Biometals (2014) 27:45–52
DOI 10.1007/s10534-013-9685-4

Micromolar copper modifies electrical properties and spontaneous discharges of nodose ganglion neurons in vitro

Fernando C. Ortiz · Cecilia Vergara · Julio Alcayaga

Received: 5 July 2013 / Accepted: 4 November 2013 / Published online: 10 November 2013

Abstract Copper plays a key role in aerobic cell physiology mainly related to mitochondrial metabolism. This element is also present at higher than basal levels in some central nuclei and indeed, current evidence support copper's role as a neuromodulator in the central nervous system. More recent data indicate that copper may also affect peripheral neuronal activity, but so far, there are not detailed descriptions of what peripheral neuronal characteristics are targeted by copper. Here, we studied the effect of physiological concentration of $CuCl_2$ (μM range) on the activity of peripheral neurons using a preparation of nodose ganglion in vitro. By mean of conventional intracellular recordings passive and active electrical membrane properties were studied. Extracellular copper modified (in a redox-independent manner) the resting membrane potential and the input resistance of the nodose ganglion neurons, increasing the excitability in most of the tested neurons. These results suggest that Cu^{2+} modulates the activity of nodose ganglion neurons and support nodose ganglion in vitro preparation as a simple model to study the subcellular mechanisms involved in the Cu^{2+} effects on neuron electrical properties.

Keywords Copper · Excitability · Primary sensory neurons · Nodose ganglion · Vagal afferents

F. C. Ortiz · C. Vergara (✉) · J. Alcayaga
Laboratorio de Fisiología Celular, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile
e-mail: cvergara@uchile.cl

Present Address:
F. C. Ortiz
Neurophysiology and New Microscopes Laboratory, INSERM U603, CNRS UMR 8154, University of Paris Descartes, 45 rue des Saints-Pères, 75006 Paris, France
e-mail: fernando.ortiz-cisternas@parisdescartes.fr

Introduction

Ionic copper (Cu^{2+}) plays an essential role in cell physiology acting as cofactor of enzymes related to mitochondrial metabolism and therefore it is present in every aerobic cell (Osiewacz and Borghouts 2000; Rossi et al. 2004). This element is also present in several regions of the central nervous system (CNS) located within synaptic or somatic vesicles. Higher than "basal" copper levels are present mainly in the cerebellum, olfactory bulb, hippocampus and cortical areas (Kardos et al. 1989; Sato et al. 1994; Trombley and Shepherd 1996; Tarohda et al. 2004), suggesting some specific role of cooper in these neural circuits. In agreement with this, there are reports on the effects of copper on several voltage and ligand gated channels (Mathie et al. 2006; Huidobro-Toro et al. 2008; Gaier et al. 2013). Also, patients suffering from Menkes and Wilson diseases, that are related to

a deficit and an increase in copper levels, respectively, present severe neurological damage (see de Bie et al. 2007; Kodama et al. 2011). Copper dyshomeostasis has also been associated with several other neurodegenerative pathologies, like Alzheimer (AD), Amyotrophic Lateral Sclerosis (ALS) or Inclusion Body Myositis that share several aspects with AD (Aldunate et al. 2012; Eskici and Axelsen 2012; Roos et al. 2013). This evidence has led to the proposal that copper acts as a neuromodulator in the CNS, but its action mechanisms are not completely understood (Trombley et al. 2000; Horning and Trombley 2001; Aedo et al. 2007; Gaier et al. 2013). Evidence from our group indicates that in the CNS copper modulation of excitability is a complex phenomenon. In the CA1 area of the hippocampus, nanomolar exogenous copper enhances cellular and network excitabilities and improves temporal processing (Maureira et al. 2006). On the CA3 area, copper also triggers increases on spontaneous discharges but in the micromolar range (Ignacio Diaz, unpublished observations). On the other hand, more recent data indicate that peripheral copper may also affect neuronal and motor activity. Two relevant examples: (a) there is evidence that copper dyshomeostasis in motor neurons at the periphery can be the cause of distal hereditary motor neuropathy and probably other related motor neurons diseases (Merner et al. 2011), and (b) copper in the micromolar range can alleviate some of the symptoms in a *Caenorhabditis elegans* model of Inclusion Body Myositis (Rebolledo et al. 2011). Interestingly, copper concentration in the CNS oscillates from 0.2–1.7 μM in the extracellular compartment and estimations for the synaptic cleft are up to 200 μM (Mathie et al. 2006); nevertheless in the peripheral nervous system (PNS) neurons are exposed to different copper levels, namely: plasmatic copper is around 15 μM (Mathie et al. 2006). To our knowledge, there are no detailed reports of which could be the effect of copper on PNS neurons excitability.

Considering that there are different levels of copper in the PNS compared to CNS it appears important to characterize Cu effects in PSN neurons. Here we report that copper, in concentrations within the physiological range, modifies the electrical properties of sensory neurons from the rabbit nodose ganglion-in a redox-independent way- increasing the excitability in most of the tested neurons.

