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Voltage-Dependent Effect of Al^{3+} on Channel Activities in Hippocampal Neurons

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The effects of Al^{3+} on Na^+ and K^+ channel activities in rat and rabbit hippocampal neurons have been examined. Al^{3+} mediated a pronounced voltage-dependent inhibition of single Na^+ channel activities in inside-out patches excised from neuronal membranes. The reduction in channel activity was more substantial under defined conditions, particularly when changing the holding potential from −120 to −80 mV. This voltage-dependent block indicates that Al^{3+} can mainly inhibit the excitation of neurons with less negative resting potentials. The observed effect may be related to the pathogenetic mechanism of the specific action of Al^{3+} on defined types of neurons affected in neurodegenerative disorders such as Alzheimer's disease.

Previous studies using microprobe analysis and related methods have shown an increased content of aluminum in neurofibrillary tangles and the potential role of this metal in the pathogenesis of Alzheimer's disease has been examined (1-6). Despite some controversial findings (7,8), there is evidence that when administered into the brain ventricles of experimental animals aluminum can cause neurofibrillary degeneration as well as some symptoms related to Alzheimer's disease and other neurodegenerative disorders (9-17). There is also substantial evidence showing the involvement of aluminum as neurotoxic and environmental agent in the development of dialysis encephalopathy (17-19) and of amyotrophic lateral sclerosis-parkinsonism of Guam (20,21). Aluminum was also found to inhibit excitability of lobster axons (22) as well as calcium uptake into synaptosomes (23-25). Another study has shown that aluminum can modulate excitability of cholinergic synapses (26). Aluminum chloride was also found to induce formation of tangle-like neurofibrils in cultured rat brain neurons (27).

Thus previous electrophysiological studies indicate that aluminum can induce significant alterations of different electrical parameters of nerve membranes. Its actions on specific channel activities, however, are not well understood.

In this study the effects of aluminum on single Na^+ and K^+ channel activities in hippocampal neurons have been examined. It was found that Al^{3+} can induce a voltage-dependent inhibition of Na^+ channel activity in these neurons.

MATERIALS AND METHODS

All reagents were purchased from Sigma Chemical Co. (St. Louis, MO). Neuronal cultures were prepared from hippocampal tissue of 20-day-old rat embryonic- or 2-3 day-old rabbit newborn brains using procedures described elsewhere (28,29). Most of the experiments were performed on neurons which were prepared as explant cultures without using any proteolytic enzymes to avoid proteolytic cleavage of membrane proteins. The cells remained viable for 1-4 weeks but were usually employed in electrophysiological experiments 3-8 days after their dissociation.

Channel activities were studied at 21-23°C using the patch-clamp method (30). The hanging-drop patch-clamp technique was employed for studying single channel activities in inside-out patches excised from neuronal membranes (28). The micropipettes were prepared from 7052 Corning glass capillaries. They were fire-polished to a diameter of less than 1um and were coated with Sylgard (Dow Corning Co., Midland, MI).

Solutions contained (in mM): intracellular, 105 CsF, 40 CsCl, 10 NaF, 5.0 EGTA, 5.0 HEPES, adjusted to pH 7.2 with CsOH; extracellular, 150 NaCl, 1.5 $CaCl_2$, 1.0 $MgCl_2$, 5.0 glucose, 5.0 HEPES, adjusted to pH 7.4 with NaOH.

Data acquisition and analysis were performed using programs based on the Fastlab system (Indec Systems). Pulses were generated and current traces were sampled on line at 50 us per point. The single channel currents were filtered at 3 kHz through an 8-pole Bessel filter. Partial compensation of leakage and capacity current was obtained by subtraction from the experimental records of exponentials fitted to blank traces which do not contain channel openings or to scaled records from hyperpolarizing pulses.

