F.Ü.Sağlık Bil.Dergisi 1999, 13 (1), 65-70

PHARMACOLOGICAL CHARACTERISTICS AND PHYSIOLOGICAL ROLES OF CALCIUM-ACTIVATED CHLORIDE CONDUCTANCE IN NEURONS

Ahmet AYAR, Kadir SERVİ

Fırat Üniversitesi, Veteriner Fakültesi, Elazığ / TÜRKİYE

Geliş Tarihi: 01.11.1998

Nöronlarda Kalsiyum-Bağımlı Klor⁻ Kanalı Akımlarının Farmakolojik Özellikleri ve Fizyolojik Rolleri

ÖZET

Nöronal hücre membranları çeşitli voltaj ve ligand kapılı iyon kanallarına sahiptir. Bu iyon kanallarının tipi ve lökasyonu bir nöronun elektriksel davranışını belirler. Sodyum, potasyum, klor ve kalsiyum kanalları membranda bulunan esas iyon kanallarıdır. Bu kanallardan bazıları kalsiyum-bağımlı olarak aktive olur ki bunlar; kalsiyum-bağımlı klor, kalsiyum-bağımlı potasyum, kalsiyum-bağımlı secici olmayan katyon kanallarıdır. Bu derlemede, nöronal kalsiyum-bağımlı klor kanallarının bazı farmakolojik ve biyofiziksel özellikleri ile fizyolojik rolleri tartışıldı.

Anahtar Kelimeler: Kalsiyum-bağımlı klor kanalları, klor kanalı inhibitörleri, nöron

SUMMARY

Neuronal cell membranes contain several ligand and voltage-gated ion channels. The type and location of these ion channels determines the electrical behaviour of a neurone. Sodium, potassium, chloride and calcium channels are among the main ion channels that present in cell membranes. Some of these channels are also activated by calcium; the calcium-activated chloride, calcium-activated potassium and calcium-activated non-selective cation channels are among the main Ca^{2+}-dependent ion channels of cell membranes. In this review, we discuss some of the pharmacological, biophysical properties and possible physiological roles of calcium-activated chloride channels in neurons.

Key words: Calcium-activated chloride channels, chloride channel inhibitors, neurone.

Increases in intracellular Ca^{2+}, ($[Ca^{2+}]_i$), either by entry of Ca^{2+} from the extracellular space or its release from intracellular stores can activate Ca^{2+}-activated conductances in the cell membrane. These conductances are Ca^{2+}-activated chloride, potassium, and non-selective cation conductances, which under voltage clamp recording conditions generate their respective currents ($I_{Cl(Ca)}$, $I_{K(Ca)}$, I_{CAN}). Ca^{2+}-activated conductances can be used as physiological indicators of increases in $[Ca^{2+}]_i$ close to the cell membrane. Whole cell $I_{Cl(Ca)}$ and I_{CAN} have proved to be particularly useful in studying Ca^{2+} regulation in cultured DRG neurones (1) and in this paper we aim to review their pharmacological properties and possible physiological roles.

Studies using conventional electrophysiological methods (sharp electrodes), whole-cell and single channel patch clamp techniques and ion flux measurements have indicated that many cell types express chloride channels not only in their cell membrane but also on the membranes of organelles including mitochondria and ER. Studies of their properties indicate that several chloride channel types in cell membranes are activated by Ca^{2+} (2).

The first report of $I_{Cl(Ca)}$ came from studies on amphibian tissues, salamander retina (3) and *Xenopus laevis* oocytes (4). Since then, $I_{Cl(Ca)}$ has

been identified in other cell types such as secretary cells, skeletal, cardiac and smooth muscles, and neurones (5, 6). Neurones which express Ca^{2+}-activated chloride channels include mouse spinal neurones (7), rat DRG neurones (5, 8), chick DRG neurones (9), rabbit pelvic parasympathetic ganglion neurones (10), quail cultured sensory and parasympathetic neurones (11).

