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Nitric oxide is involved in the expression of neocortical spike-and-wave spindling episodes in DBA/2J mice

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Abstract

This study investigated the possible role of nitric oxide (NO) in the development of neocortical spike-and-wave spindling episodes (S&W) of DBA/2J mice. The administration of distilled water did not modify either the number or duration of S&W in DBA/2J mice during the whole recording period (240 min). L-N^G-nitro arginine methyl ester (L-NAME) (3–300 μg/mouse, i.c.v.) dose-dependently 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). In addition, L-NAME 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 electroencephalogram desynchronized pattern was also significantly reduced. By contrast D-N^G-nitro arginine methyl ester at the same doses did not affect S&W of mice. The inhibitory effect of L-NAME on S&W of mice was dose-dependently reversed by L-arginine (L-ARG, 3–300 μg/mouse, i.c.v.) but not by D-arginine. Finally, glyceryl trinitrate on its own (3–300 μg/mouse, i.c.v.) significantly increased the S&W of mice and it was also able to reverse the inhibition on S&W of mice operated by L-NAME. These results provide evidence that NO may play a significant role in the development of brain excitability.

Introduction

DBA/2J mice spontaneously generate bilaterally synchronous spike-and-wave bursts of 7–8 cps spindles during quiet waking, active waking and slow-wave sleep but not during REM sleep (Ryan 1984). Numerous factors have led to a recent increased consideration of the DBA/2J mice as a model for studying cerebral excitability (Ryan & Sharpless 1979). The genetic contribution to epilepsy in man is becoming increasingly apparent (Newmark & Penry 1980). Consequently, genetically seizure-prone animals are assuming more importance as models of the pathophysiology of epilepsy in man.

Ryan & Sharpless (1979) suggested that DBA/2J mice may provide a useful model for studying the basis of the inheritance of a presumed epileptiform. There has been increasing biochemical evidence since 1970 that one of the targets for convulsion-induced changes is the cell membrane of neurons. 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 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 have indicated that dexamethasone, a phospholipase A_2 (PLA_2) inhibitor, attenuates bicuculline-induced free fatty acid accumulation in a dose-dependent manner (Bazan & Rakowski 1970; Bazan et al 1982, 1984). Recently, we have demonstrated that dexamethasone reduces S&W of DBA/2J mice through a protein-synthesis-dependent mechanism (Capasso et al 1994).

In this respect, since prostaglandins have been reported to be involved in the development of brain excitability (Bazan et al 1986) and glucocorticoids control

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prostaglandin biosynthesis by inhibiting the release of their common precursor, arachidonic acid, through PLA_2 inhibitory proteins (Gryglewski 1976; Di Rosa & Persico 1979; Blackwell et al 1980; Dennis 1987; Shimizu & Wolfe 1990; Barnes & Adcock 1993; Glaser et al 1993), we may suggest 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; Hirata et al 1980; Carnuccio et al 1981; Rothhut et al 1983) and it is subsequently converted to prostaglandins by the enzyme cyclooxygenase (Vane, 1971; Murphy et al 1979; Dennis 1987; Shimizu & Wolfe 1990; Glaser et al 1993).

The ability of indometacin (a well known prostaglandins inhibitor) to reduce S&W of DBA/2J mice (Capasso & Loizzo 2001) strongly supports the above hypothesis, suggesting that arachidonic acid and its metabolites (prostaglandins) are involved in the development of S&W of mice.

In recent years, a strong relationship between NO and prostaglandins has also been reported. In fact, the nitric oxide synthase inhibitor L-N^G-nitro arginine methyl ester reduces in parallel both NO and prostaglandin generation; this effect is reversed by L-arginine, the precursor for the NO synthesis, but not by D-arginine (Di Rosa et al 1996). Moreover, both sodium nitroprusside and glyceryl trinitrate enhance the production of prostaglandins, suggesting that NO stimulates prostaglandin biosynthesis through a direct interaction with cyclooxygenase enzymes (Di Rosa et al 1996).

