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PRIMARY AND SECONDARY REGULATION OF QUANTAL TRANSMITTER RELEASE: CALCIUM AND SODIUM

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SUMMARY

Calcium is the prime regulator of quantal acetylcholine liberation at the neuromuscular junction; its entry through the presynaptic membrane and the level of free $[Ca]_{in}$ most probably determine the number of transmitter quanta liberated by the nerve impulse. The level of free $[Ca]_{in}$, in turn, is controlled by a number of subcellular elements: mitochondria, endoplasmic reticulum, vesicles, macromolecules and the surface membrane.

The action potential induced calcium entry is not the only factor responsible for coupling nerve terminal depolarization with increased transmitter release; increased transmitter release occurs also in the virtual absence of calcium ions in the extracellular medium, when a reversed electrochemical gradient for calcium probably exists during action potential activity. Several lines of evidence suggest that the entry of sodium ions is responsible for this augmented transmitter release: the tetanic potentiation observed under reversed calcium gradient is blocked by tetrodotoxin; tetanic and post-tetanic potentiation are augmented and prolonged by ouabain; the amplitude of the extracellular nerve action potential is reduced with high-frequency stimulation, in parallel with increased spontaneous quantal release. In addition, sodium-filled egg-lecithine liposomes augment quantal liberation.

The augmentory effect of sodium on transmitter release is probably due to an intracellular calcium translocation, since no preferred timing after the action potential is observed. Thus the level of $[Na]_{in}$ in the presynaptic nerve terminal can control indirectly the efficiency of synaptic transmission.

INTRODUCTION

Since the fundamental works of Eccles, Katz & Kuffler (1941, 1942) the neuromuscular junction has subserved as the model system for understanding excitatory chemical synaptic transmission. It was at the neuromuscular junction that most of the basic properties of the post-synaptic membrane were elucidated (Fatt & Katz, 1951; Katz & Thesleff, 1957; Takeuchi & Takeuchi, 1960; Katz & Miledi, 1972; Magleby & Stevens, 1972; Hartzell, Kuffler & Yoshikami, 1975; Neher & Sakmann, 1976). Similarly, many of the cellular processes involved in the quantal transmitter liberation were described originally at the motor nerve endings (Fatt & Katz, 1952; Del Castillo & Katz, 1954*a*, *b*; Dudel & Kuffler, 1961; Katz & Miledi, 1965, 1967; Kuffler & Yoshikami, 1975). In this article we briefly review the factors involved in regulation of quantal transmitter release at the neuromuscular junction and then dwell, in somewhat more detail, on the primary role of calcium and the secondary role of sodium in this process.

QUANTAL RELEASE OF TRANSMITTER

Quanta are liberated at rest at a frequency of approximately 1 s^{-1}. Since each quantum consists of about 10^4 molecules of acetylcholine (Kuffler & Yoshikami, 1975) and the action lasts merely 1 ms it can be detected only by its post-synaptic action – the miniature end-plate potential (m.e.p.p.). The arrival of the action potential at the motor nerve terminal greatly increases the rate of quantal liberation and several hundred quanta are released within 1 ms. This more than 10^5-fold increase in the probability of quantal liberation produces a large post-synaptic depolarization, the end-plate potential (e.p.p.), which when suprathreshold causes an action potential in the muscle and a twitch.

The current view of the processes involved in evoked transmitter release can be summarized as follows: the invasion of the nerve terminal by the action potential causes a depolarization of the presynaptic membrane and this depolarization leads to an opening of the calcium channels; since the concentration of free calcium ions is at least 10^4 higher in the extracellular medium than inside the cell (Baker, 1976), the increase in calcium permeability leads to a calcium influx and to an increase in $[Ca]_{in}$ that in turn triggers quantal transmitter release. (For a review on the underlying processes and on the open questions see Rahamimoff, 1979; Rahamimoff, Lev-Tov, Meiri, Rahamimoff & Nussinovitch, 1980.)

Release of transmitter is a stochastic process and the number of acetylcholine quanta liberated fluctuates from trial to trial. The mean number of quanta released by a nerve impulse (quantal content, m) is modified physiologically by two main factors: the frequency of activation of the nerve terminal (Del Castillo & Katz, 1954*b*) and the degree of presynaptic depolarization (for example, in presynaptic inhibition – Dudel & Kuffler, 1961).

