Decreased K^+ Conductance Produced by Ba^{++} in Frog Sartorius Fibers

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ABSTRACT The action of Ba^{++} on membrane potential (E_m) and resistance (R_m) of frog (*R. pipiens*) sartorius fibers was studied. In normal Cl^- Ringer's, Ba^{++} (<9 mM) did not depolarize or induce contractions, but increased R_m slightly above the control value of 3.8 ± 0.6 KΩ-cm². In Cl^--free Ringer's (methane sulfonate) R_m was 28.8 ± 2.8 KΩ-cm², and low concentrations of Ba^{++} (0.05–5.0 mM) depolarized and induced spontaneous contractions (fibrillation), even in tetrodotoxin. To stop disturbance of the microelectrodes, contractions were prevented by using two Cl^--free solutions: (*a*) twice hypertonic with sucrose (230 mM), or (*b*) high K^+ (83 mM) partially replacing Na^+. In the hypertonic solution, the fiber diameters decreased, E_m increased slightly, and R_m decreased to 9.0 ± 0.6 KΩ-cm² (perhaps due to swelling of sarcotubules). Ba^{++} (0.5 mM) rapidly increased R_m to 31.3 ± 3.8, decreased E_m (e.g., to −30 mv), and induced spontaneous "action potentials;" Sr^{++} had no effect. In the high K^+ solution, the fibers were nearly completely depolarized, and R_m was decreased markedly to 1.5 ± 0.2 KΩ-cm²; Ba^{++} increased R_m to 6.7 ± 0.5 KΩ-cm². The Ba^{++} actions usually began within 0.5 min and reached a maximum within 5 min. Addition of $SO_4^{=}$, to precipitate the Ba^{++}, rapidly reversed the increase in R_m. Ba^{++} must act by decreasing K^+ conductance (g_K). In Cl^- Ringer's, the high g_{Cl}/g_K ratio masked the effect of Ba^{++} on g_K. Thus, small concentrations of Ba^{++} specifically and rapidly decrease g_K.

In many nerve and muscle cells, Ba^{++} rapidly increases membrane resistance (R_m), depolarizes, initiates automaticity, and prolongs the action potentials (5, 8, 14, 31, 39, 40, 42, 43). All these effects can be attributed to a decreased K^+ conductance (g_K). It has been postulated that Ba^{++} may plug K^+-selective channels in the membrane by virtue of both ions having nearly identical crystal radii (26, 39).

In monolayer cultures of chick heart cells, quiescent nonpacemaker cells were converted into firing pacemaker cells rapidly after addition of Ba^{++} (5 to 10 mM) (38, 39); with time, the cells became nearly completely depolarized and quiescent, concomitant with a marked increase in R_m. How-

ever, large action potentials, whose frequency was a function of the degree of repolarization, developed during the application of hyperpolarizing pulses, thus exposing the latent automaticity of the depolarized cell. Sr^{++} (5 to 10 mM) initially produced hyperpolarization, increased R_m slightly, and induced automaticity in quiescent cells. Elevated $[K^+]_o$ (20 to 30 mM) suppressed automaticity of pacemaker cells and decreased R_m. Hence, a lowered g_K is one method for inducing automaticity.

In toad spinal ganglion cells, Nishi and Soeda (31) showed that Ba^{++} (24–80 mM) rapidly produced a transient, initial hyperpolarization; subsequently, a sustained depolarization occurred and R_m increased due to a decrease in g_K. The hyperpolarization was attributed to an enhanced active extrusion of Na^+ since it was blocked by low temperature, metabolic inhibitors, or preincubation in K^+-free solutions, and was potentiated by microelectrophoretic injection of Na^+. It has also been shown that, among their many actions, local anesthetics (cocaine and procaine) and some antihistaminics decrease g_K several fold in frog sartorius fibers (2–4, 18, 37). In view of the above observations, it was interesting to determine whether small concentrations of Ba^{++} could be used to rapidly, specifically, and reversibly decrease g_K in frog sartorius fibers.

METHODS

Isolated frog sartorius muscles (*Rana pipiens*) were used in all experiments. In the few experiments in which denervated muscles were used, the denervations were carried out by removal of a 5–10 mm segment of motor nerve close to the muscle 2–5 wk prior to use. The muscles were dissected and stored at 4°C in Ringer's solution or Cl^--free Ringer's solution until used; the longest storage time was about 30 hr. Care was taken to tie threads only on the tendons: one on the tendon of insertion and two on the tendon of origin. For study, each muscle was mounted with its inside surface facing uppermost on a raised platform in the center of a circular chamber. Only fibers in the superficial visible layer were penetrated; therefore, the external resistance (r_o) was negligible. A dissecting microscope (× 32) with a calibrated ocular scale was used to measure interelectrode distance.

The bath had a volume of 20 ml and was gassed with a mixture of 5% CO_2, 95% O_2. The gassing also circulated the bath fluid through tubing connecting opposite poles of the chamber. Flow through this tube was about 20 ml/min so that the entire volume of the bath circulated through the tube once per minute. A loose plastic diaphragm at the inlet pole protected the muscle and microelectrodes from violent agitation. Chemical agents were always added to the inlet compartment by syringe "injection" of a small volume (e.g., 0.1 ml) of concentrated solution. It was estimated by use of dyes that there was a lag period of about 15 sec before any of the added agent reached the muscle on the elevated platform and that 90% mixing occurred in about 1 min.

The composition of the various solutions used is given in Table I; all solutions also contained tetrodotoxin (0.5 μg/ml). Of the four solutions, solution I is normal

Cl^--containing Ringer's, and solution II is Ringer's made Cl^--free by substituting methane sulfonate ion ($CH_3SO_3^-$) for Cl^-. Because of spontaneous contractions produced by Ba^{++} in Cl^--free Ringer's, even in the presence of tetrodotoxin, it was necessary to abolish contractions by making the Cl^--free Ringer either twofold hypertonic by sucrose addition (solution III) or high in K^+ by partial substitution of Na^+ (solution IV). The relative tonicities of the various solutions are also given in Table I. The pH of all solutions equilibrated with the gas mixture of 5% CO_2, 95% O_2 was 7.4.

