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BLOCKADE BY 9-AMINOACRIDINE OF POTASSIUM FLUXES IN FROG SARTORIUS MUSCLE*

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Abstract—Potassium fluxes of unstimulated frog sartorius muscles bathed in hypertonic, isotonic or sodium-free solutions were depressed by 9-amino-acridine (9AA; 0·1 to 1 mM). The flux ratio (efflux/uptake) of ^{42}K movement was depressed because efflux was depressed to a greater extent than was uptake. In hypertonic solutions, muscles treated with 9AA (up to 2·5 mM) had a higher potassium content than did untreated muscles. There was no detectable difference in the sodium content of treated and untreated muscles. The resting membrane potential of surface fibers was reduced by 9AA. It was concluded that 9AA caused a relatively specific blockade of potassium movement in frog muscle by direct interaction with membrane sites.

IN FROG sartorius muscle fibers, potassium fluxes are described as passive unidirectional processes that follow simple first-order permeability kinetics.[1,2] Active transport mechanisms normally contribute little to the uptake or efflux of potassium ions by the resting muscle fiber.[3] When measurements of potassium fluxes were made in muscles treated with cesium,[4] barium,[5] or tetraethylammonium (TEA),[6] it was found that potassium fluxes were depressed. A reasonable interpretation of the findings is to postulate that the blocking ions occluded sites in the membrane normally occupied by potassium to prevent the passage of potassium through the membrane.

The present findings describe the effects of 9-aminoacridine (9AA) on potassium fluxes in resting frog sartorius muscle fibers. The considerable physical and chemical data available about 9AA[7] may provide clues concerning membrane binding sites for potassium ions. Like TEA, 9AA has molecular dimensions comparable to those of hydrated potassium ions and exists in the ionized form at pH 7·0. It had been shown earlier that a derivative of 9AA caused a marked prolongation of the muscle action potential and a decrease in the resting membrane potential.[8]

Most of the experiments were made on muscles bathed in hypertonic solutions. This was done for two reasons. First, electrical recording from intracellular sites was facilitated by the uncoupling of excitation–contraction that occurred in the hypertonic solution.[9] Although the resting membrane and action potentials were nearly identical to those obtained in muscles bathed in isotonic solutions, contraction did not occur in response to direct stimulation of the muscle. Accordingly, there was a minimal amount of distortion in the electrical recording as a result of mechanical events. Second, the ratio of chloride to potassium conductance is reduced greatly in sartorius muscles equilibrated with hypertonic solutions.[10] Resting muscle fibers in hypertonic solutions

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have low membrane conductances for sodium and chloride ions but high conductance for potassium. For this reason, sartorius muscles in hypertonic solutions are ideal preparations for studying the relationship between the actions of drugs on the electrical properties of the membrane and the movement of potassium ions. The effects of 9AA on potassium fluxes in muscles bathed in isotonic and sodium-free solutions were studied also.

METHODS

The experiments were performed with sartorius muscles of *Rana pipiens*. The muscles were removed from pithed frogs, with special precautions taken to avoid damage to the pelvic end of the muscles. Unless indicated otherwise, the muscles were stored at room temperature (21–25°) in a small dish containing the appropriate solution for at least 1 hr prior to use. The standard hypertonic solution had twice the tonicity of the usual Ringer's solution, a pH of 7·1, and contained the following: 114·85 mM NaCl, 1·8 mM $CaCl_2$, 2·5 mM KCl, 2·15 mM Na_2HPO_4, 0·85 mM NaH_2PO_4 and 232 mM sucrose. Isotonic solutions were made by omitting sucrose, and sodium-free solutions were made by omitting all sodium salts. All experiments were performed at room temperature.

Electrophysiology. The muscles were fixed by miniature pins to the paraffin bed of a small bath containing 8 ml of the hypertonic solution. The pins were inserted into the connective tissues of the pelvic and tibial ends of the muscle in such a way that damage to the muscle fibers was avoided. The resting length and geometry of the muscle were maintained.

