Fish Physiology and Biochemistry **21:** 81–88, 1999.

Catfish electroreceptor organ functioning during five days exposure to different calcium environments

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Accepted: December 19, 1998

Key words: chemoreception, electroreception, hair cells, homeostasis, *Ictalurus*, Saffan, stanniocalcin, stimulus transduction, synaptic efficacy

Abstract

Catfish, *Ictalurus melas*, were pre-adapted to artificial tap water with 1.2 mM Ca^{2+} for two weeks, and subsequently transferred to artificial tap water with 0.6, 1.2, or 2.2 mM Ca^{2+} for one week in order to investigate the effect of the environmental Ca^{2+}-concentration on stimulus encoding and the frequency characteristics in ampullary electroreceptor organs. Within 30 minutes after transfer, the spontaneous activity of the primary afferents, as well as gain and phase of the stimulus induced responses changed transiently corresponding to the Ca^{2+}-concentration. One day after transfer the Ca^{2+}-induced changes of the spontaneous activity had disappeared as well as the differences between the sensitivities at frequencies of 2, 8, 12, 16 and 20 Hz in 0.6 and 1.2 mM Ca^{2+}, whereas at 16 and 20 Hz in 2.2 mM Ca^{2+} the sensitivity was still elevated. The Ca^{2+}-induced phase shift was strongly frequency dependent. At 2 Hz no Ca^{2+} effect on the phase was observed, whereas at 12, 16 and 20 Hz significant effects could be demonstrated up till three days after transfer. The latency was not affected by the transfer.

The Ca^{2+}-induced effects on the primary afferent spontaneous activity are probably related to a Na^{+}/Ca^{2+}-exchanger at the basal faces of the receptor cells. The frequency dependent effects on gain and phase are concluded to relate to properties of the apical membrane, most likely to Ca-dependent K-channels. These findings further support the concept that ampullary electroreceptor might serve as chemoreceptor organs.

Introduction

The biological role of ampullary electroreceptor organs in the life of freshwater catfish (*Ictalurus sp, Clarias sp)* is electroreception. Evidence for this was provided by behavioural experiments, which, in addition to electrophysiological experiments, revealed the part played by electroreceptor organs in predation and orientation (e.g., Parker and van Heusen 1917; Kokubo 1934; Dijkgraaf 1968; Roth 1968, 1969, 1972; Peters and Meek 1972; Peters and van Wijland 1974; Kalmijn et al. 1976a, b; Peters et al. 1995; Moller 1995; Hanika 1997). On the other hand, ampullary electroreceptor organs are also 'sensitive' to the chemical composition of the environment, among other things to the free Ca^{2+}concentration (Roth 1971, 1982; Peters et al. 1975, 1989; Zhadan and Zhadan 1975; Bauswein 1977). This feature has also been demonstrated in other parts of the octavolateralis system like the mechanoreceptive hair cells of the lateral line organ of *Necturus* (Sand 1975; Sand et al. 1975; Baumann and Roth 1986). Changing the concentrations of Na^{+}, K^{+} or Ca^{2+} ions by a few millimoles affects the spontaneous and stimulus induced spike rate of the electroreceptor primary afferents (Roth 1971). One of the most striking effects of changing the ionic composition of the environmental freshwater is its relatively long lasting duration. Changes of stimulus induced and spontaneous spike rates last up to one hour, the duration of an electrophysiological observa-

Table 1. Composition of artificial tapwater

Substance	(mM) concentration
Na^+	0.65
K^+	0.025
Mg^{2+}	0.15
Cl^-	1.20
HCO_3^-	0.63
PO_4^{3-}	0.035
SO_4^{2-}	0.16
Ca^{2+}	0.6, 1.2, or 2.2

tion. The slow adaptation contrasts strongly with the rapid adaptation of the receptor organs to the biologically adequate electrical stimuli (Roth 1971; Peters et al. 1989).