RESULTS

In this study, the effects of aluminum on Na+, and K+ channel activities in hippocampal neuronal membranes were examined using the whole cell- as well as

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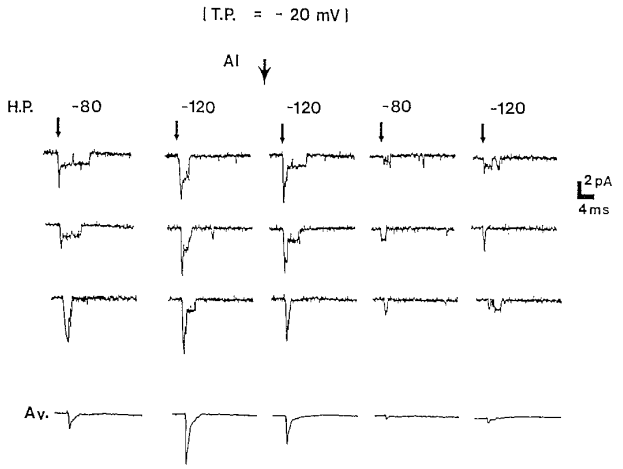

FIG. 1. Effect of aluminum on single Na^+ channel activity in inside-out patches detached from membranes of hippocampal neurons. The patch holding potential was set to −80 or to −120 mV (as indicated above each column of current sweeps) and 20 repetitive 25 ms pulses were applied to a constant test potential of −20 mV at a frequency of 1Hz. Three consecutive single channel current sweeps and an average current trace (Av) obtained from 20 consecutive ensemble events in response to these depolarizations are shown in each of the five columns. The current sweeps represented in the two columns on the left side were taken under control conditions. $AlCl_3$ was added to the intracellular solution at a final concentration of 100 uM 10 min before taking the current sweeps represented in the middle column. The addition of aluminum is indicated by a big arrow over the space between the second and the third column from the left side. The sweeps represented in the three columns on the right side were taken in the presence of aluminum. The small arrows show the beginnings of the depolarizing pulses which activate the Na^+ channels. Their openings immediately after the beginning of the pulse can be seen as downward deflections of the current traces. The channels usually return to the closed state (the background current trace) several msec after the beginning of the pulse due to the inactivation mechanism. Current traces were filtered at 3 kHz by using an 8-pole Bessel filter. No additional digital filtering was used.

the inside-out configuration of the patch clamp technique. Aluminum, at a concentration of 0.5 mM, induced a considerable block of Na^+ currents but did not affect substantially the outward delayed-rectifier K+ currents in brain cells (data not shown). Single Na^+ channel activities were examined in inside-out membrane patches excised from hippocampal neurons. Al^{3+} was added at a concentration of 0.1 mM to the solution perfusing the intracellular side of the membrane patch. A slight reduction in Na^+ channel activity was initially observed at a holding potential of −120 mV. After applying a more positive holding voltage, −80 mV, for several minutes and changing the voltage again to −120 mV a substantial decrease in Na^+ channel activity was observed as illustrated in Figure 1 where the ensemble average currents before and after the addition of aluminum are compared. This voltage-dependent effect indicates that Al^{3+} can mainly inhibit the excitation of neurons with less negative or fluctuating resting potentials which may explain its specific actions on defined types of hippocampal neurons affected in Alzheimer's disease.

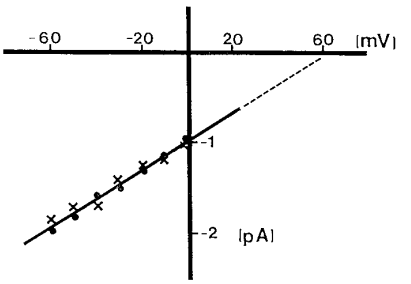

FIG. 2. Single channel current-voltage relations before (circles) and after (crosses) addition of aluminum. Single channel current amplitudes are plotted as a function of applied test potential.

