BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **220,** 47–52 (1996)
ARTICLE NO. 0354

Modulation of K^+ Channel Currents by Serum Amineoxidase in Neurons

Lingyun Wu,* Mircea-Alexandru Mateescu,† Xin-Tao Wang,†,‡ Bruno Mondovi,‡ and Rui Wang*,[1]

Department of Physiology and Research Center of Hôpital du Sacré Coeur de Montréal, Université de Montréal, CP 6128, Succ. A, Montréal, Québec, H3C 3J7 Canada; †Department of Chemistry-Biochemistry, Université du Québec à Montréal, CP 8888, Succ. A., Montréal, Québec, H3C 3P8 Canada; and ‡Department of Biochemical Sciences and CNR-Center of Molecular Biology, University of Rome "La Sapienza", 00185, Rome, Italy

Received January 23, 1996

The influence of bovine serum amineoxidase (SAO), a circulating copper-enzyme, on neuronal K^+ channels is described. Bovine SAO enhanced K^+ channel currents in N1E-115 neuroblastoma cells in a time-dependent manner. Unlike ceruloplasmin (another copper-protein, shown as depolarizing factor in neurons), SAO had no effect on resting potential of neurons. However, pretreatment of cells with SAO inhibited ceruloplasmin-induced membrane depolarization. Although ceruloplasmin alone inhibited K^+ channel currents, it further enhanced K^+ channel currents in the presence of SAO. Therefore, SAO may be another endogenous modulator of neuronal K^+ channels with effect and mechanisms different from those of ceruloplasmin.

Both serum amine oxidase (SAO) and ceruloplasmin are circulating endogenous copper-proteins. Bovine SAO (180 kDa) is a typical oxidase (EC 1.4.3.1) catalysing the oxidation of alipathic primary polyamines, with release of related aldehydes, ammonia and hydrogen peroxide (1–3). In the presence of spermine or spermidine SAO could present cytotoxicity (4–6) and antitumoral activity (6,7).

Recent data show that kidney diamineoxidase/histaminase (an enzyme closely parented to SAO), is the membrane amiloride-binding protein (8) and that this protein has a NH_2-terminal sequence identical to that of placenta diamine oxidase. In fact, more than 56% identity has been found between copper amineoxidases from different tissues of the same species (9). These data on protein homology and the fact that amiloride is known to act at the level of ionic channels (10), suggest that copper-amineoxidases may be involved in the regulation of membrane electrical activities and that their functions as proteins may be affected by these electrical activities. Ceruloplasmin (132 kDa) is a multifunctional blue-copper plasma protein involved in copper transport and in some antioxidative processes. As oxidase (EC 1.16.3.1), also known as Ferroxidase I, ceruloplasmin is involved in the oxidation $Fe^{2+} \rightarrow Fe^{3+}$ and of several aromatic amines and phenols. In previous studies (11) we have established that ceruloplasmin exhibits an important antiarrhythmic action protecting isolated ischemic hearts against reperfusion fibrillation and that ceruloplasmin can induce a prolongation of refractory periods and of action potential duration. We have recently advanced the hypothesis that ceruloplasmin could be an endogenous neuronal depolarizing factor since this copper protein depolarized N1E-115 cells and inhibited K^+ channels in these neurons (12). This previous report provokes several interesting questions; one of them is whether other endogenous copper oxidases would act on neuronal ionic channels. In the present study, the effect of SAO on membrane potential and K^+ channel currents, was investigated. The interaction of SAO with ceruloplasmin and their effects on the electrical properties of neuronal membrane were also examined.

MATERIALS AND METHODS

Enzyme purification. Bovine SAO and ceruloplasmin were purified from serum following a recent method (13) for joint

[1] To whom correspondence should be addressed at Department of Physiology, Université de Montréal, CP 6128, Succ. A, Montréal (Québec), H3C 3J7 Canada. Fax: 1-514-338 2694.

