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Short communication

Systemic administration of lithium chloride and tacrine but not kainic acid augments citrulline content of rat brain

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Abstract

The effects of tacrine (5 mg/kg i.p.) in lithium chloride (LiCl; 12 mEq/kg i.p.)-pretreated (24 h beforehand) animals and of kainate (10 mg/kg i.p.) on brain citrulline, the co-product of nitric oxide (NO) synthesis, were studied in rats. High performance liquid chromatography analysis of whole brain tissue homogenates from rats treated with LiCl and tacrine revealed a significant increase in citrulline content before the onset of seizures. This effect was prevented in a stereoselective manner by N^{ω}-nitro-L-arginine methyl ester (10 mg/kg i.p., given 20 min before tacrine), an inhibitor of NO synthase. By contrast, kainic acid (10 mg/kg i.p.) did not affect significantly brain citrulline during the pre-convulsive period. In conclusion, our data indicate that in rats seizures induced by LiCl and tacrine but not kainic acid are triggered by excessive NO production in the brain.

Keywords: Lithium chloride; Tacrine; Seizure; Nitric oxide (NO); Citrulline; L-NAME (N^{ω}-nitro-L-arginine methyl ester)

1. Introduction

Nitric oxide (NO) is a labile radical species endowed with neuronal messenger functions in the central nervous system (CNS). Increases in intraneuronal Ca^{2+} stimulate NO synthase, which oxidizes L-arginine with stoichiometric production of L-citrulline and NO (see Knowles and Moncada, 1994). In vitro experiments have shown that stimulation of *N*-methyl-D-aspartate (NMDA), non-NMDA or muscarinic acetylcholine receptors leads to NO production and cGMP accumulation (see Garthwaite and Boulton, 1995; Arroyo et al., 1990). Activation of the L-arginine-NO-cGMP pathway is also involved in the pathophysiology of cell death produced by excessive stimulation of NMDA receptors in rat cortical neurones (Dawson et al., 1991) and neuroblastoma cells (Corasaniti et al., 1993) in culture. Recently, it has been reported that intracerebroventricular administration of N^{ω}-nitro-L-arginine methyl ester (L-NAME), an inhibitor of NO synthase (see Knowles and Moncada, 1994), prevents limbic seizures and related hippocampal damage produced by tacrine, an anticholinesterase agent, in rats pretreated with lithium chloride (LiCl; see Bagetta et al., 1992) but not the seizures and hippocampal damage evoked by systemic administration of kainic acid in rats (Bagetta et al., 1995). These data suggest that excessive NO production may be the trigger for seizure onset and consequent neurodegeneration seen in rats receiving LiCl and tacrine but not for those elicited by kainic acid. In support of this hypothesis, here we now report original data demonstrating that systemic administration of LiCl and tacrine but not kainic acid increases the brain content of citrulline, the co-product of NO synthesis, during the pre-convulsive period in rat.

