Minireview

Modulation of Calcium Channel Function in Nerve Cell Membrane

P. G. KOSTYUK, P. A. DOROSHENKO

A. A. Bogomoletz Institute of Physiology, Acad. Sci. of the Ukr. SSR, Bogomoletz Str. 4, Kiev, USSR

The "classical" concept concerning the functioning of ion channels considers them as being operationally independent from cellular metabolism. This concept seemed to be quite universal; however, during recent years it has become more and more obvious that it considerably simplifies the reality and that channel function can be under direct control of intracellular metabolic processes; this is especially true for calcium channels.

The first indications of metabolic modulation of calcium channel function have been obtained from cardiac muscle fibers. It is well known that in cardiac muscle fibres the calcium-dependent plateau of the action potential is prolonged under the action of catecholamines; this prolongation is of major importance during sympathetic enhancement of cardiac activity (Reuter 1974; 1979). Based on data about the functional role of cyclic nucleotide metabolism in activation of protein phosphorylation (e. g. Greengard 1978), it was suggested that the catecholamine-induced potentiation of the calcium component of the cardiac action potential is mediated by increased synthesis of cyclic AMP by adenylate cyclase and by subsequent phosphorylation of the proteins responsible for calcium conductance via the cAMP-dependent protein kinase. Suggestions concerning a possible role of cAMP in the modulation of the calcium conductance in neuronal membrane were made by Shimachara and Tauc (1977) and Klein and Kandel (1978). They recorded in Aplysia the synaptic action exerted by a defined neuron (or directly the slow inward current in that neuron) and observed potentiation under external application of serotonin (which is a common neurotransmitter in mollusc ganglia). The same effect could be obtained by injecting cAMP into the cell or by incubating the ganglion in a solution containing phosphodiesterase inhibitors (phosphodiesterase destroys intracellular cAMP). The data reported by these authors stimulated more detailed investiga-

Presented at the microsymposium "Calcium Transport Systems in Excitable Cells", Bratislava, February 7, 1990.

tions of the possible metabolic modulation of calcium channel function, using direct recordings of calcium currents.

cAMP-dependent modulation of calcium currents in neuronal membrane. The high-threshold calcium channels in the neuronal membrane possess an important characteristic which makes them very convenient for the study of the metabolic dependence of their function. During intracellular dialysis the corresponding calcium currents rapidly decrease in amplitude: in large mollusc neurons this takes several tens of minutes (Kostyuk and Krishtal 1977; Byerly and Hagiwara 1982), in smaller mammalian neurons only few minutes (Kostyuk et al. 1981). Obviously, a cytoplasmic factor which can easily be washed out from the cell or destroyed during its dialysis is necessary for normal functioning of calcium channels. Both in mollusc and mammalian neurons the introduction into the dialysis solution of cAMP together with ATP and Mg^{2+} (the cofactor necessary for ATP hydrolysis) not only prevented a further decrease of calcium currents in many cells, but sometimes restored them to their initial levels. Separate introduction of each of these substances had only a weak stabilizing effect (Fedulova et al. 1981; Doroshenko et al. 1982). With snail neurons, the maximal effect was observed at cAMP concentration of approx. 10^{-4} mol/l, although partial restoration could be observed even at micromolar concentrations. Optimal concentrations of ATP and Mg^{2+} were 2 mmol/l and 3 mmol/l, respectively. After reaching the maximal effect, the amplitude of the calcium current started to decrease again; however, the decrease was less rapid than during dialysis with simple saline solution. The introduction of cGMP was not associated with any effect on the "wash-out" of calcium currents.

Obviously, the activity of membrane-bound enzymes is retained in conditions of intracellular dialysis or perfusion, and they can be activated by the corresponding substrates introduced into the cell. This has been supported by a series of other experiments. The addition of fluoride ions into the perfusate in concentrations that activate membrane adenylate cyclase (several mmol/l) together with ATP and Mg^{2+} also restored calcium currents. On the contrary, the addition of Cu^{2+} (adenylate cyclase inhibitor) speeded up the "wash-out".

The described mechanism of the cytoplasmic control of membrane calcium conductance is characteristic only for the high-threshold channels. The low-threshold calcium channels, as already mentioned, are very resistant to alterations of the intracellular processes; they can retain their function in isolated membrane patches for a long time. The rapid inactivation of low-threshold channels is not connected to the action of intracellular calcium ions (Carbone and Lux 1984; Fedulova et al. 1985). Intracellular introduction of fluoride or cAMP did not modulate the activity of low-threshold channels either (Dupon et al. 1986; Carbone and Lux 1984).