CALCIUM IONS AND TRANSMITTER RELEASE

The finding that an entry of calcium through the presynaptic membrane and the resulting increase in $[Ca]_{in}$ are presumably responsible for evoked transmitter release (Katz & Miledi, 1969; Baker, Hodgkin & Ridgway, 1972; Llinas & Nicholson, 1976) focused the attention on two interrelated problems. First, which are the subcellular structures and organelles participating in the regulation of intracellular free calcium? Second, is there an interaction between calcium influx and steady state $[Ca]_{in}$ in the regulation of transmitter release?

(1) *The surface membrane*

The surface membrane participates in at least three different processes that can modify $[Ca]_{in}$:

(*a*) *Voltage-dependent calcium channels.* These channels open on depolarization and calcium ions flow along their electrochemical gradient. Under normal conditions, the concentration of calcium outside the cells is approximately 10^{-3} M and the free $[Ca]_{in}$ is about 10^{-7} M, and there is therefore a very large inward chemical gradient. In addition, there is the membrane potential that when negative inside creates an additional inward driving force for calcium ions. In the squid, two types of voltage

dependent calcium channels have been described (Baker *et al.* 1971). The first is similar (or identical) to the sodium channel and is blocked by tetrodotoxin. The second channel is blocked by a variety of ions such as Mn^{2+}, Co^{2+}, Mg^{2+} in a way which is pharmacologically similar to the calcium activation of quantal acetylcholine liberation at the neuromuscular junction (Dodge, Miledi & Rahamimoff, 1969; Meiri & Rahamimoff, 1971; Weakly, 1973). The voltage-dependent calcium channels are obviously the main determinants of evoked transmitter release.

(*b*) *Na^+–Ca^{2+} exchange.* For the nerve terminal $[Ca]_{in}$ to remain in a steady state over long periods of time the entry of calcium through the surface membrane must be followed by an efflux. Part of the efflux takes place through the Na^+–Ca^{2+} exchange, where the extrusion of calcium ions is coupled by the entry of sodium ions (Baker, Blaustein, Hodgkin & Steinhardt, 1969). Thus, the nerve terminal utilizes the inward sodium electrochemical gradient to transport calcium against its own very large gradient. For this process to occur no intracellular components are needed, since recently it was possible to demonstrate Na^+–Ca^{2+} exchange in membrane vesicles isolated from rat brain synaptosomes (Rahamimoff & Spanier, 1979).

(*c*) *ATP-driven calcium extrusion.* Calcium can be extruded from nerve cells not only at the expense of the sodium gradient, but also by an ATP-driven process (DiPolo, 1978; DiPolo & Beaugé, 1979). Since ATP-dependent calcium pump has been studied extensively in erythrocytes (see Schatzmann & Bürgin, 1978), it will be of interest to see whether RBC regulatory molecules (such as calmodulin) act also at the synapse.

The total calcium concentration inside nerve terminals is several orders of magnitude larger than the free $[Ca]_{in}$. It is therefore of considerable interest to identify the intracellular structures involved in calcium uptake and release and to study their properties. Obviously, a release of calcium from intracellular stores can elevate the level of free $[Ca]_{in}$; if transmitter liberation responds to $[Ca]_{in}$ and not only to the influx, then intracellular calcium translocation should affect neurotransmitter outpour. Release of calcium is expected to augment acetylcholine liberation; conversely, an increased uptake by intracellular structures can lower $[Ca]_{in}$.

(2) *Mitochondria*

One of the obvious candidates for participation in the regulation of $[Ca]_{in}$, and thus in transmitter release, is the mitochondrion. In a large variety of cells it has been shown that mitochondria are capable of taking up calcium ions against very large concentration gradients (Lehninger, 1970).

Several lines of evidence suggest that mitochondria are important in regulating transmitter release at the neuromuscular synapse. First, the mitochondria are abundant in the nerve terminal; in some cross-sections they occupy up to 40% of the cross-sectional area, while the average mitochondrial area is about 7%. Secondly, in isolated presynaptic nerve terminals (synaptosomes), up to 80% of the intracellular calcium is accounted for by the mitochondria. Finally, inhibition of the mitochondria and their calcium uptake causes, as expected, an increase in spontaneous and evoked transmitter release (Rahamimoff, Erulkar, Alnaes, Rotshenker, Meiri & Rahamimoff, 1976).