The muscle were mounted in the chamber with the inner surface facing upward. Only those muscle fibers lying in the superficial layer of the muscle were used for study. The resting membrane potentials were recorded with standard techniques. High resistance glass capillary electrodes (filled with 3 M KCl) coupled to a capacity neutralization amplifier were used to detect intracellular events. The electrodes were selected with resistances ranging from 10 to 20 megohms and with tip potentials of less than 5 mV. The amplification system (Pico-metric 181 amplifier and Tektronix 502 oscilloscope) had a drift of less than 1 mV per 24 hr. The potentials were displayed on an oscilloscope for visualization and permanent records were made on photographic paper with a kymographic camera. Under these experimental conditions, there was no evidence of deterioration of the resting membrane potential of the muscles bathed in hypertonic solutions for as long as 8 hr.

Isotope studies. Measurements of the influx and efflux of isotopic potassium (^{42}K) in sartorius muscle were made with procedures described by Sjodin and Henderson.[1] The plan of the investigation was to measure ^{42}K-influx and efflux in the same muscle. Fine threads were tied to the connective tissues of the pelvic and tibial ends of the muscle. The muscles were allowed to equilibrate in the appropriate solutions and were then weighed. The muscles were tied to a specially constructed platinum frame in a manner that preserved the geometry of the muscles. The framework holding the muscle was made to fit into the test tubes used for determinations of the uptake and efflux of the isotope. It is possible with this arrangement to move the framework and muscle from tube to tube for measurement of isotope efflux or to place the muscle (air-suspended) in the counter for measurements of isotope uptake. At the beginning of the

uptake–efflux sequence, the muscles were placed in 25 ml of isotope-containing hypertonic solution for 10 sec, removed from the solution and placed in the counter. The radioactivity detected from the air-suspended muscle was taken as representing solution adhering to the muscle.

Paired muscles from the same frog were used for the measurement of the effects of the drugs on the uptake and efflux of ^{42}K. One muscle served as a control and uptake and efflux were measured in the absence of drugs. The other muscle was bathed in a solution containing 9-aminoacridine hydrochloride.

The muscles were placed in the radioactive solution for known periods of time. Between each time period, the muscle was placed in an empty efflux tube by means of the platinum holder and the tube was then placed in the well of the counter (Packard Auto-Gamma spectrometer). The muscle was counted for 1 min and returned to the labeled soak solution. Initially, the time periods of uptake were 5 and 10 min, and were increased to 20 and 30 min in the latter part of the uptake measurements. In most experiments, uptake was carried on for 1 hr. At the termination of uptake, the muscles were placed in a series of efflux tubes, each containing 5 ml of the desired isotope-free solution. The time periods for which the muscle was in each tube were generally 20 min. One to 2 hr of efflux proved a more than adequate time for the accurate determination of a rate constant for ^{42}K efflux. At the termination of efflux, muscles were given a final count in the manner employed for uptake (air-suspended in the counter) and then prepared for analysis. Radioactivity (efflux counts) appearing in the efflux solutions was determined at this time.

At the end of the uptake–efflux sequence, the muscles were placed in 5 ml of sodium-free sucrose solution for 10 min. The sodium was replaced by tris (hydroxymethyl) aminomethane neutralized to pH 7·1. This procedure removed extracellular sodium from the muscle and allowed for the estimation of the extracellular space. The assumption was made that this procedure removed all of the extracellular sodium from the muscles and that sodium efflux from the muscles was negligible. Sodium was determined by flame photometry.

The muscles were then removed from the platinum frames, blotted gently on filter paper and wet weight was determined. Ashing of the muscles was performed in tared platinum crucibles at 550° for 10 hr. The material was diluted to a volume of 10 ml by the addition of glass-distilled water. Five ml was used for flame photometric determinations of potassium and sodium content of the muscles, and 5 ml was used for a final muscle count of radioactivity. Counting was carried out in an efflux tube so that the volume and geometry of the system were identical to those of the system used in the uptake–efflux sequence. The final count plus all of the efflux counts gave the tracer content of the muscle at the end of the influx period.

