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SPECIAL REPORT

Inhibition by N^{ω}-nitro-L-arginine methyl ester of the electrocortical arousal response in rats

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In rats chronically implanted with cannulae into one lateral cerebral ventricle and recording electrodes onto the fronto-parietal cortex, the effects of systemic or intraventricular administration of the nitric oxide (NO) synthesis inhibitor, N^{ω}-nitro-L-arginine methyl ester (L-NAME), on electrocortical (ECoG) arousal response evoked by sound stimulation were studied. In control animals, a single acoustic stimulation (80 dB for 15 s) produced a significant decrease in ECoG total voltage power lasting approximately 25 s. No tolerance developed after repeating the same sound stimulation at 15, 30, 60 min and 24 h intervals. Under these experimental conditions, pretreatment with L-NAME, given systemically (10 mg kg^{-1}, i.p.) or intracerebroventricularly (300 μg), significantly reduced the sound-evoked arousal response 1 h and 15 min later, respectively. In conclusion, the present data are in favour of a physiological role of NO in the control of arousal mechanisms.

Keywords: Nitric oxide (NO); electrocortical arousal; N^{ω}-nitro-L-arginine methyl ester

Introduction Nitric oxide (NO) has been shown to occur in the mammalian CNS (Bredt *et al.*, 1991) where it has messenger properties (Garthwaite, 1991; Moncada *et al.*, 1989). Despite its involvement as a putative trans-synaptic messenger in long-term depression (Shibuki & Okada, 1991) and potentiation (O'Dell *et al.*, 1991), the physiological significance of NO in the brain remains obscure. *In vitro* experiments have shown that receptor agonists (e.g. N-methyl-D-aspartate, NMDA) which enhance intracellular Ca^{2+}, can activate NO synthase to produce NO (see Garthwaite, 1991) by converting the endogenous substrate, L-arginine, into citrulline (Moncada *et al.*, 1989). Recently, it has been reported that intracerebroventricular (i.c.v.) microinfusion of L-arginine produces a marked fall in the amplitude of electrocortical (ECoG) activity and that this is abolished by previous microinfusion of N^{ω}-nitro-L-arginine methyl ester (L-NAME) (Mollace *et al.*, 1991), an inhibitor of NO-synthase (Moncada *et al.*, 1991). This suggests that NO formed from L-arginine may produce ECoG arousal. In addition, the short half-life of NO (Moncada *et al.*, 1991) led us to hypothesize that it may act in the brain as a signalling molecule mediating rapid and short-lasting cortical neuronal activation. Under normal conditions, discrete peripheral stimuli produce a widespread, quantifiable ECoG desynchronization (arousal or alert response) (Kiloh *et al.*, 1972). Therefore, to test our hypothesis, we have studied the effects of the NO synthesis inhibitor, L-NAME, on sound-evoked ECoG desynchronization in rats.

Methods Adult male Wistar rats (250–280 g), housed in a temperature- (22 ± 2°C) and humidity- (60 ± 5%) controlled colony room, with tap water and laboratory food available *ad libitum*, were used for the study. Under chloral hydrate (400 mg kg^{-1}, i.p.) anaesthesia, surface cortical electrodes ($n = 4$) were implanted onto each fronto-parietal region (Bagetta *et al.*, 1988) to permit ECoG recording. The animals were allowed one week for recovery before testing. In awake, freely moving animals ECoG activating response was evoked by an acoustic stimulus (80 dB) of 15 s duration, delivered at 0, 15, 30 and 60 min and then at 24 h. The sound-evoked response, occurring in the domain of the total ECoG spectrum power (0.25–16 Hz) was monitored each second after the stimulus was delivered; computerized quantitation of the sound-evoked decrease in the ECoG signal amplitude (μV) was obtained with the aid of a Berg-Fourier analyzer (OTE Biomedica, Florence). Online, qualitative assessment of the ECoG activity was obtained with an 8-channel EEG machine interfaced with the Berg-Fourier analyzer. The data are expressed as mean ± s.e.mean.

