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Effect of Cromakalim, a Potassium Channel Opener, on Experimentally Induced Convulsions in Albino Rats.

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Abstract
This study aims to study the effect of cromakalim on experimentally induced convulsion in albino rats using maximal electro-shock (MES) & pentylenetetrazol (PTZ) induced seizure models. For this study 100 albino rats of either sex weighing 100 to 150 grams were divided into 10 equal groups, 5 groups were used for each model. Rats of group 1 in both models received placebo (0.5ml of distilled water) intraperitoneally (ip), while groups 2,3 and 4 of both the models were administered three doses of cromakalim, i.e. 0.25, 0.5 and 1 mg/kg ip respectively. Rats of group 5 received phenytoin 40 mg/kg ip in the MES model and ethosuximide 100mg/kg ip in PTZ model, which served as positive controls respectively. In MES model, 150mAmp current was administered by electro-convulsiometer through ear electrodes to rats and presence of hind limb extension was recorded as the end-point, at different time intervals. In the second model, PTZ was administered at the dose of 60mg/kg ip, 60mins after cromakalim/ control drug pre-treatment. Onset and duration of clonic convulsion was recorded. In MES induced seizure model, cromakalim at the doses of 0.5 and 1 mg produced significant protective effect which was comparable to phenytoin 40mg/kg. In PTZ induced seizure model, cromakalim pretreatment in the dose of 0.25mg increased onset time, decreased duration of convulsion in 70% of animals, while the doses of 0.5 and 1 mg/kg had significant anticonvulsant activity comparable to ethosuximide 100mg/kg. Cromakalim possesses significant anticonvulsant property both on electroshock and chemoshock induced seizure models.

Key Words
MES, PTZ, cromakalim, phenytoin, ethosuximide, electroconvulsiometer.

Introduction
Epilepsy is a chronic convulsive disorder characterised by periodic, unpredictable and paroxysmal occurrence of seizure and has a poor prognosis. The available antiepileptic drugs control the fits in only two-third of patients after years of continued therapy, which is usually associated with diverse complications and manifestations of toxicity[1]. The rest may remain uncontrolled or partially controlled and have to bear the ordeal of protracted therapy with multiple antiepileptic drugs, at the cost of suffering from iatrogenic complications. Hence, the search for the ideal antiepileptic drugs continues[2]. Almost all antiepileptic drugs act by preventing neuronal discharge which produce seizure. Though the neurophysiological and biochemical mechanism at cellular or sub-cellular level affected by the different drugs may be diverse, their ultimate effect is to stabilise the neuronal membranes against the onslaught of abnormal epileptic discharge and suppress neuronal hyperexcitability[3].The K^+ channel openers like nicorandil, cromakalim, pinacidil, diazoxide and minoxidil are a group of drugs which produce stabilizing action on cell membranes through membrane hyperpolarisation via increased transmembrane K^+ conductance[4]. They have been found to exert various neuro and psychopharmacological effects and modulate neurotransmitter release. VandenBussche and associates (1985)[5] first suggested that they might inhibit seizure spread in epilepsy by blockade of neuronal Ca^{++} channels. Later other workers (Gandolgo G et al1985[6] and Biddar JN et al, 1989[7]) showed that the K^+ channel openers prevent or ameliorate seizure induced by mast cell degranulating peptide (MCDP). However, they have no effect in seizure induced by other K^+ channel blockers. They also attenuated seizures in genetically

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epileptic rats[8]. These findings suggest that K^+ channel openers may have a potential role in prophylaxis of epilepsy since MCDP has endogenous equivalent in human brain[9]. They hyperpolarise the cells by excess efflux of K^+ which causes decrease of influx of Ca^{++} leading to more intracellular negativity and decreased neurotransmitter release[10]. With this background it was considered worthwhile to undertake this study to evaluate the effect of Cromakalim, a K^+ channel opener, on seizure threshold, in standard experimental models of seizures in rats.

Materials and Methods

Experimental models

Two animal models of seizures were used in this study to evaluate the effect of cromakalim on seizure threshold, the maximum electroshock seizure (MES) model (Goodman, Toman, Swinyard, 1949 and modified by Everett et al, 1965[11]) and the pentylenetetrazol (PTZ) induced convulsions model (Swinyard et al, 1952[12]), in rats[13]. The former depicted the animal equivalent model for generalized tonic-clonic seizure of humans, for which phenytoin was used as the standard drug, while the latter represented a model for petit-mal epilepsy (absence seizure), for which ethosuximide was used as the standard drug.

