Amino Acid-Activated Channels in the Catfish Taste System

T. Kumazawa,* J. G. Brand,*#§ and J. H. Teeter*
*Monell Chemical Senses Center, #Department of Biochemistry, School of Dental Medicine, University of Pennsylvania, and §Veterans Affairs Medical Center, Philadelphia, Pennsylvania 19104 USA

ABSTRACT Membrane vesicles derived from external taste epithelia of channel catfish (*Ictalurus punctatus*) were incorporated into lipid bilayers on the tips of patch pipettes. Consistent with previous experiments (Teeter, J. H., J. G. Brand, and T. Kumazawa. 1990. *Biophys. J.* 58:253–259), micromolar (0.5–200 μM) concentrations of L-arginine (L-Arg), a potent taste stimulus for catfish, activated a nonselective cation conductance in some bilayers, which was antagonized by D-Arg. Two classes of L-Arg-gated receptor/channels were observed in reconstituted taste epithelial membranes: one with a unitary conductance of 40–60 pS, and the other with a conductance of 75–100 pS. A separate class of nonselective cation channels, with a conductance of 50–65 pS, was activated by high concentrations of L-proline (L-Pro) (0.1–3 mM), which is the range necessary to elicit neural responses in catfish taste fibers. The L-Pro-activated channels were not affected by either L- or D-Arg, but were blocked by millimolar concentrations of D-Pro. Conversely, neither L- nor D-Pro altered the activity of either class of L-Arg-activated channels, which were blocked by micromolar concentrations of D-Arg. These results are consistent with biochemical, neurophysiological, and behavioral studies indicating that taste responses of channel catfish to L-Arg are mediated by high-affinity receptors that are part of or closely coupled to nonselective cation channels directly gated by low concentrations of L-Arg, while responses to L-Pro are mediated by distinct, low-affinity receptors also associated with nonselective cation channels.

INTRODUCTION

Taste reception in vertebrates is mediated by specialized neuroepithelial receptor cells, typically grouped together in intraepithelial end organs, the taste buds. Interaction of sapid chemicals with receptors or ion channels primarily in the exposed, apical surfaces of taste receptor cells is coupled through cellular processes to the modulation of release of neurotransmitter at basolateral synapses with afferent taste fibers or adjacent taste bud cells. Recent efforts to characterize the molecular mechanisms that couple taste stimulus recognition with the generation of a cellular response and changes in the rate of release of neurotransmitter indicate that taste cell responses are mediated by several fundamentally different processes, depending upon the nature of the stimulus, the "type" of taste cell, and the species being studied (see Avenet et al., 1993; Kinnamon and Margolskee, 1996; Lindemann, 1996 for recent reviews).

Patch-clamp studies of taste receptor cells from amphibians (Avenet and Lindemann, 1987a; Bigiani and Roper, 1993; Bigiani et al., 1996; Fujiyama et al., 1994; Kinnamon and Roper, 1987, 1988a; McPheeters et al., 1994; Miyamoto et al., 1991; Sugimoto and Teeter, 1990), catfish (Tecter et al., 1993; Miyamoto, Sato, and Teeter, 1998, submitted for publication), and rodents (Akabas et al., 1988; Béhé et al., 1990; Cummings et al., 1993; Herness and Sun, 1995; Miyamoto et al., 1996; Spielman et al., 1989; Sun and Herness, 1996) have revealed a variety of voltage-dependent Na^+, Ca^{2+}, and K^+ currents. The roles of these conductances in generating and shaping taste cell responses are only beginning to be understood. However, it is clear that many taste cells generate action potentials in response to taste stimulation (Roper, 1983; Avenet and Lindemann, 1987b; Kinnamon et al., 1988; Béhé et al., 1990; Gilbertson et al., 1992, 1993).

Although differing in details, taste receptor mechanisms may be divided into two general categories: those involving the direct action of taste stimuli on ion channels, typically in the apical (exposed) membranes of the receptor cells, and those involving G-protein-coupled receptors, indirectly linked to changes in activity of ion channels in the basolateral membrane via second messenger cascades. Amiloride-sensitive, epithelial-type sodium channels in the apical membranes of some taste cells in mammals mediate responses to Na^+ and Li^+ salts (Schiffman et al., 1983; Heck et al., 1984; Brand et al., 1985; Avenet, 1988, 1992; Avenet and Lindemann, 1991; Hettinger and Frank, 1990). In hamster taste cells, sour (acid) taste appears to involve influx of protons through amiloride-sensitive sodium channels (Gilbertson et al., 1993). Apical K^+ channels have been implicated in taste cell responses to sour and some bitter-tasting stimuli in *Necturus* (Kinnamon and Roper, 1988b; Kinnamon et al., 1988; Cummings and Kinnamon, 1992; Kinnamon, 1992). Regulation of intracellular concentrations of cAMP and IP_3 by G-protein-linked receptors has been implicated in taste responses to sweeteners and some bitter substances (Avenet et al., 1988; Tonosaki and Funakoshi, 1988; Striem et al., 1989, 1991; Cummings et al., 1993,

Received for publication 22 December 1997 and in final form 7 September 1998.

Address reprint requests to Dr. John Teeter, Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104. Tel.: 215-898-5311; Fax: 215-898-2084; E-mail: teeter@pobox.upenn.edu.

T. Kumazawa's present address is Department of Environmental Engineering, Saitama Institute of Technology, 1690 Fusaijii, Okabe Saitama 369-02, Japan.

0006-3495/98/12/2757/10 $2.00

1996; Herness et al., 1997; Naim et al., 1991; Ruiz-Avila et al., 1995; Spielman et al., 1992, 1994, 1996; Bernhardt et al., 1996; Wong et al., 1996).

In the channel catfish, extraoral taste buds, present over most of the skin surface, have been shown by ligand-binding (see Krueger and Cagan, 1976; Cagan, 1986; Kalinoski et al., 1989a) and neural cross-adaptation (Caprio, 1975, 1978, 1982; Wegert and Caprio, 1991) studies to possess several independent classes of amino acid taste receptors. High-affinity receptors for short-chain neutral amino acids, the L-Ala receptors (L-AlaRs), appear to be coupled via G-proteins to the formation of both cAMP and IP_3 (Kalinoski et al., 1989b; Brand et al., 1991), although the subsequent steps leading to neurotransmitter release have not been characterized. In contrast, high-affinity receptors selectively activated by the basic amino acid L-arginine (L-ArgRs) do not appear to be coupled to second messenger formation (Brand et al., 1991), but rather appear to contain integral cation channels that open when L-Arg binds to the receptors (Teeter et al., 1990). In addition, low affinity receptors for L-proline (L-ProRs) have been proposed on the basis of neural recordings under cross-adaptation conditions (Wegert and Caprio, 1991). Single fiber recordings indicated that the responses to L-Pro were carried by a subset of the taste fibers selectively activated by L-Arg, suggesting either that L-ArgRs and L-ProRs are co-expressed in some of the same receptor cells or that L-Arg- and L-Pro-selective receptor cells synapse with the same fibers (Kohbara et al. 1992). Recent studies using PHA-E lectin, which interferes with L-Arg binding, and a monoclonal antibody (G-10), which inhibits L-Ala binding, as probes have shown differential localization of the corresponding putative receptors to separate classes of cells in the catfish taste bud (Finger et al., 1996).

In this paper, we present evidence that high concentrations of L-Pro, in the range necessary to elicit discharge in taste nerve fibers and trigger behavioral responses, activate a class of low affinity receptors, which are part of or closely associated with nonselective cation channels. These channels are similar to, but not identical with, channels coupled to a different class of receptors activated by low concentrations of L-Arg. In addition, we present evidence for two types of L-Arg-activated cation channels, displaying different unitary conductances and kinetics, and both differing from the receptor/channels activated by L-Pro. Preliminary reports of some of this work have been published (Teeter et al., 1990, 1992; Caprio et al., 1993).

METHODS

Membrane vesicles

A sedimentable fraction (P2), which was enriched in amino acid binding activity and marker enzymes for plasma membranes, was prepared by differential centrifugation of homogenates of taste epithelium, scraped from excised barbels of channel catfish as described previously (Krueger and Cagan, 1976; Cagan, 1986). A nucleotidase enriched fraction (QB0) of plasma membranes was prepared from fraction (P2) as described previously (Teeter et al., 1990). Briefly, P2 was centrifuged on a 30% (wt/wt) sucrose cushion at 36,000 *g* for 20 min. Samples of QB0 were washed to remove residual sucrose and sonicated briefly (< 30 s). All procedures were carried out at 4°C. Samples of QB0 could be stored for up to 3 d at 4°C with only gradual loss in the frequency with which amino acid-activated channels were encountered.