Methods

Animals and surgery

White New Zealand male rabbits (2.2 ± 0.1 kg; $N = 7$) were anesthetized with ketamine/xylacin (75/7.5 mg/Kg, i.m.). Nodose ganglia (NG; $n = 11$) were excised and placed in ice-cold Hanks balanced salt solution (HBSS). The capsule and connective tissue were carefully removed from over the ganglion before it was placed in a chamber and continuously superfused (1.2–1.5 mL/min) with HBSS, supplemented with HEPES buffer (5 mM, pH 7.43, 22 °C), equilibrated with air at room temperature (22 °C). At the end of surgery, animals were sacrificed with a sodium pentobarbital overdose (120 mg/kg, i.p.). All experimental procedures were approved by the Bioethical Committee of the Facultad de Ciencias of the Universidad de Chile, and were conducted in accordance to the guidelines of the Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT, Chile).

Experimental preparation and Intracellular recording

Conventional intracellular electrodes were pulled (P-87 Puller, Sutter Instruments, USA) from borosilicate glass and backfilled with KCl 3 M (25–30 MΩ). The electrodes were connected through an Ag/AgCl electrode to an electrometer (IE-210, Warner Instruments, USA). The signal was amplified, filtered (DC-10 kHz), acquired at 10 kHz (Axotape, Molecular Devices, USA) and recorded using an analog-to-digital converter (Digidata 1200, Molecular Devices, USA). A reference (Ag/AgCl) electrode was connected to the recording bath, and junctional voltage, electrode series resistance and capacity were compensated in the chamber before cell impaling. The microelectrode was located over the ganglion and advanced through the tissue with a hydraulic micromanipulator (Narishige, Japan) until a neuron was reached. Impalement was accomplished by a brief decompensation of the electrode capacitance. Once a stable recording (at least 5 min) of a neuron was obtained, resting membrane potential (V_{rest}) was measured and seven depolarizing current pulses (1 ms, 1 Hz) were applied in order to trigger action potentials (AP), from which amplitude (AP_a), duration

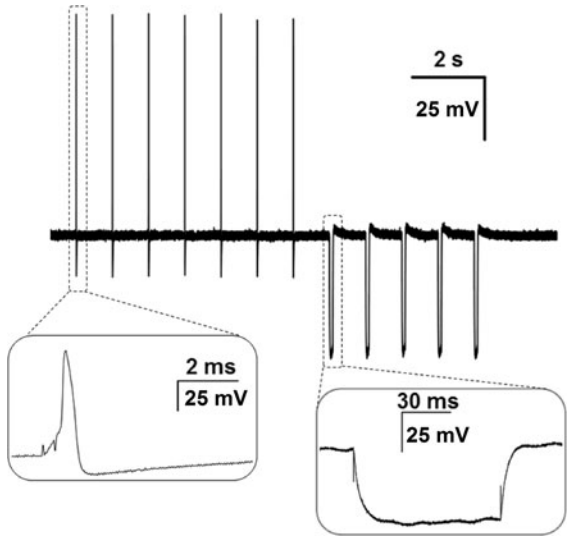

Fig. 1 Intracellular recording from a nodose neuron showing evoked activity in response to depolarizing (1 ms, 1 Hz) and hyperpolarizing (90 ms, 1 Hz) currents. *Insets* show the time course of the action potential (*left inset*) and hyperpolarizing response (*right inset*)

(AP_d) and threshold (AP_{th}) values as well as afterhyperpolarization amplitude (Hyp_a) and duration (Hyp_d) were obtained. To measure input resistance (R_{in}) and membrane capacitance (C_m), five long-lasting hyperpolarizing pulses (90 ms, 1 Hz) were applied after the depolarizing pulses (Fig. 1). R_{in} was calculated from the voltage change induced by the current at the end of the pulse. The membrane time constant (τ) was calculated from the simple exponential curve fitted to the trace of the voltage change, and C_m calculated from the relation $\tau = R_{in}C_m$. Only cells presenting a stable initial $V_{rest} \leq -40$ mV and an action potential overshoot $\geq +10$ mV were included in the analyses.

Increasing concentrations of $CuCl_2$ (0.1, 0.2, 0.5, 1, 10, 20, 50, 70 and 100 μM) were applied over the recording site through a gravity perfusion system.

We performed an estimation of the effective Cu^{2+} concentration sensed by the recorded cells by comparing the experimental curve for V_{rest} changes induced by known different extracellular KCl concentrations with the theoretical expected curve, when KCl was applied with the same system. Considering that reported diffusion coefficients for KCl (1.8–1.9 m^2/s^{-1}) and $CuCl_2$ (1.2–1.6 m^2/s^{-1}) are similar in our experimental conditions (Harned and Nuttall 1947; Lobo et al. 1998; Ribeiro et al. 2005), we obtained a dilution factor of 7. The values for copper shown are not corrected by this dilution factor.

