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SOME PHARMACOLOGICAL STUDIES ON THE OPTICALLY ACTIVE ISOMERS OF HYOSCINE AND HYOSCYAMINE

BY

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Differences between optically active isomers of hyoscine or hyoscyamine were demonstrated by Cushny (1926) who concluded that in both cases the laevo form was more effective than the corresponding dextro-isomer at peripheral parasympathetic effector sites. Although experiments in animals failed to demonstrate similar differences on the central nervous system, clinical observations by Moir (1925) suggested that the dextro-isomer of hyoscine was less effective than the laevo form. The much greater peripheral activity of laevo-hyoscine and hyoscyamine compared with their dextro-isomers was confirmed by Kroneberg (1955) but tests assumed to measure central activity such as the abolition of morphine-induced Straub reaction and ability to protect against nicotine-induced convulsions did not distinguish between isomers, the very high doses of dextro and laevo forms of the two alkaloids needed being comparable.

Bradley & Elkes (1957) observed that the electroencephalogram of the conscious cat remained unaffected by 5 and 10 mg/kg of (+)-hyoscyamine sulphate given intraperitoneally, whereas the laevo form in doses from 1 to 4 mg/kg by the same route gave similar effects to atropine, that is an increase in amplitude and frequency of slow waves in the electroencephalogram from all parts of the brain, resembling the pattern for the drowsy state. These observations were confirmed and extended by Domino & Hudson (1959) using the electroencephalogram in dogs and monkeys, and studying conditioned avoidance responses in rats treated with (+)- and (−)-hyoscyamine. All their results confirmed the observation of Bradley & Elkes (1957) that (+)-hyoscyamine was less active in the central nervous system than the laevo form. Using a very pure sample, Domino & Hudson (1959) estimated a ratio of 1 : 50 for relative potency of dextro and laevo forms of hyoscyamine centrally.

Our interest lay in the possible use of (+)-hyoscine in motion sickness and, preliminary to its trial in man, experiments were carried out to compare the activity with that of (−)-hyoscine in a battery of animal tests, some of which were expected to measure an action on the central nervous system. Because of the difficulty experienced by us in attempting to relate the activity of hyoscine with that of hyoscyamine in our review of the literature, the dextro and laevo forms of hyoscyamine were also included in most tests.

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METHODS

Acute toxicity. Determinations of the LD50 and the ED50 for convulsions by each isomer were carried out using albino mice weighing 18 to 22 g. Compounds were dissolved in 0.9% (w/v) saline and 0.1 ml. of solution was administered for each 10 g body weight by slow intravenous injection.

Antiacetylcholine activity. pA_2 values were determined by the method of Schild (1947) on guinea-pig isolated ileum suspended in oxygenated Tyrode solution at 36° C.

Mydriasis. The mydriatic properties of compounds were estimated by the method described by Ing Dawes & Wajda (1945). Systemic effects were determined in groups of six albino mice and local effects in groups of ten by measuring the pupil diameters before and 30 min after subcutaneous injection or application of a solution of the drug in 0.9% (w/v) saline to the cornea for 15 sec. The dose or concentration required to increase the pupil diameter by 200% was determined graphically. Duration studies were effected by measuring the pupil diameters every 10 min after administration of drug.

Antitremorine activity. Tremor was recorded by a method similar to that described by Ahmed & Taylor (1959). A mouse was placed in a plastic box and its movements were recorded with the aid of a gramophone pick-up head resting on the box. The output of the pick-up was amplified and fed to a low inertia motor electrical integrator (Electro Methods Ltd.). Tremor was induced by injecting male albino mice weighing 16 to 22 g with 1,4-dipyrrolidin-1'-ylbut-2-yne (tremorine), 30 mg/kg, intraperitoneally. Preliminary experiments showed that maximum intensity of tremor was obtained 10 to 20 min after injection. Therefore each animal received a subcutaneous injection of protective drug or vehicle and 15 min later tremorine was given. After a further 15 min, tremor was recorded for 1 min. The total tremor for each group of ten receiving drug treatment was calculated as a percentage of that obtained with the control group and the dose of drug required to produce a 50% reduction was determined graphically.

