Suppression of Spreading Depression of Leão in Neocortex by an N-Methyl-D-Aspartate Receptor Antagonist

Richard S. McLachlan

ABSTRACT: Spreading depression has been implicated in the pathophysiology of a number of diseases such as migraine, stroke and epilepsy. The characteristics of this phenomenon were explored in neocortex of anesthetized rats. Spreading depression was produced in 10 of 15 animals using mechanical, electrical and chemical stimulation. Mean amplitude of the DC shift was –9.3 mV, mean duration at any one electrode 65 sec and rate of spread 2-5 mm/min. Spreading depression was facilitated by focal interictal spike activity induced by penicillin and completely blocked by the N-methyl-D-aspartate (NMDA) receptor antagonist, DL-2-aminophosphonovaleric acid (APV), providing further evidence that excitatory amino acid neurotransmission is a critical element in the development or propagation of the phenomenon.

RÉSUMÉ: Suppression de la dépression propagée de Leão dans le néocortex par un antagoniste du récepteur du N-méthyl-D-aspartate. La dépression propagée a été impliquée dans la physiopathologie d'un certain nombre de maladies, dont la migraine, l'accident cérébro-vasculaire et l'épilepsie. Ce phénomène a été exploré au niveau du néocortex de rats anesthésiés. Une dépression propagée a été produite chez 10 animaux sur 15 en utilisant une stimulation mécanique, électrique et chimique. L'amplitude moyenne du déplacement DC a été de –9.3 mV, la durée moyenne à n'importe laquelle électrode de 65 sec et la vitesse de propagation de 2 à 5 mm/min. La dépression était facilitée par une activité de pointes intericales focales induites par la pénicilline et bloquée complètement par un antagoniste du récepteur N-méthyl-D-aspartate (NMDA), l'acide DL-2-aminophosphonovalérique (APV), appuyant l'hypothèse que la neurotransmission par les acides aminés excitateurs est un élément critique du développement ou de la propagation du phénomène.

Can. J. Neurol. Sci. 1992; 19: 487-491

Spreading depression was defined and characterized by A.A.P. Leão in his Ph.D. thesis in 1944, although its effects had been observed as early as the late 19th century.[1] Leão demonstrated that focal stimulation of the cerebral cortex of rabbits resulted in an attenuation of local spontaneous activity in the electrocorticogram (ECoG) as well as evoked cortical potentials, in association with an increase in cerebral blood flow due to dilation of pial arterioles. In addition, he noted that these effects lasted tens of seconds to minutes in any one area and that they had the unique property of spreading slowly through the cortex like ripples on the surface of a pond at a rate of 2-6 mm/min. Subsequently, a distinctive slowly spreading DC potential shift was also shown to occur.[2]

Spreading depression has been suggested as a pathophysiological mechanism which might explain certain aspects of a number of disease states including migraine,[3,4] stroke,[5] epilepsy,[6] transient global amnesia[7] and head injury.[8] Although excitatory amino acid neurotransmitters have been suggested as possible mediators of spreading depression,[9,10] this concept has been disputed.[11] However, recent evidence suggests that glutamate in particular is important in the production of spreading depression.[12-14] In preparation for a study of spreading depression in human cortex, the following experiments were done in rats to confirm that recording techniques were satisfactory and that appropriate stimuli were being used to induce spreading depression. In addition, the effect of the N-methyl-D-aspartate (NMDA) receptor antagonist, DL-2-aminophosphonovaleric acid (APV) on spreading depression was assessed in these experiments.

Method

Acute experiments were carried out in 15 Wistar rats (250-350 g) of both sexes anesthetized with urethane (1.15 g/kg IP) and placed in a Kopf stereotaxic frame. Body temperature was maintained at 37.5 +/–0.3°C using a heating pad and rectal

From the Departments of Clinical Neurological Sciences and Physiology, The University of Western Ontario, London
Received September 3, 1991. Accepted in revised form June 10, 1992.
Reprint requests to: Dr. R.S. McLachlan, Epilepsy Unit, University Hospital, 339 Windermere Rd., London, Ontario, Canada N6A 5A5

probe. A scalp incision was made and a 4x8 mm craniotomy opened over the left hemisphere. The dura was reflected over the somatosensory cortex and using the stereotaxic device, a linear array of three or four platinum ball electrodes (0.5 mm diameter, 1.5 mm interelectrode distance) was applied to the cortical surface. A reference electrode was placed in the posterior neck muscles. The signal from each recording electrode led through a silver/silver chloride half cell to a Grass Model 8 DC/AC amplifier and was stored on magnetic tape (TEAC) for off line analysis.