Before accepting the generality of mitochondrial involvement in neuronal intra-

cellular calcium regulation, it is worthwhile to examine the experimental evidence in the squid. There, only about 1 % of the cross-section is occupied by mitochondria (DiPolo, 1973) and the initial direct measurements of changes in $[Ca]_{in}$ following mitochondrial inhibition did not reveal any substantial increase in the absence of $[Ca]_o$ (Requena *et al.* 1977). However, recent elegant experiments of Mullins & Requena (1979) showed a definite increase in the submembranal $[Ca]_{in}$ after inhibiting the mitochondria.

There are at least two possible ways of involvement of the mitochondrion in $[Ca]_{in}$ and in transmitter release. The first is a transient; one can envisage that the arrival of the action potential at the presynaptic nerve terminal signals the mitochondrion to release calcium which in turn activates transmitter release. Such a hypothesis does not seem very plausible in view of the distance of several hundred nanometers between the mitochondria and the active sites where transmitter release occurs. For a signal (and for calcium) to cover this distance by diffusion, a much longer time period is needed than the several hundred microseconds synaptic delay (Katz & Miledi, 1965) between the arrival of the action potential and the evoked quantal transmitter release. An alternative involvement of the mitochondrion is by changing the level of $[Ca]_{in}$.

Although the mitochondria are very important elements in intracellular calcium sequestration, there are certainly other participants in the regulation of $[Ca]_{in}$. This was shown convincingly in synaptosomal preparations (Blaustein, Ratzlaff & Kendrick, 1978) where ATP-dependent calcium uptake persists even after a very substantial inhibition of the mitochondria by a number of metabolic poisons. There are several extramitochondrial calcium buffers.

(3) *Vesicles*

Early morphological studies by Politoff, Rose & Pappas (1974) showed that the vesicles of nerve terminals are able to bind calcium when exposed to calcium concentration in the range of 5–90 mM. Later, Blitz, Fine & Toselli (1977) demonstrated that coated vesicles are effective in calcium uptake. Recently it was shown that a vesicular fraction of rat brain synaptosomes is capable of concentrating calcium at least 400 times by a process utilizing ATP. These vesicles operate at a low range of [Ca] (0·1 μM was the lowest concentration examined), suggesting that they can be of importance in the normal physiological control of $[Ca]_{in}$ (Rahamimoff & Abramovitz, 1978*a*). The process of ATP-depedent calcium uptake in the vesicles is coupled by a Mg^{2+}-activated ATPase (Rahamimoff & Abramovitz, 1978*b*). Recently the calcium transporter was purified and incorporated into artificial lipid vesicles (Papasian, Rahamimoff & Goldin, 1979). In these experiments a functional reconstruction of the calcium transporter was achieved, and analysis of the proteins participating in calcium transport showed two components with molecular weights of about 90000 and 140000 daltons.

(4) *Endoplasmic reticulum*

Electron-probe analysis enabled Henkart, Reese & Brinley (1978) to show that the endoplasmic reticulum inside axons is capable of concentrating calcium. Enzymic markers do not yet have the necessary specificity to show whether the endoplasmic reticulum contributes to the vesicular calcium uptake described in the previous section.

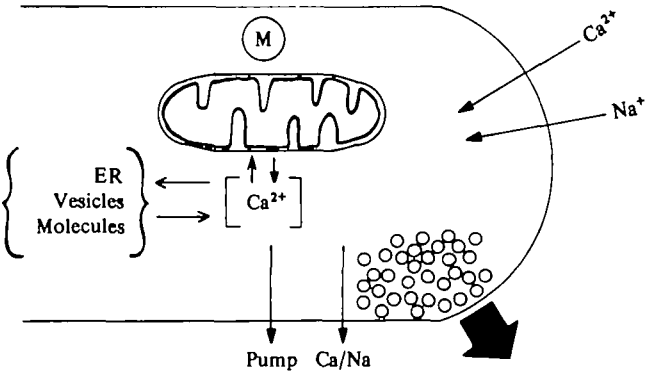

Fig. 1. Schematic representation of the $[Ca]_{in}$ controlling processes at the presynaptic nerve terminal (see text).