Ca^{2+}-Dependence and Some Biophysical Properties of $I_{Cl(Ca)}$

There are ample evidences to suggest that an increase in $[Ca^{2+}]_i$ is an essential factor for activation of $I_{Cl(Ca)}$ in every type of tissue. Under current clamp conditions, the Ca^{2+}-activated chloride conductance can be identified as an action potential after- depolarization (5, 12) following a mixed action potential which has a component of Ca^{2+} entry (13). Repeated activation of action potentials or bursts of action potential induced larger after-depolarizations than those induced by a single action potential in the same neurone (5). However, Ca^{2+} entry can also activate $I_{K(Ca)}$ and action potentials may be followed by initial hyperpolarization and then slow development of the after depolarization. The dominance of one of these two opposing Ca^{2+}-dependent conductances depends on the relative degree of activation and differences in the driving force for the two ions. Pharmacological isolation can be helpful and $I_{K(Ca)}$ can be blocked by charybdotoxin (14) . By using whole cell configuration of patch clamp technique (15) and blocking K^+ and Na^+ currents, including $I_{K(Ca)}$, with a combination of Cs in the patch pipette solution and TEA and TTX in the external recording medium, the $I_{Cl(Ca)}$ can be observed in isolation as a slowly decaying inward tail current, following voltage activated I_{Ca} (15).

Simultaneous patch clamp recording and measurement of $[Ca^{2+}]_i$ levels with fluorescence dyes has provided further convincing evidence in favour of a prerequisition of $[Ca^{2+}]_i$ for activation of $I_{Cl(Ca)}$ (16).

The $I_{Cl(Ca)}$ currents can be completely blocked by increasing cells Ca^{2+} buffering capacity by using 10 mM EGTA in the patch pipette solution.

Ca^{2+} release from intracellular stores can also activate $I_{Cl(Ca)}$. Experiments with patch clamp recording have shown that several Ca^{2+} releasing agents acting on agonist receptors release Ca^{2+} from intracellular Ca^{2+} stores, and that increased levels of intracellular Ca^{2+} can activate $I_{Cl(Ca)}$.

Caffeine is a well known intracellular Ca^{2+}-releasing agent and caffeine-induced Ca^{2+} release and subsequent activation of $I_{Cl(Ca)}$ is a widespread and a well studied phenomenon found in bullfrog sympathetic neurones (17), cultured rat DRG neurones (12), and in chick DRG neurone (9).

The other well known intracellular releasing agent I-1,4,5-P_3 has also been found to activate $I_{Cl(Ca)}$, but only in some cell types such as oocytes (19) and smooth muscle (20).
Recently, novel second messengers such as cADPR (21), ryanodine (22) and caged dihydrosphingosine (1) has been found to release Ca^{2+} from intracellular stores and activate $I_{Cl(Ca)}$.

Ion selectivity of $I_{Cl(Ca)}$

Permeability to anions and cations is an important criteria for determining individual membrane conductance. Identification of chloride conductance can easily be done by changing extracellular chloride concentration and comparing the values for E_{Cl} calculated from the Nernst equation. By changing extracellular chloride ions for relatively more permeant or impermeant ions the ion selectivity of Ca^{2+}-activated chloride channels can be studied. When chloride was replaced in extracellular solutions with glutamate, isothionate, aspartate or sucrose (impermeant ions or molecules) (23-25) the amplitude of $I_{Cl(Ca)}$ was increased at hyperpolarized potentials as E_{Cl} was shifted to more positive potentials. Furthermore, the use of relatively impermeant cations such as choline and $NMDG^+$ in place of Na^+ in the external solution did not effect the reversal potential of $I_{Cl(Ca)}$ (25). On the other hand replacing external chloride with more permeant thiocyanate shifts the reversal potential of $I_{Cl(Ca)}$ and E_{Cl} to more negative potentials as predicted from the Nernst equation (26). The permeability of Ca^{2+}-activated chloride channels to small anions has the following sequence: $SCN^->I^->NO_3^->Br^->Cl^->F^-$ and is consistent for several preparations including lachrymal gland cells (27) rabbit ear artery cells (28) and *Ascaris* muscle (29).