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2. Materials and methods

Adult male Wistar rats (250–280 g), housed under standard conditions for temperature (20 ± 2°C) and humidity (65%), were anaesthetized with chloral hydrate (400 mg/kg i.p.) to enable cortical stainless steel screw electrodes to be chronically implanted under stereotaxic guidance for recording electrocortical (ECoG) activity (see Bagetta et al., 1995). For experimentation, the doses of tacrine and LiCl used here were chosen on the basis of previous data demonstrating that systemic administration of 5 mg/kg (i.p.) tacrine in rats pretreated (24 h beforehand) with 12 mEq/kg (i.p.) LiCl produces in 90% of the treated animals ECoG seizures after a latency of approximately 15 min (see Bagetta et al., 1995). In addition, the dose of kainic acid was 10 mg/kg (i.p.) and this is known to produce seizures in 83% of rats after a latency of approximately 20 min (Bagetta et al., 1995). Therefore, brain tissue homogenates were prepared from animals which had not experienced ECoG epileptogenic discharges for approximately 15 min and 20 min after administration of tacrine (in LiCl-pretreated rats) and kainic acid, respectively. Brain tissue homogenates were also prepared from rats which had received 15 min before death an injection of double-distilled pyrogen-free H_2O (1 ml/kg i.p.); this group was taken as control. The brain levels of citrulline were determined by high-performance liquid chromatography (HPLC). After the animals were killed, the brains were rapidly removed, weighed, immersed in liquid nitrogen and stored at −70°C until analysis. After thawing, the brains were homogenized in 0.4 N perchloric acid containing the internal standard (L-Norvaline) and centrifuged (10000 × g for 15 min at 4°C). The resulting supernatant was passed through a 0.45 μm filter unit (Millipore), diluted with methanol and injected into the chromatographic column (Waters Nova Pak C18, 150 × 3.9 mm, particle size 4 μm, combined with a Waters C18 Guard-Pak) following OPA derivatization. An HPLC System Gold (Beckman) and a Shimadzu RF 530 fluorimetric detector with filters for excitation at 334 nm and emission at 440 nm were used. The mobile phase consisted of (1) solvent A (methanol/tetrahydrofuran/water (2:2:96, v/v)) containing 0.05 M disodium phosphate, 0.05 M sodium acetate, pH = 6.98 with acetic acid; (2) solvent B (methanol/water (65:35, v/v)). A linear gradient was employed in which solvent B in the mobile phase passed from 45% (initial conditions) to 100% (final conditions) in 20 min. The flow rate was 0.8 ml/min at 25°C. The data are expressed as nmol of citrulline/g of wet tissue weight. The resulting means were evaluated statistically for differences using the Student t-test. Tacrine and LiCl were from Sigma (Milan, Italy); L-NAME was a gift of Dr A.E. Higgs (Wellcome Res. Lab., Beckenham, UK). L-NA and D-NAME were purchased from Inalco (Milan, Italy). The experimental procedures used meet the guidelines of the Ministry of Health (G.U. No. 40, February 18, 1992) and have been approved by the Animal Care Committee (University of Rome 'Tor Vergata').

3. Results

HPLC measurement of brain citrulline content before the onset of ECoG discharges yielded a significant ($P < 0.01$) increase in the co-product of NO synthesis in rats ($n = 8$) receiving combined administration of LiCl (12 mEq/kg i.p.) and tacrine (5 mg/kg i.p., given 24 h after LiCl) as compared to the results obtained in vehicle (H_2O, 1 ml/kg i.p.; $n = 6$)-treated rats. Given individually, LiCl and tacrine did not affect significantly brain citrulline (Table 1). The increase in brain citrulline was prevented by L-NAME ($n = 6$), given 20 min before tacrine at a dose (10 mg/kg i.p.) which per se reduced by 12% the basal citrulline level ($n = 6$); D-NAME (10 mg/kg i.p.; $n = 6$) was without effect on LiCl-tacrine-enhanced brain citrulline (Table 1). Preliminary experiments in which LiCl-tacrine-treated rats ($n = 2$) received L-NA (10 mg/kg i.p.) instead of L-NAME (data not shown) yielded similar effects. At variance with the results obtained with combined administration of LiCl and tacrine, treatment with kainic acid (10 mg/kg i.p.; $n = 6$) did not affect significantly the brain citrulline content (Table 1).

Table 1
Effects of systemic administration of tacrine (THA) in lithium chloride (LiCl)-pretreated (24 h before) rats and of kainic acid on the brain content of citrulline, the co-product of NO synthesis

Treatment (i.p.)	No. of rats	Brain citrulline (nmol/g of tissue) Mean ± S.E.
Vehicle (H_2O) 1 ml/kg	6	104.0 ± 2.5
THA (5 mg/kg) + H_2O	6	103.0 ± 9.4
LiCl (12 mEq/kg) + H_2O	6	111.5 ± 15.5
LiCl + THA	8	130.0 ± 4.0[a]
L-NAME (10 mg/kg)	6	93.3 ± 1.9
LiCl + L-NAME + THA	6	91.6 ± 5.7
LiCl + D-NAME + THA	6	133.0 ± 3.0[a]
Kainic acid (10 mg/kg)	6	103.0 ± 9.4

The concentration of citrulline was determined in individual brain tissue homogenates prepared from rats in which ECoG activity had been continuously monitored for approximately 15 min and 20 min after tacrine or kainate injection, respectively, and in no instance were ECoG epileptogenic discharges recorded. For the antagonism study, L-NAME, an inhibitor of NO synthase, and the less active isomer, D-NAME, were administered 20 min before tacrine. [a] $P <$ 0.01 vs. control (Student's t-test).

