Modulation of Cl^-, K^+, and Nonselective Cation Conductances by Taurine in Olfactory Receptor Neurons of the Mudpuppy *Necturus maculosus*

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ABSTRACT Odors are transduced by processes that modulate the membrane conductance of olfactory receptor neurons. Olfactory neurons from the aquatic salamander, *Necturus maculosus*, were acutely isolated without enzymes and studied with a resistive whole-cell method to minimize loss of soluble intracellular constituents. 55 of 224 neurons responded to the test compound taurine at concentrations between 10 nM and 100 μM. Four different conductance changes were elicited by taurine: an increased Cl^- conductance (33%), an increased nonselective cation conductance (15%), a decreased Cl^- conductance (15%), and a decreased K^+ conductance (15%); in addition, responses too small to be characterized were elicited in some neurons. In most cases, taurine appeared to modulate only a single conductance in any particular cell. Modulation of each conductance was dose dependent, and each response ran down quickly in the normal whole-cell mode, presumably due to washout of a diffusible component in the transduction pathway. Modulation of taurine-sensitive conductances caused either inhibitory or excitatory responses. A similar diversity of responses in vivo would produce a complex pattern of electrical activity that could encode the identity and characteristics of an odor.

INTRODUCTION

Odorants induce electrical signals in olfactory receptor neurons by modulating the membrane conductance. In the olfactory epithelium, each neuron is sensitive only to a subset of possible odors, so that a single odor can elicit activity in only a fraction of the cells (Gesteland, Lettvin, and Pitts, 1965; Sicard and Holley, 1984). This feature of differential chemosensitivity underlies odor discrimination by producing unique patterns of sensory cell activity for different odorants. Although it has been widely held that the transduction processes for all odors converge to activate a cAMP-gated nonselective cation channel (Firestein, Darrow, and Shepherd, 1991), several reports have suggested that other conductances might also be affected (McClintock and

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Ache, 1989; Restrepo, Miyamoto, Bryant, and Teeter, 1990; Michel, McClintock, and Ache, 1991; Dionne, 1992; Miyamoto, Restrepo, and Teeter, 1992), and that odors can both enhance and suppress cell excitability (Gesteland et al., 1965; Derby, Girardot, and Daniel, 1991; Michel et al., 1991; Dionne, 1992). If odor transduction is produced by modulating several different conductances in different cells, the impact on our understanding of how odor discrimination occurs would be substantial. Excitation and inhibition elicited in different cells by the same odorant might result in a sharpening of the "odor image" which is conveyed to the olfactory bulb as a pattern of activity of receptor neurons. In the visual system, a similar sharpening is accomplished by "on" and "off" bipolar neurons and lateral inhibition. Thus, to understand olfactory information processing, it is important to determine whether odor transduction involves one or more than one membrane conductance and to examine the complexity of odor-induced changes in electrical excitability of olfactory receptor neurons.

We report here that taurine (2-aminoethyl sulfonate) modulates Cl^-, K^+, and nonselective cation (NSC^+) conductances in largely separate populations of olfactory receptor neurons from the mudpuppy, *Necturus maculosus*. The predominant effect of taurine, observed in a third of all taurine-sensitive cells, was to activate a Cl^- conductance causing depolarization. Depending on the magnitude and rate of activation of this conductance, cells showed either increases or decreases in excitability. By contrast, in a separate 15% of the sensitive cells taurine reduced the Cl^- conductance. This resulted in hyperpolarization and inhibition of spontaneous action potentials, although the increased input resistance caused the number of action potentials elicited by depolarization to be enhanced. In addition to these effects on the Cl^- conductance, taurine appeared to activate a NSC^+ conductance in 15% of sensitive cells, and to suppress a K^+ conductance in another 15% of sensitive cells. The implications of this multiplicity of chemically induced conductance and excitability changes on olfactory information processing will be discussed.

Some of these results have been reported previously (Bahnson, Dubin, Artieres-Pin, and Dionne, 1992).

METHODS

Acutely dissociated, visually identified, ciliated olfactory receptor neurons from the mudpuppy, *Necturus maculosus*, were studied with patch clamp techniques (Hamill, Marty, Neher, Sakmann, and Sigworth, 1981) using whole-cell methods as described in Dionne (1992) with minor modifications. Briefly, strips of olfactory tissue were cut from dorsal and ventral epithelia, placed in amphibian physiological saline (APS), chopped with iridectomy scissors to maintain connected pieces, and then dissociated in a low-calcium solution (DS) at pH 10.3 for 20 min. The treated tissue was transferred to APS containing ~0.5 μg/ml DNAse (Sigma Chemical Co., St. Louis, MO), gently stirred to mechanically separate the cells, and maintained on ice throughout the experiment (3–6 h). During recording, cells were continuously perfused with APS using a gravity-fed inflow system and an aspirated outflow (Dionne, 1992). Taurine was dissolved in perfusion buffer (APS unless otherwise noted) and applied by pressure ejection from a puffer pipette positioned ~50 μm from the dendritic knob. Fast green dye (0.05%) was dissolved with taurine to provide a visual indication that stimulus solutions reached the cell. Stimulus solutions were ejected from the puffer for 8 s with low pressure (<0.2 kg/cm^2), resulting in a delay of a few tenths of a second between activation of the puffer and exposure of

the cell to the taurine stimulus. The actual delay was variable due to the release characteristics of different puffers, to different puffer-to-cell separations, and to possible redilution of the contents of the puffer pipette tip. Thus, the rise times of our responses are limited primarily by the rapidity of stimulus application and not by the biological transduction processes.

