BRIEF COMMUNICATIONS

Toxic Effects of Silver-Silver Chloride Electrodes on Vascular Smooth Muscle

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SUMMARY. We found that silver, either as silver metal or silver chloride, exerted toxic effects on the smooth muscle of isolated cannulated hamster cheek pouch arterioles. Silver initially stimulated the smooth muscle, producing a marked vasoconstriction. The vessels then dilated back to control diameters. Once the arterioles began to dilate, they became refractory to norepinephrine or potassium stimulation. We caution the use of silver in the presence of smooth muscle, especially when tissue mass is small or free protein concentration is low. (Circ Res 53: 105-108, 1983)

SILVER or silver chloride electrodes are commonly used as reference electrodes in electrophysiological studies, with ion-selective electrodes and with oxygen electrodes. It has generally been assumed that these electrodes are biologically inert. However, while studying the oxygen sensitivity of isolated arterioles, we discovered that the Ag-AgCl reference electrodes, used in conjunction with oxygen microelectrodes, exerted marked toxic effects on arteriolar vascular smooth muscle. Due to the common usage of these electrodes, we felt that a brief investigation and report of this phenomenon was warranted.

Methods

Arterioles with inner diameters of 30-90 μm were hand-dissected from hamster cheek pouches and cannulated as blind sacs with glass micropipettes, as described by Duling et al. (1981). The vessels were pressurized to 60 mm Hg with physiological salt solution buffered with morfolinopropanesulphonic acid (MOPS) (PSS, in mm: NaCl, 145; KCl, 4.7; CaCl₂, 2; MgSO₄, 1.2; glucose, 5; pyruvate, 2; Na-EDTA, 0.02; MOPS, 2) and maintained in a 3-ml chamber filled with PSS at 37°C. The arterioles were viewed with a Nikon Diavert microscope and video system at a final magnification of 2000X at the monitor face. Vessel internal diameters were measured with a video micrometer accurate to 1 μm.

Silver-silver chloride reference electrodes were constructed from 5.0 cm Ag wires, 1.0 mm in diameter. Two such wires were placed in 0.1 N HCl and polarized to approximately 4 V until a uniform dark coating was observed on the Ag wire connected to the positive pole. The electrodes then were rinsed in running distilled water for several minutes. If the electrodes were not used immediately, they were stored in light-tight containers. Electrodes were reused three or four times before they were rechlorided. After use, electrodes were rinsed in running distilled water and stored as indicated above.

Vessels were cannulated and pressurized at ambient temperature (17-20°C). These vessels display little or no spontaneous tone at temperatures below 34°C (Duling et al., 1981); therefore, the diameters of the arterioles at ambient temperature were taken as estimates of maximal diameters. The vessels were then washed with at least 10 ml of fresh PSS, vessel chamber temperature was elevated to 37°C, and the vessels were allowed to equilibrate for 30 minutes. Our initial observations (data in Fig. 1) were made during experiments designed to measure periarteriolar Po₂ using recessed tip microcathodes (Whalen et al., 1967) and a Keithly 602 electrometer. Vessels were either exposed to an Ag-AgCl reference electrode connected to an earth ground for the duration of the experiment (n = 12 vessels), or were not exposed to the reference electrode at any time (n = 12 vessels). In both cases, the arterioles were challenged at 1-hour intervals either with a dose of norepinephrine (levophed bitartrate, Sterling) that reduced diameter approximately 50% to 70%, or with solutions containing 140 mM K+ (KCl substituted for NaCl in PSS). In other experiments (data in Figs. 2 and 3), two or three consecutive responses to norepinephrine were determined, and the results from these trials were pooled for each vessel. Then, an ungrounded chlorided silver wire (n = 4 vessels), or simply a silver wire (n = 4 vessels) was placed in the vessel chamber, and after a 2-hour exposure period, the arterioles were challenged with the same dose of norepinephrine used before silver exposure. In all experiments, the solution in the vessel chamber was replaced with fresh, 37°C PSS every 10-15 minutes.