The bath temperature was monitored by a small mercury thermometer and

TABLE I

COMPOSITION OF THE FOUR SOLUTIONS USED TO BATHE THE FROG SARTORIUS MUSCLES

Components	I Normal Cl^--Ringer's	II Cl^--free Ringer's	III Cl^--free, sucrose hypertonic	IV Cl^--free, high K^+
	mM	*mM*	*mM*	*mM*
Cl^-	103	0	0	0
$CH_3SO_3^-$	0	89.3	89.3	86.8
$CO_3^=$	0	8.8	8.8	8.7
HCO_3^-	20	0	0	0
CH_3COO^-	4	0	0	0
Na^+	120	101.0	101.0	17.4
K^+	3	2.5	2.5	83.4
Ca^{++}	1	1.7	1.7	1.7
Mg^{++}	1	0	0	0
Sucrose	0	0	230.0	0
pH Ungassed	8.9	6.8	6.8	6.8
Gassed with 5% CO_2, 95% O_2	7.4	7.4	7.4	7.4
Anion/cation (equivalent)	127/127	106.9/106.9	106.9/106.9	104.2/104.2
Tonicity (relative to 230 milliosmolar) *per cent*	110	93	193	91

automatically controlled by a thermistor and water-cooled Frigistor (Frigistor, London, England) Peltier effect units. The bath could be cooled or heated according to the direction of the electric current flow.

The experiments were done using two intracellular glass (Pyrex) capillary microelectrodes. The membrane potential-recording microelectrodes were filled with 3 M KCl, and the current-injecting microelectrodes were filled with 3 M K citrate so as not to cause electrophoresis of Cl^- when using the Cl^--free media. Both voltage and current microelectrodes had resistances between 20 and 50 MΩ. The two microelectrodes were mounted on the separate arms of a Zeiss sliding micromanipulator. Ag:AgCl wires were used as the reversible half-cells, and the external reference electrodes were large agar-Ringer's or agar–Cl-free–Ringer's salt bridges. Cathode followers were used as preamplifiers, and a calibrator was placed in series in the voltage-recording channel. The intensity of current injected was calculated from the measured voltage drop across a known resistance (50 KΩ) in series with the current

electrode. Both current and voltage were monitored on a dual-beam Tektronix 502 oscilloscope and photographed. In addition, continuous time tracings of resting potentials and electrotonic potentials were recorded on a single-channel Kent rectilinear penrecorder.

The membrane resistance of each fiber was calculated from the decay of electrotonic potentials as a function of interelectrode distance; i.e., using the conventional square pulse method (15). A high resistance (50 MΩ) was placed in series with the output of a rectangular pulse stimulator to give current pulses which were relatively constant. Two stimulators were used in parallel for experiments in which paired hyperpolarizing and depolarizing pulses were applied. The duration of the current pulses was 1 to 2 sec, and all resistances refer to the plateau of the electrotonic potential. The electrotonic potentials were measured over as long a length of fiber as practical without losing the fiber; in some cases readings were taken at interelectrode distances of up to 8 mm. Readings were usually made at three to five distances in any fiber. In addition, in some fibers the voltage/current relationship ($\Delta E_m/I_o$) was determined near the input; i.e., at short interelectrode distances. All calculated values of membrane resistance were taken from measurements of chord resistances using relatively small hyperpolarizing currents (10–40 na). Because of the relatively small degree of curvature of the $\Delta E_m/I_o$ relationship in the hyperpolarizing quadrant even in Cl^--free media, the values obtained for membrane resistance were essentially independent of the magnitude of the applied current. (In the presence of Cl^-, the large contribution of Cl^- current to the total current masks the curvature of the K^+ voltage/current relation (22)). Therefore, the alternative methods for determining membrane resistance were not necessary (2, 3, 9, 18). The semilogarithmic plots of the electrotonic potentials as a function of interelectrode distance were usually linear, and the input resistances (R_{in}) were obtained by extrapolation to zero distance. The diameters of fibers in isotonic solutions were calculated from the internal resistance per unit length (r_i) assuming the myoplasmic resistivity (R_i) to be an exponential function of temperature, and taking values of 250 Ω-cm for 19°C and 370 Ω-cm for 4°C (10). Conversion from membrane resistance of a unit length (r_m) to membrane specific resistance (R_m) was done using the calculated diameters. In many instances, the R_m of a given fiber was measured before and after Ba^{++} addition.

RESULTS

In the Cl^--free solutions, the measured membrane conductance, G_m, reflected K^+ conductance without being influenced by a parallel Cl^- conductance. Although Ba^{++} had only slight effects in the presence of Cl^-, it had pronounced effects on R_m and E_m in the absence of Cl^- (see Table II).

I. *Normal Cl^--Containing Ringer's*

For the muscle studies in Cl^--containing Ringer's, 5 mM $BaCl_2$ was added initially, but determinations of R_m were made after the Ba^{++} concentration was increased to 9 mM. There was no change in resting potentials or electrotonic potentials, and spontaneous "action potentials" or contractions were

not produced. There was no significant change in space constant (λ) or in input resistance (R_{in}) (Table III), and the R_m values of individual fibers are listed in Table IV. Ba^{++} produced no statistically significant change in the mean R_m: before Ba^{++}, the mean was 3.8 $\pm$ 0.6 KΩ-cm^2 (mean $\pm$ SE), whereas after Ba^{++}, it was 5.1 $\pm$ 0.9 KΩ-cm^2 ($p > 0.2$). Ba^{++} addition did not significantly change the resting potential in either of the two fibers from which E_m was simultaneously recorded. However, in 41 fibers sampled before Ba^{++}, the mean E_m was 79 $\pm$ 2 mv, whereas in 32 fibers after Ba^{++}, it was 70 $\pm$ 2 mv ($p < 0.005$).

II. *Cl^--Free Ringer's*

The mean R_m of 19 fibers measured in Cl^--free Ringer's (solution II) was 27.1 $\pm$ 2.1 KΩ-cm^2; their mean diameter was 84 $\pm$ 2 μ (Tables III and IV).

