Sumatriptan blocks spreading depression in isolated chick retina

PA Maranhâo-Filho[1], H Martins-Ferreira[2], MB Vincent[1], LJC Ribeiro[2], SAP Novis[3]

Headache Unit[1] and Department of Neurology[3] , Hospital Universitàrio Clementino Fraga Filho[1] , Instituto de BiofIsica Carlos Chagas Filho[2]; Universidade Federal do Rio de Janeiro, Brazil

Cephalalgia

Maranhâo-Filho PA, Martins-Ferreira H, Vincent MB, Ribeiro LJC, Novis SAP. Sumatriptan blocks spreading depression in isolated chick retina. Cephalalgia 1997;17:822–5. Oslo. ISSN 0333–1024

Spreading depression is a neurohumoral phenomenon that has been related to the pathophysiology of migraine. The recently introduced $5HT_{ID}$ agonist anti-migraine compound sumatriptan blocks neurogenic extravasation and induces cerebral vasoconstriction, but the actual mechanism of action against migraine remains obscure. Retinal spreading depression (RSD) velocity has been measured in isolated chick retinas in the presence of 0.05–2.00:nM sumatriptan. This drug reversibly blocks RSD in a concentration-dependent manner. Since the preparation is blood-vessel free, this effect must be related to the nervous tissue. *Migraine, retina, spreading depression, sumatriptan*

Maurice Vincent, Av. das Amèricas, 1155/504, 22631-000 Rio de Janeiro Brazil. 'Tel. +5521 4399245, fax. +5521 4943648, e-mail: vincent @ unisys.com.br. *Received 14 March 1997, accepted 9 July 1997*

Spreading depression (SD) is a neurohumoral phenomenon (1), present in various animal species including primates (2), which has been implicated in the pathophysiology of migraine (3–7). Although there is as yet no definite proof that SD occurs in humans, convincing evidence supports the possibility (6–11). As observed by Lashley (12), the scotoma propagation during migraine visual aura seems to correspond to Leâo's SD in the visual cortex (4). The spreading hypoperfusion velocity detected caring migraine attacks also seems to correlate with SD (13).

Isolated chick retina is a suitable model for studying SD (14). The advantages of using avian retina instead of cerebral cortex are mainly twofold: the spread may be visually observed (15); and the neuronal aspects may be approached more specifically as the isolated retina does not contain blood vessels.

Sumatriptan, a recently introduced $5HT_{1D}$ agonist (16), has proven to be effective against migraine and cluster headache (17). Sumatriptan may cause vasoconstriction by activating receptors within the arterial wall or may block neurogenic inflammation through pre–junctional trigeminal receptors, but its

mechanism of action against migraine is not fully understood (18).

In the present study the effects of sumatript an on retinal spreading depression (RSD) velocity were studied in isolated chick retina.

Methods

Twenty chicks (134.6+24.1 g; range 46–500 g) were enucleated immediately after decapitation and the eyes sectioned in the equator. The vitreous humor was removed and the posterior eyecup immersed in buffer solution (NaCl: 120; KCl: 4; $CaCl_2$: 1; $MgSO_4$: 1; $NaHCO_3$: 30; NaH_2PO_4: 1; glucose: 20. Values in mM) aerated with 95% O_2-5% CO2 to maintain pH between 7.8 and 8.0, as in previous experiments (19). Eyecup fragments (4X10 mm) were cut into strips, the nervous tissue layer isolated from other ocular structures, and mounted on strips of filter paper (retinal vitreous layer facing upwards). The preparations were placed in 0.2 ml plexiglass chambers and superperfused with 3 ml 30°C buffer solution using an infusion pump (1.5 ml/min). Two 1 µm o.d. glass microelectrodes containing 150mM NaC1 were inserted perpendicularly 4 mm apart in the inner plexiform layer of the retina. The microelectrodes were connected to a dual channel electrometer (World Precision Instruments, Inc.) and a Grass polygraph.

A 100 µm tungsten rod was used to induce RSD mechanically, the propagation being contrary to the flux of superfusion. Thus, substances eventually released during the RSD reaction were washed using a liquid movement contrary to the RSD propagation. The reaction was observed visually under an optical microscope and the typical negative voltage variation of the Leâo wave was recorded.