Single Channel Studies

It has been shown that unitary conductance for $I_{Cl(Ca)}$ in most preparations is low, and there is rapid rundown of channel activity following the removal of the patch from a cell. There have only been a limited number of single channel studies on $I_{Cl(Ca)}$ and very few in neurones. Interestingly,

cultured *Xenopus* spinal neurones express a large 300pS and intermediate 50-60 pS conductance channel (30) however these unitary conductance values are unusual. In cultured endocrine cells the unitary conductance is 2-5 pS (31). In rat lachrymal gland cells, noise analysis with whole cell recording predicts a unitary conductance of 1-2 pS at -60 mV (32). In *Xenopus* oocytes the single channel conductance is 3 pS measured from cell attached patches (33).

Pharmacology of $I_{Cl(Ca)}$

Inhibition of membrane currents by pharmacological agents is an important property which can be used to provide identification and evidence for a particular ion channel. Pharmacological tools can also be used to determine the physiological role of a particular membrane conductance. Chloride channels are ubiquitously distributed and there are reports of as many as 75 chloride channel subtypes with different biophysical properties (34). Although all aspects are not known yet, there are several important physiological and pathological conditions such as electrolyte absorption and secretion, diarrhoea (35) and cystic fibrosis (36) involve chloride channel modulation. Unfortunately, high affinity, and selective pharmacological antagonists for chloride channels are yet to be developed. Most of the available chloride channel blockers not only suffer from a low degree of selectivity but also affect cation conductances. However, niflumic acid, in micromolar concentrations has been found to be a potent and selective inhibitor of $I_{Cl(Ca)}$ in different cells (26, 37, 38).

Niflumic acid and flufenamic acid have been shown to be potent inhibitors of $I_{Cl(Ca)}$ in *Xenopus* oocytes (39). In cultured DRG neurones, 10 μM niflumic acid blocked $I_{Cl(Ca)}$ tail currents by 49%, and the amplitude of $I_{Cl(Ca)}$ inward currents activated by flash photolysis of DM-nitrophen were inhibited by 69% (26). The effects of another widely used chloride channel blocker, NPPB varies from tissue to tissue. It has been found to block $I_{Cl(Ca)}$ in *Xenopus* oocytes in a voltage dependent manner, producing greater inhibition at more depolarized potentials (40). This may also be the case in cultured rat DRG neurones where current duration is decreased more than current amplitude (12). Recently, chlorotoxin a peptide toxin from scorpion venom (41) and the polyamine spider toxins, argiotoxin-636 (42) have been found to block $I_{Cl(Ca)}$.

Since $I_{Cl(Ca)}$ can be activated by release of Ca^{2+} from internal Ca^{2+} stores, the amplitude of $I_{Cl(Ca)}$ may be reduced by the agents that deplete stores. Caffeine (43) and ryanodine (9) in addition to activating currents by releasing Ca^{2+} can also when repeatedly applied reduce $I_{Cl(Ca)}$ as stores become depleted or Ca^{2+} release channels are blocked.

The other well-known pharmacological agents, that acts as Cl^- channel blockers in non-neuronal preparations can also be used to block neuronal Cl^- channels. These include "the loop diuretic" furosemide and mefenamic acid, both acts on smooth muscle cells and neuronal cells (44, 45), the anti-helmintic drug suramin, which acts on *Xenopus oocytes* (46). A-9-C (antracene-9-carboxylic acid) can potently block $I_{Cl(Ca)}$ channels in mouse sympathetic ganglion cells (47). The stilbene derivatives, DIDS (4, 4'-diisothiocyanostilbene-2, 2'-disulfonic acid) and STITS (4-acetamido-4'-isothiocyanostilbene-2-2'disulphonic acid) are mostly used in smooth muscle cells to block $I_{Cl(Ca)}$ channels (37). The other chloride channel blockers include IAA-94 (6, 7-dichloro-2-cyclopentyl-2, 3-dihydro-2-2methyl-1-oxo-1 H-index-5 yl(oxy) acetic acid) (48) and DPC (3', 5-dichlorodiphenylamine-2-carboxylate) (49) are also potential candidates of $I_{Cl(Ca)}$ channel blockers to be used in neuronal preparations.

