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FUNCTIONING OF CATFISH ELECTRORECEPTORS: INFLUENCE OF CALCIUM AND SODIUM CONCENTRATION ON THE SKIN POTENTIAL

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Abstract—1. The skin potential of catfish was measured in order to test the hypothesis that it controls electroreceptor sensitivity.

2. The skin potential depends on the "milieu extérieur" in the same way as reported for goldfish (Fig. 2).

3. The variation of the skin potential is very large compared with the normal stimulus range of electroreceptors.

4. Calcium strongly influences the skin potential, but the latter "adapts" to calcium concentrations of 0.3–3.0 mM (Fig. 3).

5. Ion-dependence of the skin impedance cannot seriously alter receptor sensitivity.

INTRODUCTION

Influences of environmental ionic composition on the functioning of lateral-line mechanoreceptors and electroreceptors have been studied by several authors (e.g. Katsuki *et al.*, 1970; Roth, 1971; Hashimoto & Katsuki, 1972; Sand, 1975; Zhadan & Zhadan, 1975; Peters *et al.*, 1975; Bauswein, 1977). The sensitivity of these receptors to the ionic composition of the water made some of the authors regard them as chemoreceptors, whereas others regarded this ion-sensitivity as a factor impairing the response of these receptors to electric current.

Nevertheless, this phenomenon can be used to study receptor functioning. Roth (1971) and Bauswein (1977) found that catfish electroreceptors functioned properly only in relatively calcium rich fresh water (2 mM Ca^{2+}). In other media, e.g. 0.5 mM Ca^{2+} or 2 mM Na^{+}, the electrosensitivity was severely impaired. Comparable results were obtained by Peters *et al.* (loc. cit.). In addition they found that, even in deionized water, the electrosensitivity could be restored by an anodal direct current. Therefore they suggested that receptor functioning is influenced by an electrical effect of the ionic composition, i.e. by the potential difference across the skin. The influences of water composition and direct current are very long lasting: only after some tens of minutes does some adaptation process restore the sensitivity (Peters *et al.*, loc. cit.).

Potential differences across the skin, usually called "skin potential", are extensively used in studies of ion metabolism of aquatic animals. In fish, skin potentials ranging from −50 mV up to +20 mV (inside versus outside) have been found (Potts & Eddy, 1973; Maetz, 1974; Evans *et al.*, 1974; Eddy, 1975; Evans & Cooper, 1976; Carrier & Evans, 1976; Marshall, 1977). Fewer data are available concerning the long-term influence of any particular solution on the skin potential. Dietz *et al.* (loc. cit.) adapted larval salamanders to deionized water and different NaCl concentrations for 1 week and found the skin potentials to be substantially different from those of animals that had been kept in the normal medium. In a similar way the adaptation of electroreceptors may depend upon adaptation of the skin potential. No skin potential data are so far available for the catfish.

This paper deals with the relation between the skin potential of *Ictalurus nebulosus* and the concentration of some important electrolytes in the water. If the reported changes in skin potential are (partially) due to changes in the ion-permeability of the skin, then the electrical resistance of the skin is also dependent on the water composition. As the sensitivity of electroreceptors will depend on the overall skin resistance, we also paid attention to this parameter.

MATERIALS AND METHODS

The experiments were carried out on 31 adult catfish (*Ictalurus nebulosus* LeS., length 12–17 cm, weight 30–70 g), captured in a pool near Tilburg, The Netherlands. They were kept in 250 l. stock tanks with a constant inflow of tap-water and at a temperature of 13–18°C, or in circular basins containing 500 l. of stagnant, occasionally renewed tap-water and at a temperature of 18–20°C. An experimental animal was anaesthetized in tricaine (MS 222; Sandoz, Basel; 0.5 g/l tap-water) and subsequently immobilized by an intramuscular injection of gallamine triethiodide (Flaxédil; Specia, Paris; 0.07 mg/g body weight).

