Effects of Cr^{3+} ions on electrophysiological parameters of isolated skin of toad *Pleurodema thaul*

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Abstract

In view of the toxicity of chromium (Cr^{3+}) ions, it was explored the damaging effects that this ion could induce in cell membranes. The measurement of the effects induced by Cr^{3+} ions on electrophysiological parameters of short-circuit current and on the potential difference were investigated using the outer side (mucosal) and the inner side (serosal) of toad *Pleurodema thaul* skin. The results showed a decreased on electrophysiological parameters when it were administered concentrations of 33, 100 and 200 μM of Cr^{3+}, the results also suggest that the administration of Cr^{3+} inhibits the ion transport in toad skin by the interaction of Cr^{3+} with lipid bilayers or protein constituents of membrane, and not by an inhibition of the active transport of ions across Na^+ channels.

Key words: Amiloride, chromium, electrophysiological, membrane

INTRODUCTION

Heavy metals are one of the major waste in chemical processes and the most toxic are chromium (Cr^{3+}), nickel, copper, zinc, mercury and lead,[1] these heavy metals have a potential toxic effect on organisms when are present above their permissible concentration,[2] one of the most toxic is Cr^{3+}, which exist in two states in aqueous medium: Cr^{3+} and Cr^{6+}, affecting human physiology and causing severe health problems.[3] The toxicity levels of heavy metals may vary, but in case of Cr^{3+} the United States Environmental Protection Agency, has set a maximum level of 0.015 mg/L in drinking water[4,5] and The World Health Organization, set the maximum permissible limit in drinking water of 0.05 mg/L,[6] this pollution comes from Cr^{3+} salts in dyes and pigments, leather tanning, electroplating, wood preservation, effluent disposal, and fertilizer manufacture.[5,7]

For its part, Cr^{3+} is a stable element, passively absorbed and retained by cation-exchange in cell walls,[8] moreover, is considered as a trace element in human and animal nutrition,[9] being involved in the activation of membrane phosphotyrosine in mammals, however, the complete structure of the complex has not been identified. The distribution of Cr^{3+} occurs through the cell membrane proteins, but its mechanisms of absorption and transport of Cr^{3+} ions are still uncertain.[10] The cell membrane is one of the major cell components, constituting a diffusion barrier and protection; therefore, their structure and function are susceptible to changes as a result of interactions with heavy metals.[11] For these reasons, it is of interest to determine the functional and structural effects caused by Cr^{3+} ions on electrophysiological parameters of isolated of toad skins.

MATERIALS AND METHODS

Preparation of Ringer's solution

The solution was prepared in flask of 100 mL with mili-Q water and millimolar (mM) concentration of: Na^+ 114, K^+ 2.5,

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Cl^- 117.5, Ca^{2+} 2.0, HCO_3 − 2.3, glucose 11; then stored at 4°C until the time of use.

Electrophysiological measurements in isolated toad skin

All the experiments were performed with samples obtained from the abdominal skin of toads (*Pleurodema thaul*) of either sex (1.0–3.0 g). The amphibians collected were kept in tap water for 24 h before use. The skins were mounted in a system of two halves of a using Perspex chamber,[12] the circular area was 1.0 cm^2 and was exposed to 3.0 mL of Ringer's solution on each side, the system was oxygenated with an equipment model Elite Hagen aerator. The electrophysiological parameters were monitored with nonpolarizable Ag/AgCl electrodes for short-circuit current (SCC) [Figure 1a], with a distance of 15 mm from the epithelium, and connected to a voltage-clamp circuit (G. Metraux Electronique, Crissier, Switzerland). The potential difference (PD) [Figure 1b] was measured with calomel-agar electrodes at intervals of 5 min for 4 s, then were monitored on a 2-channel recorder (Cole-Parmer) and connected to a voltage-clamp circuit (G. Métraux Electronique), for the study of Cr^{3+}, was added after 30 min of steady readings, concentrations of 33, 100 and 200 μM of Cr^{3+}, either the outer or the inner surface of the skin.

Test of amiloride

For the determination of transport by stimulating the driving potential of Na^+, was used the Isaacson's amiloride (Am) test, drew on port mechanism by an equivalent electrical circuit in which can be evaluated the electromotive force of sodium-transporting mechanism ($V\text{-}E_{Na^+}$), the resistance in series of this force represent the path through which the sodium ions are actively transported. Amiloride (a gift from Merck, Sharp and Dohme) was applied to the solution bathing the outer surface of the skin (final concentration 8 μM) and the concentration of Cr^{3+} used was 100 μM.

