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Neuroscience Letters 276 (1999) 49–52

Neuroscience Letters

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Nitric oxide synthase inhibitor aminoguanidine potentiates iminodipropionitrile-induced neurotoxicity in rats

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Received 12 August 1999; received in revised form 9 September 1999; accepted 23 September 1999

Abstract

This investigation was undertaken to study the effect of nitric oxide synthase inhibitor, aminoguanidine on iminodipropionitrile (IDPN)-induced neurobehavioral and vestibular toxicity in rats. The dyskinetic syndrome was produced in male Wistar rats by i.p. injections of IDPN (100 mg/kg) for 6 days. Aminoguanidine was administered orally in the doses of 50, 150 and 300 mg/kg, 60 min before IDPN in three different groups. Control rats received vehicle only, whereas another group was treated with 300 mg/kg of aminoguanidine alone (without IDPN). Our results showed that aminoguanidine significantly and dose dependently exacerbated the incidence and intensity of IDPN-induced dyskinetic head movements. Aminoguanidine potentiated IDPN-induced loss of air righting reflex. The histopathological examination of inner ear showed aggravation of IDPN-induced degeneration of sensory hair cells in the crista ampullaris by aminoguanidine. These results suggest the role of nitric oxide in IDPN-induced neurobehavioral and vestibular toxicity.

Keywords: Iminodipropionitrile; Aminoguanidine; Nitric Oxide; Neurotoxicity; Vestibular toxicity

Exposure to β,β'-iminodipropionitrile (IDPN) results in permanent neurological syndrome in experimental animals which is characterized by spasmodic horizontal and vertical head movements, hyperactivity, circling, backward walking and swimming deficits [8]. IDPN-induced behavioral syndrome has been designated as ECC (excitation with choreiform and circling movements) syndrome [22] or waltzing syndrome [6]. The exact mechanism of IDPN-induced neurotoxicity is not clear. Recent studies have shown the involvement of vestibular hair cell degeneration in the development and persistence of IDPN-induced ECC syndrome [12–14]. Several investigators have suggested the role of neurotransmitters/neuromodulators in IDPN-induced neurotoxicity [5,18,26], whereas the role of oxygen derived free radicals (ODFR) has also been observed [15,24–26].

Nitric oxide (NO) is an important physiological signaling molecule which acts as a second messenger, neurotransmitter and neuromodulator in central nervous system, peripheral nervous system and cochlea [7,10,23,28]. NO is synthesized on demand by the conversion of L-arginine to L-citrulline by the enzyme nitric oxide synthase (NOS). NOS is localized in both, the CNS [3] and the inner ear [28]. Several investigators have used NOS inhibitors to study the role of NO in drug induced neurotoxicity [1,21,30]. The present investigation was undertaken to study the effect of NOS inhibitor aminoguanidine on IDPN-induced neurobehavioral and vestibular toxicity in rats.

Male Wistar rats (270–300 g) were divided into six groups of six animals each. The animals were housed in a temperature controlled room with 12:12 h light/dark cycles. Standard laboratory food and water were freely available ad libitum throughout the study. Neurobehavioral and vestibular toxicity in rats was produced by intraperitoneal injection of IDPN in the dose of 100 mg/kg for 6 days. The animals in control group received normal saline instead of IDPN. Aminoguanidine was administered orally in the doses of 50, 150 and 300 mg/kg body weight in three different groups of rats, respectively, 60 min before daily dose of IDPN during the first 6 days and was further continued on day 7 and day 8 at the same time. A separate group of rats received aminoguanidine (300 mg/kg) only for 8 days.

The animals were carefully observed for any behavioral abnormalities before the daily administration of the drugs. The complete behavioral studies were undertaken on days 7, 8 and 9 between 8:00 and 11:00 h according to the method described by Tariq et al. [24,25]. The rats were placed individually in a testing area (50 × 50 cm) covered with similar

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0304-3940/99/$ - see front matter
PII: S0304-3940(99)00789-2

sawdust bedding as used in home cages. The intensity of abnormal vertical and horizontal head movements was recorded for a period of 5 min using a digital cell calculator (Marbel Blood Calculator Company, USA). For the measurement of air righting reflex, the animal was held supine and dropped from a height of 30–40 cm onto a foam cushion. The presence of normal (landing squarely on the four paws) or abnormal righting reflex was recorded.

For inner ear histology the rats were anesthetized with diethyl ether and perfused (intracardiac) with 2.5% glutaraldehyde in 0.2 M phosphate buffer (pH 7.4). Temporal bones were removed and postfixed in 10% neutral buffered formalin for 15 h. The bony labyrinth was decalcified by placing it in a decalcifying agent, Cal-Ex (Fisher Scientific, USA) for 48 h. The specimens were then processed overnight for dehydration with increasing concentrations of alcohol and clearing with acetone and chloroform using automatic processor (Shandon Southern 2L Processor MkII, England). The specimens were embedded in paraffin blocks and sections of 5 μm thickness were stained with 1% toluidine blue for light microscopy observations.