(5) *Cytosolic molecules*

In addition to subcellular organelles, $[Ca]_{in}$ can be regulated by a wide variety of cytoplasmic molecules. Some of them can bind calcium directly such as ATP and calcium binding proteins and thus affect its concentration. Other molecules can activate or inhibit one of the already mentioned calcium controlling processes. Of special interest are the calcium binding modulator proteins, which can activate and control a large number of calcium dependent enzymes (Larsen & Vincenzi, 1979; Niggli *et al.* 1979; Cheung, 1980).

A diagram of the various known processes taking part in the regulation of intracellular calcium and thus of transmitter release is shown in Fig. 1. We can see that the free $[Ca]_{in}$ is a very tightly controlled variable. Free $[Ca]_{in}$ is only a very small fraction of the total intracellular calcium. Each of the controlling components may have a calcium content exceeding the amount found in a free form. A modification in one of the processes will result in a substantial net increase in $[Ca]_{in}$ only after a saturation of the calcium-binding capacity of the remaining processes. Hence, knowledge of the calcium-binding capacity and of the kinetic parameters is a prerequisite for any worthwhile kinetic model of regulation of $[Ca]_{in}$ and transmitter release.

LIPOSOMES

The nerve terminals at the frog neuromuscular junction are too small to be impaled successfully with a microelectrode. This precludes the possibility of a direct intracellular injection of calcium. Such an experiment is of a substantial interest since it would have allowed the examination of the question of whether changes in $[Ca]_{in}$ affect evoked transmitter release. Fortunately one can bypass this experimental difficulty by using ion-containing liposomes. In a number of cells it has been shown that such artificial phospholipid vesicles are able to transfer their content to the cell interior by fusion with the cell membrane or by endocytosis.

Fig. 2 shows that liposomes containing calcium ions are able to increase both evoked and spontaneous quantal transmitter release (Rahamimoff, Meiri, Erulkar & Barenholz 1978). This effect is not due to some unspecific effect of the liposomes on the presynaptic membrane, since liposomes containing KCl only do not produce

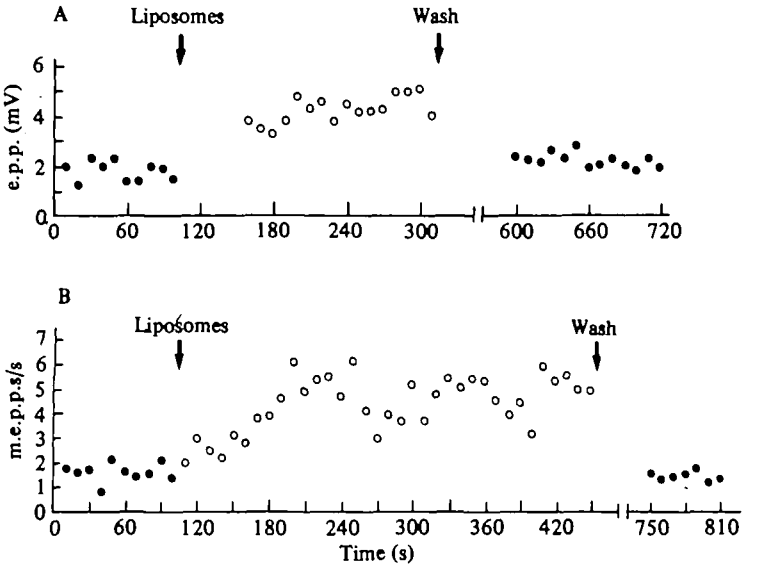

Fig. 2. Changes in transmitter release by calcium containing phosphatydylcholine liposomes. (A) Evoked transmitter release. Since the m.e.p.p. amplitude did not change significantly after the addition of the liposomes, the end-plate potential (e.p.p) amplitude is a reliable measure of the mean number of quanta released by the nerve impulse. (B) Spontaneous transmitter release.

these changes (Barenholz, Erulkar, Meiri & Rahamimoff, 1978). These experiments strongly suggest that the evoked transmitter release is due to the *sum* of calcium entering through the presynaptic nerve membrane following the action potential and the existing resting $[Ca]_{in}$. Hence a link can be established between the intracellular metabolic machinery and the synaptic efficiency. Modification of calcium uptake processes inside the nerve terminal can change the level of free $[Ca]_{in}$ and thus the amount of transmitter liberated by the nerve impulse and the probability of a successful trans-synaptic information transfer.

