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Antimetrazol Action of Two Potential Anticonvulsants, CM 40907 and SR 41378, in Immature Rats

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Summary

The action of two potential anticonvulsants, CM 40907 (10-50 mg/kg i.p.) and SR 41378 (1.25-20 mg/kg i.p.) against metrazol-induced seizures was studied in rats 7, 12, 18 and 25 days old. Two types of motor seizures - minimal, clonic and major, generalized tonic-clonic - were elicited by a 100-mg/kg dose of metrazol (s.c.) and their incidence and latency were evaluated. The severity of seizures was expressed as a score on a 5-point scale. Dimethylsulfoxide, an organic solvent, exhibited anticonvulsant action only in doses far exceeding those used for dissolving the two anticonvulsants. Both drugs suppressed minimal as well as major seizures in all age groups studied in a dose-dependent manner, SR 41378 being approximately four times more potent than CM 40907. The latencies could be measured only in animals given low doses of anticonvulsants. CM 40907 did not change the latencies whereas SR 41378 prolonged them. The severity of seizures was decreased again in a dose-dependent manner. There were only minor changes in the efficacy of CM 40907 among the four age groups. On the contrary, SR 41378 exhibited an extreme efficacy in 7-day-old rat pups, where even the 1.25 mg/kg dose signifcantly decreased the incidence and severity of seizures. The efficacy in the remaining three age groups was approximately at the same level as in adult rats.

Key words

Anticonvulsants – Motor seizures – Pentylenetetrazol – Ontogenetic development – Rat

Introduction

Preclinical testing of new antiepileptic drugs is usually performed only in adult animals (Meldrum and Porter 1986), in spite of the fact that the incidence of epilepsies in infants and children is substantially higher than in adults (O'Donohoe 1981) and that there are developmental changes in the pharmacokinetics as well as the pharmacodynamics of antiepileptic drugs (for review see Morselli *et al.* 1983, Mareš 1991). We are therefore studying the action of antiepileptic drugs against metrazol-induced seizures in immature rats.

Metrazol-induced seizures have a great advantage when testing anticonvulsant drugs: metrazol (pentamethylenetetrazol, PTZ) can elicit two different seizure patterns. Lower doses induce minimal metrazol seizures, i.e. predominantly clonic seizures involving muscles of the forelimbs and the head, whereas higher doses elicit a sequence of minimal and major, i.e. generalized tonic-clonic seizures (Swinyard and Woodhead 1982, Swinyard *et al.* 1989). Minimal seizures were taken as a model of human absences, but their adequacy has been questioned (Löscher and Schmidt 1988, Mareš and Zouhar 1988).

We have at our disposal data for classical antiepileptic drugs – clonazepam, ethosuximide and valproate (Mareš *et al.* 1981, Mareš and Velíšek 1983), carbamazepine and phenytoin (Mareš *et al.* 1983), phenobarbital and primidone (Kubová and Mareš 1991) and we can thus compare new potential antiepileptic drugs in this context. Due to the courtessy of Sanofi Recherche Ltd, two potential anticonvulsant drugs – CM 40907 and SR 41378 – were available and we used metrazol-induced motor seizures as the first test.

Material and Methods

Experiments were performed on 546 male albino Wistar rats from a specific pathogen-free colony. Four age groups were studied – animals 7, 12, 18 and 25 days old. The day of birth was counted as 0. The experiments were performed at room temperature (19-23 °C). The body temperature of the rat pups was maintained by means of a pad heated electrically to 35 °C.