The velocity of the RSD propagation was calculated from the time required by the waves to spread from the first to the second microelectrode. The preparations were equilibrated in order to obtain two successive RSDs with the same velocity (RSDi). Sumatriptan was added (0.05, 0.10, 0.25, 0.37, 0.62, 0.75, 1.00, 1.50 or 2.00; values in mM), three new RSds were elicited, and the corresponding velocities recorded (V_1, V_2 and V_3). The preparations were then washed and a final RSD (RSD_f) obtained. All RSDs were induced during 10 min intervals (Fig. 1A).

Values are given as the % RSD velocity reduction (mean±SE), where $6<n<9$ for each concentration,

[Fig. 1]

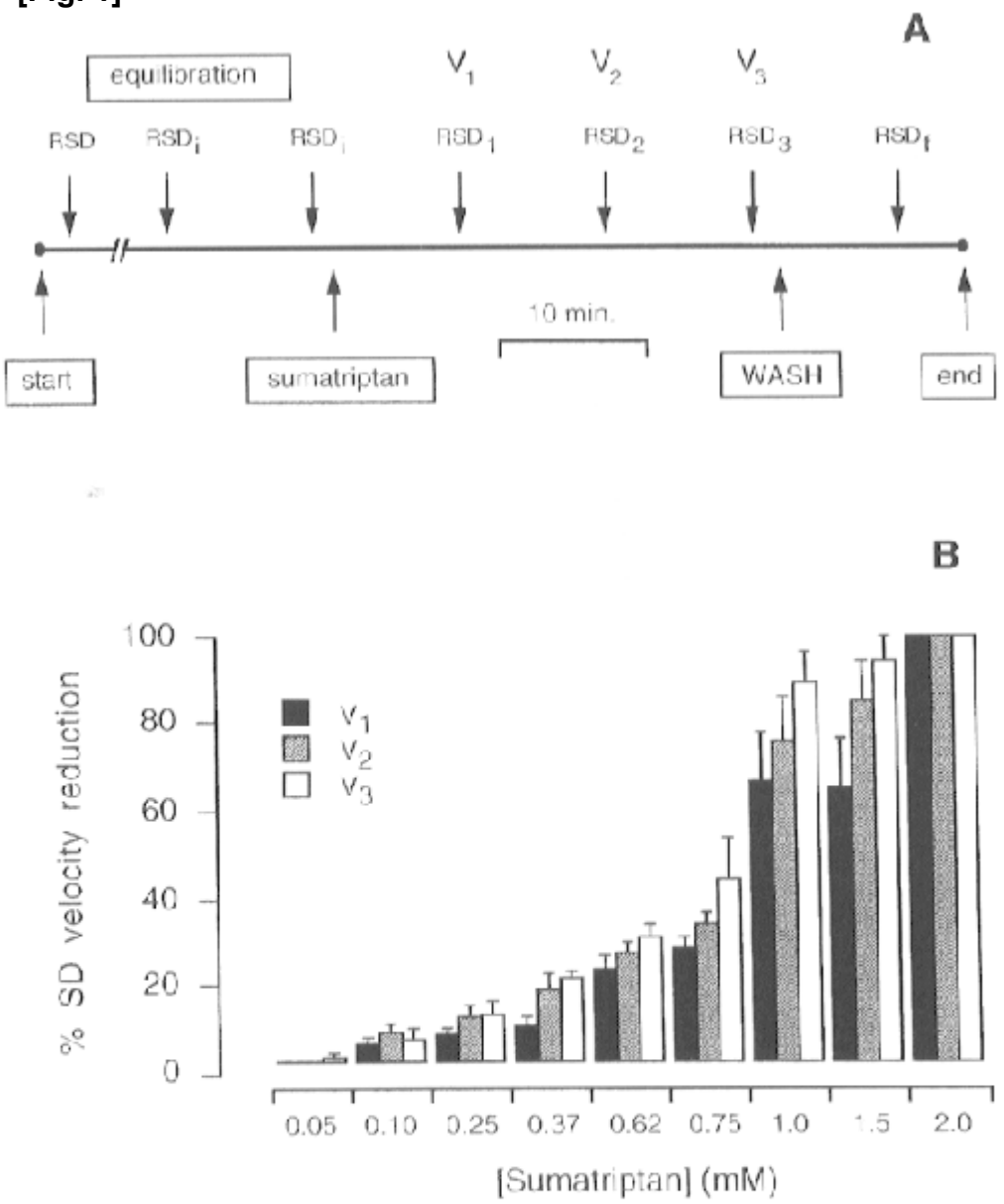

Fig. 1. Panel A: After two successive spreading depressions with the same velocity (RSD$_i$) were obtained in isolated chick retina strips, sumatriptan was added (0.05–2.00 mM), three new RSD were induced at 10 min intervals, and the corresponding velocities (V_1, V_2 and V_3) were recorded. A final retinal spreading depression (RSD_f) was obtained after washing. *Panel B*: Percentage reduction in retinal spreading depression velocity induced by different concentrations of sumatriptan (0.05–2.00 mM). Values corresponding to the first (V_1), second (V_2) and third (V_3) RDSs following sumatriptan addition are represented. Values shown as mean±SEM, $6 \leq n \leq 9$.

ANOVA and t-test were used for statistical analysis, p-values <0.05 being considered significant.

Results

Sumatriptan reversibly blocked RSD in a concentration-dependent manner (Fig. 1B). A reduction in velocity was first recorded at a concentration of 0.1 mM. With 1.5 mM sumatriptan, RSD velocity was reduced by 81.12±7.20%. A concentration of 2.0 mM sumatriptan blocked RSD completely (100±0.0%).

Further stimuli tended to progressively decrease RSD velocity, although V_1, V_2, and V_3 were not statistically different at any sumatriptan concentration except 0.37 mM (Fig. 1B, p=0.023).

After washing, the velocities tended to return to pre-sumatriptan values. With 0.05 mM sumatriptan, RSD_f was significantly lower than RSD_i (5.76 ± 0.22 and 6.58±0.39 respectively, p=0.042). With 1.5 mM sumatriptan, RSD velocity was reduced by 81.12±7.20%, and the final velocity was not statistically different from the initial measurement RSD_i=6.83±0.86; RSD_i=5.58±0.43, p=0.144). For sumatriptan concentrations of 0.05, 0.1, 0.25 and 2.0, RSD_f was significantly lower than RSD_i. The highest sumatriptan concentration used (2.0mM), which produced a total RSD block, did not induce a permanent lesion since the reactions could be obtained after washing, although final velocities were lower than initial velocities (RSD_i=5.33±0.16; RSD_f=4.98±0.14, p = 0.009).