Recordings were made using the resistive whole-cell method (Dionne, 1992) to reduce loss of normal intracellular constitutents. Patch electrodes (fabricated from Corning 8161 tubing; Garner Glass, Claremont, CA) were sealed to the dendritic ending of ciliated olfactory neurons, and the patch was permeabilized by applying a few brief pulses of high voltage (1.5 V, 0.5 ms) to the pipette. Series resistance (R_s) was determined by measuring the peak of the capacitative transient current elicited by small voltage steps. In the best cases, R_s was reduced to a stable value of 60–80 MΩ. Fast capacitative transients were electronically compensated but R_s was not. Occasionally the high voltage breakdown method caused the patch to rupture completely, resulting in a normal whole-cell recording (R_s < 25 MΩ). When R_s was <25 MΩ, chemosensitivity appeared to run down quickly. Rundown was observed in the normal whole-cell mode for each of the different taurine effects described here. A systematic comparison of rundown in normal and resistive whole-cell modes was made with cells showing taurine-induced conductance increases. The time between achieving the whole-cell mode and loss of chemosensitivity was significantly less ($P < 0.04$) for cells with R_s < 25 MΩ (6 ± 1 min; mean ± SEM, $n = 12$) than for cells in the resistive whole-cell mode (18 ± 5 min, $n = 14$); when the normal whole-cell mode was produced by suction rather than electrical breakdown, chemosensitivity disappeared within seconds. When R_s was >100 MΩ, adequate electrical control was difficult to maintain, and we avoided this condition. In addition to retarding the loss of important cytoplasmic constituents (presumably proteins), the high series resistance ought to have slowed the equilibration between pipette and cytoplasm of small ions such as Na^+, K^+, and Cl^-. We assumed that these ions were at equilibrium by the time the measurements were taken. This assumption was supported by the similarity of reversal potentials estimated in normal and resistive recordings and by measurements of the resting membrane potential. The average resting membrane potential for all cells studied in the resistive mode (−77 ± 2 mV; $n = 129$) was no different than that measured in cells in the normal whole-cell mode (−76 ± 2 mV; $n = 81$). Occasionally cells were spontaneously active, but this was rare at hyperpolarized resting potentials.

We were able to work with large series resistances because the input resistance of our unstimulated neurons (1.66 ± 0.09 GΩ, mean ± SEM, $n = 55$) was ~20-fold higher than R_s, and we avoided experimental protocols that relied on rapid voltage or current changes. In some cells taurine activated large conductance increases which reduced the input resistance to values close to R_s. Under these conditions voltage drops across R_s would introduce an error in the membrane potential estimated in the current clamp mode. For the typical 20-pA test pulses which were used and a worst case R_s of 100 MΩ, the error would be no larger than 2 mV. Comparable errors would accompany steady holding currents. These small errors would have shifted estimates of reversal potentials to slightly more positive values in the affected cells, but since their magnitude appeared to be less than the uncertainty in our measurements, we have not corrected for them.

Signals were sampled at 100 μs (voltage step protocols), 300 μs (voltage ramp protocols), or 1,000 μs (current clamp protocols), low-pass filtered at 5 kHz (4-pole Bessel), and digitally recorded with a PDP-11/23 laboratory computer system (Digital Equipment Corp., Maynard, MA) running software written in BASIC-23. Chemically induced conductances were detected by injecting constant current pulses of 15–65 pA (usually 20 pA) and 400 ms duration into the neuron. The resulting membrane voltage pulses were measured before, during, and after chemical exposure. The interstimulus interval was usually 1–2 min. Action potentials could be elicited by the injection of depolarizing current pulses or upon repolarization from hyperpolar-

izing pulses (anode-break excitation). To quantify taurine's effect on action potential frequency, action potentials were marked and the time base was divided into 30-ms-wide bins. The frequency of action potentials was computed using a 2-s-wide sliding window, and the number of action potentials within each window was plotted. The calibrations on the ordinates of the rate plots are in 1-Hz increments.

The reversal potential of taurine-elicited conductance changes was estimated in three ways. First, repetitive applications of taurine were made under current clamp conditions at various potentials and the magnitude of the induced voltage deflections were plotted versus membrane potential. In some cells this method was adequate, but changes in chemosensitivity that occurred during successive measurements, for example, due to rundown, confused many estimates. Our second approach was to monitor both the membrane potential and conductance during a single 8-s application of taurine. The reversal potential of the odor-sensitive conductance could be calculated from the magnitude and changes in these parameters (see below). The third approach to determine the reversal potential was to use voltage ramp current–voltage curves. Voltage ramps lasting 54 ms were applied from 0 to −100 mV every 400 ms to elicit ramp currents before, during, and after recovery from taurine exposure. At the beginning of each ramp, the voltage was held at 0 mV for 100 ms to inactivate Na^+ currents. Averages of 2–12 ramp currents were subtracted to isolate the taurine-induced current and to compare the pre- and postrecovery responses. Ramp currents were an effective way to examine taurine-sensitive changes in the K^+ conductance, but the large magnitude of the K^+ currents made it difficult to detect effects on other current components when the K^+ currents were activated.

Quantitative Derivation

The single exposure method for estimating the reversal potential of the odor-induced current is illustrated in Fig. 1. The essential idea is that when an odor activates (or inhibits) ion channels, it will cause a change in the membrane conductance that is revealed in two ways: first by a change in the resting potential of the cell, and second by a change in the magnitude of a voltage pulse elicited by a test current pulse. If the amount of odor-induced deflection in the resting potential is measured, this should predict the corresponding change in the voltage pulse when the reversal potential, E_T, of the odor-sensitive conductance is known. We used E_T as an adjustable parameter to fit the voltage pulse amplitudes and in this way estimated the reversal potential. It was assumed that the odor-sensitive and -insensitive conductances were not voltage dependent.

