

气相色谱法分析大鼠不同脑区脂肪酸组成及含量

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[摘要] 目的:测定分析大鼠不同脑区脂肪酸组成及含量,为阐明脂肪酸在脑内的分布特征提供依据。方法:用甲醇/二氯甲烷法提取组织脂肪酸,用甲醇/乙酰氯法进行脂肪酸甲酯化,用气相色谱法检测脂肪酸组成与含量。结果:小脑蚓部、纹状体、海马、大脑皮质和大脑白质均含有至少31种脂肪酸,其中10种脂肪酸(C20:0、C22:0、C23:0、C24:0、C20:1、C22:1、C24:1、C20:5n-3、C22:5n-6、C22:6n-3)含量在脑区之间存在显著差异($P < 0.05$)。结论:大鼠不同脑区脂肪酸组成基本一致,一些脂肪酸含量存在差异。

[关键词] 大鼠;脂肪酸;气相色谱法;脑区

[中图分类号] Q547

[文献标志码] A

[文章编号] 1007-4368(2018)08-1085-04

doi:10.7655/NYDXBNS20180814

Analysis of fatty acid composition and content in different brain regions of rats by gas chromatography

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[Abstract] **Objective:** To compare the differences of fatty acid composition and content in different brain regions of rats, and to provide experimental evidence for clarifying the distribution of fatty acids in the brain. **Methods:** Lipids were extracted from the brain regions with methanol/dichloromethane, and fatty acids were methylesterified using methanol/acetyl chloride. Fatty acid methyl esters were then assayed by gas chromatography. **Results:** Five brain regions including cerebellar vermis, striatum, hippocampus, cerebral cortex and white matter, contained at least 31 fatty acids. There were significant differences in the contents of the ten kinds of fatty acids (including C20:0, C22:0, C23:0, C24:0, C20:1, C22:1, C24:1, C20:5n-3, C22:5n-6 and C22:6n-3) among the five brain regions. **Conclusion:** The fatty acid compositions in the five brain regions of rats are basically the same and some fatty acid contents are significantly different.

[Key words] rat; fatty acid; gas chromatography; brain regions

[Acta Univ Med Nanjing, 2018, 38(08):1085-1088, 1139]

脂肪酸是机体的重要能源物质,也是各种组织细胞结构的重要组成成分,一些脂肪酸能转变生成多种生理活性物质,参与及调节体内重要的生理病

理过程^[1]。近年研究表明,脑内脂肪酸成分与阿尔茨海默病、抑郁症、精神分裂症等神经精神疾病的发生有密切的关系^[2-4]。由于脑结构较为复杂,体内脂肪酸也种类繁多,测定不同脑区脂肪酸组成及含量有助于阐明不同类型脂肪酸在脑功能及相关疾病中的作用。但是现有关于脑脂肪酸的研究主要关注脑内某一区域脂肪酸的成分,较少对不同脑区进行对比分析,目前对脑内不同部位脂肪酸的分布特征尚不十分清楚。大鼠是复制人类疾病动物模

[基金项目] 国家自然科学基金(81500984);河南省重点科技攻关项目(152102310112);河南省教育厅科技研究重点项目(14A310027)

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型的常用模式动物,国内外关于大鼠不同脑区脂肪酸组成及含量的研究报道并不多见^[5]。本实验用气相色谱法对大鼠不同脑区(小脑、纹状体、海马、大脑皮质、大脑白质)脂肪酸组成及含量进行检测分析,为阐明脂肪酸在不同脑区的分布特点及其与脑功能的关系提供实验依据。

1 材料和方法

1.1 材料

2月龄健康雄性SD大鼠5只(新乡医学院实验动物中心),体重270~320 g。适应饲养1周后,禁食水1夜,用3 mg/kg水合氯醛腹腔注射麻醉,股动脉放血处死大鼠,迅速剥脑,在冷生理盐水中快速分离小脑(蚓部)、纹状体、海马、皮质(额叶)及大脑白质,将各脑区组织迅速转移到-80℃冰箱中保存备用。

