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Role of Intracellular Calcium in Renal Nerve-Mediated Renin Release (42511)

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Abstract. We evaluated the role of intracellular calcium in renal nerve-mediated renin release in four groups of anesthetized dogs. Each dog received renal nerve stimulation (RNS) (0.5 Hz) twice as follow: control, RNS, recovery; control, RNS, recovery. Group 1 served as time control. Group 2 received Ca ionophore A23187 (Io) and Groups 3 and 4 received verapamil at 2.5 and 5 μg/kg · min respectively during the second RNS. In Group 1, renin secretion rate (RSR) increased from 95 ± 22 to 223 ± 73 ($P < 0.05$) and from 13 ± 5 to 108 ± 20 ngANG I/hr · min ($P < 0.005$) during the first and second RNS, respectively. In Group 2, RSR increased from 210 ± 85 to 402 ± 118 ($P < 0.02$) and from 88 ± 11 to 157 ± 39 ngANG I/hr · min (NS) during the first and second RNS, respectively. In both groups, systemic and renal hemodynamics and UNaV did not change. In Group 3, verapamil alone did not increase RSR. During RNS, RSR also did not increase. In Group 4, verapamil alone increased RSR from 42 ± 12 to 273 ± 71 ngANG I/hr · min ($P < 0.03$) despite a similar reduction in systemic blood pressure as in Group 3. RNS did not increase RSR further during verapamil infusion. The present study suggests that increased intracellular Ca by Io inhibits renal nerve-mediated renin release. A low dose of verapamil has no effect on renin release and does not augment renal nerve-mediated renin release. A high dose of veparamil increases renin release but does not enhance RNS-mediated renin release. We conclude that intracellular calcium plays an important role in renin release and may be the final messenger in renal nerve-mediated renin release.

Renal sympathetic nerves play an important role in mediating renal renin release (1). Electrical stimulation of the renal nerves (RN) at a level that does not alter renal hemodynamics or sodium excretion increases renin release (2). In addition, subthreshold stimulation of the renal nerve, that by itself has no effect on renin release, augments renin release associated with furosemide administration or aortic constriction (3). Since renal nerve-stimulated renin release is blocked by β-1 adrenoceptor antagonists (2) but not by β-2 or α-adrenoceptor antagonists (2, 4), it has been suggested that RN-stimulated renin release is mediated by β-1 adrenoceptors located on the juxtaglomerular cells of the afferent arterioles (2). Because all known cellular responses to β-adrenoceptor activation are mediated by an increase in intracellular cAMP through activation of adenylate cyclase (5), it has been further suggested that the intracellular second messenger mediating renin release is cAMP. There are several findings that support this. Isoproterenol (6), glucagon (7), and forskolin (8) increase cAMP stimulate renin release. In addition dibutyryl cAMP (9, 10) and phosphodiesterase inhibitor stimulate renin release (9). Conversely, renin release is inhibited by agents known to decrease cAMP, namely α-adrenoceptor (11) and adenosine A1 receptor agonists (12, 13). However, recent studies suggested that intracellular calcium may be an important second messenger in mediating renin release (14) and that cAMP may function indirectly to modulate this Ca-dependent process (9, 15). In kidney slice preparations, both isoproterenol- and cAMP-stimulated renin releases are blocked by agents (vasopressin, angiotensin II, high K, and ouabain) known to increase intracellular calcium (9). In other studies, both verapamil and trifluoperazine which decrease Ca uptake increase renin release in the absence of an elevated cAMP (15–17). This finding argues against cAMP as second messenger in mediating renin release and favors intracellular calcium as final mediator of renin release. However, it is possible that both cAMP and intracellular Ca^{2+} may be important in mediating renin release. cAMP and Ca^{2+} may influence renin release independent of each other. Alternatively, the two may sequentially influence renin release.

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To explore further the interrelationship between cAMP and intracellular calcium in mediating renin release, we evaluated the role of calcium in renal nerve-stimulated renin release. We reasoned that, if intracellular calcium is the final messenger, alteration of intracellular calcium may affect renin release associated with renal nerve stimulation. In this regard, increased intracellular calcium by Ca ionophore (Io) should block renal nerve-stimulated renin release. Conversely, decreased intracellular calcium by verapamil should enhance RN-stimulated renin release.

