Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochimica et Biophysica Acta 1618 (2003) 33–38
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
www.bba-direct.com

Action of hypoxia on different types of calcium channels in hippocampal neurons

Elena A. Lukyanetz[a,b,*], Vyacheslav M. Shkryl[b], Olga V. Kravchuk[a], Platon G. Kostyuk[a]

[a] *Bogomoletz Institute of Physiology, Bogomoletz str. 4, Kiev 01024, Ukraine*
[b] *International Center of Molecular Physiology, Kiev 01024, Ukraine*

Received 1 July 2003; received in revised form 17 September 2003; accepted 14 October 2003

Abstract

Whole-cell patch clamp and polarographic oxygen partial pressure (pO_2) measurements were used to establish the sensitivity of high-voltage-activated (HVA) Ca^{2+} channel subtypes of CA1 hippocampal neurons of rats to hypoxic conditions. Decrease of pO_2 to 15–30 mm Hg induced a potentiation of HVA Ca^{2+} currents by 94%. Using selective blockers of N- and L-types of calcium channels, we found that inhibition of L-type channels decreased the effect by 54%, whereas N-type blocker attenuated the effect by 30%. Taking into account the ratio of currents mediated by these channel subtypes in CA1 hippocampal neurons, we concluded that both types of HVA Ca^{2+} channels are sensitive to hypoxia, however, L-type was about 3.5 times more sensitive to oxygen reduction.

Keywords: Calcium; Calcium channel; Calcium channel type; Hippocampal neuron; Hypoxia; Rat

1. Introduction

Voltage-operated calcium channels are essential for Ca^{2+} signalling in excitable cells [1–3]. They can also play a significant role in the development of several brain pathologies including those at ischemic/hypoxic conditions. Several types of voltage-operated calcium channels have been described: L-, N-, P-, Q-, R- and T-types (see Refs. [4–6] for review). As it has been shown in numerous experiments, different cell types can express various combinations of Ca^{2+} channel subtypes and in different proportions. This determines the precise characteristics of the voltage dependence and kinetics of the integral calcium currents. Thus, predominance of L- and N-type Ca^{2+} channels has been previously shown in CA1 hippocampal neurons of rats [7,8]. In our previous investigations, we have shown that HVA Ca currents of CA1 hippocampal neurons were sensitive to hypoxia at definite experimental conditions [9]. We found that the degree of Ca channel sensitivity to hypoxia depended on intracellular and extracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) [7,9,10]. However, in those experiments, we tested the sensitivity of total HVA Ca^{2+} current to hypoxia without separation of the current into the subtypes—its specific components. In the present study, we investigated how Ca channel subtypes of freshly isolated CA1 hippocampal neurons respond to hypoxic exposure.

2. Materials and methods

Hippocampal neurons were dissociated with a modification of the method described previously [7]. Wistar rats at postnatal day P14 were used. At this age, hippocampal neurons already express high-voltage-activated (HVA) Ca^{2+} channels, electrophysiological properties of which are similar to those in older animals. The rats were deeply anaesthetized with ether and decapitated. The brain was removed and the hippocampus was isolated. Hippocampal slices (500 μm) were cut with a blade and transferred to a chamber containing trypsin (0.02% Type XI, Sigma). Following 30 min of enzymatic treatment, the slices were washed (30 min) before being prepared for recordings. Immediately before recordings, a hippocampal slice was removed from the storage chamber and the CA1 region was cut away from

* Corresponding author. National Academy of Sciences, A.A. Bogomoletz Institute of Physiology, Bogomoletz str. 4, Kiev 01024, Ukraine. Tel.: +380-44-256-2430; fax: +380-44-253-6458.
E-mail address: elena@serv.biph.kiev.ua (E.A. Lukyanetz).

0005-2736/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamem.2003.10.003

the remaining tissue. CA1 neurons were acutely isolated by using a modified vibro-dissociation technique [11]. The cell suspension was plated on a coverslip in the recording chamber.