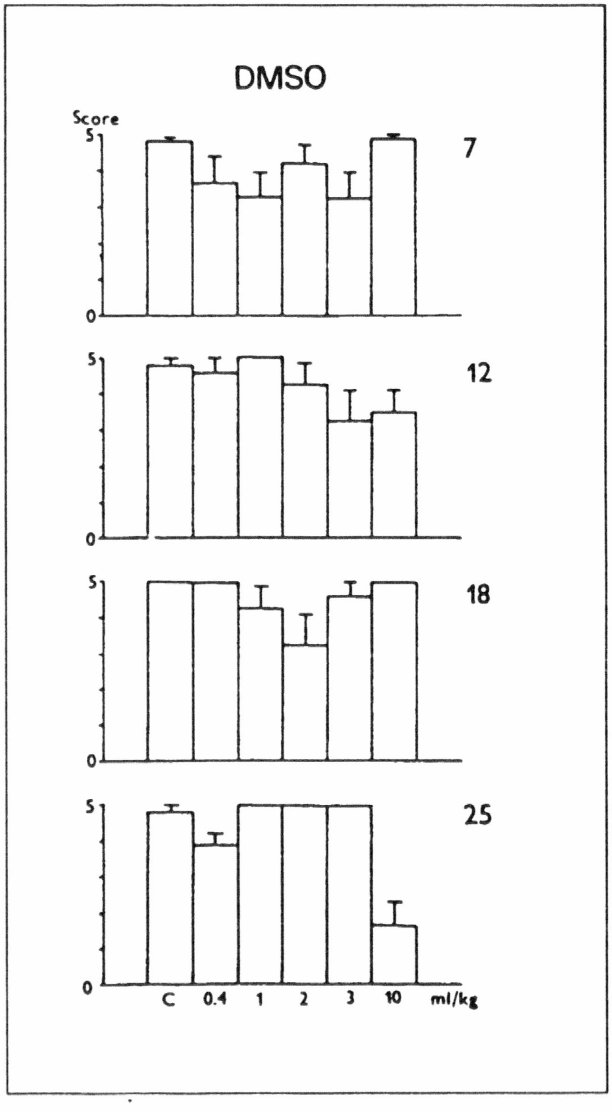

Fig. 1
Influence of dimethylsulfoxide on the severity of metrazol-induced seizures. From top to bottom:rats 7, 12, 18 and 25 days old. In each graph: abscissa – doses of the drug, c – control animals receiving only metrazol; ordinate – score (see Methods). Results significantly different from controls are marked by an asterisk.

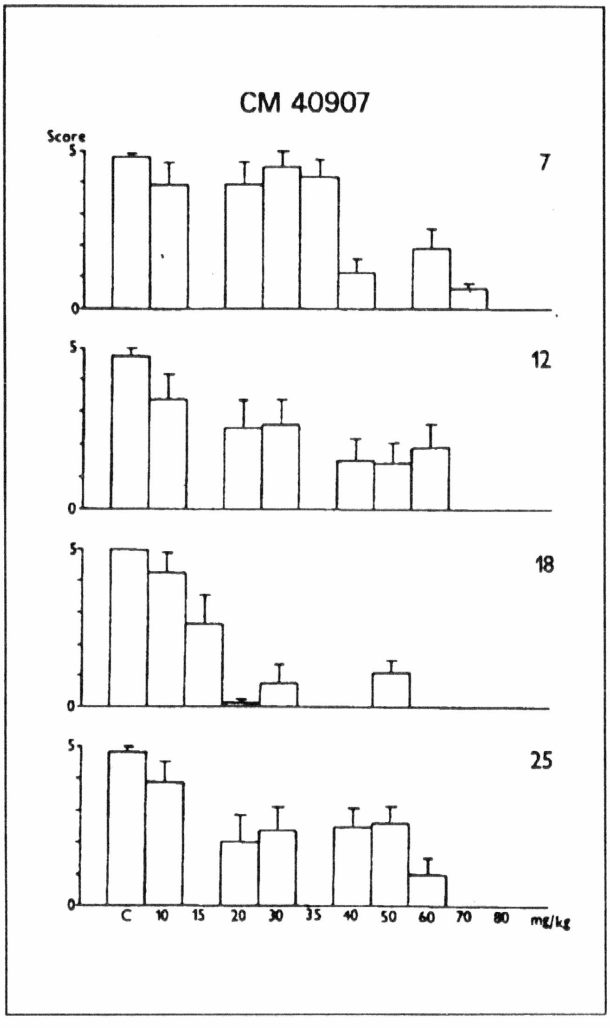

Fig. 2
Influence of CM 40907 on the severity of metrazol-induced seizures. Details as in Fig. 1, omitted columns – the doses were not tested.