TABLE II

SUMMARY OF THE EFFECT OF Ba^{++} (0.05–9.0 mM) ON FROG SARTORIUS FIBERS BATHED IN THE VARIOUS SOLUTIONS AND IN THE PRESENCE OF TETRODOTOXIN (10^{-6} g/ml)

Parameter	I Normal Cl^--Ringer's	II Cl^--free Ringer's	III Cl^--free, sucrose hypertonic	IV Cl^--free, high K^+
Spontaneous contractions	Absent	Present	Absent	Absent
Spontaneous action potentials	Absent	Present	Present	Absent
Depolarization	Slight	Present	Present	Absent (already depolarized)
R_m	Slightly increased	Increased (?)	Increased	Increased

In contrast to its slight effects in Cl^--Ringer's, Ba $(CH_3SO_3)_2$ (0.5–5 mM) added to muscles in Cl^--free Ringer's had dramatic effects. Within 1 min, spontaneous fibrillations were observed; i.e., almost all fibers were contracting irregularly without any apparent synchronization. The spontaneous fibrillation occurred even in the presence of tetrodotoxin. Thus, although tetrodotoxin did suppress the automaticity and hyperexcitability of frog sartorius fibers bathed in Cl^--free Ringer's, it was not capable of suppressing the spontaneity induced by Ba^{++} in the same fibers. In skeletal muscle and nerve, tetrodotoxin blocks the regenerative g_{Na} increase during activation without affecting the resting or active g_K (28, 29); however, tetrodotoxin does not affect the rate of rise or magnitude of the action potential in either smooth muscle or certain cardiac muscle cells (39). In several experiments, it was visually observed that neither *d*-tubocurarine (5×10^{-5} g/ml) nor denervation (4 wk prior) prevented the spontaneous contractions produced by 0.5 mM Ba^{++}; therefore, the contractions were not due to acetylcholine

release from nerve terminals. The addition of Cl^-, to muscles contracting in Cl^--free solution containing Ba^{++}, abolished the contractions.

Due to the vigorous contractions with Ba^{++}, it was impossible to maintain the two microelectrodes in a fiber for a sufficient time to make determinations of membrane resistance. However, using short duration penetrations with a single voltage-recording microelectrode, it was possible to measure membrane potentials. The mean E_m for 10 fibers in the presence of 0.5 mM Ba^{++} was 43 ± 3 mv, whereas that for 21 fibers in the absence of Ba^{++} was 75 ± 1 mv ($p < 0.001$). Since visible fibrillation of the superficial fibers continued for

TABLE III

SUMMARY OF THE EFFECT OF Ba^{++} ON THE CABLE CONSTANTS OF FROG SARTORIUS FIBERS

The values given are the means ± SE; N is the number of experiments in each category. R_i was assumed to be 250 Ω-cm at 19°C

	I Normal Cl^- Ringer's		II Cl^--free Ringer's	III Cl^--free, sucrose hypertonic		IV Cl^--free, high K^+	
	Control	+9 mM Ba^{++}	Control	Control	+0.5 mM Ba^{++}	Control	+0.5 mM Ba^{++}
N	12	10	19	26	25	7	7
λ, mm	1.58±0.16	1.72±0.11	4.88±0.21	2.47±0.11	4.55±0.30	1.17±0.30	2.85±0.18
R_{in}, MΩ	0.52±0.03	0.66±0.11	1.04±0.05	1.03±0.04	1.43±0.08	0.24±0.04	0.45±0.03
r_i, MΩ/cm	7.15±0.97	7.85±1.09	4.41±0.24	8.87±0.58	7.45±0.84	5.24±1.12	3.69±0.33
r_m, MΩ-cm	0.15±0.02	0.27±0.05	1.02±0.07	0.50±0.03	1.33±0.10	0.06±0.10	0.24±0.02
Diameter, μ	75±5	72±5	84±2	61±2 (68±2)*	66±3 (73±3)*	81±8	92±3
R_m, KΩ-cm²	3.8±0.6	5.1±0.9	27.1±2.1	9.7±0.6 (10.8±0.7)*	29.5±3.0 (32.7±3.1)*	1.5±0.2	6.7±0.5

* Values corrected by increasing the R_i values on the basis of published data for the osmometer behavior of frog sartorius fibers.

periods longer than 30 min (i.e., long after Ba^{++} should have equilibrated in the extracellular fluid space), it is likely that most fibers contracted while partially depolarized. The contractions of individual fibers appeared to be fast and twitch-like. Prolonged action potential–like responses occurred concomitant with contraction in individual fibers; these potential changes often showed large overshoots. (Since the action potentials also occurred in the sucrose hypertonic solution in which there were no contractions, they were not artifacts produced by the micropipette moving in and out of the muscle fiber.) Their rate of rise was unknown (due to the penwriter recording), and it is not known whether propagation occurred. It is possible that these responses were due to a regenerative decrease in g_K rather than a regenerative increase in g_{Na}, i.e. to K^+ inactivation rather than Na^+ activation (27), although these may only occur with applied electrical stimuli (17).

III. *Cl⁻-Free, Sucrose Hypertonic Ringer's*

EFFECT OF SUCROSE The addition of sucrose to produce twofold hypertonicity caused a pronounced fall in the electrotonic potentials at all interelectrode distances; the space constant and the input resistance were both in-

TABLE IV

EFFECT OF Ba^{++} ON MEMBRANE RESISTANCE OF FROG SARTORIUS FIBERS BATHED IN VARIOUS SOLUTIONS

I Normal Cl-Ringer's		II Cl-free Ringer's	III Cl-free, sucrose hypertonic		IV Cl-free, high K^+	
Control	+Ba^{++} (9 mM)	Control	Control	+Ba^{++} (0.5 mM)	Control	+Ba^{++} (0.5 mM)
2.9	2.3	22.2	5.7	14.1	0.8	5.9
3.0	3.5	29.5	2.8	14.3	1.2	6.6
1.6	2.1	33.4	8.6	28.3	1.6	7.3
3.8	2.8	25.3	7.0	69.6	1.4	5.2
2.2		16.0	10.0	28.2	1.6*	6.6*
1.5*	2.5*	20.2	9.8	16.1	2.2*	8.5*
9.2	7.2	16.1	10.8	18.6	1.8*	8.7*
3.1	7.8	39.4	10.1	22.0		
5.5	9.5	22.2	8.4	25.7		
4.1	4.6	23.8	9.9	20.0		
2.8		42.2	9.8	15.1		
5.9*	9.2*	47.3	4.4	45.0		
		37.3	8.0	41.2		
		19.9	13.7	44.3		
		24.9	11.3	46.9		
		18.8	11.9	60.6		
		21.6	10.7	26.3		
		22.5	7.3	27.0		
		32.1	11.8			
			10.4*	26.6*		
			11.0*	34.2*		
			12.9*	23.2*		
			5.9*	10.5*		
			9.8*	16.4*		
			14.0*	22.5*		
			17.1*	40.7*		
Mean ± SE 3.8±0.6	5.1±0.9	27.1±2.1	9.7±0.6	29.5±3.0	1.5±0.2	6.7±0.5
p	>0.2		<0.005		<0.001	

R_m values calculated from hyperpolarizing pulses and expressed as $K\Omega$-cm². All measurements were made in the presence of tetrodotoxin (0.5×10^{-6} g/ml) except for the last three fibers in solution IV, and at 19–25°C except for the last six fibers in solution I which were made at 6°C. The second row from the bottom gives the mean and standard error for each column. The p values compare the means before and after Ba^{++} addition in each solution.

