药 学 学 报
Acta Pharmaceutica Sinica 1985;20(10):721～725

研 究 论 文

大鼠脑内阿片受体的溶脱及稀释对其结合活性的影响*

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提要 用氚标记依托菲（^{3}H-etor）或氚标记二苯羟乙酸喹咛酯（^{3}H-QNB）与大鼠脑（去小脑）P_2膜制备共同保温后，以 Triton x-100 溶脱，再用 Sepharose 6 B 层析，阿片受体及 M 胆碱受体洗脱峰位置相同，分子量皆约为 450,000。以 CHAPS** 溶脱 P_2 制备，可获得有活性的阿片及 M 胆碱受体。溶脱液被 Tris 缓冲液（50 mM，pH 7.5）稀释后，阿片受体结合能力可提高 25 倍，如稀释液中含 CHAPS（1 mM）结合能力变化不大。

关键词 阿片受体；乙酰胆碱受体；受体溶脱

自 1973 年确证脑组织中存在阿片受体以来，该受体的溶脱、纯化及性质的研究引起人们极大兴趣。近 10 几年来，国外一些实验室[1~6]不断报道采用不同溶脱剂溶脱阿片受体—配基复合物或有活性的阿片受体，以及有关阿片受体性质的研究。

CHAPS 是一种新型离子型溶脱剂，用作阿片受体的溶脱，国内尚未见报道。本文对用 CHAPS 溶脱获得的阿片受体及用 Triton x-100 溶脱获得阿片受体—配基复合物的性质做了研究。

材 料 及 方 法

1. 药品 ^{3}H-etor(40 Ci/mmol)，etor 均由上海第一医学院药学系合成并标记。^{3}H-QNB (17 Ci/mmol) 是军事医学科学院合成。QNB 是解放军药物化学研究所合成。

2. P_2 膜制备与 ^{3}H 标记配基的结合及溶脱 将 ^{3}H-etor (6.5 nM) 或 ^{3}H-QNB (4 nM) 与大鼠脑（去小脑）P_2 膜制备[7]（简称 P_2 制备）分别加入（非特异性结合管）或不加（总结合管）etor (10 μM) 或 QNB (4 μM) 然后加 Tris 液，使总体积为 25 ml（含蛋白 10～15 mg），30°C保温 15 min，加 2.5 ml 冷 Tris 液，3000 r/min 离心 10 min 沉淀中再加入同样体积冷的 Tris 液，重复离心后，弃去上清，加 1% Triton x-100 溶液 1.5 ml，4°C，搅拌 20 min，100,000 g 离心1 h (4°C)，测上清液蛋白浓度 (Lowry[8]法)，并取 0.2 ml 加 10 ml 闪烁液[7]，用液闪仪测放射计数。

3. P_2 膜制备受体的溶脱及结合活性的测定 溶脱方法参考文献(6)，在 P_2 制备（蛋白浓度 12 mg/ml）中加入 CHAPS (10 mM，终浓度)。4°C搅拌 1 h，100,000 g 离心 1 h。上清液 0.6 ml 中，加入 ^{3}H-etor(3.1 nM)或 ^{3}H-QNB(2nm)，再分别加入(非特异结合管)或不加（总结合管）非标记的 etor (5 μM) 或 QNB (2 μM) 加 Tris 液使体积为 1 ml，30°C保温 15 min，加入饱和度硫酸铵溶液 1 ml，在冰浴上放置约 2 min 后，用 7101 玻璃纤维滤纸

本文于 1985 年 1 月 9 日收到

* 本文初步工作曾在 1981 年全国药理学会会议上报告；部分工作曾在 1984 年第九届世界药理学会 (IUPHAR 9 th International Congress of Pharmacology, london. Abstracts 1407 p) 上报告

** CHAPS=3-[(3-cholamidopropyl)-dimethylammonio]-1-propane-sulfonate

抽滤，用 5 ml 50%饱和度硫酸铵溶液洗涤，将滤膜放在计数杯底部，加 10 ml 闪烁液计数。

结 果

(一) ^{3}H-etor 或 ^{3}H-QNB 受体复合物的溶脱

以 Triton x-100 溶脱 P_2 膜制备与 ^{3}H-etor 或 ^{3}H-QNB 复合物。蛋白回收率为 39±8.7%。标记的阿片受体的回收率(67.6±16%)高于M胆碱受体溶脱的回收率(36±1.4%)。

将溶脱的受体配基复合物用 Sephadex G 200 层析分离，在 280 nm 监测，得到两个峰。峰 I 较小，有特异性放射结合，峰 II 较大，无放射活性，如洗脱液中加入 Triton x-100，峰 II 增高，可见它是 Triton 峰（图略）。