Consider the case where the residual membrane conductance, G_R (with reversal potential E_R), is odor insensitive, and where odor activates an additional conductance G_T (with reversal potential E_T). An expression for the potential seen during voltage pulses in the presence of odor (V_{PULSE}) in terms of the zero-current potential in the absence and presence of odor and the voltage pulse magnitude in the absence of odor can be derived as follows. In the absence of odor, the resting potential V equals E_R; at this potential no net current flows through G_R. In contrast, the addition of odor will activate the conductance G_T, causing the zero-current potential to shift to a new value V', and causing a current i_R to flow through G_R:

$$i_R = G_R(V' - E_R)$$

This current will be equal and opposite to the current i_T flowing in G_T at the same potential:

$$G_R(V' - E_R) + G_T(V' - E_T) = 0 \quad (1)$$

If, in addition, constant magnitude current pulses, $\blacktriangle i$, are applied throughout the recording (before, during, and after the application of odor), a family of voltage pulses will be produced superimposed on the resting potential (Fig. 1). Before application of the odor, the magnitude

of the voltage pulses will be $\blacktriangle v = \blacktriangle i/G_R$, but when odor activates the conductance G_T, a new voltage pulse magnitude will be seen: $\blacktriangle v' = \blacktriangle i/(G_R + G_T)$. Call the potential seen during the applied current pulse V_{PULSE}; between pulses, the potential will settle back to the baseline V'. These potentials are related by $V_{PULSE} = V' + \blacktriangle v'$. Using these expressions together with Eq. 1, the following equation for V_{PULSE} can be derived:

$$V_{PULSE} = V' + \blacktriangle v(V' - E_T)/(V - E_T) \quad (2)$$

This expression was fitted to the hypothetical data in Fig. 1. An identical expression for V_{PULSE} can be derived in the case where odor inhibits a portion of the residual conductance, and Eq. 2 is valid whether or not a steady bias current is applied to shift the membrane potential.

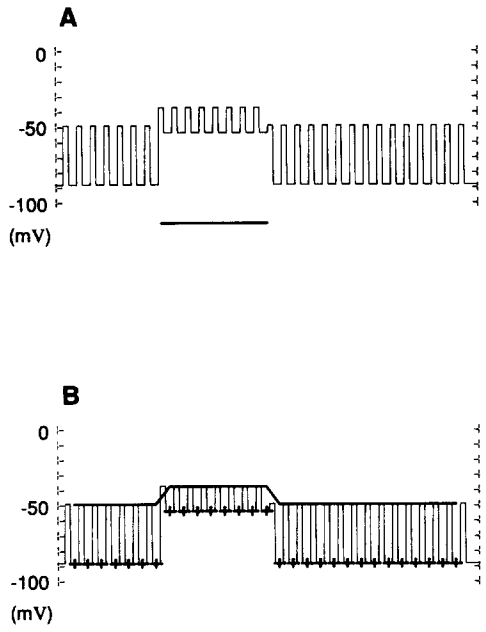

FIGURE 1. The single-application method for estimating the reversal potential of the odor-sensitive conductance. (*A*) This graphical illustration shows hypothetical voltage measurements from a model cell stimulated with small depolarizing current pulses of uniform amplitude throughout the record. During the interval marked with the horizontal bar, an odor-sensitive conductance was "activated" that caused the zero-current resting potential to depolarize and the amplitude of the voltage pulses to diminish. (*B*) The hypothetical data were fitted with Eq. 2 to estimate E_T. Cursors mark the zero-current resting potential before, during, and after application of odor. Before application of odor, the baseline voltage was $V = -88$ mV and the magnitude of the voltage pulses was $\blacktriangle v = 39$ mV. During application of odor, the baseline voltage shifted to $V' = -53$ mV and the voltage pulse magnitude decreased to $\blacktriangle v' = 16$ mV. Eq. 2 was fitted to the peak pulse voltages (V_{PULSE}) in the presence of odor and during recovery (*heavy line*) by adjusting the value of E_T. The estimate for E_T was -29 mV.

To estimate the reversal potential E_T, we recorded the membrane potential of cells while applying small current pulses (1 Hz, each ~400 ms duration) either in the presence or absence of a steady bias current. Typically, a 7–10-s recording before the addition of odor was made, followed immediately by an 8-s puff of odor and a 12–15-s period of recovery. The average amplitude of voltage pulses before the addition of odor ($\blacktriangle v$) was measured together with the baseline potential between each pulse (~30 separate values). Eq. 2 was then used with these data to predict a set of values for V_{PULSE} corresponding to each of the baseline voltage measurements. The estimate for the reversal potential of the odor-sensitive conductance, E_T, was obtained by treating E_T as an adjustable parameter to optimize the fit of the V_{PULSE} values.

Solutions

The compositions of the solutions used during this study were as follows. APS contained (mM): 130 NaCl, 2.5 KCl, 3 $CaCl_2$, 1 $MgCl_2$, 10 NaHEPES (hemi-Na salt), 5 glucose, and 5 Na-pyruvate, pH 7.4. Low Cl^- APS was identical to APS except that NaCl was replaced by Na-isethionate. DS contained (mM): 90 NaCl, 4 NaOH, 2 KCl, 1 $CaCl_2$, 10 Na_2CO_3, 10 $NaHCO_3$, 10 Na_3-citrate, and 1% wt/vol bovine albumin, pH 10.3. The intracellular saline (IS) contained (mM): 90 K-gluconate, 25 KCl, 5 $CaCl_2$, 3 $MgSO_4$, 10 NaHEPES, and 10 K_4BAPTA, pH 7.4 (0.1 μM free Ca^{2+}). All tested compounds were maintained as stock solutions (10 mM) in APS, stored at 4°C, diluted, and their pH checked before use. Experiments were performed at 22–25°C. All numerical values cited with uncertainties are means ± SEM. All compounds were purchased from Sigma Chemical Co.

RESULTS

Taurine was buffered to a neutral pH and applied at concentrations of 10 nM–100 μM. At the highest concentrations tested (10 or 100 μM), taurine elicited responses from 55 of 229 (24%) freshly isolated olfactory receptor neurons. In 37 responsive cells, the major effect of taurine was to increase membrane conductance, while in 16 cells it diminished membrane conductance. At least three different conductances were modulated by taurine. Of the cells responding primarily with a conductance increase, 18 exhibited an increased Cl^- conductance and 9 exhibited an increased NSC^+ conductance. The conductance change in the remaining 10 cells was small, and the ionic basis could not be determined. Of the cells responding with a conductance decrease, half showed a decreased Cl^- conductance and half a decreased K^+ conductance. The magnitude of each type of response varied widely from cell to cell, and each response type exhibited rundown.

