Abstract The non-selective cation channel (NSCC) in the larval bullfrog skin contributes to the short-circuit current (SCC) across the skin. The effects of amiloride and acetylcholine on the SCC were examined in the presence or absence of Cu²⁺ to determine whether the amiloride binding site mediating activation or that mediating inhibition of the channel is blocked by Cu²⁺ and whether amiloride and acetylcholine share a common binding site on the NSCC. The skin of tadpoles raised in aldosterone was examined with K-Ringer present on the apical side to potentiate the SCC. Amiloride (10⁻⁴ M) transiently increased SCC in the absence of Cu²⁺. Apical application of 500 µM Cu²⁺ increased the SCC. In the presence of Cu²⁺, amiloride decreased the SCC. In contrast, acetylcholine (1 mM) transiently increased SCC whether Cu²⁺ was present or not. These results suggest that there are two binding sites for amiloride on the NSCC, whereby the site that activates the channel is blocked by Cu²⁺ while the site that inhibits it is not, and that the binding sites for acetylcholine and amiloride may be different.

Keywords Cu²⁺ · Amiloride · Non-selective cation channel · Frog skin

Introduction

The larval bullfrog skin contains a non-selective cation channel (NSCC) that contributes to the short-circuit current (SCC) across the skin. The effects of amiloride on this channel can be viewed as two concurrent processes, activation (agonist effect) and inhibition (antagonist effect) [3, 5]. In practice, when amiloride is applied there is a rapid increase in SCC followed by a slower decline toward baseline. Acetylcholine increases the SCC transiently, like amiloride. Neither the mechanisms underlying this amiloride-induced activation/inhibition nor the details of the interactions between these two agents (amiloride and acetylcholine) and the channel have yet been elucidated. For instance, whether a single amiloride receptor or two different ones mediate activation and inhibition of the channel, and whether or not acetylcholine and amiloride bind to the same population of receptors are both unanswered questions.

In the bullfrog, the epithelial sodium channel (ENaC) contributes to the SCC across the skin of the adult. Since amiloride blocks this channel, it decreases the SCC. In contrast, Cu²⁺ stimulates the channel and increases the SCC. Although the effects of Cu²⁺ and amiloride are opposite, these two agents are thought to compete for the same binding site on the ENaC [4].

Here, we report the effects of amiloride and acetylcholine on the SCC across larval bullfrog skin in the presence and absence of Cu²⁺. The study was performed to determine whether the amiloride binding sites that participate in the activation and inhibition of the NSCC are the same or different and whether or not amiloride and acetylcholine share a common binding site.

Materials and Methods

Animals

Tadpoles of Rana catesbeiana at TK stages XI-XV were purchased from a local animal supplier in Misato City (Saitama, Japan). They were maintained in a 10⁻⁶ M solution of aldosterone in tap water for 8–12 days. They were then anaesthetized with iced water supplemented with MS-222 (Sankyo, Japan) and portions of dorsal body skin were dissected out.

Measurement of short-circuit current (SCC)

The dissected skin was mounted in an Ussing-type chamber with silicon gaskets (inner diameter 5 mm) to minimize edge damage. After a 20-min period for equilibration with Na-Ringer’s solutions on both sides, that on the apical side was replaced with K-Ringer’s solution. The skin potential was then clamped to zero using a short-circuit current amplifier (CEZ-9100; Nihon Kohden, Tokyo). The composition of the solutions (mM) was as follows. K-
percentage terms as the difference between the baseline SCC before its application and the peak value obtained after its application. Thus, in the case of data obtained after treatment with Cu²⁺, which itself increased the SCC, the responses to amiloride and acetylcholine were expressed relative to the new, raised, baseline.

Results and Discussion

Figure 1 shows typical examples and Fig. 2 a summary of the effects of Cu²⁺ on the changes in SCC induced by amiloride and acetylcholine in the larval bullfrog skin. Amiloride and acetylcholine both increased SCC transiently in the absence of Cu²⁺, as reported previously [1, 2, 5, 6, 7]. Cu²⁺ itself greatly increased the SCC. In the presence of Cu²⁺, amiloride no longer activated the SCC; only inhibition was observed at amiloride concentrations of 10⁻⁶, 10⁻⁵ and 10⁻⁴ M. The effects of amiloride on the SCC in symmetrical Na-Ringer were qualitatively similar to those seen with K-Ringer on the apical side (data not shown).

In addition, the effects of amiloride on the SCC in bicarbonate-free HEPES-buffered solution were similar to those described above (which were induced in bicarbonate-buffered solution). In bicarbonate-free HEPES-buffered solution amiloride (10⁻⁴ M) increased the SCC by 117.6±4.8%, n=4. Cu²⁺ (500 µM) increased SCC by 307.1±70.6%, n=6 whereas subsequent application of amiloride (10⁻⁴ M) in the presence of Cu²⁺ decreased the SCC (with respect to the raised baseline seen in the presence of Cu²⁺) by 52.2±8.5%, n=6. Unlike amiloride, acetylcholine transiently increased the SCC both in the presence and absence of Cu²⁺, thus showing that the SCC could increase further from the high level induced by the presence of Cu²⁺.

Our experiments were designed with the following facts in mind. First, the NSCC of bullfrog tadpoles is potentiated when tadpoles are raised in aldosterone [7]. Second, the changes in the SCC induced by amiloride and acetylcholine are larger when K-Ringer’s solution rather than Na-Ringer’s bathes the apical side [2, 5, 7].