Statistical analysis

All the experiments were expressed as mean ± standard error with $n = 5$ and Student's *t*-test was used to calculate the statistical significance and a $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Evaluation of the effects of chromium on the electrical properties of Na^+ transporting membrane as model of Na^+-absorbing epithelium

The isolated toad skin was used, due to Na^+ diffuses into the cell across the apical (outer, mucosal) membrane and is actively extruded across the basolateral (inner, serosal) membrane by Na^+-K^+-ATPase in exchange for K^+.[13] The results showed a significant decrease in the PD and in the SCC after the application of Cr^{3+} on toad skin, which can be interpreted as reflecting inhibition of the active transport of ions,[14] the observed effects could be the result of an interaction of the Cr^{3+} ion with the Na^+-K^+-ATPase channels or with the bilayers of cell membranes, resulting in a decreased transport of Na^+ through the skin.[15] The same reduction in the Na-K-ATPase was found by Maiti *et al*., 2009,[16] in fish brains with Cr^{3+} exposure which also induced oxidative stress, they proposed that a direct interaction of the metal with -SH group of the Na-K-ATPase enzyme cannot be ruled out and that can be also aggravated by a vulnerable mitochondrial electron transport chain activity, a decrease in the SCC has also been found with other metals as uranyl nitrate and mercury chloride.[17] Figure 2a and b, shows that a concentration of 33 μM there was a decrease in the SCC and PD of the outer side of 14.6% and 14.2%,

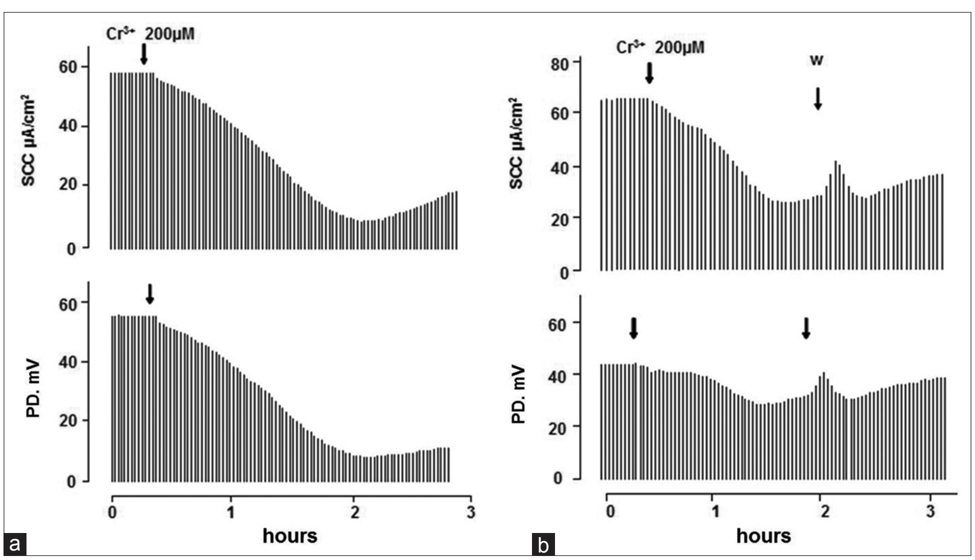

Figure 1: Effect of chromium 200 μM on the short-circuit current and potential difference added to (a) the outer side and (b) inner side surface of the isolated toad skin (W = washout)

and for the inner side was observed a decrease of 8.2% and 7.4%, respectively; when the concentration was increased to 200 µM the percentage of decrease of the electrical parameters was 68.8% for SCC and 68.4% for PD in the outer side and in the inner side the decrease was 60.3% and 41.5% for SCC and PD, respectively, the results showed that the addition of Cr^{3+} in both sides (outer y inner) of the toad skin caused a concentration-dependent change in the electrical parameters and the decrease in SCC and PD can be interpreted as a decrease in the Na^+ absorption.[14]

Comparative effects of amiloride and chromium (outer surface)

Cr^{3+} was applied to the outer surface of the skin and their effect was investigated by the Am test. Na^+ channels Am-sensitive were used as control elements for the regulation of Na^+ transport into cells across epithelia.[18] The Am test showed an increase principally to the stimulation of the driving potential of Na^+ ($V-E_{Na^+}$), also, by a significant decrease in the skin resistance, this is possible to the disruption of the membrane and/or cell integrity decreasing the SCC when added Am (concentration 8 µM) this blocks the Na^+ channels in the outer surface of the toad skin[19] and was reversible after the wash of the skin [Figure 3a]. When 100 µM of Cr^{3+} was applied to the outer surface of the toad skin after the Am no changes were observed on the SCC, effect that can be interpreted as a possibility that Cr^{3+} acts on a different binding site of the Na^+ channel. Figure 3b shows that when Am is added, a rapid decrease of SCC was observed, and its effect was reversible after a washout, then was added the ion Cr^{3+} at a concentration of 100 µM and a decrease in the SCC was also observed, demonstrating the disruption of the membrane and/or cell integrity by Cr^{3+} and not an inhibition of the active transport of ions across Na^+ channels, because once Am was added the SCC low again.

