The Effects of Externally Applied Lithium and Strontium on the Arsenazo-Monitored Cytosolic Calcium Signal of the *Limulus* Ventral Photoreceptor

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The intracellular arsenazo signal indicating the transient light-evoked change in cytosolic Ca^{2+} (or Sr^{2+}) concentration was measured in *Limulus* ventral photoreceptor simultaneously with the receptor potential at 15 °C. The decline of the arsenazo signal has two phases (D1 and D2) when the photoreceptor is bathed in physiological saline.

1. When calcium is replaced by strontium in the superfusate both receptor potential and arsenazo signal are markedly increased in amplitude and the membrane potential is hyperpolarized. The decline of the arsenazo signal is prolonged and becomes monophasic; the fast phase D1 of the decline disappears.

2. In strontium saline under voltage clamp conditions the slope of the monophasic decline of the arsenazo signal is the steeper the more negative the membrane voltage.

3. After replacing sodium by lithium in the superfusate the rise of the receptor potential and of the arsenazo signal are not much altered. The decline of the arsenazo signal, however, is slowed down more than 3-fold; this is due to the complete suppression of the fast phase D1 and the retardation of the slow phase D2 of the calcium re-decline.

Interpretation:

1. The Na–Ca exchanger can accept strontium as a calcium substitute. Strontium has a weaker desensitizing action than calcium. Strontium is not – or only very little – taken up by the endoplasmic cisternae.

2. In sodium-free lithium saline the Na–Ca exchanger, the Na–K ATPase and the calcium uptake system of the endoplasmic cisternae do not function. Therefore the intracellular calcium level rises.

Abbreviations: Arsenazo III, 2,2′-(1,8-dihydroxy-3,6-disulfo-2-naphthalene-bis(azo)dibenzenearsonic acid; AS, arsenazo signal; cGMP, guanosine 3′:5′-cyclic monophosphate; D1, D2, fast and slow phase of decline of the arsenazo signal; EGTA, ethylene glycol-bis(β-aminoethylether)-N,N,N′,N′-tetraacetic acid; calcium chelating agent; F, area, time integral of light-evoked signal; Hmax, peak height of a light-evoked signal; IP_3, inositol 1,4,5-trisphosphate; Mi, initial slope, mean slope of rise from end of latency until half height of the signal; Mm, median slope of rise, slope of rise at half height of the signal; PMP, prestimulus membrane potential; PS, physiological saline; ReP, receptor potential, light-evoked membrane voltage signal; SRC, subrhabdomeric cisternae; Tlat, latency, time from stimulus onset to first significant start of the response; Tmax, time-to-peak from stimulus onset; Tr, rise time (Tmax – Tlat); T2, decay time, time during which the response decays from its maximum to 50% of that value.

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1. Introduction

In a former publication (Deckert and Stieve, 1991) we have shown that the initial rising phase of the intracellular calcium transient is insensitive to membrane voltage. This indicates that at least 90% of the calcium rise is not due to a light-induced calcium influx through the cation channels in the plasma membrane. The re-decline of the calcium transient proceeds in – at least – two phases, a faster one (D1) with a time constant τ_1 in the order of 1 to 2 s and a slower one (D2) with a time constant τ_2 in the order of 10–20 s. The faster phase D1 was found to be independent of the membrane voltage, whereas the slower phase D2 is membrane voltage-dependent. Therefore Deckert and Stieve (1991) concluded that the second phase D2 of the calcium re-decline is based on the action of the Na–Ca exchanger across the plasma membrane. Na–Ca exchangers have been extensively studied in other systems, especially in

the squid axon and the heart of vertebrates (Blaustein, 1977; Philipson, 1985; Allen *et al.,* 1989). A Na–Ca exchanger had been suggested for the *Limulus* photoreceptor by Lisman and Brown (1972), and Ivens and Stieve (1984) and was demonstrated by O'Day and Gray-Keller (1989). The fast component D1 is probably based on a rapid calcium uptake into the specialized endoplasmic reticulum (SRC, subrhabdomeral cisternae).

Stieve and Benner (1992) have shown that if the external calcium is replaced by strontium, the arsenazo signal becomes greatly enlarged both in amplitude and in duration. Arsenazo is an indicator of calcium and of strontium about equally well (Kendrick *et al.,* 1977). This means that there seems to be a strontium transient in the cell which is five times larger than the calcium transient under physiological conditions. Strontium can be handled by Na–Ca exchangers as a substitute for calcium. This has been shown *e.g.* for the Na–Ca exchanger of the squid axon (Blaustein and Russell, 1975) and the Na–Ca–K exchanger of the toad photoreceptor cell (Yau and Nakatani, 1984).

Lithium ions can pass through all the sodium channels studied so far (Hille, 1992). However, lithium is not accepted as a sodium substitute by sodium–calcium exchangers or by sodium–potassium ATPases (Blaustein, 1977; Skou and Norby, 1979; Glynn and Karlish, 1975; O'Day and Phillips, 1991).

In this paper we report on experiments in which we replaced the external calcium by strontium or the external sodium by lithium in order to investigate the mechanisms generating the rise and decline of the calcium transient. Our results are consistent with the following interpretation:

1. The initial rising phase of the light-induced increase in the cytosolic calcium concentration is not much sensitive to lithium.

2. The light-induced rise in cytosolic strontium is – compared to that of calcium – greatly prolonged. Its second phase – which is much more pronounced than that of calcium – depends clearly on the membrane voltage. This could be easily explained if the main source of rise in cytosolic strontium is not the cisternae but the extracellular space.

3. The uptake of calcium into the cisternae, indicated by the faster component D1 of the calcium re-decline, is reduced or abolished in lithium saline. There is also no significant D1 signal to indicate an uptake of strontium into the cisternae.

4. The activity of the sodium–calcium exchanger, which is responsible for the slow calcium export D2 through the plasma membrane, is reduced to *ca.* 10% when the external sodium is replaced by lithium. But the activity of the exchanger is not much changed when calcium is replaced by strontium. The decline of the cytosolic strontium concentration depends on the membrane voltage similar to the slow decline D2 of cytosolic calcium.

2. Materials and Methods

The experimental techniques were similar as described by Nagy and Stieve (1983), Deckert and Stieve (1991), Stieve and Benner (1992), and Rüsing (1989) (see there for further details). The ventral nerve of *Limulus polyphemus* was isolated, and stored for 7–30 h in physiological saline. Before the experiment the nerve was treated by collagenase (2 mg/ml) for 10 min, then fastened in a glass chamber which was continuously perfused with saline of constant temperature (15 °C). The perfusion rate was *ca.* 1 ml/min, resulting in a 90% exchange of saline within 3 min. The superfusates used are listed in Table I.

The photoreceptor cell was impaled by a glass micropipette filled with 0.5 mol/l KCl solution containing 20 mmol/l of the indicator dye arsenazo III. The tip resistance was between 4 and 18 MΩ. After pressure injection of arsenazo-containing saline into the cell, the electrical light response (receptor potential, ReP) and the arsenazo signal (AS) were recorded in parallel.

The *arsenazo signal* is a transient transmission decrease of the arsenazo-loaded photoreceptor cell in response to a light stimulus. It indicates a transient increase in cytosolic Ca^{2+} (or Sr^{2+}) concentration. It was monitored by a continuous measuring light (λ_{max} 645 nm, half widths 9 nm, intensity 0.05–0.5 mW/cm^2). The intensity of the measuring light beam after passing the photoreceptor cell was measured by a photomultiplier. The photometric beam was adjusted in diameter to the size of the cell (between 75 and 150 μm) and positioned on the photoreceptor so as to evoke a maximum electrical light response. When switched on it caused a transient initial depolarization of

Table I. Composition of salines used.

mmol/l	Physiolog. saline	Li saline	Sr saline	Sr–Li saline	Sr–low Ca saline
NaCl	481		481		481
LiCl		486		486	
KCl	10	5	10	5	10
$MgCl_2$	25	25	55	55	45
$MgSO_4$	30	30			
$CaCl_2$	10	10			0.25
$SrCl_2$			10	10	10
NaOH	5		5		5
KOH		5		5	
HEPES	10	10	10	10	10

the membrane by 5–20 mV and a steady state depolarization of maximally 1–8 mV. Under the conditions applied by us, the absorption increase ($\Delta I/I_o$) at 645 nm results specifically from the binding of calcium (or if present, strontium) to arsenazo in the photoreceptor cell (Brown *et al.*, 1977; Kendrick *et al.*, 1977; Bauer 1981). The photomultiplier current was converted to a voltage signal and using a sample-and-hold circuit amplified 100× over the base line measured in the beginning of the recording period (Nagy and Stieve, 1983; Ivens and Stieve, 1984). The arsenazo signal was then filtered by a 100 Hz low pass filter (–3 dB point), digitized and stored on tape or disc.

The receptor cell was stimulated every 60 s by a light flash of 5 ms half time (Metz Megablitz 60 ct-1), which had passed through a wide band filter (Schott BG 18, 515 nm maximum). The flash energy of a maximum of 9×10^{14} photons/cm^2 was attenuated by neutral density filters. The glass micropipette was connected to a PCM amplifier by an Ag–Ag/Cl electrode *via* an impedance converter (input resistance 10^{14} Ω). The accuracy of measurement was 1 mV and 1 ms respectively. In one set of experiments the receptor current was measured under voltage clamp conditions with a single electrode voltage clamp (SC-100, VF-180 and CA-100 Biologic, France) in a similar way as described in Deckert and Stieve (1991) and Stieve and Benner (1992).

