文章编号: 1006-2858(2006) 11-0715-05

地卓西平全身和局部给药对小鼠前皮层多巴胺及其代谢产物和行为学的影响

左代英, 李 霞, 任利翔, 王佳平, 吴英良
(沈阳药科大学 药学院, 辽宁 沈阳 110016)

摘要: 目的 研究地卓西平(MK-801)全身和局部给药对小鼠前皮层(PFC)中多巴胺(DA)及其代谢产物二羟苯乙酸(DOPAC)和高香草酸(HVA)的影响及行为学改变。方法 通过微透析结合高效液相色谱-电化学法(HPLC-ECD)检测DA及其代谢产物, 同时监测小鼠的自主活动。结果 腹腔注射MK-801 (0.6 mg·kg^{-1})能明显增加小鼠PFC中DA、DOPAC和HVA的水平, 并明显增加小鼠自主活动次数; 向小鼠PFC中局部灌注MK-801(10、50和250 μmol·L^{-1})可以剂量依赖性地增加PFC中DA的水平和小鼠的自主活动次数, 但对DOPAC和HVA没有明显的影响; 无论全身给药还是局部灌注给药, 小鼠PFC中DA的水平与自主活动呈明显的正性相关。结论 MK-801至少部分上是直接作用于PFC的DA能神经元促进DA的释放, DA参与MK-801诱导的行为反应。

关键词: 地卓西平; 微透析; 多巴胺; 自主活动; 精神分裂症
中图分类号: R 964 文献标识码: A

精神分裂症(schizophrenia)是危害人类健康的一大顽疾, 其发病机制目前尚不完全清楚。近年来, 精神分裂症的NMDA受体功能低下假说得到了前所未有的关注, 一些NMDA受体拮抗剂如苯环己哌啶(phencyclidine, PCP)能够诱导人和动物产生精神分裂症样症状[1,2]。地卓西平(dizocilpine, MK-801)与PCP类似, 是NMDA受体的非竞争性拮抗剂。作者前期的研究表明, MK-801能够诱导小鼠产生运动亢进、定型反应和共济失调等精神分裂症样行为表现, 与文献[3]报道相一致。有研究认为, NMDA受体拮抗剂所诱导的行为反应至少部分上有多巴胺(dopamine, DA)能机制的参与, 因为一些基于DA能假说的抗精神病药如氟哌啶醇能够阻断PCP所诱导的活动亢进和定型行为[4,5]、DA耗竭可以拮抗MK-801诱导的行为反应[6], 并且MK-801诱导运动亢进的阈剂量在单胺能系统完整的动物要比单胺耗竭的动物低10倍左右[7]。鉴于二者之间的密切联系, 而现有这方面的研究多集中于全身给药对大鼠不同脑区单胺递质的考察, 且结果存在不一致性[8,9], 所以作者进行以下一些实验来系统考察MK-801全身给药以及前皮层(Prefrontal cortex, PFC)局部灌注给药对小鼠PFC中DA及其代谢产物水平的影响, 希望能为本模型的行为机制提供更进一步的实验依据。

1 仪器与材料

EP-10型高效液相色谱仪(日本Eicom公司), ECD-100电化学检测器(日本Eicom公司), S-10型自动进样器(日本Eicom公司), EP-50型微透析泵(日本Eicom公司), JS-3050色谱工作站(大连江申分离科学技术公司), 江湾Ⅰ型C07-2B脑立体定位仪(第一军医大学), 佳能摄像系统(日本Canon公司)。

MK-801(美国Sigma公司), Ringer's液: 140 mmol·L^{-1} NaCl, 3.35 mmol·L^{-1} KCl, 1.26 mmol·L^{-1} $CaCl_2$, 1.15 mmol·L^{-1} $MgCl_2$, 1.35 mmol·L^{-1} Na_2HPO_4, 0.3 mmol·L^{-1} NaH_2PO_4, pH 7.4, 临用前用0.22 μm微孔滤膜抽滤。