Liquid junction potentials

Prior to the skin potential measurements, the reliability of the salt bridge chain was tested by measuring liquid junction potentials and comparing them with predicted, calculated values. The measuring equipment, depicted in Fig. 1a, consisted of a pair of Ag/AgCl electrodes (E) fitted with two pairs of salt bridges (C and P) and connected via a preamplifier (P.A.R. 113) to a chart recorder (Hewlett Packard 7702B). The 3 M KCl bridges (P) could be short-circuited by a glass tube (S), also filled with 3 M KCl, so that the electrode polarization could be measured. The short-circuit potential was always used as zero reference; when it exceeded 2 mV the electrodes were discarded. The pipettes ended in two test tubes, one filled with a Ringer's

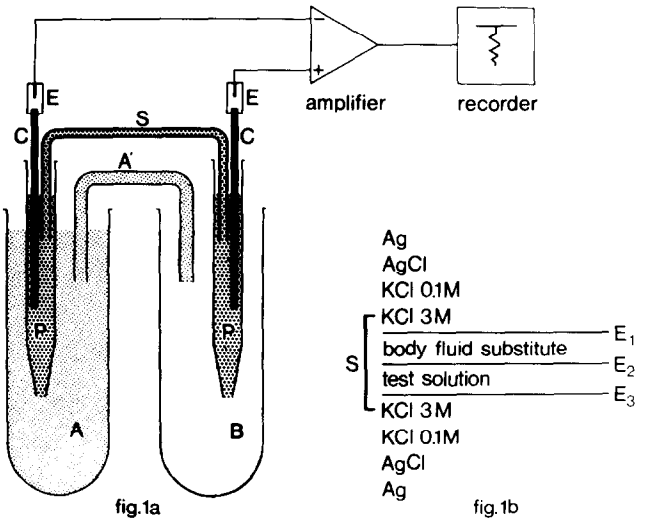

Fig. 1. A—Apparatus for the measurement of liquid junction potentials A: vessel with body fluid substitute (NaCl or Ringer); A': tube with the same fluid as in A and agar; B: vessel with test solution; C: cannula with 0.1 M KCl and agar; E: AgCl-electrode; P: pipette containing 3 M KCl, with agar in the tapered end; S: short-circuiting salt bridge (3 M KCl and agar). B—Electrochemical chain. E_1, E_2 and E_3: liquid junction potentials. S: short circuiting salt bridge. In skin potential measurement the body fluid substitute is replaced by the real fish; in this case E_2 is the skin potential.

solution (brown trout Ringer; Wolf, 1963) or 0.15 M NaCl (A), and the other one with the test solution (B). The tubes were interconnected via an agar bridge (A') containing the same solution as tube A.

Test solutions were deionized water (resistivity over 1 MΩ.cm), and solutions of NaCl or $CaCl_2$ with concentrations of 0.1 mM–100 mM, prepared by adding analytical grade salt to deionized water. The total electrochemical chain is depicted in Fig. 1b. The measured potential difference is the algebraic sum of the junction potentials between 3 M KCl bridge and Ringer (E_1), Ringer and test solution (E_2), and test solution and the second 3 M KCl bridge (E_3).

In principle these potentials can be calculated using Henderson's formula (see MacInnes, 1961), but we decided to measure them for two reasons. Firstly, the calculated values are approximations, as the ion mobilities are not known exactly at the ionic strengths used. Secondly, the ionic composition of the fish body fluid is not known exactly, nor is that of the Ringer's solution because of the unknown dissociation of the phosphate buffer. Therefore, measured and calculated values were compared only for the case of the 0.15 M NaCl body fluid substitute.