* In these fibers, measurements were made on the same fiber before and after Ba^{++} addition; i.e., each fiber served as its own control.

creased (Table III). These effects reflect changes in R_m and in fiber diameter. Compared to the mean R_m of 27.1 ± 2.1 for 19 fibers before sucrose addition, that after sucrose addition, assuming R_i to be unaltered, was 9.7 ± 0.6 KΩ-cm² ($p < 0.005$) for 26 fibers (Tables III and IV).

The calculated value of the mean fiber diameter was reduced from 84 ± 2 to 61 ± 2 μ ($p < 0.001$) as a result of the sucrose hypertonicity (Table III). If the fibers behaved as perfect osmometers, a doubling of external tonicity

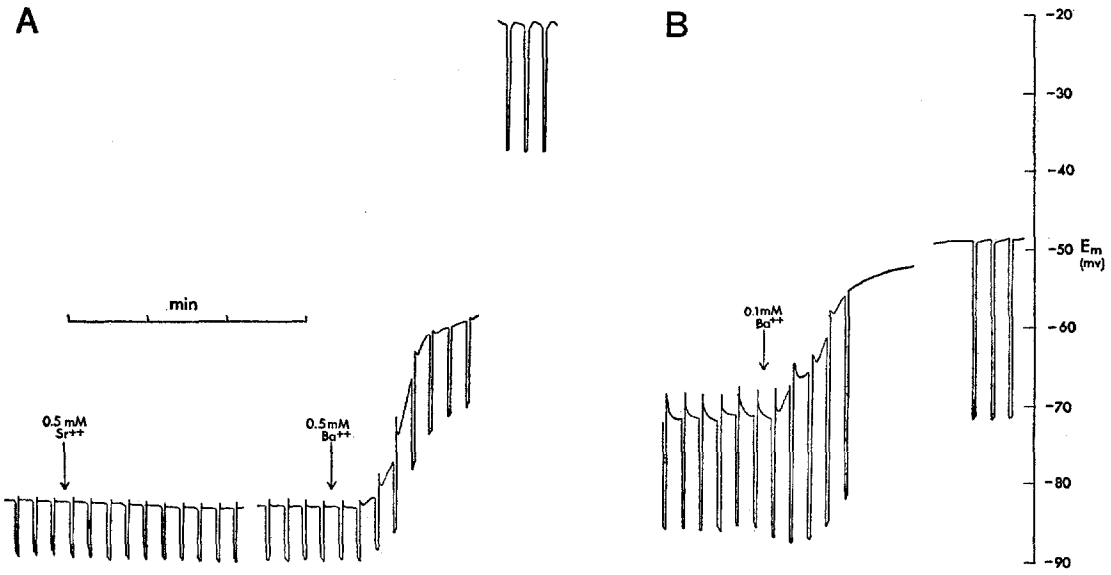

FIGURE 1. Effect of Sr^{++} and Ba^{++} on resting potentials and electrotonic potentials in two frog sartorius fibers bathed in Cl^--free, sucrose hypertonic solution (19–21°C). Recordings made by rectilinear penwriter; time and membrane potential axes apply to both A and B. *A*, Sr^{++} (0.5 mM) and Ba^{++} (0.5 mM) addition indicated by arrows. Deletions of 3 and 10 min in length were made in the record and are represented by the first and second gaps, respectively. The interelectrode distance was 3.3 mm, and the current pulses applied were 21 na. *B*, Ba^{++} (0.1 mM) added at arrow. A deletion of 1½ min in length was made at the gap in the record. The interelectrode distance was 2.2 mm and the current pulses were 20 na.

should cause their diameter to decrease to $\frac{1}{2}^{0.5}$ or 0.707 times its initial value giving 59 μ, which is close to the calculated experimental value of 61 μ. The fact that the calculated diameters varied as predicted for a perfect osmometer (i.e., no osmotically inactive space) is puzzling.

However, Reuben et al. (36) and Blinks (6) reported ratios of fiber volumes in twice hypertonic solution relative to those in isotonic of 0.67 and 0.66, respectively. The data of Dydynska and Wilkie (11) and of Bozler (7) give ratios of fiber volumes, in twice hypertonic solution relative to isotonic, of 0.62 and 0.68, respectively, assuming that 25% of the fiber volume in isotonic solution

is osmotically inactive because it is occupied by solid material (density of 1 g/ml). Using the mean of 0.66 from the four values in the literature, the ratio of mean fiber diameter in these solutions would be 0.81, the square root of the ratio of volumes. Although the above data were obtained in Cl^--containing solutions, the osmotic behavior of frog skeletal muscle in hypertonic solutions appears to be the same in the presence or absence of Cl^- (36, and unpublished observations). The ratio of mean fiber diameter in twice hypertonic solution to that in isotonic would be 61 μ/83 μ or 0.73; therefore, to produce the proper ratio of diameters, R_i in the sucrose hypertonic solution should be increased to $(0.81/0.73)^2$ or 1.23 times its value in the isotonic solution. If such a correction were made to increase the R_i values, then in the sucrose hypertonic solution, before and after Ba^{++}, respectively, the mean diameters would be 68 and 73 μ and the mean R_m values would be 10.8 and 32.7 KΩ-cm^2 (Table III) or 1.11 (0.81/0.73) times the value calculated assuming constant R_i.

The mean value of the average resting potentials of six muscles was 68 $\pm$ 3 before sucrose addition and 78 $\pm$ 6 mv after sucrose addition ($0.1 < p < 0.2$). The slightly higher resting potentials could be due to the higher $[K^+]_i$ in the shrunken fibers.