混合脂肪酸甲酯标准品含40种脂肪酸(C4:0、C6:0、C8:0、C10:0、C11:0、C12:0、C13:0、C14:0、C14:1、C15:0、C15:1、C16:0、C16:1、C17:0、C17:1、C18:0、C18:1、TRANS、C18:1、CISC、18:2、TRANS、18:2、CISC、18:3 n6、C18:3 n3、C20:0、C20:1 n9、C20:2、C21:0、C20:3 n6、C20:4 n6、C20:3 n3、C20:5、C22:0、C22:1、C22:2、C23:0、C22:4 n6、C22:5 n6、C24:0、C22:5 n3、C22:6 n3、C24:1)浓度为10 mg/mL(Sigma公司,美国)。甲醇、二氯甲烷、乙酰氯、正庚烷、乙腈、正己烷均为国产色谱纯;无水硫酸钠、碳酸钾、二丁基羟基甲苯(BHT)为国产分析纯。气相色谱仪(7890A, Agilent公司,美国)。

1.2 方法

1.2.1 脂质提取及脂肪酸甲酯化

参照文献[6]报道的方法,先提取各脑区(小脑蚓部、纹状体、海马、额叶皮质、大脑白质)脂质,再采用甲醇/乙酰氯法进行脂肪酸甲酯化。取50 mg组织用2 mL甲醇/二氯甲烷混合溶液(V:V, 3:1;含0.05% BHT)冰浴匀浆,匀浆液中加入无水硫酸钠2 g,震荡混匀,2 500 r/min离心,向上清液中缓慢加入乙酰氯200 μ L,80℃避光水浴3 h,冷却后加入7% K₂CO₃ 4 mL,震荡混匀;加入正己烷2 mL,震荡混匀、2 500 r/min离心,上清液加入乙腈,震荡混匀、离心;上层液置于尖底磨口玻璃管中氮气吹干,加入100 μ L正庚烷溶解,直接用于气相色谱分析或者-20℃保存。

1.2.2 色谱分析条件及定性定量方法

色谱柱采用DB-23石英毛细管柱(60.00 m \times 0.25 mm \times 0.15 μ m, Agilent公司,美国),载气为氮气(99.999%),分流比60:1,1 μ L进样;检测器温度

280℃,进样口温度250℃,空气流速450 mL/min,氢气流速40 mL/min。升温程序为:起始柱温50℃,保持1 min,25℃/min升温至185℃后,2.7℃/min升温至230℃,保持10 min,总分析时间33 min。按照脂肪酸标准品的保留时间对样品各个色谱峰进行定性,用峰面积归一化法计算脂肪酸百分含量。

1.3 统计学方法

把色谱图数据导入Excel,各脂肪酸含量用均值 \pm 标准差($\bar{x} \pm s$)表示,用SPSS 17.0软件进行统计分析。采用单因素方差分析(one-way ANOVA)比较各组差异,若差异有统计学意义,则进行两两比较(方差齐使用LSD检验,方差不齐使用Tamhane's T2检验), $P \leq 0.05$ 为差异有统计学意义。

2 结果

2.1 脂肪酸组成分析

本实验分析条件下,40种脂肪酸甲酯标品得到了完全分离(图1)。通过与各标品保留时间相比较,鉴定各脑区脂肪酸种类。结果显示,小脑(蚓部)、纹状体、海马、皮质(额叶)、大脑白质5个脑区的脂肪酸组成基本一致,均至少含有31种脂肪酸,其中饱和脂肪酸(saturated fatty acid, SFA)12种,单不饱和脂肪酸(monounsaturated fatty acid, MUFA)8种,多不饱和脂肪酸(polyunsaturated fatty acid, PUFA)11种。进一步通过峰面积归一化计算出不同脂肪酸的百分含量(表1)。

2.2 脂肪酸含量分析

2.2.1 脂肪酸含量的总体情况

本实验中5个脑区SFA含量在54.4%~60.0%之间,不饱和脂肪酸(unsaturated fatty acid, UFA)含量在40.0%~45.6%之间,SFA含量约是UFA的1.2~1.5倍。MUFA含量在18.8%~29.0%之间,PUFA含量在15.8%~21.6%之间,MUFA/PUFA比值在0.9~1.8之间,白质中比值最高,皮质最低。各脑区PUFA n-6含量均较PUFA n-3多,是n-6/n-3比值的1.6~2.2倍,海马中比值较高(表1)。

各脑区脂肪酸含量差别很大,C16:0、C18:0、C18:1是各脑区含量位居前3的脂肪酸,含量分别为24.6%~29.5%、24.0%~27.7%、15.9%~20.9%,然后依次是C20:4(4.6%~8.1%)、C22:6(4.0%~8.0%)、C18:2(2.4%~4.1%)、C22:4(1.5%~2.4%);不同脑区还有其他含量大于1.0%的脂肪酸,小脑中有C20:1、C24:0、C24:1,纹状体有C24:0、C24:1,白质有C20:0、C20:1、C24:0、C22:5n-3、C24:1;其余脂肪酸在各脑

