Zinc and Copper Influence Excitability of Rat Olfactory Bulb Neurons by Multiple Mechanisms

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Horning, Michelle S. and Paul Q. Trombley. Zinc and copper influence excitability of rat olfactory bulb neurons by multiple mechanisms. J Neurophysiol 86: 1652–1660, 2001. Zinc and copper are highly concentrated in several mammalian brain regions, including the olfactory bulb and hippocampus. Whole cell electrophysiological recordings were made from rat olfactory bulb neurons in primary culture to compare the effects of zinc and copper on synaptic transmission and voltage-gated ion channels. Application of either zinc or copper eliminated GABA-mediated spontaneous inhibitory postsynaptic potentials. However, in contrast to the similarity of their effects on inhibitory transmission, spontaneous glutamate-mediated excitatory synaptic activity was completely blocked by copper but only inhibited by zinc. Among voltage-gated ion channels, zinc or copper inhibited TTX-sensitive sodium channels and delayed rectifier-type potassium channels but did not prevent the firing of evoked single action potentials or dramatically alter their kinetics. Zinc and copper had distinct effects on transient A-type potassium currents. Whereas copper only inhibited the A-type current, zinc modulation of A-type currents resulted in either potentiation or inhibition of the current depending on the membrane potential. The effects of zinc and copper on potassium channels likely underlie their effects on repetitive firing in response to long-duration step depolarizations. Copper reduced repetitive firing independent of the initial membrane voltage. In contrast, whereas zinc reduced repetitive firing at membrane potentials associated with zinc-mediated enhancement of the A-type current (−50 mV), in a significant proportion of neurons, zinc increased repetitive firing at membrane potentials associated with zinc-mediated inhibition of the A-type current (−90 mV). Application of zinc or copper also inhibited voltage-gated Ca2+ channels, suggesting a possible role for presynaptic modulation of neurotransmitter release. Despite similarities between the effects of zinc and copper on some ligand- and voltage-gated ion channels, these data suggest that their net effects likely contribute to differential modulation of neuronal excitability.

INTRODUCTION

Zinc and copper are important trace metals that are differentially distributed throughout the mammalian CNS (Donaldson et al. 1973; Ono and Cherian 1999; Slomianka 1992). A variety of chemical and anatomical techniques have shown that pools of zinc and/or copper are stored in synaptic vesicles and the terminals of some, mostly glutamatergic, neurons (Friedman and Price 1984; Holm et al. 1988; Ibata and Otsuka 1969; Kardos et al. 1989; Perez-Clausell and Danscher 1985; Sato et al. 1994; Schrder et al. 2000; Slomianka et al. 1990). Such pools of zinc and/or copper can be released following membrane depolarization or neural activity in a calcium-dependent manner (Assaf and Chung 1984; Hartter and Barnea 1988; Howell et al. 1984; Kardos et al. 1989).

The mammalian olfactory bulb has one of the highest concentrations of zinc and copper in the CNS (Donaldson et al. 1973; Ono and Cherian 1999). In the olfactory bulb, zinc, as identified by a variety of histological methods, is contained primarily in olfactory sensory neuron terminals in the glomerular layer and neuron terminals of unknown origin in the granule cell layer (Friedman and Price 1984; Jo et al. 2000; Perez-Clausell and Danscher 1985). Furthermore, it has been recently reported that zinc-containing neurons are also immunoreactive for ZnT-3, a putative zinc transporter localized to synaptic vesicles (Jo et al. 2000; Palmiter et al. 1996; Wenzel et al. 1997), and for metallothionen-3 (MT3), a zinc binding metalloprotein (Cole et al. 2000; Masters et al. 1994) that can also bind copper (Chen et al. 1996; Sewell et al. 1995; Winge et al. 1994). In contrast to zinc, little is known about the precise location of copper in the olfactory bulb. However, many of the histological techniques used to identify zinc (e.g., Timm’s stain) cross-react with copper and, therefore, may reflect both zinc and copper. Furthermore, it remains unclear whether ZnT-3 can transport copper in addition to zinc. Therefore as is the case for zinc, some of the copper in the olfactory bulb may be in synaptically releasable pools as occurs in other brain regions (Assaf and Chung 1984; Hartter and Barnea 1988; Howell et al. 1984; Kardos et al. 1989).

