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Arachidonic acid and its metabolites are involved in the expression of neocortical spike-and-wave spindling episodes in DBA/2J mice

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Abstract

This work was undertaken to study the effects of dexamethasone, indometacin and mifepristone plus dexamethasone on the neocortical spike-and-wave spindling episodes (S&W) in the electrocorticogram of DBA/2J mice. Our data indicate that both dexamethasone and indometacin (1, 10, 100 μg kg^{-1}, i.p.) reduced the S&W of DBA/2J mice. This effect appeared 30 min after drug administration and lasted for the duration of the recording period (240 min). Mifepristone, a glucocorticoid receptor antagonist (1, 10, 100 μg kg^{-1}, i.p.), injected 2 h before dexamethasone, totally blocked the steroid effect. These results indicate that both dexamethasone and indometacin significantly reduce the S&W of DBA/2J mice, suggesting a possible involvement of arachidonic acid and its metabolites in the development of brain excitability.

Introduction

DBA/2J mice spontaneously generate bilaterally synchronous spike-and-wave spindle bursts (S&W) of 7–8 cycles s^{-1} during quiet waking, active waking and slow-wave sleep but not during REM sleep (Ryan 1984). Recently, DBA/2J mice have been considered as a model for studying cerebral excitability (Ryan & Sharpless 1979) due to a number of factors. Genetic contributions to human epilepsy are becoming increasingly apparent (Newmark & Penry 1980). Consequently, genetically seizure-prone animals are assuming more importance as models of the pathophysiology of human epilepsy. Ryan & Sharpless (1979) suggested that DBA/2J mice may provide a useful animal model for studying the basis of an epileptiform inheritance model.

Studies on animal models of epilepsy and in human epilepsy have established a modulatory role for corticosteroids (Woodbury & Vernadakis 1967). Since 1970 there has been increasing biochemical evidence that the neuronal cell membrane is one of the targets for convulsion-induced changes. This is partly based on the observation that following seizures, there are increased levels of diacylglycerols and free fatty acids, which are products of the degradation of the major component of cell membranes, phospholipids. In addition, the production of prostaglandins from the free fatty acid, arachidonic acid, is activated after convulsions. This implies that alterations in the metabolism of lipids in the brain are a major effect of seizures, and that further study of these biochemical pathways may reveal important information for defining the basic mechanism of seizures. Several investigations indicated that

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dexamethasone, an indirect phospholipase A_2 (PLA_2) inhibitor known to inhibit transcription of the gene for PLA_2 and to induce the formation of lipocortin-1 (an anti-inflammatory protein mediator, which acts by inhibiting tha activity of PLA_2), attenuates bicuculline-induced free-fatty-acid accumulation in a dose-dependent manner, and inhibits the production of free arachidonic acid in experimental brain damage (Bazan et al 1982, 1984; Tang & Bazan 1984).

Recently, we have demonstrated that dexamethasone reduces S&W of DBA/2J mice through a protein synthesis-dependent mechanism (Capasso et al 1994a, b). In this respect, since prostaglandins have been reported to be involved in the development of brain excitability (Bazan et al 1986) and glucocorticoids control prostaglandins biosynthesis by inhibiting the release of their common precursor, arachidonic acid, through PLA_2 inhibitory proteins (Blackwell et al 1980; Barnes & Adcock 1993; Glaser et al 1993), we may hypothesize that dexamethasone reduces S&W of DBA/2J mice by blocking the release of the prostaglandin precursor, arachidonic acid. In fact, arachidonic acid is released by the enzyme PLA_2 (Vane 1971; Flower & Blackwell 1979; Murphy et al 1979) and is subsequently converted to prostaglandins by the enzyme cyclooxygenase (Vane 1971; Dennis 1987; Shimizu & Wolfe 1990; Glaser et al 1993). Recent papers finally showed that cyclooxygenase inhibitors may aggravate kainic acid-induced seizures in laboratory animals (Baik et al 1999; Najbauer et al 2000).

Therefore, to verify whether arachidonic acid and its metabolites are involved in the expression of S&W of DBA/2J mice, we have studied the effect of dexamethasone and indometacin in the S&W of DBA/2J mice.