Physiological Roles of Ca^{2+}-activated Chloride Channels

The calcium-activated chloride channels play important roles in a variety of physiological process including osmoregulation, salt secretion and absorption, and neurotransmission. The physiological roles of calcium-activated chloride channels in neurones are not clear as their roles in other cell types, such as smooth muscle cells. Activation of calcium-activated chloride channels can result in membrane depolarisation if the chloride equilibrium potentials more positive than resting membrane potential (50). Activation of $I_{Cl(Ca)}$ following Ca^{2+} entry via voltage-gated Ca^{2+} channels can induce after-depolarisation following an action potential (5) or change action potential duration by altering Ca^{2+}-influx (51). These effects are closely related to the intracellular Ca^{2+} homeostasis mechanisms of cells and location of the ion channels in the cell membrane. In secretary cells, $I_{Cl(Ca)}$ alongside with $I_{K(Ca)}$ play important roles in fluid secretion (52). As Cl^- and monovalent cations leaves the cell water follows them and this determines the physicochemical characteristics of fluid secreted.

On the other hand, activation of $I_{Cl(Ca)}$ conductances may lead to hyperpolarisations of membrane depending on Cl^- equilibrium potential (23). Activation of ligand-gated Cl^- channels in brain and spinal cord by glycine or GABA usually produces inhibitory postsynaptic potential, because in these neurones the Cl^- equilibrium potential is close to or even more negative than resting membrane potential. Therefore, applications of agonists like glycine and GABA to this neurones causes either stabilization or hyperpolarisation of the membrane potential. Consequently, the opening of $I_{Cl(Ca)}$ in these neurones will result in reduced excitability.

CONCLUSIONS

$I_{Cl(Ca)}$ has been identified in a wide variety of neuronal cells as well as İN cardiac myocytes, smooth muscle cells including those vascular and non-vascular smooth muscle cell types. Their activation is Ca^{2+}-dependent, and can be achieved either by Ca^{2+}-entry from extracellular space or by the release of Ca^{2+} from intracellular stores. They have a certain role in control of neuronal excitability but their participation in many physiological and pathophysiological events yet to be clarified. There is a great need for development of selective and putative pharmacological blocker of $I_{Cl(Ca)}$ and studies that would provide information about molecular structure of $I_{Cl(Ca)}$ channels. Molecular and functional properties of $I_{Cl(Ca)}$ also need further clarification. This would not only help the better understand physiological roles of this channels but also be useful on therapy of pathophysiological conditions that involves upnormal functioning of $I_{Cl(Ca)}$ channels.

REFERENCES

1. Ayar A, Thatcher NM, Zehavi U, et al. Mobilization of intracellular calcium by intracellular flash photolysis of caged dihydrosphingosine in cultured neonatal rat sensory neurones. Acta Biochimica Polonica 1998; 45(2): 311-326.
2. Alvarez-Leefmans FJ and Russel JM. Chloride channels and carriers in nerve and glial cells. 1990; New York . Plenium Press.
3. Bader CD, Bertrand D, Schwartz EA. Voltage activated and calcium-activated currents studied in solitary rod inner segments from the salamander retina. J Physiol 1982; 331: 253-284.
4. Barish ME. A transient calcium-dependent chloride current in the immature *Xenopus* oocyte. J Physiol 1983; 342: 309-325.
5. Mayer ML. A calcium activated chloride current generates the after depolarization of rat sensory neurones in culture. J Physiol 1985; 364: 217-239.
6. Scott RH, Sutton KG, Griffin A, et al. Aspects of calcium activated chloride currents: a neuronal perspective. Pharmac Ther 1995; 66: 535-565.
7. Owen DG, Segal M, Barker JL. A Ca-dependent Cl^- conductance in cultured mouse spinal neurones. Nature 1984; 311: 567-570.
8. Scott RH, McGuirk SM, Dolphin AC. Modulation of divalent cation-activated chloride ion currents. Br J Pharmacol 1988; 94: 653-662.
9. Ivanenko A, Baring MD, Airey JA, et al. A caffeine-and ryanodine sensitive Ca^{2+} stores in avian sensory neurons. J Neurophysiol 1993; 70: 710-722.
10. Akasu T, Nishimura T, Tokimasa T. Calcium-activated chloride current in neourones of the rabbit pelvic parasympathetic ganglia. J Physiol 1990; 422: 303-320.
11. Bader CR, Bertrand D, Schlichter R. Calcium-activated chloride current in cultured sensory and parasympathetic quail neurones. J Physiol 1987; 294: 125-148.
12. Currie KPM and Scott RH. Calcium activated currents in cultured neurones from rat dorsal root ganglia. Br J Pharmacol 1992; 106: 593-602.
13. Dichter MA and Fischbach GD. The action potential of chick dorsal root ganglion neurons maintained in culture. J Physiol 1977; 267: 281-298.
14. Alger BE and Williamson A. A transient calcium-dependent potassium component of the epileptiform burst after-hyperpolarization in rat hippocampus. J Physiol 1988; 399: 191-205.
15. Hamill OP, Marty A, Neher E, et al. Improved patch- clamp techniques for high- resolution current recording from cells and cell free membrane patches. Pflugers Archiv 1981; 391: 85-100.
16. Pacaud P, Loirand G, Mironneu C, et al. Calcium dependence of the calcium activated chloride current in smooth muscle cells of rat portal vein. Pflugers Archiv 1992; 421: 125-130.
17. Akaike N and Sadoshima J. Caffeine effects four different ionic currents in bull-frog sympathetic neuron. J Physiol 1989; 412: 221-224.