Materials and Methods. The experiments were performed in adult female mongrel dogs weighing 18–25 kg. Food was withheld for 18 hr prior to the study but the animals were allowed free access to water. On the day of the experiment, the animals were anesthetized with pentobarbital (30 mg/kg), intubated, and ventilated with a Harvard respirator. Light anesthesia was maintained throughout the experiment with periodic injections of sodium pentobarbital. Polyethylene catheters were inserted into both ureters and the left renal vein through a flank incision and retroperitoneal approach. A polyethylene catheter was inserted into a brachial artery for continuous measurement of systemic blood pressure with a Statham transducer and a Gilson recorder. A 23-gauge needle was inserted into the left renal artery and kept open by an infusion of 2.5% dextrose in water at 0.5 ml/min. An electromagnetic flow probe was placed around the left renal artery for the continuous monitoring of renal blood flow.

After surgery, an intravenous infusion of 0.9% saline (0.5 ml) containing sufficient inulin was begun. Each animal also received maintenance infusion of 2–4 ml/min of 2.5% dextrose in water. A 60- to 90-min stabilization period was allowed before the experiment was started. The experiments were performed according to the following groups of dogs.

Group 1: Renal nerve stimulation (RNS) in vehicle-treated dogs (seven dogs). In this group of dogs the surgery was the same as described above except that the left renal nerve was carefully isolated from the left renal artery and vein, tied with a silk ligature, and cut as near to the aorta as possible. An electrode connected to a Grass electric stimulator (Model SD9 square wave stimulator, Grass Instrument Co.) was placed around the renal nerve bundle. An oscilloscope connected to the Grass stimulator continuously monitored the frequency, intensity, and duration of nerve stimulation. In this group of dogs, after the stabilization period, three successive 5-min urine collections were made (control period). After this, RNS was begun with 10 V, 0.5 Hz, and 0.5 msec and continued for 45 min. Three clearance periods were obtained during the last 15 min of RNS (RNS period). Three 5-min recovery clearance periods were obtained 30 min after discontinuation of RNS. Fifteen minutes after the recovery period, clearance periods consisted of control, RNS, and recovery as described were repeated. In this group of dogs normal saline was infused intrarenal arterially throughout the experiment. In each dog RNS was done twice. The first RNS was done to assure that each dog did respond to RNS prior to the subsequent administration of either the inhibitor (Ca Io) or the stimulator (verapamil). In addition, the first RNS also served as vehicle control for agents administered during the second RNS of each group.

Group 2: Renal nerve stimulation in Ca ionophore-treated dogs ($n = 6$). Experiments in this group of dogs were the same as in Group 1 except that the dogs received Ca ionophore A23187 at a dose of 0.6 μg/kg · min and its vehicle intrarenally during the second and the first halves of the experiments, respectively. Ca ionophore was dissolved in warm alcohol and diluted with normal saline.

Group 3: Renal nerve stimulation in low dose verapamil-treated dogs ($n = 5$). The experiments in this group of dogs were the same as in Group 1 except that verapamil at a dose of 2.5 μg/kg · min was infused during the second half of the experiment.

Group 4: Renal nerve stimulation in high dose verapamil-treated dogs ($n = 5$). The experiments in this group of dogs were the same as in Group 3 except that verapamil at a dose of 5 μg/kg · min was infused.

Blood was drawn during the middle of the first and third clearance periods for plasma renin activity (PRA), inulin, and Na determinations. PRA was determined by radioimmunoassay (18). Renin secretion rate (RSR) was calculated from (renal venous PRA − arterial PRA) × renal plasma flow and expressed as ng ANG I/hr · min. Renal plasma flow

TABLE I. EFFECTS OF RENAL NERVE STIMULATION (RNS)

