Brain Research, 169 (1979) 91–102

EFFECT OF TEA ON LIGHT EMISSION FROM AEQUORIN-INJECTED APLYSIA CENTRAL NEURONS

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(Accepted October 5th, 1978)

SUMMARY

Aplysia central neurons were injected with the calcium-sensitive photoprotein aequorin and stimulated with trains of identical depolarizing voltage-clamp pulses. The light emissions grew and the outward currents declined in successive pulses.

Tetraethylammonium (TEA) enhanced the light emissions to single depolarizing pulses and suppressed the outward current. The remaining net inward current is carried primarily by calcium ions and does not facilitate. The aequorin emissions were larger at all amplitudes of depolarizing pulses that elicited emissions, and the facilitation of emissions in a train of pulses was reduced. The effect of TEA on outward current was nearly maximal when sodium ions were partially replaced with 0.1 M TEA, while the aequorin emissions were further enhanced by increasing the TEA concentration to 0.459 M. TEA enhanced the aequorin emissions at all voltages. These observations suggest that the action of TEA on aequorin emissions is not strictly a consequence of its better known outward current blocking action. The effects of TEA could be partly due to the lowered sodium concentration of these solutions. Replacement of sodium by Tris, sucrose or mannose, however, all produced no enhancement of emissions. Tetramethylammonium (TMA) replacement of sodium had effects similar to those of TEA. Thus TEA and TMA appear to have a specific effect.

Part of the enhancement of light emissions by TEA is due to the removal of a series resistance error in the voltage clamp, and this may also account partly for the reduced facilitation of aequorin emissions in TEA. The remainder of the action of TEA on aequorin emissions evidently reflects a specific but previously unrecognized action on the cellular metabolism of calcium ions or on the voltage-dependent calcium channels.

INTRODUCTION

The cell bodies of gastropod molluscan central neurons exhibit inward calcium current in response to depolarization[16,17,22]. Calcium currents appear to influence the

shape of individual action potentials in these cells, as well as the temporal pattern of both elicited and spontaneously fired action potentials[8,15,20,27,28]. Moreover, the somatic calcium currents appear similar to those at nerve terminals responsible for triggering transmitter release[18]. Hence molluscan soma calcium currents may be useful as a model system for studying the properties of calcium channels relevant to synaptic transmission.

The quantitative study of calcium currents in molluscan somata by conventional ionic-substitution current-separation techniques has led to conflicting conclusions[8,23] and required interpretation of results obtained under conditions of drastic pharmacological modification. The use of intracellular calcium indicators, such as the photoprotein aequorin, has provided an alternate means of assessing calcium influx in molluscan somata[25].

Two recent studies of aequorin emissions from voltage-clamped molluscan neurons have identified an interesting phenomenon[10,19]. When identical, depolarizing voltage-clamp pulses are delivered in repetitive trains, a frequency-dependent facilitation, or progressive growth, of the emissions to successive pulses is observed. The facilitation of aequorin emissions occurs concurrently with frequency-dependent depression of the late outward voltage-clamp current[9,27]. These phenomena have similar time courses: they both occur maximally at frequencies of about 1 Hz, and they both reach steady-state after a few stimuli at 1 Hz. These similarities suggest that a common mechanism might underlie both phenomena.

One way that membrane currents and aequorin emissions might be related is through the effect of resistance in series with the cell membrane. Such a series resistance leads to an error in the control of the membrane potential under voltage clamp. For outward currents, the actual membrane potential will be less positive than the command potential by an amount equal to the product of the series resistance and the membrane current. Electrical measurements indicate that the magnitude of such errors may be considerable, in the order of several millivolts. Thus depression of outward current during a pulse sequence will be accompanied by a reduction in this voltage-clamp error, and hence the actual potential to which the membrane is clamped will become more positive. Facilitation of emissions could thus result from the increasingly positive potentials seen by the voltage-dependent calcium channels during the later pulses in a sequence. The present report describes one effort to test for such a mechanism.

Tetraethylammonium (TEA) has a well known effect of reducing outward potassium currents in many preparations. In sufficiently large doses TEA reduces the magnitude of the net membrane current in voltage-clamped gastopod neurons to a small fraction of normal values, and eliminates the outward current depression phenomenon virtually completely[21,26,27]. TEA should therefore eliminate any contribution of a series resistance error to the facilitation of aequorin emissions. In experiments designed to reveal such an effect, we found that TEA enhanced aequorin emissions and reduced their facilitation. The effects were greater than would be expected from the removal of a series resistance error, suggesting that TEA has an additional effect on calcium influx or its metabolism.