We have already shown that physiologically relevant concentrations of zinc or copper can modulate amino acid receptors and synaptic transmission and, in some cases, with differential effects (Trombley and Shepherd 1996; Trombley et al. 1998). However, the neuromodulatory role(s) of zinc and copper in the olfactory bulb have yet to be completely defined. In the present report, we used whole cell electrophysiology and primary neuronal cultures of olfactory bulb neurons to demonstrate that the net effects of zinc and copper on neuronal excitability are distinct and therefore may contribute to selective modulation of synaptic circuits involved in olfactory information processing.

METHODS

Cell culture

Olfactory bulbs were prepared for primary dissociated cultures using methods previously described in Trombley and Westbrook.
(1990) and Trombley and Blakemore (1999). The olfactory bulbs were isolated from 0- to 3-day-old rat pups and cut into small pieces after removing the meninges. The tissue was incubated at 37°C for 60 min in a calcium-buffered solution containing 20 U/ml papain (Worthington, Freehold, NJ) and 1 mM cysteine. The tissue was gently triturated with a fire-polished Pasteur pipette after inactivating the enzyme with excess media containing serum. The cell suspension was plated at a density of approximately 260 cells/mm² in 35-mm culture dishes (Corning, Fisher) containing a confluent layer of astrocytes prepared previously. The neuronal growth medium contained 93% minimal essential medium (MEM, Gibco), 7% horse serum, 6 g/l glucose, and a nutrient supplement (Serum Extender, Collaborative Research, Bedford, MA). Electrophysiological recordings were conducted after the neurons were in culture for 2–14 days.

Astrocyte feeder layers were prepared by plating olfactory bulb cells, as described in the preceding text, in a 75-cm² poly-L-lysine-coated flask, containing MEM with 7% fetal calf serum and 6 g/l glucose. After reaching confluence, the astrocytes were removed with 0.125% trypsin, resuspended, and plated on 35-mm culture dishes coated with poly-L-lysine. After reaching confluence, the astrocytes were treated with a mitotic inhibitor, 5-fluoro-2'-deoxyuridine, and uridine (7 μg/ml) to prevent overgrowth.

Electrophysiology

All whole cell voltage-clamp recordings were made at room temperature. The 35-mm culture dish was used as the recording chamber and perfused at 0.5–2.0 ml/min with a bath/control solution containing (mM) 162.5 NaCl, 2.5 KCl, 2 CaCl₂, 10 HEPES, 10 glucose, and 1 or 0 MgCl₂ and 1 μM glycine. The pH was adjusted to 7.3 with NaOH, and the osmolarity was adjusted to 325 mosM. Patch electrodes were pulled from filamentous borosilicate glass to a final electrode resistance of 4–6 MΩ. Electrodes were filled with a solution containing (mM): 145 KMeSO₄ or CsCl, 1 MgCl₂, 10 HEPES, and 1.1 EGTA. The final pH was adjusted to 7.2 with KOH or CsOH, and the final osmolarity was 310 mOsm. Drugs were diluted in recording solution and applied to the cultured neurons using a gravity-fed flow-pipe perfusion system, assembled from an array of 600-μm-ID square glass barrels. The barrels were positioned 100 μm from the neuron using an electronic micromanipulator (Warner Instrument, Hamden, CT), and the flow was controlled using pinch clamps. Complete solution exchanges occurred within 100–200 ms of application. Neurons were always perfused with a control solution via flow pipes except during drug application. Substances applied were ZnCl₂, CuCl₂, CdCl₂, and TTX (Sigma).