The arsenazo signal (AS) and the receptor potential were recorded simultaneously on a pulse-code modulation (PCM) system for a period of between 9 and 18 s starting 0.4 s before the test stimulus (sample frequency 1 kHz). The time resolution of the data recording system was 1 ms. All signals were stored on tape and disc and evaluated later with an IBM-compatible AT personal computer. (For further details see Deckert and Stieve, 1991.) From the recorded arsenazo signals and receptor potentials several parameters were determined with a computer program. Before analysis, the arsenazo signals were filtered with a digital 10th order low pass Butterworth filter. This filtering does not result in a significant loss in time resolution of this signal (see Deckert and Stieve, 1991).

A stimulus artefact observed in the unfiltered arsenazo signal was caused by the scattering of the stimulating light at the glass pipette. It masked the origin of the arsenazo signal. For the determination of the latency of the arsenazo signal the 8–12 ms lasting stimulus artefact was replaced by the baseline signal of the same record just prior to the stimulus (see Deckert and Stieve, 1991 and Stieve and Benner, 1991 for details).

The absolute maximum of the arsenazo signal, Hmax AS (Fig. 1 a), was determined by averaging the amplitude values of the filtered signal for 100 ms around the largest value.

The latency Tlat AS is the time from the onset of the light stimulus to the first significant start of the response (see Stieve and Benner, 1992).

The time-to-peak Tmax AS is the time from the onset of the light stimulus to the absolute maximum of the filtered arsenazo response. Each Tmax AS value was verified by visual control of the signal. If the largest value of the response appeared to be caused by a superimposed electrical interference, an appropriate value was chosen by eye. The time to the maximum was determined with an accuracy of ±10 ms.

The rise time Tr is the time-to-peak Tmax minus the latency Tlat.

The decay time T2 AS is the time during which the response decays from its maximum to 50% of this value. The initial slope of rise Mi is the average slope from the end of the latency to half of the signal height, and the median slope of rise Mm is the slope of rise in half height of the signal.

The two phases of the decay of the arsenazo signal were fitted by the function

$$H_{AS}(t') = \alpha \exp(-t'/\tau_1) + \beta \exp(-t'/\tau_2) + C.$$

α and β are scaling factors, τ_1 is the time constant of the fast phase, τ_2 that of the slow phase.

t′ is defined as $t - (t_{max} + \sigma)$, where σ, the start of the fit, was chosen by eye; C is the steady-state level of the signal; it is usually set to zero (see Deckert and Stieve, 1991, for further details).

The *receptor potentials* were processed accordingly, but without filtering; this is described in detail elsewhere (Stieve, Gaube and Klomfaß, 1986).

An experiment lasted up to 7 h. After arsenazo injection into a photoreceptor cell the nerve was superfused for 15–30 min with physiological saline (PS) before the actual measuring phase began. It started with a preperiod of 30 to 60 min in which the ventral nerve was superfused with physiological saline. One or up to 3 test periods followed in which the effects of different superfusates were tested. Finally in an afterperiod of at least 25 min the reversibility of the effects was checked by again superfusing the nerve with physiological saline.

The length of the individual periods was varied between the different sets of experiments in order to reach stable responses. The measuring phase in the lithium experiments lasted 1.5–2 h, in the strontium experiments 3.5–4 h, and in the strontium–lithium experiments 4–4.5 h.

3. Results

Due to the continuous photometric background light the studied photoreceptors were in a steady state of light adaptation which corresponded to a 5- to 40-fold diminution in sensitivity (Stommel, unpublished).

A stimulating light flash of the strong energy applied by us evokes two delayed signals (Fig. 1 and 2): an electrical response, the receptor potential or the receptor current, and a slower transient rise in the cytosolic calcium ion concentration (here measured as arsenazo signal). The electrical light response under physiological conditions has been described elsewhere in greater detail. It can be shown to consist of three components (Nagy, 1991; Deckert *et al.*, 1992; Nagy, 1993). During the preperiod of the experiments described here, while the photoreceptor was superfused with physiological saline, the receptor potential had a latency Tlat of 10 to 25 ms; a rise time Tr of 15 to 30 ms and an overall half-time of decline T2 between 0.6 and 1.7 s (Table II).

Table II. Average parameters of arsenazo signal and receptor potential of 3 experiments in which the external calcium was replaced by strontium. a) Receptor potential; b) arsenazo signal; testperiod; 65–115 min in Sr saline; afterperiod 45–50 min in PS (AGR47, 55, 57). Average ± standard error of the mean at the end of each period; c) (AGR57); kinetic parameters of the decline of the arsenazo signal, α, β scaling factors, τ_1, τ_2 time constants of faster and slower decay component.

a)

	PS	Sr	PS
PMP	−27 ± 2.5 mV	255 ± 38%	139 ± 25%
Hmax	44 ± 1.5 mV	192 ± 28%	119 ± 15%
Tlat	15 ± 0.6 ms	197 ± 21%	115 ± 15%
Tr	17 ± 2.0 ms	573 ± 108%	250 ± 73%
T2	632 ± 86 ms	555 ± 166%	392 ± 191%

b)

	PS	Sr	PS
Hmax	2.8 ± 0.08 $\Delta I/I_o \cdot 10^3$	440 ± 122%	110 ± 21%
Tlat	48 ± 3.0 ms	140 ± 8.8%	105 ± 5.4%
Tr	345 ± 102 ms	519 ± 74%	140 ± 57%
Mi	55 ± 23 $\Delta I/I_o \cdot 10^3 \cdot s^{-1}$	49 ± 6.8%	66 ± 22%
Mm	20 ± 2.5 $\Delta I/I_o \cdot 10^3 \cdot s^{-1}$	67 ± 19.6%	78 ± 5.4%
T2	1870 ± 451 ms	474 ± 89%	183 ± 28%

c)

	α $\Delta I/I_o \cdot 10^3$	τ_1 s	β $\Delta I/I_o \cdot 10^3$	τ_2 s
Preperiod PS	2.2	0.84	1.3	7.3
20 min Sr	6.8	0.78	5.4	4.6
36 min Sr	0	–	13	6.0
52 min Sr	0	–	18	7.6
86 min Sr	0	–	21	5.8
107 min Sr	0	–	17	5.4
Afterperiod 45 min PS	2.3	1.3	1.1	10.0

The arsenazo signal starts 20 to 30 ms later than the receptor potential (Tlat 30 to 50 ms), rises for 150 to 550 ms. The decline has an overall half-time of 1.5 to 4.5 s. After the maximum of the arsenazo signal the decline begins with a phase in which the slope of decline increases for 0.5 to 1 s. Then, after the point of inflection, the signal falls monotonously approaching zero between 3 and 6 s after the maximum. The decline after the point of inflection can be described as the sum of two exponentials (Fig. 4a, c; see also Deckert and Stieve,

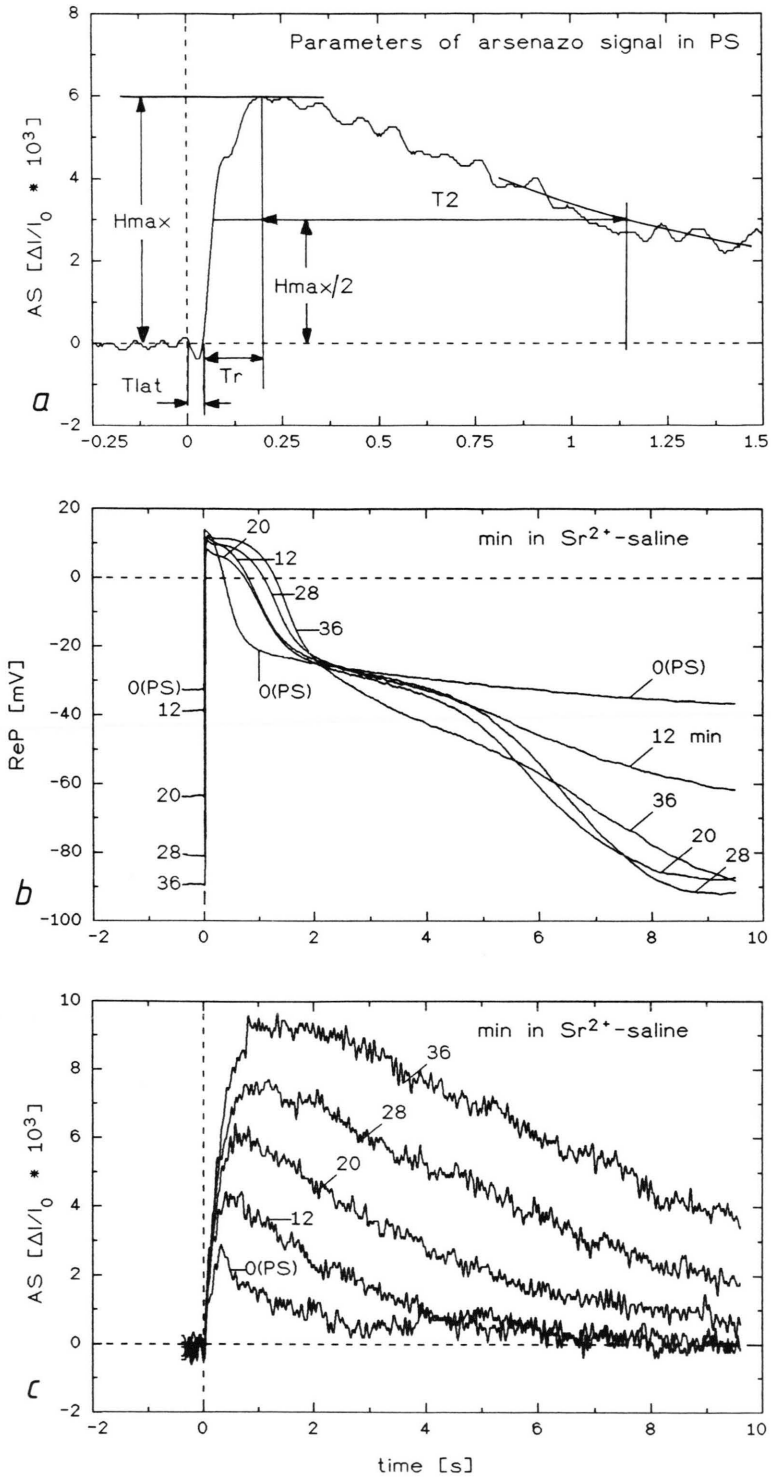

Fig. 1. Light responses of *Limulus* photoreceptor after replacing calcium by strontium ions. a. Arsenazo signal in physiological saline to demonstrate evaluated parameters. b. Receptor potentials; c. arsenazo signals, each signal the average of 4 responses. 0: Last signal in physiological saline; 12, 20, 28, 36 min after exchange of superfusate; experiment number AGR57.

