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Single Taste Stimuli Elicit Either Increases or Decreases in Intracellular Calcium in Isolated Catfish Taste Cells

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Abstract. Taste cells are specialized epithelial cells that respond to stimulation with release of neurotransmitters onto afferent nerves that innervate taste buds. In analogy to neurotransmitter release in other cells, it is expected that neurotransmitter release in taste cells is dependent on an increase in intracellular Ca^{2+} ($[Ca^{2+}]_i$). We have studied changes in $[Ca^{2+}]_i$ elicited by the taste stimuli L- and D-arginine in isolated taste cells from the channel catfish (*Ictalurus punctatus*). In a sample of 119 cells, we found 15 cells responding to L-arginine, and 12 cells responding to D-arginine with an increase in $[Ca^{2+}]_i$. The response to L-arginine was inhibited by equimolar D-arginine in cells where D-arginine alone did not cause a change in $[Ca^{2+}]_i$, which is consistent with mediation of this response by a previously characterized L-arginine-gated nonspecific cation channel antagonized by D-arginine [31]. However, we also found that these taste stimuli elicited *decreases* in $[Ca^{2+}]_i$ in substantial number of cells (6 for L-Arg, and 2 for D-Arg, $n = 119$). These observations suggest that stimulation of taste cells with sapid stimuli may result in simultaneous excitation and inhibition of different taste cells within the taste bud, which could be involved in local processing of the taste signal.

Key words: Catfish—Taste—Receptor—Calcium—Amino acids

Introduction

Taste receptor cells are specialized epithelial cells that possess receptors for sapid stimuli in their apical membrane, and respond to taste stimuli with modulation of release of neurotransmitter onto a synapse with afferent fibers and/or other cells in the taste bud [10, 12, 16, 19]. Because neurotransmitter release is typically dependent on an increase in intracellular calcium ($[Ca^{2+}]_i$) [6], it is expected that the responses of taste cells to stimuli involves at least local increases in $[Ca^{2+}]_i$. Studies from several laboratories indicate that bitter and sweet taste stimuli elicit an increase in $[Ca^{2+}]_i$ in isolated taste cells [1, 3, 22, 23]. However, the mechanisms that regulate $[Ca^{2+}]_i$ in taste cells, which may involve both release of $[Ca^{2+}]_i$ from intracellular stores [1, 3], and influx of Ca^{2+} across the plasma membrane [2, 3, 23, 27, 30], remain poorly defined [12, 19].

The extra-oral taste system of the channel catfish (*Ictalurus punctatus*) is a widely used model system for studies of taste transduction because of the abundance of easily accessible taste buds and because of the high sensitivity of this system to certain amino acids [8, 9, 10, 18]. Competition binding studies with membranes prepared from catfish taste epithelium [4, 7, 14], as well as neuronal cross-adaptation studies with intact fish [18, 20, 36], indicate the existence of several classes of amino acid receptor sites. One class of receptors, which interacts with L-alanine and other short-chain neutral amino acids, displayed an affinity of 1.5 μM for L-alanine [4]. The L-alanine receptors are coupled via a G-protein dependent process to the formation of both cyclic AMP (cAMP) and inositol-1,4,5-triphosphate (IP_3) [15]. How these second messengers are coupled to regulation of neurotransmitter release from the receptor cells remains to be established.

In contrast, a class of receptors specifically activated by L-arginine does not appear to be coupled to formation of second messengers (cAMP or IP_3) [15]. Two binding sites were identified for L-Arg, one with apparent affinity (Kd_{app}) of 20 nM, the other with an affinity of 1.3 μM [5,

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14]. A separate binding site was defined for D-Arg [5, 14, 36], but D-Arg cross-adapts with L-Arg, suggesting that both bind to at least one common site [14]. Teeter et al. [31] reconstituted nonselective cation channels from catfish epithelium membrane which were directly and selectively activated by L-Arg at concentrations between 0.5 and 1 μM. The L-Arg induced conductance was competitively blocked by micromolar concentrations of D-Arg, consistent with the binding studies. These results indicate that D-Arg binds to one class of L-Arg receptors, but does not activate the associated cation channel.

Release of neurotransmitter at the taste receptor synapse is presumed to be a calcium-dependent process, requiring an influx of Ca^{2+} or perhaps release of Ca^{2+} from internal compartments. In addition L-Arg activated cation channels in catfish taste membranes are permeable to Ca^{2+} [31]. Consequently, we have examined the changes in $[Ca^{2+}]_i$ in catfish taste cells elicited by either a cocktail of stimuli known to produce responses in taste fibers [9] or with the taste stimuli L- and D-arginine. We find that catfish taste cells respond to taste stimuli, not only with increases in $[Ca^{2+}]_i$, as expected from previous work, but in some cells with decreases in intracellular calcium. Because the decreases in $[Ca^{2+}]_i$ could depress the basal rate of neurotransmitter release resulting in inhibition of resting activity in afferent nerve fibers, our results suggest that simultaneous inhibitory and stimulatory responses of taste cells to a single taste stimulus may occur. Such responses could play a role in increasing the sensitivity of the taste system at low stimulus concentrations.