Experimental animals

Healthy albino rats weighing 150 to 250 gms, of either sex, housed in the Departmental animal house were used for this study. The animals were preliminarily screened for the occurrence of convulsions on induction, and those which responded positively to the respective stimuli, were included into the study. Fifty such animals were selected and divided into 5 equal groups for MES test, and another fifty rats were selected for PTZ test. They were housed in separate polypropylene cages (10 in each group) and maintained at a temperature of 28±1ºC and relative humidity 45-55%, in a 12:12 light: dark cycle. They were fed with standard diet and given water ad libitum, food was withdrawn 6hrs before and during experimentation, which was performed during 1200-1400hr. The study protocol was approved by the Institutional animal ethics committee.

Drugs and Chemicals

Pentylenetetrazol (Hi-Media Laboratory), cromakalim (Smith-kline Beechem Laboratories), phenytoin (Cadila Pharmaceuticals Ltd., Ahmedabad), and ethosuximide (Parke-Davis Ltd.), were used for the study.

Experimental procedure

MES Test: The rats were exposed to an electroshock of 150 mA for 0.2 secs. through ear electrodes from an Electro-convulsiometer (M/S Techno Electronics, Lucknow), after administration of test and standard drugs to the different groups of rats as follows. At 0 hr, rats of group 1 served as placebo control and were administered normal saline 0.5ml ip, groups 2, 3, and 4 were administered cromakalim 0.25, 0.5 and 1 mg/kg ip respectively, while group 5 served as positive control and was administered phenytoin 40 mg/kg ip. MES was elicited by exposing all the rats to electroshock after 30, 60, 90 and 120 mins of drug administration, tested at intervals of 1 week for each observation time. The presence or absence of hind limb extension was taken as the endpoint in all animals. All the animals were observed for development of any complications or mortality over a period of 10 days.

PTZ Test: Pentylenetetrazol in a dose of 60mg/kg was injected intraperitoneally, 60 mins after administration of the control and test drugs in the same manner as in MES test. The onset and duration of clonic convulsions was noted for 30 mins. The animals were observed for 10 days for any complications or mortality.

Statistical Analysis: The Chi-Square test and Student's t test was used for analysis of results.

Result

Cromakalim in the dose of 0.25 mg/kg had no effect on MES induced seizure at all the observation periods, similar to that observed with normal saline (placebo) treated group. Cromakalin in doses of 0.5 and 1.0 mg /kg, prevented tonic hind limb extension of rats in the MES test, at all times of observation i.e. 30, 60, 90 and 120 mins of drug administration (Table – 1, Fig 1). These effects were similar to that observed with phenytoin 40mg/kg, as there was no statistical difference between the protective action of the two higher doses of cromakalim and phenytoin ($P < 0.05$). Maximum protection (100%) was observed at 60mins, with both the drugs. In the PTZ administered rats, convulsion occurred in 100% animals, in the normal saline treated group. In the rats pretreated with cromakalim 0.25 mg/kg, convulsion occurred in only 30% of animals and the onset of convulsion was delayed appearing after 7 mins (of PTZ inj.) in comparison to after 3.16 mins

in the normal saline treated group. There was no significant difference in the duration of clonic convulsion. There occurred complete abolition of PTZ induced seizure in the rats treated with cromakalim 0.5 and 1.0 mg/kg (Table 2, Fig 2). Ethosuximide (100 mg/kg) prevented seizure induced by the chemo-convulsant pentylenetetrazol in 100% of animals. The effects seen with the two higher doses of cromakalim was statistically similar to that observed by ethosuximide. No mortality was however observed in any of the groups.

Discussion

The potassium channel openers induce hyperpolarization through potassium efflux, and by stabilizing the cell membrane might raise the seizure threshold. There have been interesting documented evidences in literatures revealing that K^+ channel openers especially cromakalim and RP49356 increased the threshold of seizure and when administered prior to convulsant substance like Mast cell degranulating peptide (MCDP) prevented epileptiform seizures[6]. Hence this study was undertaken to observe the effect of cromakalim on experimentally induced seizures in animals and compare it with known anticonvulsants like phenytoin and ethosuximide. It was observed from this study that cromakalim in the dose range of 0.5 to 1.0 mg/kg. possessed significant anti-convulsant activity both in maximal electro-shock and pentylenetetrazol induced seizure models, comparable to phenytoin 40mg/kg and ethosuximide 100mg/kg, respectively. Our findings are in corroboration with the findings of Ghasemi et al[14] who observed that cromakalim (0.1-30microg/kg, i.p.) significantly increased the seizure threshold in control mice, and also in 8week diabetic mice cromakalim (10microg/kg, i.p.) significantly increased the seizure threshold which was inhibited by pre-treatment with glibenclamide (1mg/kg, i.p.). Also reports of Shafaroodi et al[15] support that cromakalim through opening of K+ channels reverse the pro-convulsant effects of morphine. There has been several studies to explain the probable mechanism underlying the anti-convulsant action of cromakalim. Changes in membrane potential usually affect a variety of voltage dependent channels and the recorded current across the membrane is a summation of inward Na^+, Ca^{++} and outward K^+ current. If inward current predominates a membrane depolarisation result, whereas if outward current predominates, the membranes hyperpolarise (Prince DA, 1985) [16,17]. A large number of different voltage-sensitive K^+ channels can influence the expression of epileptiform activity and their activation may prevent the triggering of spontaneous epileptiform activity by maintaining membrane potential and limiting the action potential duration and neurotransmitter release, and also may contribute to the termination of ongoing epileptiform activity[18]. The importance of these channels in regulating a normal level of activity is demonstrated by the number of convulsant that block these channels[19].