昆明种小鼠, 雄性, 体质量25~30 g, 沈阳药科大学实验动物中心提供, 动物合格证号: SCXK(辽)2003-008。

收稿日期: 2006-02-08
作者简介: 左代英(1978-), 女(汉族), 黑龙江依兰人, 博士研究生; 吴英良(1957-), 男(汉族), 辽宁本溪人, 教授, 博士生导师, 主要从事神经精神药理学方面的研究, **Tel**. 024-23986278, **E-mail** yingliang_1016@163.com。

© 1994-2010 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net

2 方法

2.1 脑微透析方法

小鼠腹腔注射水合氯醛(350 mg·kg^{-1})麻醉后,固定于脑立体定位仪上。切开头部皮肤,暴露颅骨,在冠状缝水平向前1 mm,左右各3 mm处,用牙科钻在左右两侧各钻一孔,将自制的透析管横向植入前皮层内,用牙科水泥固定。术后18~24 h待小鼠苏醒恢复正常后进行灌流实验。将透析管与微透析泵相连,用Ringer's液以4 μL·min^{-1}恒速灌流。弃去前30 min的流出液,以后每20 min收集一个样品,待给药前连续3个样品中所测得的样品含量基本稳定后,进行全身给药(MK-801,0.6 mg·kg^{-1},腹腔注射)或局部灌注给药(MK-801 10、50或250 mmol·L^{-1}分别溶解于Ringer's液中)。测定给药后200 min内前皮层DA及其代谢产物DOPAC和HVA含量的变化。实验结束后,将动物断头,检查透析管埋植位置是否正确,位置不正确的动物,其数据弃之[10]。

2.2 DA及其代谢产物的含量测定

精确吸取透析液20 μL,立即注入HPLC-ECD系统中,对各个单胺递质的含量进行检测。色谱条件:Wakosil-Ⅱ5 C_{18}(4.6 mm×150 mm,5 μm)色谱柱(日本,和光纯药株式会社),流动相:体积分数为10%的甲醇溶液,0.1 mol·L^{-1}柠檬酸,0.1 mol·L^{-1}醋酸钠,0.05 mol·L^{-1}乙二胺四乙酸二钠(EDTA-Na_2),65 mg·L^{-1}辛基磺酸钠,pH=3.8,流速:1.0 mL·min^{-1}。电化学检测器工作电压:+0.75 V。

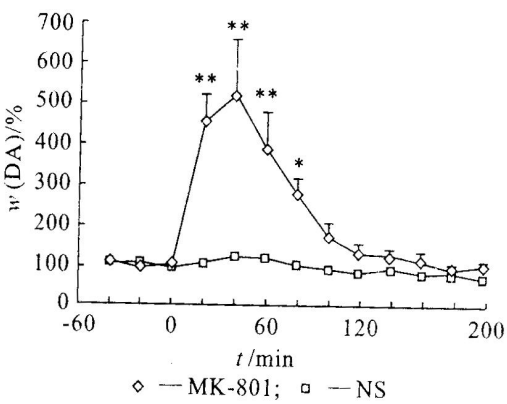

Fig. 1 Effect of systemic administration of MK-801 (0.6 mg·kg^{-1}, ip) on extracellular DA levels in the PFC of mice * $P<0.05$, * * $P<0.01$ compared with the corresponding vehicle group

2.3 行为学实验

在脑内微透析的同时监测小鼠自主活动,将小鼠放于一旷野箱中,旷野箱规格为40 cm×30 cm×20 cm,底部划分为20个等格,用摄像机记录小鼠的自主活动。在给药前15~5 min间及从给药5 min后开始每隔10 min记录1次,直至给药后200 min。回放计数小鼠自主活动次数,以小鼠四肢走到同1格内为走动1格。

2.4 统计学处理

将给药前3个连续样品测定值的平均值作为基础值,数据以$\bar{x}$±S.E.M表示。给药后每20 min的变化以基础值的百分数表示,绘制曲线图,采用反复测定方差分析判断组间总体显著性和交互作用;各时间点的数值与生理盐水组相应值采用Student's t-test进行统计分析,同时计算每只小鼠的DA水平与自主活动的Pearson's相关系数。$P<0.05$判定有统计学意义。