Given the above evidence, we cannot exclude the possibility that prostaglandins involved in the development of brain excitability (Bazan et al 1986, Capasso & Loizzo 2001) may be also related to NO activity. Therefore, in this study, we considered the possible role of nitric oxide (NO) on S&W of DBA/2J mice.

Materials and Methods

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 electrodes (of 0.3 mm diameter) were implanted and fixed with acrylic resin, in a position corresponding to the anterior and posterior sensorimotor cortex bilaterally. Since S&W cortical electroencephalogram (ECoG) spindles are invariably expressed bilaterally in close synchrony, records from only the left hemisphere were analysed. The mice were allowed at least 4 days to recover and on the day of experiment they were placed in a sound-proof room. Each mouse was placed in a lighted and shielded recording chamber, then attached to the EEG machine by long flexible wires that did not hinder the free movements of the mouse, which was allowed 2 h to adapt to the recording chamber. An ECoG bipolar tracing from the left anterior to posterior sensorimotor cortex was recorded on paper by using a poligraph Ote Biomedica Neurograph 18.

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

Animal care, environmental conditions and use followed the rules of the Council of European Communities. The experimental procedures were approved by the Bioethical Committee of the Italian National Health Institute.

ECoG evaluation

Three parameters were evaluated: the number of S&W – reduction or increase of number of spindles was expressed as a percentage of the 60 min control period; the cumulated duration of S&W in s – reduction or increase of the spindle duration was expressed as a percentage of the 60-min control period; ECoG desynchronized pattern — in each mouse, the time spent in ECoG desynchronized activity, 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 the beginning of 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 ECoG was recorded beginning at approximately 0900 h. ECoGs were recorded on paper according to the following schedule: a control EEG was recorded for one consecutive hour, then the mice were injected with L-NAME or L-arginine or D-arginine or glyceryl trinitrate (3–300 μg/mouse, i.c.v.) and the ECoG 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%.

Results

Sleep–wakefulness cycle and S&W in control mice

The administration of distilled water did not modify either the number or the duration of S&W in DBA/2J mice during the whole recording period (240 min). The ECoG desynchronized pattern, however, showed consistent fluctuations during the whole recording period as expected (Table 1). In fact, in the post-drug period, the ECoG desynchronized pattern fluctuated in the range of $56.8 \pm 5.4\%$ up to $65.5 \pm 5.9\%$.

Table 1 The effect of distilled water (10 μL/mouse) on number and duration of S&W, and ECoG desynchronized pattern, in mice.

	Before drug	After drug			
		1 h	2 h	3 h	4 h
S&W number	66.5 ± 5.4	60.4 ± 7.2	63.6 ± 7.6	64.9 ± 5.6	60.5 ± 6.2
S&W duration (s)	69.5 ± 4.9	77.1 ± 5.1	75.6 ± 6.8	74.3 ± 6.4	73.4 ± 3.5
EcoG desynchronized pattern duration (% of control)	56.8 ± 4.4	58.5 ± 6.1	57.6 ± 5.2	62.5 ± 4.2	65.5 ± 5.9

Data are means ± s.e.m.

L-NAME influence on S&W in DBA2J mice

L-NAME (3–300 μg/mouse, i.c.v.) dose-dependently reduced the S&W in the ECoG of DBA/2J mice (Figure 1). L-NAME reduction appeared 30 min after administration and lasted throughout the recording period (240 min). In addition, stereotyped behaviour, such as licking, sniffing, chewing or tremors of the head and body, and behavioral excitability, was not significantly modified by L-NAME treatment (data not shown), whereas the ECoG desynchronized pattern was significantly reduced (Figure 1). By contrast, D-NAME (3–300 μg/mouse, i.c.v.) did not modify the S&W of DBA/2J mice (data not shown). L-Arginine (3–300 μg total, i.c.v.) was able to revert the inhibitory effect of L-NAME (3–300 μg total, i.c.v.) on S&W of mice (Figure 2). Finally, glyceryl trinitrate on its own (3–300 μg total, i.c.v.) significantly increased S&W of mice (Figure 3) and it was also able to reverse the inhibition on S&W of mice operated by L-NAME (3–300 μg total, i.c.v.) (Figure 4).