Materials and Methods

Mice

Adult (60–110 days old) male DBA/2J mice were used. They were maintained 2–4 per cage on a 12-h light–dark cycle with free access to food and water. Mice were anaesthetized with pentobarbital 35 mg kg^{-1} intraperitoneally and 4 stainless steel electrodes (0.8 mm diameter) were implanted and fixed with acrylic resin, in positions corresponding to the anterior and posterior sensorimotor cortex bilaterally. Since S&W spindles are invariably expressed bilaterally in close synchrony, electrocorticographic records from only the left hemisphere were analysed. The mice were allowed at least 4 days to recover and on the day of the experiment they were placed in a sound-proof room. Each mouse was placed in a lighted and electrically shielded recording chamber, then attached to the EEG machine by long flexible wires which did not hinder free movement, and allowed 60 min to adapt to the recording chamber. An electrocorticographic bipolar tracing from the left anterior to posterior sensorimotor cortex was recorded on paper by using a poligraph Ote Biomedica Neurograph 18 (for details see Capasso et al 1994a, b).

The electrocorticogram was recorded with a 0.1-s time constant, with the filter off and a chart speed of 7.5 mm s^{-1}. Each animal was monitored continuously for 1 h in basal conditions and for at least 4 h after drug administration.

Electrocorticographic evaluation

Three parameters were evaluated. Firstly, the number of S&W was recorded; reduction or increase of number of spindles was expressed as a percentage of the 60-min control period. Secondly, the cumulated duration of S&W (s) was recorded; reduction or increase of the spindle duration was expressed as a percentage of the 60-min control period. Finally, the electrocorticographic desynchronized pattern was observed; in each mouse the time spent in electrocorticographic desynchronized activity (i.e., active wakefulness), corresponding to low amplitude and fast frequency waves in the cortical leads, was calculated as a percentage of the 60-min control period.

Treatments

Before beginning the recording session, the mice were connected to the recording cables and allowed to adapt to the recording chamber for at least 2 h. The electrocorticogram was recorded beginning at approximately 09 00 h. Electrocorticograms were recorded on paper according to the following schedule: a control EEG was recorded for one consecutive hour, then the mice were injected with dexamethasone (1, 10, 100 μg kg^{-1}, i.p.) or indometacin (1, 10, 100 μg kg^{-1}, i.p.) or mifepristone (1, 10, 100 μg kg^{-1}, i..p.) and the electrocorticogram was again recorded for 240 min.

Data analysis

The three parameters evaluated were statistically analysed by one-way analysis of variance to assess differences between the pre-drug and the post-drug periods. Significance was assumed at 5%. Time-dose 50 (i.e., the

time (min) from treatment to 50 % of effect) was computed through the Probit method.

Results

Sleep-wakefulness cycle and S&W in control animals

The administration of saline did not modify either the number or duration of S&W in DBA/2J mice during the whole recording period (240 min). The electrocorticographic desynchronized pattern, however, showed some fluctuations during the whole recording period as expected (Table 1), from 79.1 ± 8.9 % to 85.1 ± 4.3 %.

Influence of dexamethasone and mifepristone on S&W in DBA/2J mice

The administration of the higher dexamethasone doses (10, 100 μg kg^{-1}, i.p.) reduced the neocortical spike-and-

Table 1 The effect of saline on number and duration of S&W (s), and electrocorticographic desynchronized pattern in mice.

	Before saline	After saline			
		1 h	2 h	3 h	4 h
S&W number	55.3 ± 7.8	50.3 ± 8.2	52.7 ± 5.1	54.8 ± 2.4	50.3 ± 2.7
S&W duration	75.5 ± 3.4	80.1 ± 7.1	71.2 ± 6.1	74.3 ± 6.4	82.4 ± 3.1
Electrocorticographic desynchronized pattern duration (% of control)	45.8 ± 2.3	44.5 ± 4.7	42.5 ± 2.2	47.5 ± 3.7	45.7 ± 3.7

Values are presented as means ± s.e.m.

Table 2 The effect induced by dexamethasone, indometacin and mifepristone + dexamethasone on number, duration and electrocorticographic desynchronized pattern duration of S&W of DBA/2J mice, measured in the whole period of 240 min.