Taurine-induced Conductance Increases

Taurine caused an increase in conductance accompanied by depolarization from the resting potential (Figs. 2–4) in 16% (n = 37) of the cells tested. 10 μM taurine was as effective as 100 μM (three of three cells), and 10 nM taurine, the lowest concentration tested, caused an increased conductance in two cells. The magnitude of the conductance change to maximal doses of taurine (10 or 100 μM) varied widely (708 ± 305 pS, n = 30; range: 10–9,300 pS). Dose dependence of the response was observed in 16 cells where taurine was tested at concentrations < 10 μM and at 10 or 100 μM.

The specific ionic conductance that was activated by taurine could, in principle, be a NSC^+ conductance, a Cl^- conductance, or novel conductances for Na^+ or Ca^{2+}. Under our experimental conditions, each of these possible conductances would have a unique equilibrium potential: $E_{NSC} \sim 0$ mV; $E_{Cl} = -35$ mV; $E_{Na} = 84$ mV; $E_{Ca} >$ 100 mV. To help identify the ionic basis of the response, the reversal potential of the taurine-sensitive conductance was determined. These data, obtained from 27 cells and described below, suggest that taurine activated Cl^- and NSC^+ conductances in different cells. In cells where the response was to increase either a Cl^- or NSC^+ conductance, there was no detectable effect on the voltage-activated K^+ or Na^+ currents.

Increased Cl^- conductance. In 18 cells a mean reversal potential of −29 ± 1 mV was measured for the taurine-activated conductance (Fig. 2, *A* and *B*). This is close to the Nernst potential for Cl^- (−35 mV), assuming that Cl^- in the recording electrode equilibrated with the intracellular solution. The mean conductance change induced

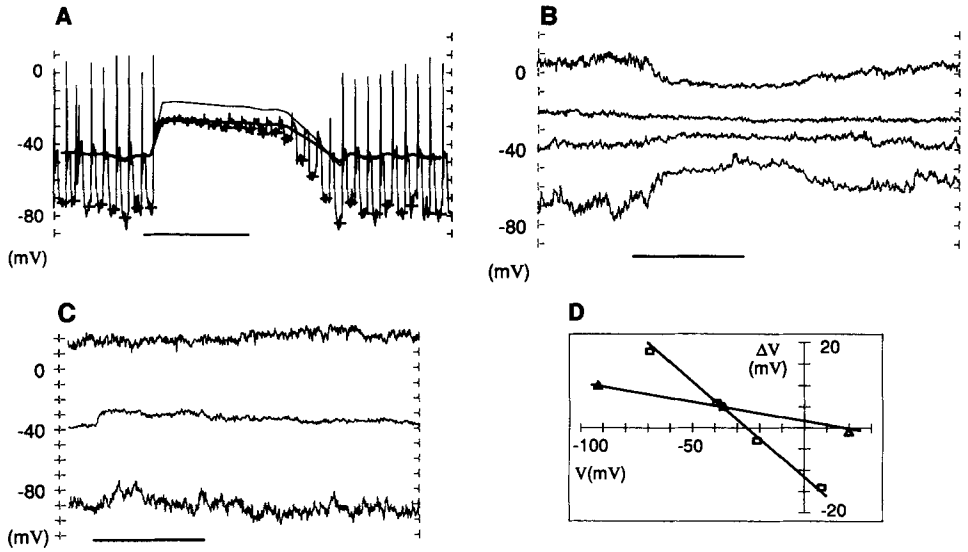

FIGURE 2. Increased Cl^- conductance. (*A*) Taurine (100 μM; *horizontal bar*, 8 s) induced an increase in the membrane conductance from a basal value of ~0.7 nS to a peak value of ~10 nS and caused the membrane to depolarize to −28 mV. Depolarizing current pulses (20 pA) were used to measure the membrane conductance. The reversal potential of the taurine-induced conductance was estimated with the single-application method (see Methods for details), giving a best fit (lower of the two lines drawn through the data) of −26 mV. The upper line that does not fit the data is the prediction of Eq. 2 when E_T was set at 0 mV. The data trace is 30.7 s long. (*B*) The reversal potential of the taurine-induced conductance was also estimated by applying 100 μM taurine (*horizontal bar*, 8 s) in the current clamp mode and recording the voltage shift induced at different membrane potentials. Extracellular [Cl^-] was 140.5 mM. The measurements are plotted as squares in *D*. (*C*) Taurine-induced responses from another cell bathed in normal APS were elicited by 100 μM taurine dissolved in low Cl^- APS (10.5 mM Cl^-; *horizontal bar*, 8 s). During application of the low Cl^- solution, the bath was continuously perfused with normal APS with the puffer positioned distant from the bath electrode to prevent changes of Cl^- activity in its vicinity. This cell responded to taurine in 140.5 mM Cl^- with an estimated E_T of −28 mV. Other experiments were done with the cells bathed continuously in low Cl^- APS. (*D*) Measurements from the data in *B* and *C* illustrate the shift in reversal potential that occurred with the change in [Cl^-]. The estimated reversal potentials of −26 mV (normal Cl^-, *squares*) and +20 mV (low Cl^-, *triangles*) were obtained by linear regression.

by 10 or 100 μM taurine was 1,021 ± 564 pS (n = 16). Lowering the extracellular [Cl^-] to 10.5 mM from 140.5 mM shifted the calculated Nernst potential to +30 mV and caused the reversal potential to shift to positive voltages (n = 2). The best example is shown in Fig. 2 *C*; reversal potentials could not be measured with the

same accuracy in low Cl^- saline as in normal APS. Based upon reversal potential and sensitivity to changes in extracellular [Cl^-], this taurine-sensitive conductance was identified as a Cl^- conductance.

To investigate whether taurine could be acting through a glycine receptor in these cells, we tested whether glycine could elicit responses and whether strychnine could block the taurine-induced Cl^- conductance increase. 100 μM glycine elicited little or no response compared with that produced by an equal concentration of taurine (Fig. 3). Strychnine was applied at 100 μM, a concentration at least three orders of magnitude above its K_i for adult strychnine-sensitive glycine receptors and fivefold above that for low-affinity strychnine receptors (Kuhse, Schmieden, and Betz, 1990); it did not block the taurine response. These results indicate that the response was not mediated by a glycine receptor.