Standard patch clamp method was used to record Ca currents with PC-ONE amplifier, Dagan, USA) as was described previously [9]. Standard P/2 pulse protocol was used to subtract the linear leak and capacity currents on-line: the protocol using the average of one depolarizing and two half-magnitude hyperpolarizing pulses. Recordings were filtered at 2 kHz and digitized at 10 kHz using a multichannel interface controlled by a computer. Ca channel activity was recorded by 2–4 MΩ micropipettes manufactured from borosilicate glass capillary tubes with Micropipette Puller P-97, Sutter Instrument, USA. Recordings were made from cells which had the seal resistance greater than 1 GΩ. All values are given as means ± S.E. Statistical differences were assessed by analysis of variance (ANOVA) with the level of significance set at P pointed in the text.

We used an experimental system for combined measurements of transmembrane ionic currents in whole-cell patch clamp configuration and measurements of pO_2 in bath solution by polarographic method. The pO_2 recordings were filtered at 1 kHz and digitized at 9 Hz using second current amplifier and the same multichannel interface as above. We used platinum electrodes with 0.3 mm active area and Ag–AgCl reference electrode. The recording potential corresponded to the diffusion plateau of electrode polarogram at −700 mV. The pO_2 was adjusted by displacement of O_2 by N_2 in the solution by bubbling gas mixture. For calibration of pO_2 values, we used one-point method described previously in detail [7,9,12].

The composition of external solution used in this study was (mM): tetraethylammonium chloride—25, 4-AP—5, $MgCl_2$—2, KCl—3, $CaCl_2$—2, HEPES—20, NaCl—100, TTX—0.001, pH 7.4; pipette solution (mM): CsCl—56, Cs_2SO_4—47, HEPES—20, $MgCl_2$—1, ATP—4, GTP—0.3, EGTA—0.5, $CaCl_2$—0.2, pH 7.2. All used chemicals were purchased from Sigma, USA.

3. Results

Freshly isolated hippocampal neurons were voltage clamped at −80 mV holding potential, and were depolarized to membrane potentials −60 to +40 mV by pulses with duration 50 ms. Fig. 1 shows the voltage protocol of these experiments. The neurons exhibited a slow inactivating integral inward current, carried out by Ca^{2+} ions which were tested (Fig. 1A). The peak magnitude of Ca^{2+} current was variable (ranging between −150 and −300 pA) and the peak of current–voltage relation corresponded to 0 or +5 mV in different tested cells. Fig. 1B shows the mean of current–voltage relationship of HVA Ca^{2+} current obtained from eight cells. The decrease of pO_2 to mean value 25.4 ± 2.5 mm Hg (n = 8) from the value measured in control conditions (pO_2 = 151 mm Hg) induced a remarkable and reversible increase of the magnitude of Ca^{2+} currents in all tested hippocampal neurons (n = 8) (Figs. 1 and 2). Fig. 2A shows an example of measurements of peak Ca^{2+} current (lower part) recorded at the same time as pO_2 values in bath solution (upper part). As it can be seen, the curve of Ca^{2+} current magnitude is precisely a mirror image of the trace showing changes in pO_2. In this particular cell, the initial current was −229 pA, during exposure to hypoxic solution (pO_2 = 25 mm Hg), the current reached value of −515 pA. During the reoxygenation with the normoxic solution, Ca^{2+} current magnitude returned back to its initial level (Fig. 2A). Fig. 2B shows the statistical data of stimulatory action of hypoxia on HVA Ca^{2+} current. The mean increase of Ca^{2+} current was equal to 94.1 ± 8.9%, n = 8 (Fig. 1B).

In the next experiments, we tested the effect of hypoxia in the presence of selective blockers of HVA Ca^{2+} channels. Our previous experiments have shown that in freshly isolated hippocampal neurons, mainly two types of HVA channels are expressed: N- and L-types which accounted for about 48% and 25%, respectively (73% together) of total HVA Ca^{2+} current [8]. Therefore, we focused on the effects of hypoxia on main