In order to gain a better insight in how ampullary electroreceptor organs cope with exposure to long lasting exposure to a different Ca^{2+} concentration, we studied the time course of the effects induced by environmental Ca^{2+}concentration changes. Exposing the subjects to a changed environment for a period longer than a mere 30 min, seems a proper way to probe the nature of the electroreceptor's biochemical mechanisms involved in adaptation to such a change, and its possible biological relevance. This paper describes the influence of a five days lasting exposure of the ampullary electroreceptor organ of the fresh water catfish *Ictalurus melas* to a steplike change of environmental Ca^{2+} concentration.

Materials and methods

Animals, exposure to calcium, and protocol

The experiments were performed on 24 adult male freshwater catfish (*Ictalurus melas*; see Spillman 1967) weighing 100 to 250 g, and having a length of 18 to 24 cm. The fish were obtained from a fish farm (Peschkes, München-Gladbach, Germany), kept in 250 l glass containers in Utrecht copper-free tapwater with 1 mM Ca^{2+} at about 15 °C, and fed on minced beef and Trouvit elite-response (Trouw Nederland B.V.). At the beginning of an experiment the fish were removed from the stock tanks and transferred to experimental tanks with artificial standard tap water of 1.2 mM Ca^{2+} (Table I) for one week to acclimate to the new electrochemical environment at 18 °C. In the first week no recordings were performed. In the second week the fish were transferred to smaller containers with a total buffer volume of 135 l and filled with fresh artificial standard tap water. Control electrophysiological recordings were performed 3 and 5 days before transfer. In the third week the fish were transferred again to small containers with artificial test water with either normal (1.2 mM), high (2.2 mM), or low (0.6 mM) Ca^{2+} levels. Three groups of four fish were recorded three times, namely at days 1, 3, and 5 after transfer. Three other groups of fish were recorded only once, within 30 min after transfer. These latter recordings were necessary for comparison of our results with earlier studies (Roth 1971), but they had to be performed on a separate set of fish because we wanted the subjects to recover from their narcosis for at least one full day. The fish were fed with minced beef only when they had been transferred to another tank, and after the electrophysiological recordings had been made. When the recordings were finished the fish were returned to the stock tanks. In each fish recordings were carried out on the same ampullary organ to reduce the variation in data (Heijmen and Peters 1995).

Electrophysiology and signal processing

Prior to recording from the ampullary electroreceptor organs, the fish were anesthetized with Saffan (Pitman-Moore, Harefield, U.K.; Oswald 1978), 36 mg kg^{-1} body $weight^{-1}$ i.m., then placed in a small perspex tray, volume 1.8 l, where artificial respiration was given at 300 ml min^{-1}. If necessary additional anesthesia was given. During the electrophysiological recordings the water temperature was kept at 18 °C. Single unit afferent activity was recorded extracellularly from organs at the dorsal head region by means of a tungsten microelectrode. A stainless steel rod electrode in the corner of the tray served as a reference. The stimulus electrode was a horseshoe shaped silver wire placed around the recording electrode at 2 to 5 mm from the skin. In this way a local uniform stimulus field was created, independent of the electrode to skin distance, because the shunt resistance to ground determines the potential at the skin surface (Peters et al. 1997). In order to overcome the polarization effects of the stimulus electrode, the stimuli were delivered via a constant current source (voltage to current converter). Sinusoidal stimuli had amplitudes of 2 to 8 nA at 2, 8, 12, 16 and 20 Hz. Square pulses of 98 ms positive followed by 98 ms negative at a repeti-

tion rate of 2 Hz, had amplitudes of 24 nA. Care was taken that the amplitudes of the sinusoidal stimuli did not saturate the electroreceptor organ. Square pulses on the other hand were made so strong that the latency of the electroreceptor organ response was independent of the stimulus strength. Amplified spike trains were processed by a window discriminator to render uniform pulses to be converted by a computer into peristimulus histograms (PSH). Sine curves were fitted through these PSHs by means of the method of least squares in order to estimate amplitude (spikes s^{-1}), phase shift with respect to the input signal (deg), and offset (spikes s^{-1}) of the modulated spike train. Sensitivity was defined as amplitude of the spike frequency modulation divided by stimulus strength. Spontaneous activity was quantified by mean and standard deviation of the corresponding interspike interval histogram.

