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Multitude of ion channels in regulation of transmitter release

Rami Rahamimoff, Alexander Butkevich, Dessislava Duridanova, Ronit Ahdut, Emanuel Harari and Sylvia G. Kachalsky

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Multitude of ion channels in the regulation of transmitter release

Rami Rahamimoff*, Alexander Butkevich, Dessislava Duridanova†, Ronit Ahdut, Emanuel Harari and Sylvia G. Kachalsky

Department of Physiology and the Bernard Katz Minerva Centre for Cell Biophysics, Hebrew University Hadassah Medical School, PO Box 12272, Jerusalem 91120, Israel

The presynaptic nerve terminal is of key importance in communication in the nervous system. Its primary role is to release transmitter quanta on the arrival of an appropriate stimulus. The structural basis of these transmitter quanta are the synaptic vesicles that fuse with the surface membrane of the nerve terminal, to release their content of neurotransmitter molecules and other vesicular components. We subdivide the control of quantal release into two major classes: the processes that take place before the fusion of the synaptic vesicle with the surface membrane (the pre-fusion control) and the processes that occur after the fusion of the vesicle (the post-fusion control). The pre-fusion control is the main determinant of transmitter release. It is achieved by a wide variety of cellular components, among them the ion channels. There are reports of several hundred different ion channel molecules at the surface membrane of the nerve terminal, that for convenience can be grouped into eight major categories. They are the voltage-dependent calcium channels, the potassium channels, the calcium-gated potassium channels, the sodium channels, the chloride channels, the non-selective channels, the ligand gated channels and the stretch-activated channels. There are several categories of intracellular channels in the mitochondria, endoplasmic reticulum and the synaptic vesicles. We speculate that the vesicle channels may be of an importance in the post-fusion control of transmitter release.

Keywords: ion channels; neurotransmitters; synaptic vesicles

1. INTRODUCTION

The function of the nervous system is based on the ability of nerve cells to communicate with each other. The communication is achieved primarily by synaptic transmission (Katz 1969, 1986), where the presynaptic nerve terminals release neurotransmitters that act on the postsynaptic membrane. It was the process of transmitter release that was the main interest of Bruno Ceccarelli and his colleagues. In this field they made many fundamental observations regarding structure and function of the presynaptic nerve terminal (Ceccarelli *et al*. 1972, 1973, 1979, 1988; Ceccarelli & Hurlbut 1980*b*; Fesce *et al*. 1980, 1986; Haimann *et al*. 1987; Hurlbut *et al*. 1990; Meldolesi & Ceccarelli 1981; Torri Tarelli *et al*. 1985, 1987).

Transmitter release from the presynaptic nerve terminal, following nerve stimulation, is a multistep process. In most cases it starts with the generation of an action potential in the presynaptic nerve, its conduction to the terminal, where it opens voltage-dependent ion channels. The opening of ion channels causes calcium ions to flow into the nerve terminal interior, where they activate the fusion machinery, which causes the fusion of the synaptic vesicle with the surface membrane and the release of transmitter. This review article deals with the ion channels that are involved in the process of transmitter release, from two different viewpoints: the channels participating in the triggering of quantal release of transmitter (Meir *et al*. 1998) and channels in the synaptic vesicles (Rahamimoff *et al*. 1989; Yakir & Rahamimoff 1995).

*Author for correspondence (ramir@cc.huji.ac.il).
†Present address: Institute for Biophysics, The Bulgarian Academy of Sciences, Sofia, Bulgaria.

2. SURFACE MEMBRANE CHANNELS

The surface membrane of the nerve terminal possesses a large number of different ion channels. A rough estimate is that in different nerve terminal preparations, many hundreds of different ion channel molecules have been described. For organizational convenience, the various ion channels are subdivided into categories (for example, calcium channels, non-selective channels, ligand gated channels, etc.). There are at least eight major categories of ion channels in the surface membrane. The categories are then subdivided into channel types (for example, the voltage-dependent calcium channels category in the nerve terminal comprises N, L, P, Q, R and T types). The channel types in turn consist of different channel molecules.