溶脱的受体—配基复合物用 sepharose 6 B 层析分离，放射活性测定显示两个活性峰，峰 I 与 280 nm 监测到的蛋白峰吻合（图 1），将其水解后，用双向层析分析，有 10 个氨基酸斑点（图 2），它是阿片受体峰。峰 II 在 280 nm 没有吸收，双向层析分析不含氨基酸。此外上柱液中加入自由的 ^{3}H 标记配基，则峰 II 幅度大为提高，故为游离的 ^{3}H 标记配基。小脑

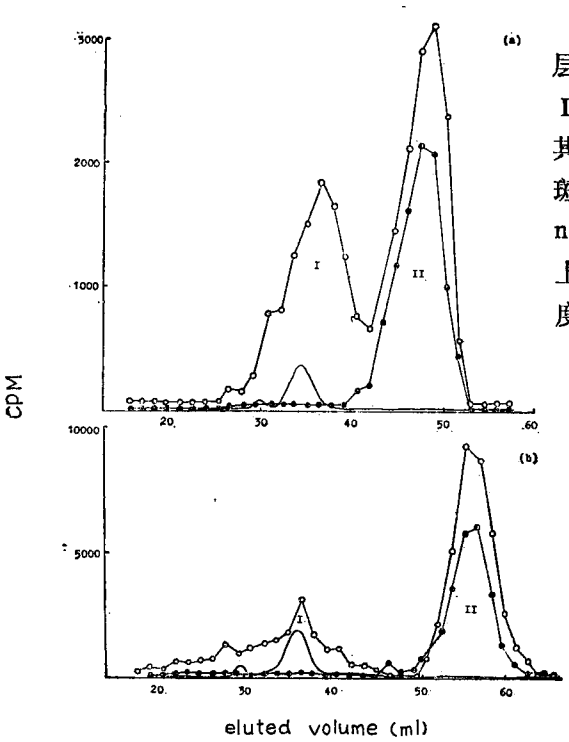

Fig 1 a. Elution profile on Sepharose 6B chromatography of Triton x-100 extract of P_2 bonnd with ^{3}H-etor. P_2 membrane preparation (13.4 mg/ml) was incubated with ^{3}H-etor (6.5 nM), with (•——•) or without (○——○) unlabelled etor (10 μM), and solubilized with Triton-x 100 (1%). After centrifugation (100,000 g, 1 h, 4℃), 1 ml of the supernatant was placed on a Sepharose 6B column (1.5×30 cm) and eluted with cold 0.05 M Tris-buffer (pH 7.4, 50 mM). Protein concentration was monitored by Uvicord (280 nM) and determined by Lowry method. Peak I was found to be a protein peak and II was a ^{3}H-etor peak; b. Elution profile of P_2 bound with ^{3}H-QNB. The procedures was the same as above except that the ligand used was ^{3}H-QNB (4 nM) and unlabelled QNB (4 μM)

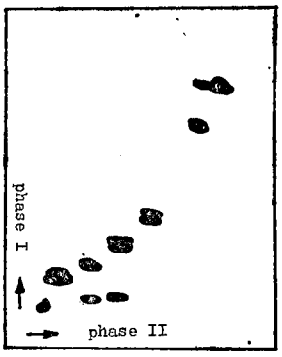

Fig 2. Paper chromatogram of radioactive peak I after acidolysis. Part of radioactive peak I was hydrolyzed by HCl (6.7 N) for 16 h. The hvdrolysate was analyzed by 2 dimensional paper chromatography at room temperature. The first dimension was performed with n-butanol—formic acid—water (15:3:2); the second dimension with n-butanol—ammonium hydroxide (12%)—ethyl alcohol (95%) (13:3:3). Color was developed by 0.5% ninhydrin spray

P_2 膜制备经标记，溶脱及层析得不到两种标记的受体。

用凝胶过滤法测得阿片受体分子量约为 450,000（图 3），M受体的分子量与此相近。

溶脱后的阿片受体—^{3}H-etor 复合物经聚丙烯酰胺凝胶电泳分离，在 Rf 0.18 处，可测到阿片受体的特异结合活性（图 4）。

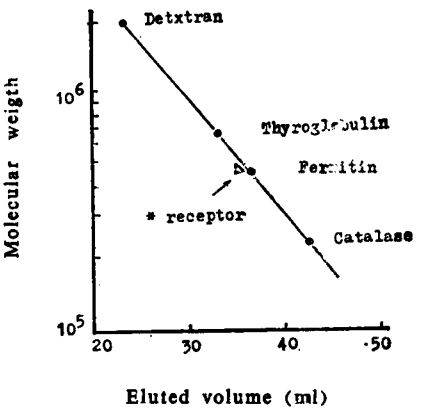

Fig 3. Estimation of molecular weight of solubilized ^{3}H-etor-bound complex on Sepharose 6 B. Sample (1 ml) was applied to a Sepharose 6 B column (1.5×30 cm) and eluted with Tris buffer (50 mM, pH 7.4) at 4°C. Protein standards were monitored by absorbence at 280 nm, and the radiolabelled complexes were monitored by liquid scintillation spectrophotometry

