated in their cross-adapting effects on the test battery and are situated in space equally from the neutral and acidic adapting compounds. Similarly, the acidic adapting compound L-glutamic acid is spaced equidistant from both the neutral and basic adapting amino acids. The neutral adapting compounds are situated together within the neutral space. In both A and B, the adapting effects of the neutral amino acids are clearly separated from the effects of the charged (acidic and basic) amino acids by principal axis I. Principal axis II
separates the adapting effects of amino acids with relatively short side chains from those with longer side chains.

**Cross-Adaptation: Relationship of Test Stimuli**

A source of much additional information is a principal-components ordination that relates every test substance to all others on the basis of similarities of the EOG responses to the test stimuli across the eight different adapting regimes. An important feature of this analysis is that it also provides a basis to place the test amino acids not used as competing stimuli into a coordinate system based on the test response similarity across the eight competition regimes. In this analysis, shown in Fig. 4, two vectors account for 71% of the data variance; again, principal axis I orders the items by charge, whereas principal axis II separates
them by amino acid side chain length. The basic amino acids L-arginine and L-
lysine are grouped together; also, the acidic amino acid L-aspartic acid resides
close to L-glutamic acid, its predicted position being due to the similar side chains.
A third pattern results for the neutral amino acids. The hydrophilic, short side
chain neutral amino acids (SCN) are separated from the more hydrophobic, long
side chain neutral (LCN) stimulus compounds. The SCN stimuli are glycine and
the L-isomers of alanine, serine, glutamine, and cysteine; the LCN stimulus
compounds include the L-isomers of methionine, ethionine, valine, norvaline,
leucine, norleucine, histidine, phenylalanine, and GME. Although the olfactory
receptors of the channel catfish are more responsive (i.e., larger magnitude EOGs
to equimolar stimulus concentrations) to the LCNs having unbranched side
chains (methionine, ethionine, GME, norvaline, norleucine) (Caprio, 1978),
hydrophobic neutral amino acids having branched side chains (leucine and valine)
and ringed structures (phenylalanine and histidine) appear to interact with the
same LCN transduction process. The difference in the relative stimulatory
effectiveness of these compounds in the unadapted state, however, may be due
to different affinities and/or numbers of membrane binding sites. Curiously, L-
cysteine, an SCN according to side chain structure, acts somewhat like an LCN
(Figs. 3 and 4) and possibly interacts with both processes; although some cysteine
molecules could possibly have oxidized to cystine, the dimer is relatively nonstim-
ulatory and could not have accounted for this result.
L-Proline, an imino acid, may also be distinct from the other L-neutral amino
acids. The pattern of responses seen for L-proline under the different adapting
regimes, however, is somewhat similar to those for the SCNs and is less related
to the acidic and basic patterns (Fig. 4). A most fascinating occurrence is the

![Figure 4](https://jgp.rupress.org/content/84/4/805/f4.large.jpg)
position of the test amino acid D-alanine, which is displaced far from its L-enantiomer (Fig. 4); thus, D-amino acids may interact with transduction processes separate from those for the L-isomers.

**DISCUSSION**

*The EOG and Olfactory Transduction Process Considerations*

The EOG, a negative slow potential caused by summed current flow through the extracellular resistance of the olfactory epithelium (Ottoson, 1956, 1971; Getchell, 1974), was used as an indicator of the cross-adapting effects of various amino acid stimuli on the olfactory receptors of the channel catfish. The EOG has been assumed to be the population average of receptor potentials responsible for initiating the neural impulses (Ottoson, 1971) and accordingly has been shown, especially for amino acid responses in fishes, to be a reliable indicator of olfactory receptor regenerative neural activity (Caprio, 1978; Silver, 1982) and electroencephalic bulbar responses (Byrd and Caprio, 1982).