	GFR (ml/min)						Renal plasma flow (ml/min)					
	C	RNS	R	C	RNS	R	C	RNS	R	C	RNS	R
Group 1 Renal nerve stimulation in vehicle-treated dogs ($n = 7$)												
Mean	26.9	25.5	21.9	22.9	23.2	23.4	101	100	92	87	87	92
±SEM	3.4	2.5	1.9	1.1	1.9	1.7	13	12	14	12	11	12
P		(NS)	(NS)		(NS)	(NS)		(NS)	(NS)		(NS)	(NS)
Group 2 Renal nerve stimulation in Ca ionophore-treated dogs ($n = 5$)												
Mean	28.5	27.8	25.9	24.5	23.4	23.5	145	139	137	124	129	132
±SEM	4.5	4.5	3.4	2.9	2.5	1.8	20	16	16	17	22	25
P		(NS)	(NS)		(NS)	(NS)		(NS)	(NS)		(NS)	(NS)
Group 3 Renal nerve stimulation in verapamil-treated dogs (2.5 μg/kg · min) ($n = 5$)												
Mean	21.6	21.1	20.7	22.9	21.9	21.1	100	89	90	106	109	113
±SEM	2.6	1.9	1.7	1.4	2.1	1.1	8	9	10	13	14	15
P		(NS)	(NS)		(NS)	(NS)		(0.04)	(NS)		(NS)	(NS)
Group 4 Renal nerve stimulation in verapamil-treated dogs (5 g/kg · min) ($n = 5$)												
Mean	27.5	27.5	27.9	31.5	29.8	27.8	132	121	123	124	114	118
±SEM	0.9	1.0	1.7	2.0	2.9	2.1	10	13	13	10	9	6
P		(NS)	(NS)	(0.04)	(NS)	(NS)		(NS)	(NS)		(NS)	(NS)

Note. Abbreviations used: C = control, R = recovery, RVR = renal vascular resistance.

(RPF) was determined from renal blood flow (RBF) where RPF = (1 − HCT) × RBF. Renal vascular resistance (RVR) was calculated from the ratio of renal perfusion pressure/renal blood flow and expressed as mm Hg/ml · min. The data from each three clearance periods were averaged and treated as control, RNS, and recovery. The Student paired and unpaired *t* tests were used for statistical analyses where applicable. A *P* value of <0.05 was considered significant for two-tailed analysis. The results were expressed as means ± SE.

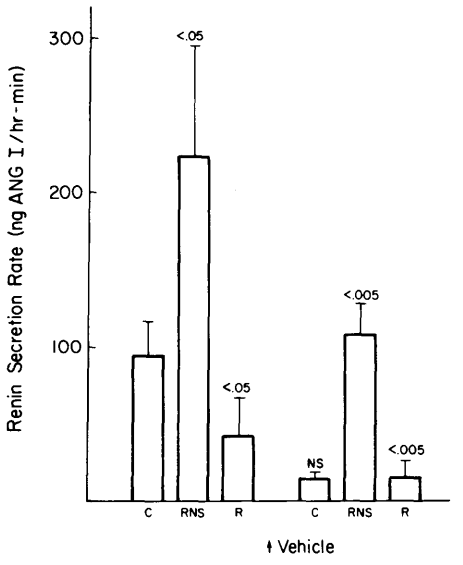

FIG. 1. Renin secretion rate in response to renal nerve stimulation in vehicle-treated dogs (c = control, RNS = renal nerve stimulation, R = recovery).

Results. *Effects of renal nerve stimulation in vehicle-treated dogs (Table I, Group 1).* In this group of seven dogs, during the first RNS, renin secretion rate (RSR) increased from 95 ± 22 to 223 ± 73 ($P < 0.05$) and returned to 42 ± 25 ($P < 0.05$) ng ANG I/hr · min. During the second stimulation, RSR increased from 13 ± 5 to 108 ± 20 ($P < 0.005$) and returned to 13 ± 11 ($P < 0.005$) ng ANG I/hr · min (Fig. 1). GFR, renal plasma flow, renal vascular resistance, systemic blood pressure, and urinary Na excretion did not change significantly in both RNS.