Results The background ECoG activity in an awake rat is shown in Figure 1 (upper panel, trace a). Application of a 15 s acoustic stimulus (80 dB) induces ECoG desynchronization which is reproducible (Figure 1, upper panel, traces b and c). The evoked desynchronization started 6.6 ± 1.8 s after the delivery of the stimulus had been completed and this yielded a 69.6 ± 5.1% decrease in the ECoG signal amplitude. The reduction in amplitude reached its maximum 18.6 ± 4.2 s later and complete recovery to baseline level was obtained 23.0 ± 6.9 s after the stimulation had been completed. Repeated application of the acoustic stimulus (15 min, 30 min, 60 min and 24 h after the first stimulus; $n = 12$ experiments) did not produce significant changes in the latency to onset of ECoG response, time to peak or recovery time (Figure 2). Under these experimental conditions, systemic administration of L-NAME (10 mg kg^{-1}, i.p.; $n = 8$ rats) significantly reduced the sound-evoked ECoG response from 75.2 ± 5.6% to 39.7 ± 15.5% ($P < 0.05$; Student's t test) 60 min later, in comparison to controls (vehicle-treated) (Figures 1 and 2). No significant effects were evident 15 min and 30 min after L-NAME administration. In addition, this treatment doubled the latency of ECoG response onset (13.0 ± 2.5 s vs control 6.7 ± 2.2 s; $P < 0.05$) and significantly shortened the recovery time (15.0 ± 6.7 s vs control 30.0 ± 10.2 s; $P < 0.05$). A dose of 1.0 mg kg^{-1}, i.p. L-NAME was ineffective ($n = 6$). A similar inhibition of sound-evoked ECoG response was obtained with a shorter latency (15 min) after microinfusion of L-NAME (300 μg; $n = 6$) into one lateral cerebral ventricle (Figure 2).

Discussion The present data demonstrate that sound-evoked ECoG arousal is prevented by systemic injection of L-NAME, a potent inhibitor of NO synthesis. This effect does

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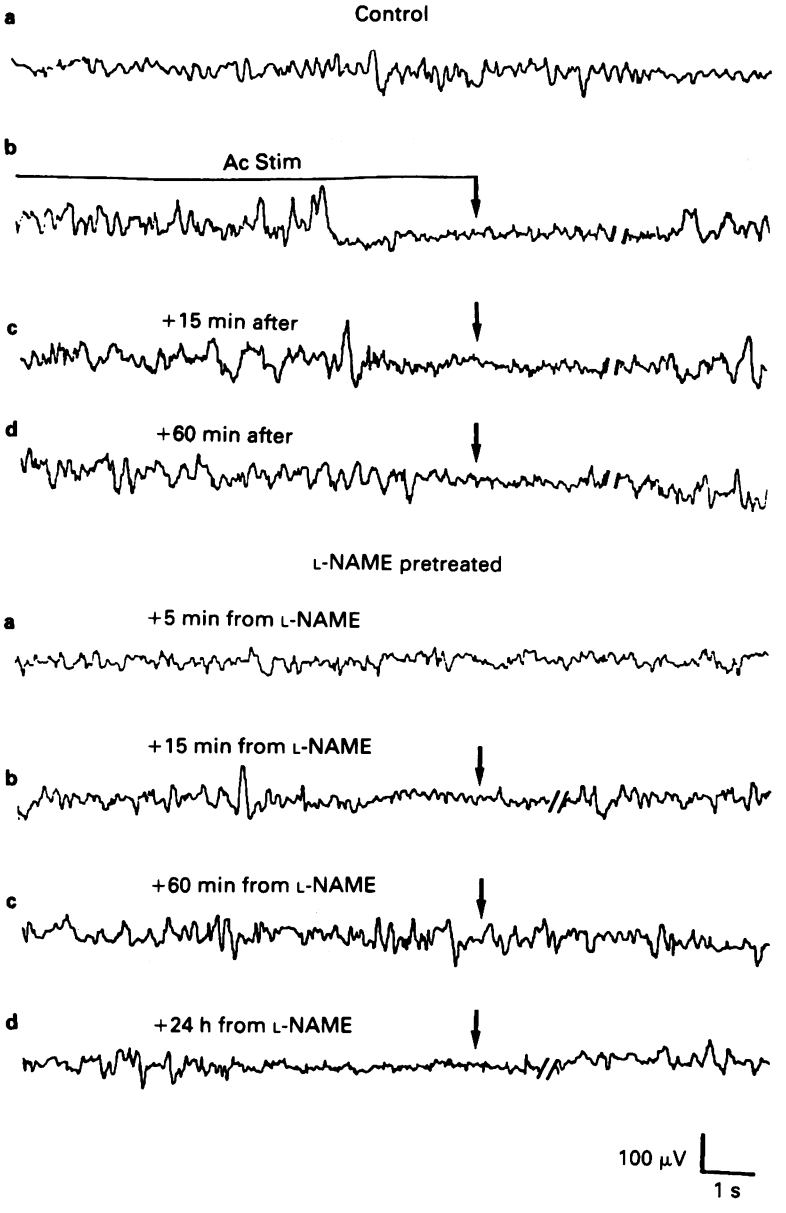