Bovine serum albumin was used in four experiments (Fraction V, Sigma). The albumin was first dissolved in distilled water to give a concentration of 25 g/100 ml. This solution then was dialyzed against at least a 50-fold excess of distilled water and then PSS. The pH of the concentrated albumin solution was then adjusted to 7.4 with NaOH, and the albumin solution was diluted to appropriate concentration with PSS when ready for use.

Statistical analysis was performed using a paired Student's t-test (Sokal and Rohlf, 1969). The n values in the
Results

We found that, whenever silver was present in the vessel chamber, either as silver or silver-silver chloride, the arterioles lost all responsiveness to vasoactive stimuli (0.59–15 μM norepinephrine or 140 mM K⁺) within 2 hour after cannulation (Figs. 1 and 2). This observation did not depend on the presence or absence of an oxygen electrode in the vessel chamber, connection of the electrode to an earth ground, or whether or not the silver had been previously chlorided (Fig. 2). In one experiment, a freshly chlorided electrode was preequilibrated in 50 ml of 37°C PSS for approximately 4 hours and then used. Similar results to those in Figure 2 were obtained. Also, we found that new electrodes and electrodes that had been used up to 4 times produced similar effects. Thus, it would appear that the observed effects depended on the physical presence of silver in the vessel chamber and not on the age or past history of the electrode.

Vessels not exposed to silver remained responsive to vasoactive stimuli (0.59 μM norepinephrine or less, or 140 mM K⁺) for at least 2 hours in vitro (Fig. 1), and most vessels not exposed to silver retained reactivity for 4 hours or more.

The response of arterioles to silver was consistently biphasic, although the timecourse of the events were highly variable. A typical record is shown in Figure 3. Within 4 to 15 minutes after silver was introduced into the bath, the arterioles would constrict to approximately 30% of their pre-exposure diameters. The vessels would then remain constricted for 8 to 60 minutes after which time they would dilate to their approximate pre-exposure diameter. Once the vessels began to dilate, they were refractory to vasoactive stimuli for at least 4 hours, even after the source of silver had been removed from the vessel chamber and the arterioles had been vigorously washed with fresh PSS. In two of the eight vessels exposed to silver or chlorided silver wires, removal of the silver, as soon as vessel constriction could be detected, reversed the effects.

We tested to see if the vasodilator adenosine (167 μM) or Ca²⁺-free salt solutions would relax the arterioles while it was in the silver constricted state, and we found that both treatments were without effect.

Because the toxic species of silver compounds has been attributed to free silver ions (Cooper and Jolly, 1970; Klein, 1978; Petering, 1976), and because the affinity of Ag⁺ for protein is very high (Klein, 1978; Petering, 1976), we tested to see whether protein in the PSS in the vessel chamber would protect against the toxic actions of silver.

We found that, in the four vessels tested, 0.5–1.5 g/100 ml albumin eliminated the effects of silver on arterioles for at least 2 hours (Fig. 2). In additional
experiments, 0.1 g/100 ml albumin was used, and we found that this concentration of protein did not eliminate the constrictor action of silver. However, the arterioles remained responsive to norepinephrine during the 2-hour exposure, in spite of being constricted. Thus, 0.1 g/100 ml albumin in the bath solution protected the vessel from the effects of silver but did not eliminate the effects entirely.

We also attempted to isolate electrodes from vessels by a 3.5-cm salt bridge filled with PSS, as suggested by Marshall (1959). This increased the time of onset of the stimulatory phase to 30–60 minutes but did not eliminate the effect.

Platinum wire electrodes were also tested in three vessels, and we found no deleterious effects, in terms of vessel constriction and norepinephrine reactivity, after up to 4 hours of exposure.

Discussion

Dilute solutions of a variety of silver compounds have strong bacteriocidal properties (Klein, 1978) and have been shown to inhibit enzyme activity in vitro (Klein, 1978; Petering, 1976). Also, Fisher et al. (1957) have shown that silver or silver-silver chloride electrodes implanted in cat brains cause pathological changes in tissue surrounding the electrodes. These actions of silver compounds have been attributed to the free Ag⁺ released in solution (Cooper and Jolly, 1970; Klein, 1978; Petering, 1976) and interaction of this ion with sulphydryl, amino, imidazole, phosphate or carboxyl groups of proteins (Klein, 1978; Petering, 1976).