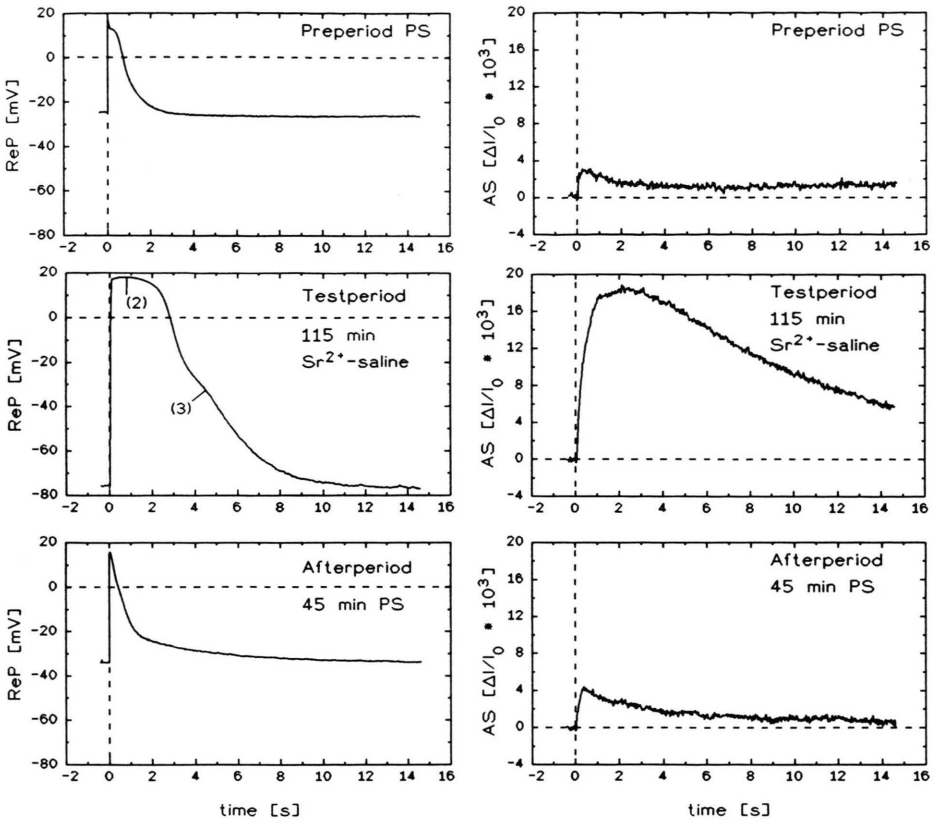

Fig. 2. Receptor potentials (left) and arsenazo signals (right) before and after changing the bath from physiological saline to strontium saline. Preperiod, physiological saline; testperiod, after 115 min in strontium saline; afterperiod, after 45 min in physiological saline. In strontium saline additional components (2) and (3) of the receptor potential become obvious. AGR55.

1991). The two phases of decline have time constants τ_1 of 0.5 to 1.5 s (fast phase D1) and τ_2 of 4 to 10 s (slow phase D2) (Tables II, III, IV).

3.1 Replacement of the external calcium by strontium

In this group of 5 experiments during the test period all the added calcium in the superfusate was substituted by strontium (Table I). Fig. 1, 2, and 4 show records from two representative experiments, Fig. 3 shows the changes in the parameters of arsenazo signal and receptor potential in the course of an experiment; Table II shows the average results from three comparable experiments of that group.

When the external calcium is replaced by strontium the arsenazo signal and receptor potential change slowly and gradually, they become more and more enlarged and prolonged. The testperiod lasted 65 to 115 min. Within 30 to 60 min many parameters of the receptor potential became stable (except T2 which could change for 90 to 100 min) whereas the arsenazo signal often did not stabilize during the testperiod. Switching back to superfusion with physiological saline results in reversion of the changes of both signals (Fig. 2, 3, and 4, Table II).

3.1.1 Receptor potential

When the superfusion is switched from physiological saline to a saline in which calcium is replaced by strontium, the changes of the electrical light response develop gradually. The various parameters of the receptor potential change accord-

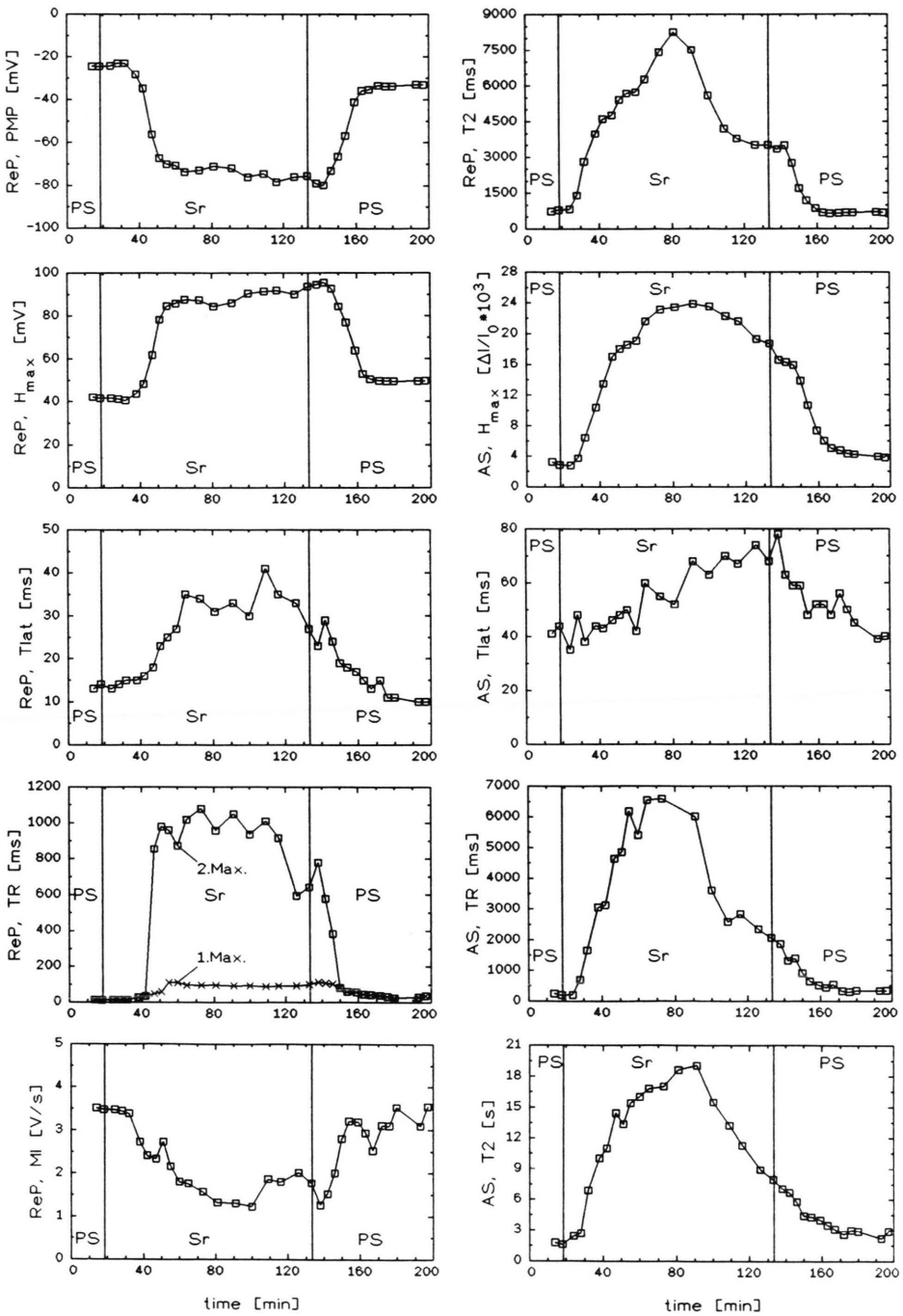

Fig. 3. Parameters of receptor potentials and arsenazo signals are shown in the course of an experiment with calcium- (PS) or strontium-containing (Sr) superfusate. ReP: PMP, prestimulus membrane potential; Hmax, absolute maximum; Tlat, latency; Tr, rise time; Mi, initial slope of rise; T2, half-time of decay. AS: Hmax, Tlat, Tr, T2. AGR55.