The incidence of dyskinesia and air righting reflex were evaluated by χ^2 test. Dunnett's multiple comparison test was used to analyze animal body weights (BWt) and the intensity of dyskinetic head movements. A value of $P < 0.05$ was considered as statistically significant.

Administration of IDPN significantly reduced the animal BWt (Fig. 1) which is in agreement with earlier reports from our laboratory [26] and by other investigators [16,17]. Administration of aminoguanidine alone had no effect on animal BWt, it also failed to alter IDPN-induced reduction in BWt. The animals treated with IDPN produced characteristic behavioral syndrome with dyskinetic head movements (Table 1) and loss of air righting reflex (Table 2). Although the animals treated with aminoguanidine alone did not show any abnormal behavior, co-treatment of

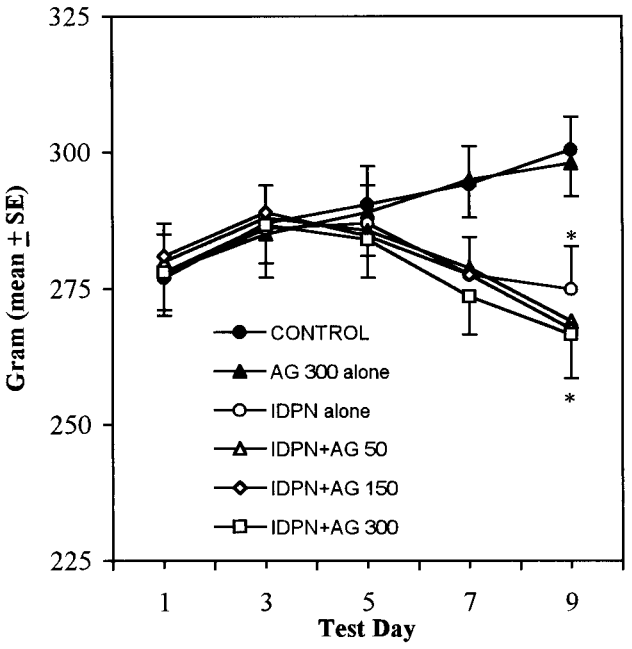

Fig. 1. Effect of different treatments on animal body weight. *$P < 0.05$ vs. control group using Dunnett's test.

Table 1
Effect of aminoguanidine on the intensity and incidence of IDPN-induced dyskinetic head movements[a]

Treatment	Animal number						Intensity (Mean ± SE)	Incidence (%)
	1	2	3	4	5	6		
Day 7								
Control	0	0	0	0	0	0	0.00 ± 0.00	0.00
AG alone	0	0	0	0	0	0	0.00 ± 0.00	0.00
IDPN alone	0	16	0	0	0	0	2.66 ± 2.66	16.66
IDPN + AG 50	0	19	0	0	0	28	7.83 ± 5.08	33.33
IDPN + AG 150	0	29	11	0	0	0	6.66 ± 4.81	33.33
IDPN + AG 300	38	13	0	0	20	2	15.66 ± 6.72	50.00
Day 8								
Control	0	0	0	0	0	0	0.00 ± 0.00	0.00
AG alone	0	0	0	0	0	0	0.00 ± 0.00	0.00
IDPN alone	0	40	0	0	0	0	6.66 ± 6.66	16.66
IDPN + AG 50	0	11	35	0	0	24	11.66 ± 6.06	50.00
IDPN + AG 150	0	19	48	25	25	0	19.50 ± 7.37	66.66*
IDPN + AG 300	28	38	29	0	31	27	25.50 ± 5.34*	83.33**
Day 9								
Control	0	0	0	0	0	0	0.00 ± 0.00	0.00
AG alone	0	0	0	0	0	0	0.00 ± 0.00	0.00
IDPN alone	0	30	0	28	23	0	13.50 ± 6.10	50.00
IDPN + AG 50	0	19	33	40	0	15	17.83 ± 6.47	66.66
IDPN + AG 150	39	29	42	41	43	0	32.33 ± 6.79*	83.33
IDPN + AG 300	34	40	33	28	32	30	32.83 ± 1.68*	100.00*

[a] *$P < 0.05$ and **$P < 0.01$ vs. IDPN alone group.