Experimental procedure

To examine membrane currents mediated by K⁺, Na⁺, and Ca²⁺ voltage-gated ion channels, whole cell voltage-clamp recordings were made using an AxoClamp 2B amplifier (Axon Instruments, Foster City, CA) in either discontinuous mode (switching frequency of 10–15 kHz) or continuous mode. Membrane currents were filtered at 1–3 kHz, digitized at 5–10 kHz, and analyzed using AxoData and AxoGraph software (Axon Instruments). Current-clamp data from studies designed to examine single action potentials, repetitive firing, or input resistance were collected unfiltered using the same amplifier and software.

Data analysis

Data are expressed as means ± SD. P values were determined by paired t-tests, and values ≤0.05 were considered significant.

RESULTS

Zinc and copper inhibit spontaneous excitatory and inhibitory synaptic transmission

We have previously reported that zinc and copper can modulate glutamate, GABA, and glycine receptors on olfactory bulb neurons (Trombley and Shepherd 1996). Here, we examined the effects of zinc and copper on spontaneous excitatory and inhibitory synaptic activity. To facilitate robust spontaneous activity, the neurons were plated in 35-mm culture dishes at a density of approximately 260 cells/mm², and the recordings were made after the neurons were in culture for approximately 1 wk. Spontaneous inhibitory synaptic activity was recorded in current-clamp mode from mitral/tufted cells, the primary targets of olfactory bulb inhibitory interneurons. The large amplitude of the inhibitory synaptic potentials in Fig. 1A was due to a low intracellular chloride concentration. Flow-pipe application of either metal resulted in complete suppression of inhibitory synaptic transmission in all neurons examined (copper, n = 4; zinc, n = 6; Fig. 1A). In these experiments, the inhibitory postsynaptic potentials were mediated by GABAₐ receptors, as confirmed by their inhibition in response to application of 3 μM bicuculline (data not shown).

In contrast to the similarity of the effects of zinc and copper...
on inhibitory transmission, zinc and copper had quantitatively different effects on excitatory transmission. Whereas application of 100 μM zinc only reduced glutamate-mediated excitatory transmission in mitral/tufted cells (n = 7), 30 μM copper completely eliminated all excitatory activity (n = 9; Fig. 1B). The effects of either metal on inhibitory or excitatory transmission were rapidly reversible on washing with control solution.

**Zinc and copper inhibit inward Ca\(^{2+}\) currents**

The modulatory effects of zinc and copper on voltage-gated calcium-channel currents were examined to explore whether differential effects on these currents could contribute to their differential effects on excitatory transmission, possibly through effects on transmitter release. For ease of analysis, 2 mM calcium in the bath solution was substituted with 10 mM Ba\(^{2+}\) to increase the amplitude of the current. Calcium-channel currents were evoked in voltage-clamp by holding the potential at −60 mV and stepping to 0 mV for a duration of 50 ms. Data were collected in the presence of 1 μM TTX and a CsCl-based electrode solution to block Na\(^+\) and K\(^+\) currents, respectively.

Calcium-channel currents with kinetic profiles typical of high-threshold Ca currents were induced in 15 of 16 neurons. The peak amplitude of these currents ranged between 179.3 and 673.9 pA (mean = 293 ± 232 pA). Measurements of the currents were made at the peak and steady-state components of the current. Zinc application inhibited the Ca\(^{2+}\) channel currents at both time points by 63 ± 33 and 63 ± 13%, respectively (n = 16; P < 0.001; Fig. 2). Copper application reduced the inward current at both time points by an average of 52 ± 27 and 54 ± 22%, respectively (n = 12; P < 0.001; Fig. 2).

**Differential effects of zinc and copper on input resistance**

We next hypothesized that the differential effects of zinc and copper on basic membrane properties may have contributed to their quantitatively different effects on excitatory transmission. In current-clamp mode, the effects of zinc and copper on input resistance were determined using intracellular injections of 250-ms hyperpolarizing steps of −50 pA. Hyperpolarizing pulses were used to reduce any contribution from activation of voltage-gated ion channels on input resistance. The prepulse membrane potential was maintained near −50 mV with a small amount of current injection (±10 pA). Measurements for analyses were made after the membrane voltage reached steady-state.