Cation analyses were performed with a specially constructed flame photometer. The flame photometer consisted of a Beckman atomizer and flame unit and an American Instrument Company photometer equipped with an RCA 1P22 photomultiplier tube. Potassium and sodium interference filters of 2 mμ band width allowed analyses to be made with no detectable interference.

The rates of ^{42}K uptake and efflux were calculated in the following way. The tracer uptake points were converted to micromoles of potassium per gram of muscle by knowledge of the specific activity of the uptake solution and the counts/min in the muscle at the end of the uptake period. The radioactivity of the muscle at the end of

uptake was determined by adding all efflux counts to the final muscle count. As mentioned before, in an uptake experiment, the muscle was first dipped in the radioactive solution for a 10-sec period and counted. This count was taken as representing solution adhering to the muscle. This count and the extracellular space counts were always subtracted from each uptake value to obtain the kinetics of cellular penetration. Extracellular space counts were obtained from a knowledge of the volume of the space, determined by the method mentioned, and the activity of the uptake solution. Applying this procedure yielded initial linear portions of the uptake curves which passed through the origin. The slope of the curve indicated the rate of influx of ^{42}K in units of μmoles per gram-hour.

The rate of efflux was determined by plotting the radioactivity in the muscle as a function of time, starting with the end of the uptake phase of the measurement and ending with the final muscle count. The slope of the curve obtained is the rate constant for the efflux of ^{42}K (k) and is expressed as the reciprocal of time (hr^{-1}). The rate of efflux (μmoles/g-hr) was determined by multiplying the rate constant (hr^{-1}) by the internal concentration of potassium ions (μmoles/g).

For a detailed account of the mathematical and conceptional basis of the foregoing, see Sjodin and Henderson.[1]

RESULTS

The mean resting membrane potential of muscle fibers bathed in the hypertonic sucrose-containing solutions was 92 mV (56 cells in 10 muscles; range, 85–100 mV). This value was the same as that obtained in muscles bathed in isotonic solutions (sucrose removed) where the mean resting membrane potential was 92·6 mV (18 cells in three muscles; range, 88–100 mV).

TABLE 1. DEPOLARIZATION OF SARTORIUS FIBERS BY 9-AMINOACRIDINE

No. of fibers	9AA (mM)	E_m* (mV)	Depolarization (mV)
56		92 (85–100)	
15	0·05	82 (80–85)	10
19	0·10	77 (50–90)	15
33	0·25	67 (60–85)	25
14	0·50	25 (20–30)	67

* Membrane potential of surface fibers recorded 15 min after application of 9-aminoacridine. Muscles were bathed in hypertonic solutions. Values are expressed as averages with the range in parentheses.

9-Aminoacridine (0·05–1·0 mM) caused a depolarization of the fibers that depended upon the duration of exposure and the concentration of the drug applied. With the application of 1·0 mM 9AA, there was an almost immediate (within 30 sec) depolarization of all surface fibers to values ranging between 20 and 30 mV. When lower concentrations (0·1 mM) were applied, depolarization occurred after 10 min of exposure and increased in intensity for the next 20 min. There was usually no effect on the resting

membrane potential for the first 5–7 min. The extent of depolarization caused by graded concentrations of 9AA recorded 15 min after application of the drug is given in Table 1.

The depolarization caused by 9AA was reversed readily when exposure of the muscle to the drug was limited to 10 min or less. After that time, repeated washings for several hours resulted only in partial restoration of the resting membrane potential.

The mean resting membrane potential of 20 fibers in four muscles bathed for 2 hr in a sodium-free, chloride-deficient solution was 80·0 mV (70–85 mV). After 15 min of exposure to 0·5 mM 9AA, the mean resting membrane potential for 40 fibers of the same four muscles was 37·5 mV (20–55 mV).