Effects of renal nerve stimulation in dogs treated with Ca ionophore A23187 (Table I, Group 2). In this group of dogs, the animals received vehicle and Ca ionophore during the first and second renal nerve stimulations, respectively. RSR increased from 210 ± 85 to 402 ± 118 ($P < 0.02$) and returned to 148 ± 52 ($P < 0.05$) ng ANG I/hr · min during the first stimulation. RSR was 88 ± 11 before, 157 ± 39 (NS) during, and 51 ± 17 ($P < 0.05$) ng ANG I/hr · min after the second renal nerve

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RVR (mm Hg/ml · min)						$U_{Na}V$ (μEq/min)						Blood pressure (mm Hg)					
C	RNS	R	C	RNS	R	C	RNS	R	C	RNS	R	C	RNS	R	C	RNS	R
0.785	0.823	0.904	0.981	1.030	0.944	34	20	16	16	16	24	128	129	133	136	140	139
0.082	0.100	0.098	0.099	0.118	0.131	18	9	6	5	5	9	5	5	4	5	6	6
(NS)	(NS)	(NS)	(NS)	(NS)		(NS)	(NS)	(NS)	(NS)	(NS)		(NS)	(NS)	(NS)	(NS)	(NS)	
0.508	0.547	0.574	0.638	0.659	0.650	42	15	10	12	13	21	123	126	127	128	130	129
0.060	0.070	0.097	0.097	0.113	0.107	24	6	3	2	3	5	6	7	8	7	6	5
(NS)	(NS)	(NS)	(NS)	(NS)		(NS)	(NS)	(NS)	(NS)	(0.05)		(NS)	(NS)	(NS)	(NS)	(NS)	
0.76	0.91	0.914	0.713	0.674	0.633	14	7	11	57	53	83	146	148	147	135	132	127
0.04	0.10	0.131	0.083	0.094	0.092	3	3	4	35	25	43	6	8	12	10	10	9
(NS)	(NS)	(.052)	(NS)	(NS)		(NS)	(NS)	(NS)	(NS)	(NS)		(NS)	(NS)	(NS)	(NS)	(NS)	
0.582	0.644	0.66	0.57	0.60	0.56	31	15	10	130	124	123	140	140	142	131	128	127
0.042	0.080	0.091	0.070	0.081	0.032	12	7	5	39	40	40	5	5	6	7	7	6
(NS)	(NS)	(NS)	(NS)	(NS)		(NS)	(NS)	(0.03)	(NS)	(NS)		(NS)	(NS)	(0.013)	(NS)	(NS)	

stimulation (Fig. 2). GFR, renal plasma flow, renal vascular resistance, and systemic blood pressure did not change with either RNS. Likewise, there were no significant changes in Na excretion during both renal nerve stimulations.

Effects of renal nerve stimulation in low dose verapamil-treated dogs (Table I, Group 3). In this group of dogs, during the first RNS, GFR, RPF, RVR, UNaV, and BP did not change significantly but RSR increased from 98 ± 29 to 200 ± 34 ($P = 0.006$) and returned to 42 ± 14 ($P = 0.012$) ng ANG I/hr · min. Verapamil alone increased RSR from 42 ± 14 to 68 ± 16 ng ANG/I hr · min (NS), increased RPF from 90 ± 10 to 106 ± 13 ml/min ($P = 0.02$) and decreased RVR from 0.91 ± 0.13 to 0.71 ± 0.08 mm Hg/ml · min ($P = 0.052$). During the second RNS, GFR, RPF, RVR, UNaV, and systemic blood pressure did not change significantly. RSR also did not increase significantly. During verapamil infusion, RSR was 68 ± 16 before, 140 ± 13 during (NS), and returned to 43 ± 16 ng ANG I/hr · min (NS) after RNS.

Effects of renal nerve stimulation in high dose verapamil-treated dogs (Table I, Group 4). In this group of dogs, during the first renal nerve stimulation, GFR, RPF, RVR, blood pressure, and UNaV did not change significantly. RSR increased from 63 ± 17 to 109 ± 14 ($P < 0.004$) and returned to 42 ± 12 ($P < 0.03$) ng ANG I/hr · min. After administration of verapamil at 5 μg/kg · min, GFR increased from 27.9 ± 1.7 to 31.5 ± 2.0 ml/min ($P = 0.04$), systemic blood pressure decreased from 142 ± 6 to 131 ± 7 mmHg ($P = .013$), and UNaV increased significantly from 10 ± 5 to 130 ± 39 ($P = 0.03$) μEq/min. Renin secretion rate increased from 42 ± 12 to 273 ± 71 ($P = 0.03$) ng ANG I/hr · min (Fig. 3). During the second renal nerve stimulation, GFR, RPF, RVR, systemic blood pressure, and UNaV did not change significantly. Likewise, RSR was 273 ± 71 before, 272 ± 85 during, and 164 ± 74 ng ANG I/hr · min after renal nerve stimulation.