Although olfactory receptors are slowly adapting (Ottoson, 1956; Getchell and Shepherd, 1978; Baylin and Moulton, 1979), this characteristic is based on the tonic or steady state response, which continues throughout the stimulus presentation. The initial transient or phasic response adapts rapidly and it is the adaptive effects on this component of the EOG response on which the present experiments are based. Phasic olfactory responses to alpha amino acids having similar side chain structures are much reduced when the olfactory receptors are adapted with a similarly structured compound; this is evidence that these amino acids share the same olfactory transduction process. α-Amino acids with dissimilar side chain structures result in minimal olfactory cross-adaptation and therefore are assumed to interact with relatively independent transduction processes and thus different receptor sites.

A concern, however, with respect to the present electrophysiological data (Fig. 2) is the criteria for assignment of subgroups of the 20 test amino acids to different olfactory transduction processes when (a) all the adapting compounds affect to varying degrees the EOG responses to the test amino acids, (b) full reciprocal cross-adaptation does not occur for particular pairs of stimuli assumed to share a specific transduction process, and (c) specific adapting regimes affect differently the responses to amino acids assigned to the same transduction process. Observation a is evidence for the presence of multiple binding sites for different amino acids on the same olfactory neuron (i.e., different transduction processes sharing the same "neural channel"). Support for this is the finding that individual olfactory neurons respond to a number of different stimuli (Getchell, 1974; Blank and Mozell, 1976; Baylin and Moulton, 1979; Revial et al., 1978, 1982). Thus, olfactory receptor neurons that are partially depolarized by the adapting stimulus cannot respond fully to any of the test stimuli, whether they share the same transduction process or not.

Observations b and c may be inconsistent with the hypothesis that those amino acids involved share the same membrane receptor site according to a simple model of olfactory stimulus-receptor interaction, but they are compatible with
an allosteric binding model and the concept of transduction processes. According to this model, different amino acids that share a particular transduction process may bind to the same site or to two or more different sites associated with an acceptor molecule. The multiple sites per acceptor molecule are capable of operating a membrane ion gate that participates in the initiation of the receptor current for that process. Also, the binding of an amino acid stimulus to each site allosterically affects the binding properties of the other site(s), even though the actual binding sites on the molecule may be relatively independent. Thus, if the various amino acids that share the same transduction process bind to different sites on the same acceptor molecule, they may differentially affect both the binding affinity of the “other” site(s) and conductance of the associated ion channel, resulting in examples of b and c. Finally, specific amino acids could interact with different affinities to several transduction processes, resulting in observations a–c.

Biochemical Support for the Electrophysiologically Determined Catfish Olfactory Transduction Processes

The question as to whether the identified transduction processes are synonymous with the different olfactory receptor binding site types for amino acid stimuli cannot be precisely answered as yet; however, evidence to support this view is the similarity between the present electrophysiological results and the data obtained from biochemical binding experiments of tritiated amino acids on isolated membrane fractions of the olfactory mucosa of the catfish (Cancalon, 1978), rainbow trout (Cagan and Zeiger, 1978; Brown and Hara, 1981, 1982), skate (Novoselov et al., 1980; Fesenko et al., 1983), and a cilia preparation from the olfactory rosettes of rainbow trout (Rhein and Cagan, 1980, 1983). The SCN transduction process is analogous to site TSA proposed by Cagan and Zeiger (1978) and Rhein and Cagan (1980, 1983), which shows greatest binding specificity for L-threonine, L-serine, and L-alanine. The present electrophysiological results also directly indicate that glycine and L-glutamine may interact with this site. On the basis of the similarity in side chain structure, both L-asparagine and L-α-aminobutyric acid should also be recognized by this site, and there is some biochemical evidence for this (Brown and Hara, 1981). Additionally, some binding at site TSA of L-valine and L-histidine (Cagan and Zeiger, 1978), L-leucine, L-tyrosine, L-isoleucine, and L-phenylalanine (Brown and Hara, 1981) is indicative that these neutral amino acids with longer side chains are also partial agonists of site TSA (= SCN).