(a) *Calcium channels*

It is undoubtedly well established that calcium ions are crucial for transmitter release (e.g. Augustine & Charlton 1986; Augustine *et al*. 1985*a*,*b*). It is therefore reasonable to conclude that calcium channels, being the major route for calcium entry into nerve terminals, play a key role in regulation of synaptic transmission.

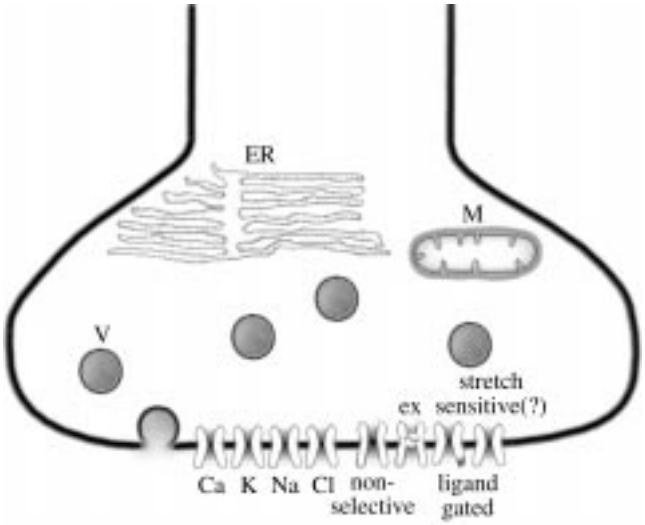

Figure 1. Schematic drawing of the presynaptic nerve terminal and different categories of ion channels in the surface membrane.

A huge number of calcium channel exist in the nervous system. Most of the channels are voltage-dependent and are classified as low-voltage activated (LVA) and high-voltage activated (HVA) channels. The threshold of activation in the former group is slightly above the resting potential, whereas in the latter group it is substantially higher (towards 0 mV). The pharmacological classification arranges the channels into six types: L, N, P, Q, R and T. At the molecular level, there may be hundreds, if not thousands of different subunit compositions of calcium channels (see Meir *et al*. 1998). We speculate that such a large number of molecules regulating calcium entry into the nerve terminal allows for a great plasticity in transmitter release, and thus in synaptic communication.

(b) *Potassium channels*

Opening of these channels produces an outward current causing the hyperpolarization of the surface membrane. Consequently, these channels are involved mainly in repolarization of the membrane during action potential; in after-hyperpolarization, which follows an action potential; and in maintaining the resting potential of the plasma membrane. They are also involved in ending periods of strong activity of the nerve terminals, such as bursts, and in modulation of firing rates.

At least six different types of potassium channels exist in nerve terminals. These include the fast, transient A-type channels; slowly activating KS channels, delayed rectifier channels, which activate rapidly but exhibit little or no inactivation; calcium- and ATP-gated channels, and S channels, blocked by the neurotransmitter serotonin.

A large number of different channel molecules have been described in various nerve terminals. For example, at least nine different calcium-gated potassium channels varying in their electrophysiological characteristics have been directly recorded in nerve terminals. There are at least 12 different mammalian genes encoding A-type channels. Taken into account that different types of potassium channels can coexist at the same terminal (e.g. Bartschat & Blaustein 1985; Bielefeldt *et al*. 1992), one realizes that the number of possible combinations of potassium channels that can be present in the surface membrane of nerve terminal is immense. This large variety in the armamentarium of potassium channels at nerve terminals probably serves to tailor each terminal to the specific functions of the synapses in different locations in the nervous system in different species.