3 结果

3.1 全身给药和局部给药对小鼠DA及其代谢产物的影响

如图1所示,MK-801(0.6 mg·kg^{-1},ip,n=9)能明显增加小鼠PFC细胞外液中DA水平(20~200 min内,$F_{1,16}$=13.890,P=0.002)。给药后细胞外液中DA水平即开始升高,在20~40 min达到峰值(521.8%),120 min左右回到基础水平。DA的代谢产物DOPAC(图2,20~200 min内,$F_{1,18}$=11.918,P=0.003)和HVA水平(图3,20~200min内,$F_{1,18}$=6.756,P=0.018)在PFC

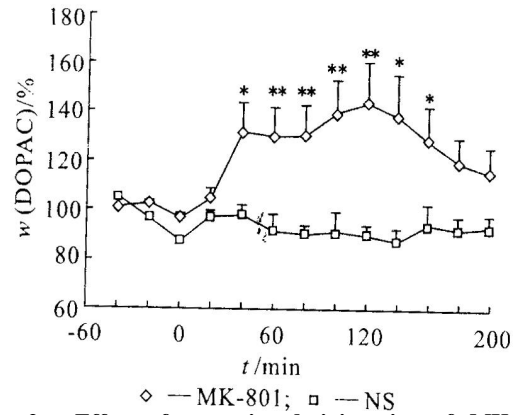

Fig. 2 Effect of systemic administration of MK-801 (0.6 mg·kg^{-1}, ip) on extracellular DOPAC levels in the PFC of mice * $P<0.05$, * * $P<0.01$ compared with the corresponding vehicle group

© 1994-2010 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net

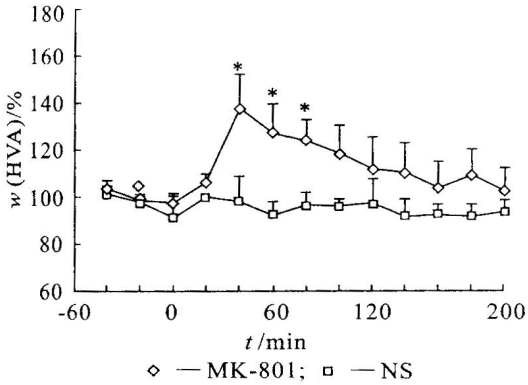

Fig. 3　Effect of systemic administration of MK-801 (0.6 mg·kg^{-1}, ip) on extracellular HVA levels in the PFC of mice. * $P<0.05$ compared with the corresponding vehicle group

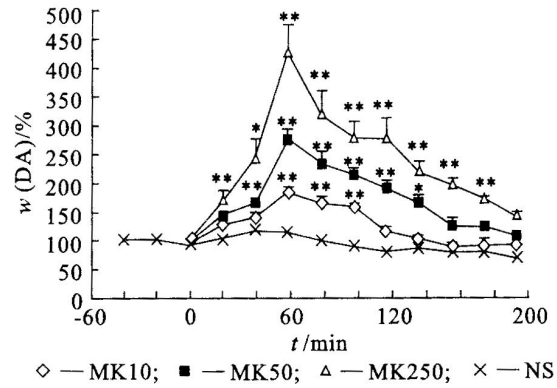

Fig. 4　The time course for the extracellular levels of DA after perfusion of 10, 50, 250 μmol·L^{-1} MK-801 into the mice PFC. The arrow indicates the time of MK-801 perfusion. * $P<0.05$, ** $P<0.01$ compared with the corresponding vehicle group

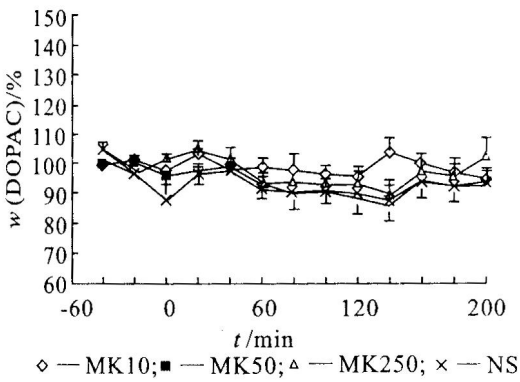

Fig. 5　The time course for the extracellular levels of DOPAC after perfusion of 10, 50, 250 μmol·L^{-1} MK-801 into the mice PFC. The arrow indicates the time of MK-801 perfusion