The present results, which suggest that there are dual transduction processes (SCN and LCN) showing some cross-reactivity for neutral amino acids based on the hydrophobicity of the side chain, have support from the biochemical studies. BCH [β-2-aminobicyclo (2,2,1)-heptane-2-carboxylic acid] totally inhibited the binding of leucine, tyrosine, and isoleucine, while only reducing by 50% the binding of the more hydrophilic amino acids alanine, serine, glutamine, and threonine to isolated membrane fractions of rainbow trout olfactory mucosa.
(Brown and Hara, 1981). Also, a portion of the binding of the hydrophilic amino acids was Na$^+$ dependent, whereas the longer side chain amino acids were relatively Na$^+$ independent (Brown and Hara, 1982). The LCN olfactory transduction process in the catfish recognizes all of the more hydrophobic amino acids (Fig. 4). The independence of site H, which binds L-histidine, was tentatively postulated by Cagan and Zeiger (1978), but it is not supported by the present results and falls within the LCN transduction process in the catfish. The suggestion that valine binds to both site TSA and an additional site (Cagan and Zeiger, 1978) may be indicated by the present results. EOG responses to valine across the different adapting regimes are similar to the responses to the amino acids included in both the SCN and LCN processes, as evidenced by the position of valine in Fig. 4. A similar situation occurs for L-proline, which may share the SCN process and an additional imino (for proline and hydroxyproline) process. Interestingly, protease treatment of palatine taste buds of the Japanese eel, Anguilla japonica, also indicated that the receptor protein for L-proline was different from that for other amino acids (Yoshii et al., 1979). Site $A_d$ for D-alanine (Cagan and Zeiger, 1978) has some support from the present experiments, as D-alanine does not fall within any of the four proposed transduction processes (Fig. 4).

The olfactory transduction process $B$ in the catfish for the basic amino acids lysine and arginine is probably analogous to site L (lysine) in the rainbow trout (Cagan and Zeiger, 1978), and is consistent with the evidence that arginine and lysine do not compete with tritiated alanine binding to a cilia preparation from rainbow trout olfactory rosettes (Rhein and Cagan, 1980) or to a membrane fraction of skate olfactory tissue (Novoselov et al., 1980; Fesenko et al., 1983). Evidence for stimulus channel $A$ in the catfish for two acidic amino acids, L-aspartic and L-glutamic acids, is seen in the skate, where L-glutamic acid also failed to interfere with tritiated alanine binding (L-aspartic acid was not tested) (Novoselov et al., 1980; Fesenko et al., 1983). Of interest in the present experiments is the observation of the relative independence of transduction processes for the compounds L-glutamic acid, L-glutamine, and L-GME. By altering the side chain charge and the molecular configuration, these compounds interact with three different transduction processes, processes $A$, SCN, and LCN, respectively.

There are, however, some discrepancies with specific amino acids when comparing binding and olfactory electrophysiological data (Brown and Hara, 1981). This may in part be the result of the widely differing experimental methods utilized in the two types of experiments. The electrophysiological data were obtained in vivo under more natural conditions than the biochemical studies, where disruption of the tissue might affect the binding properties (Cagan and Zeiger, 1978). However, the results with isolated cilia (Rhein and Cagan, 1980, 1983) were similar to those with the membrane preparation from homogenates. It is nevertheless striking that similarities in the electrophysiological and biochemical results do exist between the two widely differing experimental techniques.
Comparison of Amino Acid Specificities of Catfish Olfactory Receptors to Those of Invertebrate and Mammalian Transport Systems