Still, experimental data do not prove directly the suggestion that an increase in intracellular cAMP levels affects the calcium conductance through activation of the cAMP-dependent protein kinase (cAMP-PK), which in turn phosphorylates some proteins important for the functioning of calcium channels; nonetheless, this seems highly probable. More direct evidence has been obtained from experiments with the catalytic subunit (CS) of the cAMP-PK. The injection into Aplysia neurons (through a microelectrode) of the cAMP-PK CS purified from bovine myocardium facilitated the generation of "calcium" action potentials (Kaczmarek et al. 1980). When introduced into a dialysed neuron, it stopped the "wash-out" of high-threshold calcium currents and restored them, sometimes up to the initial levels (Doroshenko et al. 1984). The presence of ATP, not cAMP, was necessary for the effect. Stable calcium currents of constant amplitude could be recorded during long lasting (several hours) cell dialysis. Removal of ATP from the cell resulted in rapid deterioration of the currents.

All the above can be considered as supporting the concept suggesting that calcium conductance in the neuronal membrane is modulated by the phosphorylating activity of cAMP-PK. Natural inactivation of calcium channels may be also connected to channel dephosphorylation, as it is slowed down as a result of the above interferences (see also Armstrong and Eckert 1985; Eckert et al. 1986; Chad and Eckert 1986; Armstrong and Kalman 1988). The parallelism of intracellular calcium increase and protein phosphorylation depression led to the suggestion that the blocking effect of intracellular calcium on calcium channels is also mediated through a metabolic link, namely via potentiation of the channel-forming protein dephosphorylation.

One of the points of interaction of Ca^{2+} and cyclic nucleotides in their recurrent action on membrane channels could be the system of cellular phosphodiesterases (PDE). The activity of PDE is highly dependent on Ca^{2+} ions which activate it already in micromolar concentrations through the formation of complexes with calmodulin (see Rasmussen et al. 1979). An increase in intracellular calcium levels will trigger, through this mechanism, a decrease of cAMP levels and correspondingly switch calcium channels into inactive state; on the contrary, low calcium levels will substantially depress the activity of PDE.

In parallel it has been shown that stimulation of proteolysis and decrease in intracellular ATP levels can also participate in calcium current "wash-out" (Chad and Eckert 1986; Eckert et al. 1986; Belles et al. 1988). In certain cases introduction of cAMP into the dialysed neurons did not prevent the "wash-out", but some positive effects were observed with ATP (Byerly and Yazejian 1986) or AMP (Kononenko and Shcherbatko 1988). Recently, a special factor has been found in the cytoplasm of cardiomyocytes which prevented calcium

channels from "wash-out"; the activity of this factor (m. w. 20—30 kD) could be abolished by the application of trypsin or by heating (Kameyama et al. 1988). It is quite possible that different types of neurons differ in their mechanism and degree of cAMP-dependent control of calcium channels. There may be a correlation between the presence of this factor and some functional properties of the neuron: calcium currents were strongly potentiated by intracellular introduction of cAMP into cells in which a similar potentiation could be produced by extracellular application of serotonin. Cells which were insensitive to introduction of cAMP did not respond to serotonin either. Possibly, in some neurons the mechanism of cAMP-dependent phosphorylation serves to mediate the natural modulatory action of serotonin on calcium channels, and this mechanism is not expressed in cells in which the function of serotonin and the corresponding receptors are absent (Kostyuk et al. 1990).

Adenylate cyclase is a complex system of membrane proteins in which an important role is played, in addition to the external receptor and internal hydrolytic units, by the intermediate GTP-binding (*G*) regulatory proteins. Up- and down-regulation studies of the enzymatic activity and later direct biochemical investigations have revealed that as a matter of fact, the *G*-proteins represent a complex of substances some of which transmit the activating (G_s) and others the inhibitory (G_i) signal.

Depression of calcium currents due to changed adenylate cyclase activity through G_i proteins is well known to occur in cardiomyocytes during the action of acetylcholine on M-cholinoreceptors (Hescheler et al. 1986; Fischmeister and Hartzell 1986). There are no direct data about possible down-regulation of calcium conductance in neuronal membranes through the cAMP-PK system, although there are numerous examples of a similar regulation under the action of physiologically active substances, operating on other principles (see below).