0006-291X/96 $18.00

chromatographic purification of ceruloplasmin and SAO. Bovine plasma after fractionation with ammonium sulphate (35%–55% saturation) was applied onto an Aminoethyl (AE)-Agarose column for the separation of ceruloplasmin. The not-retained fraction was loaded onto Q-Sepharose and then on Con A-Sepharose (Pharmacia Uppsala, Sweden) chromatographic columns. The purified SAO was electrophoretically homogeneous and the specific activity, determined by the method of Tabor et al. (14) with benzylamine as substrate, was of 0.32 EU/mg protein. Ceruloplasmin (a pool of 13 mg/mL) was electrophoretically homogeneous and with a high molecular integrity ($A_{610}/A_{280} = 0.060$). Phosphate buffer used to prepare the stock solutions of SAO and ceruloplasmin had no effect on membrane potential and K^+ channel currents in N1E-115 cells (data not shown).

Cell preparation. Undifferentiated neuroblastoma cells (N1E-115), obtained from Dr. E. Karpinski's laboratory (University of Alberta, Canada), were cultured at 37°C in a humidified atmosphere containing 5% CO_2 in room air. The medium (Dulbecco's Modified Eagle medium, Gibco BRL, Burlington, Canada) containing 10% fetal calf serum was changed every 3 or 4 days and the cells were used before they became confluent. The N1E-115 cells cultured as described above (15) were spheroidal in shape and had no visible neural outgrowths.

Electrical recording. An Axopatch-1D (Axon Instruments, Inc., USA) patch-clamp amplifier was used to record membrane potential and currents. At the beginning of each experiment, the junctional potential between the pipette and the bath solution was electronically adjusted to zero. The bath solution contained (in mM) NaCl 130, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.2, Hepes 10 and Glucose 10. The pipette solution was composed of (in mM): KCl 130, Hepes 10, $MgCl_2$ 1, EGTA 10 and $CaCl_2$ 1. The pH and osmolality of all solutions were adjusted to 7.4 and 300 mOsm, respectively.

All the experiments were conducted at room temperature (20–22°C).