Examples exist in unicellular organisms and the multicellular invertebrates of relatively independent transport systems for the acidic, basic, and neutral amino acids; further, the neutral amino acids are recognized by at least two partially interacting systems, a transport system with the highest affinity for short chain neutral compounds (analogous to the SCN olfactory transduction process) and another system primarily for the more hydrophobic amino acids (analogous to the LCN olfactory transduction process). Some specific examples include a “leucine” carrier in a marine pseudomonad that recognizes all hydrophobic neutral amino acids, but not the more hydrophilic neutral, acidic, or basic compounds with shorter side chains (Pearce et al., 1977). A similar situation exists for a protozoan flagellate where a “methionine” carrier was shown to have an affinity comparable to that of the “leucine” carrier described above (Simon and Mukkada, 1977; see Aomine, 1981, for a review of amino acid transport systems in protozoa). This “methionine” system also has little affinity for the basic or acidic amino acids. Numerous examples also exist of amino acid transport system specificities in multicellular invertebrate species (Asch and Read, 1975; Manahan et al., 1983; see review by Stewart, 1979), many of which show specificities similar to those found for the present olfactory data. Additionally, studies in both bacteria (Israeli et al., 1977) and invertebrates (Asch and Read, 1975) report separate transport sites for proline, which was tentatively assigned to a separate olfactory stimulus process in the present study. Other transport similarities to the olfactory system of the catfish include in bacteria the placement of aromatic (phenylalanine, tyrosine, and tryptophan) compounds with the linear and branched chain hydrophobic amino acids (Pearce et al., 1977), which is similar to what has been found in mammalian tissues (Oxender and Christensen, 1963). Although phenylalanine was the only one of three aromatic amino acids listed in Fig. 4 for catfish olfaction, preliminary unpublished data (J. Dudek and J. Caprio) indicate that both tyrosine and tryptophan are affected in a way similar to other hydrophobic amino acids during the adaptation experiments and have been tentatively assigned to the LCN transduction process. It is important to emphasize here that only the “specificities” of various amino acid transport systems are being compared with the olfactory specificity of the identified transduction processes of the catfish and not the “transport” process as a whole.

A number of amino acid competition studies involving mammalian transport systems also support the relative independence of the carriers for the acidic, basic, and neutral amino acids. These studies indicate further that the neutral amino acids are carried in a multiplicity of tissues by at least two (Oxender and Christensen, 1963; Oxender et al., 1977; Betz and Goldstein, 1978; Sepulveda and Smith, 1978; Sepulveda and Pearson, 1982) or three (Enders et al., 1976; Handlogten et al., 1981; Bass et al., 1982) transport systems, which have been termed, or at least are somewhat analogous to, the L, A, or ASC systems. The L system has a high affinity for the hydrophobic amino acids and corresponds to the specificity of the LCN olfactory transduction process in the catfish. However, the L-system is characterized by being relatively Na⁺ insensitive and BCH
inhibitable, and it is presently unknown whether these characteristics apply to the LCN process. The neutral hydrophilic amino acids (alanine, serine, threonine, and glutamine) are recognized by the Na⁺-dependent A and ASC transport systems in different tissues (Christensen et al., 1967; Enders et al., 1976; Sepulveda and Pearson, 1982), whose substrate specificities parallel those for the SCN olfactory transduction process. The distinguishing characteristics of these transport systems are that the ASC system is intolerant to N-methylation of its substrates (Christensen et al., 1967) and has strict Na⁺ specificity (Li⁺ cannot replace Na⁺ to any great extent) (Christensen and Handlogten, 1977). Whether the specificity observed for the SCN olfactory process indicates that the stimulus channel should be separated into two subsystems, analogous to the A and ASC transport systems, or to transport system specificities that do not exactly conform to the classical A and ASC pathways (Mircheff et al., 1982; Stevens et al., 1982), cannot be answered at present.

Since amino acid transport systems are found to be associated with numerous tissue types, including olfactory mucosa (Gross and Beidler, 1973), previous olfactory biochemical studies (Cagan and Zeiger, 1978; Cancalon, 1978; Rhein and Cagan, 1980, 1983; Brown and Hara, 1981, 1982) may have included an unknown percentage of binding of tritiated amino acids to transport molecules rather than olfactory receptor sites. However, the order of maximal binding for amino acids in the biochemical studies (Cagan and Zeiger, 1978; Cancalon, 1978) does reveal a significant correlation with amino acid rank order of effectiveness, as determined electrophysiologically (Hara, 1973; Caprio, 1978). Thus, even if olfactory receptor sites for amino acids are different from transport sites for these amino acids, as indicated for proline in bacteria (Ordal et al., 1978), then transport and olfactory acceptor site specificities are highly similar. Thus, the relatively simple and long-lasting in vivo olfactory receptor preparation described here may be utilized to investigate further the substrate specificities and effects of chemical agents on olfactory receptors in fishes and to predict the effects of these agents on amino acid transport systems in a wide variety of systems and organisms.

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