At least 20 minutes following electrode placement, a stimulus was applied to the cortex 1-2 mm from the first recording electrode in an attempt to induce spreading depression. The stimulus was mechanical (M) in 12 rats (pressure with a 1 mm blunt probe for 1 second), chemical (C) in 9 (intracortical injection of 0.5-1.0 µl of 15% KCl) and electrical (E) in 7 (0.8 mA, 50 Hz, 2 ms cathodal current for 1 second). Animals were given one or more types of individual stimuli in the following combinations: M = 2, C = 2, E = O, M + E = 4, M + C = 4, E + C = 1 and M + E + C = 2. Spreading depression was considered to be present if there was 1) transient attenuation of background ECoG activity lasting more than 15 seconds, 2) a simultaneous negative shift in the cortical surface DC potential and 3) a sequential appearance of one or both of these over time at adjacent electrodes.

In 6 of 8 animals from which spreading depression could not be elicited, topical sodium penicillin G 100 000 IU/ml was applied to the exposed cortex using a 1 mm pledget of litmus paper followed by a retrial of stimulation. Focal spike activity induced by penicillin is known to facilitate spreading depression.[15] In the 7 animals which had spreading depression without the enhancing effect of penicillin, topical 500 µM APV was applied to the cortex prior to additional stimulation. Topical normal saline was used as an additional control in four of these animals.

Results

Spreading depression occurred in 10 of the 15 rats. If it occurred in an animal, it was reproducible using any of the three types of stimulation as long as there was a latent period of at least 10 minutes between stimuli. Within one minute of the stimulus application, a bi- or triphasic primarily negative DC shift occurred at the first electrode followed by slow spread to the other electrodes at a rate of 2-5 mm/min (Figure 1). The duration of the negative wave was from 45-80 seconds ($\bar{x}$ = 65 seconds) and the amplitude of the negative potential change from 5-12 mV($\bar{x}$ = 9.3 mV). A low voltage short duration prepositivity occurred in some animals and the negative DC shift was always followed by a more prolonged after-coming positive wave lasting up to five minutes. In 3 animals, KCl application was followed by recurrent waves of spreading depression every three to five minutes lasting up to 15 minutes.

The spreading DC shift was associated with a simultaneous spreading attenuation of the ECoG (Figure 2). Although this was observed in 7 animals, low voltage recordings in 3 animals before stimulation did not change during spreading depression probably because any further attenuation would not have been noticeable.

Local application of penicillin to the cortex in 6 animals produced typical interictal penicillin spikes with an associated transient standing DC shift.[16] Spreading depression could not be induced in any of these animals prior to the establishment of a penicillin focus but occurred readily in 3 animals after interictal spike activity appeared.

Figure 3 shows that the application of APV to the cortex of seven rats completely blocked spreading depression ($p < 0.001$, Student's T test using either paired or independent variables), an effect which was reversible in 4 of the 7 animals after irrigation of the cortex with saline to remove APV. Saline application by itself prior to APV in 4 animals had no effect on spreading depression.

Discussion

Spreading depression was produced in the neocortex of two-thirds of the animals tested using various stimuli. The reason spreading depression did not occur in some of the animals is not clear but this is a recognized phenomenon particularly in animals with less lissencephalic brains which are higher in the phylogenic scale.[17] For example, it is more difficult to obtain spreading depression in the monkey than in the cat, in the cat than in the rat and so forth. The results of these experiments in rats provide a basis for comparison when the same methodology is used in forthcoming studies of spreading depression in human cortex.