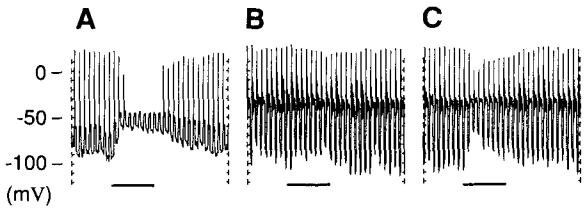

FIGURE 3. Low glycine potency. These three panels are from a cell in which taurine elicited a Cl^- conductance increase. (*A*) Taurine (100 μM; *horizontal bar*, 8 s) increased the membrane conductance ~740 pS, depolarized the membrane potential, and caused suppression of action potentials elicited by depolarizing pulses applied at the resting potential. E_T for the taurine-activated conductance was −33 mV. (*B*) Hyperpolarizing current pulses were subsequently applied to test the response to glycine (100 μM; *horizontal bar*, 8 s) since the rate of action potentials elicited by anode-break excitation was often a more sensitive indicator of changes in excitability than the response measured with depolarizing pulses. Glycine had very little effect on either the membrane conductance or the firing properties of the cell. (*C*) The cell was tested with taurine after the glycine test using the same hyperpolarizing pulse protocol. It responded with an increase in membrane conductance of ~310 pS, a decrease in action potential height and a substantial reduction in anode-break activity. The responses in *B* and *C* were obtained 24 min after that in *A*. Even at the beginning of recording from this cell there was no significant response to glycine.

Increased nonselective cation conductance. In nine cells from the increased-conductance group, a mean reversal potential of −4 ± 2 mV was measured for the taurine-activated conductance. On the basis of this reversal potential, the taurine-activated conductance was tentatively identified as a NSC^+ conductance. These cells showed a mean conductance increase to 10 or 100 μM taurine of 550 ± 103 pS (n = 7). A cell with a typical response of this type is shown in Fig. 4. In this cell, 10 nM taurine caused a conductance increase together with depolarization and a change in excitability that appeared to depend on membrane potential (Fig. 4, *A* and *B*). Changes in the NSC^+ conductance tended to be biphasic with large, transient components followed by long-lasting smaller increases. Under voltage clamp conditions in the example shown, voltage ramps between 0 and −100 mV elicited linear currents, and taurine reversibly enhanced the control currents by ~10%. The current–voltage plot in Fig. 4 *C* shows the taurine-induced current after subtraction of the control current and indicates a reversal potential near −2 mV.

Taurine-induced Conductance Decreases

Taurine caused a decrease in conductance in 7% of cells tested (n = 16). The mean resting conductance of this group of cells was 668 ± 77 pS, a value not significantly different from that seen in isolated olfactory neurons regardless of chemosensitivity (633 ± 50 pS; n = 65) or in the taurine-sensitive cells showing only a conductance increase (820 ± 113 pS, n = 37). The taurine-induced response was accompanied by hyperpolarization of the resting potential in half the cells and by depolarization in the other half. This indicated that at least two different components of the membrane conductance were altered. Examination of the reversal potentials of the responses

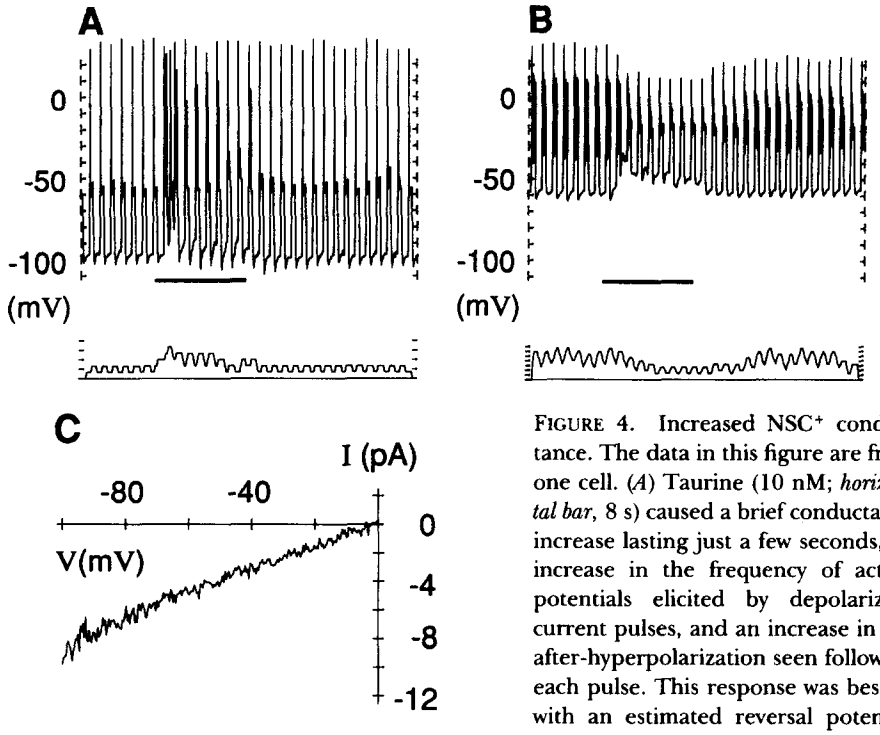

FIGURE 4. Increased NSC^+ conductance. The data in this figure are from one cell. (*A*) Taurine (10 nM; *horizontal bar*, 8 s) caused a brief conductance increase lasting just a few seconds, an increase in the frequency of action potentials elicited by depolarizing current pulses, and an increase in the after-hyperpolarization seen following each pulse. This response was best fit with an estimated reversal potential for the taurine-induced conductance of about −10 mV and was poorly fit with −30 mV (not shown). The rate of action potentials elicited by the pulses is shown in the lower trace (calibration increments = 1 Hz); the response was phasic-tonic. (*B*) The cell was then tested at a membrane potential of −59 mV where the number of action potentials elicited by depolarizing current pulses was enhanced. At this potential, the effect on firing behavior shown in the lower trace was considerably altered from that seen at the more hyperpolarized resting potential. Compared with the response in *A*, the pulse-induced after-hyperpolarization during the taurine response was reduced substantially. (*C*) The taurine-sensitive conductance was measured in the voltage clamp mode by injecting voltage ramps between 0 and −100 mV. The difference between the averaged ramp currents measured in the presence of 10 μM taurine and the averaged initial control and recovered currents is plotted. A taurine-activated inward current reversed at about −2 mV. It was rare to observe these small taurine-induced currents because most cells had large, voltage-activated, noninactivating K^+ currents; the K^+ currents were unusually small in this cell.

