

Dihydroetorphine-induced place preference was mediated by dopamine D₁ receptors in rats

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KEY WORDS dihydroetorphine; dopamine D₁ receptors; dopamine D₂ receptors; nucleus accumbens; psychological adaptation; animal behavior; haloperidol; Sch-23390; sulpiride; spiperone

ABSTRACT

AIM: To study the influence of dopamine (DA) receptor antagonists upon the rewarding property of dihydroetorphine (DHE). **METHODS:** Conditioned place preference (CPP) paradigm was used to characterize the rewarding effect of DHE. DA receptor antagonists were injected administered subcutaneously or pentoneally and microinjected into nucleus accumbens (NAcc). **RESULTS:** DHE (0.05, 0.5, and 5.0 $\mu\text{g}\cdot\text{kg}^{-1}$, sc) produced place preference ($P < 0.01$). Both the DA receptor antagonist haloperidol and the selective D₁ receptor antagonist Sch-23390 attenuated the place preference produced by DHE (0.5 $\mu\text{g}\cdot\text{kg}^{-1}$, sc). *l*-Sulpiride and spiperone, selective D₂ receptor antagonists, had no such effects. **CONCLUSION:** The D₁ (but not D₂) receptors in NAcc are crucial in the mediation of the rewarding effect of DHE.

INTRODUCTION

The conditioned place preference (CPP) paradigm has been used to study the reinforcing properties of drugs abused^[1]. Studies suggest that dopaminergic system plays a critical role in motivational effects of drugs abused. The D₁ receptor antagonist attenuates

opioids-induced place preference, but the role of D₂ receptors in opioids reward remains in controversy^[2]. The mesolimbic dopamine system (MLDS) and, in particular, its terminals in nucleus accumbens (NAcc), were involved in the motivational effects of drugs abused. Microinjections of opioids into NAcc produced place preference^[3]. Bilateral 6-hydroxydopamine (6-OHDA) lesions of NAcc blocked morphine-induced place preference^[4,5]. *In vivo* microdialysis studies suggest that the rewarding properties of opioids were mediated by increase in extracellular DA in the NAcc^[6]. These studies have led to the hypothesis that the NAcc is a critical neural site for the rewarding effects of opioids. Dihydroetorphine (DHE) is a relative μ -receptor selective ligand^[7]. DHE produced strong place preference^[8]. But whether the dopaminergic system is involved in the rewarding effect of DHE remains unknown. Accordingly, in the present study, the different DA receptor antagonists were systemically administered and microinjected into NAcc before DHE sc to examine the role of specific DA receptor types of NAcc in the mediation of the place preference of DHE.

MATERIALS AND METHODS

Materials Wistar rats ($\hat{\sigma}$, $n = 160$, weighing 220–270 g, Certificate No 01-3056) were supplied by Department of Laboratory Animal Science, Beijing Medical University. DHE (Beijing Sihuan Pharmaceutical Plant, China), haloperidol (Shanghai No 13 Pharmaceutical Plant, China), and Sch-23390 (RBI, USA) were dissolved in 0.9 % saline. *l*-Sulpiride (Sigma, USA) and spiperone (RBI, USA) were dissolved in distilled water containing Me_2SO and HCl 1 $\text{mol}\cdot\text{L}^{-1}$ (pH of final solution 6.5).

Surgery Rats were anesthetized with sodium

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pentobarbital ($40 \text{ mg} \cdot \text{kg}^{-1}$, ip) and placed on a stereotaxic instrument (NaRishige, Japan). Each rat was unilaterally implanted a stainless steel guide cannula (0.65 mm) into NAcc (1.8 mm anterior to bregma, 1.2 mm lateral to midline, and 6.5 mm below dura⁽⁹⁾). The guide cannula was positioned 1.0 mm above the designated site of injection and was anchored to the skull with dental acrylic. Rats were allowed 1 week to recover before the start of place conditioning.