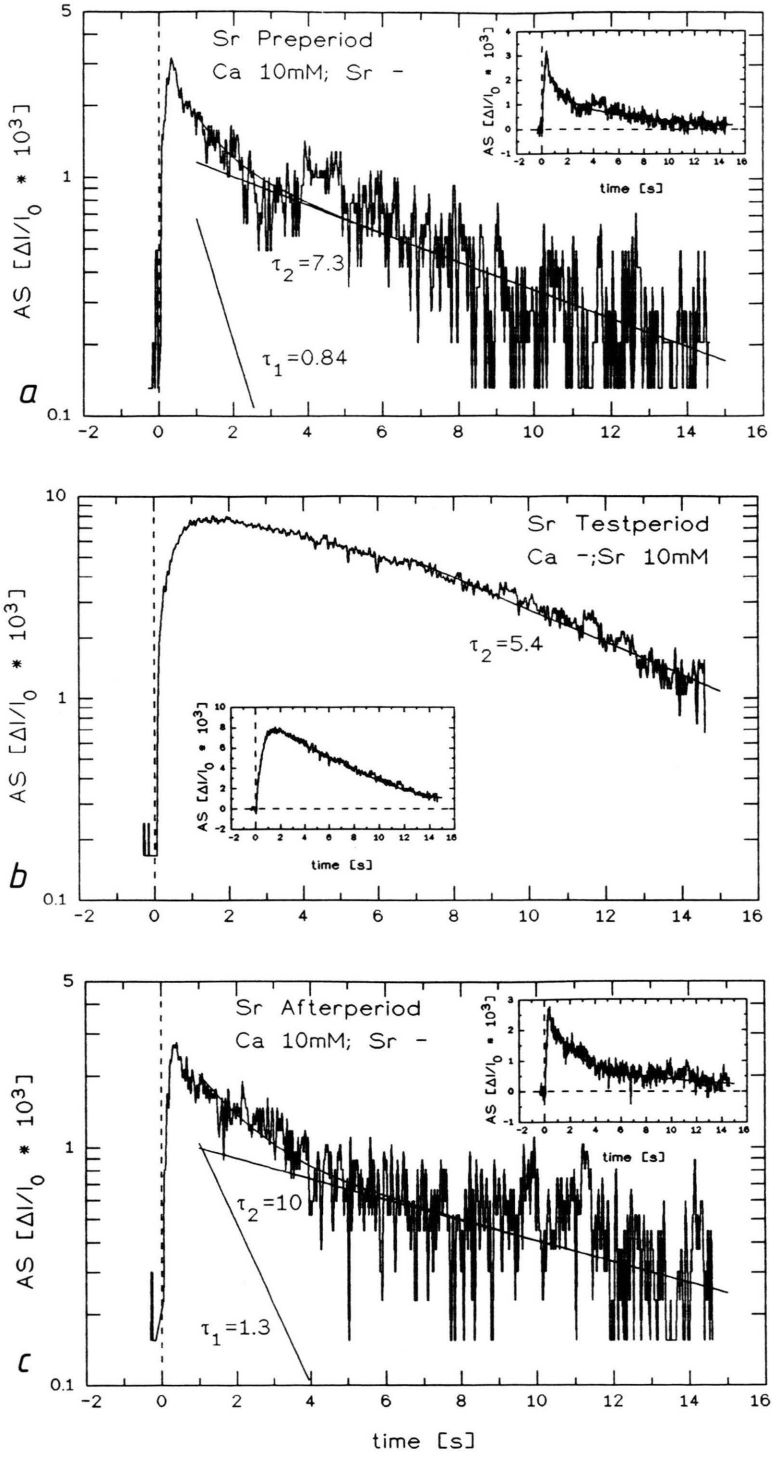

Fig. 4. Arsenazo signals and their fits in semilog plots. a. Preperiod, averaged last four responses in physiological saline; b. testperiod, averaged four responses after 107 min in strontium saline; c. afterperiod, after 45 min again in physiological saline. Insets: the same data in linear plots; AGR 57.

ing to different patterns in time. After 10 to 15 min the prestimulus membrane potential PMP becomes more negative stepwise with each light flash (Fig. 1), due to a strong after-hyperpolarization of the response. This effect almost saturates after 20–30 min with an average hyperpolarization of about 35 mV, but there is a slow further increase. Correspondingly, the amplitude Hmax of the receptor potential becomes larger by the same amount starting 20 min after the switch (Table II a, Fig. 3).

The latency Tlat grows on the average almost 2-fold (by 10–20 ms) starting to change about 20 min after the switch to strontium saline. The receptor potential shows conspicuous changes in shape (Fig. 2, Table II a). Whereas the slope of its initial rise is reduced to 50%, two additional components 2 and 3; become increasingly prominent they may correspond to the C2 and C3 components (Nagy, 1991; Deckert *et al.,* 1992; Nagy, 1993). In 4 of the 5 experiments the second maximum is highest. The rise time Tr to the first maximum is 5.7-fold prolonged. T2, the half-time of decay (which is now measured from the second maximum) of the receptor potential, starts to grow immediately after the switch to strontium superfusion. It reaches a maximum after about 60 min in strontium saline and is then shortened to a steady value 5.5-fold longer than the reference value. All the observed strontium-induced changes are distinctly, but not completely reversed in the 60 min afterperiod in physiological saline (Table II a). On the average rise time and decrease time remain larger by 2.5 and 4 times, respectively, than in the preperiod.

3.1.2 Arsenazo signal

Also the changes of the arsenazo signal develop slowly with time upon superfusion with strontium saline, but they start to change without a significant delay after the switch; they precede those of the receptor potential (except for Tlat and T2 of the receptor potential) by about 20 min (Fig. 1 and 3). Only the changes in the latency of the arsenazo signal may start more than 10 min later than the other changes of the arsenazo signal. Within 30 to 60 min, the arsenazo signal increases in amplitude and duration. After more than 60 min in strontium saline, the amplitude, rise, and decline time of the arsenazo signal are again diminished whereas the latency keeps still growing (Fig. 3). At the end of the testperiod, the average latency of the arsenazo response is longer by about the same time (20–30 ms) as that of the receptor potential. The initial slope of rise Mi of the arsenazo signal is significantly, the median rise Mm not statistically significantly reduced. After prolonged stay in strontium saline, the rising phase Tr of the arsenazo signals is on the average more than 5-fold prolonged and the amplitude Hmax more than 4-fold enlarged (Table II b).

The decline of the arsenazo signal is also much slower than in physiological saline. T2, the overall half-time of decay, is increased 4.7-fold (Table II b). The declining phase appears monophasic and almost linear in time. D1, the fast phase of decline, is no longer detectable (Fig. 4). It seems to have disappeared within the first 10–20 min after the change in the superfusate (Table II c). The time constant τ of the decline keeps growing during the first 60 min in strontium saline and decreases later in parallel to the amplitude Hmax of the arsenazo signal. The decline of the arsenazo signal after 30 to 60 min in Sr saline can be well fitted by a single exponential of about the same time constant τ (6–7.6 s) as D2 in the preperiod under physiological calcium conditions (τ_2: 7.3 s, Table II c).

These changes of the arsenazo signal are well reversed in the afterperiod in physiological saline, although the parameters do not assume the same values as in the preperiod; some of them are larger, some of them are smaller (Table II a–c). At the end of the afterperiods of all experiments the two phases of decline of the arsenazo signal were again clearly recognizable, but T2 has not completely recovered in the 30 min afterperiod (Table II b).

3.1.3 The voltage dependence of the arsenazo signal in strontium saline

In another type of experiments we tested in 4 cells whether the arsenazo signal depends upon the membrane voltage when the external calcium was replaced by strontium for at least 45 min. The membrane voltage to which the photoreceptor membrane was clamped, was varied between +30 and −60 or −80 mV (Fig. 5).

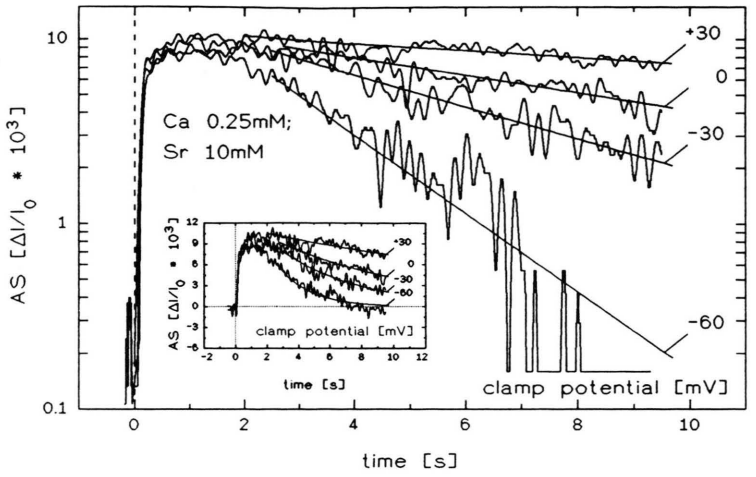

Fig. 5. Arsenazo responses (5 Hz filtered) and their fits of a photoreceptor for 60–70 min in strontium saline under voltage clamp conditions at different clamp voltages. Semilog plot. Inset: the same data in linear plot; AW231190.

In strontium saline, not only the decline but also the second phase of rise of the arsenazo signal revealed a voltage dependence.

When the photoreceptors are superfused with strontium saline for more than 60 min, the initial rise Mi and the median rise Mm of the arsenazo signal are not significantly influenced by the membrane voltage. A later phase of rise, however, becomes steeper with more negative membrane voltage (Fig. 5). In contrast to the behaviour in calcium-containing saline (Deckert and Stieve, 1991), the rise time of the arsenazo signal is enlarged when the membrane is clamped to more positive voltages. Such voltage dependence is expected if strontium uptake through ion channels in the plasma membrane, *e.g.* the light-activated cation channel, contributes significantly to the arsenazo signal.

The apparently monophasic decline of the arsenazo signal under strontium conditions, which can be fitted by a single exponential, depends strongly upon the membrane voltage. It is the steeper the more negative the membrane voltage but does not yet reverse its sign at membrane voltages up to +30 mV (Fig. 5). The monophasic decline depends on the membrane voltage in a qualitatively similar way as the slow D2 phase of the arsenazo response in physiological, calcium-containing saline (Deckert and Stieve, 1991).

3.2 Replacement of the external sodium by lithium

In 5 experiments the sodium in the superfusate was replaced by lithium (Table I) for 16–20 min. The results are summarized in Table III; representative experiments are demonstrated in Fig. 6, 7, and 8.

