ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Oct; 22 (10): 871 - 875
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Distribution of enantiomers of *trans*-tramadol and *trans*-*O*-demethyltramadol in central nervous system of rats

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KEY WORDS tramadol; central nervous system; pharmacokinetics

ABSTRACT

AIM: To investigate the distribution of the enantiomers of *trans*-tramadol (*trans*-T) and its active metabolite, *trans*-*O*-demethyltramadol (M1), in the central nervous system (CNS). **METHODS**: After a single ip dose of *trans*-T hydrochloride or M1, the rats were killed by decapitation. A high performance capillary electrophoresis (HPCE) method was used to determine the concentrations of enantiomers of *trans*-T and M1 in the serum and different brain tissues, including cerebrospinal fluid (CF), cerebral cortex (CC), corpus striatum (CS), hypothalamus (HY), cerebellum (CE), and medulla oblongata (MO). **RESULTS**: After ip *trans*-T hydrochloride, the concentrations of (+)-*trans*-T were higher than those of (−)-*trans*-T in the serum and all tested brain tissues; The concentrations of (+)-M1 were lower than those of (−)-M1 in the all tested brain tissues; The concentrations of the enantiomers of *trans*-T and M1 were the highest in the CC, the lowest in the CF. After ip M1, the concentrations of (+)-M1 were higher than those of (−)-M1 in the serum and all tested brain tissues; The concentrations of the enantiomers of M1 were the highest in the CC, the lowest in the CF. **CONCLUSION**: The concentrations of the enantiomers of *trans*-T and M1 varied in the serum and different brain tissues. The distribution of *trans*-T and M1 in the CNS of rats was stereoselective. The stereoselectivity in the distribution of M1 after M1 injection was different with that after *trans*-T injection.

INTRODUCTION

trans-Tramadol (*trans*-T), a racemic mixture of (+)- and (−)-*trans*-T, is used as a centrally acting analgesic. The enantiomers of *trans*-T display different properties for various receptors. (+)-*trans*-T preferentially inhibits serotonin reuptake, whereas (−)-*trans*-T mainly inhibits norepinephrine reuptake. Preclinical studies suggest a complementary and synergistic antiociceptive interaction between the enantiomers of *trans*-T[1]. (+)-*trans*-*O*-Demethyltramadol [(+)-M1], the active metabolite of (+)-*trans*-T, can bind to μ-opioid receptor with a higher affinity than (+)- and (−)-*trans*-T. Thus, the enantiomers of *trans*-T mediate the mononinergic component in the analgesia, whereas (+)-M1 induces the analgesic by the opioid mechanism[2]. The dual action model may contribute to its efficiency in certain pain, little respiratory depression, and little tolerance after repeated administration[1,2].

The enantiomers of *trans*-T and (+)-M1 is needed to be delivered to the central nervous system (CNS), where they induce antiociceptive actions. Frink *et al* reported that the enantiomers of *trans*-T could affect the neurotransmitters to different extents in the different brain tissues[3]. In this paper, the distribution of the enantiomers of *trans*-T and M1 in the central nervous system was investigated in rats.

MATERIALS AND METHODS

Apparatus P/ACE System 5000 high performance capillary electrophoresis (HPCE) equipped with UV detector and Gold software (Beckman, California, USA). Uncoated fused-silica capillary with a total length of 37 cm, an effective length of 30 cm, an inner diameter of 75 μm (Beckman, California, USA). DIAX 900 homogenizer (Heidolph-Elektro, Germany).

Drugs and reagents *trans*-T hydrochloride was provided by Shijiazhuang № 1 Pharmaceutical Industry (China). M1 was kindly provided by Grünenthal Gmbh (Stolberg, Germany). *cis*-T hydrochloride (internal standard) was a gift from Chemical Department of Jinzhou Medicine College (China). Sulfobutylether-β-

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Received 2001-03-26 Accepted 2001-08-08

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Oct; 22 (10)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

cyclodextrin (chiral selector) was kindly provided by Lanzhou Institute of Chemical Physical, the Chinese Academy of Sciences. Other reagents, from different commercial sources, were of analytical or HPLC grade.

Animals Sprague-Dawley rats (n = 28, both sexes, 237 g ± 9 g) were provided by Experimental Animal Center of Hebei Medical University (Grade Ⅱ, Certificate № 04057). The rats were given ip with *trans*-T hydrochloride 16.7 mg/kg or M1 5 mg/kg.

