

Ventricular arrhythmia evoked by microinjection of picrotoxin into brain areas in rabbits

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ABSTRACT To explore the main areas of brain responsible for arrhythmia evoked by icv picrotoxin (Pic), a very small dose of Pic (3 μg) was injected into different areas of brain in anesthetized rabbits. The short latency of arrhythmia was found after microinjection of Pic into posterior hypothalamus (PH, 5.6 ± 0.9 min) and the medial part of tuberal region (7.5 ± 2.9 min). The ventricular arrhythmia was abolished by vagotomy, iv phentolamine or diazepam, or pretreatment with adrenalectomy or vagotomy. Microinjection of phentolamine or diphenhydramine into PH prior to Pic postponed the arrhythmia.

The results indicate that PH and the medial part of tuberal region are the most effective areas for Pic to induce arrhythmia, activations of both sympatho-adrenomedullary and parasympathetic systems are involved in the genesis of arrhythmia.

KEY WORDS picrotoxin; arrhythmia; hypothalamus; cerebral ventricles; microinjections; phentolamine; vagotomy; adrenalectomy

Recent pharmacological evidences suggest that inhibitory neurotransmitter GABA is involved in cardiovascular regulation. However, most of those researches concentrated on GABAergic regulation of blood pressure⁽¹⁾, whereas little has been known about its influence on arrhythmia. Intracerebroventricular injection of picrotoxin (Pic) in large doses (200-600 μg) could induce ventricular arrhythmia⁽²⁾, but what area responsible for arrhythmia remained unknown. Therefore, the present study was designed to investigate: (1) the sensitive area around cerebral ventricles for GABA antagonist to induce arrhythmia, (2) peripheral mechanism of arrhythmia evoked by blockade of central GABA receptors, (3) interaction between GABA and other

neurotransmitters on arrhythmia.

MATERIALS AND METHODS

Ninety-four rabbits of either sex, weighing $2.3 \pm \text{SD } 0.2$ kg, were anesthetized with a mixture of urethane (350 mg/kg) and α -chloralose (17.5 mg/kg). The trachea was cannulated. Rabbits were immobilized with gallamine triethiodide and artificially ventilated. A femoral artery was cannulated for the measurement of arterial pressure, lead II of electrocardiogram (ECG) and blood pressure were recorded simultaneously on a polygraph. In some rabbits, cervical vagus nerves were isolated in order to be cut, or bilateral adrenalectomy was done 2 h before microinjection of Pic.

Rabbits were mounted in a stereotaxic instrument, stereotaxic coordinates were taken referring to the atlases of Sawyer and Meessen, and corrected according to the size of skull (Fig 1). After surgical operation, a period of about 30 min was allowed to stabilize before microinjection to minimize influence of injury on arrhythmia. Microinjection apparatus consisted of an injection cannula (internal diameter: 0.1 mm) which extended 2 mm beyond the end of a guide cannula. Drug solution (1 μl) or the same volume of vehicle was infused over 2 min using an infusion pump. In each rabbit, Pic was injected into one central site only. After experiments, the coronal sections were cut for verification of injection sites.

Pic (Fluka AG Chemical Co) was prepared by dissolving 15 mg in 0.5 ml of absolute ethanol and then diluted to 5 ml with saline. Phentolamine (Ciba Co), propranolol, diphenhydramine, cimetidine, atropine and

diazepam were ampules.

Statistical tests employed were paired *t* test and *F* test.

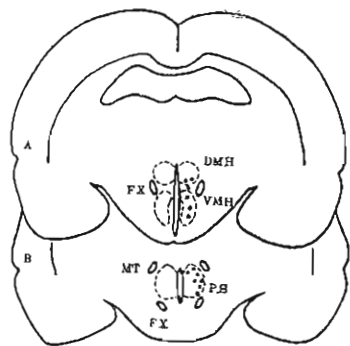


Fig 1. Site of injection of picrotoxin in ventromedial and dorsomedial hypothalamus (VMH + DMH, A), posterior hypothalamus (PH, B) of rabbits. FX = Fornix, MT = Mamillothalamic tract.