As a rule each experiment consisted of 5 recording cycles. In each cycle, recording of the spontaneous activity was followed by recording with stimulation at 2, 8, 12, 16 and 20 Hz in a pseudo random order. Each single recording lasted for about 50 s. The parameters of these single recordings (averaged over 50 s) were used as data. After completion of the sinusoidal stimulation, additional recordings were performed to quantify latency after square pulse stimulation. Latency was defined as the interval between the onset of the stimulus pulse and the moment the number of spikes bin^{-1} deviated $3 \times$ S.D. from the average number of spikes during the preceding 100 ms.

Ca^{2+} measurements

The Ca^{2+} concentrations in the tanks were measured every two days. Samples were analyzed spectrophotometrically (Beckman Synchron CX) or by means of inductively coupled plasma atomic emission spectroscopy (ICP-AES) by a third party, (Analytical Geochemical laboratory of Utrecht University). The electrical conductivities of the 0.6, 1.2, and 2.2 mM Ca^{2+} water were 230, 370, and 610 μS cm^{-1} respectively. Since the water conductivity determines the stimulus efficacy, the recorded sensitivities were corrected for the conductivities.

Statistics

A repeated measures ANOVA (within factors: time and stimulus frequency; between factor: calcium concentration) was done to determine significant differences ($p < 0.05$) in the sensitivities, spontaneous activities and phase shifts at various calcium concentrations. After having established the main and interaction effects, a plain t-test was used to find local contrasts.

Results

Pre-adaptation

Pilot experiments suggested a long duration of the calcium induced changes in receptor organ functioning. Therefore the fish were kept in strictly controlled artificial tap water during the fortnight preceding the actual change of $[Ca^{2+}]$. Since there was no significant difference between the data at day 5 and 3 before the transfer, the control data at day 3 before transfer, the last observation before the transfer, were taken as a reference set to calculate the normalized relative values.

The first 30 min after transfer

The first 30 min after transfer to the containers with the test concentrations revealed the greatest changes as expected from earlier studies (Roth 1971). The spontaneous activity rose to 110% in 2.2 mM Ca^{2+} water, and fell to 80% in 0.6 mM-Ca^{2+} water (Figure 1A). These Ca^{2+}-induced changes were statistically significant ($p < 0.05$). A more or less similar pattern could be recognized in the sensitivities at 16 and 20 Hz, but the sensitivity changes at 2, 8 and 12 Hz were less conspicuous (Figures 1C, 1E, 2A). The Ca^{2+}-related sensitivity changes were significant ($p < 0.05$) at 16 and 20 Hz and apparently frequency dependent. The Ca^{2+}-related changes in phase characteristics were also significant ($p < 0.05$) and frequency dependent. At 2 Hz there was no effect, whereas at 20 Hz the phase shift increased with 20 degrees in 2.2 mM Ca^{2+} water, and decreased with 40 degrees in 0.6 mM Ca^{2+} water (Figures 1G, 1I, 2C). The latency in response to a positive pulse like stimulus did not show any Ca^{2+} related effect. Mean latencies of the 30 min groups in standard tap water were 14.5 ± 1.0, 12.5 ± 1.9, and 14.5 ± 1.9 ms, whereas the mean latencies immediately after transfer to 0.6, 1.2, and 2.2 mM Ca^{2+} were 14.6 ± 1.1, 16.0 ± 2.8, and 13.5 ± 1.9 ms, respectively.

Days 1, 3, and 5 after transfer

From day 1 through day 5 after transfer to the test solutions the effects of a change in Ca^{2+}-concentration