METHODS

Experiments were performed on identified neurons in the abdominal ganglion of *Aplysia californica*. In all experiments the ganglion was desheathed after a brief exposure to a few grains of pronase. Most often the ganglion was then pinned to a Sylgard-bottom Petri dish, and impaled with two microelectrodes filled with 3 M KCl and beveled to a resistance of 2–5 MΩ. In some experiments, small clumps of neurons were removed from the ganglion and the neuropil was undercut to remove all but the proximal 200–300 μm of their axons[6]. The voltage-clamping arrangement was similar to that described by Connor and Stevens[6,6a] except that membrane potential was recorded differentially with respect to an external potential-sensing electrode, and the modification described by Dionne and Stevens[7] was included to improve the control of membrane potential during long depolarizing pulses. Membrane current was measured using a virtual-ground current-to-voltage converter on the current-carrying bath electrode.

Neurons were microinjected with about 0.2–1.0 nl of a solution of 0.2 mM aequorin in 0.25 M K_2SO_4, 2 μM K_2EDTA (ethylenediamine tetraacetic acid) and 10 mM phosphate buffer at pH 7.5. Light emitted as a consequence of the reaction of calcium with aequorin was collected and transmitted by a fiber optic light guide to a photomultiplier tube (EMI 9789A) run at an anode voltage of 870 V. The anode current of the photomultiplier tube was measured using a current-to-voltage converter with a time constant of 30 msec. The anode current is proportional to the intensity of the light emitted by aequorin. For further details of our methods for the preparation, storage, assay and microinjection of aequorin, and of detecting light (see ref. 24).

All experiments were performed at a controlled constant temperature, which was usually 18 °C. Our basic medium was a conventional artificial sea water. In all other solutions, the osmotic pressure of the solution was matched to that of sea water by replacing NaCl with an osmotically equivalent amount of TEA-Cl, TMA-Cl (tetramethylammonium chloride), Tris (Tris (hydroxymethyl) aminomethane), mannose or sucrose. Table I lists the compositions of solutions. Solution changes consisted of exchanging 25 bath volumes of fluid, followed by a 20 min pause for temperature re-equilibration.

TABLE I

Composition of solutions

All concentrations are in mM. Roughly half the HEPES (4.7 mM) is present in anionic form; 95% of the Tris (458 mM) is in the form Tris·HCl, from which the Cl^- dissociates in solution. Both Tris and HEPES are adjusted to pH 7.5.

Solution	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	*HEPES*	*TEA*$^+$	*TMA*$^+$	*Tris*	*Mannose*	*Sucrose*
Artificial sea water	495	10	10	50	620	10					
0.1 M TEA SW	385	10	10	50	611	10	100				
TEA-sub 0-Na SW		10	10	50	584	10	459				
TMA-sub 0-Na SW		10	10	50	633	10		508			
Tris-sub 0-Na SW		10	10	50	583				482		
Mannose-sub 0-Na SW		10	10	50	125	10				844	
Sucrose-sub 0-Na SW		10	10	50	125	10					837

RESULTS

Our usual procedure to observe facilitation was to subject a voltage-clamped neuron filled with aequorin to a series of identical depolarizing pulses repeated at 1 Hz, a frequency which yielded a nearly maximal facilitation in Aplysia neurons. In most experiments, the holding potential was set in the range of —40 to —50 mV. The pulses were 0.3 sec in duration, and their amplitude was adjusted until easily detectable submaximal facilitating aequorin light emissions were obtained. Usually pulses to a membrane potential of + 15 mV (range + 2 to + 30 mV) produced the largest facilitation. Fig. 1A is a typical record from such an experiment. Two phenomena are immediately evident: (1) the aequorin emissions facilitate, that is, they grow in intensity to successive pulses in the series. The process reaches a maximum at about the fourth pulse, and emissions decline slightly thereafter; (2) the late outward currents undergo depression, that is, the outward currents flowing during successive responses are declining.