Tranquillizing activity. The chimney test (Boissier, Tardy & Diverres, 1960) has been suggested as a convenient method of detecting tranquillizing activity. Groups of ten albino mice weighing between 18 and 22 g were used. Each mouse was introduced into one end of a copper tube 30 cm long with an internal diameter of 2.5 cm. The mouse was allowed to climb up the tube, which was held in the hand at an angle of approximately 45°, until it reached the top when the tube was immediately inverted to the vertical and the time taken for the mouse to progress backwards along the whole length of the tube was recorded. Only the animals which carried out this procedure within 30 sec were used for the test. Drugs were administered subcutaneously and the animals tested 30 min later. The proportion of mice at each dose level of drug failing to reach the top of the tube within 30 sec was recorded.

Effect on fighting mice. Fifty male albino Swiss mice were isolated in individual plastic cages for 28 days, after which time 96% exhibited aggressive behaviour when another male mouse was introduced into the cage. A fighting episode was characterized by sniffing the hind-quarters of the intruder mouse and rapid attack at the back of the neck, the nose, feet or tail. Drugs were administered subcutaneously to isolated mice 30 min before intruders were introduced into their cage. Failure to attack within 30 sec was considered to be due to a drug-induced block of the fighting response.

Effect on spontaneous locomotor activity. The effects of drugs on spontaneous locomotor activity of rats was determined by a method similar to that described by Dews (1953). The apparatus consisted of a box measuring 40 × 40 cm intersected by three equi-spaced heat-shielded beams of light parallel to each axis. A break of any light beam activated a digital counter. Groups of six Wistar rats were placed in the box immediately after subcutaneous administration of drugs in 0.9% (w/v) saline and the activity count between 20 and 40 min later was recorded and expressed as a percentage of the count obtained for a control group which had received vehicle only.

Conditioned avoidance response. Rats were trained to jump on to a pole in response to the sound of a buzzer (conditioned response) using a technique similar to that described by Cook & Weidley (1957). After the administration of suitable subcutaneous doses of arecoline hydrobromide maximal reduction of this conditioned response was obtained about 8 min later (Pfeiffer & Jenney, 1957). Each rat was injected subcutaneously with a solution of the compound under test or vehicle alone and this was followed 20 min later by a subcutaneous injection of arecoline hydrobromide (5 mg/kg). Responses to the buzzer were determined five times immediately before and three times 8 min after administration of arecoline. An ED50 for each isomer was calculated using the percentage of animals at each dose level giving three out of

three correct responses to the buzzer. In control experiments using eighty-four rats the percentage of correct responses to the buzzer was 92% after injection of vehicle and 2% following the injection of arecoline. Groups of seven animals were used.

All quantal determinations were made by the method of Litchfield & Wilcoxon (1949). Results are given in terms of anhydrous base.

Drugs. These were acetylcholine bromide (B.D.H.), arecoline hydrobromide (Macfarlan Smith), (+)-hyoscine hydrobromide, $[\alpha]_D^{20°} = +25.8°$ and $+24.7°$, (−)-hyoscine hydrobromide, $[\alpha]_D^{20°} = -25.2°$, (+)-hyoscyamine sulphate, $[\alpha]_D^{20°} = +26.7°$, (−)-hyoscyamine hydrobromide, $[\alpha]_D^{20°} = -25.7°$, and tremorine (1,4-dipyrrolidin-1′-ylbut-2-yne) dihydrochloride (E.P.I. Research Department).

RESULTS

Acute toxicity in mice. The acute intravenous toxicity of (+)-hyoscine did not differ significantly from that of (−)-hyoscine (Table 1) and values obtained for the dextro and laevo forms of hyoscyamine were very similar. However, hyoscyamine was about twice as toxic as hyoscine by this route. In all cases death was preceded by tonic convulsions, gasping respiration and exophthalmos. The intravenous ED50 for the production of convulsions was about two-thirds of the corresponding LD50.