Materials and Methods

Solutions

Fish Ringer's (FR) contained (in mM): 110 NaCl, 3 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 5 D-Glucose, 1 NaPyruvate, 10 HEPES, 20 sucrose at pH 7.5. FR without Ca^{2+} or Mg^{2+} was supplemented with 0.1 mM EDTA (No Ca/Mg FR), or with 5 mM EDTA (FR-EDTA). The papain solution was No Ca/Mg FR with 5 mM L-cysteine and 15 units/ml papain. The stop solution was No Ca/Mg FR with 10 μg/ml leupeptin. The loading solution consisted of the stop solution supplemented with 5 μM fura-2/AM and 80 μg/ml pluronic F127. The enzyme mixture solution was: FR, 1 mg/ml hyaluronidase, 1 mg/ml collagenase type II and 0.5 mg/ml trypsin inhibitor. The stimulant mixture (cocktail) contained: L-arginine, L-alanine, L-proline, L-glutamine, taurolithocholate, 100 μM ea. and 1 mM L-glutamate.

Materials

Papain (type III; EC 3.4.22.2), trypsin inhibitor (type II-S), collagenase (type II), L-cysteine, leupeptin, L-arginine, L-alanine, L-proline, L-glutamate, glutaric acid and taurolithocholic acid were obtained from Sigma chemical company (St. Louis, MO). Concanavalin A and hyaluronidase were from Worthington (Freehold, NJ). Fura-2 acetoxymethyl ester (Fura 2/AM), fura-2 potassium salt and pluronic acid F127 were obtained from Molecular Probes (Eugene, OR). Nimodipine (Bay E 9736) was obtained from the Miles Institute for Preclinical Pharmacology (West Haven, CT) and D-arginine was obtained from Aldrich (Milwaukee, WI).

Isolation of Taste Receptor Cells

Taste receptor cells were dissociated from the maxillary barbels of channel catfish (*Ictalurus punctatus*) by an enzymatic treatment followed by gentle trituration. Two enzyme treatments were examined, both resulted in viable taste receptor cells. Treatment with papain yielded cells that maintained their distinctive shape longer, compared with cells isolated using a mixture of hyaluronidase and collagenase. Barbels were removed from the fish and placed in ice-cold FR-EDTA. The skin was removed from the underlying tissue by blunt dissection and placed in fresh ice-cold FR-EDTA, where it was cut to small pieces (approximately 1 mm^2). The skin was exposed to FR-EDTA for a maximum of 10 min.

When papain was used, the FR-EDTA solution was replaced with papain solution and the skin pieces were minced. After 4 to 5 min at room temperature, the papain solution was decanted and the tissue was washed two times with the stop solution. After the second wash, the stop solution was replaced by the fura-2 loading solution. Gentle trituration with a fire polished Pasteur pipette (tip diameter approximately 0.5 mm) dissociated the tissue. Calcium and magnesium were added to final concentrations of 1 mM and 2 mM respectively.

Alternatively, cells were isolated using a mixture of collagenase and hyaluronidase. The skin of the barbels was placed in enzyme mixture for 45 min at room temperature. The solution was then replaced with No Ca/Mg FR and the tissue was incubated for additional 45 min on ice. The No Ca/Mg FR solution was decanted and the tissue placed in the loading solution, triturated, then supplemented with divalent cation and incubated at 4°C.

Dissociated cells were allowed to load with fura-2 by incubation at 4°C for at least 30 min. Taste receptor cells maintained at 4°C were easily identified by their flask or bipolar shape. However, incubation at room temperature resulted in the cells becoming round within 1-–20 min. The majority of the recordings were performed using rounded taste cells, identified as taste cells prior to rounding.