Recently, the separation of the subunits from the purified calcium channels of the skeletal muscle T-system and their phosphorylation *in vitro*, has shown that cAMP-PK phosphorylates both the α_1 subunit with m. v. 165 kD and the β-subunit with m. v. 55 kD (Curtis and Catterall 1984; Hosey et al. 1986; Imagava et al. 1987, and others). Further experiments are necessary to determine what site in the channel is really phosphorylated *in vivo* thus being important for changing the channel function; very important will be also comparison of these data from muscle fiber membrane with those concerning high-threshold neuronal calcium channels.

Direct modulation of calcium channels by GTP-binding proteins. It has been shown by many investigators that several neurotransmitters depress calcium currents in sensory neurons from the dorsal root ganglia. This effect was first described by Dunlap and Fishbach (1978, 1981) and later by Forscher and

Oxford (1984) in relation to noradrenaline which affects only high-threshold currents (McFadzean and Docherty 1987) with the secretion of substance P being depressed in parallel (Dunlap and Fishbach 1981). The calcium currents can be depressed also by GABA (Dunlap and Fishbach 1981; Okamoto et al. 1983; Deisz and Lux 1985) and its agonist baclofen (Scott and Dolphin 1986). GTP-binding proteins are involved in the above effects, as a similar depression can be produced by intracellular injection of nonhydrolysable GTP analogues (GTP-γ-S, GMP-PNP) which induce long-lasting activation of the corresponding proteins (Dolphin and Scott 1987, 1989). At the same time, the depression is not connected to any changes in the intracellular cAMP levels, althougt it could be blocked by toxins which affect the adenylate cyclase complex (pertussis toxin). Calcium currents were depressed also under the action of adenosine which affects the adenylate cyclase system through the A_1 receptors connected to the G_i-proteins (Dolphin et al. 1986; Macdonald et al. 1986).

All these data lead to the conclusion that calcium channel activity may be modulated through a short way, namely by direct interaction of the membrane *G*-proteins with the channels. This suggestion has been widely supported and is used now to explain the modulatory action of many neurotransmitters (see the review by Ewald et al. 1988). Nevertheless, the existence of transmitter receptors directly on the voltage-operated calcium channels cannot be excluded (Forscher et al. 1986).

The depressory effect of noradrenaline (and dopamine) was shown also on neurons from other structures: mammalian brain (Williams and North 1985), snail ganglia (Akopyan et al. 1985; Gerschenfeld et al. 1986), sympathetic ganglia (Horn and McAfee 1979, 1980; Marchetti et al. 1986). In the latter case the effect is mediated through α- adrenoreceptors and can be antagonized by the corresponding blockers (phentolamine). A more detailed analysis has shown that it is connected to α_2-receptors (McAfee et al. 1981); it can be reproduced in frog sympathetic neurons (Koketsu and Akasu 1982). The effects are also not connected to changes in intracellular cAMP levels, although they are sensitive to pertussis toxin and to the action of antibodies specific for the α-subunit of the *G*-protein. Injection of the purified subunit mimicked the inhibitory effect of dopamine (Harris-Warrick et al. 1988). A similar mechanism seems to mediate the inhibitory M-cholinoreceptive action of acetylcholine on calcium currents in sympathetic neurons (Wanke et al. 1987). In certain ("bursting") snail neurons the calcium currents could be inhibited also by serotonin (Kononenko and Shcherbatko 1985).