Drug (μg kg^{-1}, i.p.)	% of inhibition			TD50 (min)
	S&W number reduction	S&W duration reduction	Electrocorticographic desynchronized pattern duration	
Saline	0	0	0	
Dexamethasone				
1.0	0	0	0	
10	54.5 ± 6.7**	77.8 ± 7.7**	40.4 ± 2.7**	65 (51–69)
100	64.8 ± 5.7**	87.5 ± 5.9**	41.2 ± 1.7**	71 (60–81)
Indometacin				
1.0	0	0	0	
10	44.5 ± 2.3**	57.8 ± 4.7**	39.1 ± 1.3**	55 (51–69)
100	34.8 ± 3.7**	67.5 ± 4.9**	38.7 ± 2.2**	61 (60–81)
Mifepristone + dexamethasone				
1.0 + 1.0	14.1 ± 1.7§	17.8 ± 1.9§	19.1 ± 1.2§	
10 + 10	15.6 ± 1.4§	13.8 ± 1.1§	12.1 ± 1.6§	
100 + 100	18.5 ± 1.3§	12.8 ± 1.3§	16.1 ± 1.4§	

Results are expressed as mean ± s.e.m. (n = 9). **$P < 0.01$ vs control; §$P < 0.01$ vs dexamethasone alone. Time to 50 % of effect (time-dose 50; TD50), is expressed in min, with confidence limits within brackets.

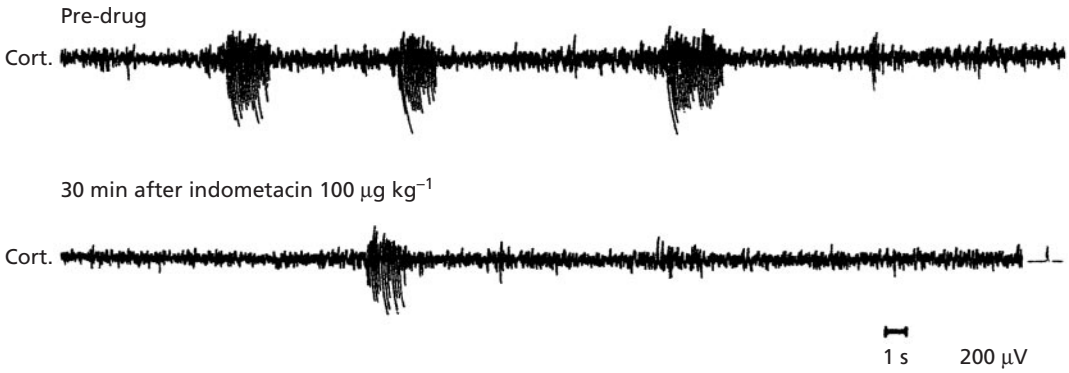

Figure 1 Example of spike-and-wave burst before and after intraperitoneal administration of indometacin 100 μg kg^{-1} to DBA/2J mice. Electrocorticographic tracing is from the anterior to posterior sensorimotor cortex.

wave spindling episodes in the electrocorticogram of DBA/2J mice. This effect appeared 30 min after administration and lasted for the duration of the recording period (240 min) (Table 2). In addition, dexamethasone treatment did not induce significant alterations of stereotyped behaviour such as licking, sniffing, chewing or tremors of the head and body and behavioural excitability, whereas the electrocorticographic desynchronized pattern was significantly reduced (Table 2).

The glucocorticoid receptor antagonist mifepristone, at the higher doses (10, 100 μg kg^{-1}, i.p.), did not modify per-se the S&W of DBA/2J mice. However, mifepristone inhibited the reducing activity of dexamethasone on S&W of mice, and the blocking effect lasted for all of the recording period (Table 2).

Influence of indometacin on S&W in DBA/2J mice

Indometacin, a prostaglandin-forming cyclooxygenase inhibitor, at the higher doses (10, 100 μg kg^{-1}, i.p.) reduced both the number and the duration of S&W of DBA/2J mice. The reduction of spindle bursts, which was significant 30 min after drug administration, was enhanced with time and lasted for the recording period (240 min). In addition, indometacin did not induce significant alterations of stereotyped behaviour such as licking, sniffing, chewing or tremors of the head and body and behavioural excitability, whereas the electrocorticographic desynchronized pattern was significantly reduced (Figure 1, Table 2).