RESULTS

Comparative cardiovascular responses to injections of Pic into different areas of brain

The short latency of arrhythmia was found after injection of Pic ($3 \mu\text{g}$) into PH and the medial part of tuberal region (VMH and DMH). The arrhythmia evoked by injection of Pic into the aqueduct usually had a long latency and occurred after a remarkable bradycardia. No arrhythmia was found within 20 min after injection of $3 \mu\text{g}$ of Pic into AH, LH and the 4th ventricle (Tab 1). Simi-

Tab 1. Cardiovascular responses to injections of picrotoxin (Pic, $3 \mu\text{g}$) into different areas of brain in rabbits. $n=6-7$, $\bar{x} \pm \text{SD}$. * $P > 0.05$, ** $P < 0.05$, *** $P < 0.01$ vs baseline. + $P > 0.05$, ++ $P < 0.05$, +++ $P < 0.01$ vs PH group.

Sites of injection	Baseline		Peak changes			Latency of arrhythmia (min)
	MAP (kPa)	HR (bpm)	MAP (kPa)	HR (bpm)	S-T segment (mV)	
PH	12.5 ± 0.8	238 ± 22	6.6 ± 1.8 ***	$-6 \pm 24^*$	0.20 ± 0.08	5.6 ± 0.9
VMH+DMH	12.9 ± 1.0	236 ± 21	5.6 ± 3.0 ***	$-19 \pm 30^*$	$0.15 \pm 0.09^+$	$7.5 \pm 2.9^+$
AH	12.0 ± 0.6	243 ± 14	$0.3 \pm 0.9^*$	$-17 \pm 10^*$	$0.02 \pm 0.02^{++}$	No arrhythmia
LH	13.5 ± 0.7	234 ± 15	2.4 ± 1.0 **	$-10 \pm 13^*$	$0.12 \pm 0.07^{++}$	$38.8 \pm 13.0^{+++}$
Aqueduct	12.8 ± 1.0	275 ± 19	6.1 ± 2.2 ***	-144 ± 47 ***	$0.07 \pm 0.05^{++}$	$9.7 \pm 3.2^{++}$
4th ventricle	13.3 ± 0.6	244 ± 22	$0.9 \pm 0.5^*$	$-39 \pm 30^*$	$0.05 \pm 0.03^{++}$	No arrhythmia

PH: posterior hypothalamus; VMH: ventromedial hypothalamus; DMH: dorsomedial hypothalamus; AH: anterior hypothalamus; LH: lateral hypothalamus.

lar injection of vehicle into any one of above areas did not elicit significant responses.

Influences of some treatments on arrhythmia evoked by injection of Pic into PH The typical results were shown in Fig 2. Vagotomy or iv atropine (0.2 mg/kg) interrupted the ventricular (not atrial) arrhythmia whereas deviation of S-T segment was aggravated. Phentolamine (2 mg/kg) or diazepam (0.5 mg/kg) iv abolished both arrhythmia and change of S-T segment. Propranolol (1 mg/kg) iv had little effect on the ventricular (not atrial) arrhythmia. Bleeding from artery only attenuated but did not abolish the arrhythmia.

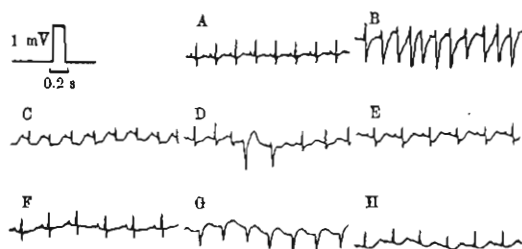


Fig 2. Influences of several treatments on the ventricular arrhythmias induced by microinjection of picrotoxin into PH in anesthetized rabbits. For each rabbit, only one treatment was given about 2 min after ventricular arrhythmia occurred A) Normal ECG; B) After Pic; C) Bilateral vagotomy ($n=6$); D) Bleeding from artery ($n=4$); E) Atropine 0.2 mg/kg iv ($n=4$); F) Phentolamine 2 mg/kg iv ($n=4$); G) Propranolol 1 mg/kg iv ($n=4$); H) Diazepam 0.5 mg/kg iv ($n=5$)

Tab 2. Influences of pretreatment with vagotomy, adrenalectomy or microinjection of some antagonists on the responses to microinjection of Pic (3 μ g) into PH. $n=5$, $\bar{x} \pm$ SD. * $P>0.05$, ** $P<0.05$ *** $P<0.01$ vs control ($n=7$).