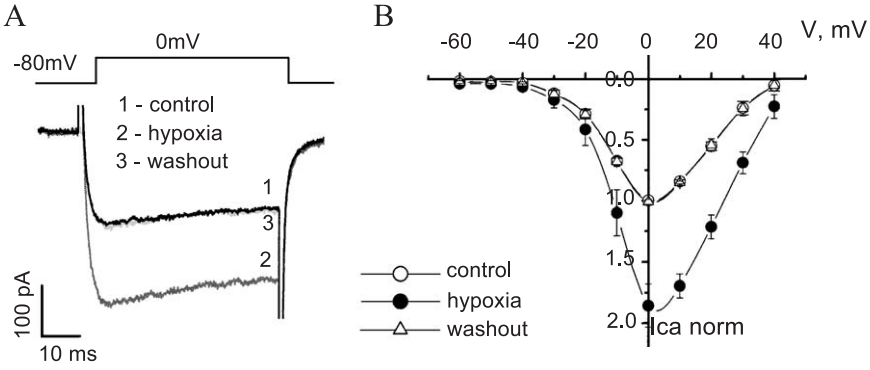

Fig. 1. Typical examples of whole-cell HVA calcium current enhancement by hypoxia in freshly isolated hippocampal neurons. (A) Typical examples of whole-cell HVA calcium currents recorded before (1), 10 min after (2) exposing the cell to hypoxic solution and after reoxygenation (3). The voltage protocol of experiment is shown in the upper part. (B) Mean current–voltage relations obtained from eight tested cells (open circles—control, dark circles—hypoxia, open triangles—normoxia). Holding potential −80 mV.

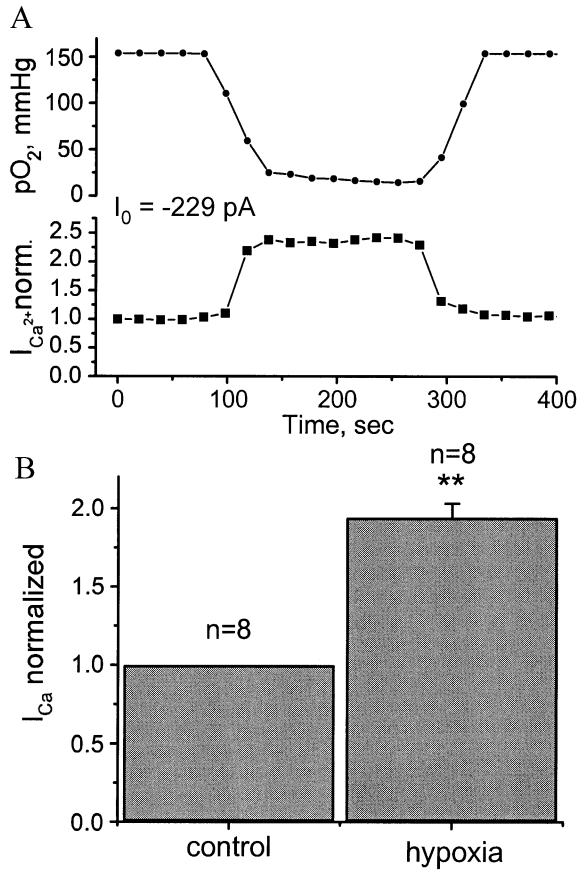

Fig. 2. Behavior of magnitude of HVA Ca^{2+} currents during hypoxia in freshly isolated hippocampal neurons. (A) Example of changes in magnitude of peak Ca^{2+} currents measured in control conditions (normalized to its initial level, the initial magnitude of which is pointed in absolute units, I_0). Holding potential was -80 mV. Upper curve represents changes in oxygen partial pressure (pO_2) in bath solution near the cell body. (B) Bar graph representing the mean values of Ca^{2+} current in control and at $pO_2 = 25$ mm Hg. The pO_2 traces here and throughout represent the solid lines through data points measured in the same time as peak Ca^{2+} current. $^{**}P < 0.01$ compared to control.

contributors to the Ca^{2+} current-L- and N-type channels. Other channels we referred to as non-L- and non-N-type. To block L-type channels, we used nifedipine, a well-known selective blocker of L-type Ca^{2+} channels in concentrations of 20 μM to maximally inhibit L-type Ca^{2+} channels. Previously, it was shown that nifedipine blocked the L-type Ca^{2+} channels with an IC_{50} about of 0.3 μM [13]. The experiments with nifedipine were carried out in reduced light in order to avoid the photolysis of this compound. Fig. 3A shows that application of nifedipine caused a decrease of hypoxic effect on Ca^{2+} current. An example of changes of Ca^{2+} current magnitude in one of the cells is presented in Fig. 3A. The Ca^{2+} current magnitude increased from -196 to -255 pA under the same hypoxic conditions in the presence of nifedipine. Statistical analysis has shown that the mean effect of hypoxia on Ca^{2+} current was $39.9 \pm 4.0\%$ ($n = 5$) in the presence of 20 μM nifedipine (Fig. 3C).