Spreading depression has been shown to result from transient changes in the apical dendrites of pyramidal cells which are triggered by a substance released from adjacent depressed neurons which excites contiguous neurons to allow the slow spread to occur.[17] Although primarily studied in cortex, spreading depression has been demonstrated in other brain areas as well using both *in vivo* and *in vitro* techniques.[18,19] The wave of depression is usually preceded by a brief period of excitation in which neuronal spike activity is increased, followed by inhibited neuron firing.[20,21] At the same time, intracellular recordings have revealed a sustained depolarization of pyramidal neurons[22] which occurs in association with a striking increase in extracellular potassium[19] and decrease in calcium.[13] Potassium efflux from neurons[23] or glia,[24] which results in extracellular potassium concentration rising above a critical level,[25] may be responsible

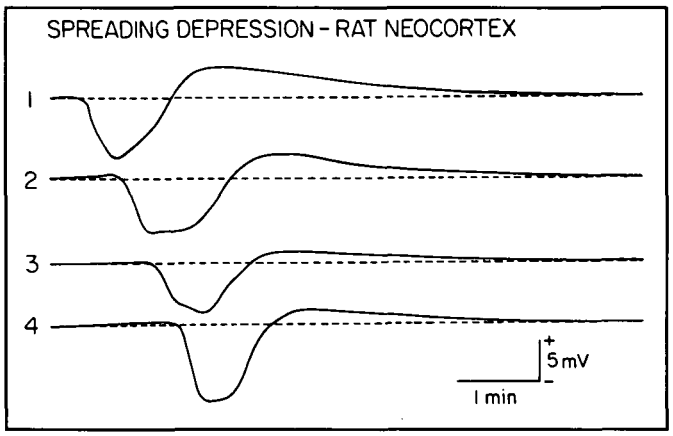

Figure 1 — A DC potential shift occurs at sequential electrodes 1.5 mm apart following mechanical stimulation of the cortex adjacent to electrode 1. The stimulus was applied 30 seconds before the first DC change appeared at electrode 1.

https://doi.org/10.1017/S0317167100041688 Published online by Cambridge University Press