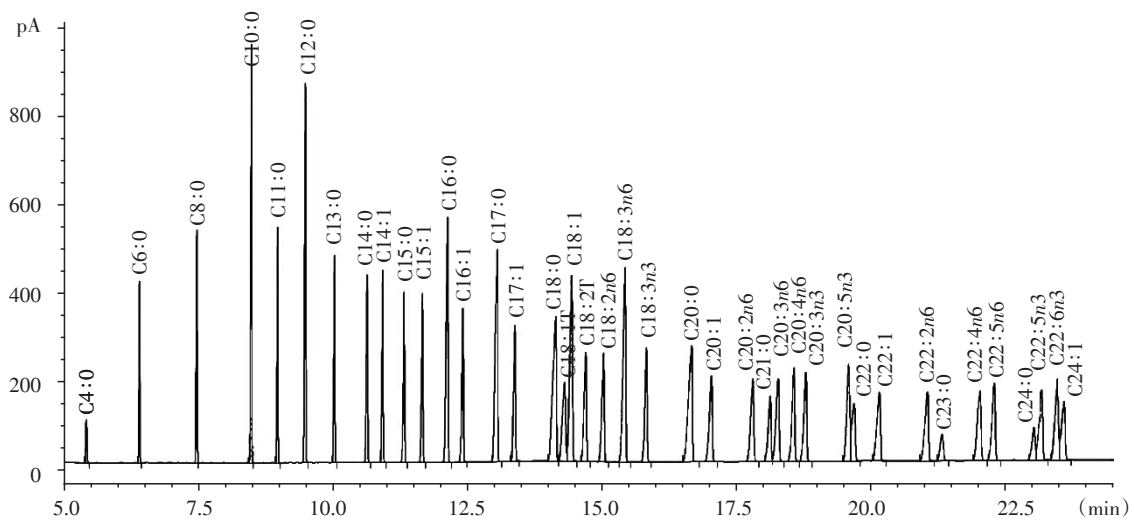


图1 脂肪酸甲酯标准品的气相色谱图

Figure 1 Gas chromatogram of fatty acid methyl ester standards

区分布较少,含量介于0.03%~0.99%。

2.2.2 脂肪酸在不同脑区的含量比较

本实验定性的31种脂肪酸中,有10种脂肪酸在各脑区含量的差异有统计学意义($P < 0.05$),其中C20:0、C22:0、C23:0、C24:0、C20:1、C22:1、C24:1和C20:5在皮质、小脑含量较高,在纹状体、海马、皮质中较低,而C22:5n-6在皮质、海马中较高,白质中最低,C22:6在皮质、海马、纹状体、小脑中较高,白质中最低。其余21种脂肪酸在各脑区含量的差异无统计学意义($P > 0.05$,表1)。

从脂肪酸类别看,不同脑区总SFA、总UFA含量差异无统计学意义($P > 0.05$),但不同脑区MUFA、PUFA、PUFA n-6、PUFA n-3的差异有统计学意义($P < 0.05$)。MUFA在皮质、小脑含量较多,而PUFA在海马、皮质较多。PUFA n-6在海马、皮质较多,PUFA n-3在皮质较多,二者在白质中均最少。

3 讨论

本实验用气相色谱法分析了大鼠小脑(蚓部)、纹状体、海马、大脑皮质、大脑白质5个脑区脂肪酸组成及含量,发现各脑区脂肪酸组成种类基本一致,均含有31种脂肪酸(由于标准品的局限,个别脂肪酸未能定性)。跟国内外分析脑脂肪酸组成的文献相比^[7-9],本实验检测到的脂肪酸种类更多,除了25种偶数碳脂肪酸外,还有6种奇数碳脂肪酸。

虽然各脑区脂肪酸组成基本一致,但一些脂肪酸含量在脑区之间有明显差异。白质中C20:0、C20:1、C22:0、C22:1、C23:0、C24:0、C24:1、C20:5含量分别是皮质的2.3、5.2、4.4、4.0、3.9、6.1、11.7、4.3倍,相