Fig. 1B depicts the effect of a medium containing 0.1 M TEA (isosmotically replacing sodium) on membrane currents and aequorin emissions from neuron R_2[11]. Net membrane current is drastically reduced, reversed in sign, and virtually constant over the pulse sequence. In some cells the inward currents decrease slightly in later

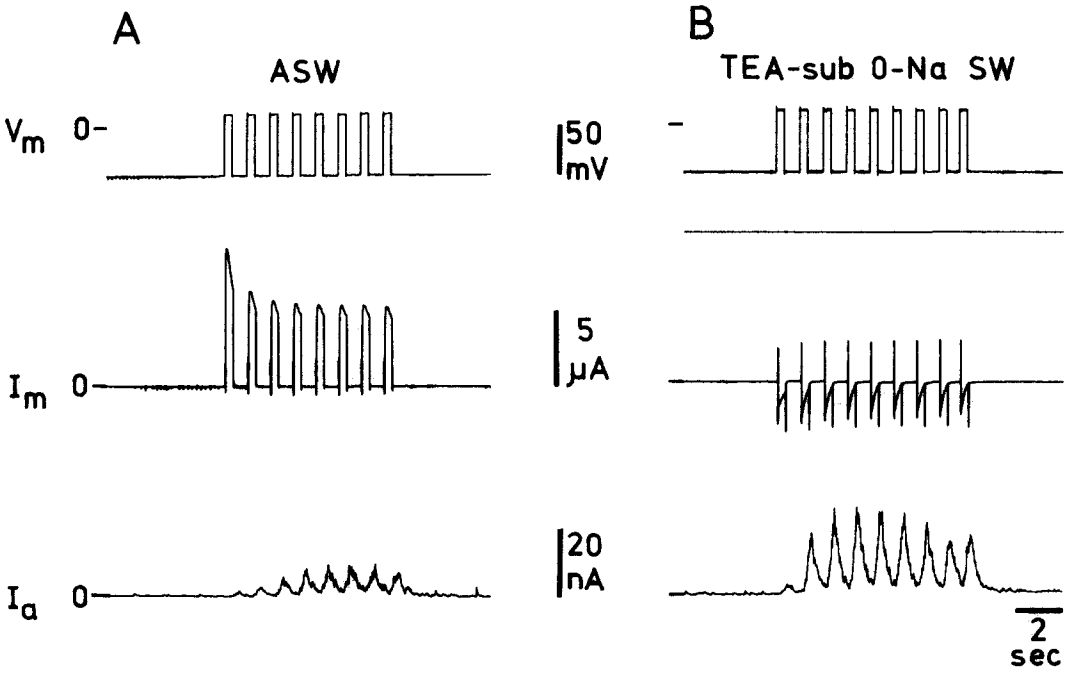

Fig. 1. Effect of TEA on membrane current (I_m) and aequorin emissions, measured as photomultiplier tube anode current (I_a), in cell R_2, where membrane potential (V_m) is voltage-clamped to a series of 0.3 sec depolarizing pulses at 1 Hz. A, sea water. B, TEA-substituted O-Na sea water. I_a filtered through a 40 msec time-constant. Temperature, 18 °C.

pulses, probably due to a summating slowly gated potassium current activated by calcium influx and not completely blocked by TEA[26]. Series resistance errors cannot contribute to aequorin facilitation under these conditions, yet it is apparent that the fractional facilitation during sequences in normal and TEA media is nearly the same. An unexpected but much more striking effect of TEA treatment is the increase in the absolute size of each emission in the sequence. In other cells studied (see Table II), TEA produced rather more of a reduction in facilitation. In every cell, TEA increased the aequorin light emissions, whether induced by brief (0.3 sec) depolarizations to about + 15 mV, or by a longer (1–2 sec) pulse of smaller magnitude. The similarity of the facilitation patterns in normal and TEA media, as shown in Fig. 1, strongly suggests that the facilitation in TEA reflects the same major process responsible for the facilitation in normal medium. We therefore conclude that depression of outward current and consequent series resistance error variation is not the major cause of aequorin facilitation under normal conditions.

In other experiments, the effects of TEA at two different concentrations, 0.1 M and 0. 46 M (totally replacing sodium) were compared. The results suggest that TEA's

TABLE II

Effects of TEA and TMA on aequorin emissions and membrane currents

I_a is the peak photomultiplier anode current corresponding to an aequorin emission. F is the facilitation of aequorin emissions, measured as the fractional increase in light emission of the fourth pulse compared to the first pulse in a 1 Hz train of 0.3 sec pulses. I_m is the net membrane current (positive outward) at the end of a pulse. In some cases, figures are the averages of measurements, up to N = 9. ASW, artificial sea water.