In some experiments dithiothreitol (DTT) 10 mM was applied in the superfusion medium. All chemicals were obtained from Sigma-Aldrich Co. (USA).

Data analysis and statistic

Data were analyzed with Clampfit (Molecular Devices, USA) and GraphPad Prism (GraphPad Software Inc., USA) software. Electrophysiological parameters were compared using paired Student's *t* test or Mann–Whitney test (for two group comparisons) or one way parametric or nonparametric ANOVA (for multiple comparisons) according to the structure of data. Curves were adjusted using a Pearson or Spearman correlation test pursuant to data structure. Significant level was set at $P < 0.05$. All values given as mean ± SEM; *n* corresponds to the number of neurons in each experimental series.

Results

Copper effect on evoked electrical activity

A total of 39 quiescent and 10 spontaneously active NG neurons were recorded. Of the quiescent neurons, 34 (87.2 %) responded to the brief (1 ms) depolarizing pulses with a broad action potential that presented a large overshoot, an inflection (hump) in the repolarizing phase and a long lasting afterhyperpolarization (Fig. 2a; Table 1). The remaining neurons (5; 12.8 %) discharged brisk action potentials with a smaller overshoot and a shorter afterhyperpolarization (Fig. 2b; Table 1).

The action potential amplitude was significantly larger ($P < 0.05$, Mann–Whitney test) and duration of the spike and of the afterhyperpolarization were significantly longer ($P < 0.05$, Mann–Whitney test) in the neurons that presented action potentials with an inflection in the repolarizing phase ("humped"). The rest of the passive (V_{rest}, R_{in}, C_m, τ) and active properties (AP_{th}, Hyp_a, Hyp_d) were no significantly different between the two neuronal populations ($P > 0.05$, Mann–Whitney test; Table 1).

The application of copper in the micromolar range (20–100 μM) to quiescent neurons affected differentially V_{rest} and R_{in}. We found no noticeable effects in

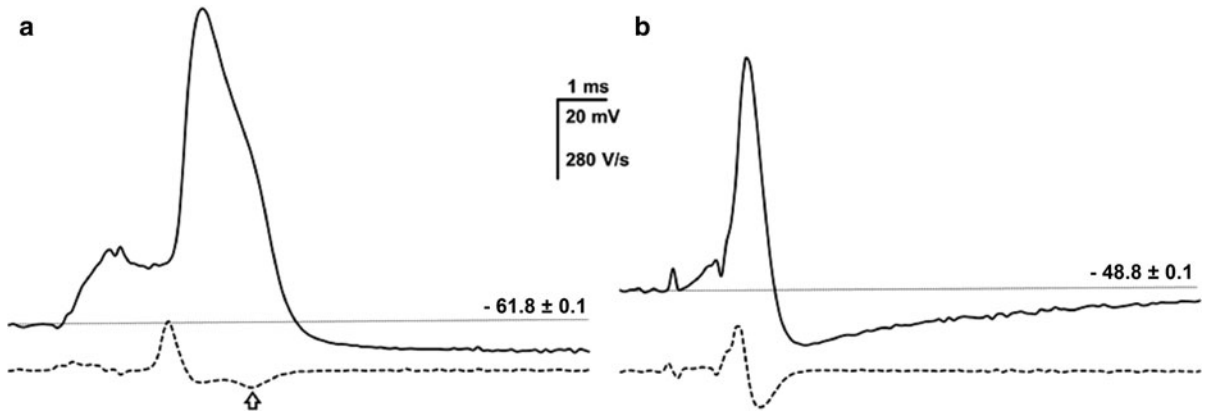

Fig. 2 Action potentials (*continuous line*) and its temporal derivative (*segmented line*) from nodose neurons evoked by brief (1 ms) depolarizing current pulses. **a** A neuron with a broad spike (> 1.5 ms) and an inflection in the repolarizing phase (*arrow* at derivative record). **b** A neuron showing a brisk action potential with a rapid repolarization and spike duration shorter than 1 ms. *Upper traces* membrane potential. *Lower traces* dV/dt. *Dotted line* V_{rest}

Table 1 Mean passive and active electrical properties of nodose ganglion neurons

Neuron AP type		V_{rest} (mV)	R_{in} (MΩ)	C_m (pF)	τ (ms)	AP_a (mV)	AP_d (ms)	AP_{th} (mV)	Hyp_a (mV)	Hyp_d (ms)
Humped ($n = 34$)	Mean	−55.1	17.4	198.4	2.2	81.2[a]	3.5[a]	−27.9	7.5	90.1[a]
	SEM	1.3	4.7	52.2	0.2	5.4	0.3	1.8	1.0	18.0
Non humped ($n = 5$)	Mean	−52.1	19.1	96.6	2.0	69.9	1.6	−28.8	8.6	23.7
	SEM	4.7	5.1	27.9	0.3	1.3	0.1	3.7	2.7	6.8

V_{rest} mean resting membrane potential, R_{in} mean input resistanc, C_m mean membrane capacitanc, τ mean membrane time constant, AP_a, AP_d, AP_{th} mean action potential amplitude, duration and threshold, respectivel, Hyp_a, Hyp_d mean afterhyperpolarization amplitude and duration, respectively

[a] $P < 0.05$, Mann–Whitney test

the low micromolar range (Fig. 3). The other passive and active parameters measured did not change significantly ($P > 0.05$, paired Student's t test; $n = 39$) during copper application.