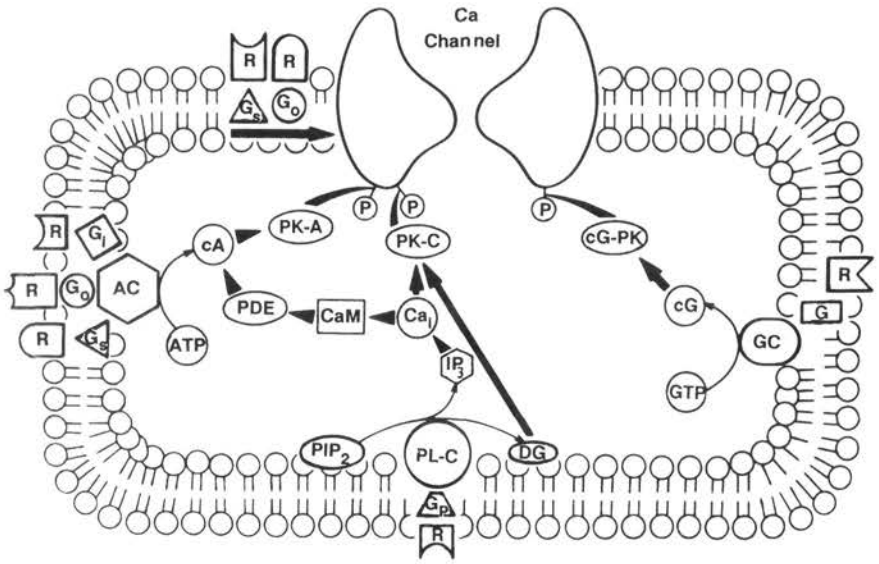
Down-regulation of neuronal calcium channels has been observed recently also under the action of endogenous peptides. Met- and Leu-enkephalins as well as morphine blocked calcium currents in the neuroblastoma X glioma hybrid cellular line (Tsunoo et al. 2986; Hescheler et al. 1987; Shimahara and Icard-

Liepkalns 1987; McFadzean 1988). The effect could be abolished by pertussis toxin and restored by intracellular introduction of the G-protein α-subunit; possibly, in this case the channels were also directly affected by *G*-proteine. In snail neurons calcium currents could be depressed by cholecystokinin (Hammond et al. 1987) and the endogenous FMRF-peptide (Brezina et al. 1987). In mice sensory neurons inhibition could be produced apart from enkephalins also by dynorphin (Werz and Macdonald 1984, 1985; Macdonald and Werz 1986); the inhibition concerned predominantly the high-threshold currents (Gross and Macdonald 1987). However, a potentiating effect of morphine, via μ-receptors, on calcium curents in cells has also been described (Lorentz et al. 1988).

Data concerning possible up-regulation of calcium channels by *G*-proteins are unequivocal. Scott and Dolphin (1987) observed potentiation of the agonist action of BAY K 8644 on calcium currents in dorsal root ganglion neurons, after intracellular introduction of a nonhydrolysable GTP-analogue. Potentiation of calcium curents in hippocampal neurons by noradrenaline was observed by Gray and Johnston (1987); however, it could be mimicked also by injection of cAMP or application of forskolin. Some increase ($\sim$ 50 %) of calcium currents in snail neurons could be induced by application of the parathyroid hormone (Kostyuk et al. 1990). This effect remained despite a progressive "wash-out" of calcium currents and the corresponding decrease of intracellular cAMP levels. It is not known whether this hormone is present in snail ganglia; data about effective binding of exogenous hormone, however, indicate the existence of receptors to a similar polypeptide which may act as a natural agonist of calcium channels.

The existence of up-regulation of calcium channels through G proteins acting in parallel with indirect modulation via cAMP-dependent phosphorylation has recently been demonstrated in cardiomyocytes (Shuba et al., 1990).

Modulation of calcium channels by other protein kinase systems. After the detection of the C-kinase protein phosphorylation pathway attempts were made to reveal its possible participation in the modulation of calcium channels. DeRiemer et al. (1985) have found that PK-C activation by phorbol esters potentiates calcium currents in Aplysia neurons. Harris et al (1986) observed also a parallel increase in norepinephrine secretion in pheochromocytoma cells. A long lasting increase of high-threshold calcium current induced by phorbol ester has been observed by Doroshenko and Kostyuk (1987) in snail neurons. In Aplysia bag-cells after treatment with PK-C activators additional calcium channels with higher unitary conductance have been observed to be involved in the activity (Strong et al. 1987). On the other hand, Hammond et al. (1987) have seen an opposite effect after direct injections of PK-C into some snail neurons: an increase of the inhibitory action of cholecystokinin. An inhibitory effect of



phorbol esters and diacylglycerol on the high-threshold calcium channels in aortic smooth-muscle fibers was noticed by Galizzi et al. (1987). A depression of both low- and high-threshold components of the calcium currents in cloned pituitary cells and chick sensory neurons under the action of PK-C activators has been described by Marchetti and Brown (1988). Finally, in mice sensory neurons a depression of the inactivating high-threshold calcium current was observed under the action of both PK-C activators and forskolin or dibutyryl-cAMP (Gross and Macdonald 1988).