(c) *Calcium-gated potassium channels*

These channels open in response to raising intracellular calcium concentration $[Ca^{2+}]_{in}$ or in response to depolarization and raising $[Ca^{2+}]_{in}$. In nerve terminals, calcium-gated potassium channels are clustered close to calcium channels (Issa & Hudspeth 1994; Roberts 1994; Roberts *et al*. 1990; Robitaille *et al*. 1993*a*,*b*). These channels are probably of importance in the regulation of transmitter release during the repetitive activity of the nerve terminal. The repetitive activity of the terminal causes an increase in $[Ca^{2+}]_{in}$ (Brethes *et al*. 1987; Jackson *et al*. 1991) and thus an increase in calcium-gated current (Hayashi & Stuart 1993; Kretz *et al*. 1982). The activated potassium current causes hyperpolarization of the membrane, reducing the release of transmitter.

(d) *Sodium channels*

Sodium channels play a pivotal role in the generation of action potential. Since the amount of transmitter liberated by an action potential depends on the shape of the depolarization (Katz & Miledi 1965, 1967*a*–*c*), sodium channels may be of significance in regulation of transmitter release. An additional role of sodium channels is regulation of the intracellular sodium concentration, which determines the $[Ca^{2+}]_{in}$ through the sodium–calcium exchanger.

Sodium channels have been found on various nerve terminals in numerous preparations (Bablito *et al*. 1986; Cazalis *et al*. 1987; Clark & Brooks 1989; Coniglio *et al*. 1993; Fatatis *et al*. 1992; Legendre *et al*. 1988; Lemos *et al*. 1986; Liao *et al*. 1991; Mason & Dyball 1986; Salzberg *et al*. 1983).

(e) *Chloride channels*

In most animal cells the equilibrium potential of chloride is close to the resting potential. Opening of chloride channels causes increase in membrane conductance and decrease in the excitability of nerve terminal (Rudomin 1990). Indeed, it has been shown that presynaptic chloride channels are involved in presynaptic inhibition (Dudel & Kuffler 1961). Chloride channels may also be of importance in determining which branch of axon will be activated by an action potential. By decreasing excitability of one branch (through localized activation of chloride channels), action potential can be directed to a specific location (Eguibar *et al*. 1994; Zhang & Jackson 1993).

Several types of chloride channels are found at presynaptic nerve terminals. These include $GABA_A$ (e.g. Finger & Martin 1989; Jackson & Zhang 1995) and $GABA_C$ (e.g. Lukasiewicz 1996) receptors, Ca^{2+}-activated Cl^- channels (Hussy 1992), glycine receptors (Engblom *et al*. 1996; Wahl *et al*. 1994) and voltage-gated chloride channels (Edry-Schiller *et al*. 1991*b*).

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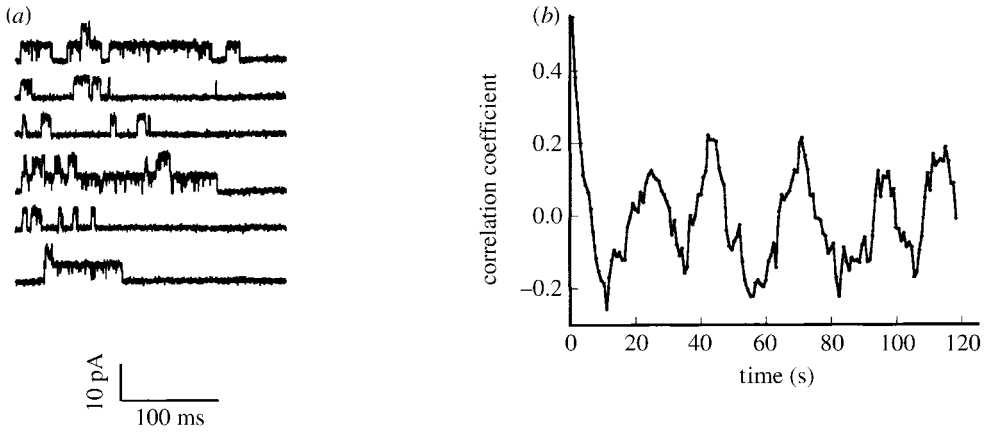