According to the effect of copper on R_{in} and V_{rest}, the recorded neurons ($n = 39$) could be ascribed to one of four different groups. In most neurons (53.8 %; $n = 21$; Fig. 3a) copper application induced a significant depolarization ($r = 0.93$; $P < 0.05$, Pearson correlation test) and a decrease of R_{in} ($r = 0.99$; $P < 0.05$, Pearson correlation test). Conversely, in 20.5 % of the neurons $CuCl_2$ application produced a significant hyperpolarization ($r = -0.91$; $P < 0.05$, Spearman correlation test) and an increase of R_{in} ($r = 0.97$; $P < 0.05$, Pearson correlation test, $n = 8$; Fig. 3b). A third group ($n = 8$; Fig. 3c), representing 20.5 %, showed a depolarization that correlated significantly ($r = 0.95$; $P < 0.05$, Pearson correlation test) with increasing copper concentration, but unlike of first group with a significant increase in R_{in} ($r = 0.91$; $P > 0.05$, Pearson correlation test). Finally, only two neurons (5.2 %, Fig. 3d) were unresponsive to the application of copper, with no significant changes in V_m ($r = -0.01$; $P > 0.05$, Pearson correlation test) or R_{in} ($r = -0.27$; $P > 0.05$, Pearson correlation test) with increasing copper concentration.

In order to explore whether redox reactions are involved in the effect of copper action, DTT a strong reducing agent, was added to the bath solution (final concentration 10 mM) before and during copper application. Fig. 4a shows almost 8 min of continuous recording of membrane potential for a NG cell. The application of 50 μM copper caused a 16 mV depolarization. Application of DTT to the superfusion medium had no noticeable effect on this new resting membrane potential (Fig. 4a) or any of the other