Cell solution	*1 Hz train of 0.3 sec pulses*					*1 or 2 sec pulse*	
	I_a *(nA)*		*F*	I_m *(μA)*		I_a *(nA)*	I_m *(μA)*
	1st pulse	*4th pulse*		*1st pulse*	*4th pulse*		
L_4 ASW						3.9	0.55
TMA-sub 0-Na SW						5.4	0.47
ASW	0.6	1.8	2.0	1.3	1.1	2.7	0.53
TEA-sub 0-Na SW	1.4	3.2	1.3	—0.80	—0.50	6.9	—0.05
R_{15} ASW	2.0	5.6	1.8	3.6	0.8	6.0	0.6
TMA-sub 0-Na SW	4.2	6.0	0.43	0.8	0.1	8.0	0.1
ASW	0.8	3.3	3.1	1.8	0.4	3.4	0.4
ASW	0.4	4.0	9.0	2.0	0.4	3.2	0.4
0.1 M TEA SW	1.5	2.4	0.6	—0.14	—0.14	4.0	—0.12
TEA-sub 0-Na SW	2.6	2.6	0	—0.20	—0.20	5.8	—0.20
0.1 M TEA SW	2.0	2.0	0	—0.15	—0.15	6.0	—0.13
ASW	2.6	2.6	0	0.20	0.18	3.5	0.12
R_2 ASW	1.2	7.8	5.5	8.5	5.5		
0.1 M TEA SW	5.0	30	5.0	0.5	0.4		
ASW	2.5	15	5.0	9.8	6.4		
0.1 M TEA SW	3.3	16	3.9	0.0	0.4		
L_4 ASW	2.0	3.3	0.67	2.2	0.8		
TEA-sub 0-Na SW	6.5	7.2	0.11	—0.05	—0.05		

enhancement of the peak aequorin emission and reduction of facilitation are both dose-dependent, larger effects being observed in the higher TEA concentration. This finding also indicates that TEA treatment has an additional effect independent of outward current suppression, since the observed effects of TEA on clamping current are already nearly maximal at the 0.1 M dose.

It seemed possible that the effects of TEA on aequorin emissions might be due to a leakage of TEA into the cell and a specific interaction between these ions and the calcium-aequorin reaction. To test this possibility, we followed a procedure described in detail elsewhere[29] and injected aequorin into a cytoplasm-like buffer containing 10 μM free calcium and in some cases TEA-Cl. The intensity of light emitted was actually reduced to about 25% in the presence of 100 mM TEA, while it was unaffected by 10 mM TEA. It is unlikely that the intracellular TEA concentration reaches even 10 mM. In any case, the only effect of even 100 mM TEA is to inhibit the aequorin emission evoked by calcium. Thus it is unlikely that the enhanced emissions of aequorin-filled neurons in TEA are due to a chemical interaction with aequorin.

Since neuronal calcium metabolism is believed to depend on the electrochemical gradient for sodium ions[2], it seemed that the TEA effect on aequorin emissions could be partly due to the reduction in sodium concentration, or its omission from the sea water. We attempted to test this idea by recording the effects on the magnitude of the aequorin emission and its facilitation of replacing all of the sodium in the sea water with TMA, a substance which does not permeate sodium channels and is without effect on potassium channels, at least at the frog node[13]. To our surprise, we found that TMA-substituted 0-Na sea water sometimes reduced the net outward current at the end of a 0.3–1.0 sec, +5–+15 mV depolarizing pulse to as little as 13% of its value in sea water. This preliminary result suggests that in Aplysia neurons TMA (Eastman) applied extracellularly has a weak blocking action on the potassium currents. The data in Table II show that TMA enhanced the aequorin emissions. In one cell (L_4), where TMA had essentially no effect on outward currents, a considerable enhancement of emissions was nonetheless observed. In another cell (R_{15}) a similar enhancement was observed, the facilitation of aequorin emissions in a train was reduced, and the late outward current was suppressed by 87%. It appears that TMA may cause enhancement of aequorin emissions apart from any effect secondary to outward current suppression, but the variability of our results prohibits any firm conclusion at this time.