Figure 2. The bursting potassium channel in the fused presynaptic nerve terminals of *Torpedo*. (*a*) Examples of the channel's activity. The membrane was depolarized from 120 mV to +20 mV for 250 s with a frequency of 1.25 Hz. (*b*) Oscillations in the activity of the bursting potassium channel. Autocorrelogram of 300 responses to depolarizatory pulses from 90 mV to +20 mV. Stimulation frequency, 1.25 Hz.

(f) *Non-selective channels*

Non-selective channels do not distinguish between anions and cations. For example, a large non-selective channel described in fused *Torpedo* synaptosomes conducts Na^+ K^+, Ca^{2+}, Cl^- and even neurotransmitter acetylcholine almost equally well (Meir & Rahamimoff 1996). At least five electrophysiologically different non-selective channels has been recorded in various terminals, ranging in conductance between 69 and 850 pS (Lemos & Nordmann 1986; Meir & Rahamimoff 1996; Stuenkel *et al.* 1990; Tarelius *et al.* 1990). The physiological role of these channels is not clear, but at least in the case of the channel in *Torpedo* electric organ, non-selective channels can provide a route for calcium entry into nerve terminals (Meir & Rahamimoff 1996).

(g) *Ligand-gated channels*

Ligand-gated channels can be subdivided into channels gated by intracellular and extracellular ligands. The latter are also called receptors. Presynaptic receptors to neurotransmitters glutamate, acetylcholine, γ-amino-butyric acid (GABA) (for a review, see McGehee & Role 1996), serotonin (Crespi *et al.* 1997; Maura *et al.* 1992; Nichols & Mollared 1996) and glycine (Engblom *et al.* 1996; Wahl *et al.* 1994) have been described in various preparations. In addition to that purinergic P_{2X} receptors gated by ATP are also found in the surface membrane of nerve terminals (Gu & MacDermott 1997; Motin & Bennett 1995; Sun & Stanley 1996). Whereas acetylcholine, serotonin, glutamate and P_{2X} receptors are cationic channels, GABA and glycine receptors are predominantly anionic channels, conducting chloride. These receptors probably function in the presynaptic modulation of transmitter release.

Channels gated by intracellular ligands include ATP-gated potassium channels (Diest *et al.* 1992; Lee *et al.* 1995, 1996; Stanford & Lacey 1997; Takata *et al.* 1993; Watts *et al.* 1995; Ye *et al.* 1997) and Ca^{2+}-gated potassium and chloride (Okada *et al.* 1995) channels. ATP-gated K^+ channels are closed by raising intracellular ATP concentration. Thus, the electrical activity of the nerve terminal and hence, transmitter release, can be regulated by the energy content through these channels.

(h) *Stretch-activated channels*

It has been known for more than 40 years that stretching of the muscle–nerve synapse increases spontaneous transmitter release (Fatt & Katz 1952). Recently, stretch-activated ion channels have been described in various preparations (Chen & Grinnell 1994, 1995, 1997; Ingber 1997; Sackin 1995). Thus, stretch-activated ion channels may provide an additional means of regulation of the activity of the nerve terminal.

(i) *The bursting potassium channel*

One of the channels we were interested in is the bursting potassium channel in the fused *Torpedo* presynaptic nerve endings (synaptosomes) (Edry-Schiller *et al.* 1991*a*) (figure 2*a*). Its features deserve a brief mention here.