As shown in Figure 2 no significant changes in single channel current-voltage relations were observed after addition of aluminum. The slope conductance measured in the absence and in the presence of aluminum was almost the same, 15.6 pS. This indicates that its blocking effect does not involve a decrease in single channel conductance but most likely Al^{3+} reduces the probability of channel opening and other channel parameters which deserve a further investigation.

Figure 3 shows the time-dependent reduction of sodium currents after addition of aluminum at different holding voltages. This figure includes data obtained from two separate experiments where initially similar current amplitudes were recorded but subsequently different experimental protocols were applied. In one

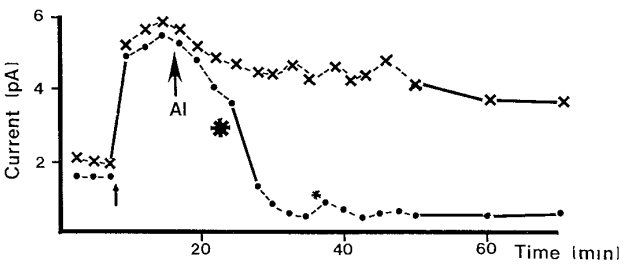

FIG. 3. Time-course of sodium current change mediated by aluminum at different holding potentials. Each point represents the amplitude of the ensemble average current (in pA) from 20 consecutive single channel sweeps taken at different time intervals after formation of the gigaseal. Depolarazing test pulses to −20 mV for 25 ms were applied from holding potentials of −80 or −120 mV. Data obtained from two separate experiments are shown. In one of them (circles) the membrane potential was initially held at −80 mV. At the time indicated by a small arrow the holding voltage was changed to −120 mV. While holding at the same potential aluminum was added to the cytoplasmic side of the membrane patch as indicated by a big arrow. Several minutes later the holding voltage was changed back to −80 mV (marked with a big asterisk). Later, the potential was changed again to −120 inV as indicated by a small asterisk. The same protocol was used in another experiment (crosses) except that the holding voltage of −120 mV was not changed after addition of aluminum.

of the experiments (circles) a drastic reduction of the current after addition of aluminum is observed only when the voltage is changed to −80 mV. The current amplitudes remain low even when later the holding potential is changed to −120 mV. In another experiment (crosses) a slight decrease of the current is observed after addition of aluminum at −120 mV but no substantial further reduction of the current amplitudes occurs when holding at the same voltage until the end of the experiment.

Al^{3+} induced similar voltage-dependent effects in six experiments. We have examined its actions in different cells and found that Al^{3+}-mediated channel inhibition is more pronounced in hippocampal than in neocortical and neuroblastoma cells. A further investigation is necessary to verify the specificity of action of aluminum on defined types of neurons.

DISCUSSION

The described effects of aluminum on Na^+ channels in hippocampal neurons may help elucidating the mechanism of its potential action in the pathogenesis of Alzheimer's disease and related neurodegenerative disorders. It is remarkable that the concentration of Al^{3+} which we have used in the described experiments is in the range of aluminum concentrations estimated to be present in brain cells pathologically affected during the development of Alzheimer's disease.

The biophysical mechanism of the voltage-dependent effect of Al^{3+} is not clear but it is possible that Al^{3+} may bind to a negatively charged group of the Na^+ channel molecule. This charged segment is probably oriented toward the cytoplasmic side of the membrane. At less negative voltages due to a voltage-dependent conformation change this group may become more accessible for polycations such as aluminum.

Although it is difficult to explain the mechanism of aluminum action on Na^+ channels, the voltage dependence of its effect indicates some role of the highly positive electrical charge of this cation. Some polycationic compounds such as cationized ferritin were found to exhibit an effect on sodium channel inactivation kinetics without altering the characteristics of K+ channels (31). Another trivalent cation, La^{3+} and other positively charged agents can also modulate the activity of Na^+ channels. However, no significant effect of La^{3+} on Na^+ channels in hippocampal neurons was found in the present study (data not shown). Therefore, the voltage-dependent actions of aluminum on Na^+ channels observed in the present study may bear some specificity in comparison to other polycationic agents. One of the possibilities, for example, is that the size of the aluminum ion may determine its selective interaction with a slowly moving negatively charged group in the Na^+ channel molecule which according to a previously proposed mechanistic model (32) moves from the extracellular to the intracellular side of the membrane during the depolarization phase. Aluminum may interact with this negatively charged group during the voltage-dependent conformational change. At less negative potentials this negatively charged group may become more accessible and after binding aluminum its movement to its original position may be impeded which may cause a blockade of the channel.