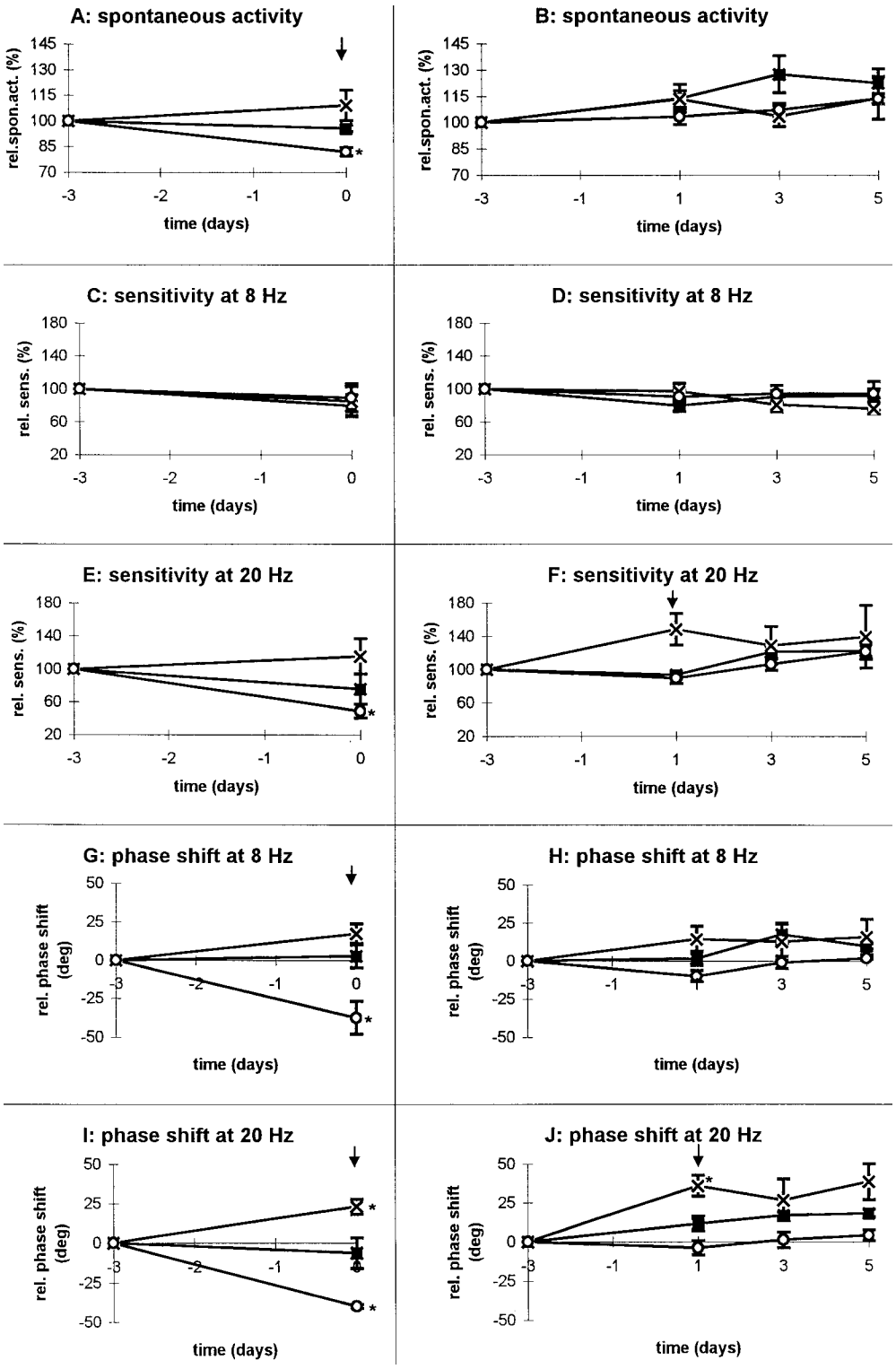

Figure 1. Examples of spontaneous activity, sensitivity and phase shift of ampullary electroreceptor afferent activity as a function of time at different Ca^{2+} concentrations, relative to the values at day 3 before transfer. At day 0 the fish were transferred from standard artificial tapwater with 1.2 mM Ca^{2+} to artificial tapwater with 0.6, 1.2, or 2.2 mM Ca^{2+}. (A, C, E, G, I) Data of day 0, at 30 min after transfer. (B, D, F, H, J) Data of 1, 3 and 5 days after transfer. Each line represents the means of data of four fish ± SEM. Solid squares 1.2 mM, crosses 2.2. mM, open circles 0.6 mM Ca^{2+}. Asterisks indicate significance relative to day 3 before transfer. Arrows indicate significant concentration effects.