This channel is the most common one in the fused presynaptic nerve endings. It is highly selective for potassium, and its activation and inactivation properties are those of the A-type potassium channel (Edry-Schiller *et al.* 1991*a*; Edry-Schiller & Rahamimoff 1993). The behaviour of the channel shows a very interesting property. The openings of the channel in response to depolarization pulses are not random, but are dependent on the 'history' of the channel. The probability of opening is greatly augmented if the channel was open by a previous pulse. We termed this property a 'statistical memory' (Rahamimoff *et al.* 1992). This memory lasts for several hundred milliseconds to several seconds. Under appropriate experimental conditions, oscillations in the activity of the channel with a period of approximately 20 s can be observed (Rahamimoff *et al.* 1995) (figure 2*b*). Experimental evidence shows that these properties are likely to result from the entry of the channel into the voltage-dependent inactivated state (Butkevich *et al.* 1997). We speculate that these properties may be of importance in frequency modulation phenomena.

3. INTRACELLULAR CHANNELS

Many intracellular organelles have ion channels. In the organelles present in the nerve terminals, ion channels have been described in the synaptic vesicles. It is well known that nerve terminals also have mitochondria and

endoplasmic reticulum, but much of the information regarding the ion channels in these organelles comes from other tissues.

(a) *Channels in synaptic vesicles*

Synaptic vesicles have a complex life-cycle, which includes synthesis, filling with transmitter (or transmitters), transport to the release part of the nerve terminal, docking at the active zone, activation, fusion with the surface membrane, release of the stored transmitter, retrieval of the vesicle into the cytoplasm and refilling with transmitters. Many of these stages were the centre of interest of Bruno Ceccarelli (Ceccarelli *et al*. 1972, 1973, 1979; Ceccarelli & Hurlbut 1980*a*,*b*; Fesce *et al*. 1980; Hurlbut *et al*. 1990; Meldolesi & Ceccarelli 1981; Valtorta *et al*. 1990). Here we would like to examine one specific aspect of the possible roles of ion channels in the life-cycle of the synaptic vesicle. Ion channels were found in synaptic vesicles isolated from nerve endings of the *Torpedo* electric organ (DeRiemer *et al*. 1988; Kelly & Woodbury 1996; Rahamimoff *et al*. 1989, 1990; Woodbury 1995; Yakir & Rahamimoff 1995) and in vesicles isolated from neurosecretory endings of the hypophysis (Lee *et al*. 1992). Six different types of ion channels have been identified to date (Woodbury 1995). For one type of channel, the molecular structure has been determined to correspond to synaptophysin (Thomas *et al*. 1988).

(b) *The post-fusion hypothesis of transmitter release*

Transmitter is released from the nerve terminals as preformed, multimolecular quanta (for a summary, see Katz 1969). This was first found at the frog neuromuscular junction and afterwards in many other synapses. After the discovery of the synaptic vesicles, it was proposed that these vesicles form the structural basis for quantal release. Many observations over the last decades confirm this hypothesis (see Ceccarelli *et al*. 1973, 1979; Heuser 1989; Heuser & Reese 1973, 1981). However, the vesicle hypothesis for secretion is not devoid of problems, as summarized recently (Rahamimoff & Fernandez 1997).

In the past, it was proposed (Uvnas & Aborg 1983, 1989) that charged transmitter molecules are stored in the synaptic vesicles in a bound form, bound to an ion exchange matrix. For positively charged transmitters, such as acetylcholine, the ion exchange matrix has a negative charge. Proteoglycans and other charged polymers that were found in the synaptic vesicle (Kiene & Stadler 1987; Kuhn *et al*. 1988; Nanavati & Fernandez 1993; Stadler & Dowe 1982; Stadler & Kiene 1987) can subserve this role. Hence, there is a need for cations in the releasing synaptic vesicle. The positively charged transmitter molecules have to be replaced with cations to be released through the fusion pore. If this is the case, then the ion channels in the vesicle membrane may have a crucial role in quantal transmitter release. During the fusion process, the membrane of the vesicle changes its membrane potential according to the potential of the surface membrane. If the voltage change is in the range of the activation of the non-selective ion channels, then they open and cations enter the vesicle, release the transmitter from the ion exchange matrix and make it available for release through the fusion pore. Such a mechanism will have a limited value if a complete fusion occurs between the vesicle membrane and the surface membrane, but will be of great importance if a temporary fusion, nicknamed 'kiss and run' (Fesce *et al*. 1994), happens. If such a sequence of events does take place, then a post-fusion control of transmitter release is possible.