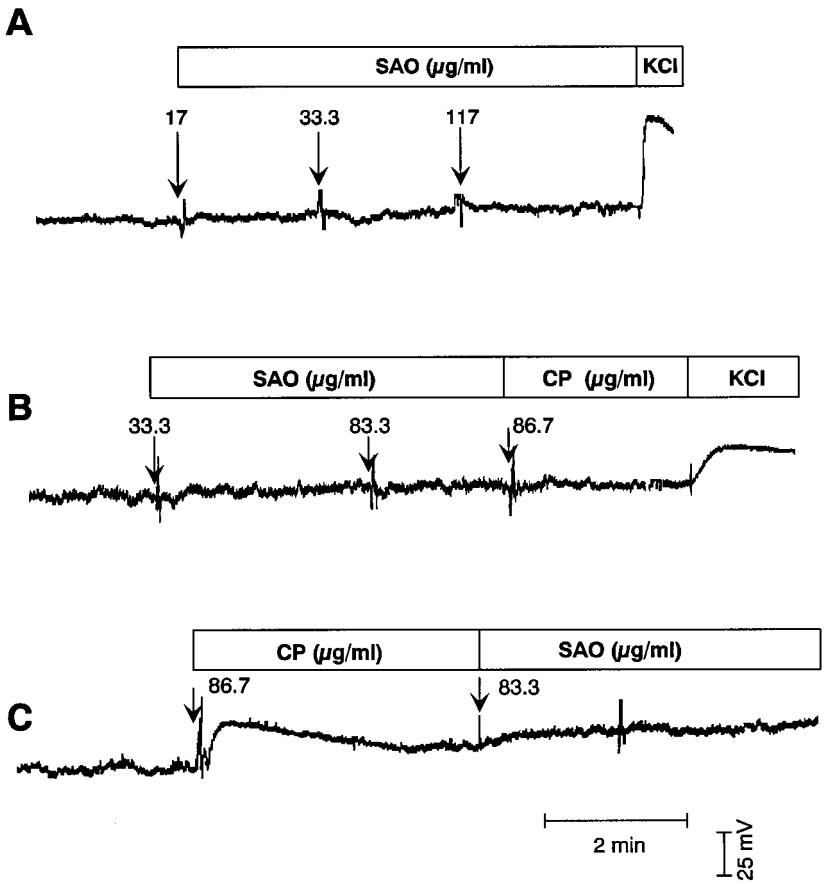

FIG. 1. Effects of SAO and ceruloplasmin (CP) on membrane potentials of N1E-115 cells. **A.** Resting membrane potentials of a N1E-115 cell before and after SAO at different concentrations. KCl (60 mM) effectively depolarized the cell in the presence of SAO. **B.** The presence of SAO (83.3 μg/mL) abolished the depolarizing effect of CP (85.8 μg/mL) on a N1E-115 cell. KCl (60 mM) effectively depolarized the cell in the presence of SAO and CP. **C.** The presence of CP (85.8 μg/mL) did not change the effect of SAO on N1E-115 cell membrane potential. The concentration values indicate the SAO and ceruloplasmin levels in the bath solution.

Membrane potential of N1E-115 cells was recorded using the whole-cell configuration of the patch-clamp technique (16) in the current-clamp mode with a holding current of 0 pA. The membrane potential was considered as stable if there was no gradual depolarization under control conditions. After an initial equilibration period of 5 min with stable recordings, resting potentials of N1E-115 cells were recorded.

Voltage-dependent K^+ channels were recorded using the whole-cell configuration of the patch-clamp technique with a holding potential of −80 mV. Test pulses were ranged with a 10 mV increment from −100 to +60 mV. Current-voltage (I–V) curves were constructed using the sustained current amplitude at the end of 800 ms test pulses. The data were collected and processed using software of pClamp (version 6.01) from Axon Instruments.

Volumes of pooled solution of SAO (5 mg/mL) or of ceruloplasmin (13 mg/mL) were directly added to the bath solution such as to achieve the desired final concentration.

Statistics. The data were expressed as means ±S.E.M. Tests of significance were performed using the Student's *t* test or, where applicable, analysis of variance in conjunction with the Newman-Keul's test. The significant level was chosen at $p < 0.05$.

RESULTS

Bovine SAO at different concentrations had no effect on the resting membrane potential (Fig. 1 A). The resting membrane potentials before and after 463 nM SAO (83,3 μg/mL) were −82.3 ± 1.2 mV and −80.2 ± 0.8 mV, respectively (n = 4, p0.05). In a previous note (12), we already shown that ceruloplasmin significantly depolarized the N1E-115 cells (Fig. 4B). In the presence of SAO, however, ceruloplasmin-induced membrane depolarization was abolished (Fig. 1B). Furthermore,

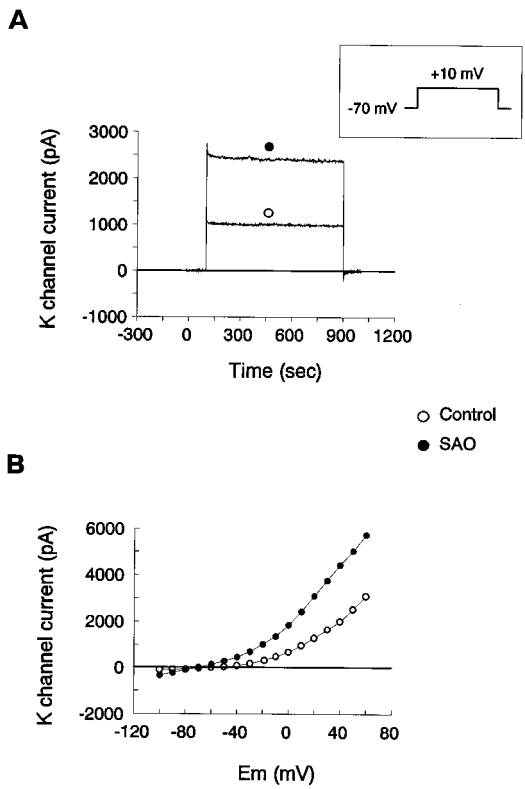

FIG. 2. The effect of SAO (83.3 μg/mL) on the voltage-dependent K^+ channel currents in N1E-115 cells. **A.** The original K^+ channel current records before and after the application of SAO in a N1E-115 cell. The test pulse was shown in the insert. **B.** The I–V relationships of the K^+ channels, recorded from the same cell as shown in A, in the absence (open circles) and in the presence (filled circles) of SAO. The outward current amplitudes were measured at the end of 800 ms depolarization pulses.