Discussion

The results of our experiments provide a strong evidence that NO may be involved in the control of brain excitability. In fact, the ability of L-NAME to reduce, and of glyceryl trinitrate to increase, S&W of DBA/2J mice suggests that during brain excitability NO may be released after NO synthase activation. This relationship between NO and brain excitability may be supported by data showing that NO synthase inhibitors abolish some aspects of the brain excitability (Homayoun et al 2002a, b; Khavandgar et al 2002).

This paper evaluated the role of NO in the expression of S&W of DBA/2J mice and our results confirm that NO may be involved in the expression of brain excitability.

It has been reported that there is a strong link between NO and prostaglandins: the nitric oxide synthase inhibitor L-NAME reduces, in parallel, both NO and prostaglandin generation: this effect is reversed by L-arginine, the precursor for the NO synthesis, but not by D-arginine (Di Rosa et al 1996). Moreover, both sodium nitroprusside and glyceryl trinitrate enhance the production of prostaglandins, suggesting that NO stimulates prostaglandin biosynthesis through a direct interaction with cyclooxygenase enzymes (Di Rosa et al 1996).

Recently, we have demonstrated that arachidonic acid metabolites are involved in the expression of brain excitability, since both cyclooxygenase and lipoxygenase inhibitors reduced brain excitability (Capasso & Loizzo 2001), thus confirming the involvement of prostaglandins in the

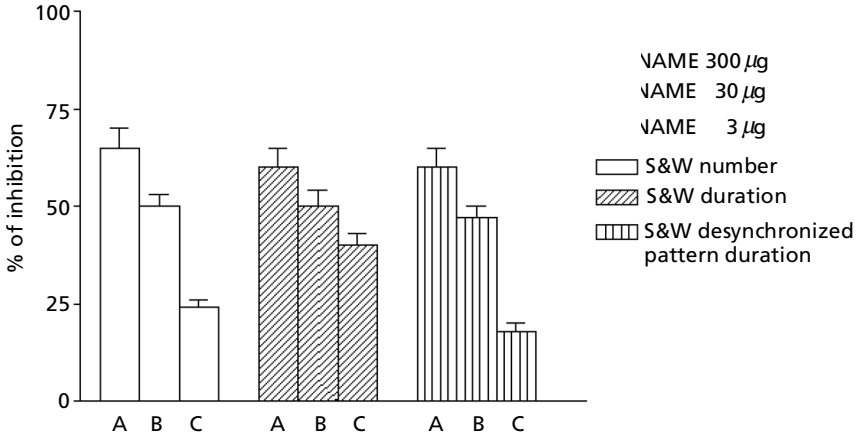

Figure 1 The effect induced by L-NAME (3, 30 or 300 μg/mouse, i.c.v.) on the S&W number, duration and desynchronized pattern duration of mice. The results are expressed as mean ± standard error of % of inhibition (n = 6). *$P < 0.05$, **$P < 0.01$ vs baseline control.