Both potential anticonvulsant drugs – products of Sanofi Recherche – CM 40907 (6-(2'chlorophenyl) 3-(4-hydroxypiperidino)pyridazine) and SR 41378 (6-(2',4'-dichlorophenyl)3-(4-hydroxypiperidino) pyridazine) were always freshly dissolved in dimethylsulfoxide (DMSO) in concentrations of either 50 or 25 mg/ml. The drugs were injected intraperitoneally in doses from 10 to 60 mg/kg (CM 40907, a total of 168 rats) and/or from 1.25 to 20 mg/kg (SR 41378, a total of 152 animals) 30 min before PTZ. We always started with doses used in previous papers in adult rodents (Chambon *et al.* 1985, 1986) and the range of the doses used was exteneded according to the results in a given age group. Thus different doses were used in various age groups. In pilot experiments, higher doses were also administered. Only the results with six 7-day-old rats given the 70-mg/kg dose of CM 40907 are shown in

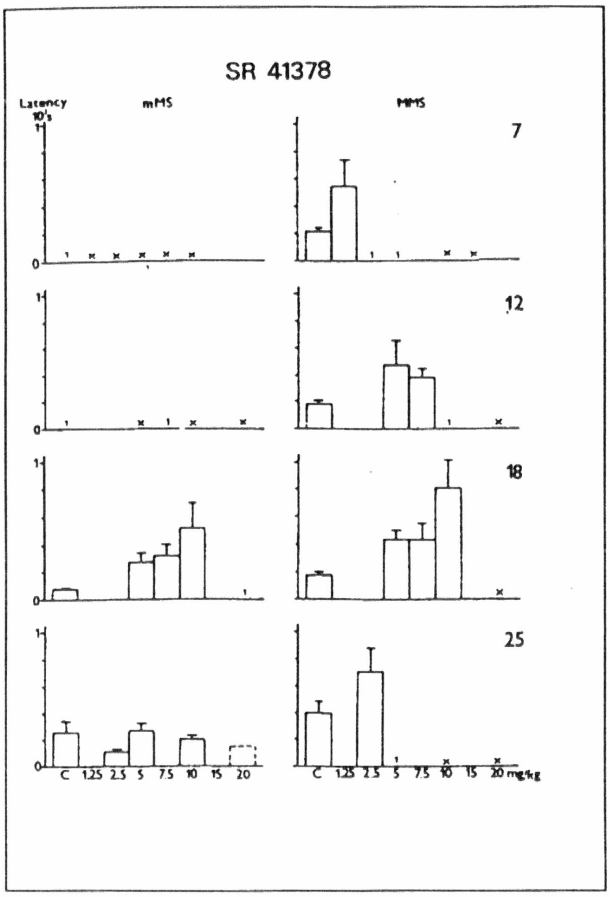

Fig. 3
Influence of SR 41378 on latencies (mean ± S.E.M.) of minimal (mMS, left) and major (MMS, right) metrazol seizures. Details as in Figs. 1 and 2, only ordinates – latencies in thousands of seconds. x means that the seizures did not appear in the actual age and dose group, 1 means that seizures were observed in only one rat and a hatched column in the 25-day-old group represents an average of two values.