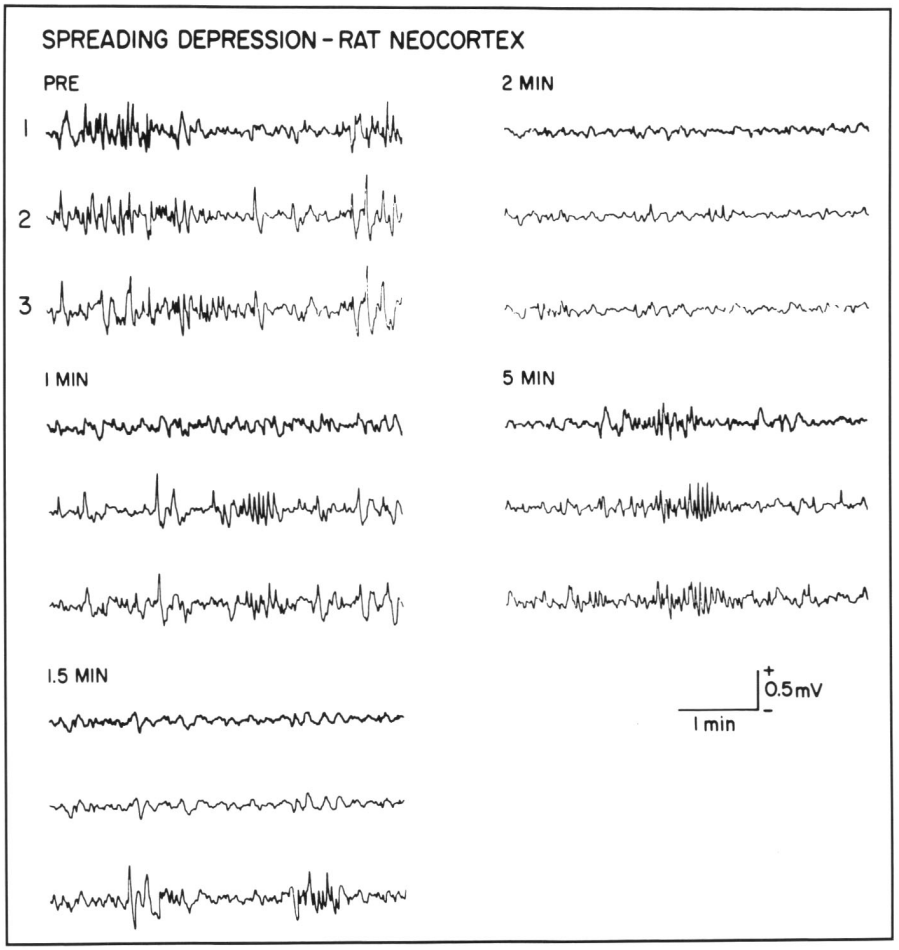

Figure 2 — ECoG at three electrodes before (Pre) and at different times after KCl application 2 mm from electrode 1. Note almost complete attenuation of both spindle activity and interictal spikes which spreads to involve all three electrodes at two minutes.

for depolarization of adjacent dendrites allowing the abnormal activity to spread.

Focal interictal spike activity increases extracellular potassium locally in the cortex.[26] If epileptiform activity is sufficiently intense, extracellular potassium levels exceed the 10-12 mM threshold for spreading depression[25] and the spikes begin to trigger waves of spreading depression.[15] Although spreading depression from extrinsic cortical stimulation was facilitated in 3 of 6 animals after a penicillin spike focus was established, spontaneous spreading depression in association with interictal spikes did not occur in this study.

Other evidence suggests that a synaptic mechanism may account for these changes. Apart from KCl, the most potent stimulus of spreading depression is NMDA.[27] In addition, spreading depression is associated with various degrees of glutamate release.[10,11,28] These findings strongly implicate amino acid neurotransmitters such as glutamate and aspartate in the pathophysi-ology of the phenomenon. Our finding that topical APV, a specific competitive NMDA antagonist completely and consistently abolished spreading depression supports this contention. It is also in agreement with the suppression of spreading depression *in vitro* by APV in hippocampal slices[13] and *in vivo* by intraperitoneal, non-competitive NMDA antagonists such as ketamine[12,14] or the competitive antagonist 2-amino-7-phosphonoheptanoate.[14] The effect of such parenteral administration is widespread throughout the brain and the concentration reaching the cortex is unknown. With topical application of 500 μM APV, the concentration acting at the cortical surface is diluted even further by cerebrospinal fluid. APV blocks responses to NMDA at concentrations as low as 10 μM with only minimal effect on quisqualate and Kainate responses (see ref. 29 for discussion). Increasing the concentration as high as 500 μM produces little additional effect.[30] Therefore it is likely that the APV-sensitive receptor affected in spreading depression is the NMDA receptor.

The reason spreading depression could not be obtained following the removal of APV in 3 animals is unclear but may relate to the phenomenon of long-term depression. This type of prolonged depression of synaptic activity has been demonstrated in neocortex and is caused by a sudden increase in intracellular calcium such as occurs in spreading depression.[31] Unlike long-term potentiation, it can persist even after NMDA receptor blockade.

NMDA antagonists have a strong antiepileptic effect when used in animal models of epilepsy[29,32] or in slices of human neocortex obtained at epilepsy surgery.[33] They have also been found

https://doi.org/10.1017/S0317167100041688 Published online by Cambridge University Press