Skin potentials

For the recording of skin potentials, the 3 M KCl bridges, instead of being suspended in test tubes, were extended by polythene cannulae (o.d. 1.0 mm, i.d. 0.4 mm, length 20–30 cm) filled with 3 M KCl and 2% agar. After the animal had been anaesthetized, the skin was perforated with a dental cutter at about 1 cm ventrally from the dorsal fin. One cannula was then inserted between the skin and the underlying muscles and pushed through the myosepts in the caudal direction until it was 5–7 cm away from the perforation. At this point the skin potential was not noticeably influenced by the short-circuiting effect of the tiny hole. This was checked by raising the piece of skin where the cannula entered above the water and drying it with a stream of air. By suspending the fish in nylon netting we were able to attach the other cannula externally, with its opening close to the skin, in the same area as the internal cannula. In most of our experiments, a second pair of cannulae was fixed in the same way at the contralateral side. Then we could record simultaneously from both sides with two preamplifiers connected to the chart recorder.

After recording in deionized water for about 30 min salt was added from a 2 M stock solution, yielding concentrations from 0.1 to 100 mM in a 1:3:10 sequence.

The measurements were performed on catfish from the tap-water described above and on fish that had been adapted to a $CaCl_2$ solution for 1–5 weeks. During adaptation the fish were kept in tanks containing 20 l. of solution, which was renewed every week (catfish were found to excrete less than 0.2 mM of the ion species concerned in a week).

Skin resistance

Skin resistance was measured by feeding current (4–40 $\mu A/cm^2$) through the experimental tank via two stainless steel gauze electrodes. In fact we measured the skin impedance with sinusoidal current at frequencies of 1–1000 Hz. In this way we avoided a possible alteration of skin properties by direct current, and were able to determine the frequency characteristic of the skin potential. Since part of the stimulus current flows round the fish, only a relative measure of impedance can be obtained from the potential difference between one external and one internal electrode (u_{skin}) and the potential difference between the two internal electrodes (u_{int}). Assuming the resistivity of the body fluid to be independent of the composition of the external medium, we can regard the impedance ratio (u_{skin}/u_{int}) as a suitable measure for comparing skin resistances in different media.

RESULTS

Liquid junction potentials

With the test solutions we used the calculated values of the l.j.p. involved in skin potential measurement ($E_1 + E_3$, see Fig. 1b) ranged from −4.0 to +1.1 mV. Unfortunately, this potential cannot be measured directly, because the electrochemical chain inevitably includes the junction potential E_2, which is not relevant in the case of skin potential measurement because it will be substituted by the skin potential.

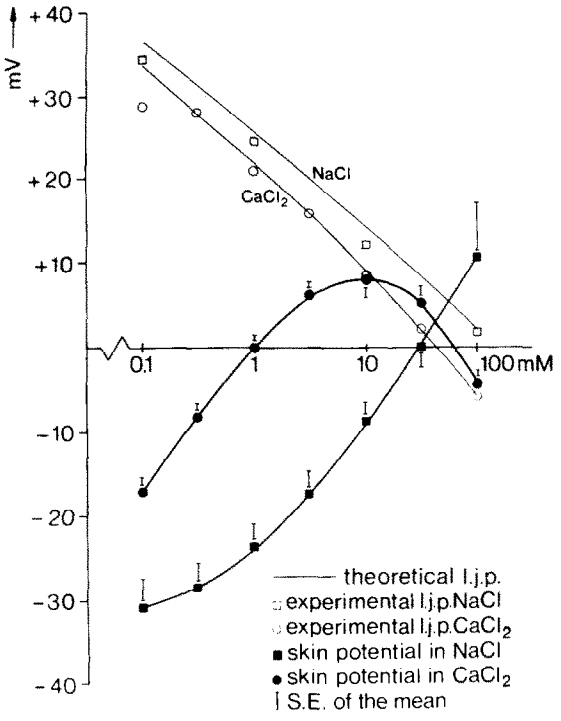

Fig. 2. Liquid junction potential and skin potential as a function of salt concentration. The thin curves represent the theoretical, i.e. calculated, junction potential (E_2 in Fig. 1b). Open circles and squares are experimentally determined values of the junction potential with $CaCl_2$ and NaCl respectively as test solution. Closed circles and squares together with the bold curves pertain to the measured skin potential of catfish. Abscissa: concentration of the test solution. Ordinate: potential. The junction potentials are taken as body fluid substitute versus test solution, the skin potentials as inside versus test solution. All potentials are corrected for the theoretical values of the junction potentials arising at the 3 M KCl bridges ($E_1 + E_3$ in Fig. 1b).