Potassium fluxes. The elevated flux ratios (^{42}K efflux/^{42}K uptake; ϕ_o/ϕ_i) of muscles bathed in hypertonic, sucrose-containing and in sodium-free, chloride-deficient solu-

TABLE 2. UPTAKE AND EFFLUX OF ^{42}K IN FROG SARTORIUS MUSCLES TREATED WITH 9-AMINOACRIDINE

Muscle no.	9AA (mM)	^{42}K uptake (μmoles/g-hr)	Na_i (μmoles/g)	K_i (μmoles/g)	k* (l./hr)	^{42}K-efflux† (μmoles/g-hr)	ϕ_o/ϕ_i‡
			Hypertonic sucrose solution§				
127A-1		10·49	16·3	75·45	0·22	16·60	1·58
127A-2	0·10	10·14	18·7	93·08	0·09	8·37	0·82
127B-1		10·81	11·3	83·96	0·21	17·63	1·63
127B-2	0·10	9·50	16·6	95·81	0·10	9·58	1·01
120A-1		14·00	22·8	118·8	0·14	16·63	1·40
120A-2	0·25	10·64	26·6	108·4	0·04	4·33	0·41
121A-1		13·20	20·6	105·9	0·15	15·89	1·20
121A-2	0·25	8·00	20·6	107·8	0·05	5·39	0·67
120B-1		13·21	24·1	121·9	0·18	21·94	1·66
120B-2	0·50	7·86	21·6	122·2	0·04	4·89	0·62
121B-1		14·23	25·2	103·8	0·20	20·76	1·46
121B-2	0·50	9·38	22·7	123·4	0·04	4·94	0·53
114A-1			14·8	86·2	0·16	13·79	
114A-2	0·50		14·9	88·6	0·06	5·32	
114B-1			29·5	96·4	0·17	16·39	
114B-2	1·0		22·3	99·6	0·03	2·99	
			Sodium-free, chloride-deficient, isotonic solution				
23A-1		9·19	2·7	71·43	0·18	12·86	1·40
23A-2	0·25	5·39	4·0	78·95	0·04	3·16	0·59
23B-1		8·26	2·5	60·24	0·24	14·46	1·75
23B-2	0·25	4·73	5·0	52·22	0·06	3·13	0·66
33A-1		8·89	7·0	89·43	0·15	13·41	1·51
33A-2	0·25	4·98	5·5	103·07	0·03	3·09	0·62
33B-1		7·21	4·7	93·71	0·11	10·31	1·43
33B-2	0·25	3·97	4·6	98·86	0·02	1·98	0·50
			Sucrose-free, isotonic solution				
24A-1		11·72	17·6	93·47	0·14	13·09	1·12
24A-2	0·25	5·50	13·4	90·28	0·02	1·81	0·33
24B-1		9·93	17·8	82·32	0·11	9·06	0·91
24B-2	0·25	5·91	17·4	85·49	0·01	0·85	0·01

* Value for rate constant of loss from final period of efflux.
† ^{42}K efflux equal to k (μmoles/g).
‡ Flux ratio of efflux (ϕ_o) divided by uptake (ϕ_i).
§ See Methods for composition of solutions.

tions indicate that the muscles lost potassium ions at a greater rate then did muscles bathed in isotonic, sodium chloride-containing solutions (Table 2). In the case of the muscles in hypertonic solutions, the increased rate of loss of ^{42}K can be attributed to a decrease in the diameter of the fibers.[1,6] The muscles in sodium chloride-deficient solutions probably lost potassium because of the redistribution of chloride that occurred in the muscles upon deprivation of external chloride ions. The loss of intracellular chloride is accompanied by a loss of intracellular potassium. The differences in solutions notwithstanding, 9AA appeared to modify potassium fluxes of the several resting muscles in the same way.