EFFECT OF BA^{++} The addition of 0.5 mM $Ba(CH_3SO_3)_2$ to muscles equilibrated in the sucrose hypertonic Ringer's caused a rapid depolarization. Depolarization usually began within 0.5 min, and a constant maximum level of depolarization was attained within 5 min (Fig. 1). (The muscle chamber had a lag period of about 15 sec, and a time for 90% mixing of about 1 min (see Methods).) The mean value of the average resting potential of each of six muscles was 32 $\pm$ 3 mv (81 fibers) in the presence of 0.5 mM Ba^{++} and 76 $\pm$ 6 mv (54 fibers) in the absence of Ba^{++} ($p < 0.001$).

Fig. 2 illustrates penwriter records of Ba^{++}-induced spontaneous action potentials which were often observed despite the presence of tetrodotoxin and the low resting potentials. The duration of the action potentials was about 15 sec, and there usually was an overshoot. A slow depolarization or "pacemaker" potential preceded some of the action potentials; during this pacemaker potential, there was a small gradual increase in the electrotonic potentials (Fig. 2 C). Despite the diminished resting potential and the spontaneous action potentials, neither tonic nor phasic contractions were observed with the dissecting microscope. The spontaneity produced by Ba^{++} may have been induced by a combination of partial depolarization and the reduced g_K (39), although E_m alone cannot be the sole determinant of automaticity because equivalent depolarization by K^+ or by electrotonic pulses did not induce spontaneity (39). Since elevated $[K^+]_o$ increases g_K and suppresses spontaneity (39, 41), these data support the hypothesis that the level of g_K (along

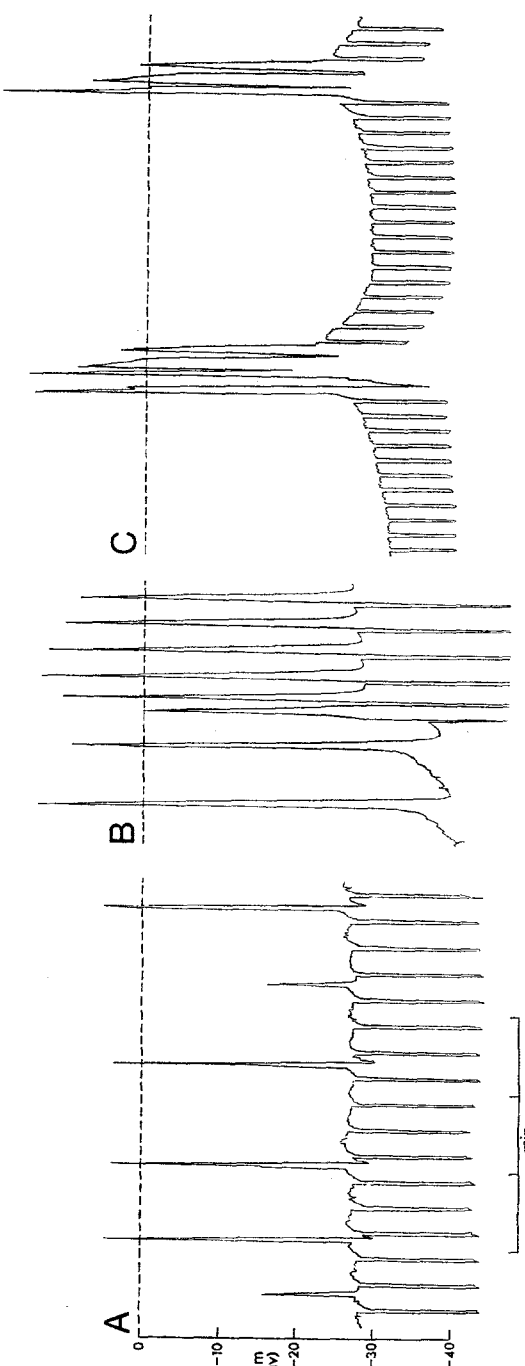

FIGURE 2. Penwriter records of spontaneous, overshooting action potentials produced by Ba^{++} in frog sartorius fibers bathed in solutions (21–23°C) containing tetrodotoxin (0.5 μg/ml). The time and membrane potential axes apply to all three figures; the dashed line indicates zero resting potential. *A*, in Cl^--free, sucrose hypertonic Ringer's. The sequence begins about 5 min after addition of 0.5 mM Ba^{++}. The interelectrode distance was 3.7 mm, and the current pulses were 21 na. The resting potential was about −28 mv. Note failure of spikes in two cases. *B*, in Cl^--free Ringer's in which four-fifth's of the $NaCH_3SO_3$ has been replaced isosmotically with sucrose. The sequence begins about 5 min after addition of 5 mM Ba^{++}. The current electrode was inserted following the second action potential. The electrotonic potentials were 37 mv (their peaks are cut off in the figure), the interelectrode distance was 0.16 mm, and the current pulses were 35 na. Note the anodal break responses produced at the cessation of each electrotonic potential. *C*, in Cl^--free, sucrose hypertonic Ringer's. The sequence begins 15 min after addition of 0.5 mM Ba^{++}. The interelectrode distance was 2.8 mm, and the current pulses were 12 na. Note the gradual increase in electrotonic potentials during the slow depolarization preceding each action potential.

with a relatively small g_{Cl}/g_K ratio) determines whether the membrane shall be unstable.