反,白质的C22:6、C22:5n-6却较低,只有皮质含量的50%和27%。值得注意的是,本实验中含有差异的脂肪酸均为20碳以上的极长链脂肪酸,提示不同极长链脂肪酸的差异性分布可能是脑区功能不同的结构基础之一。近年研究表明,一些极长链的SFA和PUFA与多种脑疾病(如脑白质脱髓鞘、阿尔茨海默病、帕金森病等)有关^[4,7,10-11],进一步探明疾病状态下脑区脂肪酸组成含量的变化将有助于阐明相关疾病的发生机制。

近年关注脂肪酸与脑功能的研究日益增多,鼠类也常用作此类研究的实验动物。一些研究者选择全脑或半个大脑作为样本进行脂肪酸测定可能并不是最佳方案,根据本实验结果显示的不同脑区存在脂肪酸的差异性分布这一特点,选择相对局限的脑区进行脂肪酸测定应该更为合理。总之,本实验阐明了大鼠不同脑区脂肪酸组成及含量特征,为进一步研究脂肪酸与脑区功能的关系提供了实验基础。

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表1 大鼠不同脑区脂肪酸组成及含量
Table 1 Composition and content of fatty acid in different brain regions of rats (% , $\bar{x} \pm s, n=5$)

| 脂肪酸种类 | 小脑 | 纹状体 | 海马 | 皮质 | 白质 |
|----------|----------------|----------------------------|------------------------------|------------------------------|-------------------------------|
| C10:0 | 0.204 ± 0.090 | 0.162 ± 0.074 | 0.215 ± 0.122 | 0.278 ± 0.151 | 0.188 ± 0.072 |
| C11:0 | 0.397 ± 0.218 | 0.324 ± 0.166 | 0.428 ± 0.263 | 0.601 ± 0.398 | 0.404 ± 0.211 |
| C12:0 | 0.115 ± 0.046 | 0.182 ± 0.089 | 0.118 ± 0.076 | 0.133 ± 0.033 | 0.180 ± 0.090 |
| C14:0 | 0.344 ± 0.074 | 0.444 ± 0.190 | 0.309 ± 0.100 | 0.334 ± 0.050 | 0.429 ± 0.161 |
| C14:1 | 0.264 ± 0.146 | 0.385 ± 0.264 | 0.263 ± 0.278 | 0.266 ± 0.074 | 0.312 ± 0.194 |
| C15:0 | 0.090 ± 0.017 | 0.177 ± 0.167 | 0.083 ± 0.021 | 0.123 ± 0.010 | 0.100 ± 0.048 |
| C15:1 | 0.319 ± 0.178 | 0.452 ± 0.372 | 0.428 ± 0.296 | 0.444 ± 0.131 | 0.503 ± 0.240 |
| C16:0 | 25.957 ± 2.229 | 28.647 ± 2.894 | 28.269 ± 1.334 | 29.490 ± 2.719 | 24.656 ± 4.178 |
| C16:1 | 0.787 ± 0.264 | 0.811 ± 0.412 | 0.737 ± 0.346 | 0.810 ± 0.097 | 0.765 ± 0.413 |
| C17:0 | 0.297 ± 0.054 | 0.233 ± 0.069 | 0.248 ± 0.049 | 0.282 ± 0.056 | 0.271 ± 0.060 |
| C17:1 | 0.462 ± 0.243 | 0.695 ± 0.423 | 0.423 ± 0.398 | 0.564 ± 0.113 | 0.789 ± 0.446 |
| C18:0 | 24.000 ± 1.432 | 27.683 ± 2.119 | 26.661 ± 2.127 | 27.570 ± 3.638 | 25.077 ± 1.450 |
| C18:1 | 20.874 ± 4.741 | 15.910 ± 1.750 | 17.286 ± 3.107 | 15.928 ± 7.899 | 20.946 ± 2.923 |
| C18:2n-6 | 4.057 ± 2.423 | 2.359 ± 1.555 | 2.609 ± 1.900 | 2.362 ± 1.659 | 2.416 ± 1.179 |
| C18:3n-6 | 0.322 ± 0.298 | 0.675 ± 0.554 | 0.464 ± 0.337 | 0.352 ± 0.151 | 0.735 ± 0.577 |
| C18:3n-3 | 0.463 ± 0.543 | 0.103 ± 0.108 | 0.136 ± 0.096 | 0.147 ± 0.113 | 0.159 ± 0.060 |