TABLE 1

ACUTE TOXICITY OF ISOMERS OF HYOSCINE AND HYOSCYAMINE IN MICE

Injections were intravenous. 95% confidence limits are shown in parentheses

Isomer	LD50 (mg/kg)	ED50 Convulsions (mg/kg)
(+)-Hyoscine	154 (134–178)	102 (90–115)
(−)-Hyoscine	163 (150–176)	110 (97–124)
(+)-Hyoscyamine	81 (78–83)	54 (47–62)
(−)-Hyoscyamine	95 (88–102)	54 (48–60)

Antiacetylcholine activity. A marked difference in activity was obtained with the two optical isomers of hyoscine. The pA_2 value (mean and standard deviation) obtained on five preparations using (+)-hyoscine was 7.39 ± 0.12 and with (−)-hyoscine 9.12 ± 0.38, representing a potency ratio of 1 : 54. Similar estimates reported by Marshall (1955) for the optical isomers of hyoscyamine gave a ratio of 1 : 32.

Mydriatic properties. Fig. 1 illustrates the large quantitative difference between the dextro- and laevo-isomers of hyoscine and hyoscyamine determined 30 min after subcutaneous injection. The dextro : laevo ratio for hyoscine was 1 : 68 and for hyoscyamine 1 : 29.

Concentrations of isomers to produce a similar effect 30 min after application to the cornea were as follows: (+)-hyoscine 42 μg/ml., (−)-hyoscine 1.6 μg/ml., (+)-hyoscyamine 220 μg/ml. and (−)-hyoscyamine 5.8 μg/ml. These results gave a dextro : laevo ratio of 1 : 27 for hyoscine and 1 : 38 for hyoscyamine. The onset and duration of action of (+)- and (−)-hyoscine at equi-effective concentrations were similar (Fig. 2).

Effects in tests presumed to measure central activity. Tests used to measure the central actions of isomers depended upon two different principles. Firstly, the drugs were compared directly for their ability to influence spontaneous activity, chimney climbing and aggression. Secondly, they were tested indirectly for their antagonism to drug-induced effects such as

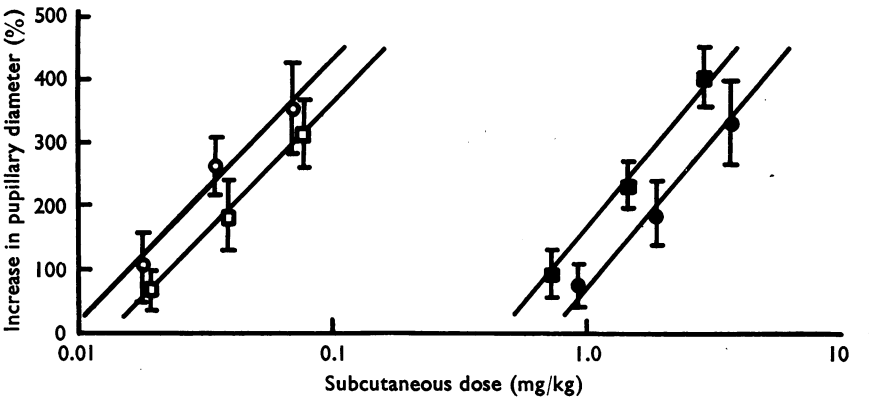

Fig. 1. Dose/response lines for mydriasis in mice produced by isomers of hyoscine and hyoscyamine. Abscissa (log scale): subcutaneous dose of isomer in mg/kg of body weight. Ordinate: mean percentage increase in pupillary diameter for groups of six mice 30 min after injection; vertical lines give standard errors. o——o, (−)-hyoscine; ●——●, (+)-hyoscine; □——□, (−)-hyoscyamine; and ■——■, (+)-hyoscyamine.

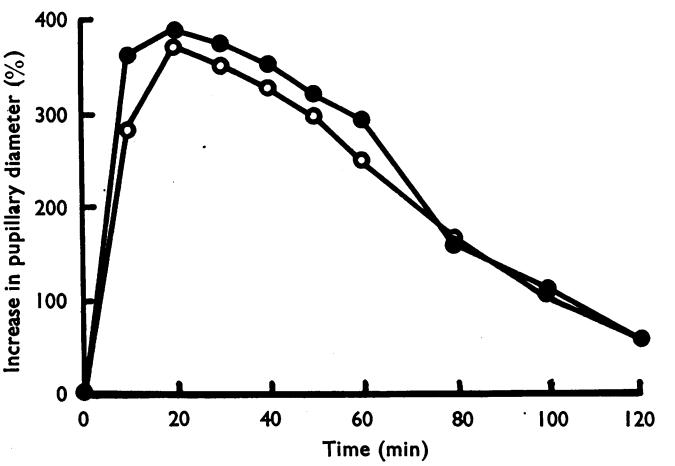

Fig. 2. Duration of mydriasis in mice after corneal application of (+)- and (−)-hyoscine. Abscissa: time in minutes. Ordinate: mean percentage increase in pupillary diameter for groups of ten mice. o——o, (−)-hyoscine; and ●——●, (+)-hyoscine.

tremor and abolition of the conditioned avoidance response. In both types of test, the laevo-isomers were found to be more active than the corresponding dextro forms (Table 2).

