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苯环利定对大鼠急性脑缺血的对抗作用[1]

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Antagonistic effect of phencyclidine on cerebral ischemic damage of rats[1]

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ABSTRACT Dynorphin and catecholamine were measured in ischemic rat produced by four-vessel (2 vertebral arteries and 2 common carotid arteries) occlusion for 10 min. The results showed that: (1) The contents of dynorphine (pg / mg tissue) in cerebral cortex were 5.5 ± 0.6 ($n=7$) in normal rats and decreased to 4.9 ± 0.5 ($n=9$, $P<0.05$) in cerebral ischemic rats; with immediate ip phencyclidine (1-(1-phenylcyclophexyl)piperidine, PCP, 1 mg · kg^{-1}), the contents of dynorphin were increased to 5.3 ± 0.4 ($n=5$, $P<0.05$ *vs* the ischemic rats). (2) The contents of DOPAC (pg / mg tissue) in cerebral cortex were 38 ± 6 ($n=7$) and increased to 120 ± 60 ($n=5$, $P<0.05$) in 10 min cerebral ischemic rats; with immediately ip PCP (1 mg · kg^{-1}), the contents of DOPAC were decreased to 26 ± 13 ($n=7$, $P<0.05$ vs the ischemic rats). (3) The release of DA (pg / mg tissue) in cortical slices *in vitro*, in high K^+ solution were 24 ± 3 ($n=5$) and significantly increased to 57 ± 15 ($n=5$, $P<0.05$) in ischemic rat brain slices; with immediate ip PCP (1 mg · kg^{-1}), the contents of DA were decreased to 38 ± 10 ($n=5$, $P<0.05$ *vs* the ischemic rats). These results suggest PCP play an antagonistic role in cerebral ischemic damage of rats.

KEY WORDS phencyclidine; dynorphin; dopamine; radioimmunoassay; high pressure liquid chromatography; cerebral ischemia

提要 本文采用结扎大鼠双侧椎动脉、双侧颈总动脉造成大鼠急性脑缺血模型来研究苯环利定(phencyclidine, PCP)对脑缺血的影响。实验结果表明：PCP 能抑制缺血后皮质组织中强啡肽含量的降低及 DOPAC 含量的增加，以及离体皮质脑片 DA 释放的增加。提示：PCP 对脑缺血有拮抗作用。

关键词 苯环利定；强啡肽；多巴胺；放射免疫测定；高压液相色谱法；脑缺血

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本实验室已经报道了强啡肽和 PCP 在调节外周血管的舒缩活动中起着相反的作用[1,2]，接着又发现脑血管上存在着 PCP 结合位点[3]，并且 PCP 能增强电场刺激引起脑血管收缩[4]，提示：脑血管上有 PCP 受体。与此同时，国外又有文献报道 PCP 及其类似物 dizocilpine maleate (MK-801)能非特异地拮抗兴奋性氨基酸[5]，而后者被认为是与脑缺血所引起的神经细胞坏死密切相关[6]，已有文献报道 MK-801 有抗脑缺血作用[7]。而 PCP 对脑缺血是否有作用，未见报道。本文采用大鼠四动脉结扎模型[8]来观察 PCP 对强啡肽以及单胺类递质含量的影响，来说明 PCP 对脑缺血的作用。

MATERIALS AND METHODS

大鼠 Sprague-Dawley 种，♂，体重 250 ± SD 40 g，由本校实验动物部提供。

药品 PCP，白色粉末，由本校药学院合成。

脑匀浆液制备 分离出大鼠双侧颈总动脉和双侧锥动脉，永久性地结扎双侧椎动脉，24 h 后再将双侧颈总动脉夹闭，造成大鼠急性脑缺血，同时注射生理盐水(1 mg · kg^{-1})或者 PCP(1 mg · kg^{-1})，10 min 后进行再灌注，2 h 后断头处死，迅速取出大脑，分四个脑区，匀浆，25 000 × g，离心 30 min，取上清液，冰冻抽干后，用放射免疫法测各个脑区的