Analyses of neuronal input resistance in the presence of 30 μM copper indicated no significant difference compared with that of the control solution (1 ± 15% increase, n = 13; P > 0.5). However, application of 100 μM zinc produced a modest (18 ± 11%) increase in input resistance (Fig. 3A, n = 10; P < 0.001).

**Zinc and copper inhibit inward Na\(^+\) currents but do not prevent evoked single action potentials**

We further hypothesized that the quantitatively different effects of zinc and copper on the postsynaptic response to excitatory transmission may be mediated, in part, by distinct effects on voltage-gated ion channels. As an initial test of this hypothesis, we examined the effects of zinc and copper on inward Na\(^+\) currents, the major component of the upstroke of an action potential. The Na\(^+\) currents were evoked by 50-ms, −70- to −20-mV steps under voltage-clamp conditions. The electrodes in this experiment were filled with a CsCl-based solution to block K\(^+\) currents. Measurements for analyses were made at the peak amplitudes of the evoked current. Individual application of 100 μM zinc (n = 13; P < 0.001) or 30 μM copper (n = 10; P < 0.005) resulted in significant decreases in the Na\(^+\) current by an average of 22 ± 17 and 20 ± 16%, respectively, compared with control currents (Fig. 3B). Current blockade with 1 μM TTX confirmed that these currents were mediated by typical TTX-sensitive Na\(^+\) channels.

Because zinc and copper reduced inward Na\(^+\) currents, we next examined whether zinc or copper could reduce the ability of olfactory bulb (OB) neurons to fire single evoked action potentials. Single action potentials were evoked by injecting incremental steps of 0.05 nA, for a 5-ms duration, until the neuron fired an action potential. The initial prestep resting potential was approximately −60 mV.

Analyses of the action potential amplitudes following metal application show that neither copper (n = 8; P > 0.3) nor zinc (n = 10; P > 0.2) induced significant changes compared with control conditions. Furthermore, data analyses show that neither zinc nor copper caused significant differences in the half-widths of the action potentials (Cu: P > 0.3; Zn: P > 0.5) nor the amplitude of the current required to initiate an action potential (Cu: P > 0.3; Zn: P > 0.3) compared with control currents (Fig. 3C). In 4 of 10 neurons, zinc application reduced the spike after-hyperpolarization by an average of 64 ± 69% (P < 0.02).

**Zinc and copper inhibit delayed-outward K\(^+\) currents**

Because the delayed outward K\(^+\) current plays a major role in the downstroke and repolarization of an action po-
potential, we examined the modulatory actions of zinc and copper on this current. Potassium currents were examined using a KMeSO₄-based electrode solution and 1 mM TTX to block Na⁺ currents. Neurons were clamped at -50 mV and stepped to +20 mV for a duration of 100 ms. Neurons responded with an outward current consistent with the kinetic profile characteristic of delayed rectifier-type currents in these neurons (Trombley and Westbrook 1991). Measurements for data analyses were made at peak and steady-state amplitudes.

Copper (30 μM) application inhibited the peak amplitudes by an average of 18 ± 9% (n = 5; P < 0.02) and the steady-state amplitudes by an average of 19 ± 9% (n = 5; P < 0.01; Fig. 4). Similarly, zinc (100 μM) application inhibited steady-state current amplitudes by an average of 21 ± 13% (n = 11; P < 0.003; Fig. 4). However, zinc potentiated the peak current amplitude in most neurons (n = 8) and had no effect in three others. In the eight neurons in which potentiation was observed, the potentiation of the peak current amplitude was due to the appearance of a transient A-type outward current component that was not present under control conditions. In these neurons, the peak current amplitude was potentiated by an average of 21 ± 16% (P < 0.007). Zinc had no significant effect on the peak current in the remaining three neurons. In these neurons, no A-type current component was apparent.