Next we tested the effect of selective blockade of N-type calcium channel subtype by ωCTx-GVIA, a peptide toxin of snail *C. geographus*. Previously it was shown that, omega-CgTx GVIA, a blocker of N-type Ca^{2+}

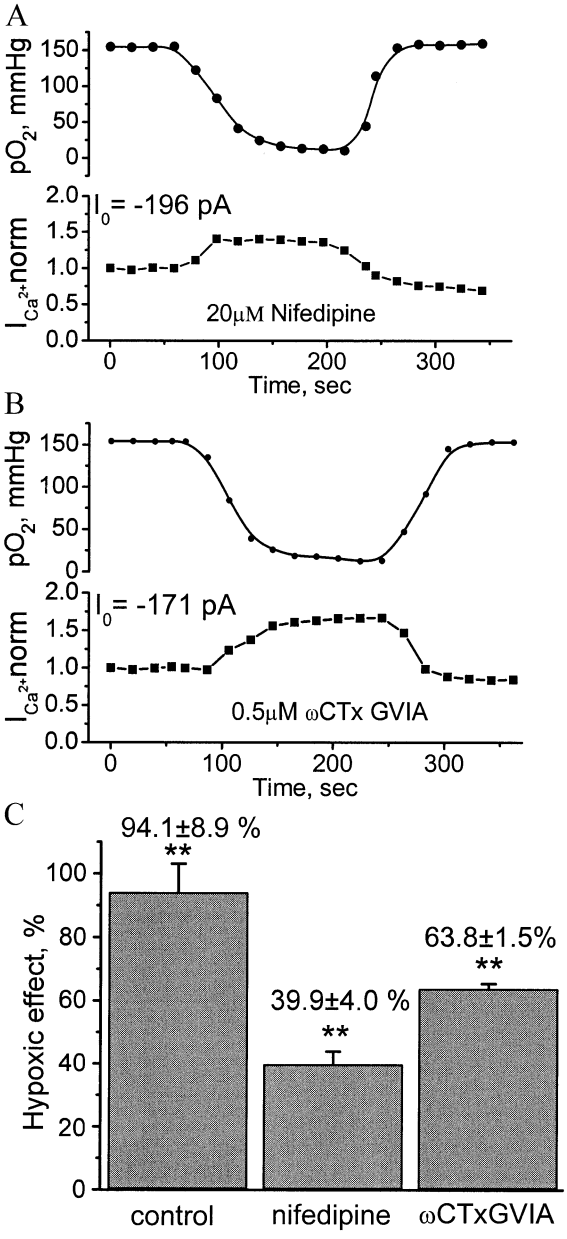

Fig. 3. Measurements of Ca^{2+} currents during hypoxia in freshly isolated hippocampal neurons in the presence of selective blockers of HVA Ca^{2+} channels. Examples of changes in magnitude of peak Ca^{2+} currents measured in the presence of 20 μM nifedipine (A) or 0.5 μM ωCTxGVIA (B). pO_2 of the solution was decreased to 25 mm Hg in both cases, and represented on corresponding upper parts. (C) Bar graph representing mean values of effect of hypoxia on HVA Ca^{2+} channels from CA1 hippocampal neurons measured in control, in conditions of blocked L-type Ca channels (in the presence of 20 μM nifedipine) or N-type (in the presence of 0.5 μM ωCTxGVIA) are shown. pO_2 of the solution was decreased to 25 mm Hg in all cases. Cells were depolarized from -80 mV holding potential to $+5$ mV. $^{**}P < 0.01$ compared to conditions before hypoxia.

channels, has an IC_{50} value of about 28 nM [14]. When N-type calcium channels were irreversibly blocked by 0.5 μM ωCTx-GVIA, the effect of hypoxia accounted for 63.8 ± 1.5%, $n=5$ (Fig. 3C). An example of measurements from one of the tested cells is presented in Fig. 3B. In this cell, initial magnitude of Ca^{2+} current (−171 pA) was increased to −291 pA by exposure to hypoxic solution containing the toxin.