In a further effort to ascertain what effects on emissions might result simply from the removal of sodium ions, we examined the effects of solutions in which all sodium was replaced by Tris, mannose or sucrose. The results of these experiments are collected in Table III, which shows that none of these sodium substitutes ever increased the aequorin response or reduced significantly their facilitation. The results with sucrose and mannose are complicated by the reduction in outward current in these solutions. This is probably caused by an increase in series resistance experienced in non-electrolytes. For this reason the results of experiments with Tris-substituted zero-sodium sea water, where the conductivity of the solution is the same as that of sea water, are probably more significant. Here, there was little effect on the outward

TABLE III

Effects of sodium removal on aequorin emissions and membrane currents

Cell solution	*0.4 or 1 Hz train of 0.3 sec pulses*					*1 or 2 sec pulse*	
	I_a *(nA)*		F	I_m *(μA)*		I_a *(nA)*	I_m *(μA)*
	1st pulse	*4th pulse*		*1st pulse*	*4th pulse*		
R_B ASW	1.6	3.2	1.0	1.8	1.6		
Tris-sub 0-Na SW	1.6	3.2	1.0	1.6	1.4		
L_4 ASW						4.6	0.90
Tris-sub 0-Na SW						4.0	0.86
ASW						5.1	1.01
R_2 ASW	2.0	15	6.5	7.2	4.0	35	2.3
Tris-sub 0-Na SW	2.0	14	6.0	6.6	4.5	30	3.4
ASW	3.0	26	7.7	10.0	7.4	38	5.0
ASW	4.0	16	3.0	14.5	8.5	32	5.2
mannose-sub0-NaSW	3.0	13	3.3	3.5	3.0	30	3.0
ASW	2.5	15	5.0	9.8	6.4	24	5.0
R_{15} ASW	2.8	6.2	1.2	8.6	4.4	5.8	6.8
sucrose-sub 0-Na SW	1.3	2.5	0.9	2.2	1.9	3.0	2.3

potassium current during the depolarizing pulses, and there was again no consistent effect on aequorin emissions. The effects of TEA and TMA on aequorin emissions therefore seem specific to these substances, and not due to the removal of sodium ions.

The aequorin emission has been shown to be dependent on the size of the depolarization[10,19], and to go through a maximum at about +30 – +70 mV in marine molluscs. The facilitation of aequorin emissions is also voltage-dependent, and is maximal for moderate depolarizations of about +10 – +50 mV[10,19]. Thus a positive shift in the effective membrane potential seen by the calcium channels could both enhance emissions and reduce facilitation. Such a shift in membrane potential could arise from differential effects of TEA or TMA and sodium ions on the membrane surface potential. The reduction of a series resistance error in TEA and TMA would have a similar effect at voltages where large currents would otherwise flow.

We examined this idea by measuring the voltage-dependence of aequorin emissions in sea water and TEA-substituted O-Na sea water. As illustrated in Fig. 2, we found that emissions were larger in TEA for depolarization to any level between —5 and +70 mV. Furthermore, the experiment summarized in Table IV showed that the reduction of facilitation seen in TEA could not be significantly restored by reducing or increasing the size of the depolarizing pulses in a train. The effects of TEA are therefore clearly not due simply to any shifts in the effective membrane potential.

DISCUSSION

We have considered several possible mechanisms by which TEA could affect aequorin emissions.

(1) There is an uncompensated series resistance in our voltage-clamp preparation. We measured this resistance by the current-step method[4,14]. The series resistance

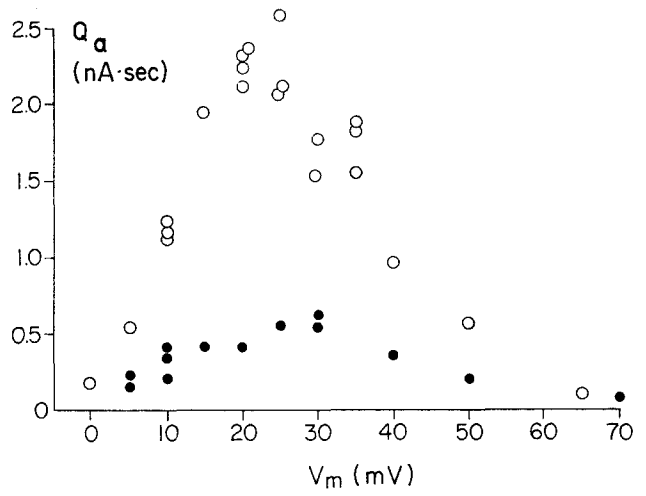

Fig. 2. Effect of amplitude of 0.3 sec depolarizing pulse (Vm) on time-integrated aequorin emission magnitude (Q_a), in sea water (●) and in TEA-substituted O-Na sea water (○). Axotomized cell L_4. Temperature, 18 °C. Use of integrated aequorin responses reduced the scatter in the data.

