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Cardiovascular effects of intracerebral injections of glutamate and kainate in rats

Enrico LAMPA, Mario CAZZOLA, Maurizio ANGRISANI, Concetta MATERA, Michele LISA, Vincenza SUSANNA, Rosanna MARRAZZO, Savino CHIEPPA, Emilio MARMO[1]

(Istituto di Farmacologia e Tossicologia, Prima Facoltà di Medicina e Chirurgia, Università di Napoli, 80138 Napoli, Italy)

ABSTRACT The intracerebral (lateral ventricle, 3rd ventricle, posterior hypothalamus and corpus striatum) administrations of sodium glutamate (G) 0.1-10 mg/rat and kainic acid (K) 0.1-10 μg/rat induced transitory and dose-dependent increases of arterial BP and bradycardia. These effects were abolished by *icv* L-glutamic acid diethyl ester hydrochloride 1-10 μg/rat. G-and K-hypertensions were reduced by catecholamine depletion, peripheral adrenergic blocks, central α_2-adrenergic stimulation, Ca^{2+} transmembrane block, ganglionic block, adrenalectomy, and spinal transection. Bilateral vagotomy and carotid sinus denervation augmented the hypertension. The bradycardia seemed to be reflexly mediated via carotid sinus and aortic pressoreceptors.

KEY WORDS glutamate; kainic acid; cardiovascular system; intracerebral injections

L-Glutamate (G) acted as an excitatory synaptic transmitter in the CNS[1]. Less information is available about the cardiovascular effects in rats[1-5]. G levels were particularly high in dorsal medial and commissural nuclei of the nucleus tractus solitarius of cats[8]. G showed a possible transmitter function as baroreceptor afferent nerve fibers[7]. Microinjection of G into intermediate tractus solitarii nucleus in anesthetized rats elicited hypotension, bradycardia, apnea, and stimulated baroreceptors reflexes[7]. The purpose of this study was to determine the effects of intracerebral injections of G and its rigid analog kainate (K)[1,8,9] on cardiovascular system in normotensive anesthetized rats.

METHODS

Experiments were performed on ethyl urethane (1.2 g/kg *ip*) anesthetized normotensive albino rats (NOS strain, 260±SD 25 g). A carotid artery was catheterized for recording blood pressure (BP) on a Hellige polygraph. ECG was registered by an Epsilon model electrocardiograph. Electrocortical activity was recorded by an Hellige poligraph. Spinal rats were prepared by a transection at C 7. Bilateral vagotomies[10] and carotid sinus denervation[11] were made at the neck.

A stainless steel cannula (OD 0.6 mm) was implanted into the 3rd ventricle, right lateral ventricle or other brain areas (corpus striatum or posterior hypothalamus)[12] under ketamine (100 mg/kg *im*) anesthesia 5-7 d previously. Solutions of 5 μl were injected in 2 min. Control injections of the vehicle solution produced no significant changes in

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[1]Reprint requests to Prof E Marmo, Chairman of the Dept of Pharmacology and Toxicology, Corso Vittorio Emanuele 168, 80121 Napoli, Italy.

BP, heart rate (HR) and in overt behaviour and electrocortical activity. Interval between injections is 30 min.

After the experiment, the stereotaxic location of the cannula was checked histologically.

Tab 1. Arterial blood pressure and heart rate in rats treated with *L*-glutamate (G) (0.1-10 mg/rat in 2 min every 30 min) or kainate (K) (0.1-10 μg/rat in 2 min every 30 min). n=10, $\bar{x}\pm$SD. *p<0.05. **p<0.01