**Zinc and copper modulate transient-outward K⁺ current**

The transient-outward K⁺ current plays a role in mediating the interspike interval and is usually activated by a depolarizing event following a hyperpolarizing event. To examine this current, both the transient and delayed outward K⁺ currents were activated by holding the neuron at a hyperpolarizing potential of -90 mV and stepping the neuron to +20 mV for a duration of 100 ms. As in the previous experiments, these K⁺ currents were isolated using 1 mM extracellular TTX and a KMeSO₄-based intracellular solution. Measurements for analyses were made at peak and steady-state amplitudes.

Zinc (n = 8; P < 0.001) and copper (n = 7; P < 0.003) application attenuated the steady-state outward K⁺ current in
all of the neurons examined by an average of 17 ± 8 and 20 ± 11%, respectively (Fig. 5). Similarly, copper (n = 7; P < 0.001) application inhibited the transient outward K⁺ current in all neurons examined by an average of 17 ± 7%. Zinc application significantly inhibited the transient outward K⁺ current in most neurons (n = 8; P < 0.005) by an average of 15 ± 11%. Two other neurons showed no effect.

**Zinc and copper modulate repetitive firing**

Although our results show that zinc or copper application did not significantly reduce the ability of a neuron to fire single action potentials, we hypothesized that the complex effects of these metals on K⁺ currents may influence repetitive firing. Repetitive firing was evoked by injecting 0.1-nA increments of 150-ms duration until trains of action potentials were fired from holding potentials of −50 and −90 mV. The application of 30 μM copper reduced neuronal firing at a holding potential of −50 mV by an average of 45 ± 28% (n = 29; P < 0.001; Fig. 6). At a holding potentials of −90 mV, copper reduced the number of evoked action potentials by 46 ± 23% in five neurons (Fig. 7, P < 0.001), but had no effect on repetitive firing in four others. In contrast to copper, zinc modulation of repetitive firing was voltage dependent in some neurons. The application of zinc reduced neuronal firing in all neurons examined at a holding potential of −50 mV by an average of 49 ± 41% (n = 9, P < 0.001; Fig. 6). At −90 mV, the majority (69%) of neurons responded to zinc application with a reduction in the number of action potentials fired (63 ± 16%; P < 0.001; n = 9). However, in contrast to copper, a significant proportion of the neurons (31%, n = 4) increased action potential frequency by 100–500% in response to application of zinc (Fig. 7).

**DISCUSSION**

The present study demonstrates that, in addition to their effects on ligand-gated ion channels (Trombley and Shepherd 1996; Trombley et al. 1998), physiologically relevant concentrations of zinc and copper can influence the neuronal excitability of olfactory bulb neurons by modulating voltage-gated ion channels. Furthermore, our results indicate that zinc and copper have distinct effects on voltage-gated K⁺ channels and input resistance, effects that may alter a neuron’s capacity to repetitively fire action potentials.
Reportedly, ≥15% of brain zinc is contained in synaptic vesicles (Frederickson et al. 1989). Particularly high concentrations of zinc are found in the vesicular compartments of the olfactory bulb, where zinc is primarily localized to the glomerular and granule cell layers (Friedman and Price 1984; Jo et al. 2000; Ono and Cherian 1999; Perez-Clausell and Danscher 1985). Jo et al. (2000) have demonstrated that the pattern of zinc visualized with zinc autometallography in the olfactory bulb is correlated with the pattern of immunoreactivity for ZnT3, a zinc transporter required for vesicular accumulation of zinc (Cole et al. 1999). Furthermore, metallothionein III, a zinc-binding protein that can also bind copper (Chen et al. 1996; Sewell et al. 1995; Winge et al. 1994), is expressed in olfactory bulb neurons that sequester zinc in synaptic vesicles (Masters et al. 1994). In a subclass of vesicular zinc-containing neurons, glutamate and zinc have been shown to be co-localized to the same synaptic terminal from which they are co-released in a Ca^{2+}-dependent manner following membrane depolarization or neural activity (Assaf and Chung 1984; Beaulieu et al. 1992; Crawford and Connor 1973; Frederickson and Moncrieff 1994; Holm et al. 1988; Howell et al. 1984; Martinez-Guijarro et al. 1991; Rubio and Juiz 1998).