Imaging of $[Ca^{2+}]_i$ Using Fura-2

Fura-2-loaded cells were allowed to attach to coverslips coated with concanavalin-A (1 mg/ml) which formed the bottom of an experimental chamber. The chamber was attached to the stage of an inverted microscope (Nikon Diaphot) and the cells were imaged using epifluorescence illumination. The cells were excited alternately at 340 nm (calcium sensitive) and 360 nm (calcium insensitive) with light emitted from a Xenon lamp and narrow band pass filters. A shutter was used to minimize exposure of the cells to UV light. Shutter state and filter switching were computer-controlled. Fluorescence emitted by the cell was viewed through a Nikon Fluor 40× 1.3 NA objective and was linearly intensified using an OPELCO KS-1380 image intensifier. Images were captured using a Sanyo CCD camera and digitized by a Quantimet 570 image analysis workstation (Leica, Deerfield, IL). Images were averaged 16 times and stored. The maximal data rate was 7 sec per ratio image. Ratio calculation of the fluorescence emitted during excitation at the two wavelengths (corrected for background fluorescence) was performed by the Quantimet workstation and produced the concentration of free Ca^{2+} at each pixel of the image [13, 34, 35]. A detailed description of the measurement system has been published [24, 25]. Estimation of Ca^{2+} concentration was performed with solu-

tions containing fura-2 free acid employing equation 5 from Grynkiewicz et al. [13] as detailed in ref. [25]. To measure the changes of $[Ca^{2+}]_i$ in response to stimuli, images were acquired sequentially at intervals of a few secs. Unless otherwise noted, the concentration of calcium described in time course plots is the average $[Ca^{2+}]_i$ in the entire cell. Solutions were changed by perfusion of the recording chamber, with a complete exchange of solution ranging from 10 to 30 secs, depending on the location of the cell in the chamber. A positive response to stimulation was defined as a significant change in baseline $[Ca^{2+}]_i$ following stimulation (Students *t*-test $P < 0.05$).

ABBREVIATIONS

HEPES	*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid
EDTA	ethylenediaminetetraacetic acid
IP_3	inositol-1,4,5-triphosphate
cAMP	adenosine cyclic,3′,5′-monophosphate
$[Ca^{2+}]_i$	intracellular calcium concentration

Results

L-AND D-ARGININE ELICIT INCREASES IN $[Ca^{2+}]_i$ IN SOME TASTE CELLS

Changes in intracellular Ca^{2+} were measured in response to stimulation with both L-arginine and D-arginine, which have been shown to elicit neural response in the taste system of the channel catfish. L-arginine induced an increase in $[Ca^{2+}]_i$ in 15 of 119 cells (Fig. 1*A* and Table 1). The increase in $[Ca^{2+}]_i$ induced by L-arginine was antagonized by equimolar D-arginine (Fig. 1*A*, representative of recordings in 10 cells), as would be expected if this response was mediated by a previously identified L-arginine-gated nonspecific cation channel, which was antagonized by D-arginine [31]. As expected from binding studies [14], and recording from taste fibers [18], the cells responded to L-arginine over a wide concentration range (10 nM to 1 mM). In some cells (n = 5), concentrations as low as 10 nM of L-arginine were sufficient to elicit an increase in $[Ca^{2+}]_i$ (Fig. 1*B*). As shown in Fig. 1, the time course varied considerably among cells; in some cells the response was slower than the exchange of solution (Fig. 1*A* and *C*) while in other cells the response appeared as rapid as the increase in stimulus concentration. A detailed study of the concentration dependence of the response was impractical in these experiments because of the small fraction of responding cells.

In addition to its effect as an antagonist to the L-arginine induced increases in $[Ca^{2+}]_i$ in some taste cells, the D- enantiomer of arginine, which elicits responses in some taste fibers [36] elicited increases in $[Ca^{2+}]_i$ in 10 of 68 cells. Unlike the responses to L-arginine, the increases in $[Ca^{2+}]_i$ induced by D-arginine were neither blocked nor enhanced by the presence of equimolar L-arginine (Fig. 1*C*). Some cells responded to only one enantiomer (Table 2) indicating that the transduction mechanisms mediating the increases in $[Ca^{2+}]_i$ produced by