Pretreatment	Maximal changes			Latency of arrhythmia (min)
	MAP(%)	HR(%)	S-T segment(mV)	
Control	46.5 \pm 13.2	-2.4 \pm 9.6	0.20 \pm 0.08	5.6 \pm 0.9
Vagotomy	39.6 \pm 9.7*	20.3 \pm 6.6**	0.30 \pm 0.10 **	> 60***
Adrenalectomy	50.7 \pm 18.1*	-0.5 \pm 5.3*	0.13 \pm 0.10*	> 60***
Phentolamine	23.8 \pm 6.7**	-11.3 \pm 10.7*	0.17 \pm 0.05 *	12.3 \pm 3.7**
Propranolol	40.8 \pm 9.4*	-5.4 \pm 20.8*	0.22 \pm 0.13*	6.5 \pm 0.8*
Diphenhydramine	20.0 \pm 4.6**	-0.2 \pm 3.0*	0.10 \pm 0.06**	14.6 \pm 6.0**
Cimetidine	36.8 \pm 6.2*	1.5 \pm 5.2*	0.14 \pm 0.10*	7.4 \pm 2.3*

The values of MAP and HR were expressed as (maximal change / baseline) \times 100. All antagonists (5–10 μ g, 1 μ l) were injected into PH 5 min prior to Pic.

Influences of some pretreatments on arrhythmia evoked by injection of Pic into PH The results were shown in Tab 2. Pretreatment with bilateral adrenalectomy or vagotomy prevented the ventricular arrhythmia with little effect on the magnitude of pressor response. Microinjection of phentolamine or diphenhydramine prior to Pic attenuated pressor response and postponed the arrhythmia. Microinjection of propranolol or cimetidine did not affect the response to Pic.

DISCUSSION

The results with Pic 3 μ g demonstrate that the most sensitive sites of brain for Pic to elicit arrhythmia locate in PH and the medial part of tuberal region, while bradycardia mainly resulted from action on periaqueductal gray. It has been known that hypothalamus around the third ventricle and periaqueductal gray are rich in GABA and its synthetic enzyme^(3,4). Bicuculline (another GABA antagonist) acted on a similar site to elicit pressor response in anesthetized cats⁽⁵⁾.

In our experiment, neither arrhythmia nor significant change of S-T segment occurred within 20 min after injection of Pic 3 μ g into lateral hypothalamus, the 4th ventricle and "the pressor area" of medulla (unpublished observation). These results seem to contradict the findings of others^(6,7). The discrepancy

may be due to large volume and dose (16–200 μ g) used in their experiments, thus, the response could have resulted from the spreading of drug to adjacent tissues, the rostral ventrolateral medulla and PH where potent GABAergic mechanisms located. Being consistent with this possibility, bicuculline injected into "the pressor area" of medulla did not elicit significant response either⁽⁸⁾.

Activations of both sympathetic and parasympathetic systems are involved in Pic-induced arrhythmia, since either bilateral vagotomy or iv phentolamine interrupted this arrhythmia. Activations of both kinds of nerves have a greater arrhythmogenic influence than activation of either one alone⁽⁹⁾. Arrhythmia did not parallel to the magnitude of pressor response judging from the results of treatments and pretreatments. Adrenalectomy did not attenuate the magnitude of pressor response but abolished arrhythmia, which suggests that catecholamines released from the adrenal glands contribute to arrhythmia, mainly through enhancing automaticity in ectopic pacemaker sites. Since the adrenal glands were excised totally in our experiment, it was impossible to determine whether modulation of GABA receptors by glucocorticoids⁽¹⁰⁾ influenced arrhythmia.

PH with its adjacent tissues contains high concentration of norepinephrine⁽¹¹⁾ and his-

tamine⁽¹²⁾. The results of pretreatments with several antagonists in PH indicate that the response to Pic may partly result from the activation of α -adrenoceptor and H₁ receptor indirectly.

Diazepam iv also interrupted the arrhythmia mentioned above, assumingly, through the effect on rostral ventrolateral medulla⁽¹³⁾, but direct activation of benzo-diazepine receptors in myocardium⁽¹⁴⁾ could not be ruled out.

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兔脑区微量注射印防己毒素诱发的室性心律失常

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提要 麻醉兔不同脑区注入微量印防己毒素(Pic, 3 μ g), 以下丘脑后区及结节区内侧部诱发心律失常的潜伏期最短, 分别为 5.6 \pm 0.9 min 和 7.5 \pm 2.9 min. 上述室性心律失常可被双侧迷走神经切除、iv 酚妥拉明或安定、预先肾上腺切除所中止或预防, 也可被预先后区注射酚妥拉明或苯海拉明所延迟. 提示下丘脑后区及结节区内侧部是 Pic 诱发心律失常最主要部位, 交感肾上腺髓质系统及副交感系统激动参与心律失常发生.

关键词 印防己毒素; 心律失常; 下丘脑; 脑室; 微量注射; 酚妥拉明; 迷走神经切断术; 肾上腺切除术