Results obtained in the chimney test illustrate most clearly the quantitative difference between isomers, the steep slope of the regression line giving narrow limits of error. At the doses used in this test no muscular incoordination was evident over three successive periods of 15 sec when the mice were placed on a 1-in. diameter rod rotating at 8 revs/min.

The results from the fighting mice test were less precise and, although a significant difference was found between the two isomers of hyoscine, the potency ratio was smaller

TABLE 2

THE ACTIVITY OF ISOMERS OF HYOSCINE AND HYOSCYAMINE IN TESTS FOR CENTRAL ACTIVITY

95% confidence limits are shown in parentheses where applicable. Compounds were administered by subcutaneous injection and tests were performed at the times shown. For spontaneous motility, the "maximal dose" is that which gave maximal increase in activity. For the antitremorine test, the "ED50" is that which caused 50% reduction in tremor

	Tests measuring direct effects			Tests measuring antagonism of drug effects	
Isomer	Chimney test (mice) at 30 min ED50 (mg/kg)	Fighting test (mice) at 30 min ED50 (mg/kg)	Spontaneous motility (rats) at 20–40 min "Maximal dose" (mg/kg)	Anti-tremorine test (mice) at 30 min ED50 (mg/kg)	Anti-arecoline test (rats) at 28 min ED50 (mg/kg)
(+)-Hyoscine	0·53 (0·44–0·65)	0·44 (0·2–0·99)	5·0	1·2	6·4 (4·4–9·4)
(−)-Hyoscine	0·012 (0·01–0·014)	0·092 (0·063–0·13)	0·25	0·019	0·08 (0·06–0·12)
(+)-Hyoscyamine	2·06 (1·65–2·57)	—	—	7·2	44 (27–70)
(−)-Hyoscyamine	0·067 (0·056–0·08)	0·093 (0·042–0·41)		0·34	0·94

than that obtained in the chimney test. (−)-Hyoscine and (−)-hyoscyamine were equally effective in suppressing fighting episodes.

The effects on the spontaneous motility of rats are seen in Fig. 3, which clearly shows the stimulant action of both (+)- and (−)-hyoscine. The approximate dextro : laevo dose ratio in this test was found to be 20.

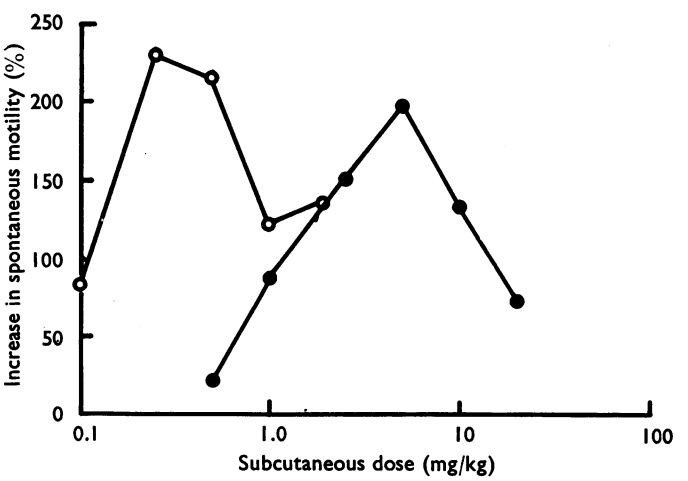

Fig. 3. Effect of (+)- and (−)-hyoscine on spontaneous motility of rats. Abscissa (log scale): subcutaneous dose of isomer in mg/kg body weight. Ordinate: mean percentage increase in spontaneous motility for two groups of six rats from 20 to 30 min after injection. o——o, (−)-hyoscine; and ●——●, (+)-hyoscine.