The olfactory bulb also contains a high concentration of copper (e.g., Donaldson et al. 1973; Ono and Cherian 1999), although the precise location of copper in the OB has yet to be determined. However, it has been shown in the hypothalamus and cortex that, similar to zinc, copper can be released by membrane depolarization or neural activity (Assaf and Chung 1984; Beaulieu et al. 1992; Crawford and Connor 1973; Frederickson and Moncrieff 1994; Holm et al. 1988; Howell et al. 1984; Martinez-Guijarro et al. 1991; Rubio and Juiz 1998).

Direct effects

The lack of an effect of applied copper on input resistance supports the notion that copper ions do not directly activate ion channels or have a significant effect on leakage channels. Our results show modest increases in membrane resistance in response to zinc, which appear to be due to blockade of leakage channels. This finding is consistent with the effects of zinc on the input resistance of hippocampal neurons, as reported by Mayer and Vylicky (1988). In contrast, Zhou and Hablitz (1993) showed that zinc application induced no effect on membrane resistance in rat neocortical slices. The differences in these results may be due to differences in the preparations (culture vs. slice) and experimental approach. For example, the application techniques used in the present culture experiments ensured that the neurons were enveloped in 100 μM zinc. The concentration of zinc reaching the cell in the slice preparation could be substantially lower than the 50- to 300-μM concentration that was applied. Differences in the expression and/or sensitivity of leakage channels between these preparations...
have not been determined but could also contribute to the observed differences.

**Sodium currents and action potentials**

We observed significant inhibition of Na\(^+\) currents with the application of either metal. In contrast, Easaw et al. (1999) observed no significant change in acutely dissociated neurons from the horizontal limb of the diagonal band of Broca in response to application of 50 \(\mu\)M zinc. Acute dissociations tend to lower the density of ion channels. This, combined with the lower concentration of zinc used (50 vs. 100 \(\mu\)M), may have contributed to the different results. Furthermore, our observation that applied copper significantly inhibits Na\(^+\) currents is consistent with the previous findings of some investigators (Flonta et al. 1998; Skulski and Lapin 1992).

Although application of either metal inhibited currents mediated by voltage-gated TTX-sensitive Na\(^+\) channels, these inhibitory effects were not sufficient to inhibit the firing of evoked single action potentials nor to significantly alter their kinetics. These results are similar to the effects of zinc on hippocampal neurons reported by Mayer and Vyklicky (1989). However, they reported that, at 50 \(\mu\)M, zinc caused a small reduction in action potential half-width with a substantial effect at 1 mM. In olfactory bulb neurons, we saw little effect of zinc at 100 \(\mu\)M (the highest concentration used) on action potential half-widths. These subtle differences may reflect differences in potassium channels between these neuronal populations, which mediate repolarization, thus influence action potential kinetics. The present experiments also suggest that the effects of zinc and copper on voltage-gated Na\(^+\) channels, at physiologically relevant concentrations, are probably not a major contributor to the observed significant reduction/elimination of spontaneous excitatory activity between olfactory bulb neurons.

**Zinc and copper have distinct effects on voltage-gated K\(^+\) channels**

Results from the present experiments suggest that the effects of zinc on K\(^+\) channels depend not only on the type of K\(^+\) channel (transient vs. delayed rectifier) but are also voltage dependent. At all voltages examined, zinc inhibited a delayed rectifier-type outward current. Delayed rectifier-type channels play a significant role in the repolarization phase of action potentials, thus facilitate recovery of Na\(^+\) channels from voltage-dependent inactivation. Their inhibition would likely lead to a reduction in the rate of repetitive firing of action potentials. Our results support this notion, as both zinc and copper reduced the amplitude of a delayed rectifier-type K\(^+\) current and also reduced the frequency of action potentials evoked from a membrane potential of \(-50\) mV by long depolarizing current steps.