强啡肽含量；或者直接用于单胺类递质的测定．脑匀浆组织称重．

脑片制备 脑区置于饱和 95% O_2 + 5% CO_2 的人工正常脑脊液中，沿着额极向枕极的方向，切成厚度为 0.5–1.0 mm 的冠状切片，放入 1 ml 饱和 95% O_2 + 5% CO_2 的人工高 K^+脑脊液中，37℃水浴 15 min 后，抽取全部人工脑脊液，25 000×g，离心 30 min，上清液用于单胺类递质的测定，脑片称重．

放射免疫测定强啡肽法[9] 测定药盒购自第二军医大学神经生物学教研室．

高压液相色谱法[10] 以 D–樟脑–β–磺酸为离子对试剂反相高压液相色谱测定大鼠脑组织及脑片中儿茶酚胺含量．

数据处理 数据用 $\bar{x}\pm$ SD 表示，用组间 t 检验显著性．

RESULTS

PCP 对脑缺血时脑内强啡肽含量的影响 大鼠大脑皮质区域，脑缺血时强啡肽的含量明显低于未缺血大鼠($P<0.05$)，而在缺血同时 ip PCP 1 mg·kg^{-1} 的大鼠，其大脑皮质强啡肽的含量要比缺血组高($P<0.05$)，与未缺血组相比，无显著差别(Tab 1)．

下丘脑，纹状体，海马区域在脑缺血组强啡肽的含量均比未缺血组略低些，但统计不显

Tab 1. Contents of immunoreactive dynorphin A1–13 (pg / mg tissue) in brain areas before and after ip PCP 1 mg·kg^{-1}. Number of rats in parenthesis. $^{*}P>0.05$, $^{**}P<0.05$ *vs* normal rats. $^{+}P>0.05$, $^{++}P<0.05$ *vs* NS group in ischemic rats.

Group	Cortex	Hypothalamus	Striatum	Hippocampus
Normal rats				
NS	5.46 ± 0.60 (7)	100 ± 60 (12)	110 ± 40 (13)	60 ± 30 (13)
PCP	5.04 ± 0.77 (10)	90 ± 60 (8)	90 ± 60 (8)	60 ± 20 (9)
Ischemic rats				
NS	4.89 ± 0.50 (11)**	70 ± 30 (9)*	90 ± 30 (13)*	46 ± 10 (13)*
PCP	5.32 ± 0.38 (9)$^{++}$	90 ± 50 (10)$^{+}$	90 ± 30 (9)$^{+}$	49 ± 7 (9)$^{+}$

Tab 2. Contents of catecholamine (pg / mg tissue) in brain areas before and after ip PCP 1 mg·kg^{-1}. $^{*}P>0.05$, $^{**}P<0.05$ *vs* normal rats. $^{+}P>0.05$, $^{++}P<0.05$ *vs* NS group in ischemic rats.

Brain region	Group (n)	DOPAC	DA	5-HIAA	HVA	5-HT
Cortex	Normal rats					
	NS (7)	38 ± 6	1 100 ± 140	69 ± 6	42 ± 20	180 ± 40
	PCP (6)	46 ± 7	860 ± 180	80 ± 4	60 ± 50	160 ± 40
	Ischemic rats					
	NS (5)	120 ± 60**	1 100 ± 40*	90 ± 9**	60 ± 40*	185 ± 13*
	PCP (7)	25 ± 13^{++}	1 700 ± 700^{+}	70 ± 40^{+}	30 ± 27^{+}	110 ± 70^{+}
Hypothalamus	Normal rats					
	NS (6)	27 ± 100	340 ± 60	210 ± 70	9 ± 6	450 ± 120
	PCP (6)	50 ± 18	360 ± 80	280 ± 60	33 ± 17	460 ± 120
	Ischemic rats					
	NS (5)	60 ± 20**	410 ± 80*	380 ± 60*	16 ± 5*	610 ± 800*
	PCP (7)	190 ± 120^{+}	372 ± 24^{+}	420 ± 70^{+}	16 ± 4^{+}	660 ± 140^{+}

Tab 3. Release of carecholamine (pg / mg tissue) from brain slices before and after ip PCP 1 mg · kg^{-1} in high potassium (40 mmol · L^{-1}) bath. $^{*}P>0.05$, $^{**}P<0.05$ *vs* normal rats. $^{+}P>0.05$, $^{++}P<0.05$ *vs* NS group in ischemic rats.