	Third ventricle	Right lateral ventricle	Posterior hypo-thalamus	Corpus striatum
G	Maximal increase of mean arterial pressure(kPa)			
0.1	2.0±0.3*	1.1±0.3*	1.1±0.3*	1.0±0.3*
1	4.1±0.9**	3.3±0.9**	2.0±0.6**	1.7±0.6**
10	5.3±0.9**	3.8±0.9**	2.8±0.6**	2.2±0.6**
G	Area of hypertension(mm²)			
0.1	179±158	90±34	83±22	80±25
1	810±568	520±214	464±218	480±227
10	1080±620	708±426	628±436	613±483
G	Duration of hypertension (s)			
0.1	260±164	97±60	150±75	103±66
1	525±464	346±240	360±309	328±237
10	642±568	530±483	508±401	463±379
G	Maximal reduction of heart rate (bpm)			
0.1	0	0	0	0
1	8±2**	9±2**	8±1**	9±1**
10	11±3**	10±6**	12±3**	13±3**
K	Maximal increase of mean arterial pressure(kPa)			
0.1	1.6±0.3*	1.0±0.3*	1.2±0.3*	1.0±0.3*
1	3.9±3.1**	2.9±0.6**	2.1±0.6**	2.0±0.6**
10	4.9±2.8**	3.5±0.6**	2.5±0.9**	2.3±0.6**
K	Area of hypertension (mm²)			
0.1	183±144	77±56	72±37	70±31
1	792±530	537±227	493±240	505±214
10	1134±663	759±461	658±445	631±426
K	Duration of hypertension (s)			
0.1	250±176	91±75	138±85	98±63
1	518±436	352±259	370±271	315±246
10	684±553	523±464	529±423	448±363
K	Maximal reduction of heart rate (bpm)			
0.1	0	0	0	0
1	8±2**	9±1**	9±1**	8±1**
10	10±3**	11±6**	13±6**	12±3**

G and K were dissolved in phosphate buffer 0.2 mol/L (pH 6.5) and the pH of the solution was adjusted to 6.5 with NaOH 12 mol/L. Other drugs were dissolved in 0.9% NaCl for systemic injection, or the artificial cerebrospinal fluid[13]. The doses referred to the free bases.

Tab 2. Effects of *L*-glutamic acid diethylester (GDEE) (1 and 10 μg/rat in 2 min 15 min before) on hypertension and bradycardia induced by K (0.1-10 mg/rat in 2 min). n=10, $\bar{x}\pm$SD. All p<0.01, except *p>0.05. Expt with GDEE †1 μg/rat, ††10 μg/rat.

G	Third ventricle	Right lateral ventricle	Posterior hypo-thalamus	Corpus striatum
	% Reduction of hypertension acme†			
0.1	98±29	100	100	100
1	64±25	64±22	60±18	66±22
10	31±14	35±13	38±13	40±14
	% Reduction of hypertension area†			
0.1	96±6	100	100	100
1	61±20	59±18	62±22	65±22
10	36.5±0.7	32±12	37±14	38±13
	% Reduction of hypertension duration†			
0.1	95±14	100	100	100
1	62±22	60±19	62±21	63±20
10	37±11	34±13	31±12	36±12
	% Reduction of hypertension acme††			
0.1	100	100	100	100
1	70±22	68±22	75±25	73±23
10	45±16	41±15	46±14	43±16
	% Reduction of hypertension area††			
0.1	100	100	100	100
1	73±23	72±22	71±23	78±24
10	47±15	41±12	45±14	44±15
	% Reduction of hypertension duration††			
0.1	100	100	100	100
1	68±20	77±22	75±23	77±22
10	40±13	45±14	44±18	41±18
	% Reduction of sinus bradycardia††			
0.1	100	100	100	100
1	90±22	87±24	90±22	86±22
10	67±19	69±21*	76±20*	63±19

Tab 3. Effects of GDEE (After 15 min,†1 and ††10 μg/rat in 2 min) on hypertension and bradycardia induced by K (1 μg/rat in 2 min). n=10, All p<0.01. K 0.1 and 10 μg/rat were also p<0.01.