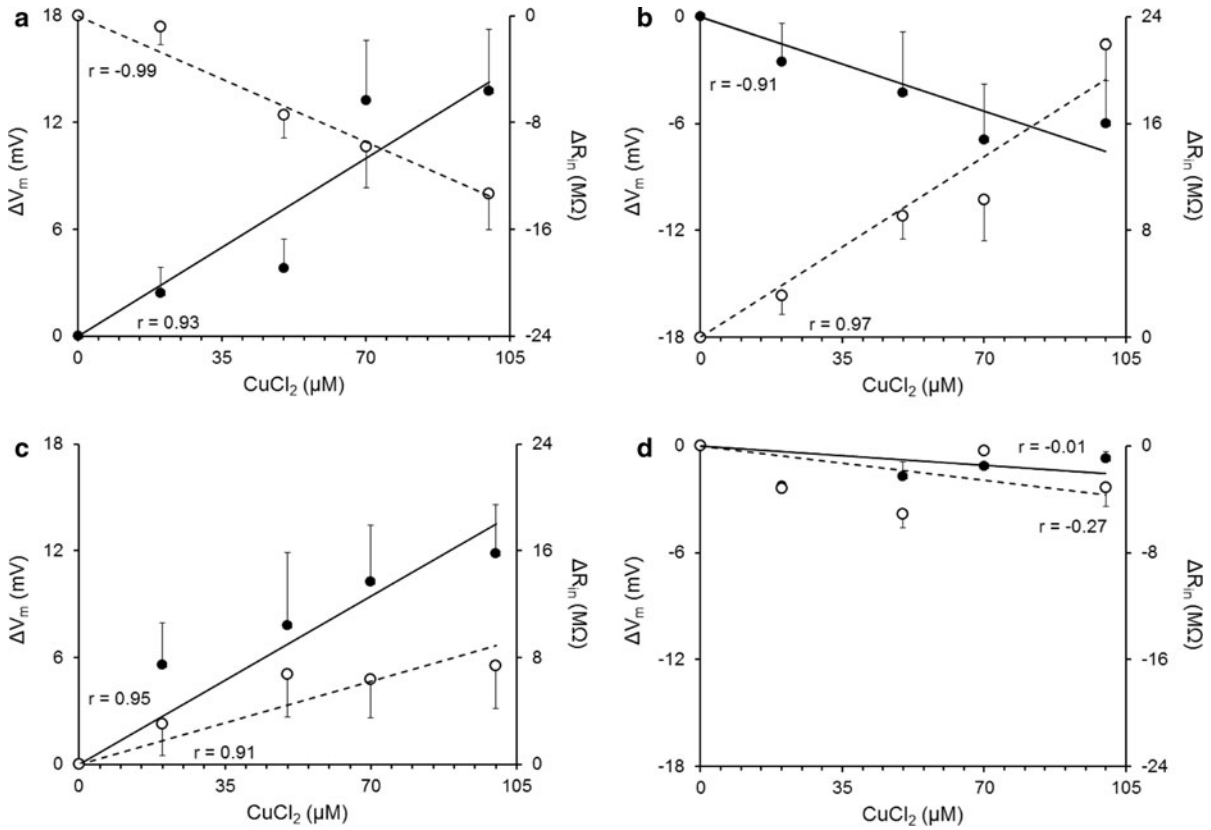

Fig. 3 Extracellular copper modifies both membrane potential (ΔV_m, *filled circles*, *solid line*) and input resistance (ΔR_{in}, *open circles*, *dashed line*). Cells were grouped according to the copper effect. Cells in **a** and **c** were depolarized by copper while cells in **b** and **d** were hyperpolarized. Group size in panels **a**–**d** were $n = 21$, $n = 8$, $n = 8$ and $n = 2$, respectively. Correlation coefficient (r) for most curves, obtained using Pearson correlation test, were significant ($P < 0.05$), except for changes in V_m and R_{in} in **d** ($P > 0.05$, Pearson correlation test)

measured parameters. Moreover, DTT was unable to prevent a further 10 mV depolarization induced by 70 μM copper (Fig. 4a, b; $P > 0.05$; Bonferroni test after Friedman test, $n = 10$).

Copper effect on spontaneous neuron activity

Copper effect was studied on ten NG neurons that presented basal spontaneous activity. In 8 (80 %) of these neurons the application of $CuCl_2$ (50 and 70 μM) induced a depolarization and increased the frequency of discharge, a proportion of neurons similar to those quiescent neurons depolarized by cooper application (29/39; 74 %; Fig. 3a, c). The frequency of spontaneous discharges increased in a dose-dependent manner after copper application (Fig. 5; $P < 0.05$; one way ANOVA). Moreover, the discharge pattern changed from an almost regular pattern in control conditions (Fig. 5ai) to a doublet burst pattern (Fig. 5aii, aiii) that was quite noticeable at the largest cooper concentration (70 μM; Fig. 5aiii). In this condition, the presence of DTT (10 mM) had no significant effect on the responses induced by maximal copper concentration (Fig. 5b; $P > 0.05$; Tukey test after one way ANOVA). The additional 2 tested neurons were unresponsive to copper.

Discussion

Several studies report that copper and zinc, among other trace metals, play a role in central nervous system as modulators of both synaptic transmission and neuronal excitability (Horning and Trombley 2001; Colvin et al. 2003; Quinta-Ferreira and Matias 2004; Delgado et al. 2006; Gaier et al. 2013). Here,

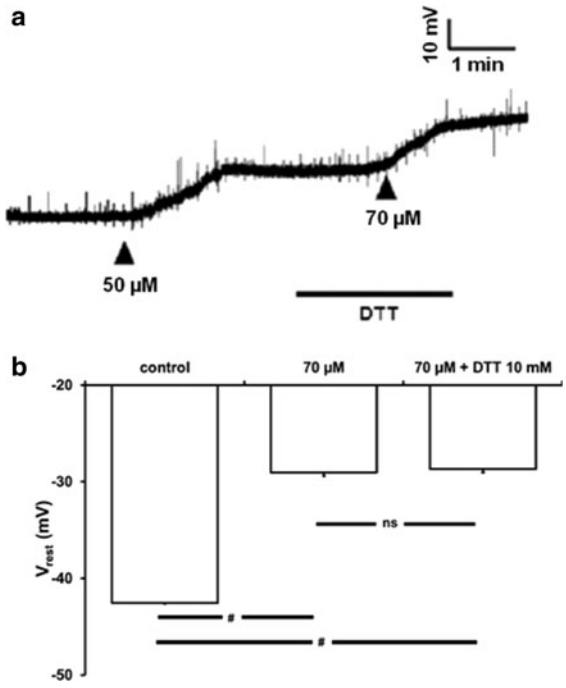

Fig. 4 Copper effects are not modified by dithiothreitol. **a** An ~8 min continuous recording of V_{rest}. Fifty micromolar copper added to the chamber (*first arrowhead*) induced a ~16 mV depolarization. DTT (10 mM) did not recover the depolarized state of the cell nor avoided a further 10 mV depolarization induced by increasing copper to 70 µM. **b** Mean effects ($n = 10$) on V_{rest} of 70 µM $CuCl_2$ either alone or in the presence of DTT (10 mM). The application of $CuCl_2$ produced a significant depolarization ($^{\#}P < 0.01$, Bonferroni test after Friedman Test), that was unaffected by DTT (*ns* no significant difference, $P > 0.05$, Bonferroni test after Friedman test)

using NG as model, we show that in neurons from the peripheral nervous system copper modifies resting membrane potential, input resistance and intrinsic burst properties in a concentration range that most probably is physiological.