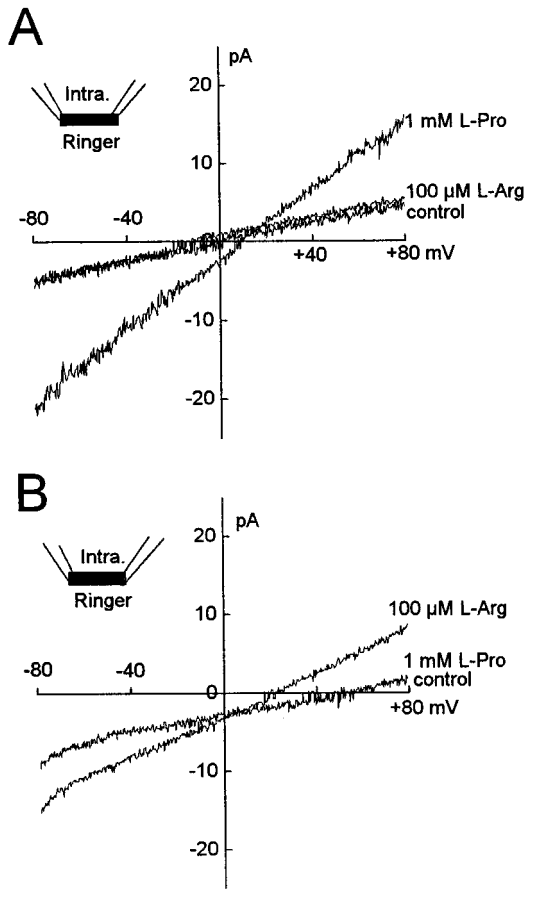

FIGURE 1 Currents elicited by voltage ramps from −80 to +80 mV across planar lipid bilayers formed on the tips of patch pipettes into which taste epithelial membranes derived from channel catfish had been fused. (*A*) With pseudointracellular solution in the pipette ("cytoplasmic" side) and FPS in the bath, the voltage ramp produced a small current. Addition of L-Arg to the bath to produce a final concentration of 100 μM had no affect on bilayer conductance. Subsequent addition of 1 mM L-Pro resulted in a marked increase in bilayer conductance. (*B*) In a different bilayer, 1 mM L-Pro produced no change in conductance, while the subsequent addition of 100 μM L-Arg resulted in an increase in bilayer conductance.

Solutions

Solutions were prepared from reagent-grade salts and filtered immediately before use (0.2 μm Acrodisc filters, Gelman Sciences, Ann Arbor, MI). Most experiments were performed with a fish physiological saline (FPS) containing (mM): 110 NaCl, 2.5 KCl, 1.6 $MgCl_2$, 1.0 $CaCl_2$, 5 MOPS, pH 7.4 in the bath and a pseudointracellular solution containing (mM): 12.5 NaCl, 85 KCl, 1.6 $MgCl_2$, 0.25 $CaCl_2$, 0.5 EGTA, 5 MOPS, pH 7.4 in the recording pipette. In some experiments various concentrations of NaCl, KCl, $CaCl_2$, $BaCl_2$, Na-gluconate, K-gluconate, or N-methyl-D-glucamine chloride (NMDG-Cl) were used in the bath and/or pipette. Azolectin (type II-S from soybeans) was obtained from Sigma Chemical Company, St. Louis, MO and palmitoyloleoyl phosphatidylethanolamine (POPE) and phosphatidylserine (PS) were obtained from Avanti Polar Lipids, Inc.,

Birmingham, AL and stored at -70°C. Lipids were prepared fresh for each experiment by dissolving dried lipid in analytical grade hexane (Nanograde, Mallinckrodt Inc., St. Louis, MO) at a concentration of 30 mg/ml for azolectin and 10 mg/ml for a 7:3 mixture of POPE:PS. Amino acid solutions were prepared from 10 mM stock solutions and filtered just before use. Bath solution changes were accomplished either using a gravity-flow perfusion system or by carefully moving the bilayer (pipette tip) to a new chamber containing the appropriate solution. Amino acid stimuli were added to the chamber with a micropipette to produce the desired final concentration. Aliquots of stimuli were sequentially added to the recording chamber to provide an ascending series of concentrations. Experiments were performed at room temperature.

Bilayer recordings and data analysis

Membrane vesicles were incorporated into high resistance bilayers on the tips of patch pipettes using minor modifications of the technique described by Coronado and Latorre (1983). A lipid monolayer was formed on the surface of a small (300 μl) plastic chamber containing membrane vesicles suspended in FPS (30–60 μg protein), by carefully adding 5–10 μl of azolectin or POPE:PS (7:3). After allowing a minimum of 10 min for the solvent to evaporate, high-resistance bilayers were formed on a pipette tip by inserting it into the chamber under positive pressure, releasing the pressure, withdrawing the pipette, then reinserting it. Bilayers with resistances of 2 to >50 GΩ were routinely obtained. The bilayers in these experiments may be thought of as "outside-out" patches because taste stimuli were added only to solutions in the bath, not to the internal solutions in the pipette. Although putative receptors for amino acids may also have been incorporated into the bilayer in the opposite polarity (into the pipette), they would not have been activated in these experiments. Spontaneous, voltage-dependent and ligand-activated conductances were detected by recording the currents produced by voltage steps or ramps across the bilayer in the absence and presence of amino acid taste stimuli. "Control" currents, resulting from voltage ramps applied before addition of amino acid taste stimuli are presented in all records, except those in Figs. 5 and 6, where they have been subtracted from conductances recorded in the presence of stimuli for clarity.

Patch pipettes were pulled from borosilicate glass capillaries (Kimex, Thomas Scientific, Philadelphia, PA) using a horizontal puller (P-80/PC, Sutter Instruments, San Rafael, CA). The pipettes had bubble numbers of 2.5–4.5. Bilayer and single channel currents were recorded under voltage-clamp using a LIST EPC7 patch amplifier (Medical Systems Corp., Greenvale, NY). Current signals were amplified and low-pass filtered at 1000 Hz (eight pole Bessel filter; Frequency Devices, Inc., Haverhill, MA). Currents were digitized (4–10 kHz) and stored on the fixed disk of a 386 or 486 computer running pCLAMP software (Axon Instruments, Foster City, CA). Voltage ramps and pulses were generated with a D/A converter under computer control.

RESULTS

L-Pro- and L-Arg-activated conductances

Lipid bilayers formed from either azolectin or POPE:PS had resistances in excess of 2 GΩ, often 30–50 GΩ. Bilayers formed in the absence of membrane vesicles (n = 8) displayed no changes in conductance in response to voltage ramps (−80 to +80 mV) when either L-Pro (1–2 mM) or L-Arg (100–500 μM) was added to the bath ("outside" the bilayer). In addition, the majority of the bilayers into which