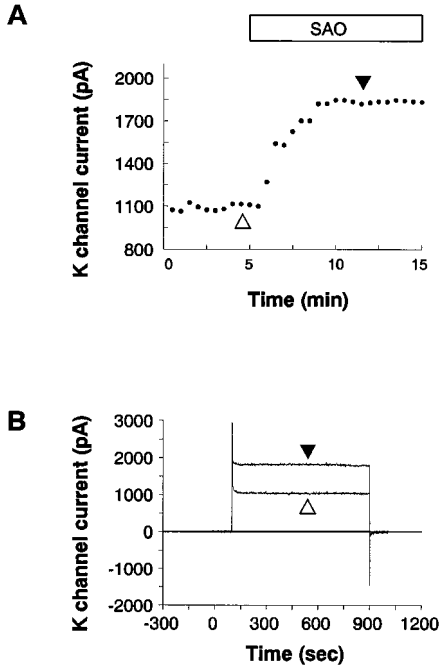

FIG. 3. The time-dependent effect of SAO (83.3 μg/mL) on K^+ channel currents in N1E-115 cells. **A.** The time course of the enhancement of K^+ channel currents by SAO in a N1E-115 cell. The outward current amplitudes were measured at the end of a 800 ms depolarization pulse from −70 mV to 0 mV. **B.** Original outward current traces taken from the same cell as shown in A. Open and filled triangles represent the currents recorded before and after the application of SAO, respectively.

SAO still had no effect on membrane potential after pretreatment (Fig. 1C) of cells with 650 nM ceruloplasmin (85.8 μg/mL).

Voltage-clamp study revealed that bovine SAO (83.3 μg/mL) significantly amplified the outwardly rectifier K^+ channel currents (Fig. 2). The effect of SAO was more significant at more depolarizing potentials. The SAO effect on K^+ channel currents was also time-dependent (Fig. 3A). The enhancement of K^+ channel currents was initiated 30 to 60 sec after the application of SAO and maximally developed around 5 min. This enhancement was maintained at the enhanced level for more than 5 min (Fig. 3).

Heat-inactivated SAO itself had no effect on membrane potential or K^+ channel currents. The presence of heat-inactivated SAO also did not interfere with the effect of native SAO (Fig. 4A). Since we have shown previously that ceruloplasmin inhibited K^+ channel currents (12), the interaction of SAO and ceruloplasmin was further investigated. It was quite amazing that ceruloplasmin (650 nM), instead of inhibiting K^+ channel currents, actually further amplified K^+ channel currents in the presence of SAO (Fig. 4C).

DISCUSSION

Serum amine oxidase (180 kDa) is a circulatory copper oxidase involved in oxidation of aliphatic amines with the main physiological role to control the level of biogenic amines. By analogy to ceruloplasmin, known as a multifunctional protein (ferroxidase with antioxidant properties, copper carrier, anti-inflammatory agent, angiogenic factor) and recently shown to induce cardioprotection in ischemic heart (11) and membrane depolarization in neurons (12), we supposed that SAO as copper protein, in addition to its major oxidase activity, may also exhibit some other functions. In fact, an antiarrhythmic cardioprotective effect of SAO at reperfusion of ischemic isolated rat heart