Because of the strong affinity of proteins and other biological anions for Ag⁺, the action of Ag⁺ on large multicellular organisms or systems is not apparent until such systems are challenged with massive doses of Ag⁺ (Cooper and Jolly, 1970; Klein, 1978). Small amounts of Ag⁺ injected into an experimental animal will bind to plasma proteins, and the resulting free Ag⁺ concentrations will be essentially zero. This is not the case with small tissues in vitro or with isolated cells where the free protein concentration is small. The arterioles we studied were only two cell layers thick, and the protein available for binding Ag⁺ would have been very limited. In this regard, it is noteworthy that protein added to the solutions surrounding arterioles eliminated the effects of silver on the arterioles. Protein added to the media surrounding bacteria has also been shown to decrease the bacteriocidal action of Ag⁺ (Klein, 1978).

No methods for Ag⁺ measurement were available to us to determine the exact silver concentrations in our solutions. However, a very rough upper limit may be estimated by computing the maximum Ag⁺ concentration consistent with the known solubility products (Castellan, 1971). In an ideal solution containing 150 mM Cl⁻, based on the solubility of AgCl in water at 37°C (21.9 μM/liter; interpolated from data in Linke, 1958), the concentration of free Ag⁺ should be no greater than 5.65 nM. However, in more complex solutions, such as the PSS used in this study, the calculation of the expected Ag⁺ concentration becomes non-trivial. In 150 mM NaCl solution at 37°C, the solubility of AgCl may be as high as 11 μM/liter (interpolated from data in Linke, 1958). Unfortunately, the methods used to determine this solubility measured total Ag concentration in the solution and not Ag⁺ concentration. Thus, the precise concentration of Ag⁺ generated in the present study cannot be calculated, but is likely to be less than 11 μM/liter.

The pattern of response that we observed is not unique to vascular smooth muscle. Lukanov and Atmadjov (1979) exposed guinea pig stomach smooth muscle to physiological salt solutions made with water containing electrolytically produced Ag⁺ (total Ag concentration, 46 μM). They found that the silver solutions produced biphasic responses that were nearly identical to our findings; silver initially stimulated the smooth muscle, and this stimulation was followed by an apparently indefinite period of refractoriness to stimuli which normally would have caused the stomach muscle to contract. The precise mechanism by which Ag⁺ exerts its effects on smooth muscle is not known, but Lukanov and Atmadjov (1979) suggested that the excitatory phase of Ag⁺ action resulted from inhibition of the "Ca-pump" and the inhibitory phase resulted from a drastic decrease in Ca²⁺ permeability.

Our observations and the results of Lukanov and Atmadjov (1979) indicate that extreme caution must be exercised when using silver compounds in the presence of smooth muscle. This is especially true when tissue mass is small and the concentration of free protein is low. In flow-through systems, or in systems with significant tissue other than smooth muscle silver toxicity may not be a problem. However, in systems where the only tissue present is smooth muscle we suggest that the use of silver compounds be avoided. If silver is to be used, protein should be added to the media surrounding the
smooth muscle preparations. Platinum electrodes do not appear to affect smooth muscle and might be substituted for silver-silver chloride electrodes where possible.

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References


INDEX TERMS: Arterioles • Silver-silver chloride electrodes • Silver toxicity • Vascular smooth muscle • Vascular reactivity

Erratum

RE: Myocardial Metabolites of Ethanol (Circ. Res. 52: 479–482, 1983)

Due to a printer’s error the structural formula in the legend of Figure 2 on page 480 is incorrect. It should read:

\[
\cdot \text{CH}_2\text{C}–\text{OC}_2\text{H}_5 \\
\| \\
\text{O}^+–\text{H}
\]
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