The thin curves in Fig. 2 represent the calculated magnitude of E_2 as a function of concentration for the test solutions NaCl and $CaCl_2$, when 0.15 M NaCl was used as body fluid substitute.

The measured values were systematically lower than the calculated ones, ranging from 3 mV at 0.1 mM to 0 mV at 100 mM, i.e. about 10% of E_2. This suggests that the measuring accuracy is limited mainly by the large E_2, and that the actual values of $E_1 + E_3$ will also deviate as little as 10% from the theoretical ones, i.e. at most 0.4 mV.

When Ringer's solution was used as body fluid substitute, the junction potentials were about 5 mV lower, independent of ion species and concentration. In view of the above, we have subtracted the calculated value of $E_1 + E_3$ from the measured skin potentials.

Skin potentials

The skin potential was measured in distilled water (eight fish) and in solutions of NaCl (six fish) and/or $CaCl_2$ (seven fish). In distilled water the skin potential was -30 to -50 mV, and drifted so much that we did not attempt to determine the proper value. Addition of a non-permeant buffer (Tris-maleic acid) did not substantially improve stability, so we proceeded with unbuffered solutions with a pH of 5.0–6.5. In all other media the skin potential was stable to within 3 mV a few seconds after each salt addition. Usually there was a small difference between the skin potentials recorded from the left and right side of the fish. The difference averaged 2.5 mV. We used the mean of the values from the left and right side as the best estimate of the skin potential. As expected, the skin potential appeared to be strongly dependent on ion concentration (Fig. 2).

There is a marked difference between the effects of NaCl and $CaCl_2$, especially at concentrations found in fresh water. At 1 mM $CaCl_2$, which is about the calcium concentration in Utrecht tap-water (Peters *et al.*, 1975), the skin potential is about 0 mV, whereas in 1 mM NaCl it is more than 20 mV negative. The skin potential also depends on the medium in which the fish is kept. This is illustrated in Fig. 3, which shows the relation of skin potentials to $CaCl_2$ concentration for fish adapted to tap-water or to various calcium concentrations.

Skin resistance

The skin resistance was determined in six fish, in tap-water and in a few other media. Between 1 and 10 Hz, the skin impedance ratio was constant, and can be regarded as the (d.c.) resistance. In tap-water, it averaged $u_{skin}/u_{int} = 12.0$. At higher frequencies the ratio declined, showing a cut-off frequency $f_c = 22$ Hz and a slope of circa -6 dB octave. With higher ion concentrations the impedance ratio became lower and the cut-off frequency higher: in 10 mM NaCl $u_{skin}/u_{int} = 12.8$ and $f_c = 45$ Hz; in 30 mM $CaCl_2$ 3.8 and 130 Hz, respectively. In more diluted solutions the impedance ratio became higher and the cut-off frequency lower: in 0.1 mM NaCl $u_{skin}/u_{int} = 15.5$ and $f_c = 9$ Hz.

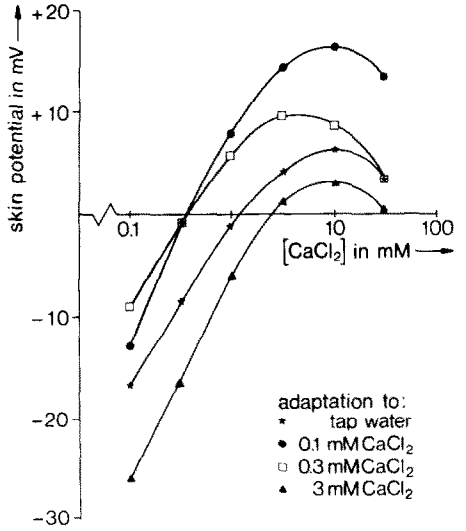

Fig. 3. Dependence of the skin potential on the calcium concentration to which the fish were adapted. The different curves represent the skin potential of fish adapted to: ● 0.1 mM $CaCl_2$ (for 5 weeks); □ 0.3 mM $CaCl_2$ (5 weeks); ▲ 3 mM $CaCl_2$ (1 week) and * tap-water (indefinite).