on ampullary electroreceptor organs were still manifest, but less pronounced, declining with time, and strongly frequency dependent. Of all properties the spontaneous activity seemed the least affected by the changed environmental concentration. It was practically normal again at day 1 after transfer, and, though it fluctuated from day to day, showed no Ca^{2+}-related pattern (Figure 1B). The sensitivity behaved asymmetrically. There was no difference between the 1.2 mM and 0.6 mM data at any day or frequency (Figure 1D, 1F, 2B). There occurred, however, a strong significant ($p < 0.05$) asymmetry at day 1 after transfer, between 2.2 mM and 1.2 mM at 16 and 20 Hz, where the sensitivity in 2.2 mM Ca^{2+} was about 50% greater than in the reference situation at 1.2 mM Ca^{2+}. The difference between sensitivity at 20 Hz in 1.2 mM and 0.6 mM calcium on the other hand was not significant (Figure 1F).

The effect of a change in Ca^{2+} concentration on the phase characteristics was different again. The phase shift at the higher frequencies changed about 10 deg in 2.2 mM Ca^{2+} on day 1 after transfer and gradually reverted through day 5. The effect of 0.6 mM Ca^{2+} on the phase shift at 20 Hz on day 1 after transfer was less drastic, but followed a similar course. The phase curves also showed a clearest Ca^{2+} effect at 12, 16 and 20 Hz (Figure 1H, 1J, 2C, 2D). At 2 Hz no effect could be demonstrated.

As for the latency no significant Ca^{+}-related latency changes were found.

Discussion

General comments and time course

Our results are in line with earlier experiments that demonstrated the ion composition of the aquatic environment, *in casu* the Ca^{2+} concentration, to affect electroreceptor organ functioning during the first 30 min after transfer to an environment with a different Ca^{2+} concentration (Roth 1971, 1982; Peters et al. 1975, 1989; Zhadan and Zhadan 1975; Bauswein 1977). Our results are also in accordance with experiments on the Ca^{2+} sensitivity of the mechanoreceptive lateral line systems of *Necturus* and *Rutilus* (Sand 1975; Karlsen and Sand 1987). Apparently this form of Ca^{2+} sensitivity is not specific for electroreceptor organs. Further our experiments reveal a frequency dependent effect (cf. also Bauswein 1977) that in part lasts up to six days. The effects are strongest immediately after transfer. The recorded parameters show considerable scatter, which might be caused among other things by blood Ca^{2+} levels oscillating with a period of 14 days as demonstrated in other species (Wagner et al. 1993). The spontaneous activity, sensitivity and phase shift follow different time courses, and are most likely related to different cellular mechanisms (cf. Sand 1975).

Cellular mechanisms that can account for the observed effects

In a way chemosensitivity of electroreceptor organs is self-evident. Both electrical and chemical stimuli (ions) can disturb the electrochemical homeostasis of the electroreceptor cell, and thus bring about changes of the membrane potential, which are thought to be reflected in the release of neurotransmitter. For this reason ampullary electroreceptor cells are also denoted 'sensor homeostats' (Andrianov et al. 1996).

More specifically Ca^{2+} can act on ampullary electroreceptor transduction in several ways: (i) uptake of Ca^{2+} through the gills would change blood Ca^{2+} levels and thus the Ca^{2+} gradient over the electroreceptor cell and its synapses; (ii) influx of Ca^{2+} into the receptor cells might raise intracellular [Ca^{2+}] and in turn boost the pump rate of the Na^{+}/Ca^{2+}-exchanger, or change the configuration of membrane bound structures such as ion channels and thus their operating characteristics; (iii) extracellular Ca^{2+} ions might somehow affect the distribution of electrical charges over the apical membrane and thus alter ion channel properties or to some extent even the thickness of the electric double layer.