Thus, taking into account that hippocampal neurons also express (in significantly less degree) Q- and P-types, we calculated the contribution of Ca^{2+} channel subtypes in the total effect of hypoxia from a system of equations:

$$\begin{cases} E_L + E_N + E_r = 94.1 \\ E_N + E_r = 39.9 \\ E_L + E_r = 63.8 \end{cases} \quad (1)$$

where E_L, E_N and E_r are the values of effect of hypoxia on corresponding L-, N- and r- (residual non-L- and non-N-) channel subtypes. The first equation represents the value of the effect in control conditions when all three components are present, and the two other equations represent the value of effect of hypoxia without N- or L-components, when those were abolished by corresponding blocker. In our calculations, we assumed that different types of Ca^{2+} channels behave independent of one another regardless of the degree of oxygenation; there is no cross-reactivity of the inhibitor between different channel subtypes, independent of the degree of oxygenation, and that there is no change in drug sensitivity with hypoxia. Solution of Eq. (1) has shown that L-type contributed 54.2% (E_L), N-type—30.3% (E_N) and other types—only 9.6% (E_r) to total effect of hypoxia (Fig. 4A).

The fact that on the one side, the hypoxic effect on Ca^{2+} current was suppressed by L-type channel blocker more significantly than by N-type blocker, and on the other side that the fraction of N-types was greater than L-type in total Ca^{2+} current (according to our previous study [8]), can point to higher sensitivity of L-type to hypoxia. Therefore, to quantitatively estimate the sensitivity of channel subtypes to hypoxia, we modified Eq. (1) by considering a ratio of channel subtypes in total Ca^{2+} current (25%, 49% and 26% for L-, N-types and residual currents, respectively, as we have shown in our previous experiments [8]):

$$\begin{cases} aE_L + bE_N + cE_r = 94.1 \\ bE_N + cE_r = 39.9 \\ aE_L + cE_r = 63.8 \end{cases} \quad (2)$$

where $a=0.25$, $b=0.49$ and $c=0.26$ are coefficients representing the fraction of corresponding channel types

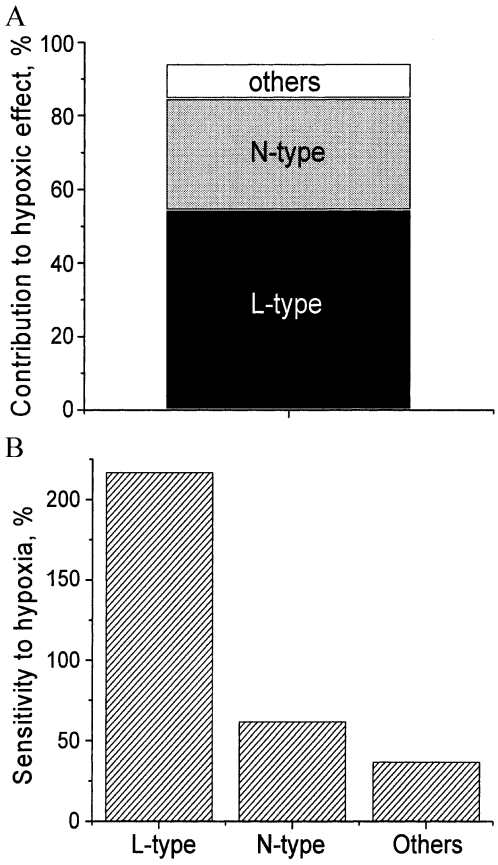

Fig. 4. Summary representation of the effect of hypoxia on HVA calcium currents. (A) Bar graph representing the mean contribution of HVA channel subtypes in effect of hypoxia on total HVA Ca^{2+} currents. (B) Sensitivity of HVA Ca^{2+} channels to hypoxia. The percentage of channel subtype expression in hippocampal neurons was taken into account.

in total Ca^{2+} current of CA1 hippocampal neurons [8]. According to the Eq. (2), we established that hypoxia induced a potentiation of L-type by 216.8%, N-type by 61.8% and residual non-L- and non-N-type Ca^{2+} currents by 36.9% (Fig. 4B).