Tissue preparation One hour after ip *trans*-T hydrochloride or M1, the animals were killed by decapitation. The blood was collected, the cerebrospinal fluid (CF) was drained out by puncturing the posterior atlanto-occipital membrane, and about 0.1 g of each solid brain tissue was dissected out over a stainless steel plate cooled with ice. The solid brain tissues were cerebral cortex (CC), corpus striatum (CS), hypothalamus (HY), cerebellum (CE), and medulla oblongata (MO). After weighting, the solid tissues were homogenized for 30 s, and diluted with 1 mL of water.

Sample extraction In a 10-mL tube, 0.2 mL of serum (SE), about 0.1 mL of CF, or 1 mL of tissue suspension was alkalized with 200 or 500 μL of sodium hydroxide 0.5 mol/L. After adding 100 μL of *cis*-T hydrochloride 1 mg/L, the samples were extracted with 5 mL of ethyl acetate and centrifuged at 2000 × g for 10 min. The organic fraction was evaporated under a gentle stream of nitrogen. The residue was redissolved in 100 μL of water and an aliquot (30 μL) was removed out for analysis.

Sample analysis *trans*-T and M1 in the tissues were determined by HPCE as described previously[4] with minor modifications. There was no interference from the all tested tissues. For the enantiomers of both *trans*-T and M1, the correlation coefficient for linear regression was more than 0.99; the relative recovery was from 94.6 % to 102.7 %; the intra-day relative standard deviation (RSD) was less than 13.5 %; the inter-day RSD was less than 18.3 %; the limit of detection (LOD) was 2 μg/L.

Data analysis The concentrations were expressed as $x \pm s$. The concentrations of the enantiomers of *trans*-T or M1 were compared through paired t test. For comparison of more than two groups, ANOVA was used.

RESULTS

Delivery to the CF and CC One hour after ip *trans*-T hydrochloride, concentrations of (+)-*trans*-T were higher than those of (−)-*trans*-T in the SE, CF, and CC, and the concentrations of the enantiomers of *trans*-T were the highest in the CC and the lowest in the CF (Tab 1). The concentrations of (+)-M1 were lower than those of (−)-M1 in the CF and CC, and the concentrations of the enantiomers of M1 were the highest in CC and the lowest in the CF (Tab 2).

Tab 1. The concentrations and ratios of enantiomers of *trans*-tramadol (*trans*-T) in the serum (SE), cerebrospinal fluid (CF), and cerebral cortex (CC) of rats at 1h after ip 16.7 mg/kg of *trans*-T hydrochloride. $n = 18$. $\bar{x} \pm s$. $^cP < 0.01$ *vs* (+)-enantiomer. $^eP < 0.05$, $^fP < 0.01$ *vs* SE. $^hP < 0.05$, $^iP < 0.01$ *vs* CF.

Tissues	(+)-*trans*-T /ng·g^{-1}	(−)-*trans*-T /ng·g^{-1}	(+)/(−)-*trans*-T
SE	735 ± 398	391 ± 185^c	1.82 ± 0.37
CF	494 ± 214^e	245 ± 130^{cf}	2.21 ± 0.70
CC	3612 ± 1737^{fi}	2166 ± 928^{cfi}	1.63 ± 0.27^h

Tab 2. The concentrations and ratios of enantiomers of *trans*-*O*-demethyltramadol (M1) in the serum (SE), cerebrospinal fluid (CF), and cerebral cortex (CC) of rats at 1h after ip 16.7 mg/kg of *trans*-T hydrochloride. $n = 18$. $\bar{x} \pm s$. $^cP < 0.01$ *vs* (+)-enantiomer. $^fP < 0.01$ *vs* SE. $^iP < 0.01$ *vs* CF.

Tissues	(+)-M1 /ng·g^{-1}	(−)-M1 /ng·g^{-1}	(+)/(−)-M1
SE	208 ± 131	220 ± 72	0.91 ± 0.45
CF	49 ± 29^f	76 ± 40^{cf}	0.66 ± 0.34
CC	361 ± 169^{fi}	469 ± 126^{cfi}	0.76 ± 0.26

One hour after ip M1, the concentrations of (+)-M1 were higher than those of (−)-M1 in the SE, CF and CC, which was different with the results founded after ip *trans*-T hydrochloride. It was also founded that the concentrations of the enantiomers of M1 were the highest in the CC, the lowest in the CF (Tab 3).

Distribution in the CNS One hour after ip *trans*-T hydrochloride, concentrations of (+)-*trans*-T were higher than those of (−)-*trans*-T in the all tested tissues, and the concentrations of enantiomers of *trans*-T were the highest in the CC and the lowest in the MO (Fig 1A). The concentrations of (+)-M1 were lower than those of (−)-M1 in the all tested tissues except HY, and the concentrations of the enantiomers of M1 were the highest in the CC and the lowest in the MO (Fig 1B).