R_{in}, λ, and R_m were increased in the presence of Ba^{++} (Table III). In addition, membrane time constant (T_m) was increased. Compared to the mean R_m of 9.7 ± 0.6 KΩ-cm² for 26 fibers before Ba^{++} addition, that after addition of 0.5 mM Ba^{++} was 29.5 ± 3.0 KΩ-cm² ($p < 0.001$) for 25 fibers (Tables III and IV). In some cases, fiber diameter and R_m were obtained from the same

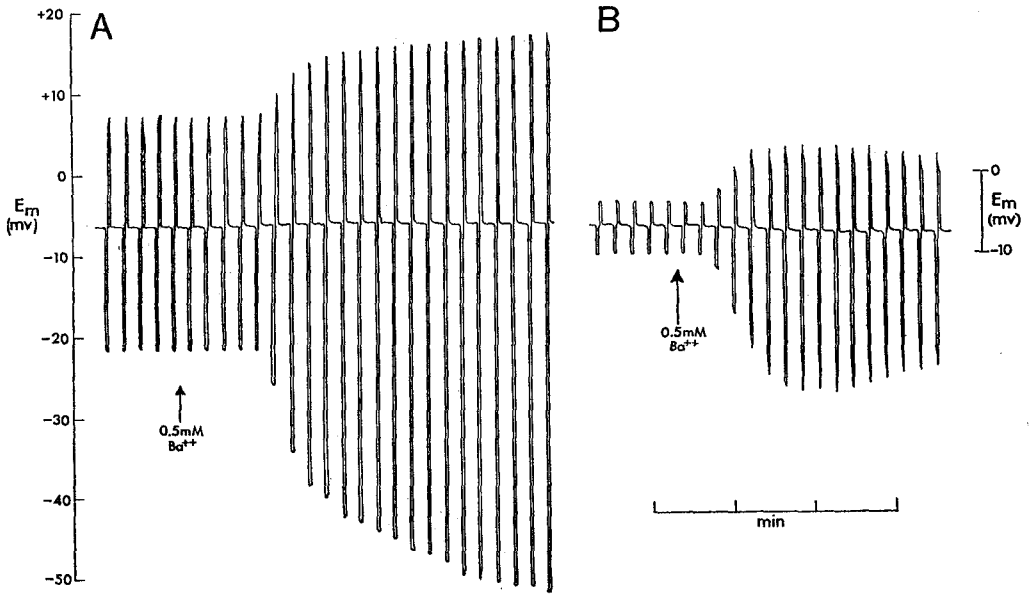

FIGURE 3. Effect of Ba^{++} on electrotonic potentials in frog sartorius fibers in Cl^--free, high K^+ solution (23–27°C). The time and membrane potential axes apply to both A and B. *A*, resting potential about 7 mv. Ba^{++} (0.5 mM) added at arrow. The interelectrode distance was 0.17 mm, and the paired current pulses were 63 na (hyperpolarizing) and 37 na (depolarizing). *B*, Ba^{++} (0.5 mM) added at arrow. The interelectrode distance was 1.44 mm, and the paired current pulses were 42 na (hyperpolarizing) and 23 na (depolarizing).

fiber before and after addition of 0.5 mM Ba^{++} (Table IV). Ba^{++} increased the mean R_m of the seven fibers used in this manner as their own controls from 11 ± 1.3 to 24.6 ± 1.2 KΩ-cm² ($p < 0.005$). Even in Ba^{++} concentrations as low as 0.05 mM, R_m was increased substantially; however, a reliable dose-response curve was not determined. Ba^{++} produced no significant change in the mean fiber diameter.

Insensitivity to Sr^{++} The addition of 1 mM $Sr(CH_3SO_3)_2$ had no effect on either R_m or resting potential. The presence of Sr^{++} did not antagonize the subsequent action of 0.5 mM Ba^{++} (Fig. 1).

IV. *Cl⁻-Free, High K⁺ Solution*

EFFECT OF K^+ In the Cl^--free, high K^+ solution (IV), both λ and R_{in} were markedly decreased relative to their values in unmodified Cl^--free Ringer's (solution II) (Table III). As indicated in Tables III and IV, compared to the mean R_m of 27.1 ± 2.1 KΩ-cm² in Cl^--free Ringer's (solution II), that of seven fibers decreased to 1.5 ± 0.2 KΩ-cm² ($p < 0.001$) as a result of elevating $[K^+]_o$ to 83 mM (solution IV). In addition, the membrane time constant was markedly decreased. There was no significant change in the mean fiber diameter. All fibers had resting potentials of 4–9 mv in this solution.

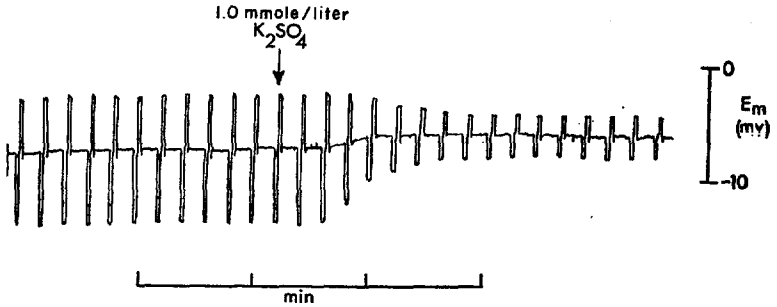

FIGURE 4. Reversal of the Ba^{++} effect on the electrotonic potentials of frog sartorius fibers bathed in Cl^--free, high K^+ solution (26°C) containing 0.5 mM Ba^{++}. At the arrow, 1 mmole/liter K_2SO_4 was added to precipitate the Ba^{++}. Interelectrode distance was 2.2 mm, and the paired current pulses were 51 na (hyperpolarizing) and 30 na (depolarizing). Resting potential was about −6 to −8 mv.

EFFECT OF BA^{++} $Ba(CH_3SO_3)_2$ (0.5 mM) produced a large increase in the electrotonic potentials usually within 0.5 min after its addition (Fig. 3). The hyperpolarizing electrotonic potentials consistently increased more than the depolarizing ones, as demonstrated in Fig. 3. The mean R_m of seven fibers, calculated from hyperpolarizing pulses, increased from the value of 1.5 ± 0.2 to 6.7 ± 0.5 KΩ-cm² ($p < 0.001$) as a result of Ba^{++} addition (Tables III and IV). Ba^{++} caused no significant change in the already diminished resting potential. The change in mean fiber diameter was not significant ($p > 0.1$).

The rapid reversibility of the Ba^{++} effect on R_m was demonstrated in several experiments in which $SO_4^=$ was added in order to precipitate the Ba^{++}. The addition of 1.0 mmole/liter K_2SO_4 to the bath containing 0.5 mM Ba^{++} caused a rapid decline of the electrotonic potentials in several fibers (Fig. 4). R_m decreased from 4.6 to 1.6 KΩ-cm² in the one fiber in which R_m was determined before and after $SO_4^=$ addition. The addition of $SO_4^=$ thus returned R_m to a value close to the mean value (1.5 KΩ-cm²) of control fibers in the absence of Ba^{++}.