Lithium cannot substitute sodium in the sodium/calcium exchanger (see above). Complete replacement of the external sodium by lithium causes a diminution of the arsenazo signal and a retardation of its declining phase, and a diminution of the receptor potential but an acceleration of its decline (Table III).

3.2.1 Receptor potential

After 10 to 15 min superfusion with sodium-free lithium saline the prestimulus membrane potential PMP is depolarized by 5–10 mV (Fig. 6a, 7, Table III a). The amplitude Hmax of the receptor potential is decreased by the same amount (on the average to 84%). The latency is not changed, the rising phase (rise time Tr) of the receptor potential is slightly, but not statistically significantly reduced, whereas the decline is substantially accelerated. The half time of decay T2 is shortened, on the average to 70%. Two components of the receptor potential become more pronounced in the testperiod in some of the experiments (not shown).

The depolarization, the diminution, and the acceleration of the decline of the receptor potential in the testperiod resemble the changes due to light adaptation or raised intracellular calcium ion concentration (Stieve, 1981; Brown, 1986). However, the latency is not shortened. All these effects are well reversed in the afterperiod (Table III a).

Table III. Average parameter of arsenazo signal and receptor potential of the 5 experiments in which the external sodium was replaced by lithium. a) Receptor potential; b) arsenazo signal. Testperiod, after 16–20 min stay in lithium saline; afterperiod, 25–35 min in PS (AGR35, 36, 37, 38, 58). Average ± standard error of the mean. c) (AGR58); kinetic parameters of the decline of the arsenazo signal; see Table II for further details.

a)

	PS	Li	PS
PMP	−33 ± 2.8 mV	72 ± 6.5%	121 ± 17%
Hmax	49 ± 2.8 mV	84 ± 4.2%	113 ± 9.1%
Tlat	14 ± 1.1 ms	99 ± 13.6%	120 ± 8.5%
Tr	39 ± 16 ms	85 ± 15%	97 ± 16%
T2	1707 ± 393 ms	71 ± 12%	140 ± 9.7%

b)

	PS	Li	PS
Hmax	3.8 ± 0.25 $\Delta I/I_o \cdot 10^3$	59 ± 7.0%	66 ± 5.2%
Tlat	42 ± 5.7 ms	121 ± 7.8%	142 ± 13%
Tr	508 ± 235 ms	95 ± 20%	154 ± 60%
Mi	47 ± 7.8 $\Delta I/I_o \cdot 10^3 \cdot s^{-1}$	102 ± 25%	74 ± 11%
Mm	25.5 ± 3.5 $\Delta I/I_o \cdot 10^3 \cdot s^{-1}$	63.2 ± 14.8%	65.3 ± 10.7%
T2	4451 ± 859 ms	318 ± 119%	159 ± 22%

c)

	α $\Delta I/I_o \cdot 10^3$	τ_1 s	β $\Delta I/I_o \cdot 10^3$	τ_2 s
Preperiod PS	1.6	1.1	2.4	6.3
3 min Li	1.4	1.0	3.0	5.2
7 min Li	2.9	1.8	1.2	33
11 min Li	0.9	2.0	2.4	36
15 min Li	0	–	2.3	28
Afterperiod 30 min PS	1.8	1.4	1.5	29

3.2.2 Arsenazo signal

The arsenazo response becomes reduced to 60% during the (*ca.* 18 min) testperiod; the rise of the arsenazo signal is not much changed, whereas the decline in 3-fold retarded (Table III b).

In detail: the latency of the arsenazo signal is prolonged to 120%. Rise time Tr and initial slope of rise Mi are unchanged, the median slope of rise Mm is reduced to 63%.

The main change, however, is a reversible retardation of the decline of the arsenazo signal. The overall half-time of decay T2, increases about 3-fold (320%). The retardation of the calcium redecline occurs both by suppression of the fast phase D1 and by a 5-fold retardation of the slow phase D2.

Fig. 6 b and Table III c demonstrate the development of the effects. During the preperiod in physiological saline one can clearly distinguish the two phases of decline of the arsenazo signal (Fig. 6, 7 and 8). In lithium saline the decline becomes monophasic with a more or less constant slope about 1 s after the maximum of the arsenazo signal. The fast phase D1 is slowed down (τ_1 is 2-fold prolonged); its weight α, however, is initially increased (about 5 min after the switch) and later gradually reduced to zero. After more than 11 min D1 has vanished. The slow phase D2 becomes gradually slower (τ_2 from 6 to 33 s). The changes of D2 start earlier than that of D1 and are practically completed within 7 min after the switch. At the end of the *ca.* 18 min test period the fast decline is no longer recognizable, whereas the time constant τ of the monophasic decline is slowed down by a factor of about 5 (Table III c, Fig. 6, 8).

The effects of the lithium saline on the arsenazo signal are less reversed in the afterperiod in physiological saline than those on the receptor potential. The amplitude does not recover significantly, but the changes in decline of the arsenazo signal are partially reversed (T2 AS recovers from 320 to 160%, Table III b) within the *ca.* 30 min afterperiod. The fast phase D1 of decline recovers much better than the slow phase D2 (Table III c, Fig. 7 and 8).

3.3 Combined replacement of calcium by strontium and of sodium by lithium

A combination of replacement of calcium by strontium and sodium by lithium in the superfusate with 3 testperiods was used in 5 experiments. In the first testperiod, which lasted 60 to 85 min, calcium was replaced by strontium; this time was not in all cases long enough for the strontium effect to develop to a steady level. In the second testperiod of 12 to 20 min, in addition the external sodium was replaced by lithium. In the third testperiod of *ca.* 30 to 40 min, the superfusate contained again normal sodium concentration, but

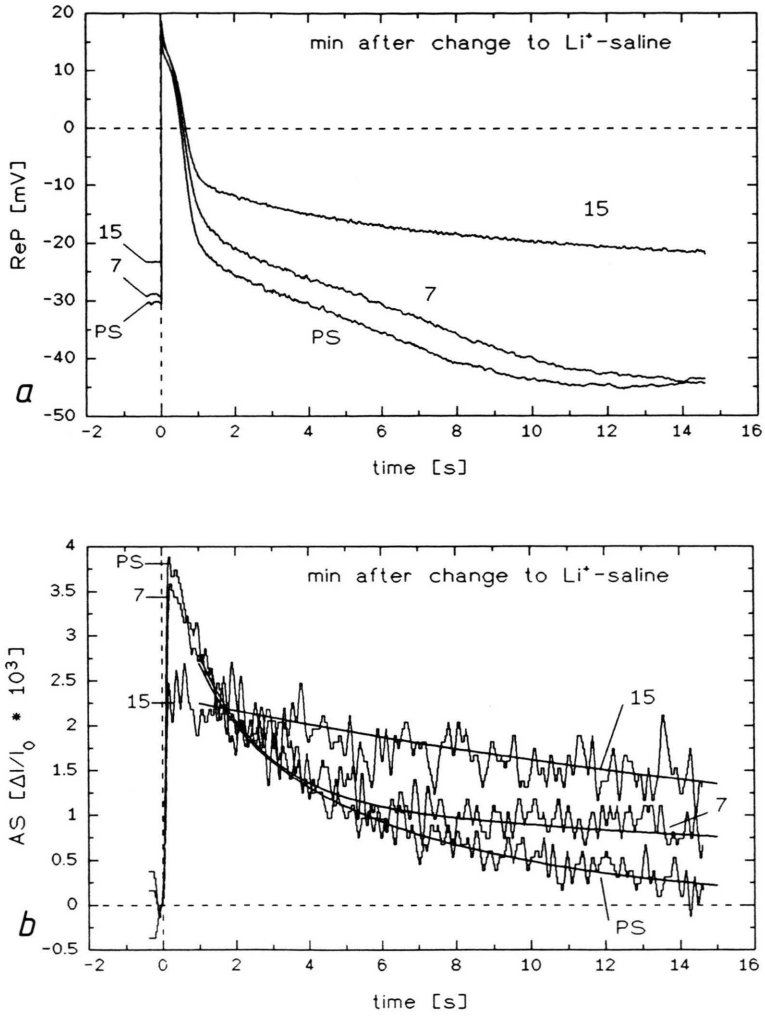

Fig. 6. Development of the change of a. receptor potential and b. arsenazo response following the change from physiological saline to a saline in which all the sodium was replaced by lithium in the superfusate. PS: response in physiological saline, and after 7 and 15 min stay in lithium saline; AGR 58.

strontium instead of calcium. Finally, in the afterperiod (*ca.* 35 min), the superfusate was physiological saline. The results are summarized in Table IV, a representative experiment is documented in Fig. 9 and 10.

When both the external calcium and sodium are replaced by strontium and lithium, the two effects described above seem to add almost independently. The combined effects of strontium and lithium make the declines of the receptor potential and of the arsenazo signal very slow; the half time of the overall decline of the arsenazo signal becomes 12-fold longer and practically monophasic and linear.

Our main interest was to see the changes induced when, in addition to the calcium replacement by strontium, all the external sodium was substituted by lithium. Due to the relative short first testperiod, the effects of the replacement of calcium by strontium are still growing during the second testperiod. The lithium effect can be judged most critically by comparing the data of testperiod 2 with those of test period 3 if we regard only those changes which were reversed in the third testperiod trustworthy as lithium effects. One has to consider, however, that not all the lithium effects are reversible after washing out the lithium (see 3.2, Table III).