confirmed this and suggested that they were mediated by a decrease in Cl^- conductance in some cells and by a decrease in K^+ conductance in others.

Decreased Cl^- conductance. In eight cells from this group, the conductance decrease was accompanied by hyperpolarization (Fig. 5). Taurine at 10 or 100 μM reduced the membrane conductance to 71 ± 8% (range: 26–92%, n = 8) of the resting value. The mean reversal potential of the taurine-induced response was −31 ± 2 mV (n = 4), suggesting that taurine reduced a resting Cl^- conductance. From cell to cell, the response appeared to be dose dependent, although data comparing maximal and submaximal doses in the same cell were obtained in only one case. There, the higher concentration produced a more robust response and

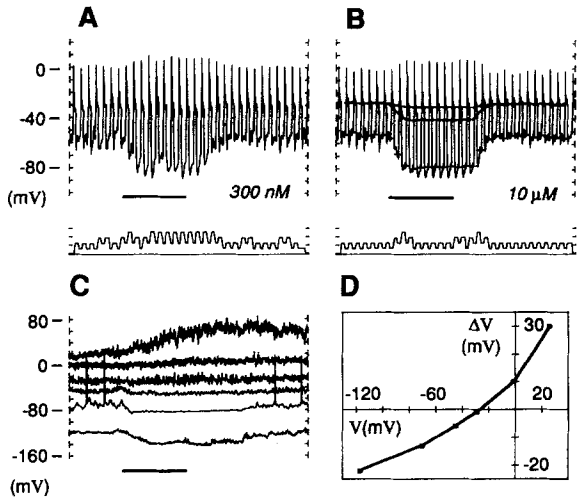

FIGURE 5. Decreased Cl^- conductance. The data in this figure are from the same cell recorded at several different membrane potentials. (*A*) Taurine (300 nM; *horizontal bar*, 8 s) produced a conductance decrease (measured with depolarizing current pulses) to 55% of the initial value, hyperpolarization of the membrane potential (initially −54 mV), an increase in action potential height, and increased firing. The rate of firing of action potentials elicited by each pulse is plotted in the bottom trace (calibration increments = 1 Hz). (*B*) Similar to *A*, but with 10 μM taurine. The higher dose elicited a more robust response and qualitatively altered the excitability of the cell. Eq. 2 was used to derive the fitted curves with reversal potentials of −25 mV (upper line, best fit) and 0 mV (lower line, systematic underestimate). (*C*) Responses induced by taurine (10 μM; *horizontal bar*, 8 s) in the current clamp mode were recorded at different membrane potentials. (*D*) The peak voltage deflections induced by taurine in *C* were plotted against membrane potential to estimate a reversal potential of −26 mV for the taurine-sensitive conductance.

appeared to have differential effects on excitability, although the response magnitudes were roughly similar (Fig. 5, *A* and *B*). In this cell the dose dependence also appeared to be affected by voltage. In contrast to the responses at −54 mV (Fig. 5, *A* and *B*), at −88 mV the conductance change induced by 300 nM taurine was only about one-third that induced by 10 μM (not shown). This suggests that the affected conductance is strongly voltage dependent, but the data are preliminary in this regard. In other cells, 10 μM taurine was as effective as 100 μM (n = 2). One cell responded robustly to 100 nM taurine, the lowest dose tested in this group.

Decreased K^+ conductance. In eight cells, taurine decreased the membrane conductance and caused depolarization. There was no systematic effect of taurine on Na^+ current activation or inactivation kinetics in this group (three of three cells). Rather, the taurine effect was associated with partial inhibition of the outward current (Fig. 6), which is carried in part through several different types of K^+ channels (Dionne, 1989). In addition to the partial block of the outward current by taurine, the

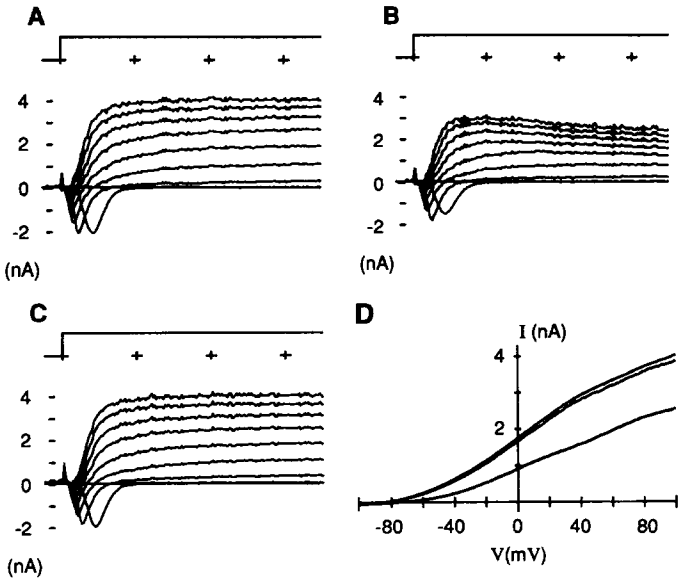

FIGURE 6. Decreased K^+ conductance. The data in this figure are from one cell. *A–C* show families of current traces elicited by voltage steps (time course diagrammed at the top of each panel) from a holding potential of −89 mV to test potentials between −80 and +100 mV (20-mV increments). The cursors mark the time base at 5-ms intervals. Each family of curves reveals transient inward (negative) voltage-gated Na^+ currents followed by sustained outward (positive) currents. (*A*) Initial control in the absence of taurine. (*B*) Currents measured in the presence of 100 nM taurine; the sustained outward currents are suppressed and show slight inactivation. Activation of the outward currents was not shifted along the voltage axis. (*C*) Currents recorded following a 1-min wash after taurine exposure. The taurine response was rapidly and completely reversible. (*D*) Ramp current–voltage curves were recorded from the same cell under the conditions in *A–C*. The controls before and after exposure to taurine appear as the upper two traces. The response in the presence of taurine (100 nM) shows the outward current reduced ~40% at all voltages. Voltage ramps from +100 to −100 mV.