Discussion

There is evidence suggesting that migraine aura is related to SD (13). Aura symptoms and cortical SD propagate with similar velocities, and regional blood flow abnormalities observed during provoked and spontaneous migraine attacks may correspond to SD (5, 20). It is possible that migraine without aura occurs with subclinical spreading hypoperfusion (20). Experimentally, SD induced activation of the trigeminovascular system (21), which may be a key phenomenon for the migraine attack (22). Evidence of SD in the human brain has recently emerged, supporting its role in migraine (23).

Sumatriptan is an anti-migraine compound and a 5HT agonist (24, 25). Its effect may be mediated through vasoconstriction (26, 27) and/or blockade of neurogenic inflammation (28–30). Although sumatriptan is comparatively effective during the headache phase (17), it has been considered ineffective against migraine aura (31), but given to a patient right at the visual aura onset this drug was reported to eliminate the visual symptoms (32).

The present model is suitable for studying drug effects in SD since the phenomenon is similar in the retina and other regions of the CNS (14, 33). The velocity of SD propagation, the variations in K+, Ca2+, Na+ and Cl as well as the SD characteristic negative slow voltage variation are similar in the retina and the cortex. Concerning energy, however, the retinal metabolism is glycolitic, whereas the cortical metabolism is mainly aerobic (33). In this preparation, drug effects are mediated by the nervous tissue, since chick retina is free of blood vessels. The present data show that sumatriptan reversibly blocks RSD in isolated chick retina,

suggesting that this effect is not necessarily mediated via blood vessels or by vessel tone. Although sumatriptan concentrations used in the present study were higher than therapeutic levels, it is tempting to speculate whether sumatriptan in lower concentrations could produce similar effects in certain areas of the human m grainous brain.

The mechanism of the present effect is obscure. The concentration range between the smallest

reduction in RSD velocity and complete blockade is comparatively narrow (0.05–2.00 mM), suggesting some sort of on-off phenomenon which it not particularly dependent on sumatriptan concentration.