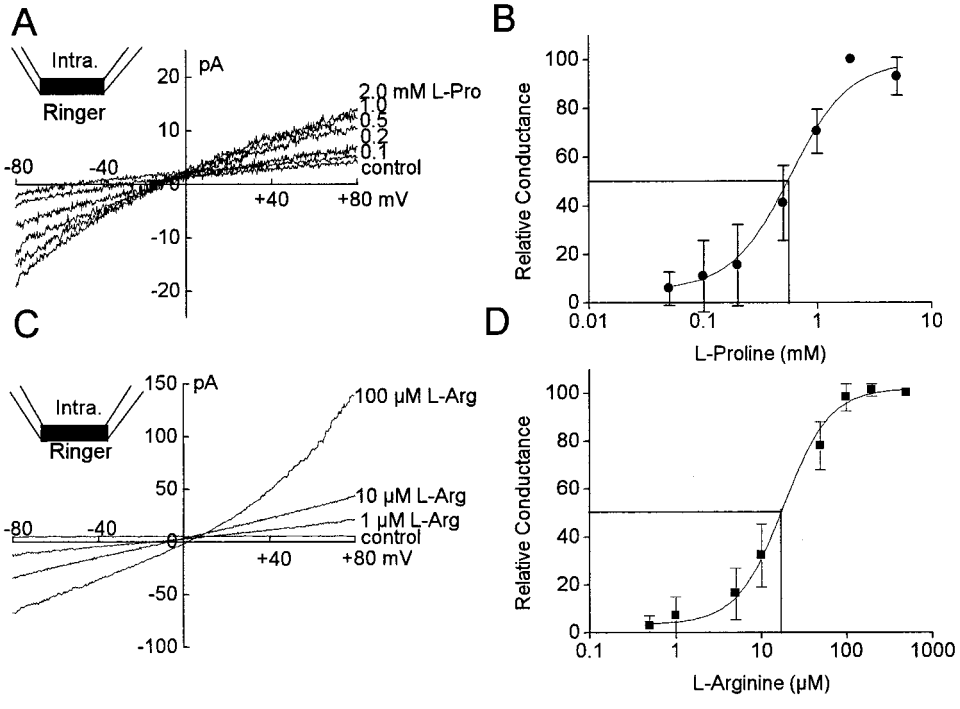

FIGURE 2 Concentration dependence of L-Pro- and L-Arg-induced conductances. (*A*) Successive addition of L-Pro to the bath, producing the concentrations shown at the right of each current record, resulted in a progressive increase in bilayer conductance. (*B*) Plot of relative conductance (as a percentage of the maximum conductance elicited by 5 mM L-Pro) as a function of log L-Pro concentration. The solid curve represents the fit of the data with the Hill equation. The conductance activated near 0.1 mM, reached half-maximal at 0.56 mM, and saturated above 2 mM L-Pro (mean ± SE, n = 3). The Hill coefficient was 1.7. (*C*) In a different bilayer, increasing concentrations of L-Arg resulted in progressive increases in bilayer conductance. (*D*) Dose-response curve for L-Arg. The solid curve represents the best fit using the Hill equation. The conductance activated above 0.5 μM, reached half-maximal at 17 μM, and saturated above 200 μM (mean ± SE, n = 5). The Hill number was 2.5.

membrane vesicles had been fused also displayed no change in conductance when either L-Pro or L-Arg was added to the bath, confirming that neither amino acid, at the concentrations used in this study, elicited a nonspecific increase in bilayer conductance. In ~15% of the taste membrane-containing bilayers exposed to L-Pro (28 of 180 bilayers), concentrations >~0.1 mM elicited increases in bilayer conductance (Fig. 1 *A*). In the example presented in Fig. 1 *A*, 100 μM L-Arg was first added to the bath solution, resulting in no change in bilayer conductance. This concentration of L-Arg has previously been shown to elicit a near-saturating increase in conductance in L-Arg-sensitive bilayers (Teeter et al., 1990, and Fig. 2 *D*). The subsequent addition of 1 mM L-Pro resulted in a significant increase in bilayer conductance. In ~25% of the taste membrane-containing bilayers exposed to L-Arg (55 of 210 bilayers), concentrations >0.5 μM produced an increase in bilayer conductance (Fig. 1 *B*), confirming our earlier observations (Teeter et al., 1990). Previous exposure of the bilayer represented in Fig. 1 *B* to 1 mM L-Pro had resulted in no change in conductance. These results indicated that the increases in bilayer conductance elicited by L-Arg and L-Pro were mediated by different receptors associated with taste epithelial membranes. Although no attempt was made to characterize in detail the effects of other amino acids on the L-Pro- or L-Arg-activated conductances, neither L-Ala nor glycine altered the conductance of any of over 25 bilayers, including those displaying increases in conductance with subsequent addition of L-Arg or L-Pro (data not shown). Binding studies have shown that the L-Arg site is highly selective for L-Arg and closely related analogs, whereas L-Ala and glycine interact with a different, apparently G-protein-coupled, site (Cagan, 1986; Bryant et al., 1989; Kalinoski et al., 1989a, b).

Both the L-Pro- and L-Arg-activated conductances were concentration-dependent, with increasing concentrations producing progressively larger increases in bilayer conductance (Fig. 2, *A* and *C*). The L-Pro-dependent conductance activated at ~100 μM, reached half-maximal activation (K_m) at 567 μM and saturated between 2 and 3 mM (Fig. 2 *B*). The Hill coefficient for the L-Pro-dependent conductance was 1.7, suggesting cooperativity in the binding to and/or opening of the associated channels. The L-Arg-gated conductance was considerably more sensitive, activating between 0.5 and 1 μM, reaching half-maximal activation at 17 μM and saturating between 100 and 200 μM (Fig. 2 *D*). The Hill coefficient for the L-Arg conductance varied from 2 to 3, depending upon the preparation, again suggesting cooperativity in channel activation.

Stereospecificity of L-Pro- and L-Arg-mediated conductances

Although the D-enantiomers of Pro and Arg produced no observable changes in bilayer conductance, even at concentrations well above those at which the L-enantiomers elicited responses, they did suppress the increases in conductance produced by their respective L-isomers (Fig. 3, *A* and *B*). The suppression of conductance was concentration-dependent, with increasing concentrations of the D-isomer resulting in successive blockage of bilayer conductance (Fig. 4). In the example illustrated in Fig. 4 *A*, the increase in conductance elicited by 1 mM L-Pro was progressively blocked by incremental increases in the concentration of D-Pro with an IC_{50} of 175 μM (Fig. 4 *B*). A similar suppression of the L-Arg-induced conductance by D-Arg was reported previously (Teeter et al., 1990). In the example shown in Fig. 4 *C*, the conductance resulting from the addition of 10 μM L-Arg was progressively blocked by increasing concentrations of D-Arg. The IC_{50} for the suppression in this example was 6 μM D-Arg (Fig. 4 *D*).

Ionic selectivity of L-Pro- and L-Arg-activated conductances

With FPS in the bath and pseudointracellular solution in the pipette, the L-Pro-dependent conductance reversed between −18 and +32 mV, with a mean reversal of +4.7 ± 6.1 mV

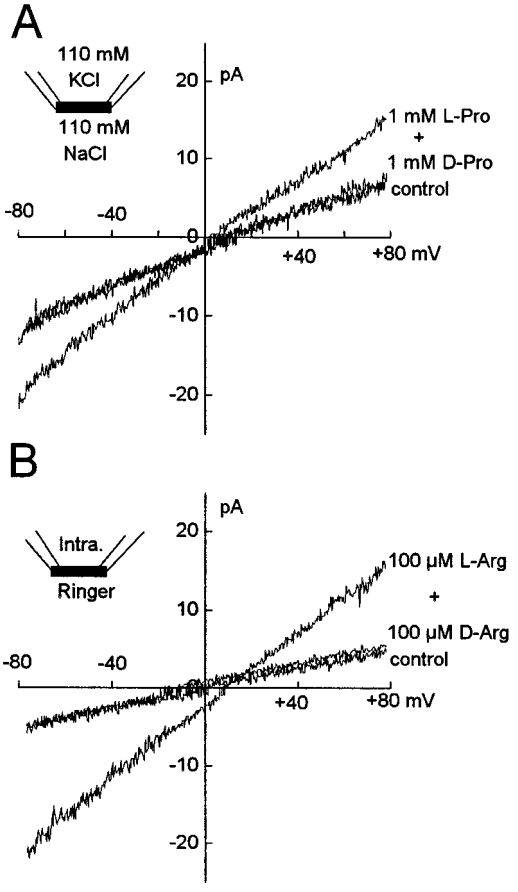

FIGURE 3 The conductances produced by L-Pro and L-Arg were blocked by the respective D-isomers. (*A*) The increase in conductance elicited by 1 mM L-Pro in this bilayer was rapidly blocked by subsequent addition of 1 mM D-Pro to the bath. (*B*) The conductance activated by 100 μM L-Arg in a different bilayer was completely suppressed by the addition of 100 μM D-Arg.