Several recent studies have provided additional evidence about the role of aluminum in the pathogenesis of Alzheimer's disease and established a link between Al^{3+} and amyloid β proteins (Aβ) which under some conditions can form aggregates with a characteristic distribution of their surface charges (33-36). The positive charges of these aggregates may also play a role in deposition of Aβ on hippocampal neurons followed by alterations in the activities of membrane receptors and channels which lead to neuronal damage typical for Alzheimer's disease. It was found that Al^{3+} and Ca^{2+} can mediate β-pleated sheet formation in phosphorylated fragments of human neurofilament proteins (35). A potential target for both Al^{3+} and Aβ or aggregates containing both agents is the recently cloned Ca^{2+}-sensing receptor (CaR) which is modulated by Ca^{2+} and a variety of polycationic agonists (37,38). In a recent study we have shown that Aβ can modulate cationic channels in hippocampal neurons through the CaR (38). Like other trivalent cations such as Gd^{3+}, Tb^{3+}, and La^{3+}, Al^{3+} may also serve as a potent agonist of the CaR and may alter channel activities in neurons through the CaR which deserves a further investigation.

In summary, the voltage dependence of the blocking effect indicates that aluminum may inhibit predominantly the excitation of neurons with less negative or fluctuating resting potentials which may be related to the mechanism of its specific action on defined types of hippocampal and other neurons during the development of neurodegenerative disorders such as Alzheimer's disease as suggested in recent studies (33-36).

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REFERENCES

1. Crapper, D. R., Krishnan, S. S., and Dalton, A. J. (1973) *Science* **180,** 511–513.
2. Perl, D. R., and Brody, A. R. (1980) *Science* **208,** 297–299.
3. Wisniewski, H. M., Sturman, J. A., and Shek, J. (1980) *Ann. Neurol.* **8,** 479–490.
4. Troncoso, J. C., Price, D. L., Griffin, J. W., and Parhad, I. M. (1982) *Ann. Neurol.* **12,** 278–283.
5. Hess-Kozlow, K. M., and Price, D. L. (1985) *Brain Research* **342,** 172–175.