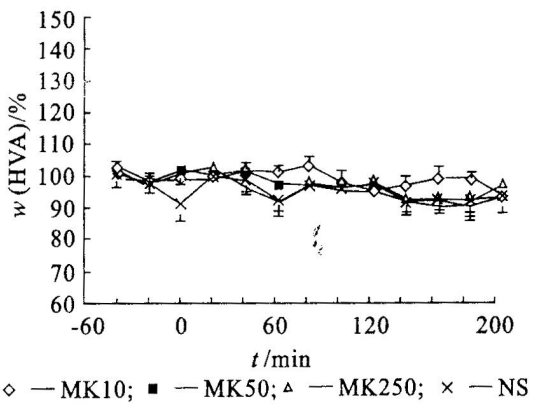

Fig. 6　The time course for the extracellular levels of HVA after perfusion of 10, 50, 250 μmol·L^{-1} MK-801 into the mice PFC. The arrow indicates the time of MK-801 perfusion

细胞外液也有不同程度的升高。MK-801(10、50 和 250 μmol·L^{-1})于小鼠 PFC 中局部灌注给药可以剂量依赖性的增加 PFC 细胞外液中 DA 水平,最大增加值分别为 183.6%、274.2% 和 425.8%(图 4)。PFC 局部灌注给药对 DA 的代谢产物 DOPAC 和 HVA 没有明显的影响(图 5、6)。

3.2　全身给药和局部给药对小鼠行为学的影响

微透析试验的同时,作者同步观察了小鼠的自主活动。结果表明,生理盐水对照组小鼠在整个实验过程中多在旷野箱内处于安静状态,睡眠或清醒,偶尔有少量的正常探求行为。MK-801(0.6 mg·kg^{-1}, ip, $n=9$)给药后能明显增加小鼠自主活动的次数(图 7; 5~195 min 时间内, $F_{1,16}=46.680$, $P<0.001$),自主活动的最大值在给药后的 25~35 min 内。而且,给药组所有的小鼠均表现有定型行为。MK-801(10、50 和 250 μmol·L^{-1})于 PFC 局部灌注给药 60 min 能够剂量依赖性地增加小鼠的自主活动次数,并延长其作用时间(图 8)。

3.3　MK-801 全身给药和局部给药后小鼠自主活动与 DA 的相关性

在所有全身给药的小鼠中,透析液中 DA 的水平与小鼠的自主活动呈正相关(r 在 0.658, $P<0.05$ 和 0.826, $P<0.01$ 之间, df=9)。不同浓度 MK-801 局部灌注给药时,透析液中 DA 水平与自主活动均呈正相关(10 μmol·L^{-1}时, r 在 0.633, $P<0.05$ 和 0.871, $P<0.01$ 之间, df=8; 50 μmol·L^{-1}时, r 在 0.682, $P<0.685$ 和 0.802, $P<0.01$ 之间, df=8; 250 μmol·L^{-1}时, r 在 0.669, $P<0.05$ 和 0.892, $P<0.01$ 之间, df=8)。

© 1994-2010 China Academic Journal Electronic Publishing House. All rights reserved.　http://www.cnki.net

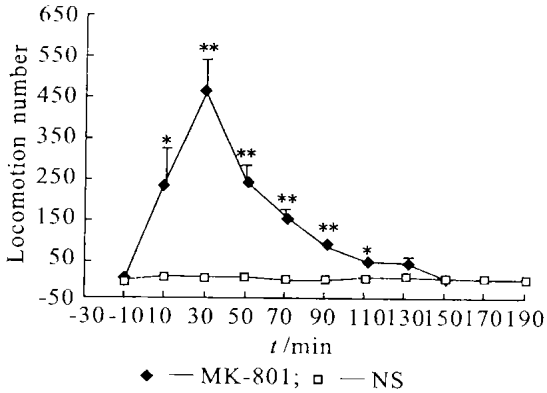

Fig. 7 Effect of systemic administration of MK-801 (0.6 mg·kg^{-1}, ip) on open-field locomotion of mice * $P<0.05$, * * $P<0.01$ compared with the corresponding vehicle group