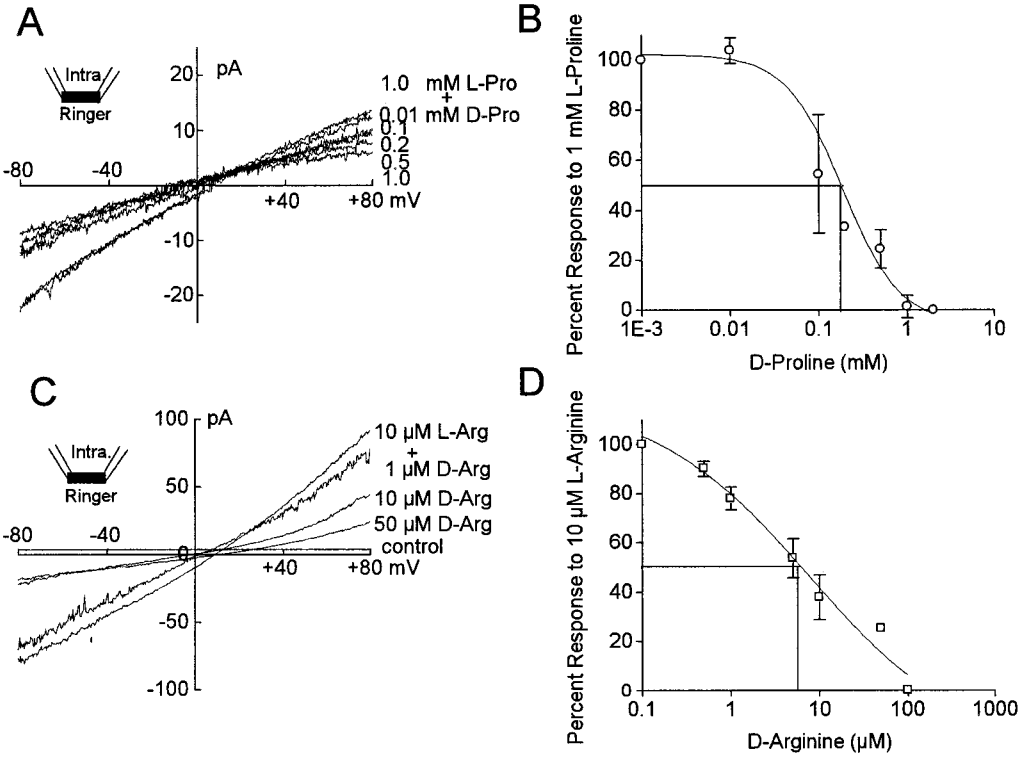

FIGURE 4 Concentration dependence of the suppression of the L-Pro- and L-Arg-dependent conductances by their respective D-isomers. (*A*) The increase in bilayer conductance produced by 1 mM L-Pro was progressively suppressed by the successive addition of increasing concentrations of D-Pro to the bath. (*B*) Plot of the percent response to 1 mM L-Pro as a function of D-Pro concentration. The dose-response curve is a Hill equation fit of the data. Half-maximal suppression (IC_{50}) was 175 μM (mean ± SE, $n = 3$). (*C*) In a different bilayer, the increase in conductance produced by 10 μM L-Arg was progressively reduced by increasing concentrations of D-Arg. (*D*) Plot of percent response to 10 μM L-Arg as a function of D-Arg concentration. The solid curve represents the best fit of the data points with the Hill equation. The IC_{50} was 6 μM (mean ± SE, $n = 4$).

(mean ± SD, $n = 13$; Figs. 1 *A* and 3 *A*), suggesting that the underlying channels were relatively nonselective between Na^+ and K^+. In the same solutions, the L-Arg-gated conductance reversed at +8.5 ± 5.3 mV ($n = 19$; Figs. 1 *B* and 3 *B*). Both the L-Pro-dependent and L-Arg-dependent conductances were permeable to divalent cations (Figs. 5 and 6,

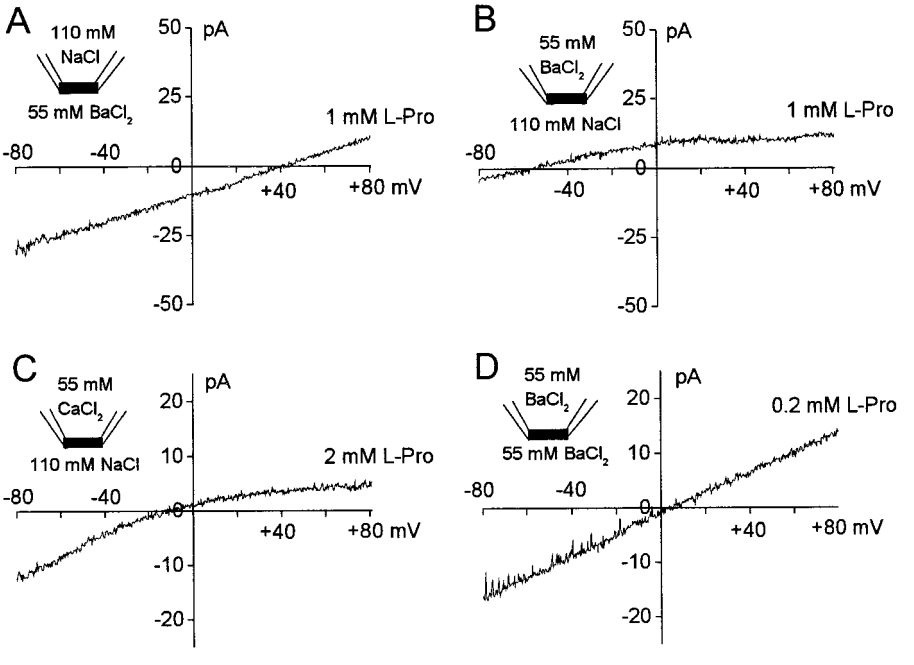

FIGURE 5 Increases in conductance produced by L-Pro under different ionic conditions. The currents elicited by voltage ramps before the addition of L-Pro have been subtracted from each trace to make a determination of the reversal potential. (*A*) With 110 mM NaCl in the pipette and 55 mM $BaCl_2$ in the bath, the conductance produced by 1 mM L-Pro in this bilayer reversed at +42 mV (39.0 ± 6.0 mV; mean ± SD, $n = 2$). (*B*) In a different bilayer, with the solutions reversed (55 mM $BaCl_2$ in the pipette and 110 mM NaCl in the bath), the L-Pro-induced conductance reversed at −55 mV (−38.2 ± 14.7 mV, $n = 4$). (*C*) With 55 mM $CaCl_2$, rather than $BaCl_2$ in the pipette, and 110 mM NaCl in the bath, the L-Pro conductance in a different bilayer reversed at −7 mV (+2.3 ± 9.3 mV, $n = 3$). (*D*) With 55 mM $BaCl_2$ on both sides of the bilayer, the L-Pro conductance reversed near 0 mV (+1.0 ± 5.0 mV, $n = 4$).

