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EFFECT OF KETAMINE ON SEIZURE ACTIVITY AND ITS INTERACTIONS WITH ANTIEPILEPTIC DRUGS IN RATS

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ABSTRACT

Introduction: There is controversy regarding use of ketamine as general anesthetic agent in patients of epilepsy. Both pro and antiepileptic effect has been documented in clinical practice. It has been shown that ketamine minimizes seizure-induced brain damage. Its combination with antiepileptic drugs also prevents degeneration of thalamic neurons induced by focal cortical seizures. It was therefore decided to explore the effect of ketamine on seizure activity and its interactions with antiepileptic drugs in rats.

Methods: The effect was assessed by methods of supramaximal electroshock seizures and chemoshock (pentylenetetrazol) seizures.

Results: In present study, ketamine showed protection against electroshock seizures whereas, it enhanced chemoshock seizures. Combined treatment of ketamine with antiepileptic drugs exerted a much stronger protective effect against electroshock seizures than either drug alone. Protective effect was significant when ketamine was combined with sodium valproate ($p<0.038$) and highly significant when ketamine was combined with phenytoin and fosphenytoin ($p<0.001$ and 0.0003respectively). In chemo shock seizures ketamine showed protection in combination with benzodiazepines (100%), antagonism with phenytoin and fosphenytoin (0%) while no change was noticed in combination with sodium valproate and phenobarbitone (20 and 30%). Apart from antiepileptic effect, neuroprotective effect of ketamine is of great importance in the management of chronic conditions like epilepsy, thus it appears that ketamine in combination with phenytoin and fosphenytoin may have definite role in management of grand mal epilepsy. In future if a long acting orally active derivative of ketamine is developed that will be an additional antiepileptic drug in the armamentarium of clinicians.

Keywords: antiepileptic drugs, chemoshock, electroshock, ketamine.

INTRODUCTION

Ketamine is an injectable rapid acting general anesthetic agent. Oral ketamine in a dose of 6 mg/kg has been used as a preanaesthetic medication in children and it produces uniform, predictable sedation without significant side effects.(Gutstein, H.B.,et al.1992) There are conflicting evidences regarding use of ketamine as general anesthetic agent in patients of epilepsy. Both

pro and antiepileptic effect has been documented in clinical practice and animal studies. (Modica, P.A., et al. 1990 part I & II). It has been shown that ketamine minimizes seizure-induced brain damage and its combination with antiepileptic drugs also protects the brain damage associated with epilepsy. (Clifford, D.B., et al. 1990 Ketamine prevents learning impairment when administered immediately after status epilepticus. (Stewart, L.S.et al. 2001) Ketamine prevents degeneration of thalamic neurons induced by focal cortical seizures. (Clifford, D.B., et al. 1989) It was therefore decided to evaluate the effect of ketamine on induced seizure activity and its interactions with antiepileptic drugs and provide further information on neuropharmacological characterization of ketamine using rat as an animal model.

MATERIALS AND METHODS

The study protocol was approved by Institutional Animal Ethics Committee of the institute. Male albino rats weighing between 150-200 gm were procured from National Institute of Nutrition, Hyderabad. Rats were housed in colony cages with free access to food and water except 4 hours prior and during experiment and were maintained on natural light and dark cycle. The rats were randomly divided into groups of 10 each (n=10) and convulsive tests were carried out between 12.00 to 14.00 hrs. Rats were repeated for convulsive tests after a gap of 7 days.

Drugs used were injections of ketamine (Ketlar, Medicare Ltd.), diazepam (Calmpose, Ranbaxy Pharma), lorazepam (Lopez, Intas Pharmaceuticals), midazolam (Mezolam, Neon Labs.), sodium valproate (Encorate, Sun Pharmaceuticals), phenobarbitone sodium (Fenobarb, Samarth Pharma), phenytoin sodium (Epsolin, Zydus Neurosciences), fosphenytoin (Fosphen, Intas Pharmaceuticals) and powder pentylenetetrazol (Penta methyl tetrazol, Himedia Labs). Solutions of these drugs were prepared freshly in desired strength in water except for midazolam, diazepam and lorazepam in which 1%, 1.5% and 2% v/v of 95% alcohol was added respectively. Drugs were injected intraperitonealy (i.p.) in a volume of 0.2ml/100 gms of rats in following groups:

Group I: Distilled water (control group)

Group II: Ketamine alone

Group III: Antiepileptic drug alone

Group IV: Ketamine + Antiepileptic drugs

To leave scope to assess potentiation as well as antagonism, all drugs were given in subtherapeutic doses (showing antiepileptic response in10-30% rats), which was decided by trial and error method. Convulsive tests were carried out by following methods 30 minutes after drug administrations.