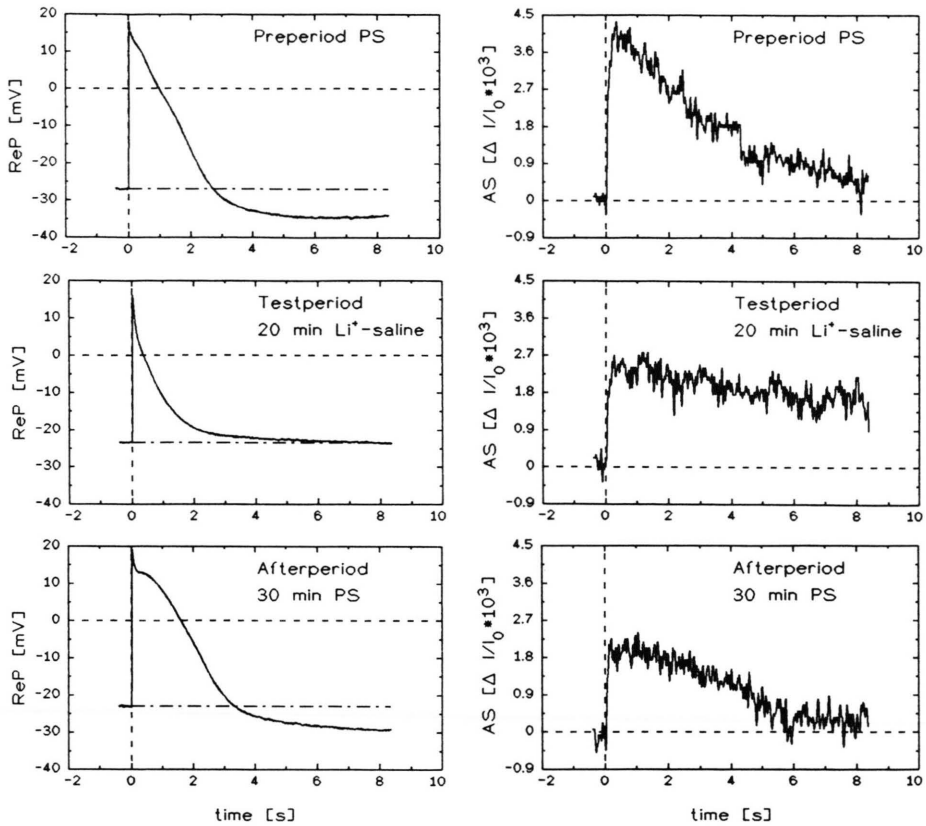

Fig. 7. Receptor potentials (left) and arsenazo signals (right) when the sodium in the superfusate was replaced by lithium. Preperiod, last four responses in physiological saline; testperiod, responses after 20 min in lithium saline; afterperiod, 30 min again in physiological saline; AGR 37.

By this criterion lithium causes similar changes of the arsenazo signal as when the external solution contains calcium, *i.e.* drastic retardation of the slow declining phase of the arsenazo signal (since D 1, the fast phase of decline, has already disappeared due to the replacement of calcium by strontium). The receptor potential, however, is diminished, but not shortened by the action of lithium in contrast to the lithium effect in normal calcium-containing superfusate (see 3.2).

The effects are only partially reversible during the 30 to 60 min afterperiod. When the cell is again superfused with physiological saline both responses recover, but not completely. (In the experiment from Fig. 10 the recovery is less than in the average of all experiments (Table IV).)

3.3.1 Receptor potential

The changes of the receptor potential when calcium in the superfusate is replaced by strontium are already described above: The prestimulus membrane potential PMP is hyperpolarized, the amplitude Hmax is increased, latency, rise time, and decline are prolonged.

Compared to test periods 1 and 3, additional replacement of sodium by lithium causes a reduction in the hyperpolarization of the prestimulus membrane potential PMP and an about equal reduction of the amplitude of the receptor potential – probably due to an inhibition of the Na–K ATPase. There is no significant change in latency and in rise time (which had been greatly prolonged due to the action of strontium). However, the half-time of decline T 2 of the receptor potential is additionally 2-fold prolonged. In strontium saline the replacement of sodium by lithium causes a prolongation of T 2 (Table IV a), whereas applied in a saline of physiological calcium concentration, it has the opposite effect, namely shortening of T 2 (Table II a).

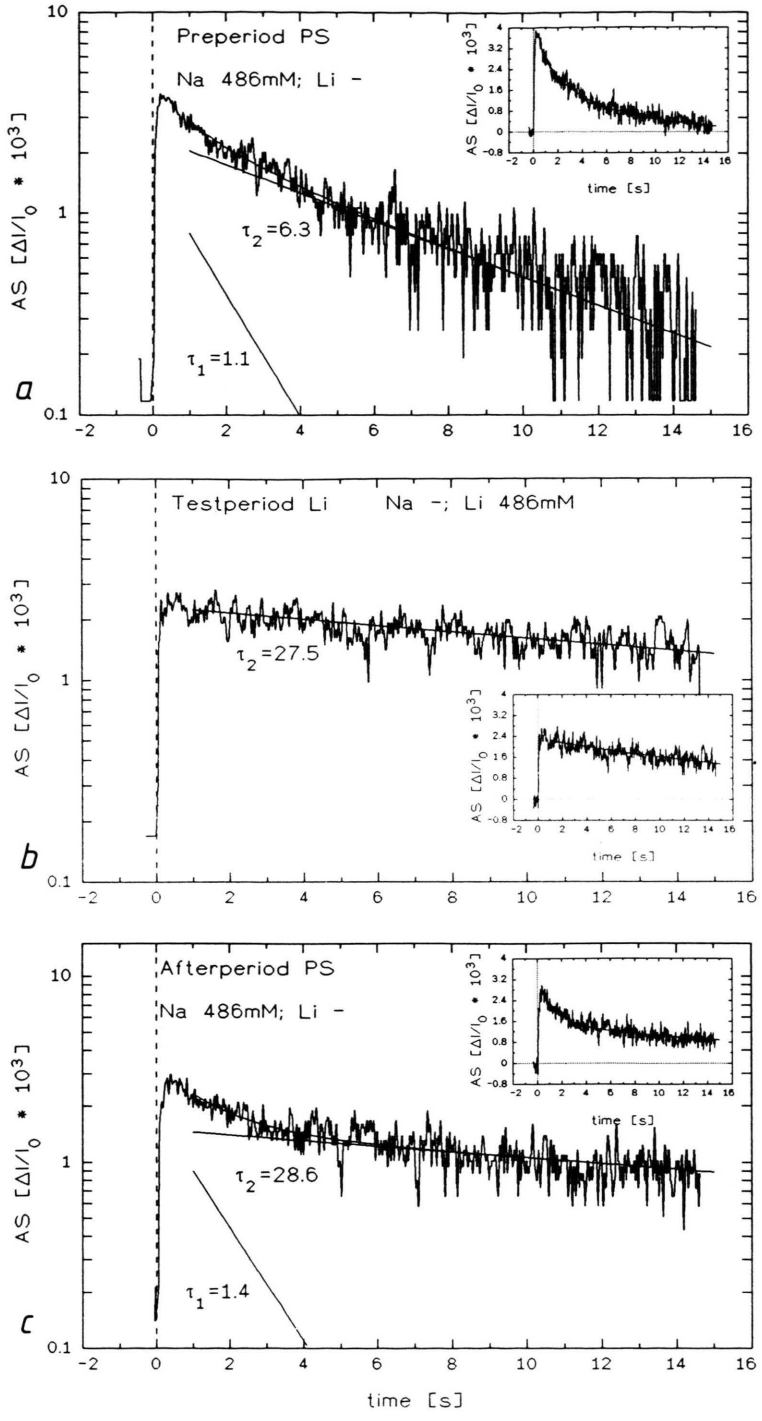

Fig. 8. Arsenazo responses and their fits when the sodium in the superfusate was replaced by lithium in semilog plot. a. Preperiod, physiological saline; b. testperiod, after 20 min in lithium saline; c. afterperiod, after 30 min again in physiological saline. Insets: the same data in linear plots; AGR 58.

Table IV. Average parameters of arsenazo signal and receptor potential of the experiments in which the external calcium was replaced by strontium in combination with a replacement of the external sodium by lithium. a) Receptor potential; b) arsenazo signal. Sr I, 75–90 min; Sr, Li, 15–20 min; Sr II, 20–30 min; PS, 25–65 min; (AGR 46, 49, 50, 53, 60). Average ± standard error of the mean; c) (AGR 53); kinetic parameters of the decline of the arsenazo signal.

a)

	PS	Sr I	Sr, Li	Sr II	PS
PMP	−33 ± 4.5 mV	202 ± 42%	150 ± 38%	152 ± 23%	165 ± 33%
Hmax	44 ± 5 mV	170 ± 27%	146 ± 29%	125 ± 10%	134 ± 14%
Tlat	15 ± 2.2 ms	250 ± 62%	269 ± 66%	272 ± 84%	218 ± 54%
Tr	49 ± 22 ms	4527 ± 2177%	4354 ± 2209%	3200 ± 1474%	783 ± 350%
T2	1146 ± 394 ms	615 ± 253%	1246 ± 616%	873 ± 440%	217 ± 62%

b)

	PS	Sr I	Sr, Li	Sr II	PS
Hmax	4.1 ± 0.54 $\Delta I/I_o \cdot 10^3$	373 ± 41%	383 ± 37%	297 ± 30%	99 ± 25%
Tlat	39 ± 3.3 ms	207 ± 44%	206 ± 45%	371 ± 95%	228 ± 66%
Tr	437 ± 178 ms	604 ± 134%	969 ± 199%	1078 ± 481%	222 ± 80%
Mi	81 ± 4.5 $\Delta I/I_o \cdot 10^3 \cdot s^{-1}$	65 ± 6.7%	52 ± 5.4%	34 ± 7.7%	51 ± 18%
Mm	36 ± 4.5 $\Delta I/I_o \cdot 10^3 \cdot s^{-1}$	120 ± 18%	94 ± 12%	63 ± 16%	57 ± 16%
T2	1755 ± 419 ms	639 ± 188%	1176 ± 362%	756 ± 182%	354 ± 110%

c)

	α $\Delta I/I_o \cdot 10^3$	τ_1 s	β $\Delta I/I_o \cdot 10^3$	τ_2 s
Preperiod PS	5.8	0.54	3.1	3.8
75 min Sr I	0	–	23	3.3
20 min Sr, Li	0	–	30	7.4
30 min Sr II	0	–	31	4.1
Afterperiod				
25 min PS	3.8	1.7	4.9	10

Two further components (see Deckert *et al.*, 1992) of the receptor potential develop more and more prominent in the strontium and in the strontium–lithium saline (Fig. 9).

