THE CARDIAC EFFECTS OF COPPER AND CERULOPLASMIN ON SPINY DOGFISH SHARK (SQUALUS ACANTHIAS)

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Copper, an essential trace element, participates in maintaining the physiological functions of many organisms. The neuronal effect of copper as well as the interaction of copper with neuronal ion channels have been noticed. The toxicity of copper on the cardiovascular system has not been explored except for the study by Evans & Weingarten (Toxicol. 61:275, 1990) indicating that the contraction of aortic smooth muscle from Squalus acanthias might not be affected by copper. The physiological range of serum copper in dogfish is currently unknown. Over 90% of copper in mammalian serum is bound to ceruloplasmin (CP), a multifunctional blue copper-protein (132 kDa) containing 5-6 copper atoms per molecule. Recently, we showed that CP depolarized neuronal membrane and suppressed neuronal K channel currents (Wang et al., Biochem. Biophys. Res. Commun. 207:599,1995). Since copper and CP are closely related to each other and they may actively participate in the regulation of cardiovascular functions under toxicological or (patho)physiological conditions, the effects of copper and CP on heart beating and on K channel currents in single ventricular myocytes from dogfish shark Squalus acanthias were examined in this study.

In the first series of experiments, the beating rate of atria isolated from pithed Squalus acanthias (male, 2-7 kg) was measured. After removal from the shark, the hearts were immediately immersed in cold elasmobranch physiological saline (EPS) containing (mM): NaCl 270, urea 350, KCl 4, $MgCl_2$ 3, HEPES 10, $CaCl_2$ 3, Na_2SO_4 0.5, KH_2PO_4 0.5, and heparin (50 units/ml). Both aorta and two coronary arteries were cannulated and the whole heart was mounted on a Langendorff device. The time from the removal of the hearts from shark to the initiation of retro-perfusion of isolated heart with oxygenated EPS (30°C) was less than 30 min. The atrial beating was counted if the beating rate was stable for 15 min. The stable basal beating rate of atria was 49±12 times/min (X±SEM, n=16). $CuSO_4$ increased the atria beating rate at different concentrations (1-100 μM) (Fig. 1) although the dose-response relationship needs to be further clarified. The increased atrium beating rate gradually slowed down toward the basal level after

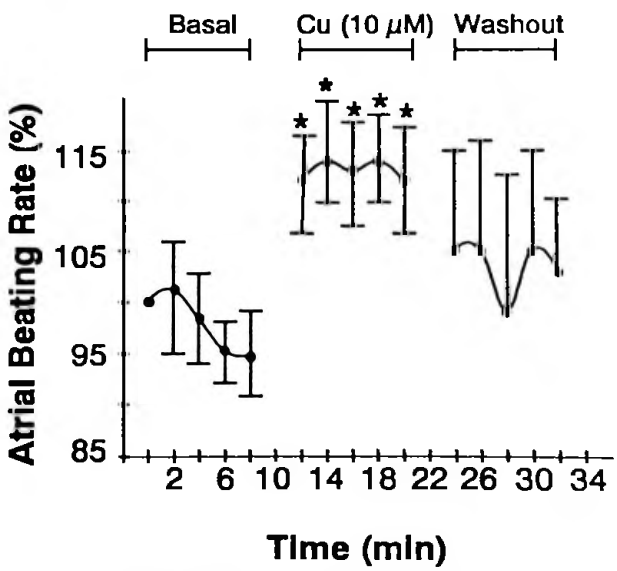

Fig. 1. Effect of copper on atrial beating rate.
* $p<0.05$ vs. basal beating rate. n= 16.

removing $CuSO_4$ from perfusing solution. It was also found that, in some cases, when atrium beating rate increased, ventricle beating rate decreased or even stopped. After the increased atrium beating rate was corrected, ventricle beating rate also recovered and synchronized with atrial beating rate. To test the effect of CP on atrial beating rate, highly purified bovine CP from our laboratory (Wang et al., Prep. Biochem. 24:237,1994) was used. At concentrations of 0.1-10 μM, CP had no effect on atrial beating rate (n=6).

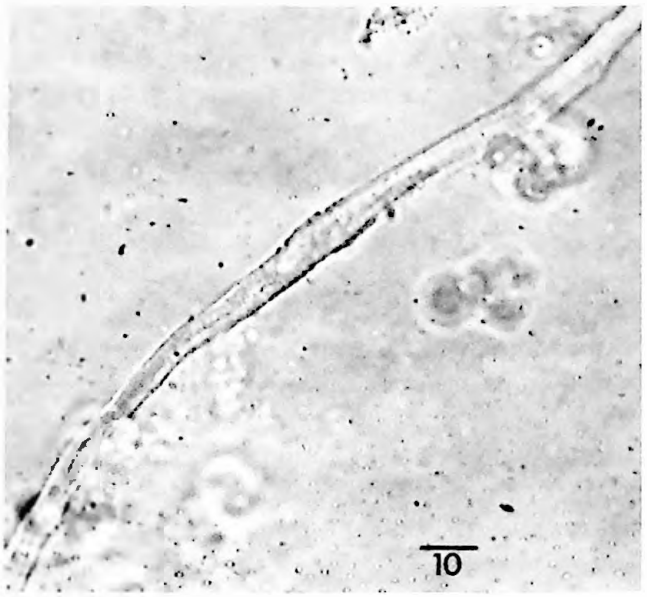

Fig. 2. The morphology of freshly isolated ventricular myocytes from shark heart. The horizontal bar represents 10 μm.