Although sumatriptan was effective in blocking RSD in this preparation, the drug poorly penetrares the blood-brain barrier (BBB) under normal conditions (34). If the aura of migraine is associated with SD, the poor penetration may explain why sumatriptan may not be effective in aborting the aura. In the present study sumatriptan was applied directly to the nervous tissue, favoring the SD blockade. This may partially be the reason why intravenous sumatriptan did not interfere with SD inrats (21). On the other hand, it is also possible that the BBB is selectively and temporarily disrupted during migraine attacks (31), but not necessarily at regions where SD-related symptoms are produced. Thus, sumatriptan could interfere with neuronal activity even though the aura would not be aborted. It has been demonstrated that sumatriptan binds to receptors in various areas of the human brain (35), Where its neuronal effects could be produced. Further more, high densities of [^{3}H]sumatriptan-binding sites were observed particularly at the visual cortex (35), an area clearly implicated with the visual aura.

Theoretically, sumatriptan could interfere with RSD through damage to the nervous tissue. Against this possibility is the comparatively normal RSDp velocity obtained after washing even in spite of the high concentrations used here. The reduction in RSDp velocity was clearly related to duration of the experiments rather than to the sumatriptan concentration (data not shown). To further test this possibility, some samples were left with sumatriptan for as long as 12 h and RSD could still be obtained after washing. The transparency and general aspect of the sumatriptan treated samples did not differ from control retinal strips (data not shown).

This is the first experimental model where sumatriptan has been shown to block RSD. Ina previous recent study, 1.5 mM sumatriptan reduced RSD velocity by around 50% (36). No other concentrations were tested and the reduction obtained was apparently lower than the effect found here. This is probably due to methodological differences.

In conclusion, chick retina seems to be a useful model for studying drug influence on RSD. Sumatriptan reversibly reduced RSD velocity in a concentration-dependent way. While the mechanism of this effect is currently unknown, the effect may have implications for the pathophysiology and treatment of migraine.

Acknowledgments.- The authors acknowledge Dr Linda White for her comments and GlaxoWellcome for supplying the drug.

References

1. Leào AAP. Spreading depression of activity in cerebral cortex. J Neurophysiol 1994;7:359–90
2. Marshall WH. Spreading cortical depression of Leâo. Physiol Rev 1959;39:633–41
3. Leâo AAP, Morison RS. Propagation of spreading cortical depression. J Neurophysiol 1945;8:33–45
4. Milner PM. Note on a possible correspondence between the scotomas of migraine and spreading depression of Leâo. Electroencephalogr Clin Neurophysiol 1958;10:705
5. 0lesen J, Larsen B, Lauritzen M. Focal hyperemia followed by spreading oligemia and impaired activation of rCBF in classic migraine. Ann Neunrol 1981;9:344–52
6. Lauritzen M, Skyhøj Olsen T, Lassen NA, Paulson OB. The changes of regional cerebral blood flow during the course of classical migraine attacks. Ann Neurol 1983;13:633–41
7. Welch KM, D'Andrea G, Tepley N, Barkley G, Ramadan NM. The concept of migraine as a state of central neuronal hyper-excitability. Neurol Clin 1990;8:817–28
8. Leâo AAP. On the inferred relationship of migraine and spreading depression. In: Clifford Rose F, editor. Advances in headache research. Chicago: John Libbey 1987:19–24
9. Avoli M, Drapeau C, Louvel J, Pumain R, Oliver A, Villemure J-G. Epileptiform activity induced by low extracellular magnesium in the human cortex maintained in vitro. Ann Neurol 1991;30:589–96
10. Barkley GL, Tepley N, Nagel-Leiby S, Moran JE, Simkins RT, Welch KM. Magnetoencephalographic studies of migraine. Headache 1990;30:428–34
11. Tepley N, Wijesinghe RS. A dipole model for spreading cortical depression. Brain Topogr 1996;8:345–53
12. Lashley KS. Patterns of cerebral integration indicated by the scotomas of migraine. Arch Neurol Psychiatry 1941;46:259–64
13. Lauritzen M. Cortical spreading depression as putative migraine mechanism. Trends Neurosci 1987;10:447–67
14. Martins-Ferreira H. Depressâo alastrante na retina. An Acad Bras Ciênc 1962;34:XLIV
15. Martins-Ferreira H. Spreading depression: a neurohumoral reaction. Brazilian J Med Biol Res 1994;27:851 63
16. Lance JW, Pfaffenrath V. Sumatriptan. From molecule to man. Eur Neurol 1991;31:279–344
17. Tfelt-Hansen P. Sumatriptan for the treatment of migraine attacks a review of controlled trials. Cephalalgia 1993;13:238–44
18. Humphrey PPA, Goadsby PJ. The mode of action of sumatriptan is vascular? A debate. Cephalalgia 1994;14:401–10
19. Ferreira-Filho CR, Martins-Ferreira H. Interstitial fluid pH and its changes during spreading depression in isolated chick retina. In: Do carmo RJ, editor.