Brain region	Group (*n*)	DOPAC	DA	5-HIAA	HVA
Cortex	Normal rats				
	NS (5)	207 ± 21	24 ± 3	102 ± 23	45 ± 6
	PCP (5)	120 ± 90	21 ± 6	96 ± 14	32 ± 11
	Ischemic rats				
	NS (5)	260 ± 80*	57 ± 15**	134 ± 19**	58 ± 21*
	PCP (5)	150 ± 40^{++}	38 ± 10^{++}	120 ± 50^{+}	33 ± 15^{+}
Hypothalamus	Nomal rats				
	NS (5)	86 ± 27	13 ± 9	230 ± 100	10 ± 13
	PCP (5)	71 ± 38	17 ± 6	190 ± 40	23 ± 11
	Ischemic rats				
	NS (7)	65 ± 21*	18 ± 5*	160 ± 40*	9 ± 6*
	PCP (8)	78 ± 30^{+}	16 ± 5^{+}	180 ± 70^{+}	16 ± 6^{+}

著($P>0.05$).

PCP 对脑缺血时脑内儿茶酚胺含量的影响 大脑皮质区域，脑缺血时双羟苯乙酸(3,4-dihydroxyphenyl acetic acid, DOPAC)的含量低于未缺血大鼠($P<0.05$)，而在缺血同时 ip PCP 1 mg · kg^{-1} 的大鼠，则皮质 DOPAC 要比缺血组大鼠高($P<0,05$)，与未缺血组相比，差别不显著.

而 5-羟吲哚乙酸(5-hydroxyindole-3-acetic acid, 5-HIAA)的含量，在缺血时明显高于未缺血大鼠($P<0.05$)，缺血后，立即给予 PCP 则 5-HIAA 的含量有所下降，但不显著($P>0,05$) (Tab 2).

PCP 对脑缺血时脑片释放儿茶酚胺的影响 脑缺血时，大鼠大脑皮质区域脑片释放多巴胺(dopamine, DA)的量高于未缺血组大鼠($P<0.05$)，而在缺血同时 ip PCP (1 mg · kg^{-1})，皮层脑片释放 DA 的量下降，与缺血大鼠相比，差别显著($P<0.05$)；在缺血时，皮质脑片释放 DOPAC 的量与未缺血相比差别不显著，而在缺血同时 ip PCP 脑片释放 DOPAC 的量减少($P<0.05$) (Tab 3).

DISCUSSION

本文在大鼠四动脉结扎造成脑缺血模型上观察到脑组织在缺血 10 min / 再灌注 2 h 后，强啡肽在皮质的含量有所下降，给予 PCP 能够抑制这一下降，Handa *et al* 报道过蒙古沙鼠脑缺血时给予强啡肽可以改善运动神经元的功能[11]，提示：脑内强啡肽含量的升高能够减轻脑缺血.

已有文献报道：脑缺血与脑内单胺类递质变化有关[12,13]，所以，我们以单胺类递质变化为另一个指标来观察 PCP 对脑缺血的影响. 结果表明：脑缺血时，大脑皮质 DOPAC 含量升高，这可能是由于缺血时，增加了 DA 的合成，释放和降解，或者是降低了 DA 的清除率. 从离体脑片释放模型中观察到：缺血后，DA 的释放明显增加，这与文献[14]报道是一致的. 而给予 PCP 后，DA 释放明显减少了，提示：PCP 可能主要是通过抑制了脑缺血时 DA 的释放，从而起到减轻脑缺血的作用.

在海马、纹状体、下丘脑区域，缺血后我们未见到明显的强啡肽和儿茶酚胺的变化，可

能是由于本实验模型缺血时间较短，并且缺血后 10 min 又立即再灌注，所以只引起轻度的急性脑缺血，表现为：大脑皮质缺血损伤明显，而其他区域则不明显，提示在脑缺血时，最先累及的是大脑皮质区域.

脑缺血后究竟是 PCP 本身有保护脑细胞作用，还是由于它引起的递质变化而起到了保护脑细胞的作用，或者是两者协调作用，还有待于研究.

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