Supramaximal electroshock seizure (MES)

Electroshock Seizure test is extremely valuable because drugs that are effective against tonic extensor phase induced by electro shock generally have been proven to be effective against tonic-clonic seizures (grand mal epilepsy) in human. Rats were tested for tonic extensor phase (TEP) of electroshock seizure by convulsiometer (Techno) using a current strength of 150 mA for 0.2 sec through ear electrodes.(Swinyard,

E.A.,et al .1952) In prior screening rats not showing typical TEP were discarded. Abolition of TEP indicates protective (antiepileptic) effect of a drug.

Chemoshock seizure induced by pentylenetetrazol (PTZ)

Seizure induced by chemo convulsant pentylenetetrazole is most useful in identifying drugs that are effective against absence seizures (petit mal epilepsy) in human.

PTZ in a dose causing tonic-clonic convulsions in all animals without mortality (70 mg/kg) was injected i.p. and the animals were subsequently placed singly in cages and observed for tonic-clonic convulsions for a period of 30 minutes. Abolition of tonic clonic convulsions indicates protective (antiepileptic) effect of a drug. Early onset of convulsions with mortality suggests proconvulsant action of a test drug. Significant lowering of the threshold dose of PTZ (convulsions in all rats without mortality) as compared to control group confirms proconvulsant action of a test drug. Subtherapeutic dose of ketamine (4 mg/kg) that was decided for MES method was kept constant for PTZ method to evaluate effect of ketamine on seizure activity.

To further explore this proconvulsant action ketamine in fixed dose of 4 mg/kg was given with graded dose of pentylenetetrazol and average time of onset of convulsion and mortality was observed.

STATISTICAL ANALYSIS

Results were expressed as percentage of animals showing protective effect. Protective effect of Group II and III was compared with Group I (control). Protective effect of Group IV was compared with addition of Group II and III. All comparisons were done by proportion test. (Sunder Rao, P.S.S., et al .2006) Data was analyzed on STATA statistical software. The value of $p<0.05$ was considered as statistically significant and $p<0.01$ as highly significant.

RESULTS

Effect of ketamine and antiepileptic drugs alone and in combination against MES are shown in Table-1. Ketamine showed protection against electroshock seizures (20%). Combined treatment of ketamine and antiepileptic drugs exerted a much stronger protective effect against electroshock seizures than either drug alone. This was significant for a combination of ketamine and sodium valproate ($p<0.038$) and highly significant with a combination of ketamine and phenytoin/fosphenytoin ($p<0.001$ and 0.0003).

Seizure producing effect of combination of ketamine (fixed dose 40 mg/kg) with pentylenetetrazol (graded doses) seen in Table-2 shows that with graded dose of PTZ alone no convulsions were observed at a dose of 30, 40 and 50 mg/kg of PTZ. Convulsions were observed in 40% of animals at a dose of 60 mg/kg and convulsions in 100% animals at a dose of 70 mg/ kg without any mortality. Ketamine in a fixed sub therapeutic dose of 4 mg/kg in combination with graded dose of PTZ showed 70% convulsions at 30 mg/kg and 100% convulsions were observed at 40, 50, 60 and 70 mg/kg dose of PTZ respectively with decreasing order of average time of onset of convulsions. This showed that ketamine has proconvulsant action. Effect of ketamine and antiepileptic drugs alone and in combination

against PTZ are shown in Table-3. Ketamine showed proconvulsant action against PTZ induced convulsions (0%) while a combined treatment of ketamine with benzodiazepines showed potentiation (80-100%), a combined treatment of ketamine with phenytoin/fosphenytoin showed antagonism (0%) and no significant change was seen when ketamine was combined with sodium valproate or phenobarbitone (20%).