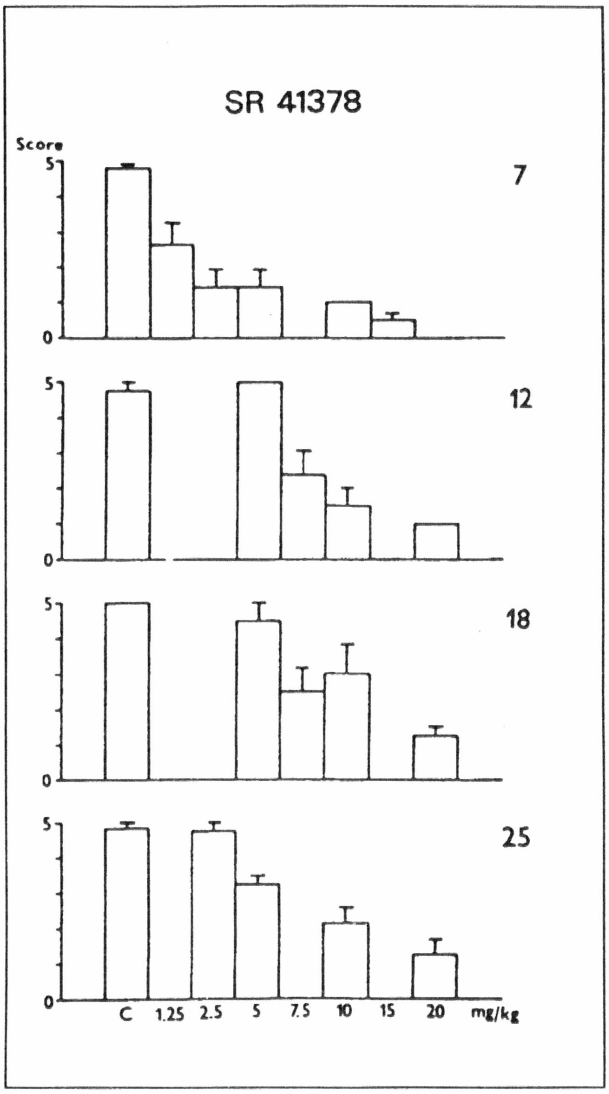

Fig. 4
Influence of SR 41378 on the severity of metrazol-induced seizures. Details as in Figs. 1 and 2.

Fig. 2. There are no data concerning the possible interaction of DMSO with metrazol-induced seizures, therefore 176 animals were pretreated with various doses of this solvent (0.4, 1, 2, 3, or 10 ml/kg). The doses of 2 ml/kg and higher exceeded the quantity of solvent administered with the two anticonvulsant drugs and the highest dose of DMSO (10 ml/kg) represents a load on the homeostasis of body fluids. Metrazol (pentylenetetrazol, PTZ, Sigma, freshly dissolved 10 % solution) was injected subcutaneously in a dose of 100 mg/kg in all age groups, besides the 18-day-old group where the 90 mg/kg dose was administered.

The animals were then observed in isolation for 30 min after PTZ administration. The incidence of abnormal behaviour, isolated myoclonic jerks, minimal and major metrazol seizures was recorded by one of us and the latencies of both types of seizures were measured. The severity of epileptic phenomena was evaluated according to the following scale (Pohl and Mareš 1987):

0 – no changes
0.5 – abnormal behaviour (e.g. orienting reaction, scratching, washing)
1 – isolated myoclonic jerks
2 – atypical minimal seizures (e.g. only some elements were present, or seizures were unilateral)
3 – minimal metrazol seizures (i.e.predominantly clonic seizures of forelimb and head muscles with preserved righting ability)

Table 1
Influence of DMSO on incidence of PTZ-induced seizures (number of rats with seizures/number of rats in a group)

Age in days	Doses of DMSO 0	0.4	1	2	3	10 ml/kg
			Minimal seizures			
71/16	0/9	0/11	2/10	0/10	1/9	
12	1/13	0/9	0/10	0/8	0/8	0/9
18	9/12	7/8	7/8	3/8	9/10	4/8
25	10/11	9/9	8/8	6/8	8/8	3/8*
			Major seizures			
716/16	7/9	7/11	8/10	6/10	9/9	
12	12/13	8/9	10/10	7/8	5/8	7/9
18	12/12	8/8	7/8	5/8	9/10	8/8
25	10/11	4/9	8/8	8/8	8/8	1/8*

*Results significantly different from the controls (Dose 0) at the 5 % level.

Table 2
Influence of CM 40907 on incidence of PTZ-induced seizures (number of rats with seizures/number of rats in group)

Age in days	Dose of CM 40907 0	10	15	20	30	35	40	50	60 mg/kg
				Minimal seizures					
7	1/16	-	-	1/8	0/8	0/8	0/8	1/8	-
12	1/13	0/8	-	1/8	0/8	-	0/8	0/9	0/8
18	9/12	4/8	3/8	-	1/8*	-	-	1/9*	-
25	10/11	6/8	-	3/8*	5/8	-	6/8	6/8	-
				Major seizures					
7	16/16	-	-	6/8	7/8	7/8	1/8*	2/8*	-
12	12/13	5/8	-	4/8	5/8	-	2/8*	3/9*	3/8*
18	12/12	7/8	4/8*	-	1/8*	-	-	1/9*	-
25	10/11	5/8	-	3/8*	2/8*	-	1/8*	1/8*	-

*Results significantly different from the controls (Dose 0) at the 5 % level.

4 – major seizures without a tonic phase
5 – complete major seizures (i.e. generalized tonic-clonic seizures with a loss of righting reflexes). Each animal was scored with the maximum value reached and the average was counted for each treatment, dose and age.