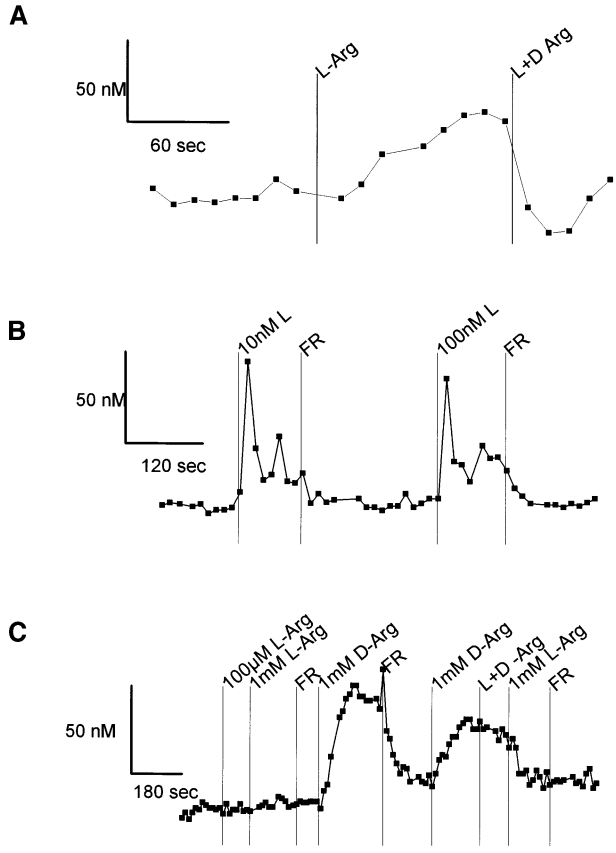

Fig. 1. Increases in $[Ca^{2+}]_i$ induced by L- and D-arginine. (*A*) L-arginine (L-arg, 1 mM) induced an increase in $[Ca^{2+}]_i$. Addition of 1 mM D-arginine (L + D Arg) induced a return to baseline $[Ca^{2+}]_i$ levels. Baseline $[Ca^{2+}]_i$ was 150 nM. (*B*) Concentrations as low as 10 nM of L-arginine (10 nM L) were sufficient to elicit an increase in $[Ca^{2+}]_i$. This figure shows the responses to 10 nM and 100 nM (100 nM L). Baseline $[Ca^{2+}]_i$ was 30 nM. (*C*) Stimulation with 100 μM and 1 mM L-arginine did not induce $[Ca^{2+}]_i$ change. Stimulation with 1 mM D-arginine induced a reversible increase in $[Ca^{2+}]_i$. This response was not antagonized by equimolar concentration of L-arginine (L + D arg). The response was washed out by removing D-arginine in the absence (FR) and the presence (1 mM L-arg) of 1 mM L-arginine. Baseline $[Ca^{2+}]_i$ was 60 nM.

Table 1. Frequencies of $[Ca^{2+}]_i$ changes in response to simulation with L- or D-arginine

	Increase	Decrease	Total tested	% Responding
L-arginine	15 (12.6%)	6 (5.1%)	119	17.6%
D-arginine	10 (14.7%)	2 (2.9%)	68	17.5%

the two enantiomers were different, and that the receptors are stereospecific. In 4 out of 8 taste cells D-arginine elicited an increase in $[Ca^{2+}]_i$, while L-arginine did not, and 6 out 9 cells responded to L-arginine but not to D-arginine with an increase an $[Ca^{2+}]_i$. Similarly, 2 out of 9 cells that responded with an increase in $[Ca^{2+}]_i$ when

Table 2. Responses to stimulation with L- or D-arginine

	L-arginine +	L-arginine −	L-arginine NR	Total	%
D-arginine +	2	2	4	8	13.79%
D-arginine −	1	1	0	2	3.45%
D-arginine NR	6	1	41	48	82.76%
Total	9	4	45	58	
%	15.52%	6.90%	77.59%		

Only cells in which all compounds were tested are reported in this table. + denotes an increase in $[Ca^{2+}]_i$, – denotes a decrease in $[Ca^{2+}]_i$ and NR denotes no change.

stimulated with D-arginine, showed an increase in $[Ca^{2+}]_i$ in response to L-arginine.

Responses to L- and D-arginine in the Absence of Extracellular Ca^{2+}

To determine the source of Ca^{2+} during the L-and D-arginine-induced increases in $[Ca^{2+}]_i$, taste receptor cells were stimulated in the absence of extracellular Ca^{2+} (with EGTA added to the fish Ringer's solution). We found that, in some cases, increases in $[Ca^{2+}]_i$ induced by L-arginine were almost completely abolished by removal of extracellular Ca^{2+} (Fig. 2*A*, representative of 4 cells). This would be consistent with influx of Ca^{2+} through the plasma membrane mediated by Ca^{2+} permeable channels such as the L-arginine-gated channel [31], or voltage-dependent Ca^{2+} channels [2, 27, 30]. However, in other cells (Fig. 2*B*, representative of 5 cells) the increases of $[Ca^{2+}]_i$ in the absence of extracellular Ca^{2+} were substantial. Similar responses in the absence of $[Ca^{2+}]_o$ were observed for D-arginine (Fig. 2*B*). These observations suggest that, in addition to influx of Ca^{2+} through Ca^{2+}-permeable channels on the plasma membrane, release of Ca^{2+} from internal stores plays a role in the response of these cells to L- and D-arginine.