Discussion

This study indicated that dexamethasone reduces the S&W of DBA/2J mice, thus confirming that corticosteroids are able to modulate brain excitability (Woodbury & Vernadakis 1967). S&W reduction by dexamethasone is evident at low doses (10 μg kg^{-1}, i.p.), and increases with the dose, suggesting that the steroid may antagonize S&W of mice via an interaction with glucocorticoid receptors. Corticosteroids exert many of their effects on target cells through intracellular receptor mechanisms (Thompson & Lippman 1974; Barnes & Adcock 1993). Two types of receptors for corticosteroids are known, type 1 and type 2 (De Kloet & Reul 1987). Type-2 receptors display a higher affinity for synthetic glucocorticoids, such as dexamethasone, than corticosterone (Reul & De Kloet 1986), and mifepristone antagonizes dexamethasone effects at the type-2 receptor binding sites (Moguilewski & Philbet 1984; Peers et al 1988).

In this study, the possible involvement of glucocorticoid receptors is confirmed by experiments performed with mifepristone which blocks dexamethasone reduction in S&W of mice. Our results support the possibility that activation by dexamethasone of glucocorticoid receptors may be involved in the concentration-related reduction in S&W of mice. The ability of mifepristone to antagonize the effect of dexamethasone in a dose-related fashion further supports this hypothesis. As previously reported (Capasso et al 1994a, b), dexamethasone's effect on S&W of mice appears to be mediated by the modulation of some intermediate mechanism, perhaps gene expression, since a period of 30 min is required to reduce significantly the S&W. Various reports in the literature support the view that the effects mediated by corticosteroids are time related and involve intracellular mechanisms or protein synthesis (Blackwell et al 1980).

Our previous paper indicates that the inhibitory effects of dexamethasone on S&W of mice are prevented

by cycloheximide, a protein synthesis inhibitor, indicating that dexamethasone may exert inhibitory effects on S&W of DBA/2J mice through a protein-synthesis-dependent mechanism (Capasso et al 1994a, b). Therefore, considering that steroid action depends on receptor occupation and protein synthesis (Blackwell et al 1980), the results obtained with cycloheximide (Capasso et al 1994a, b) and mifepristone suggest that in our experiments dexamethasone reduces S&W of mice through a synthesis-dependent mechanism via glucocorticoid receptors. Furthermore, the ability of dexamethasone to reduce S&W of mice suggests that during S&W of mice there may be a release of arachidonic acid after PLA_2 activation. This relationship between arachidonic acid and convulsion may be further supported by data showing that dexamethasone was able to reduce free fatty acids caused by bicuculline-induced status epilepticus. In this respect, we may hypothesize that PL A_2-mediated release of fatty acids may be one of the mechanisms which influence epileptiform phenomena (Tang & Bazan 1984), as happened in our mice. The possible involvement of arachidonic acid in the development of S&W of mice is further supported by the results of experiments performed with indometacin, a prostaglandin biosynthesis inhibitor, which was able to reduce S&W of mice, indicating that arachidonic acid metabolites (prostaglandins) may also be involved in the expression of S&W of mice, thus confirming an involvement of prostaglandins in the brain excitability. In fact, a relationship between increased neuronal activity and increased prostaglandins has been reported (Bradley et al 1969). Several studies indicate that electrical stimulation of the brain, either directly or through afferent stimulation, causes an increase in the level of prostaglandins. In addition, stimulation of the CNS by convulsant drugs causes increased prostaglandins (Wolfe 1975; Wolfe & Coceani 1979). Taken together, our data confirm and extend the above papers indicating that arachidonic acid and its metabolites (prostaglandins) are involved in the development of S&W of mice.

