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An endogenous ligand from human cerebrospinal fluid for the phencyclidine receptors

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ABSTRACT A fraction which potently and specifically competed with [^{3}H]PCP binding to rat brain synaptic membrane preparation was isolated from human CSF. This substance ran just before the salts in Sephadex G-25 column and was eluted with 10-40% acetonitrile gradient in reverse phase HPLC, predicating that it may be a small hydrophilic compound.

KEY WORDS phencylicdine; endogenous substances receptors; cerebrospinal fluid; competitive binding

Phencyclidine (1-1-phenylcyclohexyl piperidine, PCP) has received increasing attention recently not only because of its abuse hazards, but also its unique central nervous system (CNS) effects. PCP-induced psychosis resembles schizophrenia. Various laboratories described the specific binding sites of PCP in rat and human brain with characteristic regional distribution[1-3]. Several papers suggested that PCP receptors were well related to its psychotomimetic effects and that endogenous ligands for the receptors might be present in CNS[4]. Therefore, a search for this kind of ligand will cast some light on the pathogenesis of schizophrenia. A peptide was found in porcine brain[4]. In our laboratory, with preparative HPLC and Sephadex chromatography, a fraction which inhibited [^{3}H]PCP binding to rat brain synaptic membrane preparation was found in human brain[5]. In this paper, we reported a fraction isolated from human cerebrospinal fluid (CSF) with potent [^{3}H]PCP binding displacing activity.

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MATERIALS AND METHODS

(+)-[^{3}H]PCP (1776 GBq/mmol, New England Nuclear). PCP was synthesized by Department of Pharmaceutical Chemistry of Shanghai Medical University. CSF was collected via lumbar puncture at 8-9 AM from patients suffering from peripheral neuritis or myelopathy without any psychotropic medication. It was frozen immediately, and then lyophilized (5 ml in each tube) for use.

The lyophilized samples were resuspended in acetic acid 0.5 mol/L and chromatographed on a Sephadex G-25 fine column (1.2×60 cm, 9 ml/h) and with acetic acid 0.5 mol/L using LKB automatic collector. Aliquots were collected, lyophilized for [^{3}H]PCP receptor binding assay and salt concentrations measured by Beckman system E-4 A electrolytes analyzer. The fractions containing [^{3}H]PCP binding activity were pooled and chromatographed by reverse phase analytical HPLC. The

fractions were resuspended in 0.01% heptafluorobutyric acid (HFBA). They were chromatographed on 3.9×30 cm μ-Bondapak C_{18} column at a flow rate of 0.6 ml/min with 0–40% acetonitrile gradient and were monitored at 230 nm. The samples from analytical HPLC were further screened by [^{3}H]PCP binding assay.

Rat brain synaptic membrane preparation was prepared according to reference[4] with a few modifications. For [^{3}H]PCP receptor binding assay, duplicate assay tubes contained [^{3}H]PCP 8 nmol/L, membrane preparation of 10 mg tissue and chromatographed fractions or unlabeled PCP 20 μmol/L in a final volume of 0.5 ml buffer (sucrose 50 mmol/L in Tris-HCl 5 mmol/L, pH 7.6). Incubation took 45 min at 4 ℃, then the samples were filtered under reduced pressure through GF/C filters (Whatman) which were presoaked for at least 2 h at 4 ℃ in 0.05% polyethylenimine, then rapidly washed with 2×5 ml aliquots of ice cold 5.0 mmol/L Tris-HCl buffer. Each filter was dried by infrared lamps, then placed in vials containing 5 ml of scitillation liquid (TP 0.3%, POPOP 0.03% in toluene) and counted by liquid scitillation spectrometry. [^{3}H]PCP specific binding to synaptic membrane was defined as the difference in radioactivities bound in the absence and presence of unlabeled PCP. In a typical experiment, total binding was 2400 cpm and nonspecific binding was 800 cpm.

RESULTS

Active peaks and salt peaks screened by [^{3}H]PCP binding assay and salt analyzer, respectively Fig 1 showed that human CSF went through Sephadex G-25 fine column. As Fig 2 showed, human CSF contained an active fractions at No. 17–19 and a salt peak at No. 20–21. The pH of most fractions for binding assay were 7.5. Some were adjusted to 7.5 with 5 mmol/L Tris solution. The experiment was repeated 3 times. Since the salt will interfere the [^{3}H]PCP binding, the salt containing fractions No. 20–21 were discarded. The fractions No. 17–19 were combined and lyophilized for analytical HPLC.

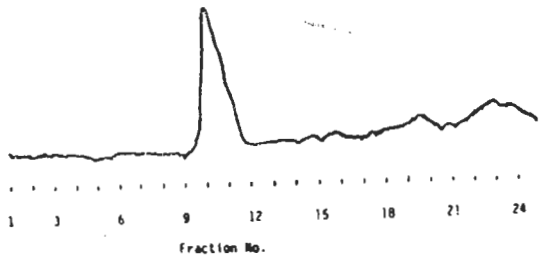

Fig 1. G-25 fine chromatography of human CSF. Column: 1.2×60 cm. Fraction volumn: 3 ml. Fractions were diluted with acetic acid 0.5 mol/L at a flow rate of 9 ml/h, and monitored at 254 nm.

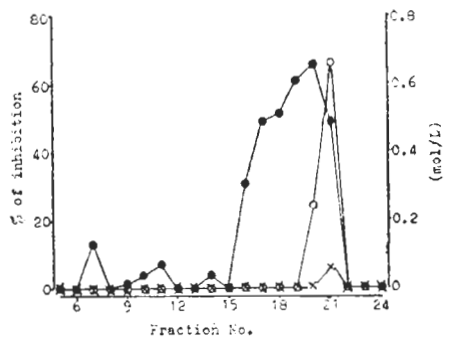

Fig 2. Active peaks and salt peaks screened by [^{3}H]PCP binding assay and salt analyzer respectively. (●), [^{3}H]PCP binding displacing activity; (○) and (×), the concentrations of Na^+ and K^+.

Analytical HPLC elution pattern of fraction No. 17–19 Fig 3 showed the peaks within 30 min elution after injection of fractions No. 17–19 (Fig 2). Eluates were collected by peaks, and screened by [^{3}H]PCP binding assay. The shaded peaks had the activity to inhibit [^{3}H]PCP binding.

DISCUSSION

According to many works including

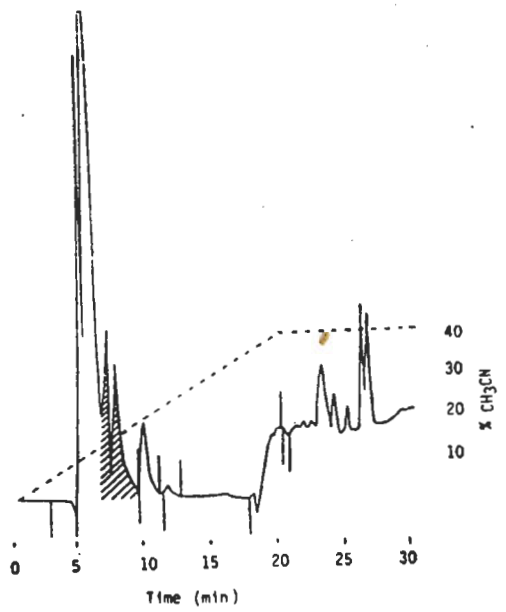

Fig 3. Analytical HPLC elution pattern of fraction No.17-19 from Sephadex G-25 chromatography. Column: μ Bondapak C_{18}. Mobile phase: 0.01% HFBA (vol/vol). Flow rate: 0.6 ml/min. The eluent was monitored at 230 nm.

ours in the past, [^{3}H]PCP binding assay was specific, and none of the known neurotransmitters and neuropeptides displaced its binding in reasonable concentrations[4]. In this experiment, we excluded the factors which might interfere with binding, such as pH and salts. Therefore, we consider that the endogenous ligand in human CSF is reliable. It may be released directly from the brain or exists as an active metabolite of some authentic forms of PCP-like substances. Unlike the neuropeptide reported by Quirion *et al*[4], our fraction may be a small hydrophilic compound, since it moved ahead the salt in Sephadex G-25 column and was rapidly eluted with about 15% acetonitrile in reverse phase HPLC.

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人脑脊液中内源性苯环利定配体

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提要　利用竞争性放射受体结合分析法，我们发现人腰穿脑脊液经 Sephadex G-25 层析柱(1.2 × 60 cm) 醋酸 0.5 mol/L 洗脱，流速 9 ml/h，每管 3 ml，在 17-21 管处得到一个较大的活性峰，其中 20-21 管为盐峰，将 17-19 管再经反相分析型 HPLC，在 15% 左右乙腈浓度时得到两个具有 PCP 受体结合活性峰。上述结果提示，得到一个小分子亲水的内源性 PCP 配体。

关键词　苯环利定；内源性物质受体；脑脊髓液；竞争性结合

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