Tab 3. The concentrations and ratios of enantiomers of *trans*-*O*-demethyltramadol (M1) in the serum (SE), cerebrospinal fluid (CF), and cerebral cortex (CC) of rats at 1h after ip 5.0 mg/kg of M1. $n = 10$. $\bar{x} \pm s$. $^{c}P < 0.01$ *vs* (+)-enantiomer. $^{f}P < 0.01$ *vs* SE. $^{i}P < 0.01$ *vs* CF.

Tissues	(+)-M1 /ng·g^{-1}	(−)-M1 /ng·g^{-1}	(+)/(−)-M1
SE	253 ± 141	113 ± 49^{c}	2.23 ± 0.56
CF	108 ± 38^{f}	63 ± 19^{cf}	1.77 ± 0.46
CC	590 ± 308^{fi}	273 ± 114^{cfi}	2.14 ± 0.52

One hour after ip M1, the concentrations of (+)-M1 were higher than those of (−)-M1 in the CC and MO, which was different with the results founded after ip *trans*-T hydrochloride. The concentrations of the enantiomers of M1 in the CC were also higher than those in the MO (Fig 2).

DISCUSSION

The blood-brain barrier (BBB) at the cerebro-vascular endothelium prevents the passage of water-soluble nonelectrolytes, electrolytes, and proteins from blood to

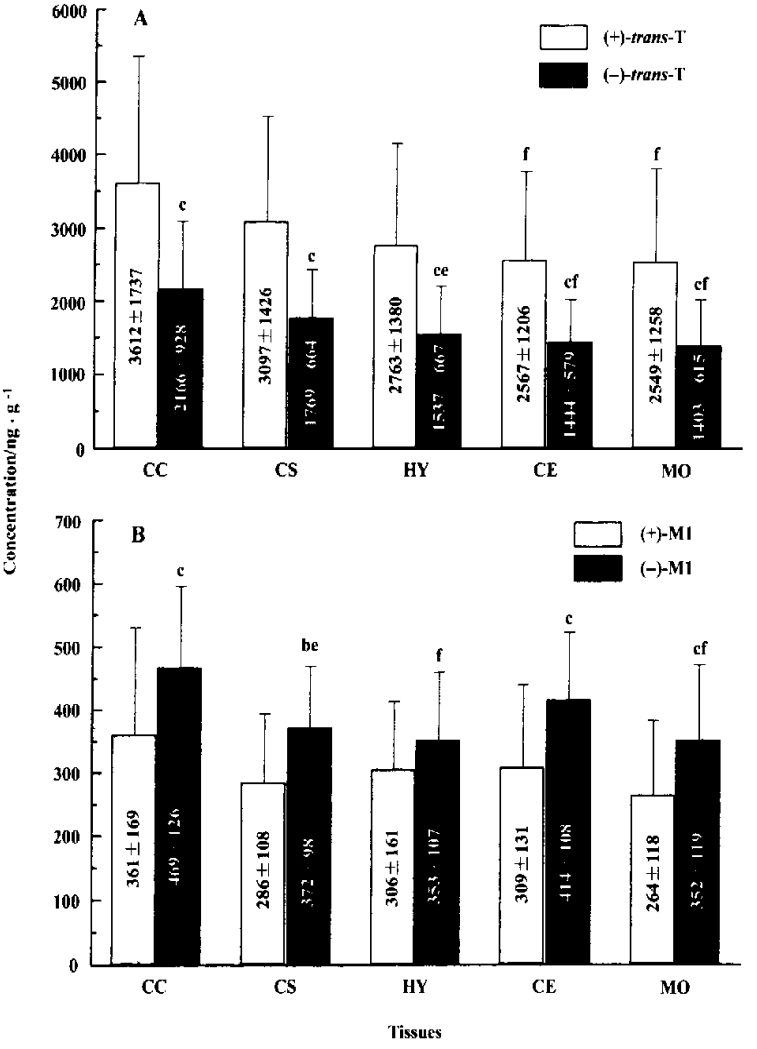

Fig 1. The concentrations of enantiomers of A) *trans*-tramadol (*trans*-T) and B) *trans*-*O*-demethyltramadol (M1) in the cerebral cortex (CC), corpus striatum (CS), hypothalamus (HY), cerebellum (CE), and medulla oblongata (MO) of rats at 1 h after ip 16.7 mg/kg of *trans*-T hydrochloride. $n = 18$. $\bar{x} \pm s$. $^{b}P < 0.05$, $^{c}P < 0.01$ *vs* (+)-enantiomer. $^{e}P < 0.05$, $^{f}P < 0.01$ *vs* CC.