Figure 1 Electrocortical (ECoG) records from a control rat (upper panel) showing the desynchronizing ECoG response to a 15 s acoustic stimulus (Ac Stim) (80 dB; trace b); similar responses, are obtained 15 min (trace c) and 60 min (trace d) after the first stimulus has been delivered. Trace (a) shows the background ECoG activity. Systemic treatment with N^{ω}-nitro-L-arginine methyl ester (L-NAME, 10 mg kg^{-1}, i.p.) prevents the sound-evoked ECoG response and this is evident 60 min after acoustic stimulation (lower panel; trace c) but not 5 min (trace a) or 15 min (trace b) after. Complete recovery is obtained 24 h after L-NAME administration (trace d). The arrowheads indicate the end of the acoustic stimulation. It is evident that ECoG arousal response starts before the end of acoustic stimulation (b, c and d of control experiments).

not seem to be due to the increase in systemic blood pressure eventually produced by L-NAME (see Moncada *et al.*, 1991) since pretreatment with adrenaline at a dose producing increase in blood pressure (1 mg kg^{-1}, s.c., given 5 min before sound stimulation) did not affect the ECoG arousal response (data not shown). The inhibitory effects induced by L-NAME are due to an action at the CNS level; in fact, i.c.v. injection of L-NAME produced a similar inhibition of sound-evoked ECoG response, though after a shorter (15 min) latency. Previous experiments have shown that intracerebral injection of nanomolar doses of NO or NO-releasing agents, e.g. SNAP (see Moncada *et al.*, 1991), produces ECoG desynchronization (Bagetta *et al.*, 1992). These effects were similar in duration and intensity to those produced by intracerebral 8-bromo-cyclic GMP and could be prevented by the NO-trapping agent, haemoglobin, given into the same site or by systemic pretreatment with methylene blue (Bagetta *et al.*, 1992), a soluble guanylate cyclase inhibitor (see Moncada *et al.*, 1991). The present experiments show that inhibition of NO synthesis in the rat brain leads to a significant reduction of the ECoG arousal response and, together with our previous findings (Mollace *et al.*, 1991; Bagetta *et al.*, 1992), suggest that NO may play a physiological role in the control of the arousal state.

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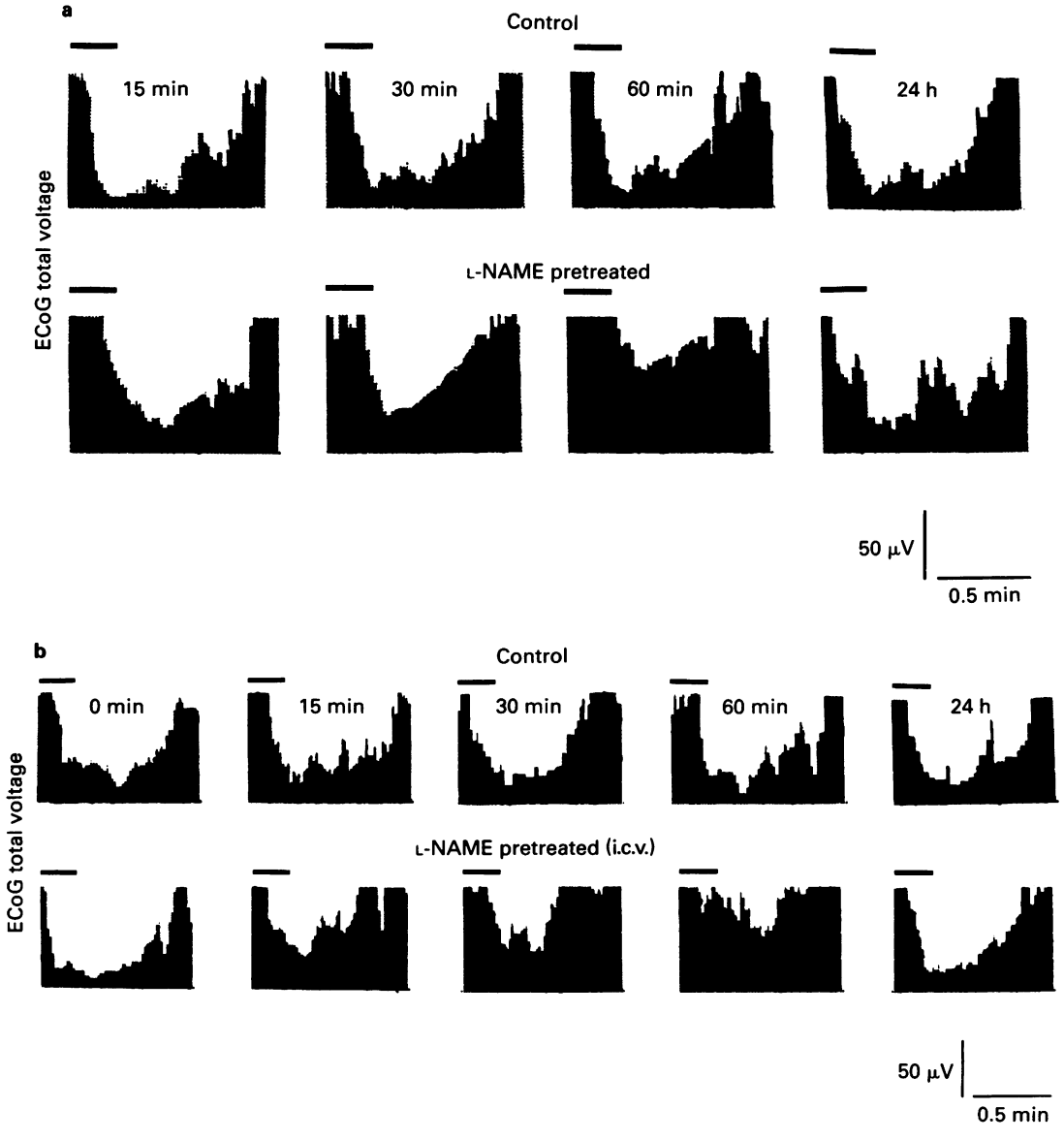