* Receptor; opiate receptor or muscarinic receptor

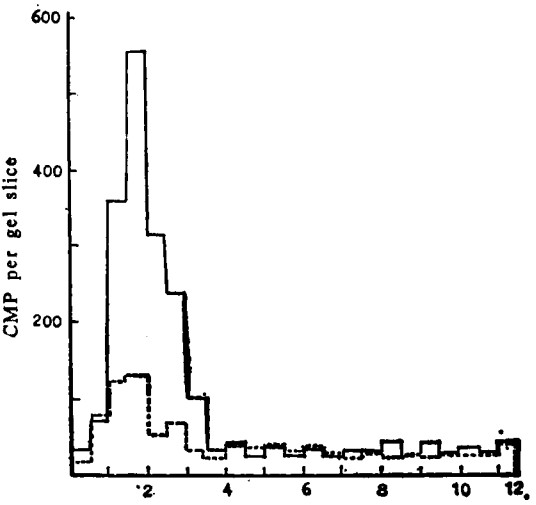

Fig 4. Electrophoretic analysis of the ^{3}H-etor-bound complex. Electrophoresis of the solubilized ^{3}H-etor-bound complex on 7.5% polyacrylamide cylindrical gel under nondenaturing conditions. Gels were prepared according to the procedure of Davies and Stark[9], and electrophoresis was carried out at 3 mA for 1.5 h. Radioactivity was monitored in 0.5 cm slices by liquid scintillation spectrophotometry. Totol binding (——) and nonspecific binding (----)

（二）膜制备的溶脱

用 10 mM CHAPS 溶脱 P_2 制备，经超速离心，蛋白回收率为 32±2.5%。溶脱液与 ^{3}H-etor 或 ^{3}H-QNB 结合后，经 Sepharose 6 B 层析分离可得到特异结合活性峰，位置与蛋白活性峰相一致（图 5）。阿片与M受体活性回收率分别为 22.8±7.1%及 11.4±2%。

（三）稀释的影响

如图 6 所示，测定管中所含溶脱液的蛋白浓度为 1 mg 时，其特异结合活性为 0.037±0.029 pmol/mg 蛋白。用 Tris 液稀释溶脱液，随着蛋白浓度的稀释，其结合活性明显增加，当稀释至 16 倍时（即蛋白浓度为 0.6 mg/ml 时），其结合活性为 0.92±0.18 pmol/mg 蛋白，提高 25 倍。稀释液中含有 CHAPS（1 mM），则稀释后活性没有明显增加。

讨　论

Püget(3) 等人用 sepharose 6 B 分离胆盐溶脱的与 ^{3}H-etor 结合的阿片受体，得到高分子量（500,000）和低分子量（洗脱位置与游离的 ^{3}H-etor 位置同）两个放射活性峰，作者认

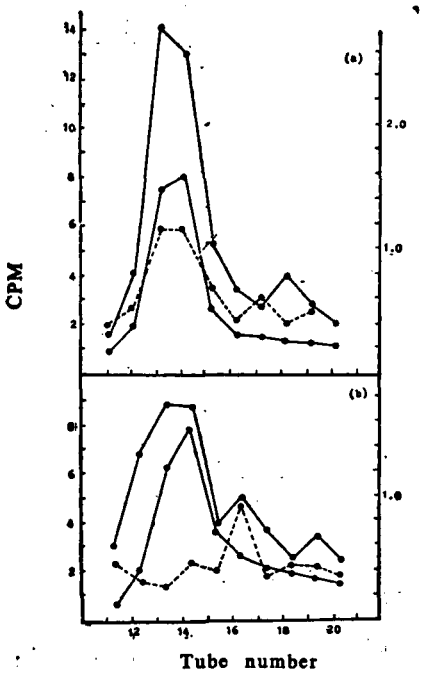

Fig 5. a. Elution profile of solubilized opiate receptor. P_2 fraction of rat brain was solubilized with CHAPS (10 mM) and centrifuged at 100,000 g for 1 h. The supernatant was incubated with 4.3 nM ^{3}H-etor with (•-----•) or without (•——•) unlabelled etor (3μM) and fractionated on Sephadex G-200 column. Protein content (•——•) was determined by Lowry method[8]; b. Elution profile of solubilized muscarinic receptor. The procedure was the same as above except that ^{3}H-QNB (4 nM) and unlabelled QNB (4 μM) were used instead of ^{3}H-etor and unlabelled etor