Fig. 4 shows the effect of hyoscine and hyoscyamine in reducing tremorine-induced tremor. (−)-Hyoscine was found to be much more effective than (+)-hyoscine and the hyoscyamine isomers. Similar results were obtained in the test where antagonism of the arecoline-induced block of the conditioned avoidance response was measured.

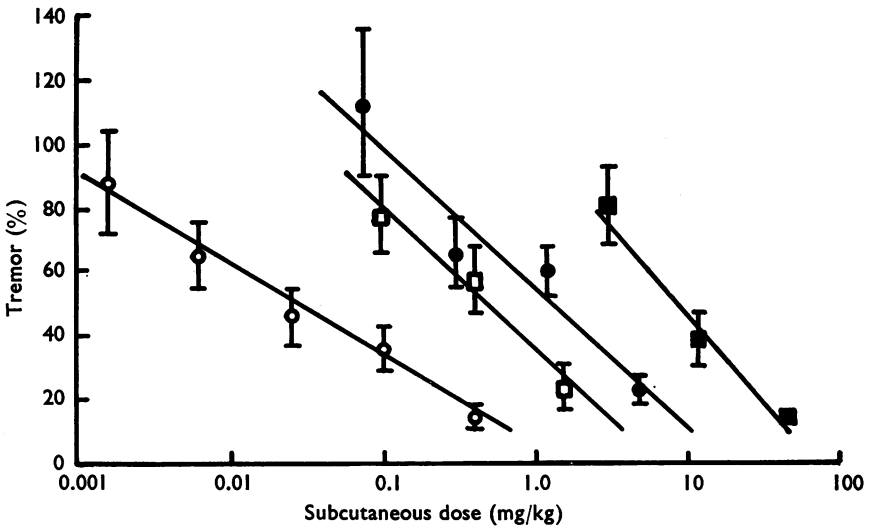

Fig. 4. The reduction of tremorine-induced tremor in mice by isomers of hyoscine and hyoscyamine. Abscissa (log scale): subcutaneous dose of isomer in mg/kg of body weight. Ordinate: mean percentage tremor of test group of ten mice compared with control group 30 to 31 min after injection of isomer and 15 to 16 min after tremorine; vertical lines give standard errors. o——o, (−)-hyoscine; ●——●, (+)-hyoscine; □——□, (−)-hyoscyamine; and ■——■, (+)-hyoscyamine.

DISCUSSION

In the case of hyoscine our results clearly show that a marked quantitative difference between dextro and laevo forms occurs both peripherally and centrally. Results obtained for both hyoscine and hyoscyamine on the guinea-pig ileum and the mouse eye confirm previous observations in regard to peripheral activity (Cushny, 1921; Kroneberg, 1955; Marshall, 1955). Moreover, in addition to the quantitatively similar effects of the isomers at toxic dose levels in mice, we have also been able to show a quantitative difference in the central activity of both isomers of hyoscine and hyoscyamine at doses far below toxic levels either by measuring direct effects or in tests involving drug antagonism in the central nervous system. (−)-Hyoscine was effective at doses from 0.01 to 0.25 mg/kg and (−)-hyoscyamine showed activity at doses from 0.04 to 0.4 mg/kg in rats and mice (Table 2).

Considering our results it appears that the dextro and laevo forms of hyoscine and hyoscyamine are equi-active only at toxic dose levels. In mice, Cushny (1921) showed the subcutaneous minimum lethal doses to be similar for (+)-hyoscine (1,800 mg/kg) and (−)-hyoscine (1,600 mg/kg), and Kroneberg (1955) obtained intravenous LD50s equal to ours. Both Cushny and Kroneberg showed the equal potency of (+)- and (−)-hyoscyamine on frog muscle endplate, and as the mode of death after toxic doses appears to be due to respiratory block the two effects may be due to a common mechanism. The Straub tail

TABLE 3

A SUMMARY OF THE RATIOS OF EQUI-EFFECTIVE DOSES OBTAINED WITH DEXTRO AND LAEVO ISOMERS OF HYOSCINE AND HYOSCYAMINE

*From Marshall (1955)