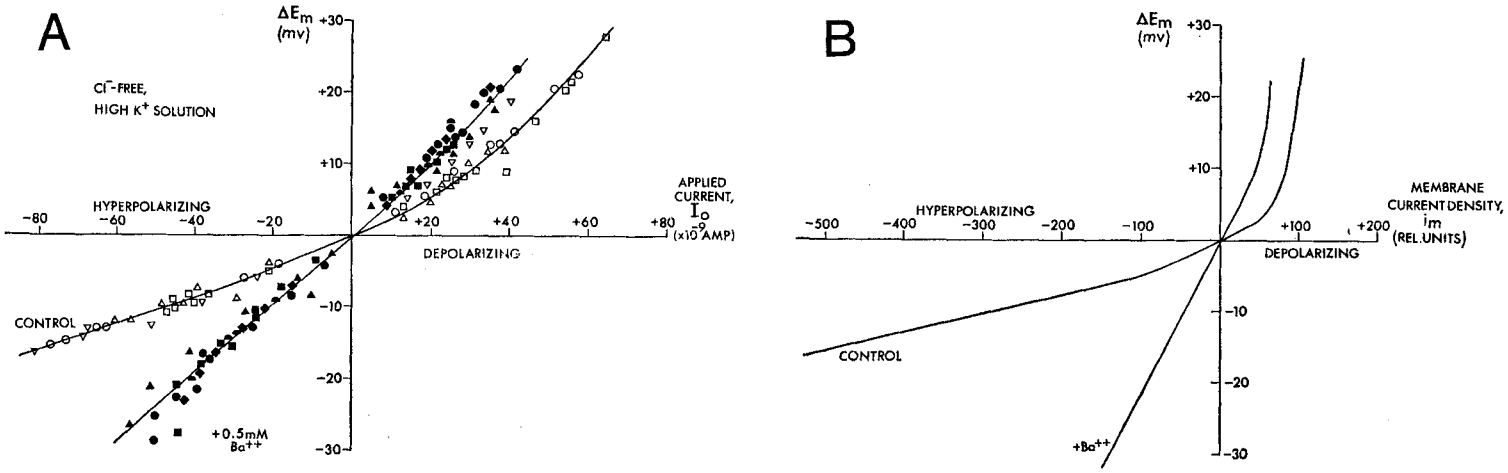

FIGURE 5. The voltage/current characteristics of frog sartorius fibers bathed in Cl^--free, high K^+ solution before and after Ba^{++} addition. *A*, the electrotonic potentials (ΔE_m) recorded at short interelectrode distances (0.16–0.5 mm) are plotted as a function of applied current (I_o). In the absence of Ba^{++}, the interelectrode distance was always less than 0.19 mm. Open symbols, control before Ba^{++}; solid symbols, after 0.5 mM Ba^{++}. In order to normalize results from fibers of differing diameters, the actual applied current was multiplied by the following factors to give I_o: 0.91 (△▲), 0.62 (□■), 1.0 (○●), 1.18 (▼), 0.58 (◆), 0.59 (*). In three fibers (△▲, □■, and ○●), data were obtained in the same fiber before and after Ba^{++}. *B*, the change in membrane potential is plotted as a function of the relative membrane current density (i_m) by modification of the curves in *A*. The relative current density is proportional to $I_o \cdot dI_o/d\Delta E_m$ (9, 22).

In two fibers from muscles denervated for 2 wk, Ba^{++} (0.5 mM) produced its usual marked increase in electrotonic potentials. Thus, the action of Ba^{++} on diminishing g_K may be additive with the action of denervation (21, 30) on diminishing g_K.

The voltage/current curves at short interelectrode distances were determined for several fibers before and after Ba^{++} addition (Fig. 5). In order to normalize the curves obtained from fibers of different diameters, the current necessary to produce each observed electrotonic potential in a given fiber was multiplied by a constant chosen so that, in the absence of Ba^{++}, a 4.5 mv hyperpolarization would occur with 20 na. In the two fibers in which control curves in the absence of Ba^{++} were not obtained, the currents were multiplied by constants chosen so that a 10 mv hyperpolarization would occur with 20 na. The normalization procedure was necessary to correct for variation in fiber diameter because, in a population of fibers of varying diameter (d) but constant R_m and R_i, the current (I_o) required to produce a given electrotonic potential (ΔE_m) is proportional to $d^{3/2}(I_o = \pi d^{3/2}(\Delta E_m/R_m{}^{0.5}R_i{}^{0.5}))$. The composite voltage/current curves obtained from six fibers before and after Ba^{++} are shown in Fig. 5 A. In Fig. 5 B, ΔE_m in these same fibers is plotted as a function of the relative membrane current density (i_m) (9). The addition of 0.5 mM Ba^{++} appeared to straighten the curves about the resting potential (origin). But this effect is only the consequence of the lower currents needed to obtain a given electrotonic potential when Ba^{++} was present; i.e., the actual nonlinearity of R_m with respect to current is the same, as will be discussed.

DISCUSSION

The results clearly demonstrate that Ba^{++} has dramatic effects on the membrane of frog sartorius fibers. In sartorius fibers bathed in Cl^--free media, in common with many other excitable cells in which K^+ movement provides a major contribution to conductance, low concentrations of Ba^{++} rapidly and reversibly increase R_m, depolarize, and initiate automaticity giving rise to prolonged action potentials. All these effects can be attributed to a specific decrease in g_K produced by Ba^{++}. Among the various alternatives concerning its mechanism of action, one possibility is that Ba^{++} may form a tight-fitting plug in K^+-selective channels in the membrane. If K^+ traverses the membrane by having part of its hydration replaced by polar groups of the membrane (26), then Ba^{++}, having nearly the same crystal or singly hydrated radius as K^+ (26, 39), would fit in the K^+ channel, but, being doubly charged, would be bound more tightly than K^+. This hypothesis would explain why Sr^{++}, having a smaller crystal radius and thus being unable to fit tightly, is ineffective. Since Sr^{++} is ineffective, one would predict that Ca^{++} and Mg^{++} would also be ineffective. However, since Rb^+, Cs^+, and tetraethylammonium ions also produce K^+ inactivation in the membranes of several preparations (see

reference 1 and the review by Grundfest (17)), this mechanical size hypothesis may be an oversimplification. Specificity for Ba^{++}, therefore, might not only be explained on the basis of a tight fit of the unhydrated or partially hydrated ion in a K^+-selective channel, but could also be explained by selective binding of Ba^{++} to negatively charged membrane sites which directly or indirectly control g_K. The specificity might then be analogous to the ability of $SO_4^{=}$ ions to clearly distinguish between the alkaline earth ions.