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Oct; 22 (10)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

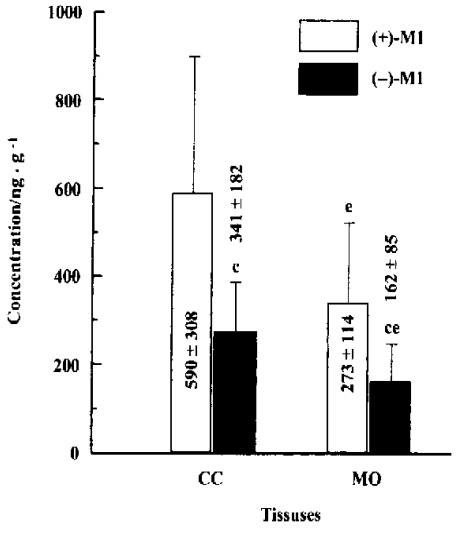

Fig 2. The concentrations of enantiomers of *trans*-*O*-demethyltramadol (M1) in the cerebral cortex (CC) and medulla oblongata (MO) of rats at 1 h after ip 5.0 mg/kg of M1. $n = 10$. $\bar{x} \pm s$. $^cP < 0.01$ *vs* (+)-enantiomer. $^eP < 0.05$ *vs* CC.

brain. *trans*-T is a basic compound, and M1 is an amphoteric one. The delivery of their enantiomers into CNS would be limited by the BBB. They must pass through the BBB to gain access to the brain tissues by passive, active, or facilitated transport. Since their concentrations were different among the SE, CF, and CC, the delivery of the enantiomers of *trans*-T and M1 into the CNS could be caused by active and/or facilitated transport.

The concentrations of the enantiomers of *trans*-T and (−)-M1 varied in different brain tissues. CC, CS, HY, CE, and MO are all the important brain tissues for pain and antinociceptive action. The different concentrations of the enantiomers of *trans*-T might be contributed to the various levels of monoamine neurotransmitters in the different brain tissues after *trans*-T injection[3].

After *trans*-T injection, the concentrations of (+)-*trans*-T were higher than those of (−)-*trans*-T in the brain tissues, and the concentrations of (+)-M1 were lower than those of (−)-M1 in some of the brain tissues. It suggested that the distribution of *trans*-T and M1 in the CNS was stereoselective. But, the stereoselectivity in the distribution of M1 was not as significant as that of *trans*-T, since the (−)/(+)-M1 ratios (Fig 2) were lower than the (+)/(−)-*trans*-T ratios (Fig 3) in the brain tissues.

Contrast to the results founded after *trans*-T injection, the concentrations of (+)-M1 were higher than those of (−)-M1 in the CC and MO after M1 injection. It could be concluded that the stereoselectivity in the distribution of M1 after M1 injection was different with that after *trans*-T injection.

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反式曲马朵及反式氧去甲基曲马朵对映体在大鼠中枢神经系统的分布

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关键词 曲马朵；中枢神经系统；药代动力学

目的：研究反式曲马朵(*trans*-T)及其活性代谢产物反式氧去甲基曲马朵(M1)对映体的中枢神经系统分布. **方法**：大鼠 ip 单剂量的盐酸 *trans*-T 或 M1 后，采用高效毛细管电泳(HPCE)法测定血清和不同脑组织中 *trans*-T 及 M1 对映体的浓度，脑组织包括脑脊液、大脑皮层、纹状体、下丘脑、小脑、延髓. **结果**：大鼠 ip 盐酸 *trans*-T 后，在血清及所有测试脑组织中，(+)-*trans*-T 的浓度均高于(−)-*trans*-T 的浓度；在所有测试脑组织中，(+)-M1 的浓度均低于(−)-M1 的浓度；*trans*-T 和(+)-M1 对映体的浓度以大脑皮层中最高，脑脊液中最低. 大鼠 ip M1 后，在血清及所有测试脑组织中，(+)-M1 的浓度均高于(−)-M1 的浓度；M1 对映体的浓度以大脑皮层中

最高，脑脊液中最低. **结论**：在血清和不同脑组织中，*trans*-T及M1对映体的浓度是有区别的；*trans*-T及M1在中枢神经系统的分布具有立体选择性；大鼠ip M1与*trans*-T后，M1在中枢神经系统分布的立体选择性是不同的.

（责任编辑 吕 静）

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