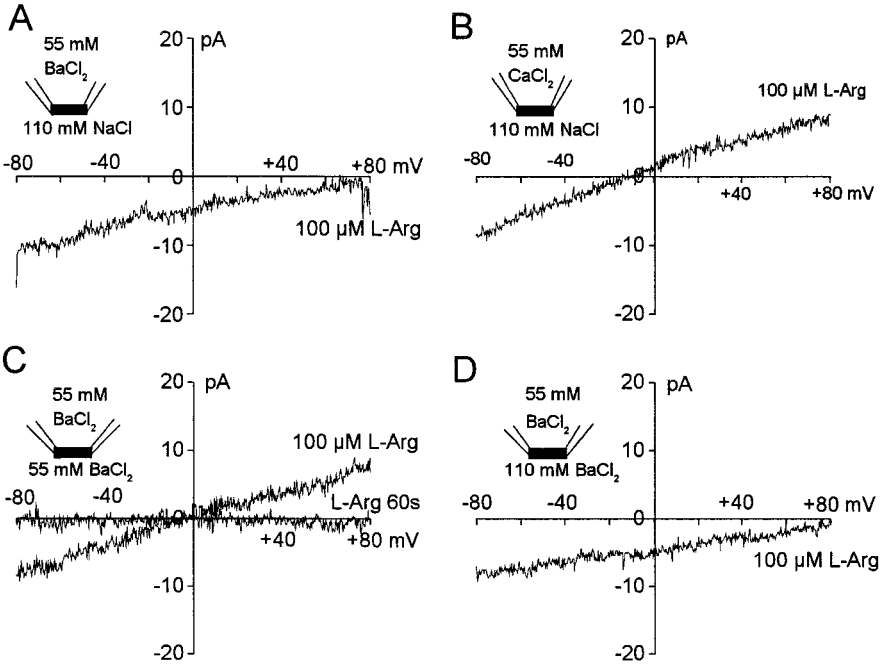

FIGURE 6 Increases in conductance elicited by L-Arg under different ionic conditions. Control currents produced by voltage ramps before the addition of L-Arg have been subtracted from each trace. (*A*) With 55 mM $BaCl_2$ in the pipette and 110 mM NaCl in the bath, the L-Arg conductance in this bilayer had an extrapolated reversal potential of +80 mV (+62.0 ± 23.1 mV, $n = 5$). (*B*) With 55 mM $CaCl_2$ rather than $BaCl_2$ in the pipette and 110 mM NaCl in the bath, the L-Arg conductance reversed at −8 mV (−7.0 ± 5.5 mV, $n = 3$). (*C*) With 55 mM $BaCl_2$ on both sides of the membrane, the L-Arg conductance reversed near 0 mV (+2.4 ± 6.8 mV, $n = 5$). A second ramp applied 60 s after the addition of 100 μM L-Arg resulted in no current, indicating that the L-Arg-dependent conductance had inactivated or desensitized. (*D*) Increasing the concentration of $BaCl_2$ in the bath to 110 mM shifted the extrapolated reversal of the L-Arg conductance to +80 mV (+80.3 ± 4.5 mV, $n = 3$).

respectively), which probably accounts for the slightly positive reversal potentials in FPS containing 1 mM Ca^{2+}. With 110 mM NaCl in the pipette ("cytoplasmic" side of the bilayer) and 55 mM $BaCl_2$ in the bath, the L-Pro-activated conductance reversed at +39.0 ± 6.0 (mean ± SD, $n = 2$), indicating that the channels were more permeable to Ba^{2+} than to Na^+ (+42 mV in the example in Fig. 5 *A*). With the solutions reversed, the L-Pro response in a different bilayer reversed at −38.2 ± 14.7 mV, $n = 4$, consistent with this interpretation (−55 mV in the example in Fig. 5 *B*). With 55 mM $CaCl_2$ in the pipette and 110 mM NaCl in the bath, the L-Pro-induced conductance reversed at +2.3 ± 9.3 mV, $n = 3$, indicating that the L-Pro-gated channels were less permeable to Ca^{2+} than Ba^{2+} (−7 mV in Fig. 5 *C*). The rectification in the current trace often observed with high divalent cations on the "external" side of the bilayer indicated some degree of divalent block of the conductance. With equimolar $BaCl_2$ on the two sides of the bilayer, the conductance produced by L-Pro reversed near 0 mV (+1.0 ± 5.0 mV, $n = 4$), as expected (Fig. 5 *D*).

In contrast to the L-Pro-activated conductance, the conductance elicited by L-Arg appeared to be more permeable to monovalent than divalent cations (compare Fig. 6 *A* with Fig. 5 *B*). With 110 mM NaCl in the bath and 55 mM $BaCl_2$ in the pipette, the current reversed at +62.0 ± 23.1 mV, $n = 5$; extrapolated to +80 mV in Fig. 6 *A*. Also, with Ca^{2+} rather than Ba^{2+} in the pipette, the current reversed at −7.0 ± 5.5 mV, $n = 3$ (−8 mV in Fig. 6 *B*) rather than +80 mV (compare Fig. 6, *A* and *B*). With equimolar $BaCl_2$ on the two sides of the bilayer, the L-Arg-induced conductance reversed near 0 mV (+2.4 ± 6.8 mV, $n = 5$; Fig. 6 *C*), and this was shifted to a much more positive potential when the concentration of $BaCl_2$ in the bath was increased to 110 mM (+80.3 ± 4.5 mV, $n = 3$; Fig. 6 *D*).

L-Pro- and L-Arg-activated single channels

Current fluctuations that appeared to represent single L-Pro-activated channels were observed in many of the bilayers displaying an increase in conductance with the addition of L-Pro. The unitary conductance of these channels averaged 59.8 ± 1.9 pS (mean ± SD, $n = 5$), with FPS in the bath and pseudointracellular solution in the pipette (48 pS in the example in Fig. 7 *A*). Under the same ionic conditions, the predominant class of L-Arg-gated channels had a mean conductance of 45.5 ± 0.7 pS ($n = 7$) (Fig. 7 *B*), although larger channels were sometimes observed (see below).

The increase in bilayer conductance elicited by L-Pro was generally smaller than that evoked by L-Arg (compare Fig. 2, *A* and *C*). This presumably resulted from incorporation of a smaller number of L-Pro-sensitive channels in each bilayer, suggesting a lower overall density of L-Pro channels in the native membrane fragments. This is consistent with the observation that L-Pro often activated one or a few channels in a bilayer, while L-Arg-sensitive bilayers often displayed multiple channels (Fig. 8). On the basis of the total L-Arg-activated current and an estimated single channel conductance of 45 pS, L-Arg-sensitive bilayers often contained >30 channels. A plot of open probability (NP_o) over time for a bilayer exposed to L-Pro is shown in Fig. 9. During the 260 s exposure to 1 mM L-Pro, the open probability displayed a significant decrease, indicating either desensitization of the receptors or "run-down" of the channels; L-Arg-activated currents often displayed even more marked desensitization. For example, the conductance elicited by 100 μM L-Arg in Fig. 6 *C* completely inactivated within 60 s of continuous exposure to the L-Arg. However, in other bilayers, L-Arg activated single-channel currents continued to burst for many minutes (Figs. 8 and 10). In the

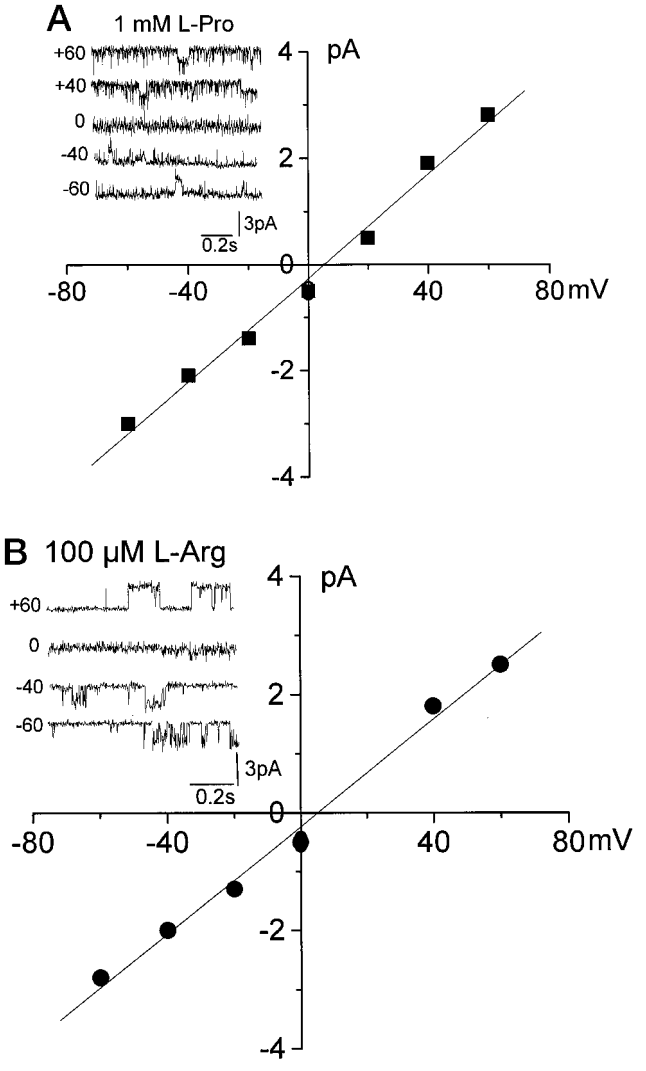

FIGURE 7 Single currents produced by L-Pro and L-Arg. (*A*) Single channel currents produced by 1 mM L-Pro (*inset*). The solid line is the least-squares fit of the current-voltage data. The slope conductance was 48 pS and the current reversed at +6 mV with intracellular solution in the pipette and FPS in the bath. (*B*) Single channel currents elicited by 100 μM L-Arg in a different bilayer. With intracellular solution in the pipette and FPS in the bath, the slope conductance was 45 pS and reversed at +5 mV.

example shown in Fig. 8, seven of the estimated 15 channels activated by L-Arg in this bilayer were observed over a period of several minutes. Occasional current transitions across multiple levels (*arrow*) were sometimes observed, suggesting cooperativity in the opening of L-Arg-gated channels. These transitions could usually be distinguished from the larger conductance L-Arg-activated channels (Figs. 10 and 11) on the basis of slight deflections in the current trace, which could be observed at high time resolution between the expected current levels.