The RSR ratios (RNS/control) during the first and second RNS for all groups were shown in Table II. As can be seen the ratio for second RNS in Group 1 was significantly higher than those for the other three groups. During the first RNS, the ratios for the four groups were not different.

Discussion. In the present study, stimulation of renal nerve at low frequency (10 V, 0.5 Hz, 0.5 msec) increases renin release significantly in the absence of significant changes in renal hemodynamics or Na excretion. This result was reproducible and occurred with both stimulations. This finding is in close agreement with that reported previously (2). In the ab-

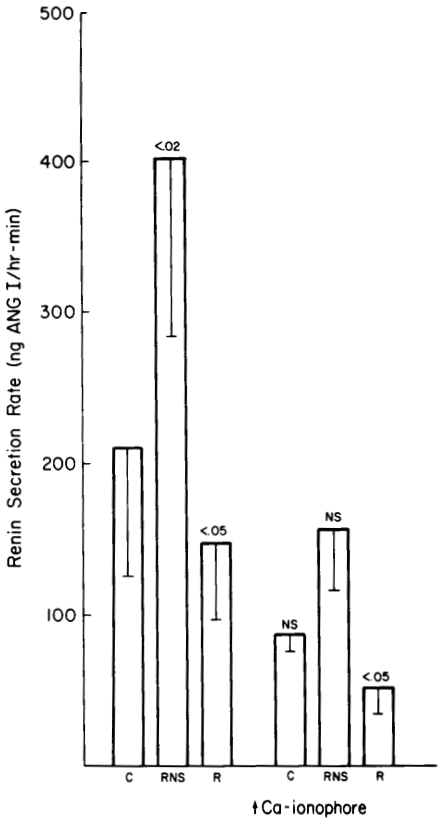

FIG. 2. Renin secretion rate in response to renal nerve stimulation in dogs treated with Ca ionophore A23187 (Io). Io was given throughout the second half of the experiments.

sence of the participation of baroreceptor, or macula densa, this finding has been interpreted as a direct effect of renal nerve on the juxtaglomerular cells. During Ca Io infusion, RN stimulation did not increase renin release.

Ca Io facilitates transmembrane calcium flux down the electrochemical gradient to increase intracellular calcium. This action of Ca Io is not dependent on voltage-operated or receptor-operated channels. Ca Io has been shown to increase or decrease renin release (19–23). This discrepancy appears to be related to the dosage, the duration of the administration, and to the presence of extracellular calcium. Small doses of Ca Io stimulate renin release through its stimulatory effect on norepinephrine and PGE_2 releases (20, 23). Ca Io in large doses and with prolonged administration inhibits renin release (20). In the presence of ECF calcium, Io inhibits renin release and vice versa (19–21).

In the present study, Ca Io was infused over prolonged periods of time. Ca Io itself had no effect on baseline renal hemodynamics, sodium excretion, or renin release, but Io inhibited renin release associated with electrical stimulation of renal nerve at low frequencies. In our laboratory, larger doses of Ca Io produced renal vasoconstriction. In the present study, the inhibition of RN-stimulated renin release by Ca Io in the absence of renal hemodynamics and sodium excretion changes suggest that intracellular calcium plays an important role in RN-stimulated renin release. Intracellular calcium may be the final messenger in mediating renin release. Our initial hypothesis is that stimulation of renal nerve increases adenylate cyclase activity and intracellular cyclic AMP which in turn stimulates calcium efflux (24), thereby reducing intracellular calcium. Decrease in intracellular calcium through some as yet unknown mecha-