DISCUSSION

Ketamine protected rats against the tonic extensor phase of electroshock seizure suggesting its anticonvulsant action. The possible explanation for anticonvulsant effect of ketamine could be non-competitive NMDA receptor antagonism. (Khanna, N., et al.1999).The anticonvulsant action may also be attributed to GABA mediated inhibition. (Manocha, A., et al. 2001) Dopaminergic receptors and serotonergic receptors are also involved in antiseizure activity (Velisek, L., et al. 1989). The inhibition of glutamate decarboxylase that antagonize excitatory phenomenon in CNS is another possible mechanism. (Taberner, P.V. 1976)

Combined treatment of ketamine with benzodiazepines (diazepam/ lorazepam/ midazolam), sodium valproate, phenobarbitone and phenytoin/fosphenytoin showed a much stronger protective effect against electroshock seizures than either drug alone. This was significant when ketamine was combined with sodium valproate and highly significant when ketamine was combined with phenytoin/fosphenytoin.
Ketamine-midazolam combination is likely to be used as premedication to control convulsions induced by electroconvulsive therapy (ECT). Also combination of ketamine with other antiepileptic is likely to be used for status epilepticus. Both drugs have same pharmacokinetic property viz. injectable route of administration, rapid onset of action and short duration of action. Intervention of ketamine in epilepsy is likely to involve its antiepileptic action, its synergism with antiepileptics, and reduction of dose of antiepileptics that in turn would reduce side effects of individual antiepileptic drug. Other benefit of ketamine will be its neuroprotective effect which limits the size of ischemic area in brain due to seizures.

Our results are in agreement with a number of previous studies. (Khanna, N., et al.1999, Manocha, A., et al. 2001, Borowicz, K.K., et al, 2003, Borowicz, K.K., et al 2004) Anticonvulsant action of ketamine may in some way be related to GABA mediated inhibition. Antiepileptic drugs potentiate GABA mediated responses i.e. both ketamine and antiepileptic drugs share the same mechanism. This may be taken as a possible explanation for potentiation of anticonvulsant action of antiepileptic drugs by ketamine.

In contrast to electroshock method, the result of the present study in chemoshock method demonstrated that ketamine alone did not afford protection against PTZ induced seizures. On the contrary, ketamine exaggerated PTZ induced seizures with early onset of seizure and significant mortality (40%). This suggested proconvulsant action of ketamine. In rats pretreated with ketamine threshold dose of PTZ which produced convulsions in all rats without mortality was low (40 mg/kg) as compared to the

control group of rats pretreated with distilled water, in whom PTZ in a dose of 40 mg/kg did not produce any convulsions.

Possible explanations for proconvulsant action of ketamine are persistent decrease in GABA mediated inhibition, interference of excitation of GABAnergic interneurons and change in effectiveness of GABA to produce inhibitory postsynaptic potential. This could be due to desensitization of GABA receptor and/or change in affinity of GABA receptor complex.(Kapur, J., et al. 1990) Ketamine has proconvulsant action against strychnine induced convulsions in rats (Kubova, H., et al. 1994) while anticonvulsant action of ketamine has been observed against chemically induced convulsions in a different animal (chick) model.(Reder ,B.S.,et al.1980)

In this study benzodiazepines, sodium valproate, phenobarbitone, phenytoin and fosphenytoin individually offered protection against PTZ induced seizures (30%) whereas some studies have demonstrated that phenytoin offered no protection against PTZ induced seizures. (Bertrum, E.H.et al.1990, Gupta, Y.K., et al .1999,)

In the present study combination of ketamine with benzodiazepines showed highly significant protection ($p<0.003$) against PTZ induced seizures. The enhancement of anti-PTZ effect of benzodiazepines with ketamine could also be related to ketamine induced increase in total number of benzodiazepine binding sites. (McNamara, J. O. 2006). This combination is likely to be indicated in petit mal status epilepticus.

There was no significant change in anti-PTZ effect of phenobarbitone and sodium valproate when ketamine was combined with them. Anti-PTZ effects of phenytoin and fosphenytoin were antagonized significantly ($p<0.04$) when ketamine was combined with them. Our results of ketamine with other antiepileptic drugs in chemoshock model are in agreement with a number of previous studies. (Taberner, P.V. 1976, Reder ,B.S, et al.1980, Koek, W. 1989, Velisek, L., et al. 1989)

CONCLUSION

In electroshock method ketamine proved to be an antiepileptic acting synergistically with sodium valproate, phenytoin and fosphenytoin. In chemoshock method ketamine actsed as proconvulsant and variably modulated the actions of other antiepileptic drugs. It acted synergistically with benzodiazepines and antagonized the anticonvulsant action of phenytoin /fosphytoin in chemoshock method.