TETANIC AND POST-TETANIC POTENTIATION: CALCIUM AND WHAT ELSE?

The nervous system typically conveys messages by a series of several action potentials elicited within a short period of time. Although the action potentials can have usually a similar amplitude, the resulting synaptic potentials do not. The main origin of the frequency modulation of synaptic activity resides at the presynaptic nerve terminal where high-frequency activation produces an increase in the number of quanta released by a nerve impulse (tetanic potentiation – TP). This high release state of the nerve terminal lasts for many seconds or minutes after the stimulation (post-tetanic potentiation – PTP). The decay of PTP shows at least two separate exponentials named augmentation and potentiation by Magleby & Zengel (1976). It has been shown (Erulkar & Rahamimoff, 1978) that the frequency of the mepps follows a similar course to the post-tetanic evoked activity (Fig. 3). This allows the creation of a rather drastic change in the ionic environment of the nerve terminal and the study of the ionic basis of potentiation.

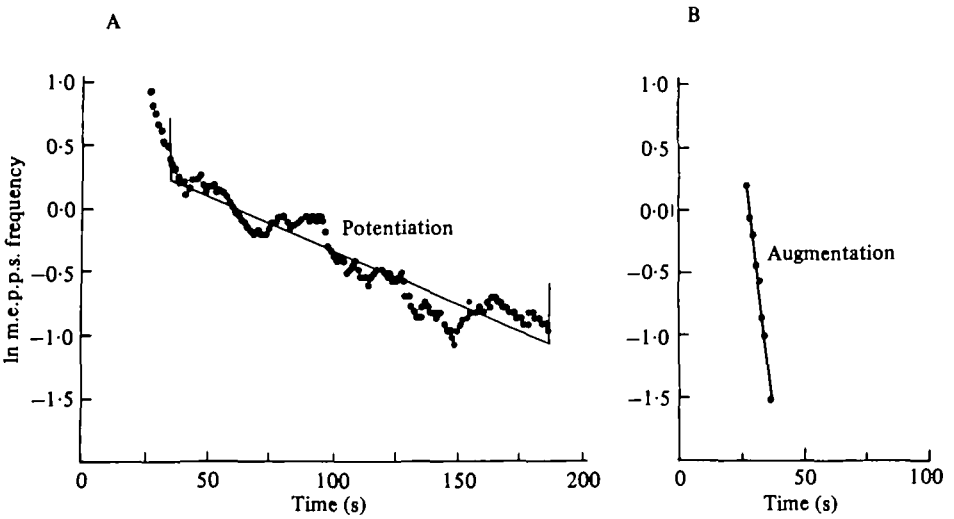

Fig. 3. The phases of post-tetanic potentiation in inward calcium gradient. The time course of post-tetanic potentiation following a tetanic train of 100 Hz for 20 s was analysed by the moving-bin method (bin 25 s, △ bin 1 s) and plotted on semilogarithmic scale after subtraction of the prestimulation m.e.p.p.s frequency. The decay shows two phases: a fast phase (augmentation) and a slower one (potentiation). The time constant of potentiation (τ_A = 156 s) and its extrapolated value at t = 0 (f_P = 1·2 m.e.p.p.s^{-1}) were obtained by a regression analysis between the two vertical lines (Fig. 3A). The time constant (τ_A = 11·6 s) and the intercept (f_A = 7·5 m.e.p.p.s^{-1}) of augmentation were obtained after subtraction of potentiation and fitting a regression line to the subtracted curve (Fig. 3B). 50 μM-$CaCl_2$ 2 mM-$MgCl_2$ frog Ringer.