4. Discussion

In previous experiments we have shown that LiCl and tacrine augment the expression of NO synthase mRNA in the rat hippocampus and this is seen before the onset of seizures (Bagetta et al., 1993a). These effects are likely to be accompanied by an increase in the gene product because during the pre-convulsive period LiCl and tacrine increase the expression of the Ca^{2+}-calmodulin-dependent NO synthase activity in the rat hippocampus (see Bagetta et al., 1993a, b). These data, together with the neuroprotection afforded by L-NAME (Bagetta et al., 1992), an inhibitor of NO synthase (see Knowles and Moncada, 1994), strongly support a role for excessive NO production in the mechanisms which trigger seizures in rats treated with LiCl and tacrine. This hypothesis is now further strengthened by the present observation of enhanced concentrations of brain citrulline, the co-product of NO synthesis (see Knowles and Moncada, 1994). Under similar experimental conditions, no significant changes have been observed in the plasma citrulline content (Bagetta et al., 1994), thus indicating that the elevation of this amino acid in the rat brain is central in origin. In addition, our original hypothesis involving excessive NO formation in the mechanism of seizure onset is further supported by the finding that pretreatment with L-NAME (present data) and L-NA (data not shown), two inhibitors of NO synthase (see Knowles and Moncada, 1994), abolished, in a stereoselective fashion, the increase in brain citrulline produced by combined administration of LiCl and tacrine. Interestingly, systemic administration of kainic acid, a well studied excitotoxin (see Bagetta et al., 1995), failed to increase brain citrulline during the pre-convulsive period. This observation is in agreement with the reported lack of significant changes in brain cortical and hippocampal NO synthase activity in conjunction with the lack of neuroprotection by intracerebroventricular administration of L-NAME in rats receiving systemic administration of this neurotoxin (Bagetta et al., 1995). Altogether, these data suggest that NO may not be involved in the mechanisms which trigger seizures in rats treated with kainic acid. Interestingly, in vivo studies have shown that inhibitors of NO synthase *enhance* rather than inhibit kainic acid neurotoxicity, though this may be due to mechanisms other than neuronal NO synthase inhibition (see Penix et al., 1994). Nonetheless, on the basis of our present data we cannot exclude that fast, transient increases in NO might have occurred in the brain in response to kainic acid. However, the results obtained for LiCl and tacrine indicate that a persistent increase is needed for NO to trigger seizures and consequent brain damage. Balcioglu and Maher (1993), using a haemoglobin trapping technique with microdialysis, have reported that in anaesthetized rats systemic administration of 13 mg/kg i.p. of kainic acid increases hippocampal NO release. However, this effect reached statistical significance 60 min after injection of kainic acid, an excitotoxin known to produce ECoG seizures in rats after a latency of approximately 20 min (Bagetta et al., 1995; Clifford et al., 1987). Therefore, the increased NO reported by Balcioglu and Maher (1993) may well be the consequence of an established epileptogenic focus and not the cause of seizures. In addition, Mülsch et al. (1994) have recently suggested that NO is causally involved in kainic acid-induced seizures in mice because administration of 10 mg/kg (s.c.) of this excitotoxin enhanced brain NO between 30 min and 60 min after treatment and this preceded the appearance of typical behavioural signs of the limbic seizure syndrome. To reconcile these data with our previous (Bagetta et al., 1995) and present negative results one can assume the existence of different mechanisms underlying kainic acid-evoked seizures in mice and rats. A more attractive explanation, however, would be that involving a different time course for the expression of electrical and behavioural seizures in mice, the former possibly being more rapid in onset, as shown in rats (see Bagetta et al., 1995; Clifford et al., 1987). If this hypothesis is correct, then it is conceivable that the increase in brain NO reported by Mülsch et al. (1994) may be the consequence of an established seizure loop generated by synchronized neurones which fire paroxysmal depolarization shifts in the absence of apparent convulsive behaviour, leading to enhanced excitatory neurotrasmitter (e.g. glutamate and acetylcholine) release, neuronal Ca^{2+} entry and subsequent NO synthase activation.

Cholinomimetics in combination with LiCl and kainic acid are useful pharmacological tools to induce limbic seizures. Although they share some similarities, these animal models of epilepsy have important anatomical, neurophysiological and neuropathological differences (see Clifford et al., 1987). Our present data indicate that one major difference is that excessive NO is involved in the mechanism of onset of seizures caused by tacrine (and possibly by other cholinomimetic agents) in LiCl-pretreated rats but not in the mechanisms triggering kainic acid-evoked seizures. In addition, we would like to speculate that selective inhibition of neuronal NO synthase may be a novel therapeutic approach for the treatment of subsets of epilepsies in humans.

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