L- and D-arginine also Induce Decreases in $[Ca^{2+}]_i$ in Some Catfish Taste Cells

In addition to causing increases in $[Ca^{2+}]_i$, we found that L- and D-arginine caused decreases in $[Ca^{2+}]_i$ in some taste cells (Fig. 3*A* and Table 1, 6 of 119 for L-arginine and 2 out of 68 for D-arginine). In one cell both L- and D-arginine caused a decrease in $[Ca^{2+}]_i$ (Fig. 3*A*), while in another one enantiomer (L-arginine) elicited a decrease, while the other elicited no response. In 3 cells, one enantiomer elicited an increase in $[Ca^{2+}]_i$, while the other elicited a decrease. This indicates that, although sometimes occurring in the same cells, the mechanisms that mediate increases or decreases in $[Ca^{2+}]_i$ upon stimulation with L- or D-arginine are independent of each other.

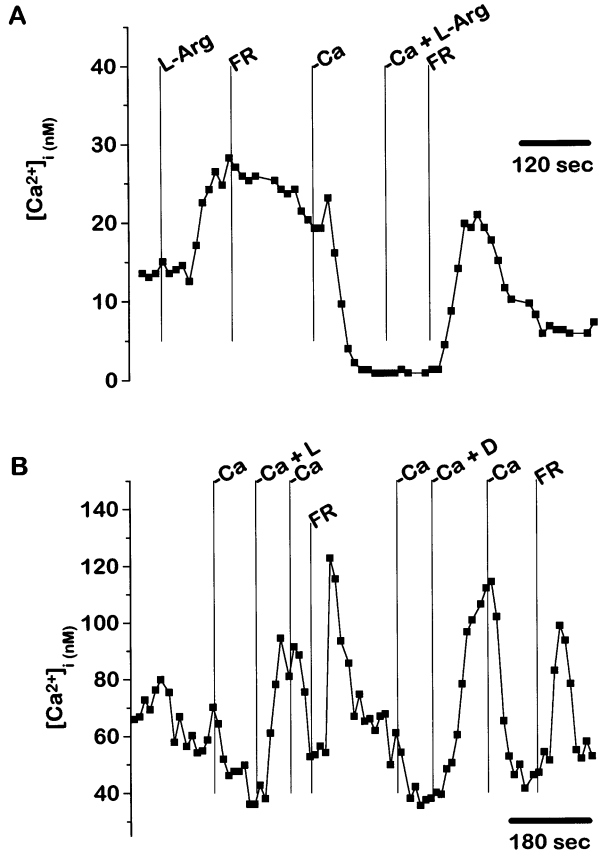

Fig. 2. Increase in $[Ca^{2+}]_i$ in the absence of extracellular Ca^{2+}. (*A*) L-arginine (100 μM) induced an increase in $[Ca^{2+}]_i$. Removal of extracellular Ca^{2+} (−Ca) was followed by a decrease in $[Ca^{2+}]_i$. Removal of Ca^{2+} was attained by addition of 1 mM EGTA in FR without added $CaCl_2$ with L-arginine in the absence of extracellular Ca^{2+} (−Ca + L-arg) did not result in increase $[Ca^{2+}]_i$. (*B*) Extracellular Ca^{2+} was removed (−Ca). The cell was stimulated with L-arginine (−Ca + L) or D-arginine (−Ca + D) (both 100 μM) in the absence of extracellular Ca^{2+}. Both stimulations resulted in reversible increases in $[Ca^{2+}]_i$ which were terminated by removal of the stimuli.

Our data do not address the nature of the mechanism mediating the taste stimulus-induced decreases in $[Ca^{2+}]_i$. However, removal of extracellular Ca^{2+} caused a decrease in $[Ca^{2+}]_i$ in thirty cells out of 54 tested (55.5%) (Fig. 3*B*). This indicates that there is a substan-