Transfer of fish to water with high or low Ca^{2+} causes a transient effect on *spontaneous activity*, which passes away within one day. Apparently the fish have acclimated to the new situation after one day. We postulate that the Ca^{2+} regulating hormone stanniocalcin must be involved in acclimation, as it was demonstrated in rainbow trout (*Oncorhynchus mykiss*) to backregulate a disturbed blood Ca^{2+} concentration within 2 h (Wagner et al. 1991; Wendelaar Bonga and Pang 1991). How Ca^{2+} affects spontaneous activity and sensitivity is not known exactly in ampullary organs of fresh water teleosts. It is unlikely that the Ca^{2+} gradient over the basal membrane of the receptor cells is the controlling factor, because the effects seem opposite to those expected if Ca^{2+} entry through voltage sensitive channels at the basal membrane of the receptor cells were crucial. Most likely the basal faces

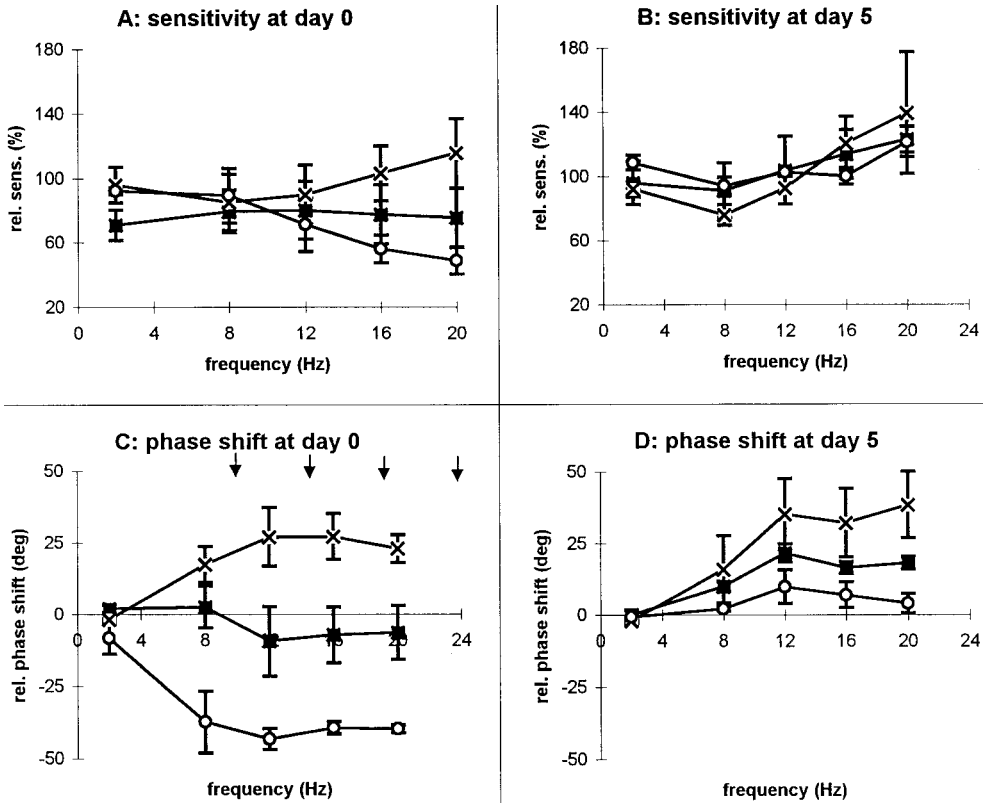

Figure 2. Examples of sensitivity and phase shift of ampullary electroreceptor afferent activity as a function of frequency at different Ca^{2+} concentrations, relative to the values at day 3 before transfer. At day 0 the fish were transferred from standard artificial tapwater with 1.2 mM Ca^{2+} to artificial tapwater with 0.6, 1.2, or 2.2 mM Ca^{2+}. (A, C) Data of day 0, at 30 min after transfer. (B,D) Data of day five after transfer. Data represent mean ±SEM. Solid squares 1.2 mM, crosses 2.2. mM, open circles 0.6 mm Ca^{2+}. Arrows indicate significant concentration effects.

of the electroreceptor cell contain a Na^+/Ca^{2+}- and Na^+/K^+-exchangers, just like the ampullary organs of the marine fishes (Sugawara 1989; Lu and Fishman 1995), which somehow control release of transmitter and thus spontaneous activity. Ca^{2+} leaking into the receptor cell would prime the Na^+/Ca^{2+}-exchanger and thus indirectly modulate spontaneous activity of the primary afferents. Disappearance of this transient effect after one day could mean that stanniocalcin closes ion channels in apical membranes, both of chloride cells and electroreceptor cells, so that Ca^{2+} fluxes have regained their original balance.