4. Discussion

Summarizing obtained data, we can conclude that in comparison to other channel types, L-type of calcium channels display particularly high sensitivity to hypoxia. Such exclusive sensitivity of L-type channels to hypoxia is not surprising, since several studies based mainly on indirect data have previously reported about the sensitivity of just these channels to oxygen deprivation. Thus, brief hypoxia enhanced the activity of L-type Ca^{2+} channels in brainstem neurons [15] and in rabbit carotid body glomus cells [16,17]. Ca^{2+} influx via L-type Ca^{2+} channels was shown to be a route of toxic Ca^{2+} influx into rat optic axons during anoxia. Immunocytochemical staining demonstrated the localization of α_{1C} and α_{1D} subunits in these axons, indicating that only L-type Ca^{2+} channels were

present there [18]. Potentiation of L-type calcium current by hypoxia was observed in experiments on pulmonary myocytes [19]. Our recent data have shown that blocking of L-type channels by nifedipine substantially reduced the hypoxia-induced Ca^{2+} growth in sensory DRG neurons [20]. L-type calcium channel blockers significantly attenuated the neuronal injury induced by oxygen deprivation in cultured hippocampal neurons [21]. Voltage-gated Ca^{2+} channel blockade with the L-type antagonist nitrendipine decreased Ca^{2+} entry and partially preserved CA1 hippocampal cell injury in slices during experimental oxygen-glucose deprivation [22]. However, results described above contradict the data obtained by Krnjevic et al. [23] in single-electrode voltage clamp experiments which have shown significant blockade (by greater than 95%) of L-type channels by hypoxia in CA1 and CA3 neurons in submerged hippocampal slices. This discrepancy may be connected with different experimental conditions in terms of ionic composition, pO_2 level and especially levels of intracellular Ca^{2+}, which is crucially important for the development of the effect of hypoxia [9]. In our previous experiments, we established that hippocampal neurons "sensed" hypoxia at $pO_2 = 90–100$ mm Hg; however, when pO_2 become lower then 40 mm Hg, the effect was critically enhanced [9]. Therefore, we can suppose that the physiological normoxic range of pO_2 for CA1 hippocampal neurons lies above 40 mm Hg.

The high sensitivity of L-type Ca^{2+} channels to hypoxia could be due to some molecular features of their α_1 and β subunits which determine higher metabolic dependence of L-type channels in comparison with other channel subtypes. Such properties as high sensitivity to intracellular Ca^{2+} [24], susceptibility to phosphorylation by protein kinases A, C, G, CaMII and others [15,17,25–32], regulation by protein phosphatases [24,33,34], sensitivity to pH [35] were described primarily for L-type channels. Probably such functional lability of L-type channels allowing their participation in delicate regulation of intracellular Ca^{2+} metabolism could make these channels extremely dangerous in conditions of oxygen deprivation. Therefore, L-type channels should not be considered as natural oxygen sensors in neurons; rather their hypoxic-sensing is a harmful side effect. Taking into account a substantial role of L-type channels in response to hypoxia, in further experiments, it would be of interest to compare the influence of hypoxia on subtypes of L-type channels formed by different α_1 subunits (α_{1S}, α_{1C}, α_{1D}, α_{1F}) expressed in other excitable tissues (cardiac, smooth muscle, endocrine cells) at appropriate experimental conditions.

Considering the possible pathophysiological role of particularly high sensitivity of L-type channels to hypoxia, one should keep in mind the extreme variability of representation of different types of HVA Ca^{2+} channels in different types of neuronal and other excitable cells. Therefore, the damaging effect of hypoxia could also be quite different in different neuronal structures, as it is indeed observed in human pathology. Such differences could be amplified by variable interactions of increased influx of Ca^{2+} via L-type channels with other factors, first of all with increased extrusion of glutamate and resulting excitoxicity produced by secondary Ca^{2+} influx. Due to their sensitivity to hypoxia, presence of L-type channels in synaptic structures may in turn significantly amplify the damaging role of glutamate-induced Ca^{2+}-overload.