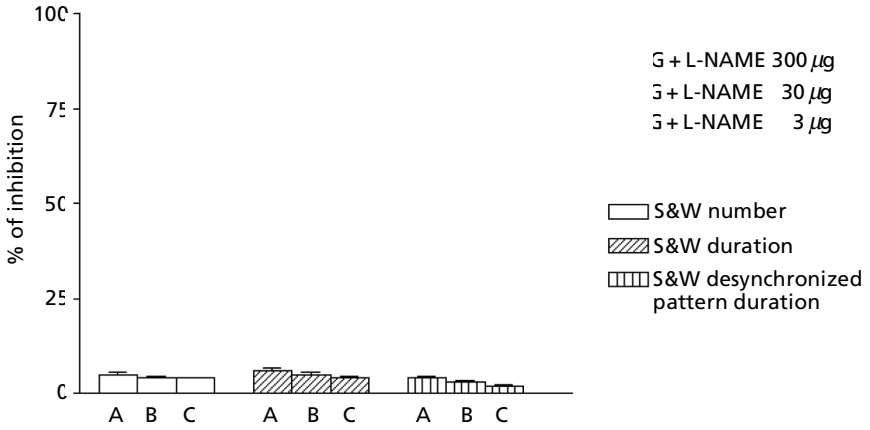

Figure 2 The effect induced by L-arginine (L-ARG) (3, 30 or 300 μg/mouse, i.c.v.) on the reducing effect of L-NAME (3, 30 or 300 μg/mouse, i.c.v.) on the S&W number, duration and desynchronized pattern duration of mice. The results are expressed as mean ± standard error of % of inhibition (n = 6).

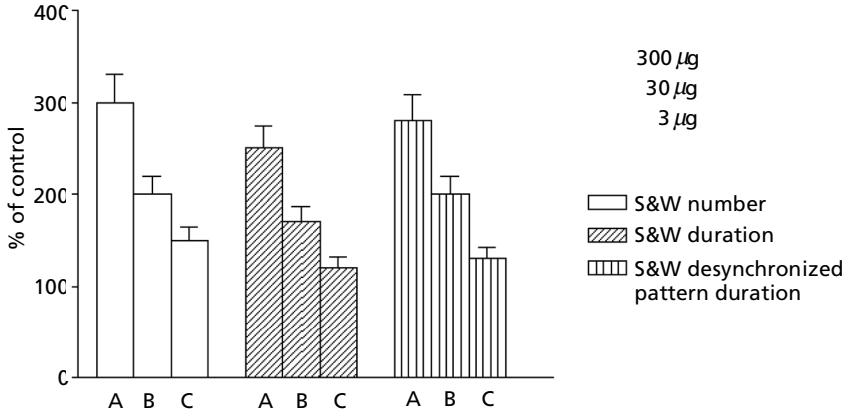

Figure 3 The effect induced by glyceryl trinitrate (GTN) (3, 30 or 300 μg/mouse, i.c.v.) on the S&W number, duration and desynchronized pattern duration of mice. The results are expressed as mean ± standard error of % of control (n = 6). *$P < 0.05$, **$P < 0.01$ vs baseline control.

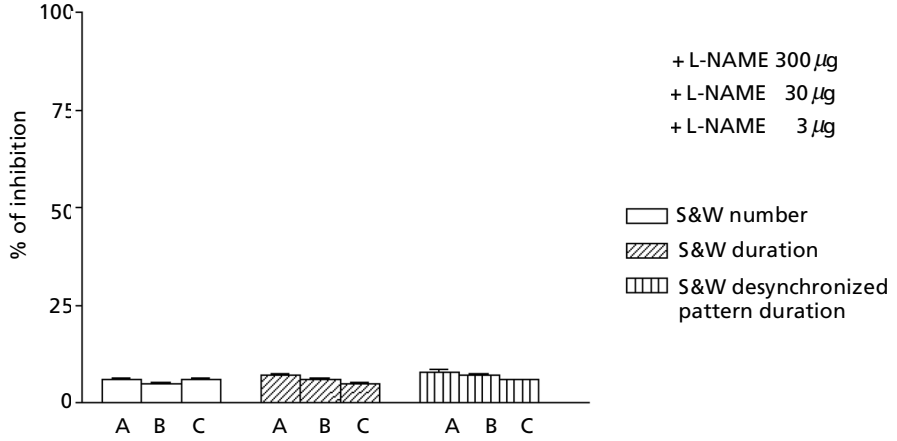

Figure 4 The effect induced by glyceryl trinitrate (GTN) (3, 30 or 300 μg/mouse, i.c.v.) on the reducing effect of L-NAME (3, 30 or 300 μg/mouse, i.c.v.) on the S&W number, duration and desynchronized pattern duration of mice. The results are expressed as mean ± standard error of % of inhibition (n = 6).

development of epilepsy (Bazan & Rakowski 1970; Bazan et al, 1984).