3.3.2 Arsenazo signal

The changes in the arsenazo signal induced by superfusing of the photoreceptor with strontium saline are already described above. In the second testperiod additional replacement of sodium by lithium causes weaker changes of the arsenazo signal in the same direction as when the external solution contains calcium. The amplitude Hmax of the arsenazo signal, the latency, the rise time, initial slope Mi, and the middle slope of rise Mm are all – according to the reversibility criterion defined above – not significantly changed.

The most significant change due to the sodium replacement by lithium is the additional retardation of the decline of the arsenazo signal (T2 is 2-fold prolonged). Both in the strontium and in the lithium–strontium saline the fast D1 phase of decline of the arsenazo signal is not recognizable. The time constant τ of the slow, monophasic decline is reversibly reduced to 7.4 s by the action of lithium (Table IV c). Lithium has caused a 2-fold retardation of D2, assuming that the contribution of the fast D1 phase of decline has already become negligible due to the strontium effect.

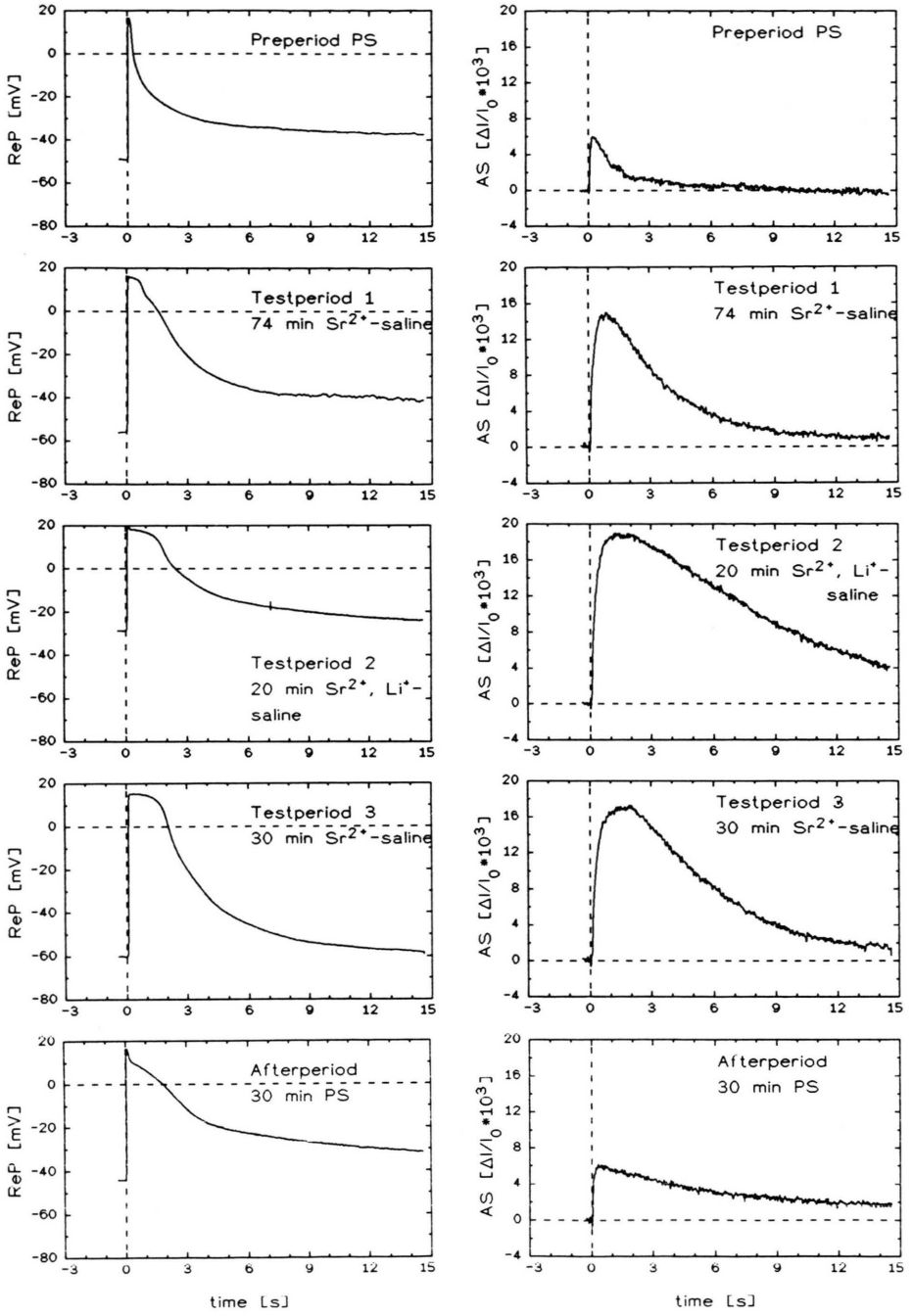

Fig. 9. Receptor potentials (left) and arsenazo signals (right) from an experiment in which the external calcium was replaced by strontium and the external sodium by lithium. Preperiod, in physiological saline; first testperiod, after 74 min in strontium saline; second testperiod, after 20 min in strontium/lithium saline; third testperiod, after 30 min in strontium saline; afterperiod, after 30 min in physiological saline; AGR53.

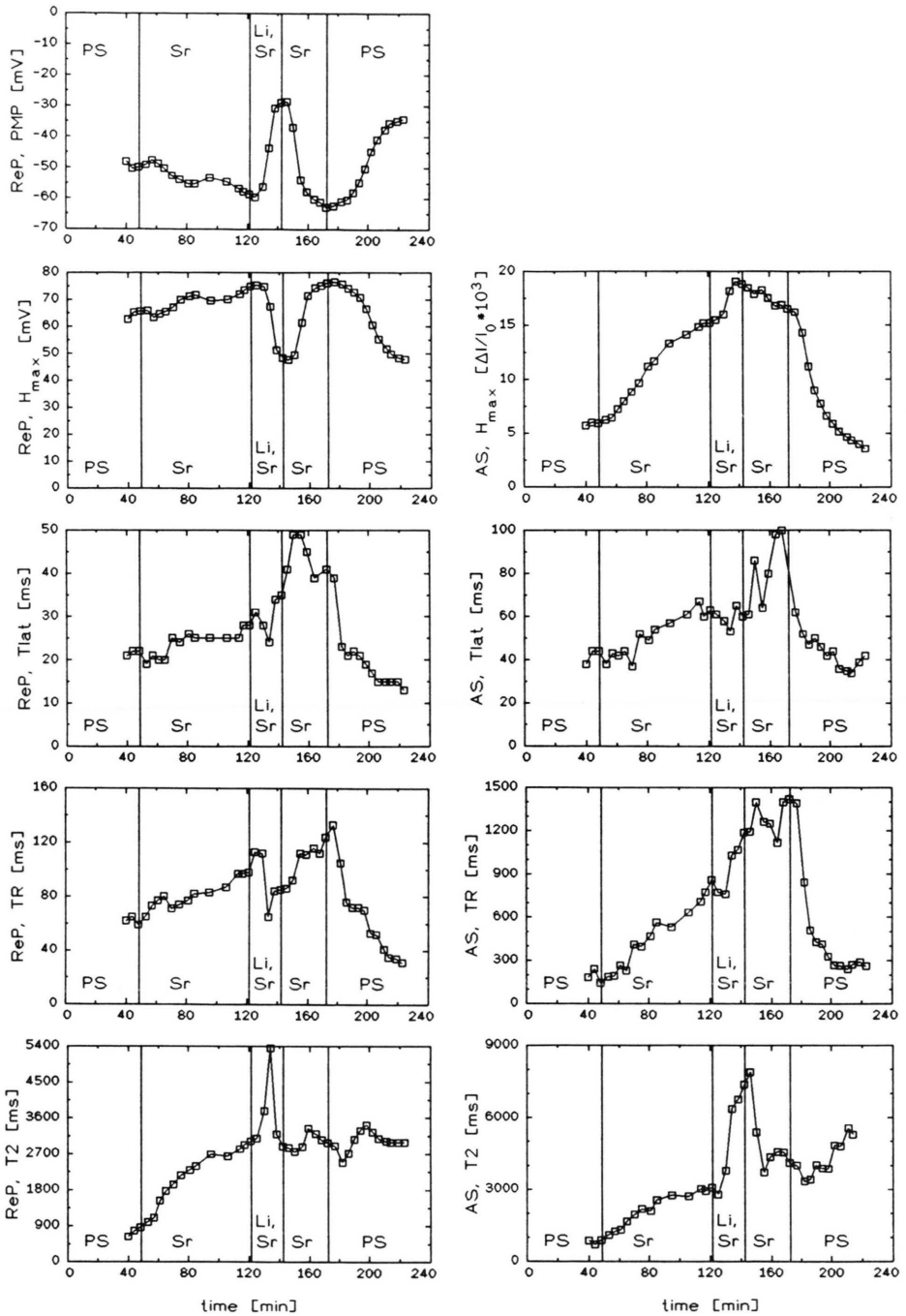

Fig. 10. Parameters of receptor potentials and arsenazo signals in the course of an experiment in which the combination of replacement of external calcium and lithium was tested. AGR53; see Fig. 3 for further details.