response showed the following features: (*a*) In voltage ramp experiments which activated the outward current at +100 mV then ramped down to −100 mV (E_K = −100 mV), the taurine-sensitive component of the outward current was suppressed at all voltages without a shift along the voltage axis, and it reversed near E_K (Fig. 6 *D*; five of five cells). (*b*) In the current clamp mode, the reversal potential of the taurine-induced response estimated with the single-application method was

−96 ± 3 mV (n = 6). (*c*) The response was dose dependent: responses of similar magnitude were elicited by ≥1 μM taurine, and smaller responses (44 ± 15% of maximum, pooled current clamp, and voltage clamp data; n = 4) were observed with 100 or 300 nM taurine. (*d*) There was a decrease in the magnitude of after-hyperpolarizations following action potentials (three of four cells). These data suggest that taurine suppressed one or more K^+ current components with predominant effects on a delayed rectifier K^+ current; the small number of cells in this group preclude a more complete identification of the sensitive components at this time.

DISCUSSION

The mechanisms underlying odor transduction in olfactory receptor neurons are only now beginning to be identified. Odors are known to stimulate the production of cyclic AMP in membranes prepared from vertebrate olfactory epithelia (Pace, Hanski, Salomon, and Lancet, 1985; Lowe, Nakamura, and Gold, 1989; Boekhoff, Tareilus, Strotmann, and Breer, 1990). In turn, cyclic AMP can stimulate NSC^+ channels in toad (Nakamura and Gold, 1987), salamander (Firestein et al., 1991; Firestein, Zufall, and Shepherd, 1991; Zufall, Firestein, and Shepherd, 1991), frog (Frings and Lindemann, 1991; Kleene and Gesteland, 1991*a*), and rat (Frings, Lynch, and Lindemann, 1992) olfactory neurons leading to the hypothesis that NSC^+ channels underlie olfactory transduction. However, odors also appear to stimulate the production of IP_3 in vertebrate olfactory membranes (Boekhoff et al., 1990), and IP_3 can activate Ca^{2+} channels in catfish olfactory neurons (Restrepo et al., 1990). In lobster olfactory neurons, chemical stimuli have been shown to activate Cl^- and K^+ conductances (McClintock and Ache, 1989; Michel et al., 1991). Thus, there may be a variety of transduction mechanisms for odorants, but whether the varied mechanisms are common among all animals or all exist within the same animal is unknown. The resolution of these questions will be important for understanding both odor transduction and discrimination.

The mudpuppy was chosen for these studies because, being an aquatic salamander, its native odorants are water-soluble compounds that are readily applied to isolated cells under our experimental conditions. Amino acids and bile acids are effective olfactory stimuli for aquatic animals (Døving, Selset, and Thommesen, 1980; Artz, Silver, Mason, and Clark, 1986). We began this work using taurine and the taurine-conjugated bile acids as chemical stimuli since these are compounds that the animals might encounter in vivo (Fuzessery, Carr, and Ache, 1978). About one-third of tested cells responded to the bile acids, but the responses were usually small even at 10–100-μM concentrations where these compounds are known to partition into cell membranes. Since we could not distinguish possible receptor-mediated responses from nonspecific effects at these concentrations, bile acids were not studied further. The work then focused on taurine which we found could elicit robust responses from some cells even at nanomolar concentrations.

Taurine elicited responses from 24% of the cells tested; it reversibly modulated at least three different components of the membrane conductance in different cells. The most prominent effects of taurine were changes in the Cl^- conductance, with 33% of the sensitive cells showing activation and 15% showing inhibition of this conductance. Activation of a NSC^+ conductance (15%) and suppression of a K^+

conductance (15%) were also observed. A prominent rundown phenomenon was observed for each of the different responses, suggesting involvement of second messengers or other soluble cytoplasmic components in generating the taurine-induced conductance changes. These data are consistent with a role for G protein–coupled receptors acting indirectly on ion channels (Bruch, 1990), but they do not speak to the variety or the identity of the soluble elements. Moreover, the limited dose-response data preclude conclusions about whether taurine acts on more than one receptor type. In some cells, secondary effects on membrane conductances caused by the pulse protocols and shifts in membrane potential were observed together with the direct taurine-induced conductance changes; these have not been studied. The two types of taurine-induced inhibition of conductance cannot be explained by a pH effect since taurine was applied in solution at physiological pH.

Identification of the different taurine-sensitive conductances was based primarily upon reversal potential measurements. Because increases in either Cl^- or NSC^+ conductance could produce qualitatively similar responses, namely, a decreased test pulse amplitude and depolarization, we were especially careful in distinguishing these two conductances. We believe that the noted differences are valid discriminating characteristics, since any NSC^+ conductance reversing at -29 mV (the Cl^- reversal potential) would require a permeability ratio $P_K/P_{Na} = 3$, which has no precedent (Nakamura and Gold, 1987; Dhallan, Yau, Schrader, and Reed, 1990; Frings et al., 1992). Furthermore, under physiological conditions, the mucosal Cl^- concentration is probably lower than that used in our extracellular saline (Chiu, Nakamura, and Gold, 1989). In this case, the Cl^- reversal potential would be more positive than -35 mV, and the depolarization induced by taurine in vivo would be larger than observed in this study. This underscores the potential physiological importance of Cl^- conductance changes.