Despite a plenty of observations about the possible role of PK-C in the modulation of calcium channel functioning, the problem of activation of this kinase in natural conditions by some external or internal factors is completely unclear. One may suggest that this mechanism can be switched on in addition to other processes, for instance by the elevation of intracellular calcium levels due to the activity of calcium channels or release from intracellular stores. In such a case PK-C can act as a supporting mechanism for cellular responses induced by other mechanisms (the so called "gain control"; see Rasmussen et al. 1985).

The cGMP-dependent phosphorylation seems to be the most seldom mechanism of modulation of calcium channels; it has been described only in some snail neurons (Paupardin-Tritsch et al. 1986).

Fig. 1 summarizes schematically the modern ideas concerning the molecular mechanisms of modulation of the voltage-operated calcium channels.

References

Akopyan A. R., Chemeris N. K., Iljin V. I. (1985); Neurotransmitter-induced modulation of neuronal Ca current is not mediated by intracellular Ca or cAMP. Brain Res. **326**, 145—148

Armstrong D., Eckert R. (1985): Phosphorylating agents prevent washout of unitary calcium currents in excised membrane patches. J. Gen. Physiol. **86**, 25a

Armstrong D., Kalman D. (1988): The role of protein phosphorylation in the response of dihydropyridine-sensitive calcium channels to membrane depolarization in mammalian pituitary tumor cells. In: Calcium and Ion Channel Modulation. Proc. Symp. Honoring R. Eckert, Febr. 26 — March 1, 1987, Los Angeles, Ca. (Eds. A. D. Grinnell, D. Armstrong and M. B. Jackson), pp. 215—227. Plenum, New York and London

Belles B., Malecot C. O., Hescheler J., Trautwein W. (1988): "Run-down" of the Ca current during long whole-cell recordings in guinea pig heart cells: role of phosphorylation and intracellular calcium. Pfluegers Arch. **411**, 353—360

Brezina V., Eckert R., Erxleben C. (1987): Suppression of calcium current by an endogenous neuropeptide in neurones of *Aplysia californica*. J. Physiol. (London), **388**, 565—595

Byerly L., Hagiwara S. (1982): Calcium currents in internally perfused nerve cell bodies of *Limnea stagnalis*. J. Physiol. (London), **322**, 503—528

Byerly L., Yazejian B. (1986): Intracellular factors for the maintenance of calcium currents in perfused neurones from the snail, *Limnaea stagnalis*. J. Physiol. (London) **370**, 631—650

Carbone E., Lux H. D. (1984): A low voltage-activated, fully inactivating Ca channel in vertebrate sensory neurones. Nature **310**, 501—503

Chad J. E., Eckert R. (1986): An enzymatic mechanism for calcium current inactivation in dialysed Helix neurones. J. Physiol. (London) **378**, 31—51

Curtis B. M., Catterall W. A. (1984): Purification of the calcium antagonist receptor of the voltage-sensitive calcium channel from skeletal muscle transverse tubules. Biochemistry **23**, 2113—2118

Deisz R. A., Lux H. D. (1985): γ-Aminobutyric acid-induced depression of calcium currents of chick sensory neurons. Neurosci. Lett. **56**, 205—210

DeRiemer S. A., Strong J. A., Albert K. A., Greengard P., Kaczmarek L. K. (1985): Enhancement of calcium current in Aplysia neurones by phorbol ester and protein kinase C. Nature **313**, 313—316

Dolphin A. C., Scott R. H. (1987): Inhibition of calcium currents by the GABA agonist baclofen: involvement of a nucleotide binding protein. Neurosci. Lett. Suppl. **29**, 85

Dolphin A. C., Scott R. H. (1989): Interaction between calcium channel ligands and guanine nucleotides in cultured rat sensory and sympathetic neurones. J. Physiol. (London) **413**, 271—288

Dolphin A. C., Forda S. R., Scott R. H. (1986): Calcium-dependent currents in cultured rat dorsal root ganglion neurones are inhibited by an adenosine analogue. J. Physiol. (London) **373**, 47—61

Doroshenko P. A., Kostyuk P. G. (1987): Enhancement of calcium current in the somatic membrane of snail nerve cells by phorbol ester. Biol. Membrany, **4**, 1160—1163 (in Russian)

Doroshenko P. A., Kostyuk P. G., Martynyuk A. E. (1982): Intracellular metabolism of adenosine 3′, 5′-cyclic monophosphate and calcium inward current in perfused neurons of *Helix pomatia*. Neurosci. **7**, 2125—2134