Considering that our in vitro experimental preparation is the whole NG composed by several layers of cells and connective tissue, the concentration of copper that the cells receive is probably lower than what is added to the chamber. From our calibration with KCl and considering that copper could be bound we estimated that the copper concentration that reached the cells is at least seven times lower than the applied concentration. Thus, our data correspond to the effects of Cu^{2+} in ~1–15 µM range. The reported values for plasmatic copper levels vary among species, ages, metabolic state and several other variables that not always are explicit. For rabbits values go from ≈4–23 µM (Reddy et al. 1965; Alissa et al. 2004). For humans the highest reported value is 51 µM (Reyes et al. 2000) and plasmatic copper is around 15 µM for the healthy normal population (Mathie et al. 2006). Based in these reports, the concentration range of Cu^{2+} used in this study would correspond to the physiological range.

According to its electrical properties, the NG is constituted by a heterogeneous intermingled population of neurons. This heterogeneity probably reflects the different sensory modalities conveyed by NG neurons to the CNS that arise from different territories such as gastrointestinal tract, cardiac and pulmonary tissue. Similar differences have been found in other visceral sensory ganglion, like the petrosal ganglion (Belmonte and Gallego 1983). The effect of copper addition was not identical for every quiescent cell tested but most neurons (29 of 39) were depolarized in response to increasing copper concentrations and 73 % of those (21 of 29) also showed a decrease in R_{in}. Likewise, in 8 out of 10 spontaneously discharging cells, copper application caused a depolarization and an increase in their frequency of action potential discharges. Taken together, our data indicate that copper's main effect is to increase NG neurons excitability.

In this regard, some reports show that copper inhibits inward synaptic receptor currents, like the ones carried by NMDA or AMPA receptors in hippocampal or cortical neurons (Weiser and Wienrich 1996; Salazar-Weber and Smith 2011) and closer to our model, enhances ATP-evoked current through P2X receptors in rat NG neurons (Li et al. 1996). Since we recorded neurons from the isolated NG in vitro, we can rule out that observed effects are explained in terms of synaptic input modulation. Thus, the underlying mechanism involved in a depolarization with a decrease in R_{in} could be the onset or increase of some inward current, like—in classical view—sodium, calcium or—at resting membrane potential—a chloride conductance. However, previous reports show that copper in the micromolar range blocks or inhibits calcium, sodium and chloride channels (Degnan 1985; SkulskiiI and Lapin 1992; Copello et al. 1993; Castelli et al. 2003; Delgado et al. 2006; Lu et al. 2009; Chen et al. 2011). Interestingly, Aedo et al. (2007) proposed that in toad olfactory neurons, where copper has a biphasic effect, there are nanomolar and micromolar

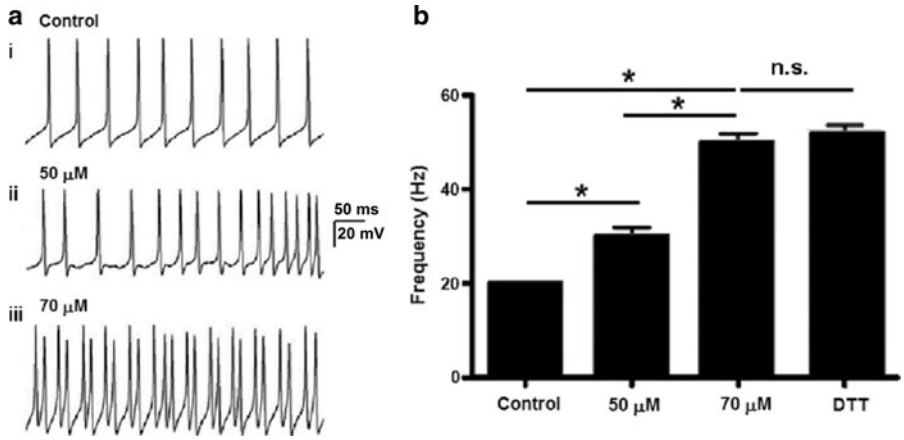

Fig. 5 Extracellular copper increased spontaneous frequency of discharge. **a** Continuous record of spontaneous activity shows that the application of $CuCl_2$ 50 μM (*ii*) and $CuCl_2$ 70 μM (*iii*) increases the frequency of discharge of this neuron. **b** Summary of effects of extracellular copper on 10 neurons, showing a significant ($P < 0.05$, one way ANOVA, Tukey test after one way ANOVA test) dose-dependent increase in discharge frequency. The responses induced by 70 μM $CuCl_2$ were unaffected by DTT. *$P < 0.05$; ns, no significant difference