All results were compared with data from 52 control rats given PTZ only. These control groups were taken from previous studies, only two to four rats were added to each age group in the course of the present study. Statistical evaluation: latencies were evaluated by means of ANOVA (BMDP Program). Direct comparisons were made using the Student-Newman-

Table 3
Influence of SR 41378 on incidence of PTZ-induced seizures (number of rats with seizures/number of rats in group)

Age in days	Doses of SR 41378 0	1.25	2.5	5	6.25	7.5	10	15	20mg/kg
				Minimal seizures					
7	1/16	0/8	0/8	0/8	-	-	0/8	0/8	-
12	1/13	-	-	0/8	0/8	1/8	0/8	-	0/8
18	9/12	-	-	7/8	3/7	4/9	5/8	-	1/8*
25	10/11	-	8/8	8/8	-	-	5/8	-	2/8*
				Major seizures					
7	16/16	4/8*	1/8*	1/8*	-	-	0/8*	0/8*	-
12	12/13	-	-	8/8	0/8*	3/8*	1/8*	-	0/8*
18	12/12	-	-	7/8	0/8*	3/9*	4/8*	-	0/8*
25	10/11	-	7/8	1/8*	-	-	0/8*	-	0/8*

*Results significantly different from the controls (Dose 0) at the 5 % level.

Keule test (Miller 1981). Scores were evaluated by means of non-parametric Kruskall-Wallis test (Lehmann and d'Abrera 1975), incidences with Fisher's F test (Kruger *et al.* 1981). The counted values of significance for the score and the incidence were treated according to Holm (1979). The level of statistical significance was set to five percent.

Results

Dimethylsulfoxide

The incidence of both types of seizures was not changed by DMSO with the only exception of the highest dose (10 ml/kg) in the 25-day-old group, where both minimal and major seizures were suppressed (three and one out of eight animals exhibited minimal and major seizures respectively – Table 1).

The latencies did not change systematically. An increase in the latency of major seizures attained statistical significance after the 2 and 3 ml/kg doses in 7-day-old rats and with the 10 ml/kg dose in both 12- and 18-day-old rats. In the oldest group studied the 10 ml/kg dose nearly abolished major seizures, so that it was not possible to assess the latencies. The unique finding which might have a meaning for the experiments with the two potential antiepileptics was the prolongation of latency of major seizures after the 1 ml/kg dose of DMSO in the 12-day-old group. As far as the minimal seizures are concerned, their latencies were prolonged with the highest doses in both 18- and 25-day-old rats.

The severity of seizures was significantly decreased only after the 10 ml/kg dose in 12-, and 25-day-old rats (Fig. 1).

It is possible to conclude that the positive findings are of little importance for our experiments because they were obtained with amounts of DMSO never administered with the two anticonvulsant drugs. The only exception which should be taken into account concerns the increase of latency of major seizures in 12-day-old animals after the 1 ml/kg dose of DMSO.

CM 40907

The incidence of major seizures (Table 2) was significantly decreased in 7- and 12-day-old pups after doses of 40 mg/kg and more, in 18-day-old animals after 12.5 mg/kg and more and in 25-day-old rats by doses of 20 mg/kg and more. Seven- and 12-day-old rat pups exhibited minimal metrazol seizures only exceptionally under control as well as experimental conditions (Table 2). Minimal seizures in 18- and 25-day-old rats were suppressed less efficiently and less consistently than major seizures. In 18-day-old rats doses of 12.5, 30 and 50 mg/kg were effective, whereas in 25-day-old rats the 20 and 80 mg/kg doses lowered the incidence of minimal seizures significantly.

The latencies of both types of seizures remained unifluenced by CM 40907, i.e. seizures were either suppressed or unchanged.

The severity of seizures was significantly decreased in all age groups, but the effective doses varied (Fig. 2): the most sensitive age group was the 18-day-old one, where the doses of 20 mg/kg and higher were effective. The highest dose for attenuation of seizure severity was necessary in 7-day-old rats - only doses of 37.5 mg/kg and more were efficient.

