

ROLE OF NITRIC OXIDE ON INSULIN INDUCED SEIZURES IN MICE

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Abstract : The role of nitric oxide (NO) on acute hypoglycemia-induced seizures in mice was investigated using insulin as the hypoglycemic agent. The NO precursor L-arginine in the doses of 150, 500 and 750 mg/kg exhibited a dose-dependent protective effect against seizures induced by 8 μ /kg insulin. The NO synthase inhibitor (L-NMMA) at the doses of 50 and 100 mg/kg potentiated the subconvulsive doses of insulin (2 μ /kg). The onset, duration, number of seizures and the mortality were noted in a 2 hr study period. The results of this study suggest than NO plays an important protective role in acute hypoglycemia induced seizures which are known to occur through the activation of NMDA receptors.

Key words : nitric oxide (NO)
insulin

hypoglycemia
L-NMMA

seizures
L-Arginine

INTRODUCTION

Nitric oxide (NO), initially identified as endothelium derived relaxing factor (EDRF), now has been recognised as a diffusible neurotransmitter and a second messenger (1-3). NO is also formed in the central nervous system (CNS) on activation of NMDA receptors (4) and existence of NO synthesis pathway has been demonstrated in various parts of CNS (5). No mediated responses to NMDA receptor activation can be inhibited by the arginine analogues L- N^G -monomethyl arginine (L-NMMA) or N-nitro-L-arginine methyl ester (L-NAME) antagonist NO mediated responses which can be reversed by an excess of L-arginine (6).

Contradictory reports on the role of NO on seizures have emerged recently (7). NO has been shown to be a proconvulsant in of seizures induced by cocaine, NMDA or tacrine plus lithium chloride which is effectively blocked by the competitive NO synthase inhibitor L-NMMA and augmented by NO donors L-arginine or sodium nitroprusside (8-10).

On the other hand investigators have also shown an anticonvulsant effect of NO. NO synthase inhibitors have been found to augment seizures induced by pilocarpine (11), icv NMDA (12) and kindled seizures both *in vivo* and *in vitro* (13, 14).

It has been shown that seizures induced by acute hypoglycemia are due to excessive

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release of excitatory amino acids with aspartate being released in larger quantities than glutamate (15), which is effectively prevented or attenuated by prior treatment with NMDA receptor antagonists (16, 17). However, the role of NO on hypoglycemia induced seizures is not clear. The present study was therefore undertaken to investigate the role of NO in insulin induced seizures using NO synthase inhibitor L-NMMA and the NO precursor L-arginine in mice.

METHODS

Animals: Inbred ICRC strain male mice weighing between 20–30 gm procured from the central animal house of the Institute were used. They were housed in colony cages in groups of 4–5 in the departmental animal house, at an ambient temperature with a 12 hr light/dark cycle. The animals had free access of standard pellet chow and tap water. All the experiments were performed between 0900 to 1200 hrs. The animals were acclimatised to the laboratory environment for at least a week before experimentation.

Drugs and Chemicals: Commercial plain insulin (BASF-Knoll Pharmaceuticals, India), L-arginine and L-NMMA (Sigma Chemical Co., U.S.A.) were freshly prepared in sterile normal saline. Insulin was injected s.c. while L-arginine and L-NMMA were administered i.p.

In a pilot study, using 8–10 mice in each group, insulin was given in graded doses to determine the dose producing seizures in 80–100% of the animals. This dose was chosen for subsequent experiments to induce

seizures. Effect of insulin on onset of seizure, duration of seizure, number of seizure episodes and mortality was studied.

The onset of seizure was noted as sudden, abnormal motor activity such as jumping, running, head twitching and/or clonic convulsion involving upper or lower limbs along with loss of righting reflex. The end point of seizure episode was the onset of post-ictal depression. Subsequent seizures follow after a time gap. The seizure-depression-seizure cycle continued until the death of the animal or recovery.

The duration of a seizure episode was noted using a stop-watch as the time gap between the onset of seizure activity to the onset of post-ictal depression. The number of seizures and outcome (mortality) were noted in a 2 hr observation period following insulin treatment.

L-arginine (150, 500 and 750 mg/kg, i.p) and L-NMMA (50 and 100 mg/kg, i.p..) were given 15 min prior to insulin administration subcutaneously. The animals were divided into the following groups.