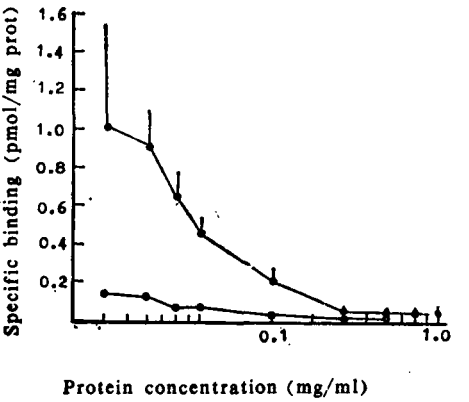

Fig 6. Effect of dilution on binding activity of opiate receptor in solubilized P_2 preparation. P_2 fraction of rat brain was solubilized with CHAPS (10 mM) and centrifuged under 100,000 g for 1 h. The supernatant was diluted with Tris-buffer (pH 7.4, 50 mM) (•——•) or Tris-buffer containing 1 mM CHAPS (○——○). Aliquots of the diluted supernatant were tested for specific binding activity with ^{3}H-etorphine (4.3 nM)

为低分子量组分是不同于高分子量组分的阿片受体或受体碎片。本文采用 Trifon x-100 溶脱获得两个洗脱峰。经对两个活性峰进行氨基酸组分分析表明，峰 I 含有十种氨基酸，为具有结合活性的阿片受体。峰 II 不含有氨基酸（见结果 1），该峰不是受体蛋白质。由于该峰与游离的 ^{3}H-etor 层析位置一致，我们认为该峰是在层析过程中从受体—配基复合物上解离下来的 ^{3}H-etor。

Simonds[6]等认为溶脱阿片受体时，去污剂的结构是关键，并认为理想的去污剂应同时带有两性离子基团和甾醇核。CHAPS 正好具有这种结构，因此用它来溶脱可获得有活性的受体。而 Triton x-100 则不能得到有活性的阿片受体，表明 CHAPS 对受体上与配基结合点的影响比 Triton x-100 小。

稀释实验结果表明，溶脱液中去污剂浓度降低，可提高受体的结合活性，部分原因可能是由于去污剂掩盖了一些结合位点，稀释可使结合位点重新暴露。如胰岛素受体[10]那样，在受体溶脱时，由于膜结构中磷脂层的破坏，亦可暴露出被掩盖的结合位点。这是于由溶脱后用 Tris 稀释，受体结合活性比未溶脱前要高很多。

实验中看到溶脱的 P_2 膜制备同时具有M受体的特异结合活性。凝胶过滤不能使两种受体分开。在放射受体结合实验中，高浓度（10^{-5}～10^{-4}M）的 etor 及 QNB 有交叉抑制作用，用 WGA-sepharose。进行亲和层析，两种受体亦在同一洗脱峰中，(结果待发表)。这提示两种受体是否有某种分子结构间的联系。对这个问题，我们还在做进一步的探讨。

致谢　索彩玲、杨惠芬、梁端等同志参加电泳、层析及放射配基结合实验；任民峰副教授对本文提出宝贵意见

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SOLUBILIZATION OF OPIATE RECEPTOR AND THE EFFECT OF DILUTION ON BINDING ACTIVITY

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ABSTRACT　Incubation of P_2 membrane preparation of rat brain homogenenate followed by solubilization with Triton-x 100, centrifugation at 100,000g for 1 hr and chromatography by Sephadex-G 200 column of the supernatant led to an elution profile in which opiate receptors and muscarinic receptors were found in the same protein peak. The molecular weight of both receptors were estimated to be 450,000 daltons.

Neither opiate nor muscarinic receptors could be obtained in the active from when Triton-x 100 was used as the solubilizing agent, but solubilization with CHAPS was successful. With CHAPS, the yield of opiate and muscarinic receptors were about 23% and 12% respectively. Dilution of the solubilized supernatant with Tris buffer (50 mM, pH 7.5) enhenced the binding capacity of opiate receptor about 25 folds, but when the dilution buffer contained CHAPS (1 mM), there was virtually no increase in binding acivity.

Key words　Opiate receptor; Muscarinic receptor; Receptor solubilization