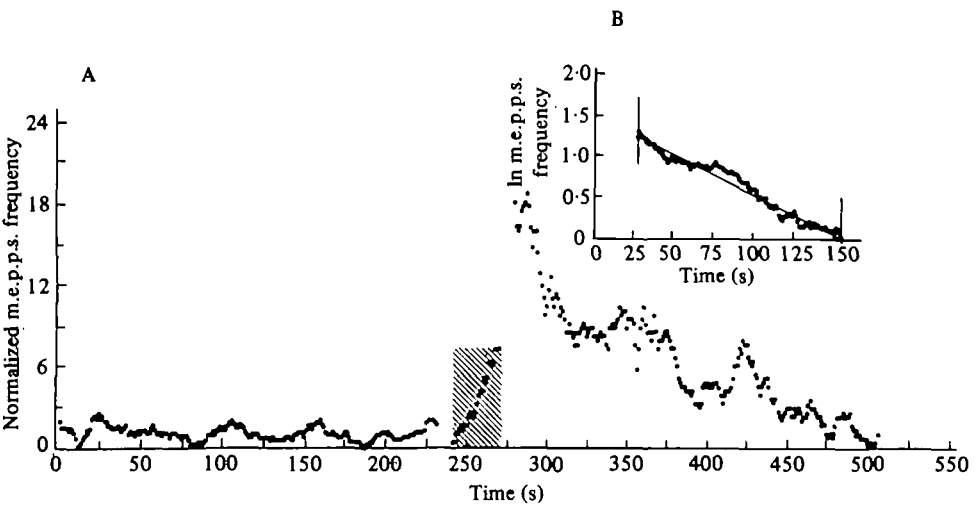

Fig. 4. Tetanic and post-tetanic potentiation in reversed electrochemical gradient for calcium ions. (A) The time course of spontaneous transmitter release before (left), during (centre) and after (right) a tetanic stimulation of 100 Hz for 40 s. Note an initial decrease in m.e.p.p. frequency during the tetanus and post-tetanic 'jump' in m.e.p.p.s frequency (see Erulkar & Rahamimoff 1978). Moving bin, bin 10 s, △ bin: 1 s. (B) The inset shows a semilogarithmic plot of PTP after subtraction of the prestimulation m.e.p.p. frequency; it follows a monophasic exponential decay (potentiation). The time constant τ_P was 105·9 s and the extrapolated value at t = 0; f_P was 23·1. Moving bin, bin = 50 s, △ bin = 1 s. (2 mM-$MgCl_2$, 1 mM EGTA frog Ringer with no added calcium).

Already earlier studies have shown that extracellular calcium plays a role in the potentiation phenomena (Rosenthal, 1969; Weinreich, 1971). A question remained however which one of the various phases of potentiation is dependent on extracellular calcium. Omission of extracellular calcium and addition of the calcium chellating agent EGTA, bring $[Ca]_o$ to levels lower than $[Ca]_{in}$ (see Hubbard, Jones & Landau, 1968). Under such conditions an increase in the membrane Ca permeability cannot induce any calcium influx. Such a treatment (Erulkar & Rahamimoff 1978) produces two very significant changes in potentiation: tetanic potentiation is greatly reduced (but not abolished!) and the augmentation phase of PTP is typically abolished (Fig. 4). Hence, an entry of calcium through the presynaptic membrane is a very important process in TP and in augmentation. However it was also very clear that entry of calcium cannot be the only process taking place during high frequency nerve activation, since a substantial increase in mepp frequency occurs even in the virtual absence of $[Ca]_o$ (Miledi & Thies, 1971; Hurlbut, Longenecker & Mauro, 1971; Rotshenker, Erulkar & Rahamimoff 1976). Moreover the slow potentiation phases also persist (Fig. 4). Therefore there must be a 'channel of communication' between the nerve terminal action potential activity and the release of neurotransmitter, in addition to entry of calcium from the extracellular medium.