Spreading depression. Berlin, Heidelberg: Springer-Verlag 1992:75–88
20. Woods RP, Iacoboni M, Mazziotta JC. Bilateral spreading cerebral hypoperfusion during spontaneous migraine headache. New Engl J Med 1994;331:1689–92
21. Moskowitz MA, Nozaki K, Kraig RP. Neocortical spreading depression provokes the expression of c-fos protein-like immunoreactivity within trigeminal nucleus caudalis via trigeminovascular mechanisms. I Neurosci 1993;13:1167–77
22. Moskowitz MA. The neurobiology of vascular head pain. Ann Neurol 1984;16:157–68
23. Mayevsky A, Doron A, Manor T, Meilin S, Zarchin N, Ouaknine GE. Cortical spreading depression recorded from the human brain using a multiparametric monitoring system. Brain Res 1996;740:268–74
24. Ferrari MD, Saxena PR. On serotonin and migraine: a clinical and pharmacological review.. Cephalalgia 1993;13:151–65
25. Wilkinson M, Pfaffenrath V, Schoenen J, Diener H-C, Steiner TJ. Migraine and cluster headache–their management with sumatriptan: a critical review of the current clinical experience. Cephalalgia 1995;15:337–57
26. Friberg I, Olesen J, Iversen HK, Sperling B. Migraine pain

associated with middle cerebral artery dilatation: reversal bv sumatriptan. Lancet 1991;338:13–17
27. Parsons AA. 5HT receptors in human and animal cerebrovasculature. Trends Pharmacol Sci 1991;12:310–15
28. Buzzi MG, Moskowitz MA. The antimigraine drug, sumatriptan (GR43175), selectively blocks neurogenic plasma extravasation from blood vessels in dura match Br J Pharmacol 1990;99:202–9
29. Buzzi MG, Moskowitz MA. Evidence for $5HT_{1B1D}$ receptors mediating the antimigraine effect of sumatriptan and dihydro-ergotamine. Cephalalgia 1991:11:165–8
30. Moskowitz MA. Neurogenic versus vascullar mechanisms of sumatriptan and ergot alkaloids in migraine. Trends Pharmacol Sci 1992;13:307–11
31. Bates D, Ashford E, Dawson R, Ensink F BM, Gilbus NE, Olesen J, et al. Subcutaneous sumatriptan during the migraine aura. Neurology 1994;44:1587–92
32. Clifford Rose E. Sumatriptan arrests migraine aura. Headache 1992;32:365–6 [Letter]
33. Martins-Ferreira H. Propagation of spreading depression in isolated chick retina. In: Lehmenkühler A, Grotemaier KH, Tegtmeier P, editors. Migraine: basic mechanisms and treatment. München, Wien, Baltimore: Urban and Schwarzenberg, 1993:533–46
34. Humphrey PP, Feniuk W, Marriott AS, Tanner RJ, Jackson MR, Tucker ML. Preclinical studies on the anti-migraine drug, sumatriptan. Eur Neurol 1991;31:282–90
35. Pascual J, del Arco C, Romôn T, del Omo E, Castro E, Pazos A. Autoradiographic distribution of [^{3}H]sumatriptan-binding sites in post-mortem human brain. Cephalalgia 1996;16: 317–22
36. Wiedemann M, de Lima VMF, Hanke H. Effects of antimigraine drugs on retinal spreading depression. Naunyn-Schmiedeberg's Arch Pharmacol 1996;353:552–6