Doroshenko P. A., Kostyuk P. G., Martynyuk A. E., Kursky M. D., Vorobetz Z. D. (1984): Intracellular protein kinase and calcium inward currents in perfused neurones of the snail *Helix pomatia*. Neurosci. **11**, 263—267

Dunlap K., Fischbach G. D. (1978): Neurotransmitters decrease the calcium component of sensory neurone action potentials. Nature **276**, 837—839

Dunlap K., Fishbach G. D. (1981): Neurotransmitters decrease the calcium conductance activated by depolarization of embryonic chick sensory neurones. J. Physiol. (London) **317**, 519—535

Dupon J.—L., Bossu J.—L., Feltz A. (1986): Effect of internal calcium concentration on calcium currents in rat sensory neurones. Pfluegers Arch. **406**, 433—435

Eckert R., Chad J. E., Kalman D. (1986): Enzymatic regulation of calcium current in dialyzed and intact molluscan neurons. J. Physiol. (Paris) **81**, 318—324

Ewald D. A., Walker M. W., Perney T. M., Matthies H. J. G., Miller R. J. (1988): Neurotransmitter modulation of calcium currents in rat sensory neurons. In: Calcium and Ion Channel Modulation. Proc. Symp. Honoring R. Eckert. Febr. 26 — March 1, 1987, Los Angeles, Ca. (Eds. A. D. Grinnell, D. Armstrong and M. B. Jackson) pp. 263—273, New York and London, Plenum

Fedulova S. A., Kostyuk P. G., Veselovsky N. S. (1981): Calcium channels in the somatic membrane of the rat dorsal root ganglion neurons, effect of cAMP. Brain Res. **214**, 210—214

Fedulova S. A., Kostyuk P. G., Veselovsky N. S. (1985): Two types of calcium channels in the somatic membrane of newborn rat dorsal root ganglion neurons. J. Physiol. (London), **359**, 431—446

Fischmeister R., Hartzell H. C. (1986): Mechanism of action of acetylcholine on calcium current in single cells from frog ventricle. J. Physiol. (London) **376**, 183—202

Forscher P., Oxford G. S. (1984):Norepinephrine modulation of calcium channels in internally dialyzed sensory neurons. Biophys. J. **45**, 181a

Forscher P., Oxford G. S., Schulz D. (1986): Noradrenaline modulates calcium channels in avian dorsal root ganglion cells through tight receptor-channel coupling. J. Physiol. (London) **379**, 131—144

Galizzi J.—P., Qar J., Fosset M., Van Rentergham C., Lazdunski M. (1987): Regulation of calcium channels in aortic muscle cells by protein kinase C activators (diacylglycerol and phorbol esters) and by peptides (vasopressin and bombesin) that stimulate phosphoinositide breakdown. J. Biol. Chem. **262**, 6947—6950

Gerschenfeld H. M., Hammond C., Paupardin-Tritsch D. (1986): Modulation of the calcium current of molluscan neurones by neurotransmitters. J. Exp. Biol. **124**, 73—91

Gray R., Johnston D. (1987): Noradrenaline and β-adrenoreceptor agonists increase activity of voltage-dependent calcium channels in hippocampal neurons. Nature **327**, 620—622

Greengard P. (1978): Cyclic Nucleotides, Phosphorylated Proteins and Neuronal Function. Raven Press, New York

Gross R. A., Macdonald R. L. (1987): Dynorphin A selectively reduces a large transient (N-type) calcium current of mouse dorsal root ganglion neurons in cell culture. Proc. Nat. Acad. Sci. USA **84**, 5469—5473

Gross R. A., Macdonald R. L. (1988): Reduction of the same calcium current component by A and C kinase: differential pertussis toxin sensitivity. Neurosci. Lett. **88**, 50—56

Hammond C., Paupardin-Tritsch D., Nairn A., Greengard, P., Gerschenfeld H. M. (1987): Cholecystokinin induces a decrease in Ca^{2+} current in snail neurons that appears to be mediated by protein kinase C. Nature **325**, 809—811

Harris K. M., Kongsamut S., Miller R. J. (1986): Protein kinase C mediated regulation of calcium channels in PC-12 pheochromocytoma cells. Biochem. Biophys. Res. Commun. **134**, 1298—1305