	Third ventricle	Right lateral ventricle	Posterior hypothalamus	Corpus striatum
Reduction of hypertension acme (%)†	62±24	65±28	60±20	68±22
Reduction of hypertension area (%)†	62±27	61±30	63±27	67±30
Reduction of hypertension duration (%)†	64±19	58±24	64±20	63±23
Reduction of hypertension acme (%)††	72±21	70±19	73±23	76±19
Reduction of hypertension area (%)††	74±24	70±20	73±26	78±22
Reduction of hypertension duration (%)††	69±26	73±26	76±22	76±23
Reduction of sinus bradycardia (%)††	88±23	83±26	87±24	88±28

RESULTS

Cardiovascular responses to intracerebral injection of G and K Intracerebral injection of G 0.1–10 mg/rat or K 0.1–10 μg/rat caused an immediate, transitory and dose-dependent increase in BP (Tab 1) accompanied by a moderate sinus bradycardia with prolongations in P–O and T–P intervals. The elevation of BP was greater after G and K were injected into the 3rd ventricle or the right lateral ventricle than that into the posterior hypothalamus and the corpus striatum (Tab 1). No significant change in maximum increase of arterial pressure (kPa), area (mm^2) and duration (s) of hypertension was seen after injections of G 1 and 10 mg/rat or K 1 and 10 μg/rat into 3rd ventricle repeated at intervals of 10 min. The higher doses of G and K produced high-voltage electrocortical spikes similar to those occurred in epilepsy without any motor convulsions.

Intracerebral injections of *L*-glutamic acid diethylester (GDEE) 1–10 μg/rat, which lowered BP 1.3–2.4 kPa but without significant change in HR, 15 min before G or K, reduced or abolished dose-dependently the cardiovascular responses to G (Tab 2–3).

Effects of several procedures on cardiovascular responses to intracerebral injections of G or K The hypertensive and bradycardic responses to intracerebral injections of G 0.1–10 mg/rat or K 0.1–10 μg/rat into the 3rd or right lateral ventricle, posterior hypothalamus and corpus striatum were reduced (p<0.01) in rats pretreated with reserpine (5 mg/kg *sc* 24 h before), guanethidine (10 mg/kg *im* 12 h before), clonidine (0.1 μg/rat intracerebrally 15 min before) or diltiazem (0.5 mg/kg *iv* in 2 min, 15 min before). See Tab 4–5–6–7.

In bilateral adrenalectomized and spinal rats the cardiovascular effects of intracerebral injection of G or K were reduced (p<0.01), See Tab 6–7.

Tab 4. Effects of reserpine† (5 mg/kg *sc* 24 h before) and guanethidine†† (10 mg/kg *im* 12 h before) on hypertension induced by G 0.1 mg/rat in 2 min. n=10, $\bar{x}$±SD, All p<0.01 *vs* basal value at 0 time. G 1 and 10 mg/rat were also p<0.01.

	Third ventricle	Right lateral ventricle	Posterior hypothalamus	Corpus striatum
Reduction of hypertension acme(%)†	89±26	83±25	80±26	84±26
Reduction of hypertension area(%)†	82±23	87±22	85±26	88±24
Reduction of hypertension redution (%)†	85±19	80±20	87±19	90±20
Reduction of hypertension acme (%)††	78±16	70±16	72±9	68±11
Reduction of hypertension area (%)††	72±19	70±18	70±19	74±23
Reduction of hypertension redution (%)††	72±13	75±21	68±20	75±25

Tab 5. Effects of reserpine† and guanethidine†† on hypertension induced by K (0.1–10 μg/rat in 2 min). n = 10, $\bar{x} \pm SD$. *p>0.05, ***p<0.01.