Two classes of L-Arg-activated channels

Two distinct classes of channels were observed in some, but not all, bilayers displaying increased conductance in the presence of L-Arg (Fig. 10). The most frequently observed channels corresponded to those described previously (Teeter et al., 1990), having a unitary conductance of 40–60 pS (smaller currents in Fig. 10). In addition, channels with a higher unitary conductance were observed in response to application of L-Arg in ~20% of the L-Arg-sensitive bilayers, often together with the lower conductance channels (Figs. 10 and 11). With FPS in the bath and pseudointracellular solution in the pipette, the higher conductance channels had unitary conductances ranging from 75 to 100 pS (81.7 $\pm$ 10.1 pS, $n = 7$). Both classes of channels were activated by similar (micromolar) concentrations of L-Arg and both were blocked by comparable concentrations of D-Arg (Fig. 11). Dose-response relationships for L-Arg activation and D-Arg suppression of the larger class of channels were not determined.

DISCUSSION

Two classes of high-affinity receptors for amino acids have been postulated in the channel catfish taste system on the basis of ligand binding (Cagan, 1986; Kalinoski et al., 1989a; Bryant et al., 1989; Brand et al., 1991) and neural cross-adaptation studies (Caprio, 1982; Caprio et al., 1993). One class of receptors binds L-Ala and other short-chain neutral amino acids and appears to be coupled via G-proteins to the formation of intracellular second messengers (Kalinoski et al., 1989b). A second class of receptors, specific for L-Arg, appears to be part of or closely associated with nonselective cation channels that are directly gated by L-Arg binding (Teeter et al., 1990, 1992; Brand et al., 1991). In addition, a low-affinity receptor system activated by high concentrations of L-Pro has been identified in neural recordings (Kohbara et al., 1992; Wegert and Caprio, 1991). Although the low sensitivity of the catfish taste system to L-Pro has precluded attempts to characterize an L-Pro receptor by ligand binding studies, recordings from single facial taste nerve fibers indicate that L-Pro taste information is carried by a subset of the L-Arg best-taste fibers (Kohbara et al., 1992).

The presence of separate classes of cation channels in bilayers into which channel catfish taste epithelial membranes have been incorporated that are activated by concentrations of L-Arg and L-Pro in the ranges necessary to elicit neural responses in channel catfish taste fibers suggests that these receptor/channels are involved in taste reception. Although the membrane preparations used in these reconstitution experiments (and in biochemical binding and second messenger studies) were not a pure preparation of taste cell membranes, the close correspondence between the results of binding studies, neural recordings, and receptor/channel reconstitution studies support the conclusion that these channels play a role in taste reception in the channel catfish. Whole-cell patch-clamp recordings (Miyamoto, Sato, and Teeter, submitted for publication) and fura-2 measurements

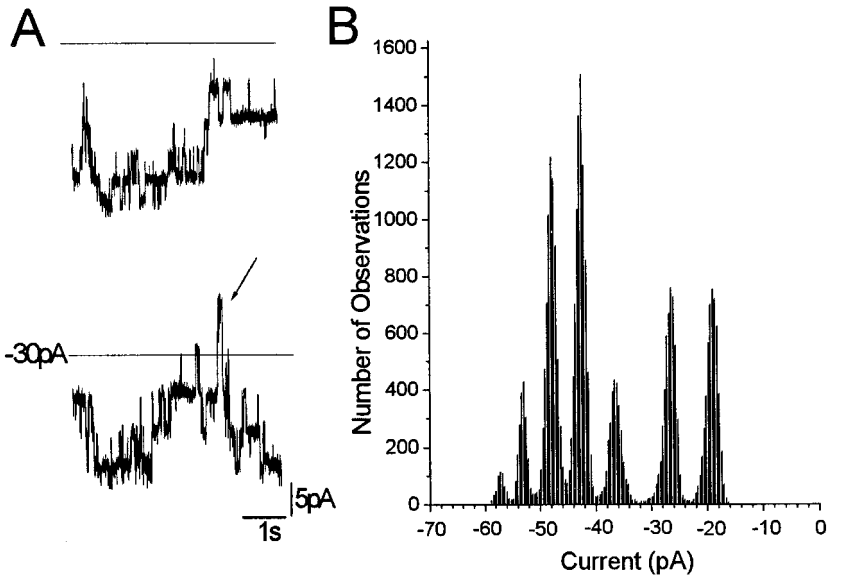

FIGURE 8 Continuous current records of channel activity activated by 100 μM L-Arg in a bilayer with 55 mM $BaCl_2$ in the bath and pipette. Seven of an estimated 15 channels are apparent in these traces. The holding potential was +80 mV.

of stimulus-induced changes in intracellular Ca^{2+} concentration (Zviman et al., 1996) in isolated channel catfish taste cells are also consistent with the presence of L-Arg-activated cation channels that are permeable to Ca^{2+} in some taste cells, but also indicate the involvement of additional mechanisms in L-Arg taste responses, including membrane hyperpolarization resulting from activation of a K^+ conductance.

The finding that L-Pro selectively activates a separate class of nonselective cation channels is consistent with the hypothesis that L-Pro receptors are expressed on a subset of the taste cells responsive to L-Arg. Although it is clear from this study that the receptors mediating the responses to L-Pro and L-Arg are different, we did not systematically investigate the frequency with which both classes of receptors were present in the same bilayers, because activation of one class of receptors may have masked the effect of the application of a second potential stimulus. Thus, if a bilayer was activated by L-Pro, it was typically not exposed to L-Arg, although on several occasions L-Pro-gated channels were exposed to D-Arg with no effect.

Ligand binding studies with taste epithelial membranes from the channel catfish indicate that there are two L-Arg binding sites (or affinity states); one with an apparent K_D of 20 nM, the other with a K_D of 1.5 μM (Kalinoski et al., 1989a; Bryant et al., 1989). Although two classes of L-Arg-sensitive channels differing in unitary conductance were observed in the present study, no clear correlation between high- and low-conductance L-Arg channels, and sensitivity to either L- and D-Arg or the rate of desensitization, were apparent.

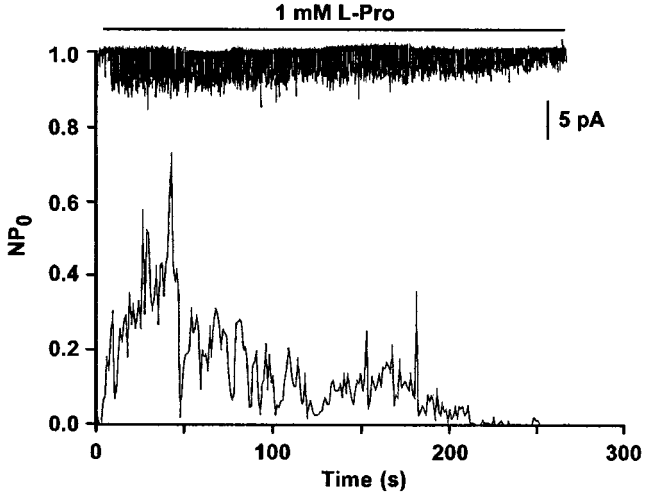

FIGURE 9 Plot of open probability (NP_o) over time for channels activated by 1 mM L-Pro (*inset*). NP_o decreased as a function of time of exposure of the bilayer to L-Pro.