Harris-Warrick R. M., Hammond C., Paupardin-Tritsch D., Homberger V., Rouot B., Bockaert J., Gerschenfeld H. M. (1988): An α_{40} subunit of a GTP-binding protein immunologically related to G_0 mediates a dopamine-induced decrease of Ca^{2+} current in snail neurons. Neuron **1**, 27—32

Hescheler J., Kameyama M., Trautwein W. (1986): On the mechanism of muscarinic inhibition of the cardiac Ca current. Pfluegers Arch. **407**, 182—189

Hescheler J., Rosenthal W., Trautwein W., Schultz G. (1987): The GTP-binding protein, G_0, regulates neuronal calcium channels. Nature **325**, 445—447

Horn J. P., McAfee D. A. (1979): Norepinephrine inhibits calcium-dependent potentials in rat sympathetic neurons. Science **204**, 1233—1235

Horn J. P., McAfee D. A. (1980): Alpha-adrenergic inhibition of calcium-dependent potentials in rat sympathetic neurones. J. Physiol. (London) **301**, 191—204

Hosey M. M., Borsotto M., Lazdunski M. (1986): Phosphorylation and dephosphorylation of dihydropyridine-sensitive voltage-dependent Ca^{2+} channel in skeletal muscle membranes by cAMP- and Ca^{2+} dependent processes. Proc. Nat. Acad. Sci. USA **83**, 3733—3737

Imagawa T., Leung A. T., Campbell K. P. (1987): Phosphorylation of the 1,4-dihydropyridine receptor of the voltage-dependent Ca^{2+} channel by an intrinsic protein kinase in isolated triads from rabbit skeletal muscle. J. Biol. Chem. **262**, 8333—8339

Kaczmarek L. K., Jennings K. R., Strumwasser F., Nairn A. C., Walter U., Wilson F. D., Greengard P. (1980): Microinjection of catalytic subunit of cyclic AMP-dependent protein kinase enhances calcium action potentials of bag cell neurons in cell culture. Proc. Nat. Acad. Sci. USA **77**, 7487—7491

Kameyama M., Kameyama A., Nakayama T., Kaibara M. (1988): Tissue extract recovers cardiac calcium channels from "run-down". Pfluegers Arch. 412, 328—330

Klein M., Kandel E. R. (1978): Presynaptic modulation of voltage-dependent Ca^{2+} current: Mechanism for behavioural sensitization in *Aplysia californica*. Proc. Nat. Acad. Sci. USA **75**, 3512—3516

Koketsu K., Akasu T. (1982): Modulation of the slow inward Ca^{2+} current by adrenaline in bullfrog sympathetic ganglion cells. Jpn. J. Physiol. **32**, 137—140

Kononenko N. I., Shcherbatko A. D. (1985): Influence of serotonin on the inward calcium current in Helix neurons. Dokl. Akad. Nauk USSR **281**, 1494—1497 (in Russian)

Kononenko N. I., Shcherbatko A. D. (1988): Effect of iontophoretic injection of AMP and cAMP on the calcium current in dialyzed snail neurons. Neirofiziologiya **20**, 769—776 (in Russian)

Kostyuk P. G., Krishtal O. A. (1977): Separation of sodium and calcium currents in the somatic membrane of mollusc neurones. J. Physiol. (London) **270**, 545—568

Kostyuk P. G., Veselovsky N. S., Fedulova S. A. (1981): Ionic currents in the somatic membrane of rat dorsal root ganglion neurons. II. Calcium currents. Neurosci. **6**, 2431—2437

Kostyuk P. G., Luk'yanetz E. A., Doroshenko P. A. (1990): Studies of the cAMP influence on calcium currents in mollusc neurones possessing different sensitivity of their calcium conduction to serotonin action. Neurofiziologiya (in press) (in Russian)

Lorentz M., Hedlund B., Arhem P. (1988): Morphine activates calcium channels in cloned mouse neuroblastoma cell line. Brain Res. **445**, 157—159

Macdonald R. L., Skerritt J. H., Werz M. A. (1986): Adenosine agonists reduce voltage-dependent calcium conductance of mouse sensory neurons in cell culture. J. Physiol. (London) **370**, 75—90

Macdonald R. L., Werz M. A. (1986): Dynorphin A decreases voltage-dependent calcium conductance of mouse dorsal root ganglion neurones. J. Physiol. (London) **377**, 237—249

Marchetti C., Brown A. M. (1988): Protein kinase activator 1-oleoyl-2-acetyl-sn-glycerol inhibits two types of calcium currents in GH_3 cells. Amer. J. Physiol. **254**, 206—210