of our cells averaged about 1.5 KΩ and this could influence the TEA results in the following manner: The average outward current during a 0.3 sec depolarization to about +15 mV in our cells was about 1—5 μA in sea water. This drops to about —0.1 μA in TEA-substituted 0-Na sea water. This effectively removes an error in voltage-clamp control of about 1.5—7.5 mV, boosting the actual membrane potential by this amount for a given command pulse. Since most of our results were obtained with pulse potentials in a range where greater depolarizations lead to greater emissions, the outward current blocking effect of TEA should lead to enhanced emissions, as we have observed. The results shown in Fig. 2, however, strongly suggest that TEA is affecting emissions in some other way besides merely reducing series resistance errors: series resistance should not greatly alter the magnitude of the maximal emission, but only the apparent membrane potential at which that emission is observed.

TABLE IV

Effects of depolarization magnitude on aequorin emissions and membrane currents in TEA

V_m is the potential to which the membrane was clamped in a 1 Hz train of 0.3 sec pulses.

Cell solution	*V_m (mV)*	*I_a (nA)*		*F*	*I_m (μA)*	
		1st pulse	*4th pulse*		*1st pulse*	*4th pulse*
R_{15} ASW	+15	0.8	3.3	3.1	1.8	0.4
TEA-sub 0-Na SW	+15	2.6	2.6	0	—0.15	—0.15
TEA-sub 0-Na SW	+10	1.0	1.0	0	—0.10	—0.10
TEA-sub 0-Na SW	+13	1.2	1.2	0	—0.12	—0.12
TEA-sub 0-Na SW	+15	1.6	2.0	0.25	—0.15	—0.15
TEA-sub 0-Na SW	+17	3.0	3.5	0.17	—0.17	—0.17
TEA-sub 0-Na SW	+20	3.8	6.1	0.62	—0.18	—0.18
TEA-sub 0-Na SW	+25	7.8	7.8	0	—0.19	—0.19

Our results also indicate that facilitation of aequorin emissions cannot be due entirely to a series resistance error in voltage control which is reduced for depressed outward current. Our major evidence against this origin of facilitation of aequorin emissions comes from two cells (Fig. 1 and Table II) in which facilitation is still prominent in TEA, where membrane currents are nearly constant. Nevertheless, we cannot discount series resistance errors from contributing in part to the process of facilitation. For example, in the cell studied in Fig. 2, the series resistance was measured as 2.0 KΩ. The depression of outward current from 2.2 μA to 0.8 μA by the 4th pulse in a train would result in a 2.4 mV increase in actual membrane potential during a pulse. From Fig. 2, this would result in a 12% increase in aequorin emissions, or a facilitation of 0.12. This is much less than the facilitation observed in this cell in sea water (0.67). Thus we feel that series resistance effects are responsible for only a small portion of facilitation of aequorin emissions in this cell. Similar analyses on other cells suggest that series resistance errors contribute a variable amount to facilitation but can never account entirely for the phenomenon.

(2) Another potential source of error in voltage-clamp control is a possible inhomogeneity of membrane potential in Aplysia cells, whose surface membranes are highly convoluted[12]. Like errors due to external series resistance, this source of error would be reduced by TEA, because the intracellular currents leading to the inhomogeneity would be reduced in TEA. However, our results with TMA, in which aequorin emissions were increased, in one case with little change in clamp current, suggests that a reduced voltage inhomogeneity is not primarily responsible for TEA's (and TMA's) enhancement of aequorin emissions.

(3) TEA may somehow result in a shift in the resting internal free calcium concentration which would affect the response of aequorin to a given calcium influx. Aequorin is known to respond in a highly non-linear fashion to the concentration of calcium[1]. To a first approximation, the aequorin light-emission follows a 2.5 power-law dependence on calcium, above a minimum calcium-independent level of emission. Thus aequorin has a threshold, with no sensitivity to changes in calcium below a certain level. In cytoplasm, with its high magnesium concentration and ionic strength, the threshold may be above 1 μM (Fig. 3)[1,5]. Noise in the detection system will raise this threshold even higher. Suppose that TEA increased the resting calcium concentration from less than 0.1—1.0 μM. Then an incremental influx of calcium might raise the local submembrane calcium concentration to 2 μM in the absence of TEA, and to 3 μM in its presence. Thus the aequorin emission in the presence of TEA would be enhanced without any change in calcium influx during a depolarization.