The effects of zinc and copper on the transient A-type current were more complex. Copper inhibited the transient current at all membrane potentials. However, zinc inhibited the current at hyperpolarized potentials but potentiated the current at membrane potentials at, or depolarized to, the typical resting potential. The effects of zinc on transient A-type currents in OB neurons are consistent with the reported effects of zinc on K\(^+\) currents in hippocampal neurons (Harrison et al. 1993), cerebellar neurons (Bardoni and Belluzzi 1994), and OB periglomerular neurons (Puopolo and Belluzzi 1998). Although the A-type current is activated by depolarizations from hyperpolarized potentials, in most neurons, including OB neurons (e.g., Puopolo and Belluzzi 1998), it is largely inactivated at the resting membrane potential. The A-type outward current slows the rate of depolarization and, hence, reduces the rate of repetitive action potential firing. The effects of zinc we observed, in a significant proportion of neurons, suggests that zinc may modulate repetitive firing in a voltage-dependent manner. That is, at hyperpolarized potentials, zinc can inhibit the A-type current and increase repetitive firing, whereas at depolarized potentials, zinc can enhance the current and reduce repetitive firing.

In a significant proportion of neurons, zinc reduced repetitive firing from a holding potential of \(-90\) mV. Copper always reduced the transient current but also reduced repetitive firing. We believe that the relative magnitude of the effects of zinc and copper on the A-type versus the delayed rectifier-type currents, as well as the variations in the relative amplitudes of A-type currents versus delayed rectifier-type currents, may explain the variation in the effects of zinc at \(-90\) mV. For example, in neurons in which the amplitude of the transient current is low, a reduction in this current at \(-90\) mV by zinc (which should lead to an increase in repetitive firing) may be overwhelmed by a reduction in the delayed rectifier current and thus produce an overall net decrease in repetitive firing.

Copper has a higher affinity than zinc for most substrates, thus affinity differences between zinc and copper could contribute to differences in their effects. However, the differences in the effects of zinc and copper discussed in the preceding text are not likely to be due simply to differences in their binding affinities because either zinc had a greater effect or zinc and copper had similar effects.

**Significance to olfactory bulb circuit function**

Zinc and copper can modulate synaptic transmission postsynaptically via direct actions on glutamate, GABA, or glycine receptors. The present results also suggest that zinc and copper could have presynaptic effects on transmitter release via inhibition of voltage-gated calcium channels. In addition to these effects on neurotransmission, zinc and copper can influence neuronal excitability by modulating voltage-gated ion channels, particularly K\(^+\) channels. The voltage-dependent effects of zinc on the A-type current suggest that the net result (a decrease or an increase in the ability to repetitively fire) is dependent on whether the neuron has just experienced a depolarizing event (e.g., an EPSP) or a hyperpolarizing event (e.g., an IPSP).

Recent reports suggest that effects of zinc (and perhaps copper) on A-type channels may have important implications for synaptic circuits that involve the two primary populations of olfactory bulb neurons, mitral/tufted cells and inhibitory interneurons (periglomerular cells and granule cells). Puopolo and Belluzzi (1998) have recently reported that periglomerular cells express prominent A-type currents that are modulated by zinc. A subset of these neurons expresses almost exclusively the A-type channel (in contrast to a combination of A-type and delayed-rectifier-type channels in other periglomerular cells). Chen and Shepherd (1997) have reported that mitral/tufted cells display a characteristic delay in the onset of action po-
tential firing, which they suggest is likely due to A-type potassium currents. Because zinc-containing olfactory sensory neurons make synaptic contact with both mitral/tufted cells and periglomerular cells, zinc may have significant effects on these neurons, hence, glomerular circuit activity. Furthermore, it has recently been demonstrated that A-type currents generate a delay in action potential firing in granule cells and play an important role in the synaptic timing of reciprocal inhibition (Schoppa and Westbrook 1999). In light of the opposing action of A-type currents, AMPA receptor-mediated events are attenuated, thus require the longer-duration NMDA receptor-mediated events for effective reciprocal inhibition.

The present results, in combination with other recent work, suggest that zinc and copper can influence neuronal excitability and synaptic transmission in the olfactory bulb by multiple mechanisms. Such modulation may contribute to odor information processing through effects on transmitter release, amino acid receptor function, and synaptic timing.

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