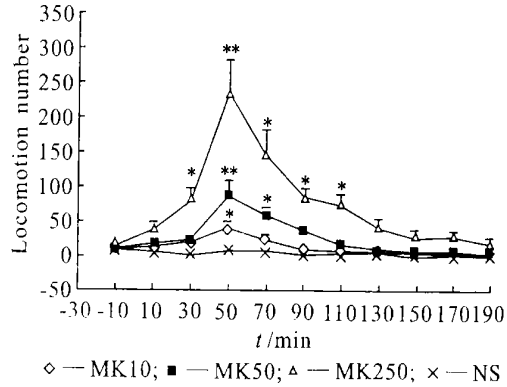

Fig. 8 The time course for the locomotion after perfusion of 10, 50, 250 μmol·L^{-1} MK-801 into the mice PFC. The arrow indicates the time of MK-801 perfusion. * $P<0.05$, * * $P<0.01$ compared with the corresponding vehicle group

4 讨论

作者首次采用微透析结合行为学的实验方法,对MK-801局部灌注或全身给药后小鼠PFC细胞外液中DA、DOPAC和HVA水平的变化及DA水平与自主活动之间的关系进行了系统的研究。结果表明,MK-801单次给药可明显增加小鼠PFC细胞外液中DA水平,其代谢产物DOPAC和HVA水平也有不同程度的升高。MK-801局部给药同样能够剂量依赖性地增加小鼠PFC中DA水平,而对DOPAC和HVA水平没有明显的影响。自主活动与PFC中DA水平的相关性研究表明,无论全身给药还是局部灌注,小鼠PFC中DA的水平与自主活动呈明显的正性相关,说明DA在MK-801所诱导的行为反应中发挥着重要的作用。

研究表明,位于DA能神经末稍突触前的NMDA受体兴奋能够促进DA的释放,而位于GABA抑制性神经元上的NMDA受体则对DA的释放发挥负反馈性抑制作用[11]。虽然在体内实验中两种作用均可以观察到,但Carrozza等人[12]认为,生理状态下NMDA受体对DA的突触前兴奋性作用有限,而抑制性作用占主导地位。NMDA受体拮抗剂可能正是减弱了NMDA受体对DA释放的抑制作用,从而促进了DA的释放,使得小鼠前皮层细胞外液中DA水平明显升高。Zhang等人[13]的研究结果也提示,非竞争性NMDA受体拮抗剂如MK-801对中脑DA能神经元的兴奋作用部分是由于GABA能中间神经元的失抑制所致。French等人[14]研究证明,MK-801能够增加腹侧被盖A10区DA能神经元的放电频率。因为神经元放电频率的增加与神经末稍DA的释放密切相关,所以在本实验中MK-801所引起的PFC中DA水平的增加应该与边缘系统DA能神经元放电频率的增加有关。

局部给药与全身给药的结果相似,MK-801能够剂量依赖性的增加小鼠PFC中的DA水平,说明MK-801能够直接作用于PFC中的DA能神经元而增加DA的释放。另外,与全身给药有所不同的是,局部给药对PFC中的DOPAC和HVA水平没有明显的影响,说明PFC中DA的代谢过程可能还有皮层以外的其他一些机制参与。

作者前期的研究结果表明,MK-801单次或多次给药能够明显改变PFC中Glu和AA水平[15],而文献报道Glu能神经传递能够影响精神分裂症患者PFC中DA系统的功能[16],所以推测MK-801对DA的作用至少部分是与Glu和AA能神经传递相关的。一些研究表明,NMDA受体主要存在于PFC的外层区域[17],而皮层的DA能神经元多数是位于PFC的内层[18],因此Glu与DA之间的作用还可能存在间接作用,其具体机制还有待于进一步的研究。

参考文献:

[1] Jentsch JD, Roth RH. The neuropsychopharmacology of phencyclidine: From NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia [J]. Neuropsychopharmacology, 1999, 20(3): 201-225.