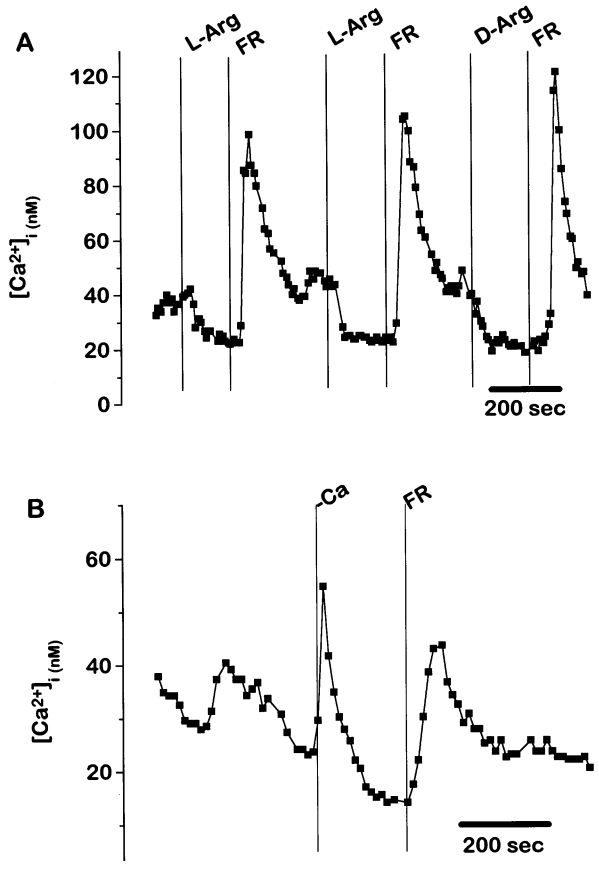

Fig. 3. Decreases in $[Ca^{2+}]_i$ induced by L and D arginine. (*A*) Stimulation with L-arginine or D-arginine (both 1 mM) induced reversible decreases in $[Ca^{2+}]_i$. Representative of 6 recordings of L-arginine and 2 for D-arginine. Upon removal of the stimuli transient increases in $[Ca^{2+}]_i$ were observed in some cells. (*B*) Transient increase in $[Ca^{2+}]_i$ were also observed in some cells when extracellular Ca^{2+} (−Ca) was removed. In almost all cells transient increases in $[Ca^{2+}]_i$ were observed when the extracellular Ca^{2+} was returned (FR) to normal (1 mM) after its removal.

tial steady state influx of Ca^{2+} in one half of the taste cells. This influx was not mediated by nimodipine-inhibitable Ca^{2+} channels because nimodipine (5 μM) did not alter the resting calcium levels in the 6 cells tested. Stimulus-induced suppression of this steady state influx of Ca^{2+} could mediate the decrease in $[Ca^{2+}]_i$ induced by L- or D-arginine. In addition, we observed that, in some cells ($n = 10$ out of 54, 18.5%), removal of $[Ca^{2+}]_o$ induced a sharp, transient increase in $[Ca^{2+}]_i$ similar to the increase of $[Ca^{2+}]_i$ observed in some cells after a stimulus-induced decrease in $[Ca^{2+}]_i$ (e.g., Fig. 3*A*) ($n = 10$).

Responses to a Cocktail of Sapid Stimuli Reveal Nonhomogeneous Increases in $[Ca^{2+}]_i$ in Taste Cells

As indicated in Materials and Methods, once the taste cells were placed in the recording chamber at room temperature they rapidly lost their characteristic elongated shape (Fig. 4*A*). This problem, compounded with relatively low percentage of taste cells responding to L- or D-arginine, made it difficult to study the spatial localization of the changes of $[Ca^{2+}]_i$ induced by the taste stimuli. To increase the chance of getting a response, we employed a stimulus cocktail containing several compounds known to be elicit robust neural response in the taste system of the channel catfish (*see* Materials and Methods for composition of cocktail). We stimulated 16 cells with the cocktail. Eleven cells exhibited an increase of $[Ca^{2+}]_i$, 4 did not respond and one showed a decrease in $[Ca^{2+}]_i$. The increase of $[Ca^{2+}]_i$ was blocked by the Ca^{2+} channel blocker nimodipine (5 μM) in 2 out of 3 cells (Fig. 4*B*).

We were able to record cocktail-stimulated increases in $[Ca^{2+}]_i$ from one taste cell that retained its characteristic elongated shape (Fig. 4*C*). Steady-state, as well as stimulus-induced levels of $[Ca^{2+}]_i$ were not uniform throughout this cell. Fig. 4*D* shows the $[Ca^{2+}]_i$ at a number of points within the taste receptor cell before and during stimulation with the cocktail. At rest the $[Ca^{2+}]_i$ was relatively uniform except for a slightly higher level at the basal end of the cell (Fig. 4*D*, 0 and 13 sec). When the cell began to respond to the stimuli (Fig. 4*D*, 28 sec), the most prominent change was an increase in $[Ca^{2+}]_i$ near the membrane at the base of the apical process and the development of gradients toward both ends of the cell. At the stimulated steady state (Fig. 4*D* 40, 54 and 66 sec), the soma displayed an elevated $[Ca^{2+}]_i$ and a gradient was observed along the apical process. The increase in $[Ca^{2+}]_i$ in this cell was inhibited by the Ca^{2+}-channel blocker nimodipine. The initial increase in $[Ca^{2+}]_i$ near the basolateral membrane at the middle of the cell is consistent with electromicroscopic localization of synapses in this region of the cell [28].