K	Third ventricle	Right lateral ventricle	Posterior hypo-thalamus	Corpus striatum	K	Third ventricle	Right lateral ventricle	Posterior hypo-thalamus	Corpus striatum
% reduction of hypertension acme†					% reduction of hypertension acme††				
0.1	87±28***	84±25***	82±27***	86±26***	0.1	76±23***	72±21***	74±20***	72±19***
1	63±20***	68±26*	68±20***	64±25***	1	53±28***	59±16***	52±16***	52±19***
10	41±23***	45±24***	42±19***	44±23***	10	31±19*	36±21***	33±16***	35±23***
% reduction of hypertension area					% reduction of hypertension area††				
0.1	80±30***	86±25***	88±23***	87±31***	0.1	74±22***	74±17***	77±17***	78±16***
1	66±25***	69±19***	70±19***	76±23***	1	50±23***	51±19***	54±15***	57±20***
10	43±29***	42±23***	47±19***	48±22***	10	28±17***	31±18***	33±16***	35±11***
% reduction of hypertension duration†					% reduction of hypertension duration††				
0.1	86±27***	79±22***	86±26***	88±26***	0.1	73±13***	70±16***	75±14*	77±22*
1	61±23***	64±27***	64±20***	68±25***	1	48±10*	52±13*	51±12*	54±14*
10	44±22*	46±26*	43±21***	43±23***	10	29±17*	33±17*	34±14*	31±10*

Tab 6. Effects of various procedures on hypertension and sinus bradycardia by G (10 mg/rat in 2 min) n = 10, $\bar{x} \pm SD$. All p<0.01 *vs* basal values at 0 time.

	% Variation of hyper-tension acme	% Variation of hyper-tension area	% Variation of hypertension duration	% Variation of sinus bradycardia
Experiments with clonidine (0.1 μg/rat *icv*)				
Third ventricle	−63±15	−70±12	−72±12	−71±12
Right lateral ventricle	−59±18	−64±15	−68±22	−68±15
Posterior hypothalamus	−65±12	−62±18	−63±18	−69±18
Corpus striatum	−66±15	−61±15	−64±15	−70±22
Experiments with diltiazem (0.5 mg/kg)				
Third ventricle	−68±9	−64±3	−67±12	−71±15
Right lateral ventricle	−72±12	−63±15	−62±18	−72±22
Posterior hypothalamus	−63±22	−62±12	−64±15	−68±18
Corpus striatum	−65±12	−65±15	−69±12	−65±12
Experiments in bisurrenectomized rats				
Third ventricle	−62±12	−65±12	−63±15	−68±12
Right lateral ventricle	−58±15	−61±15	−64±22	−70±18
Posterior hypothalamus	−53±25	−62±18	−67±18	−65±15
Corpus striatum	−61±12	−59±15	−63±12	−62±15
Experiments with spinal transection (C 7)				
Third ventricle	−66±15	−62±9	−63±12	−68±9
Right lateral ventricle	−63±9	−68±12	−62±22	−64±18
Posterior hypothalamus	−68±12	−65±25	−61±18	−65±15
Corpus striatum	−62±22	−66±18	−68±15	−63±12
Experiments with sinus carotid denervation				
Third ventricle	+89±28	+90±15	+96±12	+54±15
Right lateral ventricle	+88±22	+92±18	+94±25	+48±28
Posterior hypothalamus	+85±15	+96±22	+96±22	+42±18
Corpus striatum	+82±22	+95±15	+98±15	+46±12

Tab 7. Effects of various procedures on hypertension and sinus bradycardia by K (10 μg/rat in 2 min). n=10, $\bar{x}\pm SD$, All $p<0.01$.