6. Crapper McLachlan, D. R. (1985) *in* Normal Aging, Alzheimer's Disease and Senile Dementia (Gottfries, C. G., Ed.), p. 105, Univ. Bruxelles.
7. Candy, J. M., Klinowski, J., Perry, R. H., Perry, E. K., Fairbairn, A., Oakley, A. E., Carpenter, T. A., Atack, J. R., Blessed, G., and Edwardson, J. A. (1986) *Lancet* **2/15,** 354–357.
8. Shore, D., and Wyatt, R. J. (1983) *J. Nerv. Mental Dis.* **171,** 553–558.
9. Crapper, D. R., and Dalton, A. J. (1973) *Physiol. Behav.* **10,** 935–945.
10. Crapper, D. R., and Tomko, G. J. (1975) *Brain Research* **97,** 253–264.
11. Klatzo, I., Wisniewski, H., and Streicher, E. (1965) *J. Neuropathot. Exp. Neurol.* **24,** 187–199.
12. Ghetti, B., Musicco, M., Norton, J., and Bugiani, O. (1984) *Neuropathol. Appl. Neurobiol.* **11,** 31–35.
13. Wisniewski, H. M., Shek, J. W., Gruca, S., and Sturman, J. A. (1984) *Acta Neuropathol. (Berlin)* **63,** 190–197.
14. Triarsou, L. C., Norton, J., Bugiani, O., and Ghetti, B. (1985) *Neuropathol. Appl. Neurobiol.* **11,** 407–430.
15. Farnell, B. J., Crapper McLachlan, D. R., Bainbridge, K., Defloni, V., Wong, L., and Wood, P. L. (1985) *Exp. Neurol.* **88,** 68–83.
16. Garruto, R. M., and Gajdusek, D. C. (1985) *in* Normal Aging, Alzheimer's Disease and Senile Dementia (Gottfries, C. G., Ed.), p. 69, Univ. Bruxelles.
17. Crapper, D. R., Quittkat, S., Krishnan, S. S., Dalton, A. J., and DeBoni, U. (1980) *Acta Neuropathol. (Berlin)* **50,** 19–24.
18. Lione, A. (1986) *Pharmacol. Ther.* **29,** 255–285.
19. Arieff, A. I., Cooper, J. D., Armstrong, D., and Lazarowitz, V. C. (1979) *Ann. Intern. Med.* **90,** 741–747.
20. Perl, D. P., Gajdusek, D. C., Garruto, R. M., Yanagihara, R. T., and Gibbs, C. J., Jr. (1982) *Science* **217,** 1053–1055.
21. Carruto, R. M., Fukatsu, R., Yanagihara, R., Gajdusek, D. C., Hook, G., and Fiori, C. E. (1984) *Proc. Natl. Acad. Sd. U.S.A.* **1,** 1875–1879.
22. Blaustein, M. P., and Goldman, D. F. (1968) *J. Gen. Physiol.* **51,** 279–291.
23. Farnell, B. J., DeBoni, B., and Crapper-McLachlan, D. R. (1982) *Exp. Neurol.* **78,** 241–258.
24. Bizzi, A., Crane, R. C., Gambetti, L. A., and Gambetti, D. (1984) *J. Neurosci.* **4,** 722–731.
25. Crapper-McLachlan, D. R., and Van Berkum, M. R. A. (1986) *Prog. Brain Res.* **70,** 399–408.
26. Banin, E., and Meiri, H. (1987) *Brain Res.* **423,** 359–363.
27. Langui, D., Anderson, B. H., Brion, J.-P., and Ulrich, J. (1988) *Brain Res.* **438,** 67–76.
28. Vassilev, P. M. (1990) *Pfluegers Arch. Europ. J. Physiol.* **415,** 497–500.
29. Vassilev, P. M., Scheuer, T., and Catterall, W. A. (1989) *Proc. Natl. Acad. Sci. U.S.A.* **86,** 8147–8151.
30. Hamill, O. P., Marty, A., Neher, E., Sakmann, B., and Sigworth, F. J. (1981) *Pfluegers Arch.* **391,** 85–100.
31. Furuya, K., Hirano, H., Nishiyama, F., Kukita, F., and Yamagishi, S. (1986) *J. Membr. Biol.* **89,** 75–83.
32. Armstrong, C. M., and Bezanilla, F. (1977) *J. Gen. Physiol.* **70,** 567–590.
33. Clauberg, M., and Joshi, J. G. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 1009–1012.
34. Fasman, G. D., and Moore, C. D. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 11232–11235.
35. Hollosi, M., Shen, Z. M., Perczel, A., and Fasman, G. D. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 4902–4906.
36. Fasman, G. D., Perczel, A., and Moore, C. D. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 369–371.
37. Ye, C., Kanazirska, M., Quinn, S., Brown, E. M., and Vassilev, P. M. (1996) *Biochem. Biophys. Res. Commun.* **224,** 271–280.
38. Ye, C., Ho-Pao, C. L., Kanazirska, M., Quinn, S., Rogers, K., Seidman, C. E., Seidman, J. G., Brown, E. M., and Vassilev, P. M. (1997) *J. Neurosci. Res.*, in press.