Thus, the potassium channel blockers may alter the balance between the ingoing Na^+ and Ca^{++} and outgoing K^+ conductance in favour of the latter and so hyperpolarise the neurones and suppress the bursts of spike generation and reduce the chance of after-depolarization (by hyperpolarising the resting membrane potentials, Na^+ and Ca^{++} conductances reduces)[19,20]. They reduce the dependence on activation of Ca^{++} activated K^+ channel or activation of ATP-sensitive K^+ channels for terminating the spike generation. So hyperpolarisation occurs earlier and thus aborts the chance of generation of after-depolarisation (which continue to generate the spikes).

Conclusion

Hence, cromakalim possesses significant anticonvulsant activity against tonic-clonic and absence seizures models in animals. The anti-seizure activity is comparable to phenytoin in MES model and ethosuximide in the PTZ model. Further controlled studies are necessary using other models of convulsion for establishing the anti-epileptic property of cromakalim and confirming its mechanism of action.

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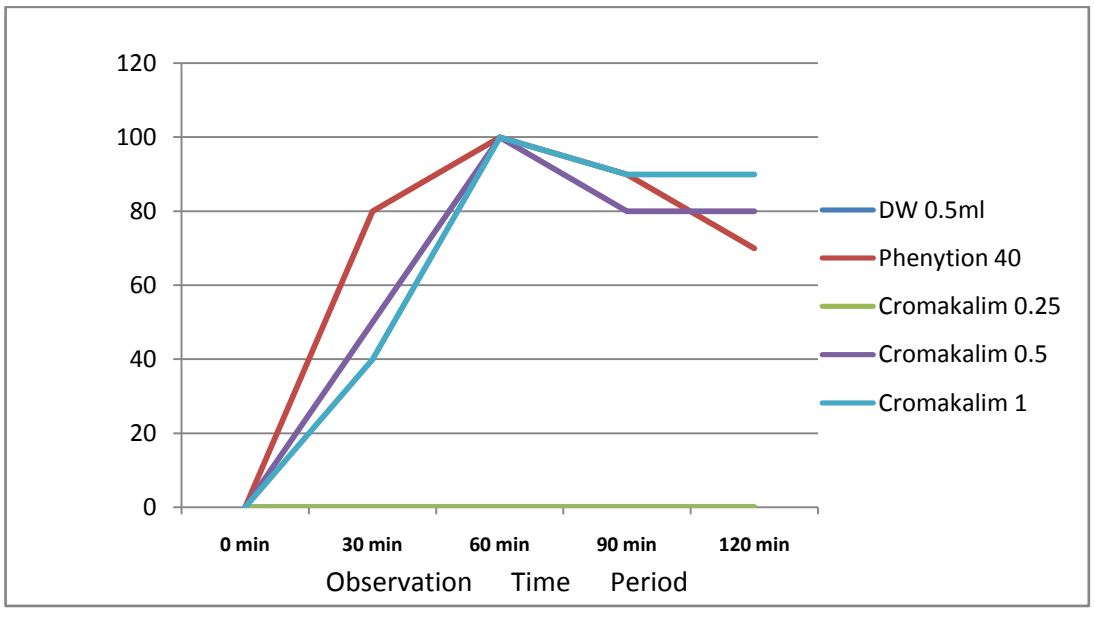

Fig 1: Effect of Drugs in MES test showing Percentage protection in rats at different time intervals.