THE SECONDARY ROLE OF SODIUM IN TRANSMITTER RELEASE

Sodium ions are obviously involved in transmitter release by generating the action potentials (Hodgkin & Katz, 1949). However, they have been implicated also by additional processes. At the vertebrate neuromuscular junction, a reduction of extracellular sodium increases the quantal content (Kelly, 1965; Rahamimoff & Colomo, 1967; Birks & Cohen, 1968*a*), while increase in intracellular sodium augments transmitter release (Birks & Cohen, 1968*b*; Baker & Crawford, 1975). In invertebrate preparations too a role for sodium in neurotransmitter liberation has been suggested (Atwood, Swenarchuk & Gruenwald, 1975). Therefore we decided to examine whether sodium ions serve as a secondary coupling mechanism between nerve terminal activity and quantal release in tetanic and post tetanic potentiation of mepp frequency (see Lev-Tov & Rahamimoff, 1980). Several lines of evidence support this hypothesis:

(1) Inhibitors of the Na^+–K^+ ATPase, such as ouabain, prevent the extrusion of sodium following nerve activity. Therefore an accumulation of $[Na]_{in}$ is expected. Such poisoning of the sodium pump causes an increase in tetanic potentiation and prolongs the duration of PTP (see Rahamimoff, Erulkar, Lev-Tov & Meiri, 1978).

(2) The degree of tetanic potentiation and the duration of PTP are dependent on the number of stimuli and their rate, in the virtual absence of $[Ca]_o$.

(3) The amplitude of the extracellularly recorded presynaptic action potential decreases concomitantly with the increase in the frequency of the mepps during tetanic stimulation.

(4) When sodium influx through the nerve membrane is inhibited by tetrodotoxin, TP and PTP are dependent on an inward calcium gradient. If the calcium gradient is reversed by EGTA, no potentiation is observed (except in high extracellular magnesium).

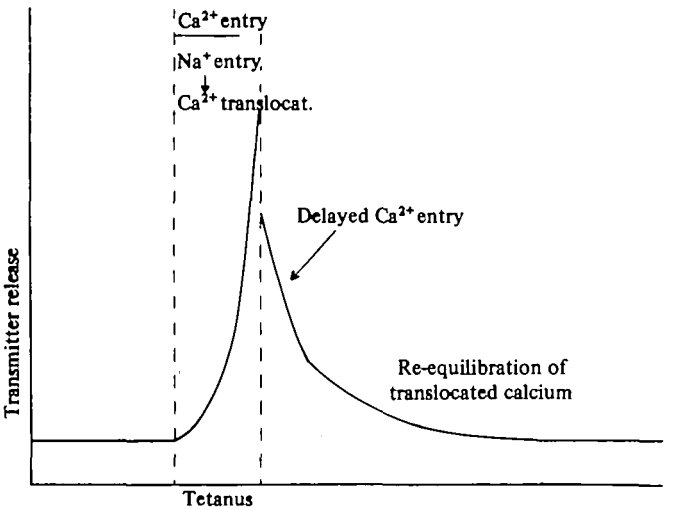

Fig. 5. Schematic representation of the primary role of calcium and secondary role of sodium ions in tetanic and post-tetanic potentiation.

(5) Veratridine, which opens sodium channels, augments tetanic potentiation (Melinek, Lev-Tov and Rahamimoff, unpublished).

(6) The sodium ionophore monensin augments tetanic potentiation (Meiri, Erulkar, Lerman and Rahamimoff, unpublished).

The above experiments strongly suggest that the accumulation of $[Na]_{in}$ which accompanies the tetanus causes an increase in transmitter release. Fig. 5 illustrates schematically the ionic dependence of the various phases of tetanic and post-tetanic potentiation.

SODIUM-INDUCED INTRACELLULAR CALCIUM TRANSLOCATION

The previous section showed that sodium ions are involved in the increase of transmitter release during and after tetanic nerve stimulation. One can envisage at least two different scenarios for this sodium action. The simplest would be a direct activation of the quantal release process. An alternative hypothesis is that the entry of sodium causes a release of calcium from intracellular stores (one or more of those depicted in Fig. 1), and the increase in quantal release reflects an increase in free $[Ca]_{in}$. There is no simple direct way to distinguish between these two hypotheses. However, the measurement of the delay between nerve activation and the time of the appearance of a quantum, permits the examination of this question in an indirect fashion. The argument is as follows: if sodium ions activate directly the release process, it is expected that immediately after the impulse the concentration of sodium near the presynaptic membrane (where the active zones for transmitter release are located) will be the highest. Hence, the rate of quantal liberation will be the largest and it will subside gradually after the action potential. On the other hand, if sodium releases calcium from intracellular stores, there will be no preferential timing after the nerve activation, since, on the average, there is no fixed distance between the store and the presynaptic membrane.