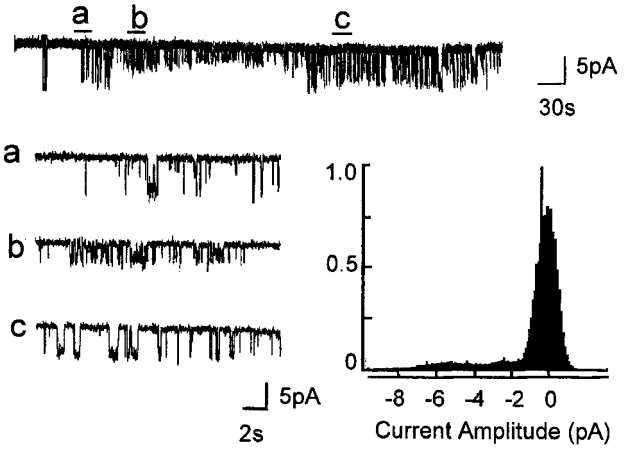

FIGURE 10 Two distinct classes of channels were activated by L-Arg in some bilayers. Both large (75 pS) and small (45 pS) single channel currents were activated in this bilayer by 100 μM L-Arg. Pseudointracellular solution in the pipette and FPS in the bath; holding potential = −80 mV.

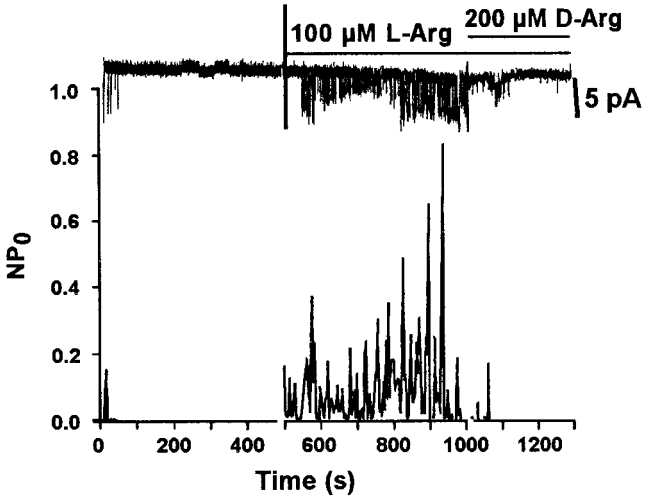

FIGURE 11 Plot of NP_o as a function of time for a bilayer containing both large conductance and small conductance channels activated by L-Arg. The addition of 100 μM L-Arg to the bath resulted in activation of both classes of channels; the subsequent addition of 200 μM D-Arg resulted in the block of both classes of channels. The intracellular solution is in the pipette and FPS in the bath. Holding potential = −80 mV.

We thank Drs. D. Restrepo and D. L. Kalinoski for many productive discussions on receptor mechanisms and for critically reading the manuscript. We thank Dr. M. Zviman for assistance with data analysis and acknowledge Drs. S. Wegert and J. Caprio for their suggestions and for sharing unpublished data with us.

This work was supported by National Institute on Deafness and other Communication Disorders Grants DC01838 and DC00356, National Science Foundation Grant IBN92-09743, and a grant from the Department of Veterans Affairs.

REFERENCES

Akabas, M. H., J. Dodd, and Q. Al-Awqati. 1988. A bitter substance induces a rise in intracellular calcium in a subpopulation of rat taste cells. *Science.* 242:1047–1050.

Avenet, P. 1988. Amiloride blockable sodium currents in isolated taste receptor cells. *J. Membr. Biol.* 105:245–255.

Avenet, P. 1992. Role of amiloride sensitive sodium channels in taste. *In* Sensory Transduction. D. Corey and S. D. Roper, editors. Rockefeller University Press, New York. 271–279.

Avenet, P., F. Hofmann, and B. Lindemann. 1988. Transduction in isolated taste receptor cells requires cAMP-dependent protein kinase. *Nature.* 331:351–354.

Avenet, P., S. C. Kinnamon, and S. D. Roper. 1993. Peripheral transduction mechanisms. *In* Mechanisms of Taste Transduction. S. A. Simon and S. D. Roper, editors. CRC Press, Boca Raton, FL.

Avenet, P., and B. Lindemann. 1987a. Patch-clamp study of isolated taste receptor cells of the frog. *J. Membr. Biol.* 97:223–240.

Avenet, P., and B. Lindemann. 1987b. Action potentials in epithelial taste receptor cells induced by mucosal calcium. *J. Membr. Biol.* 95:265–269.

Avenet, P., and B. Lindemann. 1991. Noninvasive recording of receptor cell action potentials and sustained currents from single taste bud cells maintained in the tongue—the response to mucosal NaCl and amiloride. *J. Membr. Biol.* 124:33–41.

Béhé, P. H., J. A. DeSimone, P. Avenet, and B. Lindemann. 1990. Membrane currents in taste cells of the rat fungiform papilla. *J. Gen. Physiol.* 96:1061–1084.

Bernhardt, S. J., M. Naim, U. Zehavi, and B. Lindemann. 1996. Changes in IP3 and cytosolic $Ca2^+$ in response to sugars and non-sugar sweeteners in transduction of sweet taste in the rat. *J. Physiol. (Lond.).* 490:325–336.

Bigiani, A., D.-J. Kim, and S. D. Roper. 1996. Membrane properties and cell ultrastructure of taste receptor cells in *Necturus* lingual slices. *J. Neurophysiol.* 75:1944–1956.

Bigiani, A., and S. D. Roper. 1993. Identification of electrophysiologically distinct cell subpopulations in *Necturus* taste buds. *J. Gen. Physiol.* 102:143–170.

Brand, J. G., J. H. Teeter, T. Kumazawa, T. Huque, and D. L. Bayley. 1991. Transduction mechanism for the taste of amino acids. *Physiol. Behav.* 49:899–904.

Brand, J. G., J. H. Teeter, and W. L. Silver. 1985. Inhibition by amiloride of chorda tympani responses evoked by monovalent salts. *Brain Res.* 334:207–214.

Bryant, B. P., S. Harpaz, and J. G. Brand. 1989. Structure/activity relationships in the arginine chemoreceptive taste response of the channel catfish. *Chem. Senses.* 14:805–815.

Cagan, R. H. 1986. Biochemical studies of taste sensation. XII. Specificity of binding of taste ligands to a sedimentable fraction from catfish taste tissue. *Comp. Biochem. Physiol.* 85A:355–358.

Caprio, J. 1975. High sensitivity of catfish taste receptors to amino acids. *Comp. Biochem. Physiol.* 123:357–371.

Caprio, J. 1978. Olfaction and taste in channel catfish: an electrophysiological study of the responses to amino acids and derivatives. *J. Comp. Physiol.* 123:357–371.

Caprio, J. 1982. High sensitivity and specificity of olfactory and gustatory receptors of catfish to amino acids. *In* Chemoreception in Fishes. T. J. Hara, editor. Elsevier/North Holland, Amsterdam. 109–134.

Caprio, J., J. G. Brand, J. H. Teeter, T. Valentincic, D. L. Kalinoski, J. Kohbara, T. Kumazawa, and S. Wegert. 1993. The taste system of the channel catfish: from biophysics to behavior. *Trends Neurosci.* 16: 192–197.

Coronado, R., and R. LaTorre. 1983. Phospholipid bilayers made from monolayers on patch-clamp pipettes. *Biophys. J.* 43:231–236.

Cummings, T. A., C. Daniels, and S. C. Kinnamon. 1996. Sweet taste transduction in hamster: sweeteners and cyclic nucleotides depolarize taste cells by reducing a K^+ current. *J. Neurophysiol.* 75:1256–1263.

Cummings, T. A., and S. C. Kinnamon. 1992. Apical K^+ channels in *Necturus* taste cells: modulation by intracellular factors and taste stimuli. *J. Gen. Physiol.* 99:591–613.

Cummings, T. A., J. Powell, and S. C. Kinnamon. 1993. Sweet taste transduction in hamster taste cells: evidence for the role of cyclic nucleotides. *J. Neurophysiol.* 70:2326–2336.

Finger, T. E., B. P. Bryant, D. L. Kalinoski, J. H. Teeter, B. Böttger, W. Grosvenor, R. H. Cagan, and J. G. Brand. 1996. Differential localization of putative amino acid receptors in taste buds of the channel catfish, *Ictalurus punctatus. J. Comp. Neurol.* 373:129–138.

Fujiyama, R., T. Miyamoto, and T. Sato. 1994. Differential distribution of two Ca^{2+}-dependent and -independent K^+ channels throughout receptive and basolateral membranes of bullfrog taste cells. *Pfluegers Arch.* 429:285–290.

Gilbertson, T. A., P. Avenet, S. C. Kinnamon, and S. D. Roper. 1992. Proton currents through amiloride-sensitive Na^+ channels in hamster taste cells: role in sour taste transduction. *J. Gen. Physiol.* 100:803–824.