| C20:0 | 0.952 ± 0.148 | 0.680 ± 0.093 | 0.519 ± 0.082 ^a | 0.468 ± 0.028 ^{ab} | 1.094 ± 0.349 |
| C20:1 | 2.035 ± 0.414 | 0.998 ± 0.303 ^a | 0.506 ± 0.248 ^a | 0.417 ± 0.043 ^a | 2.189 ± 0.945 |
| C20:2n-6 | 0.161 ± 0.052 | 0.051 ± 0.049 | 0.107 ± 0.099 | 0.064 ± 0.047 | 0.115 ± 0.046 |
| C20:3n-6 | 0.333 ± 0.205 | 0.281 ± 0.032 | 0.485 ± 0.337 | 0.406 ± 0.141 | 0.425 ± 0.165 |
| C20:4n-6 | 4.605 ± 0.444 | 6.081 ± 1.965 | 8.087 ± 2.010 | 6.903 ± 1.431 | 4.661 ± 0.978 |
| C20:5n-3 | 0.096 ± 0.028 | 0.052 ± 0.019 ^a | 0.037 ± 0.024 ^a | 0.028 ± 0.003 ^a | 0.121 ± 0.046 ^{bcd} |
| C22:0 | 0.595 ± 0.147 | 0.338 ± 0.104 ^a | 0.208 ± 0.141 ^a | 0.187 ± 0.056 ^a | 0.830 ± 0.338 ^{bcd} |
| C22:1 | 0.155 ± 0.045 | 0.088 ± 0.086 | 0.088 ± 0.087 | 0.067 ± 0.016 | 0.271 ± 0.141 ^{abcd} |
| C23:0 | 0.119 ± 0.053 | 0.068 ± 0.045 | 0.091 ± 0.095 | 0.050 ± 0.037 | 0.195 ± 0.086 ^{bcd} |
| C22:4n-6 | 1.547 ± 0.085 | 1.814 ± 0.542 | 2.363 ± 0.735 | 2.022 ± 0.539 | 1.926 ± 0.468 |
| C22:5n-6 | 0.236 ± 0.061 | 0.453 ± 0.111 | 0.847 ± 0.361 ^{ab} | 0.963 ± 0.215 ^{ab} | 0.262 ± 0.139 ^{cd} |
| C24:0 | 1.306 ± 0.457 | 1.079 ± 0.481 | 0.808 ± 0.183 | 0.283 ± 0.062 ^{ab} | 1.720 ± 0.622 ^{bcd} |
| C22:5n-3 | 0.234 ± 0.187 | 0.491 ± 0.313 | 0.155 ± 0.057 | 0.141 ± 0.022 | 1.021 ± 0.755 |
| C22:6n-3 | 6.441 ± 0.569 | 6.908 ± 2.141 | 6.370 ± 1.794 | 8.039 ± 1.669 | 3.981 ± 1.428 ^{abcd} |
| C24:1 | 2.206 ± 0.751 | 1.375 ± 0.404 | 0.652 ± 0.420 ^a | 0.279 ± 0.122 ^{ab} | 3.257 ± 1.209 ^{abcd} |
| ΣSFA | 54.379 ± 3.835 | 60.017 ± 4.077 | 57.957 ± 1.721 | 59.799 ± 6.508 | 55.145 ± 4.390 |
| ΣUFA | 45.596 ± 3.886 | 39.983 ± 4.077 | 42.043 ± 1.721 | 40.201 ± 6.508 | 44.855 ± 4.390 |
| ΣMUFA | 27.100 ± 4.186 | 20.714 ± 2.368 | 20.382 ± 3.746 ^a | 18.774 ± 8.073 ^a | 29.033 ± 4.298 ^{bcd} |
| ΣPUFA | 18.496 ± 3.205 | 19.269 ± 2.380 | 21.661 ± 2.596 ^a | 21.427 ± 1.932 | 15.822 ± 1.429 ^{bcd} |
| ΣPUFAn-6 | 11.261 ± 2.429 | 11.714 ± 0.667 | 14.962 ± 1.214 ^{ab} | 13.071 ± 0.570 ^{ac} | 10.540 ± 0.977 ^{cd} |
| ΣPUFAn-3 | 7.235 ± 0.867 | 7.555 ± 1.826 | 6.699 ± 1.740 | 8.356 ± 1.579 | 5.282 ± 1.416 ^{bd} |

与小脑相比,^a*P* < 0.05;与纹状体相比,^b*P* < 0.05;与海马相比,^c*P* < 0.05;与皮质相比,^d*P* < 0.05。

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[收稿日期] 2018-02-12