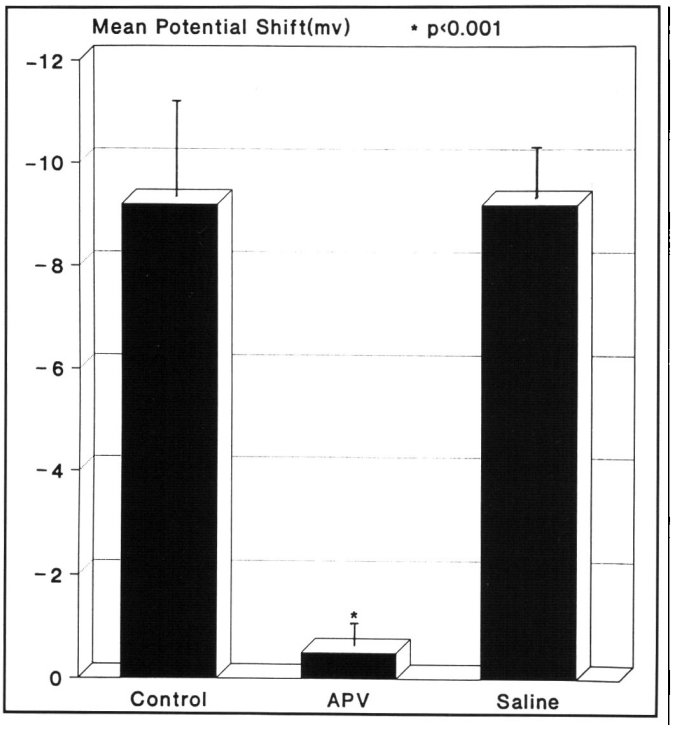

Figure 3 — The mean potential shift from baseline to maximum negativity is shown following control stimulation (n = 10), following stimulation after application of APV (n = 7) and following stimulation after application of normal saline (n = 4).

to protect against hypoxic damage to cerebral tissue of the type which occurs in stroke.[34] Since spreading depression has been suggested as a possible mechanism by which both seizures and the effects of focal ischemia might be enhanced, part of the protective effect of NMDA antagonists in these disorders may relate to their inhibition of this phenomenon. If spreading depression is also important in certain forms of migraine, then NMDA antagonists could be explored as possible therapeutic alternatives in that condition. Further research being carried out with human tissue may provide additional insight into the pathophysiology and treatment of several of these neurological diseases.

Acknowledgement

Supported by the PSI Foundation of Ontario. The author thanks Mr. Frank Bihari for expert technical assistance and Miss Heather Murray for typing the manuscript.