Albeit depressed, the uptake of ^{42}K by muscles treated with 9AA was essentially linear during the 1 hr of uptake that was studied (Fig. 1). In this regard, the effects of

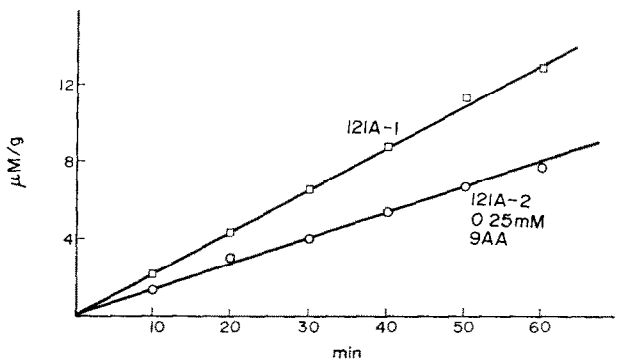

FIG. 1. Uptake of ^{42}K by frog sartorius muscle in the absence (121A-1) and presence (121A-2) of 9-aminoacridine (0·25 mM). The muscles were bathed in hypertonic sucrose solutions. Each point on the curves represents the uptake of potassium (μmoles/g) at the end of each measurement period

9AA on ^{42}K uptake were similar to those of TEA.[6] Unlike TEA, however, the concentration of 9AA required to block ^{42}K uptake was 10- to 20-fold lower than that of TEA. As shown in Table 2, a marked reduction of ^{42}K uptake occurred in all muscles treated with 0·25 to 0·50 mM 9AA, and a marginal reduction of uptake occurred in muscles treated with 0·1 mM 9AA.

A marked reduction in the rate constant of loss of ^{42}K (k) and the efflux of ^{42}K occurred in all muscles treated with 9AA (0·1 to 1·0 mM; Table 2). The time course of the depression of the rate constant of loss is illustrated in Fig. 2. In some experiments, attempts to reverse the effects of 9AA were made by washing the treated muscle with drug-free solutions. Repeated washing at 15-min intervals for 1 hr had no effect on the depression of the rate constant of loss caused by 0·25 to 1·0 mM 9AA. The effects of 9AA on ^{42}K efflux for paired muscles in the several solutions are given in Table 2. The reduction of k for muscles in which the values for rates of loss were obtained in the same muscle before and after 9AA are given in Table 3.

Sodium and potassium content. Changes in the electrolyte content of sartorius muscles at the end of the uptake–efflux sequence are presented in Table 2. The sodium content of muscles treated with 9AA (0·1 to 0·5 mM) for a total of 2·5 hr did not vary in any systematic manner. By contrast, the potassium content of muscles treated with 9AA tended to be greater than in the untreated member of the pair bathed in hypertonic

TABLE 3. EFFLUX OF ^{42}K BY MUSCLES TREATED WITH 9-AMINOACRIDINE

Muscle no.	9AA (mM)	k (l./hr)*	
		Before	During
120C-1	0·25	0·14	0·05
121C-1	0·25	0·15	0·05
120C-1	0·50	0·18	0·04
121C-1	0·50	0·20	0·05
114A-2	0·50	0·21	0·06
114B-2	1·0	0·17	0·03

* Control values of k are the average of three 15-min periods before the drugs were applied. The other values represent the last 15-min period of efflux during 60 min of exposure to the drug. The muscles were bathed in hypertonic, sucrose-containing solution.