Discussion

Taste cells are modified epithelial cells that respond to sapid stimuli with release of neurotransmitter onto afferent nerve fibers, or other taste cells [10, 12, 16]. Because synaptic vesicle release is dependent on increases in intracellular Ca^{2+} [6], we hypothesized that, as observed previously by other investigators [1, 3, 22, 23], sapid stimuli would elicit an increase in $[Ca^{2+}]_i$ in catfish cells. Although we found that the taste stimuli L- and D-arginine elicit increases in $[Ca^{2+}]_i$ in some cells, other cells responded with decreases $[Ca^{2+}]_i$. This observation raises questions about the role if any of decreases in $[Ca^{2+}]_i$ in taste transduction.

It is possible that the decrease in $[Ca^{2+}]_i$ is an epiphenomenon occurring in cells that do not make functional connections with other cells in the taste bud or with afferent nerve fibers. It is also possible that the

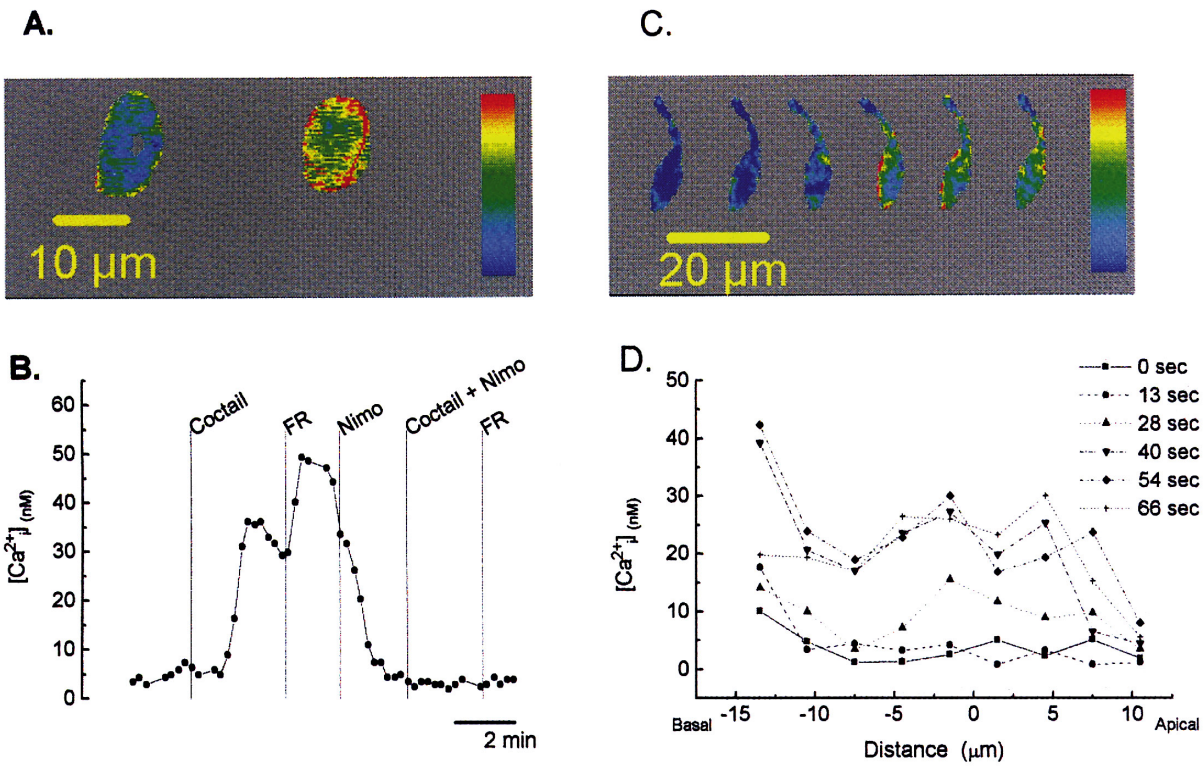

Fig. 4. (*A*) Ratio image displaying $[Ca^{2+}]_i$ changes in a taste cell that has become rounded following isolation. Left: control; right: cell after application of stimuli-cocktail. (*B, C* and *D*) $[Ca^{2+}]_i$ Changes induced by stimuli-cocktail in a cell that has kept its characteristic elongated shape of a taste cell. (*B*) Stimulation with a cocktail of sapid stimuli induced an increase in $[Ca^{2+}]_i$. The response wasabolished by μM nimodipine (Nimo). (*C*) A sequence of $[Ca^{2+}]_i$ measurements during stimulation with the cocktail (from left to right). Stimulation started shortly before the third image was captured. Scale bar is 20 μm. Pseoudocolor range is from 0 (blue) to 50 nM (red). (*D*) Concentrations of intracellular Ca^{2+} in areas along the length of the cell. The cell was divided to strips perpendicular to a line along the long axis of the cell. The $[Ca^{2+}]_i$ in each strip was averaged. The base of the apical process was chosen as zero. Stimulation was started before 28 sec.