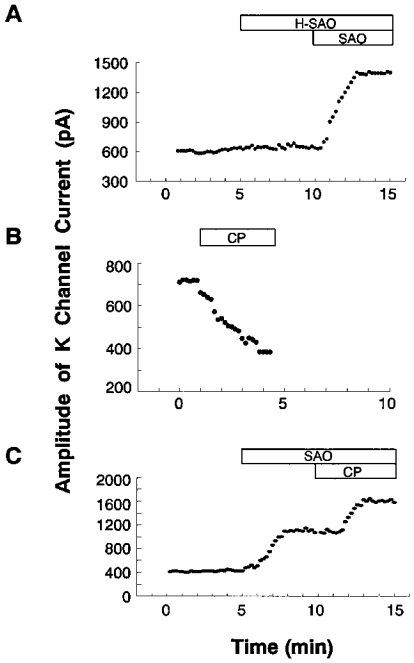

FIG. 4. Effects of SAO, heat-inactivated SAO (H-SAO), and CP on K^+ channel currents in N1E-115 cells. **A.** H-SAO (83.3 μg/mL) had no effect on K^+ channel currents and on SAO (83.3 μg/mL)-induced increase in K^+ channel currents in N1E-115 cells. **B.** Inhibitory effect of CP (85.8 μg/mL) on K^+ channel currents. **C.** In the presence of SAO (83.3 μg/mL), CP (85.8 μg/mL) further increased K^+ channel currents in N1E-115 cell. Holding potential = −70 mV; Test potential = 0 mV.

and a marked antioxidant (scavenging) activity of SAO *in vitro,* similar to that of ceruloplasmin, have been recently observed (Mateescu et al., unpublished). A similar analogy was also supposed between ceruloplasmin and SAO regarding their effects on neuronal K^+ channels.

The hypothesis followed is that SAO could be another endogenous modulator of neuronal K^+ channels. Indeed, both ceruloplasmin and SAO modulate membrane properties. However, it is obvious from the data here presented, that SAO and ceruloplasmin have opposite effects on neuronal K^+ channels. Whereas ceruloplasmin alone inhibited K^+ channel currents acting as an endogenous depolarizing factor (12), SAO enhanced K^+ channel currents in a time-dependent manner (Fig. 4). The underlying mechanisms for the effects of SAO or ceruloplasmin on K^+ channels are still unknown. In any case, these effects of SAO and ceruloplasmin are not mediated via their antioxidant or free radicals scavenging properties since conditions permitting the generation of oxygen-derived free radicals were not present in our experiments. Binding sites for SAO on hepatocytes (17) and receptors for ceruloplasmin on erythrocytes (18), brain (19), liver (20), aorta and heart (21) have already been found. The modulation of K^+ channel currents could be related to eventual SAO and ceruloplasmin interactions with relevant binding proteins (receptors) generating different second messengers. Alternatively, SAO and ceruloplasmin may directly bind to K^+ channel proteins to open or close the channel. Surprisingly, we found that ceruloplasmin further enhanced K^+ channel currents in the presence of SAO (Fig. 4C). This behaviour is the opposite of the behaviour of ceruloplasmin alone. One possibility is that SAO and ceruloplasmin bound to the same receptor or K^+ channel and that this receptor or K^+ channel might have different sub-status (or multi-phase configuration?). Furthermore, it appears that ceruloplasmin may have both inhibitory (in the absence) and excitatory (in the presence of SAO) effects on K^+ channels and that SAO can modulate its action. When the receptor and/or K^+ channel was activated by SAO first, the

inhibitory effect of ceruloplasmin may be blocked; in this case ceruloplasmin can only potentiate the SAO effect (Fig. 4C).

Another surprising aspect in favour of this reciprocal modulation is the abolishment by SAO of membrane polarization induced by ceruloplasmin (Fig. 1). This aspect fits well with recent observations showing kinetic modifications in ceruloplasmin enzyme activity induced by SAO (Mateescu et al., unpublished results).

The SAO molecular integrity is important for the modulation of cell membrane electric activity. This capacity to modulate K^+ channel currents seems to be specific for native protein. In fact, heat denatured SAO has no effect at all, on membranes. Furthermore, separate control experiments (12) shown that in similar conditions, bovine serum albumin (main circulating protein) has absolutely no effect on membrane electrical activity.