When such experiments were performed in the absence of $[Ca]_o$, no preferred time interval was observed after the nerve stimulus, in spite of the very large increase in quantal release (Lev-Tov & Rahamimoff, 1980). Therefore we conclude that the more probable explanation for the mode of action of sodium is that it causes a calcium translocation from intracellular stores.

There is only a limited amount of information regarding which one of the various intracellular calcium stores can serve as the target for this sodium action. The mitochondria (Carafoli & Crompton, 1978*a*, *b*) and the sodium calcium antiport are two obvious candidates. But of course any one of the other calcium regulatory processes can take part in this effect. In fact one does not even need a direct effect of sodium on one of the calcium regulating processes. If the entry of sodium causes an increased utilization of ATP, this by itself can transform the mitochondria from state 4 to state 3, and the resulting change in the membrane potential of the inner mitochondrial membrane can induce a passive leak of calcium.

IS THE SODIUM-INDUCED-CALCIUM-TRANSLOCATION OF ANY PHYSIOLOGICAL SIGNIFICANCE?

The experiments on tetanic and post-tetanic potentiation of mepps suggest that the entry of sodium ions causes an intracellular calcium translocation and thus an increase of $[Ca]_{in}$ and of transmitter release. These experiments were performed under low $[Ca]_o$ and thus low quantal content. Hence, although the relative contribution of sodium to potentiation is quite large, its absolute effect is rather small. If the relation between $[Ca]_{in}$ and quantal liberation is sigmoidal (similar to the relation between $[Ca]_o$ and evoked transmitter release (Jenkinson, 1957; Dodge & Rahamimoff, 1967; Hubbard *et al.* 1968; Katz & Miledi, 1969)), then even small changes in $[Ca]_{in}$ can have very substantial effects on release under more physiological conditions. If, on the other hand, one accepts the suggestion of Llinas, Steinberg & Walton (1976) that release of transmitter is a linear function of $[Ca]_{in}$, then the effect of intracellular translocation will be minimal. This suggestion of Llinas *et al.* (1976) does not seem very appealing to us, since for a 3×10^5-fold increase in rate of transmitter release between rest and activity, one needs to postulate that $[Ca]_{in}$ should increase to $10^{-7} \times 3 \times 10^5 = 30$ mM(!). Such a concentration is more than one order of magnitude larger than the normal extracellular calcium concentration. No doubt that more direct measurements are necessary to establish the $[Ca]_{in}$-transmitter release relation.

SODIUM IN OTHER SYSTEMS – FACTS AND SPECULATIONS

We suggest here that the slow modulation of transmitter release during and after tetanic stimulation is achieved in part by an accumulation of intracellular sodium leading to intracellular calcium translocation. It is of interest to examine whether a similar mechanism may occur in other systems.

Calcium and sodium ions seem to be involved in the process of acetylcholine stimulated amylase secretion from pancreatic acinar cells. For the stimulation to be effective sodium ions have to be present in the extracellular medium. Acetylcholine augments the membrane permeability to sodium and the sodium uptake (Bobinski &

Kelly, 1979), without affecting calcium influx (Nishiyama & Petersen, 1975). However, there is an increase in calcium efflux (Case & Clausen, 1973; Mathews, Petersen & Williams, 1973), which leads to the suggestion that the entry of sodium causes an intracellular calcium release (Bobinski & Kelly, 1979). It seems therefore that slow activation of the secretion process can be achieved by sodium.

The possible involvement of sodium in slow regulation of secretion makes us wonder whether it may participate in more than one mechanism in the visual process. It is well known that at the vertebrate photoreceptor there is an inward dark current which is carried by sodium ions (Baylor & Fuortes, 1970; Tomita, 1970; Korenbrot & Cone, 1972; Baylor & Hodgkin, 1973; Brown & Pinto, 1974; Yoshikami & Hagins, 1978). Light causes a decrease in this dark current and a reduction in sodium influx, which causes a membrane hyperpolarization and a reduction in transmitter release at the synapse between the photoreceptor and the bipolar cell. It will be of interest to see whether slow adaptive processes which follow prolonged illumination may be the result of a decrease in $[Na]_{in}$ leading to changes in $[Ca]_{in}$ and transmitter release.

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