affinity Cu^{2+}-binding sites in voltage-gated sodium channels through which copper could in turn activate or inhibit this sodium current (Delgado et al. 2006; Aedo et al. 2007). In NG neurons it is possible that copper in most cells activates a sodium or calcium current; this would explain the observed depolarization with an increase in membrane conductance (decrease in R_{in}) and the increase in the frequency of action potential discharge. Our group had previously reported that copper effect on the olfactory epithelium (1–5 μM induced a reduction of the discharge rate) is reversed by DTT 1 mM (Aedo et al. 2007). In addition, in the same study, 10 mM of DTT applied alone triggered a maximal increase in the discharge rate, indicating that in this system copper effect is partly mediated by redox modifications (Aedo et al. 2007). However, in our preparation, DTT 10 mM (concentration which has been shown to have a potent reducing effect; Niu et al. 2013) modifies neither V_{rest} nor neuronal discharge frequency by itself, or the copper effects. Thus, the action mechanism of the copper effect over the majority of the tested cells reported here does not involve a redox modification of membrane conductances. Although it is still necessary to identify the copper molecular target(s) in rabbit NG, the herein reported results suggest that Cu^{2+} modulates excitability of NG neurons and also support NG experimental preparation as a simple model to study mechanism underlying physiological Cu^{2+} effect on neuron electrical properties.

Acknowledgments Financial support by Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT, Chile), project 1080670 (to CV).

References

Aedo F, Delgado R, Wolff D, Vergara C (2007) Copper and zinc as modulators of neuronal excitability in a physiologically significant concentration range. Neurochem Int 50:591–600

Aldunate R, Minniti AN, Rebolledo D, Inestrosa NC (2012) Synaptic defects associated with s-inclusion body myositis are prevented by copper. Biometals 4:815–824

Alissa EM, Bahijri SM, Lamb DJ, Ferns GAA (2004) The effects of coadministration of dietary copper and zinc supplements on atherosclerosis, antioxidant enzymes and indices of lipid peroxidation in the cholesterol-fed rabbit. Int J Exp Path 85:265–275

Belmonte C, Gallego R (1983) Membrane properties of cat sensory neurones with chemoreceptor and baroreceptor endings. J Physiol 342:603–614

Castelli L, Tanzi F, Taglietti V, Magistretti J (2003) Cu^{2+}, Co^{2+}, and Mn^{2+} modify the gating kinetics of high-voltage-activated Ca^{2+} channels in rat palaeocortical neurons. J Membr Biol 195:121–136

Chen J, Myerburg MM, Passero CJ, Winarski KL, Sheng S (2011) External Cu^{2+} inhibits human epithelial Na^{+} channels by binding at a subunit interface of extracellular domains. J Biol Chem 286:27436–27446

Colvin RA, Fontaine CP, Laskowski M, Thomas D (2003) Zn^{2+} transporters and Zn^{2+} homeostasis in neurons. Eur J Pharmacol 479:171–185

Copello J, Heming TA, Segal Y, Reuss L (1993) cAMP-activated apical membrane chloride channels in Necturus