	% Variation of hypertension acme	% Variations of hypertension area	% Variations of hypertension duration	% Variations of sinus bradycardia
	Experiments with clonidine (0.1 μg/rat *icv*)			
Third ventricle	−64±12	−72±25	−73±22	−72±25
Right lateral ventricle	−60±25	−68±22	−68±18	−66±15
Posterior hypothalamus	−63±15	−64±15	−65±15	−67±15
Corpus striatum	−62±28	−61±25	−66±25	−70±22
	Experiments with diltiazem (0.5 mg/kg)			
Third ventricle	−65±18	−66±22	−68±22	−71±22
Right lateral ventricle	−70±15	−62±18	−63±22	−73±28
Posterior hypothalamus	−63±18	−60±15	−65±25	−67±25
Corpus striatum	−62±25	−63±25	−67±18	−64±25
	Experiments in bisurrenectomized rats			
Third ventricle	−60±25	−64±25	−62±25	−65±25
Right lateral ventricle	−67±22	−60±22	−64±22	−68±22
Posterior hypothalamus	−55±25	−58±22	−67±22	−62±22
Corpus striatum	−60±18	−59±25	−60±28	−60±25
	Experiments with spinal transection (C 7)			
Third ventricle	−65±28	−63±28	−62±28	−67±28
Right lateral ventricle	−62±25	−66±15	−60±25	−65±25
Posterior hypothalamus	−64±22	−64±18	−61±28	−65±28
Corpus striatum	−61±22	−67±15	−67±28	−62±25
	Experiments with carotid sinus denervation			
Third ventricle	+90±25	+88±25	+94±22	+56±25
Right lateral ventricle	+85±28	+92±22	+98±25	+40±22
Posterior hypothalamus	+87±25	+95±25	+94±22	+44±25
Corpus striatum	+84±22	+94±22	+96±22	+42±28

DISCUSSION

The elevation of BP after G and K suggests that this response ensue from the activation of central glutamergic receptors that could induce an enhancement in sympathetic tone at some extracerebral levels. G injected into the cisterna magna of dogs also produce a dose-dependent elevation of BP and a slowing of HR[14].

In normotensive and hypertensive rats the hypertension caused by G applied to rostral ventrolateral medulla was associated with moderate sinus bradycardia[2-4]. The local administration of GDEE reduced the hypertensive response to G or K[4]. Microinjection of GDEE into the glutamatesensitive sites produced a lowering of BP. In the present study GDEE antagonized the action of G and K at the cerebral level.

Systemic administrations of many drugs that deplete catecholamine stores (reserpine, guanethidine) agonize α_2-adrenergic receptors (clonidine), and block calcium channels (diltiazem), reduced the hypertension due to G or K. These data confirm the augmentation in sympathetic efferent activity. The increase of G and K hypertension by atropine, bivagotomy and carotid sinus denervation and the reduction by intracerebral clonidine, bilateral adrenalectomy or spinal transection at C 7 confirm the increase of the sympathetic efferent activity. The partial antagonism of hypertension triggered by

intracerebral G suggests that: in addition to an increase in sympathetic efferent activity (and eventually a decrease in vagal efferent activity), some other substances (eg, vasopressin) released from brain may probably be responsible for the hypertensive response. This study is now in progress in order to clarify this hypothesis.

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大鼠脑内注射谷氨酸和海藻酸的心血管效应

Enrico LAMPA, Mario CAZZOLA, Maurizio ANGRISANI, Concetta MATERA, Michele LISA; Vincenza SUSANNA, Rosanna MARRAZZO, Savino CHIEPPA, Emilio MARMO[1]

(Istituto di Farmacologia e Tossicologia, Prima Facoltà di Medicina e Chirurgia, Università di Napoli, 80138 Napoli, Italy)

摘要　脑内(侧脑室、第三脑室、下丘脑后部和纹状体)注射谷氨酸(G) 0.1-10 μg/大鼠和海藻酸(K)0.1-10 μg/大鼠引起短暂的动脉血压升高和心率徐缓，此作用呈量-效依赖关系，并可被 *icv* *l*-谷氨酸二乙酯盐酸盐 1-10 μg/大鼠所取消．通过耗竭儿茶酚胺，使用外周肾上腺素能拮抗剂，中枢 α_2-肾上腺素能激动剂，Ca^{2+} 透膜阻断剂，神经节阻断剂，肾上腺切除术和脊髓横切等可降低 G-和 K-的升血压作用．双侧迷走神经切除术和颈总动脉窦去神经术后使高血压加剧．心率徐缓似乎是反射地通过颈总动脉窦和主动脉体而介入的．

关键词　谷氨酸；海藻酸；心血管系统；脑内注射

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