The lithium-induced change of the decline of the arsenazo signal is not completely reversed in the third testperiod, when lithium is replaced by sodium, and in the afterperiod in physiological saline (Table IV, Fig. 9 and 10).

4. Discussion

Our discussion is based on the following assumptions (Fig. 11):

1. The light-induced rise in cytosolic calcium is due to two processes: a) Under physiological conditions the rise in calcium is due to a great deal to the calcium release from the intracellular cisternae. It is mediated by the metarhodopsin-activated PLC-IP_3 cascade. b) In addition, there is a light-induced inward current of calcium from the extracellular space (Stommel *et al.*, 1994).

2. The re-decline in the cytosolic calcium concentration depends at least on two processes: a) a fast calcium-reducing process, the re-uptake of calcium into the subrhabdomeric cisternae; b) a slower process due to the activity of a Na–Ca exchanger in the plasma membrane, which transports calcium out of the cell.

3. The calcium export should lead to an intracellular calcium deficit. This is compensated by a subsequent slow refilling of the intracellular cisternae by extracellular calcium.

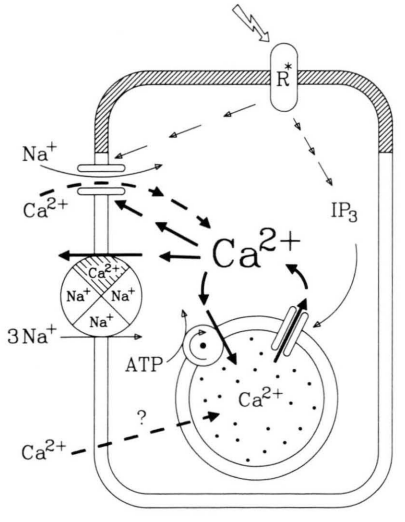

Fig. 11. Scheme of the pathways of calcium in the photoreceptor. R* light-activated rhodopsin causes the opening of the "light-activated" cation channels in the plasma membrane *via* different cascades. Calcium is pumped back into the cisternae by a Ca ATPase and exported out of the cell by a Na–Ca exchanger in the plasma membrane. The cisternae are refilled from extracellular sources – possibly *via* reverse transport of the Na–Ca exchanger into the cytosol and Ca ATPase-mediated active transport into the cisternae.

4.1 Strontium

Strontium as a substitute for calcium can replace many but not all properties of calcium. Omission of the external calcium alone has different effects on receptor potential and arsenazo signal than its replacement by strontium: if calcium is only omitted in the superfusate and not replaced by strontium, the arsenazo signal disappears and the receptor potential becomes as in dark adaptation, larger and slower (Maaz and Stieve, 1980; Ivens and Stieve, 1984; Brown, 1986, for further references).

1. The arsenazo signals in strontium saline are larger and longer than under physiological calcium conditions. This, and the prolongation of the receptor potentials are probably consequences of a weaker desensitizing action of strontium as compared to calcium; the negative feedback is weaker.

2. The fast phase D1 of decline of the arsenazo signal fades during prolonged stay in strontium saline, presumably because no, or only very little, strontium is taken up by the intracellular calcium stores. Under these conditions only one strontium-decreasing process is recognizable. This seems to be D2, which is due to the strontium export out of the cell by the sodium–calcium exchanger. The time constant of D2 is not much different in strontium than in calcium saline. This indicates that the exchanger exports strontium or calcium with about the same efficiency out of the cell. A Na–Ca exchanger can also run a Ca–Ca exchange or a Sr–Ca exchange (Blaustein, 1984). Thus it can contribute to replace cytosolic calcium by strontium even without illumination.

3. The effects of strontium develop slowly: Size, rise, and decline of the arsenazo signal keep growing during the first 60 min stay in strontium saline; hereafter they become shorter again. However, D1, the fast phase of decline disappears within 10 min, again indicating that there is no or only very little uptake of strontium by the endoplasmic cisternae. The intracellular calcium is washed out

gradually. In the first 5 to 10 min in strontium saline there is still enough calcium in the cytosol to be pumped back into the cisternae, and for about 60 min there may be still some calcium left in the cisternae, which is released induced by light.

4. The latency increase of both receptor potential and arsenazo signal in strontium saline is probably due to a different action of strontium than that which causes the changes in size and time course of the arsenazo signal (and of T2 of the receptor potential). The change of the latencies starts later and grows longer than other strontium-induced changes of the arsenazo signal. It seems that the diminution of cytosolic calcium is responsible for the prolongation of the latency but it is still not known which process in the transduction cascade is so calcium sensitive (Stieve and Benner, 1992).

5. The rise of the arsenazo signal is prolonged in strontium saline and has a lower steepness. Two processes may contribute to this effect: a) The slope of rise of the arsenazo signal is reduced in strontium saline since strontium is not or not much taken up by the cisternae. Therefore the cisternae become gradually emptied which causes a reduction of the IP_3-induced calcium release. b) The rise of the arsenazo signal is prolonged because strontium has a weaker desensitizing action than calcium. Therefore the negative feedback, which reduces the IP_3-induced Ca release, is weaker.

6. The strong after-hyperpolarization in strontium saline causes the enlargement of the receptor potential. The after-hyperpolarization is due to the activity of the Na–K ATPase in the plasma membrane (Brown and Lisman, 1972). The hyperpolarization in the calcium-deprived, strontium-containing medium is probably caused by a desinhibition of the Na–K ATPase. Calcium ions competitively inhibit the Na–K ATPase of kidney and red blood cells (Robinson, 1985; Fujise and Lauf, 1988). Strontium seems not to inhibit the ATPase as much as calcium.

4.2 Lithium

Lithium has many poisonous actions in the cell.

1. The initial slope of rise of the cytosolic calcium or strontium concentration is not changed by the action of lithium, but the amplitude of the calcium transient is reduced under the action of lithium. The initial rise in cytosolic calcium, mainly due to an IP_3-induced release from intracellular stores and to calcium influx, is not lithium-dependent when lithium is applied for a period of only 16–20 min.

2. The main action of lithium is to slow down the decline of the arsenazo signal. a) The fast phase D1 of decline of the arsenazo signal vanishes within 15 min in lithium saline. This seems to be an intracellular action of lithium on the calcium ATPase of the endoplasmic cisternae. b) D2, the exchanger-mediated slow decline of the cytosolic calcium, is slowed down 5-fold. Lithium is not accepted as a sodium substitute by the Na–Ca exchanger, but may stimulate the Ca–Ca exchange (Blaustein, 1984). When the extracellular sodium is replaced by lithium, the exchanger should not be able to reduce the cytosolic calcium concentration. Lithium affects D2 faster than D1, within 7 min. This is expected since lithium can act on the Na–Ca exchanger from the extracellular side. But D2, the slow phase of decline of the arsenazo signal, is not abolished, only reduced. Either the Na–Ca exchanger or another slow calcium-reducing mechanism is still active to some extent. The lithium effect on the exchanger is only poorly reversible.

Due to the inhibition of the calcium-decreasing processes the level of cytosolic calcium should rise. This could cause – as in light adaptation – the observed acceleration and diminution of the receptor potential in lithium saline and the reduction of the amplitude of the arsenazo signal.

3. Lithium is also not accepted as a sodium substitute by the Na–K ATPase (Skou, 1975; Glynn and Karlish, 1975). Since the Na–K ATPase needs the sodium on its intracellular site, its activity should only be reduced (not abolished) when the extracellular sodium is replaced by lithium. An impaired electrogenic, active sodium export should cause the observed reduction of the prestimulus membrane potential.

4.3 Combined action of lithium and strontium

If lithium is applied after calcium has already been replaced by strontium in the superfusate, the already monophasic slow decline of the arsenazo signal is two times prolonged. The sodium-driven strontium export by the exchanger is inhibited,

causing a rise in cytosolic strontium. Lithium can activate the Ca–Sr exchange by the Na–Ca exchanger and thus further accelerate the calcium reduction in the cytosol. However, the rise and decline of the receptor potential is not shortened by the action of lithium as in calcium-containing saline, presumably since strontium in the cytosol has a weaker desensitizing (light adapting) action than calcium.

4.4 Concluding remarks

Our results are in line with the assumption that the rise in cytosolic calcium is due to a calcium release from endoplasmic cisternae and a calcium inward current through the plasma membrane. The rapid phase of decline is due to a (membrane voltage-insensitive) uptake mechanism of calcium into the endoplasmic subrhabdomeral cisternae. This mechanism seems to be not or only barely able to transport strontium instead of calcium; therefore, after prolonged stay in strontium saline without calcium, the cisternae are progressively emptied. This causes a reduction in the IP_3-induced calcium release and an increase in the contribution of light-induced strontium influx through the plasma membrane.

The calcium uptake into the cisternae is also slowly but reversibly abolished by the action of lithium – or at least substantially inhibited if sodium is replaced by lithium. The slow decline (D2) of the arsenazo signal is based on the sodium–calcium exchanger in the plasma membrane. As expected it is able to export strontium; but lithium is no – or only a very poor – sodium substitute to drive the calcium export *via* the exchanger.

The larger arsenazo signal, representing a larger increase in cytosolic strontium as compared to calcium, indicates that strontium is weaker in desensitizing the photoreceptor cell and in reducing calcium release from the cisternae as compared to the intracellular calcium. Since strontium seems not to be taken up into the cisternae, strontium may be used to isolate the pathway of calcium through the plasma membrane, *i.e.* the light-induced influx and the export through the Na–Ca exchanger. The progressive reduction in the amplitude of the arsenazo signal after more than 60 min stay in strontium saline occurs probably when the cisternae are emptied of calcium.

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