Limitations and suggestions

Result of an animal study cannot be fully extrapolated to human epilepsy. It has to be concluded by a clinical study. Short duration of action and parenteral route of administration of ketamine are main limiting factors for clinical use of ketamine as antiepileptic drug. Ketamine is naturally available as powder and oral bioavailability of ketamine is 16.5%.(Grant, I.S. 1981) If hurdles of short duration of action and parenteral route of administration can be overcome by designing a sustain release formulation or a long acting ester of ketamine for oral administration In future if such a

long acting orally active derivative of ketamine is developed, it might become a milestone in drug therapy of epilepsy since neuroprotective effect of ketamine would be of great importance in the management of chronic condition like epilepsy.

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Table 1: effect of ketamine and antiepileptic drugs (AED) alone and in combination against maximal electro shock (MES) in rats.

			Percentage of animals showing abolition of TEP (n=10)				p Value		
No	Drugs	Dose (mg/kg)	Group I Control	Group II Ketamine	Group III AED	Group IV Ketamine + AED	Group I Versus Group II	Group I Versus Group III	Group IV Versus Group II + III
1	Ketamine	4	0	20	-	-	0.136	-	-
2	Diazepam	2	0	20	30	70	0.136	0.0603	0.2974
3	Lorazepam	2	0	20	20	70	0.136	0.136	0.1213
4	Midazolam	2	0	20	30	70	0.136	0.0603	0.2974
5	Sod.valproate	75	0	20	20	80	0.136	0.136	0.0384*****
6	Phenobarbitone	8	0	20	30	80	0.136	0.0603	0.1138
7	Phenytoin	5	0	20	10	90	0.136	0.3049	0.0019******
8	Fosphenytoin	7.5	0	20	10	100	0.136	0.3049	0.0003******

***** p Value < 0.05 is significant , ******p Value< 0.01 is highly significant.

Table 2: seizure producing effect of combination of ketamine (fixed dose) with pentylenetetrazol (graded doses)

Sr. No.	Ketamine (mg/kg)	PTZ (mg/kg)	Animals convulsing (%)	Average time of onset of convulsion (min)	Mortality
1	--	30	0	--	0
2	--	40	0	--	0
3	--	50	0	--	0
4	--	60	40	12.5	0
5	--	70	100	10.0	0
6	4	30	70	14.5	0
7	4	40	100	9.0	0
8	4	50	100	9.5	1
9	4	60	100	4.1	2
10	4	70	100	2.1	4

Table 3: effect of ketamine and antiepileptic drugs (AED) alone and in combination against pentylenetetrazol (PTZ)

			percentage of animals showing abolition of tonic clonic Phase (n=10)				p Value		
No	Drugs	Dose (mg/kg)	Group I Control	Group II Ketamine	Group III AED	Group IV Ketamine + AED	Group II Versus Group I	Group III Versus Group I	Group IV Versus Group II + III
1	Ketamine	4	0	00[#]	-	-	Ψ	-	-
2	Diazepam	0.5	0	0	30	80	Ψ	0.0603	0.0097**
3	Lorazepam	0.5	0	0	30	100	Ψ	0.0603	0.003**
4	Midazolam	0.5	0	0	30	100	Ψ	0.0603	0.003**
5	Sod.valproate	50	0	0	20	20	Ψ	0.136	1
6	Phenobarbitone	5	0	0	30	30	Ψ	0.0603	1
7	Phenytoin	6	0	0	30	0	Ψ	0.0603	0.0493*
8	Fosphenytoin	9	0	0	30	0	Ψ	0.0603	0.0493*

***** p Value < 0.05 is significant , ******p Value < 0.01 is highly significant.

[#] Early onset of convulsions with 40% mortality suggesting proconvulsant action. Threshold dose of PTZ was lowered to 40 mg/kg.

Ψ p value of zero response versus zero response which cannot be calculated