[2] Newcomer JW, Farber NB, Jevtovic-Tedorovic V, *et al*. Ketamine-induced NMDA receptor hypofunction as

© 1994-2010 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net

a model of memory impairment and psychosis [J]. Neuropsychopharmacology, 1999, 20(2): 106– 118.

[3] Brosnarr-Watters G, Wozniak DF, Nardi A, *et al*. Acute behavioral effects of MK-801 in the mouse [J]. Pharmacol Biochem Behav, 1996, 53(3): 701– 711.

[4] Ouagazzal A, Nieoullou A, Amalric M. Effects of dopamine D1 and D2 receptor blockade on MK-801-induced hyperlocomotion in rats [J]. Psychopharmacology(Berl), 1993, 111(4): 427– 434.

[5] Gattaz WF, Schummer B, Behrens S. Effects of zotepine, haloperidol and clozapine on MK-801-induced stereotypy and locomotion in rats [J]. J Neural Transm Gen Sect, 1994, 96(3): 227– 232.

[6] Mele A, Castellano C, Felici A, *et al*. Dopamine-N-methyl-D-aspartate interactions in the modulation of locomotor activity and memory consolidation in mice [J]. Eur J Pharmacol, 1996, 308(1): 1– 12.

[7] Carlsson ML, Carlsson A. The NMDA antagonist MK-801 caused marked locomotor stimulation in monamine-depleted mice [J]. J Neural Transm, 1989, 75: 221– 226.

[8] Löscher W, Armies R, Htinack D. The N-methyl-D-aspartate receptor antagonist MK-801 induces increases in dopamine and serotonin metabolism in several brain regions of rats [J]. Neurosci Lett, 1991, 128(2): 191– 194.

[9] Gandolfi O, Rimondini R, Dall' Olio R. The modulation of dopaminergic transmission in the striatum by MK-801 is independent of presynaptic mechanisms [J]. Neuropharmacology, 1992, 31(1): 1111– 1114.

[10] Wu CF, Bertorelli R, Sacconi M, *et al*. Decrease of brain acetylcholine release in aging freely moving rats detected by microdialysis [J]. Neurobiol Aging, 1988, 9(4): 357– 361.

[11] Whitton PS. Glutamatergic control over brain dopamine release *in vivo* and *in vitro* [J]. Neurosci Biobehav Rev, 1997, 21(4): 481– 488.

[12] Carrozza DP, Ferraro TN, Golden GT, *et al*. *In vivo* modulation of excitatory amino acid receptors: microdialysis studies on N-methyl-D-aspartate-evoked striatal dopamine release and effects of antagonists [J]. Brain Res, 1992, 574(1– 2): 42– 48.

[13] Zhang J, Chiodo LA, Freeman AS. Effects of phencyclidine, MK-801 and 1, 3-di(2-tolyl)guanidine on nondopaminergic midbrain neurons [J]. Eur J Pharmacol, 1993, 230(3): 371– 374.

[14] French ED, Mura A, Wang T. MK-801, phencyclidine (PCP), and PCP-like drugs increase burst firing in rat A10 dopamine neurons: comparison to competitive NMDA antagonists [J]. Synapse, 1993, 13(2): 108– 116.

[15] Zuo DY, Zhang YH, Cao Y, *et al*. Effect of acute and chronic MK-801 administration on extracellular glutamate and ascorbic acid release in the prefrontal cortex of freely moving mice on line with open-field behavior [J]. Life Sci, 2006, 78: 2172– 2178.

[16] Bunney BG, Potkin SG, Bunney WE Jr. New morphological and neuropathological findings in schizophrenia: a neurodevelopmental perspective [J]. Clin Neurosci, 1995, 3(2): 81– 88.