Since *latency* is not influenced by environmental Ca^{2+}, changes in phase shift are not due to latency. Therefore the cause of action of Ca^{2+} on phase must not be sought in the synapse, but probably presynaptically, i.e., in the receptor cell. *Phase shifts* are in general tightly coupled to the timing of physiological processes, and in this case also to the electrical capacitance of membranes and the kinetics of ion channels. The model calculations of Heijmen and Peters (1995) can account for the observed frequency dependent effects (Figures 2A, C, D) if it is assumed that due to high Ca^{2+} the apical surface area shrinks, and that at the same time the conductivity of the apical membrane increases. A more feasible explanation however is that a rise in Ca^{2+} concentration activates ion channels in the apical membrane. Frequency dependent interaction between stimulus and channel kinetics has been described earlier by Debus et al. (1995) who pointed out that measuring cell membrane capacitance's through sinusoidal currents in patch clamp experiments can cause misleading results if the angular frequency of the sine wave is close to the kinetic rate constant of the membrane channels. If this finding also applies to catfish electroreceptor cells, the Ca^{2+} induced phase shifts and Ca^{2+} related *sensitivity* could represent kinetics of channels and reveal the rate constant of the channels involved. A rough estimate of the rate constants would be 20 per s. Since

the effect is Ca^{2+} dependent, we postulate the channel to be Ca^{2+} controlled. Since Zhadan and Zhadan (1975) discovered that the sensitivity of catfish ampullary electroreceptor organs disappeared at 100 mm environmental K^+, we propose that K^+ ions carry the transduction current and that the ion exchangers are K^+-gradient controlled. Ca^{2+}-controlled K^+ channels at the apical membrane could thus explain the observed phase shifts. There is no reason to expect that osmolarity plays a part in the observed effects (cf. Zhadan and Zhadan 1975; Heijmen et al. 1996), or that Ca^{2+} affects the efficacy of the glutamatergic synapse (Andrianov et al. 1997).

An increase or decrease of the electrical sensitivity can also represent alterations of the voltage divider provided by the ensemble of the apical and basal membranes of the receptor cell. High Ca^{2+} raising the sensitivity suggests a reduction of the apical impedance relative to the basal membrane. A conductivity increase of the ion channels at the apical membrane as proposed earlier would also explain the sensitivity changes.

Biological relevance, Ca^{2+} sensor

As the spontaneous activity is affected by changes in Ca^{2+} concentration between 0.6 and 2.2 mM during at least 30 min, it is feasible that ampullary electroreceptor organs in catfish can serve as Ca^{2+} sensors. The long lasting offset of the spontaneous activity induced by the change in ion composition might signal to the central nervous system that 'something has happened' to the electrochemical balance between body fluids and environment. A catfish that passes local low or high Ca^{2+} plumes in its natural environment might thus experience a chemical stimulus via the ampullary electroreceptor organs. Within certain time limits the spontaneous activity follows the Ca^{2+} concentration of the aquatic environment. Whether such stimuli can be called 'behaviorally adequate' remains to be tested. Another consequence of the Ca^{2+} dependent frequency characteristics is that the water composition modulates the response of the electroreceptor system to electrical stimuli. A rise in environmental Ca^{2+} e.g., would temporarily increase the overall sensitivity, and enhance the higher frequencies. This effect would, to a certain extent, last even five days. Whether or not this phenomenon has adaptive value is beyond the scope of the present experiment.

Acknowledgements

The experiments were approved by the Ethical Committee of Utrecht University and conducted according to the 'Wet op de Dierproeven *anno* 1979'. The authors gratefully acknowledge the invaluable assistance of Herman Wijnne of the 'Centrum voor Biostatistiek', the zootechnical staff of the Hugo Kruyt Building, the analytical support of Wim Loos, the many discussions with our colleagues Franklin Bretschneider and Mieke Struik, and the academic freedom provided by the aegis of professor Wim van de Grind.

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