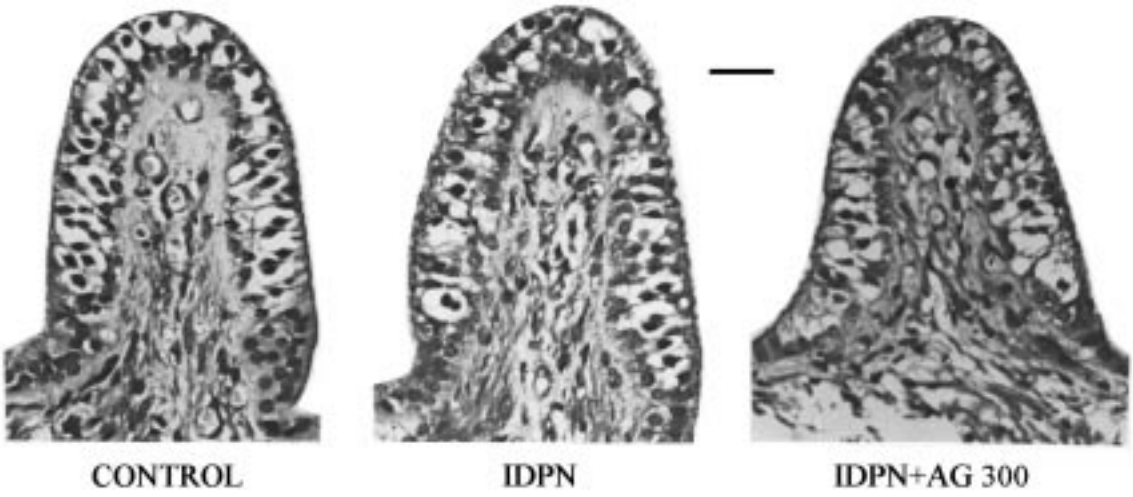

Fig. 2. Crista ampullaris of rat treated with saline (control), IDPN alone and IDPN plus aminoguanidine (300 mg/kg). Administration of aminoguanidine exacerbated the degeneration of hair cells in sensory epithelium of IDPN treated rats. Scale bar, 20 μm.

aminoguanidine with IDPN significantly and dose-dependently exacerbated the incidence and intensity of IDPN-induced abnormal head movements (Table 1).

Our histopathological studies showed a significant exacerbation of IDPN-induced degeneration of sensory hair cells by aminoguanidine (Fig. 2). The mechanism of IDPN-induced neurobehavioral syndrome is far from clear. Recent studies attributed IDPN-induced dyskinesia to degenerative changes in the crista ampullaris including cytoplasmic vacuolation, detachment of hair cell-nerve terminal contact and loss of synaptic densification [12–14]. The degeneration of vestibular hair cells following IDPN treatment sufficiently account not only for induction of the behavioral syndrome but also for its permanence [13]. A close similarity between behavioral deficits following bilabyrinthectomy or IDPN treatment further supports the involvement of vestibular system in IDPN toxicity [13]. Earlier studies from our laboratory suggest that IDPN-induced neurotoxicity is accompanied by ischemic changes in the brain and increasing the levels of endogenous vasodilator may significantly attenuate IDPN-induced neurotoxicity [24]. Recent studies have suggested that NO plays important roles in the regulation of vestibular microcirculation [2,4] as well as in vestibular compensation mechanism [11]. The donor of NO, sodium nitroprusside has been shown to improve blood flow and to reduce brain damage after focal ischemia [29]. However, inhibition of NOS has been shown to inhibit postural recovery following vestibular lesions [9]. Thus, inhibition of endogenous NO production by aminoguanidine may adversely affect compensatory mechanism to combat IDPN-induced dyskinesia.

The role of oxygen derived free radicals (ODFR) in IDPN-induced neurotoxicity has also been suggested by several investigators [15,24–26]. Nitric oxide has been shown to protect tissue against ODFR induced lipid peroxidation and cellular damage [20,27]. The cytoprotective property of NO has been attributed to its ability of blocking the hydroxyl radical formation and terminating the free radical chain reaction within the lipid membrane [27]. However, inhibition of NO synthesis by aminoguanidine has been shown to enhance the generation of hydrogen peroxide and to reduce the activity of catalase [19]. These findings suggest the involvement of ODFR in aminoguanidine-induced exacerbation of IDPN toxicity. However, further studies are warranted to determine the role of NO in nitrile induced neurobehavioral and vestibular toxicity in rats.

Table 2
Effect of aminoguanidine on IDPN-induced loss of air righting reflex[a]

Treatment	Day 7	Day 8	Day 9
Control	0/6	0/6	0/6
AG 300 alone	0/6	0/6	0/6
IDPN alone	1/6	1/6	3/6*
IDPN + AG 50	2/6	3/6	4/6
IDPN + AG 150	2/6	4/6	5/6
IDPN + AG 300	3/6	5/6##	6/6#

[a] Data shows the number of animals with loss of air righting/total number of animals in each group. *$P < 0.05$ vs. control group and #$P < 0.05$ and ##$P < 0.01$ vs. IDPN alone group (χ^2 test).

This work was financially supported by a grant from Research and Ethical Committee, Armed Forces Hospital, Riyadh, Saudi Arabia. The authors are thankful to Mrs. Anita Mabel and Mr. John Paul for technical assistance and Miss Tess Jaime for typing the manuscript.

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