DISCUSSION

The relationship between skin potential and $CaCl_2$ or NaCl concentration of the water is similar to that found by Eddy (1975) in the goldfish. Apparently the skin potential may vary over tens of mV, both in a random way and as a function of the concentration of certain ions. Despite the small standard error of the mean (Fig. 2), there was a scatter of some 5–10 mV in the individual skin potential measurements. Changes in sodium or calcium concentration of a factor of three, which are not unlikely to occur in the habitat of the fish, also change the skin potential by about 10 mV.

With concentrations normally found in fresh water, the skin potential in NaCl is far more negative than in $CaCl_2$: at 1 mM the difference is 24 mV. At first sight this might seem to represent the "offset voltage" which Peters *et al.* (1975) suggested to explain the reduced sensitivity of catfish electroreceptors in calcium deficient water. However, the polarity is opposite to the expected one, because an anodal current, which has been shown to restore receptor sensitivity in calcium deficient water, makes the skin potential more negative, instead of reducing it to the value found in water containing calcium. Presumably it is not the skin potential as such which acts as a bias potential for the receptors, but it is a small difference between the skin potential and the potential generated by the receptor epithelium (cf. Bretschneider *et al.*, 1979). If stimulated directly by the skin potential electroreceptors would be overstimulated so frequently that they would hardly ever function at all.

The skin potential appears to "adapt" to calcium concentrations of 0.3–3 mM in such a way as to approach zero at the concentration to which the fish is adapted (Fig. 3). Probably the hypothetical difference between skin potential and receptor epithelium potential is also zero under these conditions, so that it is only a sudden change of the composition of the water which temporarily inhibits receptor functioning. This might explain why electroreceptors of catfish adapted to 0.4 mM calcium function properly at that concentration (Zhadan & Zhadan, 1975), whereas in fish adapted to 2 mM calcium the response was inhibited by reducing the Ca^{2+} concentration to 0.5 mM (Bauswein, 1977). Of course, this adaptation of the skin potential does not preclude any adaptation of the receptors themselves to a constant current stimulus.

The resistance ratio and cut-off frequency of the skin we found confirm measurements by Peters *et al.* (1974) of the electrical loading of the water by an intact catfish. They are also comparable with the corresponding values found in the freshwater ray *Potamotrygon* (Szabo *et al.*, 1972) and are consistent with the current theory on the effective stimulus of ampullary electroreceptors (reviewed by Kalmijn, 1974). The cut-off frequency of the skin is higher than that of the electroreceptor response (Peters & Buwalda, 1972) and does not depend very much on water composition.

Therefore we can safely conclude that the rapid decline of receptor sensitivity above about 5 Hz will be caused by mechanisms other than the skin impedance. At low salt concentrations, the polarity of the skin potential is opposite to that of the liquid junction potential. This indicates that the skin potential originates in the membranes of the epidermis. It is worth noting that in media containing both calcium and sodium the skin potential is almost exclusively dependent on calcium concentration. In Utrecht tap-water, which contains 0.9 mM calcium and 0.3 mM sodium, the skin potential is about the same as in 1 mM $CaCl_2$. Probably the skin is substantially less permeable to sodium than to calcium. With sodium or calcium concentrations higher than 10 mM, the skin potential approaches the junction potential.

As the skin resistance declines at high concentrations, the membranes and/or tight junctions of the epidermis apparently begin to leak more with increasing concentration. With regard to freshwater catfish this will, however, be only of theoretical interest, since ion concentrations in fresh water seldom exceed a few mM.

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