References

1. Leão AAP. Spreading depression of activity in the cerebral cortex. J Neurophysiol 1944; 7: 359-390.
2. Leão AAP. Further observations on the spreading depression of activity in the cerebral cortex. J Neurophysiol 1947; 10: 409-414.
3. Welch KM, D'Andrea G, Tepley N, et al. The concept of migraine as a state of central neuronal hyperexcitability. Neurol Clin 1990; 8: 817-828.
4. Lauritzen M. Cortical spreading depression as a putative migraine mechanism. Trends Neurosci 1987; 10: 8-12.
5. Somjen GG, Aitken PG, Balestrino M, et al. Spreading depression-like depolarization and selective vulnerability of neurons. A brief review. Stroke 1990; 21: 179-183.
6. Marshall WH. Spreading cortical depression of Leão. Physiol Rev 1959; 39: 239-279.
7. Olesen J, Jorgensen MB. Leão's spreading depression in the hippocampus explains transient global amnesia. A hypothesis. Acta Neurol Scand 1986; 73: 219-220.
8. Oka H, Kako M, Matsushima M, et al. Traumatic spreading depression syndrome. Review of a particular type of head injury in 37 patients. Brain 1977; 100: 287-298.
9. Van Harreveld A, Kooiman M. Amino acid release from the cerebral cortex during spreading depression and asphyxiation. J Neurochem 1965; 12: 431-439.
10. Van Harreveld A, Fifkova E. Glutamate release from the retina during spreading depression. J Neurobiol 1970; 2: 13-29.
11. Gardino PF, DoCarmo RJ. Glutamate and spreading depression in chick retina. An Acad Bras Ciênc 1983; 55: 297-307.
12. Gorelova NA, Koroleva VI, Amemori T, et al. Ketamine blockade of cortical spreading depression in rats. Electroencephalogr Clin Neurophysiol 1987; 66: 440-447.
13. Mody I, Lambert JDC, Heinemann U. Low extracellular magnesium induces epileptiform activity and spreading depression in rat hippocampal slices. J Neurophysiol 1987; 57: 869-888.
14. Marrannes R, Willems R, DePrins E, et al. Evidence for a role of the N-methyl-D-aspartate (NMDA) receptor in cortical spreading depression in the rat. Brain Res 1988; 457: 226-240.
15. Koroleva VI, Bures J. Cortical penicillin focus as a generator of receptive spike-triggered waves of spreading depression in rats. Exp Brain Res 1983; 51: 291-297.
16. Mahnke JH, Ward AA. Standing potential characteristics of the epileptogenic focus. Epilepsia 1961; 2: 161-169.
17. Ochs S. The nature of spreading depression in neural networks. Int Rev Neurobiol 1962; 4: 1-69.
18. Vinogradova LV, Koroleva VI, Bures J. Re-entry waves of Leão's spreading depression between neocortex and caudate nucleus. Brain Res 1991; 538: 161-164.
19. Haglund MM, Schwartzkroin PA. Role of Na-K pump potassium regulation and IPSPs in seizures and spreading depression in immature rabbit hippocampal slices. J Neurophysiol 1990; 63: 225-239.
20. Goldensohn ES. Experimental seizure mechanisms. *In*: Jasper HH, Ward AA, Pope A, eds. Basic Mechanisms of the Epilepsies. Boston: Little, Brown & Co. 1969; 289-298.
21. Sugaya E, Takato M, Noda Y. Neuronal and glial activity during spreading depression in cerebral cortex of cat. J Neurophysiol 1975; 38: 822-841.
22. Snow RW, Taylor CP, Dudek FE. Electrophysiological and optical changes in slices of rat hippocampus during spreading depression. J Neurophysiol 1983; 50: 561-572.
23. Grafstein B. Mechanism of spreading cortical depression. J Neurophysiol 1956; 19: 154-171.
24. Gardner-Medwin AR. Possible roles of vertebrate neuroglia in potassium dynamics, spreading depression and mygraine. J Exp Biol 1981; 95: 111-127.
25. Vyskocil F, Kruz N, Bures J. Potassium-selective microelectrodes used for measuring the extracellular brain potassium during spreading depression and anoxic depolarization in rats. Brain Res 1982; 39: 255-259.
26. Heinemann U, Lux HD, Gutnick MJ. Extracellular free calcium and potassium during paroxysmal activity in the cerebral cortex of the cat. Exp Brain Res 1977; 17: 237-243.
27. Curtis DR, Watkins JC. Analogs of glutamic and gamma-amino-N-butyric acids having potent actions on mammalian neurons. Nature 1961; 191: 1010-1011.
28. Szerb JC. Glutamate release and spreading depression in the fascia dentata in response to microdialysis with high K^+: role of glia. Brain Res 1991; 542: 259-265.
29. Anderson WW, Swartzwelder HS, Wilson WA. The NMDA receptor antagonist 2-amino-5-phosphonovalerate blocks stimulus train-induced epileptogenesis but not epileptiform bursting in the rat hippocampal slice. J Neurophysiol 1987; 57: 1-21.
30. Davies J, Francis AA, Jones AW, Watkins JC. 2-amino-5 phosphonovalerate (2APV), a potent and selective antagonist of amino acid induced and synaptic excitation. Neurosci Lett 1981; 21: 77-81.

https://doi.org/10.1017/S0317167100041688 Published online by Cambridge University Press

31. Brocher S, Artola A, Singer W. Intracellular injection of Ca^{2+} chelators blocks induction of long-term depression in rat visual cortex. Proc Natl Acad Sci 1992; 89: 123-127.
32. Croucher MJ, Collins JF, Meldrum BS. Anticonvulsant action of excitatory amino acid antagonists. Science 1982; 216: 899-901.
33. Avoli M, Drapeau C, Louvel, et al. Epileptiform activity induced by low extracellular magnesium in the human cortex maintained *in vitro*. Ann Neurol 1991; 30: 589-596.
34. Simon RP, Swan JH, Griffiths T, et al. Blockade of N-methyl-D-aspartate receptors may protect against ischemic damage to the brain. Science 1984; 226: 850-852.

https://doi.org/10.1017/S0317167100041688 Published online by Cambridge University Press