gallbladder epithelium. Conductance, selectivity, and block. J Gen Physiol 102:177–199
de Bie P, Muller P, Wijmenga C, Klomp LW (2007) Molecular pathogenesis of Wilson and Menkes disease: correlation of mutations with molecular defects and disease phenotypes. J Med Genet 44:673–688
Degnan KJ (1985) The role of K^+ and Cl^- conductances in chloride secretion by the opercular epithelium. J Exp Zool 236:19–25
Delgado R, Vergara C, Wolf D (2006) Divalent cations as modulators of neuronal excitability: emphasis on copper and zinc. Biol Res 39:173–182
Eskici G, Axelsen PH (2012) Copper and oxidative stress in the pathogenesis of Alzheimer's disease. Biochemistry 32:6289–6311
Gaier ED, Eipper BA, Mains RE (2013) Copper signaling in the mammalian nervous system: synaptic effects. J Neurosci Res 91:2–19
Harned HS, Nuttall RL (1947) The diffusion coefficient of potassium chloride in dilute aqueous solution. J Am Chem Soc 69:736–740
Horning MS, Trombley P (2001) Zinc and copper influence excitability of rat olfactory bulb neurons by multiple mechanisms. J Neurophysiol 86:1652–1660
Huidobro-Toro JP, Lorca RA, Coddou C (2008) Trace metals in the brain: allosteric modulators of ligand-gated receptor channels, the case of ATP-gated P2X receptors. Eur Biophys J 3:301–314
Kardos J, Kovács I, Hajós F, Kálmán M, Simonyi M (1989) Nerve endings from rat brain tissue release copper upon depolarization. A possible role in regulating neuronal excitability. Neurosci Lett 103:139–144
Kodama H, Fujisawa C, Bhadhprasit W (2011) Pathology, clinical features and treatments of congenital copper metabolic disorders—focus on neurologic aspects. Brain Dev 33:243–251
Li C, Peoples RW, Weight FF (1996) Cu^{2+} potently enhances ATP-activated current in rat nodose ganglion neurons. Neurosci Lett 219:45–48
Lobo V, Ribeiro A, Verissimo L (1998) Diffusion coefficients in aqueous solutions of potassium chloride at high and low concentrations. J Mol Liq 78:139–149
Lu L, Wang C, Gao X, Xu P, Wang J, Wang Q, Cheng J, Xiao H (2009) Effects of copper on T-type Ca^{2+} channels in mouse spermatogenic cells. J Membr Biol 227:87–94
Mathie A, Sutton GL, Clarke CE, Veale EL (2006) Zinc and copper: pharmacological probes and endogenous modulators of neuronal excitability. Pharmacol Ther 111:567–583
Maureira CH, Delgado R, Wolff D, Vergara C (2006) Submicromolar concentrations of Cu^{2+} and Zn^{2+} upregulate electrical activity of CA1 neurons in rat hippocampal brain slices. Program No. 432.9. 2006 Neuroscience Meeting Planner. Society for Neuroscience, Atlanta. Online
Merner ND, Dion PA, Rouleau GA (2011) Recent advances in the genetics of distal hereditary motor neuropathy give insight to a disease mechanism involving copper homeostasis that may extend to other motor neuron disorders. Clin Genet 79:23–34
Niu X, Liu G, Wu RS, Chudasama N, Zakharov SI, Karlin A, Marx SO (2013) Orientations and proximities of the extracellular ends of transmembrane helices S0 and S4 in open and closed BK potassium channels. PLoS ONE 8:e58335. doi:10.1371/journal.pone.0058335
Osiewacz HD, Borghouts C (2000) Cellular copper homeostasis, mitochondrial DNA instabilities, and lifespan control in the filamentous fungus *Podospora anserine*. Exp Gerontol 35:677–686
Quinta-Ferreira ME, Matias CM (2004) Hippocampal mossy fiber calcium transients are maintained during long-term potentiation and are inhibited by endogenous zinc. Brain Res 1004:52–60
Rebolledo DL, Aldunate R, Kohn R, Neira I, Minniti AN, Inestrosa NC (2011) Copper reduces Aβ oligomeric species and ameliorates neuromuscular synaptic defects in a *C. elegans* model of inclusion body myositis. J Neurosci 31:10149–10158
Reddy BS, Pleasants JR, Zimmerman DR, Wostmann BS (1965) Iron and copper utilization in rabbits as affected by diet and germfree status. J Nutrition 87:189–196
Reyes H, Báez ME, González MC, Hernández I, Palma J, Ribalta J, Sandoval L, Zapata R (2000) Selenium, zinc and copper plasma levels in intrahepatic cholestasis of pregnancy, in normal pregnancies and in healthy individuals, in Chile. J Hepatol 32:542–549
Ribeiro ACF, Esteso MA, Lobo VMM, Valente AJM, Simoes SMN, Sobral AJFN, Burrows HD (2005) Diffusion coefficients of copper chloride in aqueous solutions at 298.15 K and 310.15 K. J Chem Eng Data 50:1986–1990
Roos PM, Vesterberg O, Syversen T, Flaten TP, Nordberg M (2013) Metal concentrations in cerebrospinal fluid and blood plasma from patients with amyotrophic lateral sclerosis. Biol Trace Elem Res 2:159–170
Rossi L, Lombardo MF, Ciriolo MR, Rotilio G (2004) Mitochondrial dysfunction in neurodegenerative diseases associated with copper imbalance. Neurochem Res 29:493–504
Salazar-Weber NL, Smith JP (2011) Copper inhibits NMDA receptor-independent LTP and modulates the paired-pulse ratio after LTP in mouse hippocampal slices. Int J Alzheimers Dis 2011:864753
Sato M, Ohtomo K, Daimon T, Sugiyama T, Iijima K (1994) Localization of copper to afferent terminals in rat locus ceruleus, in contrast to mitochondrial copper in cerebellum. J Histochem Cytochem 42:1585–1591
SkulskiiI A, Lapin AV (1992) Highly selective blockade of the frog skin sodium channels by monovalent copper cations. Biochim Biophys Acta 1112:27–28
Tarohda T, Yamamoto M, Amamo R (2004) Regional distribution of manganese, iron, copper, and zinc in the rat brain during development. Anal Bioanal Chem 380:240–246
Trombley PQ, Shepherd GM (1996) Differential modulation by zinc and copper of amino acid receptors from rat olfactory bulb neurons. J Neurophysiol 76:2536–2546
Trombley PQ, Horning MS, Blakemore LJ (2000) Interactions between carnosine and zinc and copper: implications for neuromodulation and neuroprotection. Biochemistry (Mosc) 65:807–816
Weiser T, Wienrich M (1996) The effects of copper ions on glutamate receptors in cultured rat cortical neurons. Brain Res 742:211–218