CONCLUSION

The experimental results of the inhibitory effects of Cr^{3+} on the electrical properties of PD, SCC and Am test, support the conclusion that the inhibition of the ion transport in

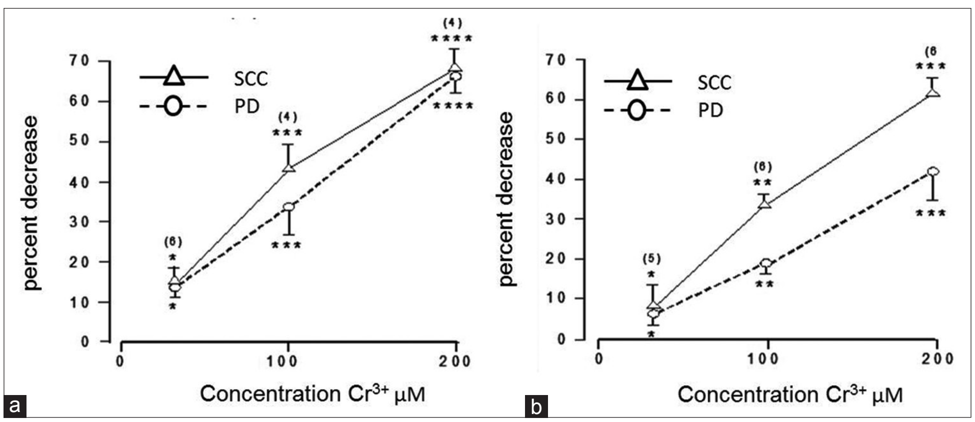

Figure 2: Inhibitory effects of increasing concentrations of chromium versus percent of decrease of the electrical properties of potential difference and short-circuit current on isolated toad skin. (a) Outer surface 33, 100, and 200 µM of chromium, (b) inner surface 33, 100, and 200 µM of chromium (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$)

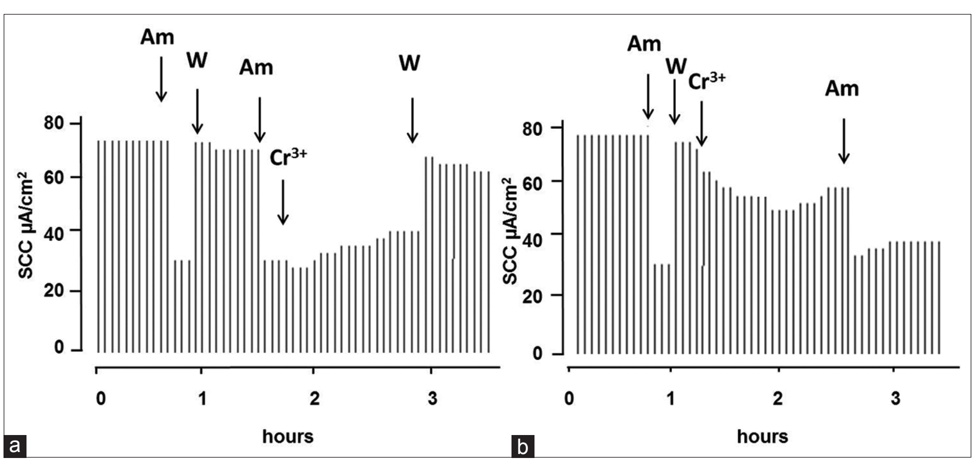

Figure 3: Comparative effects of amiloride (Am, 8 µM) and chromium (Cr^{3+}, 100 µM), on the short-circuit current of the isolated toad skin. (a) Amiloride and (b) chromium added to the outer surface; W = Washout

toad skin, could be due to the interaction of Cr^{3+} with lipid bilayers or protein constituents of membrane, and not by an inhibition of the active transport of ions across Na^+ channels.

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Conflicts of interest

There are no conflicts of interest.

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