Gilbertson, T. A., S. D. Roper, and S. C. Kinnamon. 1993. Proton currents through amiloride-sensitive Na^+ channels in isolated hamster taste cells: enhancement by vasopressin and cAMP. *Neuron.* 10:931–942.

Heck, G. L., S. Mierson, and J. A. DeSimone. 1984. Salt taste transduction occurs through an amiloride sensitive sodium transport pathway. *Science.* 223:403–405.

Herness, M. S., and X.-D. Sun. 1995. Voltage-dependent sodium currents from dissociated rat taste cells. *J. Membr. Biol.* 146:73–84.

Herness, M. S., X-D. Sun, and Y. Chen. 1997. CAMP and forskolin inhibit potassium currents in rat taste receptor cells by different mechanisms. *Am. J. Physiol.* 272:C2005–C2018.

Hettinger, T. P., and M. Frank. 1990. Specificity of amiloride inhibition of hamster taste responses. *Brain Res.* 513:24–34.

Kalinoski, D. L., B. P. Bryant, G. Shaulsky, J. G. Brand, and S. Harpaz. 1989a. Specific L-arginine taste receptor sites in the catfish, *Ictalurus*

punctatus: biochemical and neurophysiological characterization. *Brain Res.* 488:163–173.

Kalinoski, D. L., T. Huque, V. L. LaMorte, and J. G. Brand. 1989b. Second-messenger events in taste. *In* Chemical Senses: Receptor Events and Transduction in Taste and Olfaction. J. G. Brand, J. H. Teeter, R. H. Cagan, and M. R. Kare, editors. Marcel Dekker, New York. 85–101.

Kinnamon, S. C. 1992. Role of K^+ channels in taste transduction. *In* Sensory Transduction. D. P. Corey and S. D. Roper, editors. The Rockefeller University Press, New York. 261–270.

Kinnamon, S. C., V. E. Dionne, and K. G. Beam. 1988. Apical localization of K+ channels in taste cells provides the basis for sour taste transduction. *Proc. Natl. Acad. Sci. USA.* 85:7023–7927.

Kinnamon, S. C., and R. F. Margolskee. 1996. Mechanisms of taste transduction. *Curr. Opin. Neurobiol.* 6:506–513.

Kinnamon, S. C., and S. D. Roper. 1987. Passive and active membrane properties of mudpuppy taste receptor cells. *J. Physiol. (Lond.).* 383: 601–614.

Kinnamon, S. C., and S. D. Roper. 1988a. Membrane properties of isolated mudpuppy taste cells. *J. Gen. Physiol.* 91:351–371.

Kinnamon, S. C., and S. D. Roper. 1988b. Evidence for a role of voltage-sensitive apical K^+ channels in sour and salt taste transduction. *Chem. Senses.* 13:115–121.

Kohbara, J., S. Wegert, and J. Caprio. 1992. Responses of single facial taste fibers in the channel catfish, *Ictalurus punctatus*, to amino acids. *J. Neurophysiol.* 68:1012–1026.

Krueger, J. M., and R. H. Cagan. 1976. Biochemical studies of taste sensation. IV. Binding of L-[3H]alanine to a sedimentable fraction from catfish barbel epithelium. *J. Biol. Chem.* 259:88–97.

Lindemann, B. 1996. Taste reception. *Physiol. Rev.* 76:719–766.

McPheeters, M., A. J. Barber, S. C. Kinnamon, and J. C. Kinnamon. 1994. Electrophysiological and morphological properties of light and dark cells isolated from mudpuppy taste buds. *J. Comp. Neurol.* 346: 601–612.

Miyamoto, T., T. Miyazaki, Y. Okada, and T. Sato. 1996. Whole-cell recording from non-dissociated taste cells in mouse taste bud. *J. Neurosci. Meth.* 64:245–252.

Miyamoto, T., Y. Okada, and T. Sato. 1991. Voltage-gated membrane current of isolated bullfrog taste cells. *Zool. Sci.* 8:835–845.

Naim, M., T. Ronen, B. J. Striem, M. Levinson, and U. Zehavi. 1991. Adenylate cyclase responses to sucrose stimulation in membranes of pig circumvallate taste papillae. *Comp. Biochem. Physiol.* 100:455–458.

Roper, S. D. 1983. Regenerative impulses in taste cells. *Science*. 220: 1311–1312.

Ruiz-Avila, L., S. K. McLaughlin, D. Wildman, P. J. McKinnon, A. Robichon, N. Spickofsky, and R. F. Margolskee. 1995. Coupling of bitter receptor to phosphodiesterase through transducin in taste receptor cells. *Nature*. 376:80–85.

Schiffman, S. S., E. Lockhead, and F. W. Maes. 1983. Amiloride reduces the taste intensity of Na^+ and Li^+ salts and sweeteners. *Proc. Natl. Acad. Sci. USA.* 80:6136–6140.

Spielman, A. I., T. Huque, H. Nagai, G. Whitney, and J. G. Brand. 1994. Generation of inositol phosphates in bitter taste transduction. *Physiol. Behav.* 56:1149–1155.

Spielman, A. I., T. Huque, G. Whitney, and J. G. Brand. 1992. The diversity of bitter taste signal transduction mechanisms. *In* Sensory Transduction. D. Corey and S. D. Roper, editors. The Rockefeller University Press, New York. 307–324.

Spielman, A. I., I. Mody, J. G. Brand, G. Whitney, J. F. MacDonald, and M. Salter. 1989. A method for isolating and patch-clamping single mammalian taste receptor cells. *Brain Res.* 502:323–326.

Spielman, A. I., H. Nagai, G. Sunavala, H. Dasso, H. Breer, I. Boekhoff, T. Huque, G. Whitney, and J. G. Brand. 1996. Rapid kinetics of second messenger formation in bitter taste. *Am. J. Physiol.* 270:C926–C931.

Striem, B. J., Naim, M., and B. Lindemann. 1991. Generation of cyclic AMP in taste buds of the rat circumvallate papilla in response to sucrose. *Cell Physiol. Biochem.* 1:46–54.

Striem, B. J., U. Pace, U. Zehavi, M. Naim, and D. Lancet. 1989. Sweet tastants stimulate adenylyl cyclase coupled to GTP-binding protein in rat tongue membranes. *Biochem. J.* 250:121–126.

Sugimoto, K., and J. H. Teeter. 1990. Voltage-dependent ionic currents in taste receptor cells of the larval tiger salamander. *J. Gen. Physiol.* 96:809–834.

Sun, X.-D., and M. S. Herness. 1996. Characterization of inwardly rectifying potassium currents from dissociated rat taste receptor cells. *Am. J. Physiol.* 271:C1221–1232.

Teeter, J. H., J. G. Brand, and T. Kumazawa. 1990. A stimulus-activated conductance in isolated taste epithelial membranes. *Biophys. J.* 58: 253–259.

Teeter, J. H., T. Kumazawa, J. G. Brand, D. L. Kalinoski, E. Honda, and G. Smutzer. 1992. Amino acid receptor channels in taste cells. *In* Sensory Transduction. D. Corey and S. D. Roper, editors. The Rockefeller University Press, New York. 291–306.

Teeter, J. H., T. Miyamoto, D. Restrepo, M. Zviman, and J. G. Brand. 1993. L-Arginine-regulated conductances in catfish taste cells. *In* Olfaction and Taste XI. K. Kurihara, N. Suzuki, and H. Ogawa, editors. Springer-Verlag, Tokyo. 195–197.

Tonosaki, K., and M. Funakoshi. 1988. Cyclic GMP injected intracellularly into mouse taste cell decreases potassium conductance. *Nature*. 331: 354–356.

Wegert, S., and J. Caprio. 1991. Receptor sites fro amino acids in the facial taste system of the channel catfish. *J. Comp. Physiol.* 168:201–211.

Wong, G. T., K. S. Gannon, and R. F. Margolskee. 1996. Transduction of bitter and sweet taste by gustducin. *Nature*. 381:796–800.

Zviman, M. M., D. Restrepo, and J. H. Teeter. 1996. Single taste stimuli elicit either increases or decreases in intracellular calcium in isolated catfish taste cells. *J. Membr. Biol.* 149:81–88.