cells displaying decreases in $[Ca^{2+}]_i$ have such a low basal rate of neurotransmitter release that the decrease in $[Ca^{2+}]_i$ does not alter the discharge of afferent nerve fibers innervating these cells; or that the taste cells showing a decrease in $[Ca^{2+}]_i$ make an inverting synapse with afferent nerve fibers, which would cause excitation of the nerve fiber thus being indistinguishable from those making excitatory synapses. Alternatively, decrease in $[Ca^{2+}]_i$ could cause a decrease in the basal rate of neurotransmitter resulting in a decreased firing rate of the innervating nerve fiber. Taste fibers generally display low levels of spontaneous activity [18]. Stimulus-induced decreases in activity have not been described. However, a small number of fibers having relatively high resting discharge rates that are decreased by some taste stimuli, such as L- or D-arginine, could easily be missed in single fiber recording. In the catfish, as well as in other species, synapses have been described between taste cells and basal cells in the taste bud [11, 16, 17, 26, 28, 29]. Thus, it is possible that the decrease in $[Ca^{2+}]_i$ could suppress transmission between cells within the taste bud. Furthermore, simultaneous stimulus-induced increases and decreases in $[Ca^{2+}]_i$ in different taste cells within a taste bud, could result in local processing of the incoming signal. Contrasting the responses of positively responding and negatively responding taste cells could result in an increased signal to noise ratio at low stimulus concentrations resulting in an effective increase in the sensitivity of the system.

L-arginine caused an increase in $[Ca^{2+}]_i$ in about 13% of the cells tested ($n = 119$). This increase in $[Ca^{2+}]_i$ was blocked by equimolar concentrations of D-arginine ($n = 6$). The blocking effect of D-arginine on these responses leads us to suggest that these increases in $[Ca^{2+}]_i$ were at least partially mediated by the L-arginine-gated cation channels described previously [31]. The L-arginine induced activation of these channels, which were permeable to Ca^{2+}, was competitively blocked by D-arginine. On the other hand, the observation that some cells responded to L-arginine with an increase in $[Ca^{2+}]_i$ even in the absence of extracellular Ca^{2+}, suggests that L-arginine taste transduction may be mediated by more than one mechanism. Additional experiments will be necessary to resolve this issue. For example, it is possi-

ble that the high concentrations of L-arginine used in the $[Ca^{2+}]_o$ removal experiments described in this paper, could have resulted in stimulation of taste receptors for other amino acids, e.g., the L-alanine receptor, which responds to high concentrations of L-arginine. These receptors are coupled to of cAMP and IP_3 [15] which could result in the later case in release of Ca^{2+} from internal stores.

D-arginine induced an increase in $[Ca^{2+}]_i$ in approximately 15% of the cells (n = 68), which was neither blocked nor enhanced by L-arginine (n = 3). Some of the cells responding to D-arginine, did not respond to L-arginine (4 out of 10), indicating that there are stereospecific responses to D-arginine in the taste system of the channel catfish (7 cells responded to L but not D-arginine (n = 13)). This observation agrees with previous cross adaptation experiments indicating that catfish possess independent taste transduction pathways for L- and D-arginine [32, 36].

Are the $[Ca^{2+}]_i$ increases involved directly in mediating taste transduction, or do they play a role in a slower process such as adaptation? Slow responses such as those shown in Fig. 1*A* and *C* would seem to suggest the latter. However, our data cannot distinguish between these possibilities because we measured average $[Ca^{2+}]_i$ throughout the cell. As shown in Fig. 4*C*, rapid changes in $[Ca^{2+}]_i$ near the membrane are not immediately reflected in the average $[Ca^{2+}]_i$ trace (Fig. 4*B*). Therefore, in order to distinguish between fast (primary) and slow (secondary) transduction processes, it would be necessary to measure *local* increases in $[Ca^{2+}]_i$ with high temporal and spatial resolution, a task that could not be performed efficiently with the apparatus we were using.

In conclusion, our study shows that the taste system of the channel catfish responds to taste stimuli with heterogeneous responses involving both increases and decreases in $[Ca^{2+}]_i$. The finding that catfish taste cells respond heterogeneously to L-arginine is consistent with recent data from our laboratory demonstrating that catfish taste cells respond with either depolarization or hyperpolarization [21, 33] to L-arginine stimulation. Excitatory and inhibitory responses to a single taste stimulus can potentially play a role in increasing the sensitivity of the taste system at low stimulus concentrations.

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