There are several facts and hypothesis issued from this work and further investigation needs to be carried out to elucidate the mechanisms of action and the role of copper proteins on the regulation of the membrane electrical activities.

ACKNOWLEDGMENTS

This work was supported by a joint MRC (Canada)–CNR (Italy) research agreement (M.A.M and B.M) and by grants from Medical Research Council (MRC), from CNR project on “Biotecnologie e Biologia Moleculare” and from MURST (Italy). R. Wang is a McDonald Scholar of the Heart and Stroke Foundation of Canada.

REFERENCES

1. Tabor, C. W., and Tabor, H. (1984) Polyamines. *Ann. Rev. Biochem.* **53,** 749–790.
2. Janes, S. M., Mu, D., Wemmer, D., Smith, A. J., Kaur, S., Maltby, D., Burlingame, A. L., and Klinman, J. P. (1990) *Science* **248,** 981–987.
3. Mondovi, B., and Riccio, P. (1990) *Biol. Metals* **3,** 110–113.
4. Averill-Bates, D., Agostinelli, E., Przybytkowski, E., Mateescu, M. A., and Mondovi, B. (1993) *Arch. Biochem. Biophys.* **300,** 75–79.
5. Agostinelli, E., Averill-Bates, D., Przybytkowski, E., Mateescu, M. A., and Mondovi, B. (1991) *Life Chemistry Reports* **9,** 193–204.
6. Mondovi, B., Agostinelli, E., Przybytkowski, E., Mateescu, M. A., and Averill-Bates, D. (1994) *Progr. Biotechnol.* **9,** 775–778.
7. Kusche, J., Menningen, R., Gunther, M., and Leisten, L. (1989) *Biogenic Amines* **6,** 15–26.
8. Novotny, W. F., Chassandes, O., Baker, M., Lazdunski, M., and Barbry, P. (1994) *J. Biol. Chem.* **269,** 9921–9925.
9. Janes, S. M., Palcic, M. M., Scaman, C. H., Smith, A. J., Brown, D. E., Dooley, D. M., Mure, M., and Klinman, J. P. (1992) *Biochemistry* **31,** 12147–12154.
10. Frelin, C., Vigne, P., Barbry, P., and Lazdunski, M. (1987) *Kidney Int.* **32,** 785–792.
11. Atanasiu, R., Chahine, R., Dumoulin, M. J., Mateescu, M. A., and Nadeau, R. (1995) *Can. J. Physiol. Pharmacol.* **73,** in press.
12. Wang, R., Zhang, L., Mateescu, M. A., and Nadeau, R. (1995) *Biochem. Biophys. Res. Commun.* **207,** 599–605.
13. Wang, X. T., Dumoulin, M. J., Befani, O., Mondovi, B., and Mateescu, M. A. (1994) *Prep. Biochem.* **24,** 237–250.
14. Tabor, C. V., Tabor, H., and Rosenthal, S. M. (1954) *J. Biol. Chem.* **208,** 645–661.
15. Wang, R., Karpinski, E., Wu, L., and Pang, P. K. T. (1990) *J. Pharmacol. Exp. Ther.* **254,** 1006–1011.
16. Hamill, O. P., Marty, A., Neher, E., Sakmann, B., and Sigworth, F. J. (1981) *Pflügers Arch.* **39,** 85–100.
17. Dini, L., Agostinelli, E., and Mondovi, B. (1991) *Biochem. Biophys. Res. Comun.* **179,** 1169–1174.
18. Fischer, A. C., and Goode, C. A. (1994) *Prep. Biochem.* **24,** 151–165.
19. Omoto, E., and Tavassoli, M. (1990) *Arch. Biochem. Biophys.* **282,** 34–38.
20. Orena, S. J., Goode, C. A., and Linder, M. C. (1986) *Biochem. Biophys. Res. Commun.* **139,** 822–829.
21. Stevens, M. O., DiSilvestro, R. A., and Harris, E. D. (1984) *Biochem. J.* **23,** 261–266.