Figure 2 Computerized analysis of the electrocorticol (ECoG) desynchronizing response evoked by a 15 s acoustic stimulus (80 dB) in control and N^{ω}-nitro-L-arginine methyl ester (L-NAME) pretreated rats. In control rats ($n = 12$) sound stimulation evokes an approximately 80% decrease in ECoG signal amplitude with no reduction in the response intensity upon restimulation 15, 30 and 60 min and 24 h after the first stimulus (time zero) has been delivered. Significant reduction of the sound-evoked ECoG amplitude is observed 60 min and 15 min after systemic (10 mg kg^{-1}, i.p.; $n = 8$) (a) or intracerebroventricular (i.c.v; 300 μg; $n = 8$) (b) injection of L-NAME, respectively. The horizontal bar above individual ECoG records indicates the duration (see calibration) of the stimulus.

References

BAGETTA, G., DE SARRO, G.B., PRIOLO, E. & NISTICÒ, G. (1988). Ventral tegmental area: site through which dopamine D_2-receptor agonists evoke behavioural and electrocortical sleep in rats. *Br. J. Pharmacol.*, **95**, 860–866.

BAGETTA, G., IANNONE, M., LOMBARDI, U. & NISTICÒ, G. (1992). Evidence that nitric oxide is the mediator of the arousal electrocortical desynchronization in rats. International Congress on *Nitric Oxide: Brain and Immune System*, Paraelios (Calabria) Italy, 16–18 September, 1992. Abstract C12.

BREDT, D.S., GLATT, C.E., HWANG, P.M., FOTUHI, M., DAWSON, T.M. & SNYDER, S.H. (1991). Nitric oxide synthase protein and mRNA are discretely localized in neuronal populations of the mammalian CNS together with NADPH diaphorase. *Neuron*, **7**, 615–624.

GARTHWAITE, J. (1991). Glutamate, nitric oxide and cell-cell signalling in the nervous system. *Trends Neurosci.*, **14**, 60–67.

KILOH, L.G., COMAS, A.J. & OSSELTON, J.W. (1972). *Clinical Electroencephalography*. London: Butterworths.

MOLLACE, V., BAGETTA, G. & NISTICÒ, G. (1991). Evidence that L-arginine possesses proconvulsant effects mediated through nitric oxide. *Neuroreport*, **2**, 269–272.

MONCADA, S., PALMER, R.M.J. & HIGGS, E.A. (1989). Biosynthesis of nitric oxide from L-arginine: a pathway for the regulation of cell function and communication. *Biochem. Pharmacol.*, **38**, 1709–1715.

MONCADA, S., PALMER, R.M.J. & HIGGS, E.A. (1991). Nitric oxide: physiology., pathophysiology and pharmacology. *Pharmacol. Rev.*, **43**, 109–142.

O'DELL, T.J., HAWKINS, R.D., KANDEL, E.R. & ARANCIO, O. (1991). Tests of the role of two diffusible substances in long-term potentiation: evidence for nitric oxide as a possible early retrograde messenger. *Proc. Natl. Acad. Sci. U.S.A.*, **88**, 11285–11289.

SHIBUKI, K. & OKADA, D. (1991). Endogenous nitric oxide release required for long-term synaptic depression in the cerebellum. *Nature*, **349**, 326–328.

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