A Cl^- flux has been suggested to underlie odor-induced inhibitory responses in frog olfactory receptor neurons (Gesteland et al., 1965), and histamine can stimulate a ligand-gated Cl^- channel in lobster olfactory cells (McClintock and Ache, 1989). It is unlikely that the taurine-sensitive Cl^- conductance described in this study is mediated by a mechanism similar to that in lobster because the taurine-sensitive conductance described here shows rundown and comparatively little desensitization or voltage sensitivity. In the central nervous system (CNS), taurine can stimulate glycine receptors; however, the CNS receptors differ from those mediating the taurine-induced conductance in olfactory neurons. Taurine was more potent than glycine in taurine-sensitive olfactory neurons, and strychnine was ineffective at a concentration that would inhibit known CNS glycine receptors (Betz, Schmitt, Becker, Grenningloh, and Rienitz, 1986; Kuhse et al., 1990). Taurine has also been shown to act on a subclass of brain $GABA_A$ (Bureau and Olsen, 1991) and $GABA_B$ (Kontro and Oja, 1990) receptors. Since taurine has a 50–100-fold lower potency for GABA receptors than for the responses we studied, and a pronounced rundown phenomenon has not been documented for brain $GABA_A$ receptors, it is unlikely that taurine is acting on GABA receptors in olfactory neurons. One possible mechanism underlying this Cl^- response in olfactory neurons may be modulation of a Ca^{2+}-activated Cl^- channel like the one found in frog olfactory receptor neurons (Kleene

and Gesteland, 1991*b*). If this were the case, then rundown could be due to chelation of intracellular Ca^{+2} under our experimental conditions.

Whether the same or different populations of Cl^- channels underlie the taurine-induced inhibition of Cl^- conductance seen in some cells and the activation seen in others cannot be determined yet. In each case, the reversal potential and rundown were similar; however, preliminary data suggest there may be differences between the responses in their voltage dependence of decay and in their rectification. The apparent voltage dependence of the Cl^- conductance decrease might be due to voltage-gated Ca^{2+} channels. L-type Ca^{2+} channels susceptible to rundown have been seen in mudpuppy olfactory neurons (Dionne, 1988).

At least 15% of taurine-sensitive cells appeared to respond with an increase in NSC^+ conductance. In the continuing presence of taurine, this conductance change in the most sensitive cells was initially large and transient followed by a sustained smaller component. This is reminiscent of phasic-tonic responses in which cyclic AMP activates a NSC^+ conductance (Frings and Lindemann, 1991).

In 15% of taurine-sensitive cells the response was caused by a decrease in an outward K^+ current. A number of different K^+ currents have been observed in mudpuppy olfactory neurons. Tetraethylammonium-sensitive delayed rectifier currents have been present in every cell tested; fast-inactivating and Ca^{2+}-dependent K^+ currents have been observed also (Dionne, 1988). The delayed rectifier K^+ current may be the dominant outward current modulated by taurine in these cells; delayed rectifier channels have a finite open probability at the resting potential that would allow taurine to elicit responses from quiescent cells. In mudpuppy taste cells, voltage-dependent delayed rectifier K^+ channels are modulated by tastants (Cummings and Kinnamon, 1992). Occasionally, N-shaped current–voltage curves were observed during application of taurine, suggesting that Ca^{2+}-activated K^+ currents were unmasked by inhibition of the delayed rectifier current. This taurine-induced decrease in K^+ current differs from the odor-induced increase of a 4-aminopyridine-sensitive, tetraethylammonium-insensitive K^+ current reported in lobster olfactory neurons (Michel et al., 1991).

In ~20% of the taurine-sensitive cells, the responses could not be clearly attributed to a single, prominent conductance change. In most of these cells the responses were too small to characterize. However, in five cases the taurine-elicited responses were large and consistent with the modulation of two or more different conductances in the same cell. Since such responses were rare, we must regard them as preliminary, but they raise a fundamental question about the response properties of receptor neurons. Do individual receptor neurons respond to various odors in a stereotyped way using transduction pathways that converge on a single sensitive conductance that differs among cells, or are there several different conductances differentially modulated by odors in most olfactory neurons? According to the latter idea, different responses from individual receptor neurons could be elicited by different odors if they modulated several conductances to varying degrees. Modulation of excitability in olfactory receptor neurons via several different mechanisms does not appear to be a unique capability of taurine. A similar diversity of responsiveness has been observed with amino acids in the mudpuppy (Dionne, 1992) and is likely to occur in other systems (Derby et al., 1991; Michel et al., 1991). Such diversity might depend on

different receptor types in different cells that respond to the same odorant, or possibly to a single receptor type coupled through different second messengers to different conductances in different cells. Whatever the foundations of this diversity, it must afford an advantage to the olfactory system in terms of odor discrimination, since the more basic functions of odor detection could be served as well by a single transduction mechanism.

The data here support conclusions from earlier work (Gesteland et al., 1965; Derby et al., 1991; Dionne, 1992) that a single odorant can have opposing effects on cellular excitability. For the most part, taurine caused increased excitability by activating a NSC^+ conductance or by inhibiting a Cl^- conductance. The activation of a Cl^- conductance by taurine transiently excited cells if the response was rapid and the depolarization was above threshold for action potential generation. However, during a sustained exposure to taurine, the ability of cells to fire action potentials was decreased because depolarization inactivated much of the Na^+ conductance. In addition, the sustained increase in input resistance would make the cell less responsive to the effects of other odorants. Finally, when taurine suppressed the K^+ conductance, it caused a sustained but smaller depolarization that enhanced excitability. The combination of various excitatory and inhibitory responses stimulated by the same odorant may sharpen the "odor image," enabling complex information patterns to be conveyed accurately to higher-order cells. In addition to inhibition at later stages of the signal processing pathways, direct inhibition of primary sensory neurons by odorants may play a significant role in odor coding.

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