18. Berridge MJ. Inositol triphosphate and calcium signaling. Nature 1993; 361: 315-325.

19. Lechleiter J, Girard S, Clapham D, et al. Subcellular patterns of calcium determined by G-protein specific residues of muscarinic receptors. Nature 1991; 350; 505-508.

20. Ito S, Ohta T, Nakazato Y. Inward currents activated by carbachol in rat intestinal smooth muscle cells. J Physiol 1983; 470: 395-409.

21. Crawford JH, Wootton JF, Seabrook GR, et al. Activation of Ca^{2+}-dependent currents in dorsal root ganglion neurones from neonatal rat rats by metabotrobic glutamate receptor activation and intracellular β-NAD^{+} and cGMP, the precursors of cyclic ADP-ribose formation. J Neurophysiol 1997; 77: 2573-2584.

22. Ayar A and Scott RH. The actions of ryanodine on Ca^{2+}-activated conductances in rat cultured DRG neurones; evidence for Ca^{2+}-induced Ca^{2+} release. Naunyn-Schmiedeberg's Arch Pharmacol 1999; 359: 81-91.

23. Owen DG, Segal M, Barker JL. Voltage-clamp analysis of a Ca^{2+} and voltage dependent chloride conductance in cultured mouse spinal neurons. J Neurophysiol 1986; 55: 1135-1135.

24. Byrne NG and Large WA. Actions of noradrenaline on single smooth muscle cells freshly dispersed from the rat anococcygues muscle. J Physiol 1987; 389: 513-525.

25. Pacaud P, Loirand G, Lavie JL, et al. Calcium-activated chloride currents in rat vascular smooth muscle cells in short term primary culture. Pflügers Arch 1989; 413: 629-636.

26. Currie KPM, Wootton JF, Scott RH. Activation of Ca^{2+}-dependent Cl^{-} currents in cultured rat sensory neurones by flash photolysis of DM-nitrophen. J. Physiol 1995; 482: 291-307.

27. Evans MG and Marty A. Calcium-dependent chloride currents in isolated cells from rat lacrimal glands. J Physiol 1986; 378: 437-460.

28. Amédée T, Large WA, Wang Q. Characteristics of chloride currents activated by noradrenaline in rabbit ear artery cells. J Physiol 1990; 428: 501-516.

29. Dixon DM, Valkanov M, Martin RJ. A patch clamp study of the ionic selectivity of the large conductance, Ca^{2+}-activated chloride channel in muscle vesicles prepared from *Ascaris suum*. J. Memb. Biol 1993; 131: 143-149.

30. Hussy N. Calcium-activated chloride channels in cultured embryonic *Xenopus* spinal neurons. J Neurophysiol 1992; 68: 2042-2050.

31. Talep O, Feltz P, Bossu JL, et al. Small conductance chloride channels activated by calcium on cultured endocrine cells from mammalian pars intermedia. Pflugers Archiv. 1988; 412: 641-646.

32. Marty A, Tan YP, Trautmann A. Three types of calcium-dependent channel in rat lacrimal glands. J. Physiol 1984; 357: 293-325.