A growing number of articles, coming from various disciplines, suggest that there is a dynamic exchange of the contents between different pools such as clathrin-coated, dense-core and synaptic vesicles, sorting endosomes and lysosomes (Artalejo *et al*. 1998; Palfrey & Artalejo 1998; Storrie & Desjardins 1996). In many cases a 'kiss and run' was postulated (Ceccarelli & Hurlbut 1980*a*; Fesce *et al*. 1994; Meldolesi & Ceccarelli 1981). The essence of the 'kiss and run' model is the possibility for immediate recycling of secretory and endocytotic vesicles after their 'emptying'.

The kiss and run model accounts for some of the important observations regarding vesicular behaviour, such as fast transient changes in the target membrane capacitance and the rapid kinetics of the vesicle recycling (Alvarez De Toledo *et al*. 1993; Henkel & Almers 1996; Rosenboom & Lindau 1994; Ryan *et al*. 1993, 1996).

(c) *Channels in mitochondria and endoplasmic reticulum*

In mitochondria, several ion channels were found, they can be divided into voltage-dependent ion channels, ATP-sensitive K^+ channels, anion channels and voltage-independent high-conductance ion channels. Voltage-dependent ion channels were found in the inner mitochondrial membrane and can be divided into channels with a conductance of around 100 pS and 40 pS (Sorgato *et al*. 1987, 1989). ATP-sensitive K^+ channels are highly selective for K^+, can be reversibly inactivated by ATP and have a conductance of 910 pS (Inoue *et al*. 1991).

Of special interest are the mitochondrial channels involved in regulation of intracellular calcium concentration ($[Ca^{2+}]_{in}$) in the presynaptic nerve terminal. The role of the mitochondrion in regulation of presynaptic $[Ca^{2+}]_{in}$ was under a substantial controversy in the last two decades. On one hand, mitochondria are abundant in the presynaptic nerve terminals and their inhibition (which in isolated systems causes a release of their calcium) augments transmitter release (Alnaes & Rahamimoff 1975); on the other hand, attempts to estimate their calcium content yielded low values (Blaustein *et al*. 1980). The controversy was resolved only recently by molecular biology (David *et al*. 1998). Imaging showed that mitochondria take a very active part in the regulation of $[Ca]_{in}$ after a small number of nerve impulses (see Melamed-Book & Rahamimoff 1998).

The endoplasmic reticulum calcium channels are very important in many cell functions, e.g. protein phosphorylation, gene expression, and probably long-term potentiation and depression (Reyes & Stanton 1996). The intracellular channels responsible for the rapid and localized release of calcium ions from intracellular stores are the inositol (1,4,5)P3 receptors (IP3Rs) and ryanodine receptors (RYRs). They belong to two different multigene families with high similarity in their general organization (for a comprehensive review, see Pozzan *et al*. 1994). Changes in the intracellular calcium concentration are affected by the temporal–spatial distribution of

intracellular calcium pools and channels. They were found in the axons of mammalian and avian Purkinje neurons and lately in the presynaptic boutons of chicken cerebellum (Ouyang *et al.* 1997).

4. CONCLUSION

In conclusion, a huge number of different ion channels regulate transmitter release from presynaptic nerve terminals. Ion channels at the plasma membrane are involved in pre-fusion control of the release, whereas channels in synaptic vesicles may be important for a post-fusion control. In addition, ion channels in mitochondria and endoplasmic reticulum may influence transmitter release through affecting intraterminal calcium concentration. We speculate that this multitude of ion channels may allow for a wide spectrum of activities of different nerve terminals and be of importance in synaptic plasticity.

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PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES