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l–Tetrahydropalmatine increases leucine enkephalin levels in corpus striatum of rats[1]

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ABSTRACT The effect of chronic *l*–tetrahydropalmatine (*l*–THP) administration on the level of leucine enkephalin (Leu–Enk) in rat corpus striatum was studied. After *l*–THP sc injection once daily for 2 wk, the striatal Leu–Enk level was elevated dose–dependently. However, a single injection of *l*–THP failed to change the Leu–Enk level. When rats received sc Sch–23390, a selective D_1 antagonist, 15 nmol · kg^{-1} tid for 2 wk, striatal content of Leu–Enk increased from 0.17± SD 0.03 ng · mg^{-1} tissue in control group to 0.23± SD 0.05 ng · mg^{-1} tissue in Sch–23390 group ($n=8$, $P<0.05$). Sulpiride (Sul), a selective D_2 antagonist, 140 μmol · kg^{-1} sc given bid for 2 wk had no significant effect on the striatal Leu–Enk content. The results suggested that the blockade of D_1 receptors by *l*–THP might be responsible for the increase of the striatal Leu–Enk content in rat.

KEY WORDS *l*–tetrahydropalmatine; Sch–23390; sulpiride; dopamine receptor blockaders; leucine enkephalin; corpus striatum

Repeated administration of dopamine (DA) antagonists, endowed with major tranquilizing actitvity, results in increases in striatal proenkephalin mRNA and enkephalins, indicating a functional relation between the DA and enkephalin system[(1)]. *l*–Tetrahydropalmatine (*l*–THP) is a potent sedative–tranquilizing agent which has been used in China in clinical alleviation of pain and anxious insomnia[(2)]. Recently *l*–THP and its

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analogues were found to be DA receptor antagonists with preferential affinity toward the D_1 receptors[3]. The present investigation was to determine the effect of chronic *l*–THP administration on the levels of leucine enkephalin (Leu–Enk) in rat striatum. The selective D_1 antagonist, Sch–23390, and the selective D_2 antagonist, sulpiride (Sul), were also used in this study.

MATERIALS AND METHODS

Drugs and animal treatment *l*–THP sulfate injection and Sul injection were obtained from Zhanjiang Pharmaceutical Co and Shanghai Tian–Feng Pharmaceutical Co respectively. Sch–23390 from Schering Corp, USA was dissolved in warm water and diluted with saline.

Sprague–Dawley ♂ rats (Shanghai Laboratory Animals Centre) weighing 210 ± SD 15 g received sc injections of *l*–THP once daily, Sul bid or Sch–23390 tid for 2 wk. Control rats received saline. Striatum was dissected according to a classical procedure[4]. Tissue was homogenized in 10 volumes of ice–cold acetic acid 1 mol · L^{-1} and the homogenate was centrifuged at 20 000 × *g* for 30 min. The supernatant was neutralized with NaOH 1 mol · L^{-1} and stored at –30℃.

Enzyme–linked immunosorbent assay (ELISA) for Leu–Enk Synthetic Leu–Enk was coupled to bovine serum albumin (BSA) with glutaraldehyde 0.1 mol · L^{-1} according to the method of Davis *et al*[5]. The recovery of reaction was about 85% and stoichiometry was in the range of 8–10 mol peptide per mol BSA. Antibody against Leu–Enk was raised in our laboratory. Polystyrene microtitre plates purchased from Shanghai No 3 Plastic Co were coated with 100 μl of carbonate– bicarbonate buffer (0.05 mol · L^{-1}, pH 9.6) containing Leu–Enk conjugate. After incubation for 18–24 h at 4℃, unbound conjugate was removed by washing once with washing buffer (Tris–HCl 1 mol · L^{-1}, Tween–20 0.05%). Dilution buffer ($Na_2HPO_4 \cdot 12H_2O$ 8 mmol · L^{-1}, KH_2PO_4 1.5 mmol · L^{-1}, NaCl 145 mmol · L^{-1}, Tween–20 0.05% and fetal calf serum 2%) was added to each well to saturate any potential binding sites not occupied by the peptide conjugate. After incubation at 37℃ for 1 h, wells were washed 3 times with washing buffer. Solutions consisting of blanks, peptide standards, or sample extracts were incubated for 24 h at 4℃ with 50 μl of a 1 : 20 000 dilution of the primary antiserum in dilution buffer. Then the solutions were removed, the plates were again washed as above, and 100 μl of goat anti–rabbit IgG conjugated to horseradish peroxidase (Shanghai Institute of Biochemistry) was then added at a 1 : 500 dilution in dilution buffer. The plates were incubated for 2 h at 37℃, after which unbound goat anti–rabbit IgG conjugated to horseradish peroxidase was removed by washing 3 times with washing buffer. Substrate solution 100 μl (o–phenylenediamine 100 mg, 30% H_2O_2 40 μl in 100 ml of citrate–phosphate buffer) was added. The reaction was carried out at 37℃ and stopped after 30 min by adding H_2SO_4 2 mol · L^{-1} 100 μl to each well. The absorbance of the resulting chromogen was read at 490 nm in an ELISA plate reader (Nanjing Hua–Dong Electronic Co).

RESULTS

ELISA of Leu–Enk When the peptide 2.5 ng / well and 1 : 20 000 dilution of Leu–Enk antiserum were used in the assay a suitable standard curve was obtained. Result from 10 different experiments indicated that the minimal detectable amount was 12 pg. The reproducibility of that assay is 3.2% with an intraassay variation of 9.8%. The Leu–Enk antibody had no measurable affinity for Met–enkephalin, β–endorphin and

dynophin, when tested competitively (Fig 1).

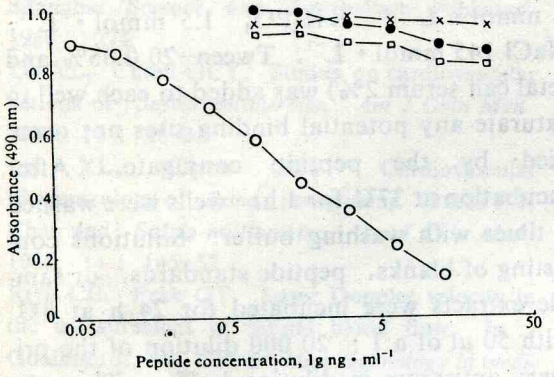

Fig 1. Specificity of Leu-Enk antisera against Leu-Enk (○), dynorphin (●), β-endorphin (×), and methionine enkephalin (□).

Effects of *l*-THP on striatal Leu-Enk level The striatal Leu-Enk level was elevated after 2 wk treatment with *l*-THP. The increase of striatal Leu-Enk was dose-dependent (Fig 2). A single injection failed to change striatal Leu-Enk content.

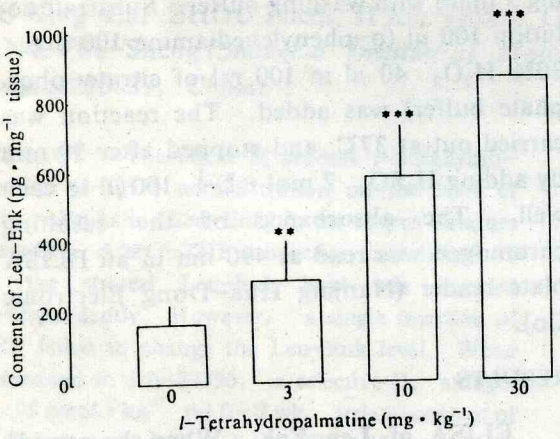

Fig 2. Effects of chronic treatment with *l*-THP on the striatal Leu-Enk content. Rats received sc injections of *l*-THP once daily for 2 wk. $n=6$, $\bar{x} \pm$ SD. $^{**}P<0.05$, $^{***}P<0.01$ *vs* control.

Effects of selective DA antagonists on striatal Leu-Enk level When rats received sc Sch-23390 75 nmol • kg^{-1} tid for 2 wk, striatal content of Leu-Enk increased from 0.17 ± SD 0.03 ng • mg^{-1} tissue in control group to 0.23 ± SD 0.05 ng • mg^{-1} tissue in Sch-23390 group ($n = 8$, $P<0.05$). On the other hand, Sul 140 μmol • kg^{-1} sc given bid for 2 wk had no significant effect on the striatal content of Leu-Enk.

DISCUSSION

Chronic administration of DA antagonists has been shown to increase the striatal content of enkephalin peptides in rat[6]. Additional studies have demonstrated that the increase in enkephalin content elicited by DA receptor blockade is preceded by the increase in the levels of striatal proenkephalin protein and preproenkephalin mRNA[7]. Evidence has shown that the actions of DA in the striatum are mediated by either D_1 or D_2 receptor. To elucidate which DA receptor subtype is operative in the regulation of the dynamic state of enkephalin, the effect of selective D_1 or D_2 receptor antagonists has been studied. However, either an increase[1] or a decrease[8] of striatal proenkephalin mRNA has been observed after chronic treatment with the D_1 antagonist Sch-23390. Our result that repeated treatment with Sch-23390 induced an increase of the striatal Leu-Enk supported the suggestion that the tonic activition of D_1 receptors decreases striatal enkephalins content and that removal of the neurally mediated regulation by a specific pharmacologic blockage of D_1 increases the level of enkephalins. *l*-THP is a DA receptor antagonist with preferential affinity toward the D_1 receptors[3], it is not surprise that chronic adiministration of *l*-THP induces an increase of the striatal Leu-Enk content in rat. Repeated administration with D_2 antagonist has been reported to induce a mild decrease of the striatal Leu-Enk content[1]. However, no change was observed in our experiments although Sul at the same dose schedule has been reported to exert behavioral signs of DA receptor

supersensitivity in the striatum[9]. Our results suggest that the *l*-THP-induced increase of striatal Leu-Enk level in rats might be mainly due to the inactivation of the tonic inhibition exerted by D_1 receptors.

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左旋四氢巴马汀增加大鼠纹状体亮氨酸脑啡肽含量

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提要 大鼠 sc 左旋四氢巴马汀(*l*-THP)两周后，纹状体内亮氨酸脑啡肽(Leu-Enk)呈剂量依赖性增加. 单剂 *l*-THP 对纹状体内 Leu-Enk 含量无影响. 大鼠 sc 选择性 D_1 受体拮抗剂—Sch-23390 两周后，纹状体内 Leu-Enk 含量也增加，但给选择性 D_2 受体拮抗剂—舒必利两周后，纹状体内 Leu-Enk 无明显改变. *l*-THP 可能通过阻断 D_1 受体使大鼠纹状体内 Leu-Enk 增加.

关键词 四氢巴马汀; Sch-23390; 舒必利; 多巴胺受体阻滞剂; 脑啡肽; 亮氨酸; 纹状体