Acknowledgements

This work was partly supported from INTAS 99-01915.

References

[1] P. Kostyuk, N. Akaike, Y. Osipchuk, A. Savchenko, Y. Shuba, Gating and permeation of different types of Ca channels, Ann. N.Y. Acad. Sci. 560 (1989) 63–79.
[2] C.M. Armstrong, B. Hille, Voltage-gated ion channels and electrical excitability, Neuron 20 (1998) 371–380.
[3] W.A. Catterall, Structure and regulation of voltage-gated Ca^{2+} channels, Annu. Rev. Cell Dev. Biol. 16 (2000) 521–555.
[4] E. Perez-Reyes, J.H. Lee, L.L. Cribbs, Molecular characterization of two members of the T-type calcium channel family, Ann. N.Y. Acad. Sci. 868 (1999) 131–143.
[5] P.G. Kostyuk, Low-voltage activated calcium channels: achievements and problems, Neuroscience 92 (1999) 1157–1163.
[6] M. Yamakage, A. Namiki, Calcium channels—basic aspects of their structure, function and gene encoding; anesthetic action on the channels—a review, Can. J. Anaesth. 49 (2002) 151–164.
[7] V.M. Shkryl, L.M. Nikolaenko, P.G. Kostyuk, E.A. Lukyanetz, High-threshold calcium channel activity in rat hippocampal neurones during hypoxia, Brain Res. 833 (1999) 319–328.
[8] E.A. Lukyanetz, V.M. Shkryl, P.G. Kostyuk, Selective blockade of N-type calcium channels by Levetiracetam, Epilepsia 43 (2002) 9–18.
[9] V.M. Shkryl, P.G. Kostyuk, E.A. Lukyanetz, Dual action of cytosolic calcium on calcium channel activity during hypoxia in hippocampal neurones, NeuroReport 12 (2001) 4035–4039.
[10] E.A. Lukyanetz, V.M. Shkryl, O.V. Kravchuk, P.G. Kostyuk, Effect of hypoxia on calcium channels depends on extracellular calcium in CA1 hippocampal neurons, Brain Res. 980 (2003) 128–134.
[11] V.S. Vorobjev, Vibrodissociation of sliced mammalian nervous tissue, J. Neurosci. Methods 38 (1991) 145–150.
[12] E.A. Lukyanetz, V.M. Shkryl, Scientific and technological aspects of oxygen-sensitive electrodes for measurements of oxygen partial pressure in patch-clamp experiments, J. Biochem. Biophys. Methods 55 (2003) 37–52.
[13] J.B. Shen, B. Jiang, A.J. Pappano, Comparison of L-type calcium channel blockade by nifedipine and/or cadmium in guinea pig ventricular myocytes, J. Pharmacol. Exp. Ther. 294 (2000) 562–570.
[14] D. Bowman, S. Alexander, D. Lodge, Pharmacological characterisation of the calcium channels coupled to the plateau phase of KCl-induced intracellular free Ca^{2+} elevation in chicken and rat synaptosomes, Neuropharmacology 32 (1993) 1195–1202.
[15] S.L. Mironov, D.W. Richter, Hypoxic modulation of L-type Ca^{2+} channels in inspiratory brainstem neurones: intracellular signalling pathways and metabotropic glutamate receptors, Brain Res. 869 (2000) 166–177.
[16] B.A. Summers, J.L. Overholt, N.R. Prabhakar, Augmentation of calcium current by hypoxia in carotid body glomus cells, Adv. Exp. Med. Biol. 475 (2000) 589–599.