There are some topics which need to be discussed. Like many other non-steroidal anti-inflammatory drugs (NSAIDs), indometacin has many effects such as uncoupling oxidative phosphorylation at supratherapeutic concentrations, depressing the biosynthesis of mucopolysaccharides, inhibiting some proteinases, and so on (Insel 1996; Banik et al 2000). In contrast with our results, many investigators have suggested that indometacin induces or facilitates experimental seizures (McGinley et al 1985; Baik et al 1999). However, there are several differences in experimental procedures adopted by these authors and by us. First, our model (spontaneous epileptiform condition in a genetically prone mouse strain) is quite different from the model used by other investigators (pentamethylenetetrazol- or kainic-acid-treated mice). Previous experiments showed that the S&W model could be extremely sensitive to drug treatment (Capasso et al 1994a, b, 1995). Drugs were effective at dosages analogous to those used in the clinical setting; the threshold active dose for both indometacin and dexamethasone (10 $\mu g\ kg^{-1}$, i.p.) was about three orders of magnitude smaller than doses used by other investigators (5 and 10 $mg\ kg^{-1}$), which dramatically reduce the biosynthesis of the prostaglandins and thromboxane B2 (McGinley et al 1985).

The physio-pathological significance of arachidonic acid metabolism in S&W of DBA/2J mice may be relevant considering the several diseases related to brain excitability. Further studies are needed to establish whether arachidonic acid is a direct modulator of the properties of excitable membranes or if the loss of arachidonic acid is a signaling mechanism.

References

Baik, E. J., Kim, E. J., Lee, S. H., Moon, C.-H. (1999) Cyclooxygenase-2 selective inhibitors aggravate kainic acid induced seizure and neuronal cell death in the hippocampus. *Brain Res.* **843**: 118–129

Banik, N. L., Matzelle, D., Terry, E., Gantt-Wilford, G., Hogal, L. (2000) Inhibition of proteolysis by a cyclooxygenase inhibitor, indomethacin. *Neurochem. Res.* **25**: 1509–1515

Barnes, J. P., Adcock, I. (1993) Antiinflammatory actions of steroids: molecular mechanisms. *Trends Pharmacol. Sci.* **14**: 436–441

Bazan, N., Morelli de Liberti, S. A., Rodriguez de Turco, E. (1982) Arachidonic acid and arachidonoyl-diglyceroles increase in rat cerebrum during bicuculline-induced status epilepticus. *Neurochem. Res.* **7**: 839–843

Bazan, N. G., Turco, E. B. R., Politi, E. (1984) Dexametasone inhibits the production of free AA in cryogenic brain edema. *Trans. Am. Soc. Neurochem.* **15**: 105

Bazan, N. G., Birkle, D. L., Tang, W., Reddy, T. S. (1986) The accumulation of free arachidonic acid, diacylglycerols, prostaglandins and lipoxygenase reaction products in the brain during experimental epilepsy. In: Delgado-Escueta, A. V., Ward, A. A., Woodbury, D. M., Porter, R. J. (eds) *Advances in Neurology*. Vol. 44, Raven Press, New York, pp 879–902

Blackwell, G. J., Carnuccio, R., Di Rosa, M., Flower, R. J., Parente, L., Persico, P. (1980) Macrocortin: a polypeptide causing the anti-phospholipase effect of glucocorticoids. *Nature* **287**: 147–149

Bradley, P. B., Samuels, G. M. R., Shaw, J. E. (1969) Correlation of prostaglandin release from the cerebral cortex of cats with the electrocorticogram, following stimulation of the reticular formation. *Br. J. Pharmacol.* **37**: 151–157

Capasso, A., Sorrentino, L., Di Giannuario, A., Palazzesi, S., Pieretti, S., Loizzo, A. (1994a) Dexamethasone and hormones related to the hypothalamic-pituitary-adrenal axis modulate inherited neocortical spindling in DBA/2J mice. *Neuropsychobiology* **29**: 143–151

Capasso, A., Di Giannuario, A., Loizzo, A., Pieretti, S., Sorrentino, L. (1994b) Phencyclidine reduces inherited neocortical spindling in DBA/2J mice. *Life Sci.* **54**: PL 235–240

Capasso, A., Cutrufo, C., Di Giannuario, A., Loizzo, A., Palazzesi, S., Pieretti, S., Sorrentino, L. (1995) Opioids regulation of neocortical spindling episodes of DBA/2J mice. *Progr. Neuropsychopharmacol. Biol. Psychiatry* **19**: 281–290