Test	Dextro : laevo dose ratio		Hyoscyamine : hyoscine dose ratio	
	Hyoscine	Hyoscyamine	(−)-Isomer	(+)-Isomer
Acute intravenous toxicity in mice	1	1	0·6	0·5
Antiacetylcholine activity on guinea-pig isolated ileum	54	32*	0·6	0·4
Mydriasis, 30 min after subcutaneous injection in mice	65	29	1·5	0·4
Mydriasis, 30 min after application to the cornea in mice	28	41	4	7
Chimney test, 30 min after subcutaneous injection in mice	46	31	6	4
Abolition of the fighting response, 30 min after subcutaneous injection in mice	5		1	
Enhanced spontaneous activity, 20 to 40 min after subcutaneous injection in rats	20			
Tremorine antagonism, 30 min after subcutaneous injection in mice	63	21	18	6
Arecoline antagonism, 28 min after subcutaneous injection in trained rats	76	47	11	7

phenomenon in mice has been shown by Bilbey, Salem & Grossman (1960) to depend in part on myoneural control since the effect of morphine can be blocked by (+)-tubocurarine. Kroneberg (1955) used antagonism of this effect of morphine as a measure of central activity, but the large doses necessary (0.6 to 12.0 mg/kg) suggest that it is partly a measure of neuromuscular blocking action. Similarly high doses (4.25 to 18.5 mg/kg) were necessary to block nicotine-induced convulsions in mice.

Cushny (1921) considered the dextro-isomer of hyoscine to be more slowly destroyed in the tissues, but our results with (+)- and (−)-hyoscine in the mydriatic tests showed similar duration of action when equi-effective doses were employed, suggesting similar rates of breakdown.

Since the doses of (−)-hyoscine and (−)-hyoscyamine required in most of our tests were of an extremely low order, the possibility exists that any effects due to the dextro-isomers are due to contamination with the laevo form, and that the dextro-isomers are inactive at all sites of action except the neuromuscular junction, which may be nonstereospecific compared with the stereospecific peripheral receptor sites and those in the central nervous system.

In addition to a comparison of the activities of the laevo and dextro forms of hyoscine and hyoscyamine, this work affords an interesting comparison of hyoscine and hyoscyamine in various pharmacological tests which may be of value in the evaluation of compounds expected to have properties similar to hyoscine (Table 3). Hyoscyamine was twice as toxic as hyoscine intravenously in mice, and the ratio has been found to be similar after subcutaneous injection (Molitor, 1936; Cahen & Tvede, 1952). Against acetylcholine-induced contractions of the guinea-pig ileum or on the mouse eye after subcutaneous injection, hyoscine and hyoscyamine were similarly active, yet after topical application to the mouse eye hyoscine was a much more effective mydriatic. A comparison of the effects of hyoscine and hyoscyamine in tests presumed to measure central activity showed hyoscine to be

considerably more active. Ahmed & Marshall (1962) found (−)-hyoscine to be more effective than atropine against tremorine-induced tremor in mice, as did Vernier & Unna (1953) in monkeys. Janssen, Jageneau & Niemegeers (1960), using a similar fighting mice test to ours, found hyoscine to be forty-times as effective in blocking the fighting episodes. Although our ED50 for (−)-hyoscine agrees with their work we found (−)-hyoscyamine equi-active, although the extremely shallow regression slopes make this test undesirable in this type of work. From our comparison of hyoscine and hyoscyamine in these tests we are in general agreement with Parkes (communication to the British Pharmacological Society, 1963) who concluded from a survey of the literature that (−)-hyoscine and atropine possessed similar activity at peripheral cholinergic sites but (−)-hyoscine was more effective in the central nervous system.

SUMMARY

1. Determinations of the potency of the dextro- and laevo-isomers of hyoscine and hyoscyamine were made before a clinical trial of (+)-hyoscine in motion sickness.

2. Methods to measure the potency of the optically active isomers at peripheral parasympathetic effector sites and in the central nervous system of rodents were used.

3. Both peripherally and centrally the isomers differed markedly in potency and possessed equal potency only at toxic dose levels.

4. Hyoscine was more active than hyoscyamine on the central nervous system irrespective of isomer considered.

5. The finding that the dextro- and laevo-isomers differ markedly in potency on the central nervous system is at variance with the classical concept of the isomers being equi-active.

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