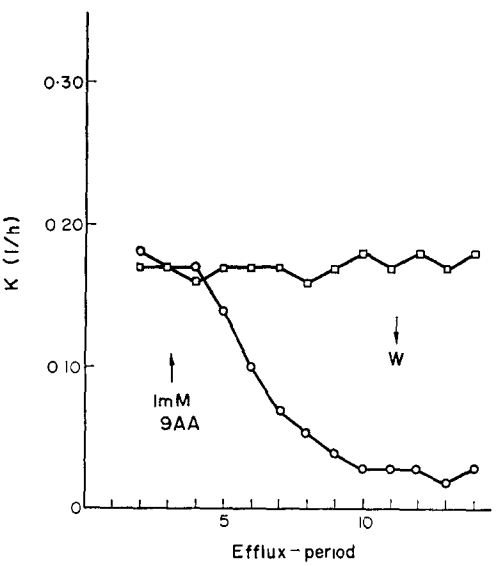

FIG. 2. Depression by 9-aminoacridine of rate constant of loss (k; l./h) in frog sartorius muscles bathed in hypertonic solutions. The k for the control muscle is designated by (□) and for the treated muscle by (○). The 9AA was added to the treated muscle after 4 efflux periods of 15 min each in drug-free solutions. The upward arrow indicates transfer of muscle to the drug-containing solution and the downward arrow indicates transfer of muscle to the drug-free wash (w) solution. The high values for k for the first period are due to washout.

solutions. Similar results were obtained with sartorius muscles soaked in hypertonic sucrose solutions for 3 hr, but not used for measurements of ^{42}K fluxes (Table 4).

DISCUSSION

The depression by 9AA of potassium movements in resting frog sartorius muscle fibers appears to be rather specific. The findings described above indicate that 9AA depressed potassium fluxes in muscles bathed in hypertonic sucrose solutions (low

TABLE 4. MUSCLE CONTENT OF POTASSIUM AND SODIUM AFTER 3 hr OF TREATMENT WITH 9-AMINOACRIDINE IN HYPERTONIC SOLUTIONS

Muscle no.	9AA (mM)	K (μmoles/g)	Na (μmoles)/g
1216A-1		70·09	14·09
1216A-2	0·25	83·88	13·28
1222A-1		99·49	20·53
1222A-2	0·25	101·84	21·28
1216B-1		66·13	12·03
1216B-2	0·5	102·30	10·58
1222C-1		105·19	23·35
1222C-2	0·5	126·26	23·46
1223A-1		101·99	17·10
1223A-2	0·5	111·52	15·22
1218A-1		90·50	11·61
1218A-2	1·0	105·40	11·37
1223B-1		94·40	20·93
1223B-2	1·0	98·68	20·79
1218B-1		92·42	13·30
1218B-2	2·5	101·60	11·12
1223C-1		94·62	17·64
1223C-2	2·5	100·22	20·82

chloride and sodium conductances but high potassium conductance), in isotonic solutions (high chloride conductance), and in sodium-free, chloride-deficient solutions. It is reasonable to conclude from these observations that the effects of 9AA on potassium fluxes were due to direct interaction with membrane processes involved with potassium movement and did not occur as secondary events related to changes either in chloride or sodium movements.

The findings in muscles soaked in sodium-free, chloride-deficient solutions militate against the possibility that the modification of active transport of potassium by mechanisms that couple sodium extrusion with potassium uptake were involved in the actions of 9AA. The coupled sodium extrusion–potassium uptake mechanism operates in frog sartorius muscles as a function of intracellular sodium.[3] When intracellular sodium is elevated, a maximum exchange of sodium and potassium occurs. However, when intracellular sodium is low, the exchange of sodium and potassium contributes little to the total movement of potassium in resting muscle fibers. The sodium content of muscle bathed in sodium-deficient solutions was reduced markedly. Nonetheless, potassium uptake and efflux were depressed by 9AA in a manner that was indistinguishable from that occurring in muscles containing normal or supranormal amounts of sodium.

By exclusion, it is proposed that 9AA impaired the uptake and efflux of ^{42}K by interfering with membrane sites involved in the transport of potassium by the resting muscle fibers. The mechanism whereby 9AA impaired potassium transport is not known. It is worth noting, however, that the dimensions of 9AA are comparable to those of hydrated potassium ions (radius of 4·45 Å) and TEA (radius of 4·6 Å).[11] 9AA is a compact, planar molecule with a surface area of 38 square Å.[7] In addition, 9AA exists almost completely (>99 per cent) in the ionized form at physiological pH. Accordingly, 9AA shares with TEA some of the physical and chemical characteristics

of hydrated potassium ions. For these reasons, TEA and 9AA are viewed as being capable of combining with membrane sites occupied by inorganic ions. Because of their similarity in size to potassium, TEA and 9AA can approach membrane sites for potassium, but are too small to make effective approaches to sites for sodium. Larger molecules such as tetrapropyl- and tetrabutylammonium ions appear to modify both the sodium and potassium movement in excitable tissues.[11,12]