[17] B.A. Summers, J.L. Overholt, N.R. Prabhakar, Augmentation of L-type calcium current by hypoxia in rabbit carotid body glomus cells: evidence for a PKC-sensitive pathway, J. Neurophysiol. 84 (2000) 1636–1644.
[18] A.M. Brown, R.E. Westenbroek, W.A. Catterall, B.R. Ransom, Axonal L-type Ca^{2+} channels and anoxic injury in rat CNS white matter, J. Neurophysiol. 85 (2001) 900–911.
[19] A. Franco-Obregon, J. Lopez-Barneo, Differential oxygen sensitivity of calcium channels in rabbit smooth muscle cells of conduit and resistance pulmonary arteries, J. Physiol. 491 (Pt 2) (1996) 511–518.
[20] E.A. Lukyanetz, R.I. Stanika, L.M. Koval, P.G. Kostyuk, Intracellular mechanisms of hypoxia-induced calcium increase in rat sensory neurons, Arch. Biochem. Biophys. 410 (2003) 212–221.
[21] M. Kimura, K. Sawada, T. Miyagawa, M. Kuwada, K. Katayama, Y. Nishizawa, Role of glutamate receptors and voltage-dependent calcium and sodium channels in the extracellular glutamate/aspartate accumulation and subsequent neuronal injury induced by oxygen/glucose deprivation in cultured hippocampal neurons, J. Pharmacol. Exp. Ther. 285 (1998) 178–185.
[22] R.M. LoPachin, C.L. Gaughan, E.J. Lehning, M.L. Weber, C.P. Taylor, Effects of ion channel blockade on the distribution of Na, K, Ca and other elements in oxygen-glucose deprived CA1 hippocampal neurons, Neuroscience 103 (2001) 971–983.
[23] K. Krnjevic, E. Cherubini, Y. Ben-Ari, Anoxia on slow inward currents of immature hippocampal neurons, J. Neurophysiol. 62 (1989) 896–906.
[24] P.G. Kostyuk, E.A. Lukyanetz, Mechanisms of antagonistic action of internal Ca^{2+} on serotonin-induced potentiation of Ca^{2+} currents in Helix neurones, Pflugers Arch. 424 (1993) 73–83.
[25] P.G. Kostyuk, E.A. Lukyanetz, P.A. Doroshenko, Effects of serotonin and cAMP on calcium currents in different neurones of Helix pomatia, Pflugers Arch. 420 (1992) 9–15.
[26] P.G. Kostyuk, E.A. Lukyanetz, A.S. Ter-Markosyan, Parathyroid hormone enhances calcium current in snail neurones—simulation of the effect by phorbol esters, Pflugers Arch. 420 (1992) 146–152.
[27] J. Yang, R.W. Tsien, Enhancement of N- and L-type calcium channel currents by protein kinase C in frog sympathetic neurons, Neuron 10 (1993) 127–136.
[28] Y. Zhu, S.R. Ikeda, Modulation of Ca^{2+}-channel currents by protein kinase C in adult rat sympathetic neurons, J. Neurophysiol. 72 (1994) 1549–1560.
[29] R.P. Xiao, H. Cheng, W.J. Lederer, T. Suzuki, E.G. Lakatta, Dual regulation of Ca^{2+}/calmodulin-dependent kinase II activity by membrane voltage and by calcium influx, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 9659–9663.
[30] E.A. Lukyanetz, P.G. Kostyuk, Two distinct receptors operate the cAMP cascade to up-regulate L-type Ca channels, Pflugers Arch. 432 (1996) 174–181.
[31] N. Sperelakis, Y. Katsube, H. Yokoshiki, H. Sada, K. Sumii, Regulation of the slow Ca^{++} channels of myocardial cells, Mol. Cell. Biochem. 163–164 (1996) 85–98.
[32] Y. Wu, L.B. MacMillan, R.B. McNeill, R.J. Colbran, M.E. Anderson, CaM kinase augments cardiac L-type Ca^{2+} current: a cellular mechanism for long Q–T arrhythmias, Am. J. Physiol. 276 (1999) H2168–H2178.
[33] E.A. Lukyanetz, Evidence for colocalization of calcineurin and calcium channels in dorsal root ganglion neurons, Neuroscience 78 (1997) 625–628.
[34] E.A. Lukyanetz, T.P. Piper, T.S. Sihra, Calcineurin involvement in the regulation of high-threshold Ca^{2+} channels in NG108-15 (rodent neuroblastoma × glioma hybrid) cells, J. Physiol. 510 (Pt 2) (1998) 371–385.
[35] S.V. Smirnov, G.A. Knock, A.E. Belevych, P.I. Aaronson, Mechanism of effect of extracellular pH on L-type Ca^{2+} channel currents in human mesenteric arterial cells, Am. J. Physiol, Heart Circ. Physiol. 279 (2000) H76–H85.