33. Takahasi TE, Neher E, Sakmann B. Rat brain serotonin receptors in *Xenopus* oocytes are coupled by intracellular calcium to endogenous channels. Proc Natl Acad Sci USA 1987; 84: 5063-5067.

34. Mayer ML, Owen DG, Barker JL. Calcium-dependent chloride currents in vertebrate central neurones. In *Chloride channels and carriers in nerve, muscle and glial cells* 1991; Alvarez-Leefmans FJ and Russel JM., pp.355-364. New York Planum Press, New York and London.

35. Levin RJ. The diarrhoea of famine and severe malnutrition: is glucagon the major culprit? Gut 1992: 33(4) 432-434.

36. Welsh MJ. Abnormal regulation of ion channels in cystic fibrosis epithelia. FASEB J 1990; 4: 2718-2725.

37. Hogg RC, Wang Q, Large WA. Effects of Cl^{-} channel blockers on calcium-activated chloride and potassium currents in smooth muscle cells rabbit portal vein. Br J Pharmacol 1994; 11: 1333-1341.

38. Leonard JP and Kelso SR. Apparent desensitization of NMDA responses in *Xenopus* oocytes involves calcium-dependent chloride current. Neuron 1990; 2: 53-60.

39. White MM and Aylwin M. Niflumic and flufenamic acids are potent reversible blockers of Ca^{2+}-activated Cl^{-} channels in *Xenopus* oocytes. Mol Pharmacol 1990; 37: 720-724.

40. Wu G and Hamill OP. NPPB block of Ca^{++} - activated Cl^{-} currents in *Xenopus* oocytes. Pflugers Archiv 1992; 420: 227-229.

41. DeBin JA, Maggio JE, Strichartz GR. Purification and characterization of chlorotoxin, a chloride channel ligand from the venom of the scorpion. Am J Physiol 1993; 264: C361-C369.

42. Scott RH, Sutton KG, Dolphin AC. Interactions of polyamines with neuronal ion channels. Trends in Neurosci. 1993; 16: 153-159.

43. Greenwood IA, Hogg RC, Large WA. Effect of furosemide, ethacrynic acid and indanyloxyacetic acid on spontaneously Ca^{2+}-activated currents in rabbit portal vein smooth muscle cells. Br J Pharmacol 1995; 115: 733-738.

44. Evans MG, Marty A, Tan YP, et al. Blockade of Ca^{2+}-activated Cl^- conductance by furosemide in rat lachrymal glands. Pflügers Archiv. 1986; 406; 65-68.

45. Sanchez-Vives MV, Gallego R. Calcium-dependent chloride current induced by axotomy in rat parasympathetic neurones. J Physiol 1994; 475; 402-417.

46. Duriex ME, Carlisle SJ, Salafranca MN, et al. Responses to sphingosine-1-phosphate in Xenopus laevis oocytes: similarities with lysophophaditic acid signalling. American J Physiol 1993; 264: C1360-1364.

47. DeCastro F, Geiogo-Barrientos E, Gallego R. Calcium activated chloride current in normal mouse sympathetic ganglion cells. J. Physiol 1997; 498; 397-408.

48. Doughty JM, Miller AL, Langton PD. Non-specifity of chloride channel blockers in rat cerebral arteries: block of the L-type calcium channel. J. Physiol 1998; 507:433-439.

49. Baron A, Pacaud G, Loirand C, et al. Pharmacological block of Ca^{2+}-activated Cl^- current in rat vascular smooth muscle cells in short-term primary culture. Pflügers Archiv 1991; 419: 553-558.

50. Aickin CC. Chloride transport across the sarcolemma of vertebrate smooth and skeletal muscle. 1990, In chloride channels and carriers in nerve, muscle and glial cells, ed. Alvarez-Leefmans EJ, Russel JM, pp. 209-249. Plenium Press, New York & London.

51. Korn SJ and Horn R. A $[Na^+]_o$ independent, pH_o-dependent mechanism for reduction of intracellular $[Ca^{2+}]$ after influx through Ca^{2+} channels in mouse pituitary cells. J Gen Physiol 1991; 98: 893-907.

52. Petersen OH. Stimulus-secretion coupling: cytoplasmic calcium signals and the control of ion channels in exocrine acinar cells. J Physiol 1992; 448: 1-51