Taken together, our data support the possibility that the reduction of S&W in DBA/2J mice induced by L-NAME is related to its ability to inhibit cyclooxygenase activity, whereas the ability of glyceryl trinitrate to increase opiate epilepsy is related to its ability to stimulate cyclooxygenase activity.

This hypothesis may be supported by our previous papers indicating that both dexamethasone and indometacin are able to reduce brain excitability (Pieretti et al 1992; Capasso et al 1994; Capasso & Loizzo 2001).

In conclusion, the results of this study indicate the involvement of NO in the expression of brain excitability and suggest that there is a link between NO, prostaglandins and brain excitability.

The physio-pathological significance of NO in brain excitability may be relevant considering the several diseases related to brain excitability. Further studies are required to establish whether NO is a direct modulator of the properties of excitable membranes or if the loss of NO is a signalling mechanism.

References

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

Bazan, N. G., Rakowski, H. (1970) Increased levels of brain free fatty acids after electroconvulsive shock. *Life Sci.* **9**: 501–507

Bazan, N., Morelli, de Liberti, S., 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

Capasso, A., Loizzo, A. (2001) Arachidonic acid and its metabolites are involved in the expression of neocortical spike-and-wave spindling episodes in DBA/2J mice. *J. Pharm. Pharmacol.* **53**: 883–888

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

Carnuccio, R., Di Rosa, M., Flower, R. J., Pinto, A. (1981) The inhibition by hydrocortisone of prostaglandins biosynthesis in the rat peritoneal leucocytes is correlated with intracellular macrocortin levels. *Br. J. Pharmacol.* **74**: 322–324

Dennis, E. A. (1987) Regulation of eicosanoids production: role of phospholipase and inhibitors. *Bio/Technology* **5**: 1294–1300

Di Rosa, M., Persico, P. (1979) Mechanism of inhibition of prostaglandin biosynthesis by hydrocortisone in rat leucocytes. *Br. J. Pharmacol.* **66**: 161–163

Di Rosa, M., Ialenti, A., Ianaro, A., Sautebin, L. (1996). Interaction between nitric oxide and cyclooxygenase pathways. In: *Prostaglandins, leukotrienes and essential fatty acids*. Churchill Livingstone, Edinburgh, UK, Vol. 54, pp 229–238

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

Gryglewski, R. J. (1976) Steroid hormones, anti-inflammatory steroids and prostaglandins. *Pharmacol. Res. Commun.* **8**: 337–348

Hirata, F., Shiffman, E., Venkatasubramanian, K., Salomon, D., Axelrod, J. (1980) A phospholipase A2 inhibitory protein in rabbit neutrophils induced by glucocorticoids. *Proc. Natl Acad. Sci. USA* **77**: 2533–2536

Homayoun, H., Khavandgar, S., Namiranian, K., Gaskari, S. A., Dehpour, A. R. (2002a) The role of nitric oxide in anticonvulsant and proconvulsant effects of morphine in mice. *Epilepsy Res.* **48**: 33–41

Homayoun, H., Khavandgar, S., Dehpour, A. R. (2002b) The involvement of endogenous opioids and nitricoxidergic pathway in the anticonvulsant effects of foot-shock stress in mice. *Epilepsy Res.* **49**: 131–142

Khavandgar, S., Homayoun, H., Dehpour, A. R. (2002) The role of nitric oxide in the proconvulsant effect of delta-opioid agonist SNC80 in mice. *Neurosci. Lett.* **329**: 237–239

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

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

Pieretti, S., Di Giannuario, A., Loizzo, A., Sagratella, S., Scotti de Carolis, A., Capasso, A., Sorrentino, L. (1992) Dexamethasone prevents epileptiform activity induced by morphine in in vivo and in vitro experiments. *J. Pharmacol. Exp. Ther.* **263**: 830–839

Rothhut, F., Russo-Marie, F., Wood, J., Di Rosa, M., Flower, R. J. (1983) Further characterization of the glucocorticoid induced anti-phospholipase protein "renocortin". *Biochem. Biophys. Res. Commun.* **117**: 878–884

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

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