[17] Cotman CW, Monaghan DT. Anatomical organization of excitatory amino acid receptors and their properties [J]. Adv Exp Med Biol, 1986, 203: 237– 252.

[18] Van Eden CG, Hoomeman EMD, Buijs RM, *et al*. Immunocytochemical localization of dopamine in the prefrontal cortex of the rat at the light and electron microscopical level [J]. Neuroscience, 1987, 22(3): 849– 862.

[19] Yonezawa Y, Kuroki T, Kawahara T, *et al*. Involvement of γ-aminobutyric acid neurotransmission in phencyclidine-induced dopamine release in the medial prefrontal cortex [J]. Eur J Pharmacol, 1998, 341(1): 45– 56.

Differential effects of systemic and local dizocilpine (MK-801) administration on dopamine and it's metabolites levels in mice prefrontal cortex on line with open-field behavior

(下转至第 738 页)

© 1994-2010 China Academic Journal Electronic Publishing House. All rights reserved.　http://www.cnki.net

[9] Pohl M, Mesch K, Rodenbrock A, *et al*. Stability investigations on the pyruvate decarboxylase from *Zymomonas mobilis* [J]. Biotechnol Appl Biochem, 1995, 22: 95-105.

[10] Gunter G, Peter L, Martinal P. Continuous production of (R)-phenylacetylcarbinol in an enzyme-membrane reactor using a potent mutant of pyruvate decarboxylase from *Zymomonas mobilis* [J]. Biotechnol Bioeng, 2001, 74: 317-325.

Molecular cloning and expression of pyruvate decarboxylase gene from *Zymomonas mobilis*

REN Jie, GUO Li-qing, JIA-Xian, JIANG Ya-hong, YOU Song

(School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang 110016, China)

Abstract: Objective To clone and express the pyruvate decarboxylase(PDCzm) from *Zymomonas mobilis*. **Methods** To clone PDCzm gene from its genomic DNA by PCR, construct its expressing plasmid and express its encoding protein by using Ion and ompT protease deficient strain *Escherichia coli* BL21(DE3). **Results** PDCzm gene(ca. 1.7kb) was obtained and its expressing plasmid pZM-22b was successfully constructed. SDS-PAGE result showed that the overexpression of PDCzm was achieved and the target protein reached 37.9% among the total proteins in its host strain. **Conclusions** The engineering strain for overexpression PDCzm was successfully constructed, which set up a base for the biotransformation using PDCzm as catalyst.

Key words: pyruvate decarboxylase; *Zymomonas mobilis;* PCR; clone; overexpression

(上接第719页)

ZUO Dai-ying, LI Xia, REN Li-xiang, WANG Jia-ping, WU Ying-liang

(School of Pharmacy, Shenyang Pharmaceutical University, Shenyang 110016, China)

Abstract: Objective To investigate the effects of systemic administration and local infusion dizocilpine(MK-801) on extracellular dopamine(DA), 3, 4-dihydroxyphenylacetic acid(DOPAC) and homovanillic acid (HVA) levels in mouse prefrontal cortex(PFC) and behaviors. **Methods** High-performance liquid chromatography and electrochemical detection(HPLC-ECD) and *in vivo* microdialysis were used on line with open-field behavior. **Results** Extracellular DA, DOPAC and HVA levels in the PFC and the locomotion numbers of mice were significantly increased after systemic MK-801(0.6 mg·kg^{-1}, ip) administration. Extracellular DA levels and the locomotion numbers of mice were dose-dependently increased after MK-801(10, 50 and 250 μmol·L^{-1}) local infusion to the PFC. DOPAC and HVA levels were unaffected after MK-801 local infusion. In all systemic and local MK-801 treatment mice, individual changes in DA dialysate levels and the numbers of locomotion were positively correlated. **Conclusions** These results suggest that MK-801 increased extracellular DA levels, at least in part, through its direct action on the PFC. DA is involved in the behavior induced by systemic and local MK-801 administration.

Key words: dizocilpine; microdialysis; dopamine; locomotion; schizophrenia

© 1994-2010 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net