In the second series of experiments, K^+ channel currents of single shark ventricular myocytes were investigated. The similar cell isolation procedure as described by Mitra & Morad (J. Physiol. 457:627, 1992) was followed with modifications. Briefly, after the isolated hearts were cannulated and mounted on a Langendorff device, they were perfused with oxygenated EPS with or without calcium for different periods. Collagenase (0.06%) and hyaluronidase (0.06%) were then added to EPS and the heart was digested for approximately 30 min. Thereafter, the heart was removed from the Langendorff device and atrium and connective tissues were removed. Gentle agitation released single myocytes from the remaining ventricular tissue. The isolated myocytes were characterized by their striated appearance, spindle shape (Fig. 2), and the contraction in response to KCl stimulation. The myocytes were kept at 4°C and used 8-24 hours after isolation for recording the delayed rectifier outward K^+ channel currents with the whole-cell patch-clamp technique. The pipette solution contains (mM): KCl 200, urea 300, trimethylamine N-oxide (TMAO) 150, NaCl 30, HEPES 20, and MgATP 2. The bath solution contains (mM): Trizma 300, KCl 5, $MgCl_2$ 3, KH_2PO_4 0.5, urea 300, HEPES 10, TMAO 70, $CaCl_2$ 0.2, TTX 1 μM. The pH and osmolality of all solutions were adjusted to 7.4 and 920 mOsm/l, respectively. $CuSO_4$ (10 μM) had no effect on K^+ channel currents (n=4). One example was shown in Fig. 3. However, CP (1 μM)

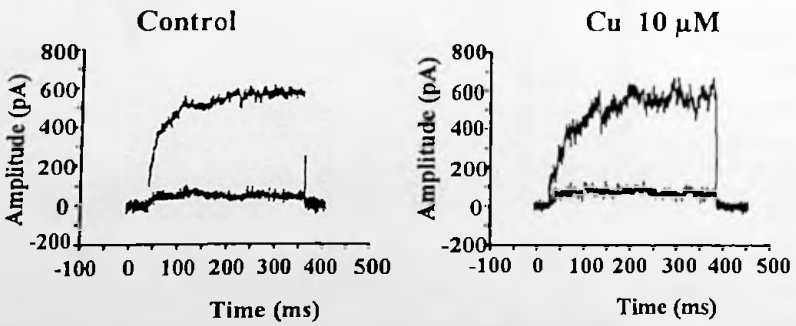

Fig. 3. Effect of copper on K channel currents in one shark ventricular myocyte. The currents were elicited from a holding potential of -80 mV to +10 and +30 mV, respectively.

significantly inhibited K^+ channel currents by 53±6% at +20 mV (n=4, $p<0.05$). This effect of CP was more pronounced at more positive membrane potentials (Fig. 4).

Our results suggest that the cardiac effects of copper and CP have different profiles. Since only atrial beating rate was measured in the present study, effects of copper and CP on the contractility of atrial and/or ventricular muscles are still unknown. The decreased atrial beating rate in the presence of copper, per se, may significantly affect the pump function of shark heart. That may form the basis of cardiac toxicity of copper in this species. CP-induced inhibition of outward K^+ channel currents in shark ventricular myocytes was not mediated by copper since this heavy metal did not affect the same ion channels. Although CP had been localized in the plasma of some fishes, such as trout (Perrier et al., Comp. Biochem. Physiol. 49B:679, 1974) and we also found the oxidative activity of shark plasma representative of CP functions, we failed to purify blue CP from shark plasma using our established purification method. This uncertainty of the presence of CP in dogfish shark plasma raised two possibilities. First, our chromatographic method for purification of bovine serum CP (very sensitive to the ionic strength) may be influenced by the unique presence of a great amount of urea in shark plasma. Second, there may be no CP in shark plasma. In the latter case, the modification of K^+ channel currents of shark ventricular myocytes by CP may indicate that the K^+ channels in shark ventricular myocytes and their mammalian counterparts share the same modification sites or mechanisms to CP. In the future we plan to study the following questions. (1) Do copper and/or CP affect the contraction force of shark cardiac muscles? (2) Does the cardiac effect of copper depend on the valency state of this ion (Tan & Roth, Neuropharmacol. 23(6):683, 1984)? (3) Is the decreased heart beating rate of shark by copper due to the inhibition of calcium channels? (4) Can the action potential and resting potential of shark ventricular myocytes be altered by copper and/or CP? (5) Are there blue CP or other CP-like proteins in shark plasma? (6) Is there any toxic effect of copper on the metabolism and proliferation of ventricular myocytes of Squalus acanthias?

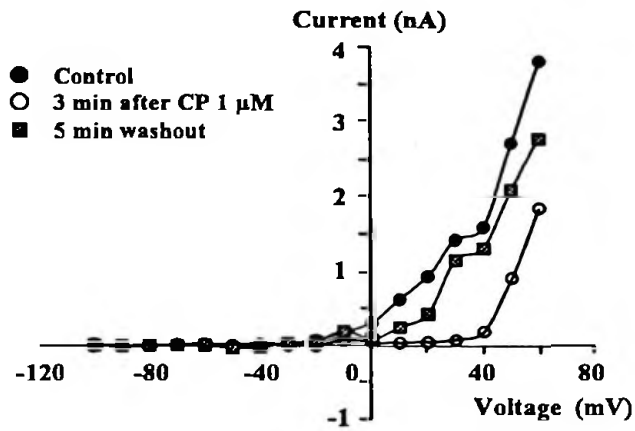

Fig. 4. Effect of CP on K channel currents in one shark ventricular myocyte.

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