At present it is not clear how TEA and TMA could cause an increase in internal free calcium. Initially we imagined that the removal of sodium ions would block the membrane sodium-calcium exchange pump[2]. This should result in an increased internal free calcium; consequently the aequorin emission to a given calcium influx would increase. However, our results with sodium-free sea water using Tris, sucrose and mannose did not show such an increase in emissions. The relatively low effectiveness of TMA-substituted zero-sodium sea water and the high effectiveness of 0.1 M TEA in which only 22% of the sodium has been replaced are also not consistent with

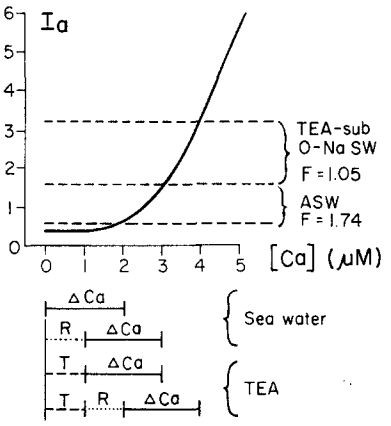

Fig. 3. Hypothetical model for facilitation and effects of TEA. The calcium-dependence of aequorin emissions (I_a, arbitrary units) represented as a 2.5 powerlaw function of calcium concentration, with a calcium-independent emission level corresponding to about 1.8 μM calcium. Constant increments of calcium on the abscissa (ΔCa) are shown in the presence of a residual calcium (R) after a previous influx, leading to a facilitated emission, and in the presence of an increased calcium in TEA (T), leading to an increased emission. The facilitation (F) is reduced. The values of R, T and Ca were chosen to account for typical results (Table II). Different values would be needed to account for any particular experiment (e.g., Fig. 1).

the idea that TEA's effects are due simply to a block of the calcium pump by sodium removal.

It might even be supposed that facilitation of aequorin emissions works in this same manner. That is, a constant increment of calcium could elicit larger emissions as a consequence of a residual subthreshold increase in submembrane calcium. If this phenomenon is superimposed on an increase in internal free calcium in TEA, then facilitation would be reduced in TEA (see Fig. 3). If this model is correct, one might expect to observe tails of emissions at the time of a facilitated emission, especially in TEA. Such tails are almost always observed (Fig. 1 and refs. 10 and 19). If the frequency of depolarizing pulses is slowed so that these tails have fully decayed, facilitation is rarely observed. In this model, facilitation is primarily a consequence of the non-linearity of the aequorin-calcium reaction, and in particular the largely undetectable residual calcium, which was not considered in earlier discussions of this problem[10,19]. The calcium current itself may not facilitate at all. That would explain why the persistent net inward currents observed in TEA-substituted sodium-free sea water (Fig. 1), which have all the properties of the inward calcium currents described by others[5,8], fail to facilitate.

(4) TEA could equally well affect the metabolism of calcium entering a cell during a depolarization. Calcium is partitioned between several intracellular compartments[2,3], including mitochondria, liposomes, cytoplasmic binding sites, and cytoplasmic free calcium. Moreover, a membrane pump operates to extrude calcium from cytoplasm. If TEA were to affect any of these elements, the proportion of calcium entering a cell that would be available to react with aequorin would change. Changes in such factors also could be involved in the facilitation of aequorin emissions to successive depolarizations.

(5) TEA could influence directly the calcium influx through voltage-dependent membrane channels.

It is impossible to say at this time to exactly what extent these various factors are involved in the effects of TEA and in the phenomenon of facilitation. Our results suggest that only a part of the growth of aequorin emissions in a train and the enhancement of emissions by TEA are due to a series resistance effect. Further experiments are needed to determine quantitatively the contributions of these and other processes, such as undetected calcium accumulation and its effect on aequorin emissions.

ACKNOWLEDGEMENTS

We are especially grateful to Dr. O. Shimomura for his generous gift of purified aequorin. We would like to thank Stuart Thompson, Peter Getting, John Connor, and Carl Scheffey for valuable suggestions.

This research was supported by NSF Grant BNS 75-20288. S.J.S. is a Miller Research Fellow and R.S.Z. is an Alfred P. Sloan Research Fellow.

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