Marchetti C., Carbone E., Lux H. D. (1986): Effects of dopamine and noradrenaline on Ca channels of cultured sensory and sympathetic neurons of chick. Pfluegers Arch. **406**, 104—111

McAfee D. A., Henon B. K., Horn J. P., Yarowsky P. (1981): Calcium currents modulated by adrenergic receptors in sympathetic neurons. Fed. Proc. **40**, 2246—2249

McFadzean I. (1988): The ionic mechanisms underlying opioid actions. Neuropeptides **11**, 171—181

McFadzean I., Docherty R. J. (1987): Noradrenaline depresses a high-threshold calcium current in a neuronal cell. Neurosci. Lett., Suppl. **29**, p. 24

Okamoto K., Kimura H., Sakai Y. (1983): Effects of taurine and GABA on Ca spikes and Na spikes in cerebellar Purkinje cells in vitro: Intrasomatic study. Brain Res. **260**, 249—259

Paupardin—Tritsch D., Hammond C., Gerschenfeld H. M., Nairn A. C., Greengard P. (1986): cGMP-dependent protein kinase enhances Ca^{2+} current and potentiates the serotonin-induced Ca^{2+} current increase in snail neurones. Nature **323**, 812—814

Rasmussen H., Clayberger C., Gustin M. C. (1979): The messenger function of calcium in cell activation. In: Symp. Soc. Exp. Biol. No. XXXIII "Secretory Mechanisms", pp. 161—197, Cambridge

Rasmussen H., Kojima I., Kojima K., Zawalich W., Apfeldorf W. (1985): Calcium as intracellular messenger: sensitivity modulation, C-kinase pathway, and sustained cellular response. In: Advances in Cyclic Nucleotide and Protein Phosphorylation Research. V. 18. (Eds. P. Greengard & G. A. Robinson) pp. 159—193. Raven Press, New York

Reuter H. (1974): Localization of beta adrenergic receptors and effects of noradrenaline and cyclic nucleotides on action potentials, ionic currents and tension in mammalian cardiac muscle. J. Physiol. (London) **242**, 429—451

Reuter H. (1979): Properties of two inward membrane currents in the heart. Annu. Rev. Physiol. **41**, 413—424

Scott R. H., Dolphin A. C. (1987): Activation of a G protein promotes agonist responses to calcium channel ligands. Nature **330**, 760—762

Shimahara T., Icard-Liepkalns C. (1987): Activation of enkephalin receptors reduces calcium conductance in neuroblastoma cells. Brain Res. **415**, 357—361

Shimahara T., Tauc L. (1977): Cyclic AMP induced by serotonin modulates the activity of an identified synapse in Aplysia by facilitating the active permeability to calcium. Brain Res. **127**, 168—172

Shuba Ya. M., Hesslinger B. Trautwein W., McDonald T. F., Pelzer D. (1990): Whole-cell calcium current in guinea-pig ventricular myocytes dialysed with guanine nucleotides. J. Physiol. (London) (in press)

Strong J. A., Fox A. P., Tsien R. W., Kacmarek I. K. (1987): Stimulation of protein kinase C recruits covert calcium channels in Aplysia bag cell neurons. Nature **325**, 714—717

Tsunoo A., Yoshii M., Narahashi T. (1986): Block of calcium channels by enkaphalin and somatostatin in neuroblastoma-glioma hybrid NG108-15 cells. Proc. Nat. Acad. Sci. USA **83**, 9832—9836

Wanke E., Ferroni A., Malgaroli A. (1987): Activation of a muscarinic receptor selectively inhibits a rapidly inactivated Ca^{2+} current in rat sympathetic neurons. Proc. Nat. Acad. Sci. USA **84**, 4313—4317

Werz M. A., Macdonald R. L. (1984): Dynorphin reduces calcium-dependent action potential duration by decreasing voltage-dependent calcium conductance. Neurosci. Lett. **46**, 185—190

Werz M. A., Macdonald R. L. (9185): Barbiturates decrease voltage-dependent calcium conductace of mouse neurons in dissociated cell culture. Mol. Pharmacol. **28**, 269—277

Williams J. T., North R. A. (1985): Catecholamine inhibition of calcium action potentials in rat locus coeruleus neurones. Neuroscience **14**, 103—109

Final version accepted April 5, 1990