Like TEA, 9AA appears to exert a preferential blocking action on the efflux of ^{42}K in the muscle. The decrease in the flux ratio for ^{42}K and the fact that the muscles treated with 9AA tend to lose less potassium than untreated muscles suggest a differential action of 9AA on potassium efflux and uptake. The reason for this differential action is unclear. It is not possible at this point to determine whether the differential effect reflects distribution of 9AA in the muscle fiber or potential asymmetry of potassium movement across the muscle membrane. It would be of interest to test the actions of 9AA in perfused axons where the inward and outward movements of potassium could be studied directly.

The present findings are somewhat paradoxical. Most agents that depolarize the muscle fiber cause a marked loss of potassium and an increase in the efflux of potassium.[12] Clearly, this was not the case for 9AA. 9AA depolarized the fibers, depressed potassium fluxes and retarded potassium efflux to the extent that an increase in internal potassium content occurred.

A possible explanation for the paradox could be the occurrence of anomalous rectification of potassium movements. In frog sartorius muscle, the potassium permeability falls when there is a net outward driving force on potassium ions. Anomalous rectification is observed over a range of depolarization up to about 20 mV,[13] which is in the range of depolarization produced by 0·1 to 0·5 mM 9AA. Such a rectification could therefore reduce efflux more than influx, and the observed effect of 9AA on fluxes might be secondary to its effect on membrane potential. However, it is unlikely that this possibility could account for the somewhat similar actions of TEA and barium ions on potassium fluxes in frog muscle.

TABLE 5. EFFECTS OF TETRAETHYLAMMONIUM, 9-AMINOACRIDINE AND BARIUM IONS ON FROG MUSCLE*

	TEA[5] (10 mM)	9AA (0·25 mM)	Barium[5] (0·25 mM)
Resting membrane potential	→	↓	↓
^{42}K-uptake (ϕ_i)	↓	↓	↓
^{42}K-efflux (ϕ_o)	↓	↓	↓
Flux ratio (ϕ_o/ϕ_i)	↓	↓	↑
K††	→	↑	↑
Na††	→	→	→

* Muscles bathed in hypertonic solutions.
† Micromoles/g of muscle.

A comparison of the actions of TEA, barium and 9AA on potassium movement and resting membrane potential is made in Table 5. Several differences should be noted among the compounds. First, barium and 9AA are effective in the same range of concentrations; TEA required a much higher concentration. Second, barium increased the flux ratio, whereas TEA and 9AA depressed the ratio. Barium depressed ^{42}K uptake more than it did efflux. Third, barium and 9AA depolarized the muscle fibers, whereas TEA (up to 40 mM) had no effect on the membrane potential. The reason for this difference is obscure. Finally, the fibers lost less potassium in the presence of 9AA and barium than did their untreated controls. There was no clear indication of any difference between TEA-treated muscles and untreated muscles, insofar as potassium content was concerned. Thus, in its actions on the muscle, 9AA appeared to be more closely related to barium ions than to TEA.

There is an additional aspect of 9AA that requires comment. Like other acridines, 9AA forms aggregates and micelles when the concentration exceeds 0·1 mM.[7] Obviously, this characteristic of 9AA complicates the evaluation of dose–response relationships, since the formation of large aggregates tends to maintain the concentration of the monomer at levels lower than those calculated. In addition, the formation of micelles has been shown to reduce the concentration of chloride ions in aqueous solutions by occluding or adsorbing the anion.[7] The occlusion of chloride by 9AA micelles does not appear to be a factor in the present study because of the low chloride conductance of muscles in hypertonic solutions and in chloride-deficient solutions. It could be a complicating factor, however, when 9AA is used to study ion movement in other tissues.

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