De Kloet, E. R., Reul, J. M. H. M. (1987) Feedback action and tonic influence of corticosteroids on brain function: a concept arising from the heterogeneity of brain receptor system. *Psychoneuroendocrinology* **12**: 83–87

Dennis, E. A. (1987) Regulation of eicosanoids production: role of Phospholipase and inhibitors. *Biotechnology* **5**: 1294–1300

Flower, R. J., Blackwell, G. J. (1979) Antiinflammatory steroids induce biosynthesis of phospholipase A2 inhibitor which prevents prostaglandins generation. *Nature* **278**: 456–459

Glaser, K. B., Mobilio, D., Chang, J. Y., Senko, N. (1993) Phospholipase A2 enzymes: regulation and inhibition. *Trends Pharmacol. Sci.* **14**: 92–98

Insel, P. A. (1996) Analgesic, antipyretic and antiinflammatory agents and drugs employed in the treatment of gout. In: Hardman, J. G., Limbird, L. E., Molinoff, P. B., Ruddon, R. (eds) *Goodman & Gilman's the Pharmacological Basis of Therapeutics*. McGraw-Hill, NY, pp 617–657

McGinley, S., Centra, M., Lysz, T. W. (1985) The effect of inhibiting brain thromboxane biosynthesis on pentylenetetetrazole-induced seizure threshold. *J. Neurosci. Res.* **13**: 563–567

Moguilewsky, M., Philbert, D. (1984) RU-38486: potent antiglucocorticoid activity correlates with strong binding to the cytosolic glucocorticoid receptor followed by an impaired activation. *J. Steroid Biochem.* **20**: 271–276

Murphy, R. C., Hammarstrom, S., Samuelsson, B. (1979) Leukotriene C: a slow-reacting substance from murine mastocytoma cells. *Proc. Natl Acad. Sci. USA* **76**: 4275–4279

Najbauer, J., Schuman, E. M., Mamelak, A. N. (2000) The aspirin metabolite sodium salicylate causes focal cerebral hemorrhage and cell death in rats with kainic acid-induced seizures. *Neuroscience* **99**: 107–117

Newmark, M. E., Penry, J. K. (1980) *Genetics of Epilepsy: a Review*. Raven Press, New York, pp 1–96

Peers, S. H., Moor, D., Flower, R. J. (1988) Reversal of anti-inflammatory effect of dexametasone by the glucocorticoid antagonist RU-38486. *Biochem. Pharmacol.* **37:** 556–557

Reul, J. M. H. M., De Kloet, E. R. (1986) Anatomical resolution of two types of corticosterone receptor sites in rat brain with in vitro autoradiography and computerized image analysis. *J. Steroid Biochem.* **24**: 269–273

Ryan, L. J. (1984) Characterization of cortical spindling in DBA/2J and C57BL/6 inbred mice. *Brain Res. Bull.* **13**: 549–558

Ryan, L. J., Sharpless, S. K. (1979) Genetically-determined spontaneous and penthylenetetrazol-induced brief spindle episodes in mice. *Exp. Neurol.* **66**: 493–508

Shimizu, T., Wolfe, S. L. (1990) Arachidonic acid cascade and signal transduction. *J. Neurochem.* **55**: 1–15

Tang, W., Bazan, N. G. (1984) Dexamethasone decreases bicuculline-induced accumulation of brain FFA. *Trans. Am. Soc. Neurochem.* **15**: 152

Thompson, E. B., Lippman, M. E. (1974) Mechanism of action of glucocorticoids. *Metabolism* **23**: 159–202

Vane, J. R. (1971) Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature* **231**: 232–235

Wolfe, L. S. (1975) Possible roles of prostaglandins in the nervous system. *Adv. Neurochem.* **1**: 1–49

Wolfe, L. S., Coceani, F. (1979) The role of prostaglandins in the central nervous system. *Annu. Rev. Physiol.* **41**: 669–684

Woodbury, D. M., Vernadakis, A. (1967) Influence of hormones on brain activity. In: Martini, L., Ganong, W. F. (eds) *Neuroendocrinology*. Vol. 2, Academic Press, New York, pp 335–375