SR 41378

The incidence of major seizures in 7-day-old rats was significantly suppressed by all doses used including the lowest one (1.25 mg/kg). Sensitivity of older animals was somewhat lower, doses of 6.25 and/or 5 mg/kg were the lowest to be effective (Table 3). Minimal metrazol seizures were effectively suppressed in 18- and 25-day-old animals with doses of 20 mg/kg and higher, i.e. substantially higher doses were necessary than for the suppression of major seizures (Table 3).

The latencies of major seizures (Fig. 3) were prolonged significantly only in 18-day-old rats by the 10 mg/kg dose. The incidence of major seizures in other age and dose groups was low so that the latencies could not be evaluated statistically. Latencies of minimal seizures were prolonged in 18-day-old rats by doses of 6.25 mg/kg and more, in 25-day-old animals the level of statistical significance was never attained (Fig. 3).

The severity of seizures was significantly decreased by a wide range of doses in all age groups due to the suppression of major seizures (Fig. 4). The corresponding lowest effective doses for 7-, 12-, 18 and 25-day-old rats were 1.25, 6.25, 6.25 and 5 mg/kg, respectively.

Discussion

Dimethylsulfoxide does not possess any anticonvulsant action in the doses used for dissolving the two potential antiepileptic drugs in our experiments. DMSO was found to increase the permeability of the blood-brain barrier (Sato *et al.* 1987), so that this action should be taken into account. The age groups used in our study differ substantially in the level of maturation of the blood brain barrier. It matures towards the end of the third postnatal week in rats (Vernadakis and Woodbury 1969), so that 25-day-old rats represent a stage, where the blood-brain barrier is developed, while 7- and 12-day-old rat pups do not possess a fully functioning barrier. The markedly higher efficacy of SR 41378 in 7-day-old rats than in all other age groups as well as the differences in developmental profiles of the two drugs speak against the possibility that the blood-brain barrier opening by DMSO plays an important role in the action of the two drugs studied.

Both potential antiepileptics were found to be efficient against both types of metrazol-induced seizures during the postnatal development in rats. A number of classical antiepileptic drugs exhibited stronger action against major seizures than against minimal metrazol seizures during the whole ontogenetic development (phenobarbital – Kubová and Mareš 1991, valproate – Mareš *et al.* 1981, clonazepam – Kubová and Mareš 1989). Only ethosuximide exhibits this action in 25-day-old and adult rats, whereas no suppression of minimal seizures was seen in 18-day-old animals (Mareš *et al.* 1981, Mareš and Velíšek 1983). Until now we have not found an antiepileptic drug exhibiting higher efficacy against minimal than against major seizures (Mareš 1991). Phenytoin, carbamazepin and primidone, drugs used also in the treatment of partial seizures (Engel 1989), exhibit specific action against the tonic phase of generalized tonic-clonic seizures (Mareš *et al.* 1983, Kubová and Mareš 1991).

An action of antiepileptic drugs against major seizures may predict their usefulness against generalized tonic-clonic seizures ("grand mal" type) in epileptic patients (Swinyard 1973, Reinhard and Reinhard 1977, Swinyard and Woodhead 1982, Swinyard *et al.* 1989). The efficacy of CM 40907 and SR 41378 against generalized tonic-clonic seizures in epileptic patients may therefore be taken for granted. Minimal metrazol seizures have been suggested to be a model of human absences (Swinyard 1973, Swinyard and Woodhead 1982), but this opinion has been subjected to serious criticism (Löscher and Schmidt 1988, Mareš and Zouhar 1988). The possible efficacy of both drugs against primary generalized seizures of the absence type as well as against partial seizures has to be examined on special electrophysiological models.

There is a clear-cut quantitative difference between the two drugs studied – SR 41378 was markedly more effective than CM 40907 in all age groups. This finding is in agreement with the published data on the action of both drugs in adult rodents (Chambon *et al.* 1985, 1986). There is also a difference in the developmental profile of action of both drugs. CM 40907 did not exhibit marked differences among the age groups studied. On the other hand, SR 41378 was found to be extremely effective in 7-day-old rat pups, the effective doses in other age groups being three to four times higher. At this moment it is impossible to say if this difference between two closely related drugs is due to pharmacokinetic or pharmacodynamic reasons. Further analysis of these drugs (or their congeners) is worthwhile, even though these drugs have not passed the preclinical and clinical testing successfully.

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