- | | | |
|-----------|---|---------------------------------------|
| Group I | - | Insulin 8 U/kg |
| Group II | - | Insulin 8 U/kg + L-arg
150 mg/kg. |
| Group III | - | Insulin 8 U/kg + L-arg
500 mg/kg. |
| Group IV | - | Insulin 8 U/kg + L-arg
750 mg/kg. |
| Group V | - | Insulin 2 U/kg + L-NMMA
50 mg/kg. |
| Group VI | - | Insulin 2 U/kg + L-NMMA
100 mg/kg. |

Statistical analysis: The data was expressed as mean \pm sem. The significance of data was analysed by ANOVA with post hoc t test. P<0.05 was taken as significant.

RESULTS

The pattern of seizures induced by insulin in this study was characteristically the same in all the animals and included 1)

increased sniffing; 2) Repeated rearing of hind limbs; 3) Increased grooming and running; 4) Splayed hindlimbs (with loss of tone of hind limbs) with dragging movement of hindlimbs while walking; 5) Extension of tail; 6) Whole body clonus; sometimes preceded by forelimb clonus lasting for 6–15; 7) Loss of righting reflex; 8) Myoclonic jerks lasting upto 20 sec (occurrence of 5, 6, 7, 8 repeatedly).

TABLE I : Effect of L-arginine (i.p.) on insulin-induced seizure in mice.
(All values in mean \pm SEM)

Treatment	n	No. of animals showing seizure	Onset of seizure (min.)	Duration of seizure (sec.)	No. of seizure	Mortality%
Insulin 8 μ /kg	10	10	83.43 \pm 8.61	65.14 \pm 14.00	2.64 \pm 0.56	50
Insulin 8 μ /kg + L-Arginine 150 mg/kg	10	5*	55.04 \pm 5.53	51.04 \pm 15.84	5.02 \pm 1.04	40
Insulin 8 μ /kg + L-Arginine 500 mg/kg	10	3*	113.00 \pm 11.63*	22.07 \pm 2.87*	3	30
Insulin 8 μ /kg + L-Arginine 750 mg/kg	10	1**	141**	4***	1	0**

*P<0.05

**P<0.01 vs insulin alone treated group.

***P<0.001

TABLE II : Effect of L-NMMA (i.p.) on insulin-induced seizure in mice.
(All values in mean \pm SEM)

Treatment	n	No. of animals showing seizure	Onset of seizure (min.)	Duration of seizure (sec.)	No. of seizure	Mortality%
Insulin 2 μ /kg	10	0	0	0	0	0
Insulin 2 μ /kg + L-NMMA 50 mg/kg	10	10	64.5 \pm 7.49***	23.1 \pm 5.76**	0.6 \pm 0.05**	100***
Insulin 2 μ /kg + L-NMMA 100 mg/kg	10	10	38.4 \pm 5.33**	21.6 \pm 8.01**	2.3 \pm 1.64**	100***

**P<0.01

***P<0.001 vs insulin alone treated animals

In the pilot study insulin 2 and 4 μ /kg failed to produce seizures in mice, while 8 μ /kg produced seizure in 86% animals.

Pretreatment with NO precursor L-arginine 150, 500 and 750 mg/kg exhibited a dose dependent protection against insulin-induced seizures. The onset of seizures was significantly prolonged at doses of 500 and 750 mg/kg. The duration of seizures and also the number of seizures decreased progressively as the dose of L-arginine was increased. Also the mortality decreased with increasing doses of L-arginine (Table I).

On the contrary, pre-treatment with NO synthase inhibitor L-NMMA 50 and 100 mg/kg potentiated the effect of sub-convulsive dose of insulin (2 μ /kg) (Table II).

DISCUSSION

The results of this study show the involvement of NO in the initiation and propagation of seizure activity. Administration of L-arginine, the precursor

of NO had a protective role, whereas the depletion of NO by L-NMMA had a proconvulsant effect on insulin-induced seizures. NO is believed to be an endogenous anticonvulsant substance (12). NO thus may have a protective role in insulin hypoglycemia induced seizures. It has been shown that dentate gyrus is the key site for hypoglycemia induced convulsions which is dependent on the activation of NMDA receptor complex due to massive release of excitatory aminoacids aspartate and glutamate. It has been shown NO synthase inhibition attenuates the vasodilation induced by hypoglycemia and that NO synthase inhibition alters the course of neuronal injury during hypoglycemia (18), especially considering that hypoglycemic neuronal injury is mediated by NMDA induced excitotoxicity (19). This can be one mechanism whereby the convulsive behaviour induced by hypoglycemia can be modulated by NO.

In conclusion, the results support the involvement of NO in insulin induced hypoglycemia.

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