In both the Cl^--free, sucrose hypertonic solution (III) and the Cl^--free, high K^+ solution (IV), the ratio of the g_K after Ba^{++} to that before Ba^{++} was about 0.2. In the high K^+ solution, g_{Na} should be negligible relative to the elevated g_K; this is supported by the fact that Ba^{++} addition (0.5 mM), although causing a 4.5-fold decrease in G_m, did not cause a decrease in E_m. Therefore, the measured G_m was entirely due to g_K, and the ratio of K^+ conductances after to those before Ba^{++} was 0.22. On the other hand, in the sucrose solution containing Ba^{++}, g_{Na} would contribute a significant part of the total membrane conductance. The relative contributions of g_K and g_{Na} to G_m can be estimated from the large displacement of E_m away from E_K following Ba^{++} addition; such depolarization would be produced if Ba^{++} were to decrease g_K but not affect g_{Na}. In Ba^{++}, the mean resting potential was -32 mv; assuming E_K and E_{Na} to remain constant and to be -93 mv and $+60$ mv, respectively, $g_K/(g_K + g_{Na})$ and $g_{Na}/(g_K + g_{Na})$ calculate to be approximately 0.6 and 0.4, respectively, from the chord-conductance equation (19). Thus, g_{Na} would be 1.3×10^{-5} mho-cm^{-2}, and g_K after Ba^{++} would be 1.9×10^{-5} and g_K before Ba^{++}, 9.8×10^{-5} mho-cm^{-2} (11.1 minus 1.3); the ratio of final to initial K^+ conductances equalled 0.19. This ratio obtained in the sucrose solution compares with that of 0.22 obtained in the high K^+ solution. Thus, despite the 33-fold elevation of $[K^+]_o$ in the high K^+ solution, Ba^{++} produced about the same proportional decrease in g_K in the high K^+ and in the sucrose hypertonic solutions. Therefore, Ba^{++} appears to act as a noncompetitive inhibitor of the movement of K^+ across the membrane. However, it is not possible to prove this unequivocally without determining complete dose-response curves. Noncompetitive inhibition would be consistent with Ba^{++} causing complete or partial blockage of some or all of the K^+-selective channels. In any case, it may be assumed that the degree of inhibition, i.e. the fractional decrease in g_K, is determined by the fraction of active sites occupied by Ba^{++}. It is not known whether Ba^{++} acts at the same sites or whether its effect is additive with the effect of other agents which decrease g_K (e.g., cocaine, denervation, and low temperature).

In Cl^--containing Ringer's, the small depolarization and the statistically insignificant increase of R_m in relatively high Ba^{++} concentrations may be explained on the basis of the large g_{Cl}/g_K ratio masking the effects of a decreased g_K. That is, depolarization is minimized because E_{Cl} clamps E_m at the

control level before the addition of Ba^{++}. Because of the large shunting by g_{Cl} (20), the decrease in g_K produces only a relatively small increase in R_m which would be difficult to prove statistically. Assuming g_{Na} to be negligibly small and g_K to be reduced to zero in 9 mM Ba^{++}, g_{Cl} is calculated to be 19.6×10^{-5} mho-cm^{-2} and the mean g_K, 6.7×10^{-6} mho-cm^{-2}, giving a g_{Cl}/g_K ratio of 2.9.

The voltage/current curves determined in the Cl^--free, high K^+ solution in the absence of Ba^{++} demonstrate anomalous rectification, which is characteristic of the movement of K^+ across the membrane, but not of Cl^- (22, 24). The presence of a relatively large g_{Cl}, thus straightens the curve by masking the K^+ behavior. Therefore, it is observed only when g_{Cl}/g_K is lowered by using Cl^--free solution, Zn^{++} (25), high CO_2, perrhenate ion, high K^+, etc. The straightening of the $\Delta E_m/I_o$ and $\Delta E_m/i_m$ relations in the presence of Ba^{++} is in agreement with the results obtained in other tissues (42, 43). (Hyperpolarizing K^+ inactivation is also eliminated by Rb^+ (1, 17).) However, if the Ba^{++} curve is adjusted by multiplying the ΔE_m values by the ratio of mean R_m values before to those after Ba^{++} (independently determined in other fibers), then Ba^{++} is observed to cause approximately the same fractional increase in ΔE_m at each i_m. The curvature of the adjusted Ba^{++} curve (extending it over the same current range as the control) is increased, eliminating the apparent straightening. Thus, rectification could be explained by a current-dependent change in g_K as well as by a voltage-dependent change.

A large decrease in the apparent R_m was unexpectedly produced in the sucrose hypertonic solution. This decrease could be explained by swelling of the transverse sarcotubules. Swelling of the sarcotubules of frog sartorius fibers in hypertonic media has been observed in electron micrographs (16, 23). Such swelling would increase shunting of the fiber surface membrane by the sarcotubules. The series resistance of the sarcotubular fluid decreases with the square of the tubular diameter, and the membrane resistance per unit length of sarcotubule may decrease as the surface area increases (as a function of diameter). Peachey (32, 33) reported a ratio of membrane areas, surface/tubular, of 7 for frog sartorius fibers of 100 μ diameter. Therefore, small changes in diameter of the sarcotubules could cause a large apparent change in R_m, even if the true resistivity of either surface or tubular membrane did not change. Several investigators have recently shown the importance of the sarcotubules in determining the resistance and capacitance of skeletal muscle fibers (12, 13, 34). However, the apparent decrease in R_m with sucrose hypertonicity may also have other explanations, especially if either sucrose or hypertonicity per se was not inert towards the electrical properties of the surface membrane (35, 36). Regardless of why the apparent R_m was decreased, the sucrose hypertonicity did not alter the effect of Ba^{++} on g_K. The rapid onset of the Ba^{++} effect does not permit localization as to the site of action,

i.e. surface and/or tubular membranes, since diffusion of Ba^{++} in the sarcotubules ought to be relatively rapid; it is possible that Ba^{++} acts at both membranes.

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