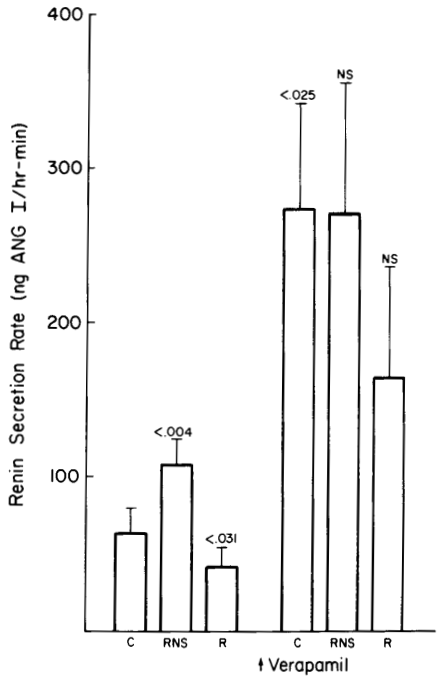

FIG. 3. Renin secretion rate in response to renal nerve stimulation in dogs treated with verapamil (5 μg/kg · min.). Verapamil was given throughout the second half of the experiment.

TABLE II. EFFECTS OF RENAL NERVE STIMULATION ON RENIN RELEASE RATIO

	First RNS RNS/Control	Second RNS RNS/Control	*P* (first vs second)
Group 1 (time control)	2.22 ± 0.29	7.66 ± 1.87	(<0.02)
Group 2 (Ca Io)	2.23 ± 0.23	1.71 ± 0.38*	(NS)
Group 3 (low dose verapamil)	3.18 ± 1.19	2.37 ± 0.68**	(NS)
Group 4 (high dose verapamil)	2.17 ± 0.45	1.00 ± 0.15*	(NS)

* $P < 0.01$, ** $P < 0.05$ compared to second RNS Group I.

nism stimulates renin release. In this regard, the intracellular messengers cAMP and Ca are in sequential control of renin release. We reasoned that if we increase intracellular calcium, we may be able to block this sequential event. The present study appears to support this hypothesis. However, this finding does not completely exclude cAMP as a final messenger since an increased intracellular calcium may inhibit adenylate cyclase and/or stimulate phosphodiesterase (25). Both actions may decrease intracellular cAMP and thereby decrease the renin release associated with RN stimulation.

To delineate further the role of calcium in RN-stimulated renin release, we examined the role of verapamil. We anticipate that verapamil, through its action in decreasing cellular calcium, may enhance RN-stimulated renin release. The major action of verapamil is to inhibit voltage-dependent calcium uptake. In intact animals, verapamil stimulates renin release probably through alteration of renal baroreceptor tones (26, 27). Thus, a normotensive dose of verapamil has no effect on renin release whereas doses of verapamil that decrease systemic blood pressure increase renin release (26). In the *in vitro* preparation, verapamil through a voltage-dependent channel inhibition stimulates renin release (28). In kidney slice preparation, K^+ depolarization inhibits renin release presumably due to voltage-dependent increases in Ca uptake. This inhibitory effect of K^+ depolarization on renin release can be blocked by verapamil, suggesting a specific voltage-dependent mechanism (28). This is also supported by the finding that verapamil has no effect on ADH or AII inhibition of renin release (29, 30). Although all three substances increase intracellular Ca, only verapamil antagonizes voltage-related Ca influx and does not antagonize receptor operated calcium influx. In the present study, verapamil at a dose of 2.5 μg/kg · min by itself did not increase renin release and did not enhance RN-stimulated renin release. At 5 μg/kg · min, verapamil alone increased GFR and Na excretion. With this dose, renin release increased. The reduction in systemic blood pressure was comparable in both groups. Thus this finding is not in agreement with those reported previously, suggesting a baroreceptor mechanism for renin release (26, 27). Our findings suggest that a high dose of verapamil may increase renin release independent of the baroreceptor mechanism. During verapamil treatment, renal nerve stimulation did not increase renin release further. These findings are not in agreement with those reported previously in rat kidney slices and isolated rat kidneys. In those studies, D-600 or methoxyverapamil did not inhibit isoproterenol-stimulated renin release when given simultaneously with isoproterenol (31, 32). We have no explanation for this discrepancy. However, it is possible that intracellular calcium efflux induced by cyclic AMP associated with the renal nerve stimulation is not further enhanced by the voltage-dependent channel inhibition of calcium influx. In the present study, it is likely that verapamil through its inhibitory effect on catecholamines may have blocked the renin release associated with renal nerve stimulation. Despite this finding, the present study suggests that intracellular calcium plays an important role in renin release and may be the final messenger in mediating renal nerve-stimulated renin release.

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