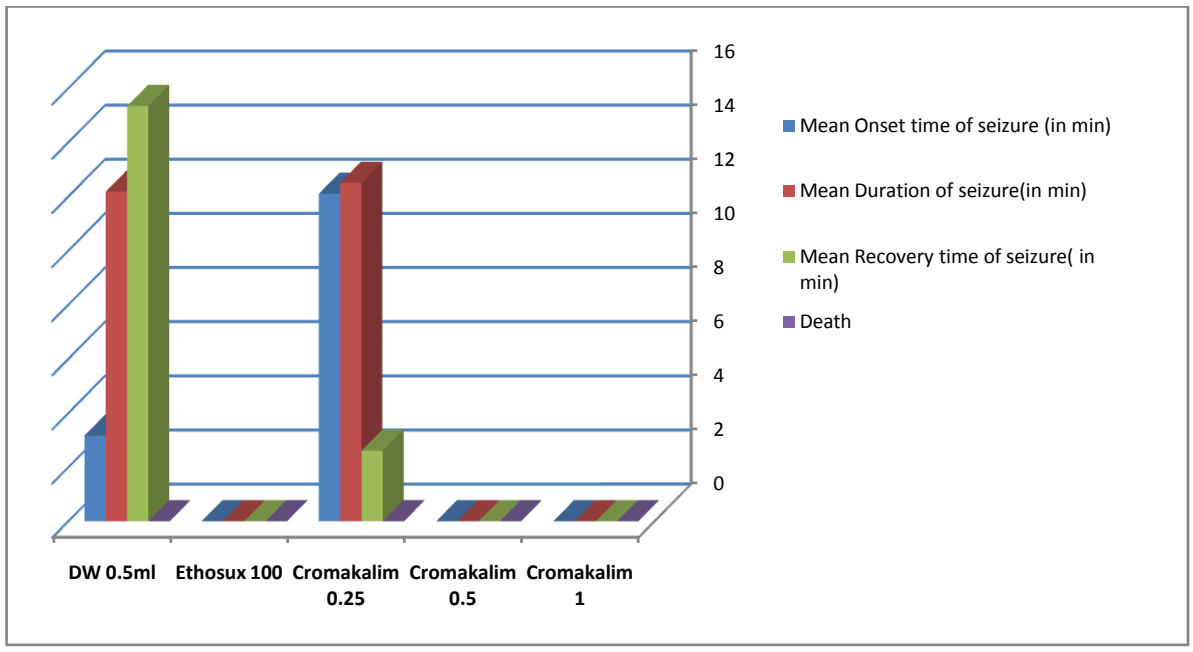

Fig 2: Effect of Drugs on PTZ induced seizure.

Table 1: Effect of Cromakalim on Maximal electroshock seizure (Chi ^{2} & "P" values).

Gr Drug	0 Min	30 Min	60 Min	90 Min	120 Min
Gr 1-Normal saline 0.5ml					
No. Protected	0	0	0	0	0
No. Unprotected	10	10	10	10	10
Comparison with Phenytoin(Chi^{2}value)	0	10.21	20.00	12.93	7.91
"P" value	> 0.05	< 0.01	< 0.001	< 0.001	< 0.01
Gr 2-Cromakalim - 0.25 mg/kg					
No. Protected	0	0	0	0	0
No. Unprotected	10	10	10	10	10
Comparison with N.saline (Chi^{2}value)	0	0	0	0	0
"P" value	> 0.05	> 0.05	>0.05	> 0.05	> 0.05
Comparison with Phenytoin(Chi^{2}value)	0	10.21	20.00	2.93	7.91
"P" value	> 0.05	< 0.01	< 0.001	< 0.001	< 0.01
Gr 3-Cromakalim - 0.5 mg/kg					
No. Protected	0	5	10	8	8
No. Unprotected	10	5	0	2	2
Comparison with N.saline(Chi^{2}value)	0	4.27	20.00	10.21	10.21
"P" value	> 0.05	< 0.05	< 0.001	< 0.01	< 0.01
Comparison with Phenytoin (Chi^{2}value)	0	0.88	0	0	0
"P" value	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
Gr 4-Cromakalim - 1.0 mg/kg (Gr 4)					
No. Protected	0	5	10	9	9
No. Unprotected	10	5	0	1	1
Comparison with N.saline(Chi^{2}value)	0	4.27	20.00	12.93	12.93
"P" value	> 0.05	< 0.05	< 0.001	< 0.001	< 0.001
Comparison with Phenytoin (Chi^{2}value)	0	0.88	0	0.56	0.31
"P" value	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
Gr 5-Phenytoin - 40 mg/kg					
No. Protected	0	8	10	9	7
No. Unprotected	10	2	0	1	3
Comparison with N.saline(Chi^2 value)	0	4.27	20.00	10.21	10.21
"P" value	> 0.05	< 0.05	< 0.001	< 0.01	< 0.01

Table 2: Effect of Drugs an PTZ induced seizure.

Group	Drug	Dose mg/kg	Occurance of Seizure in mins (Mean +SEM)		No of Deaths
			Onset	Duration	
1	N.saline	0.5 ml	3.16 ± 0.13	12.18 ± 0.63	Nil*
2	Cromakalim	0.25 ml	7.00 ± 1.08*	12.50 ± 1.28	Nil*
3	Cromakalim	0.5 ml	Nil*	Nil*	Nil*
4	Cromakalim	1 ml	Nil*	Nil*	Nil*
5	Ethosux	100 ml	Nil*	Nil*	Nil*

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