Microinjection Microinjections were made manually using a 0.3 mm stainless steel inner needle attached to a 1.0 μL microsyringe. The volume of drug given was $1.0 \mu\text{L} \cdot \text{kg}^{-1}$. Solutions were infused $0.1 \mu\text{L} \cdot \text{min}^{-1}$ and the injection needle was then left in place for an additional 1 min to ensure complete solution delivery.

Histology After the completion of testing, each rat was deeply anesthetized with sodium pentobarbital and perfused with 4% paraformalin. Brain was histologically prepared, 50 μm frozen sections were cut and stained with Nissl, and the site of injection was examined, rats with histologically incorrect cannula placements were discarded.

Apparatus⁽¹⁰⁾ The CPP apparatus was made of plexiglass. It consisted of two compartments (each 44 cm \times 28 cm \times 28 cm). One compartment was white with a textured soft floor and the other was black with a smooth floor, separated by a partition. The apparatus was placed in a soundproof room (60 cm \times 50 cm \times 50 cm), and constant light provided by a 5 W lamp was placed above the compartments.

Place conditioning A biased procedure was used. Firstly, we examined the initial preference of rats using a preconditioning test, the time spent by naive rats in the black and white compartments was $545 \text{ s} \pm 163 \text{ s}$ and $327 \text{ s} \pm 165 \text{ s}$ ($n = 50$), respectively, suggested the naive rats showing preference for black box. During the conditioning phase, the white box was served as drug-paired side, ie, the rats were confined to white box after drug administrations and to the black box after vehicle administrations. The orders of treatment (drug or vehicle) were counterbalanced across rats. Conditioning sessions were 40 min in duration and conducted twice daily (one for drug and one for vehicle) for 5 d. The interval between two administrations was more than 6 h. On the test day, the partition separating the two compartments was raised

up to 12 cm above the floor, and then the drug-free rats were placed in the middle allowing free access to both compartments for 15 min. The time spent in each box was measured. If the time spent in the white box was longer than that in the black box, it suggested that the rats produced place preference.

Statistic analysis Place conditioning scores represented the time spent in the drug-paired (ie, white) side. Data were expressed as $\bar{x} \pm s$ and analyzed by the *t*-test.

RESULTS

The place conditioning of DHE In control group, rats receiving injections of saline (sc) during each of the conditioning sessions spent in the drug-paired side $310 \text{ s} \pm 158 \text{ s}$. Rats receiving injections of DHE (0.05, 0.5, and $5.0 \mu\text{g} \cdot \text{kg}^{-1}$, sc) exhibited preference for drug-paired side (Tab 1).

Tab 1. Place conditioning produced by DHE.
 $n = 8$ rats. $\bar{x} \pm s$. * $P < 0.01$ vs NS group.

Drug	Dose/ $\mu\text{g} \cdot \text{kg}^{-1}$	Time spent in drug-paired side/s
NS		310 ± 158
DHE	0.05	$668 \pm 116^*$
	0.5	$795 \pm 49^*$
	5.0	$821 \pm 58^*$

Influence of systemically administered DA receptor antagonists upon DHE-induced place preference

Rats were pretreated with vehicle, haloperidol ($0.2 \text{ mg} \cdot \text{kg}^{-1}$, sc), Sch-23390 ($0.05 \text{ mg} \cdot \text{kg}^{-1}$, sc), *l*-sulpiride (10, 20, and $40 \text{ mg} \cdot \text{kg}^{-1}$, ip), and spiperone (0.1, 0.5, and $1.0 \text{ mg} \cdot \text{kg}^{-1}$, sc) respectively. After 30 min each rat received DHE ($0.5 \mu\text{g} \cdot \text{kg}^{-1}$, sc), the time spent in each compartment was recorded. DHE produced place preference in vehicle pretreated rats. Haloperidol and Sch-23390 attenuated the place preference produced by DHE. *l*-Sulpiride and spiperone slightly inhibited the place preference of DHE ($P < 0.05$) (Tab 2).

Influence of DA receptor blockade of NAcc upon DHE-induced place preference After pretreatments with haloperidol $5 \mu\text{g} \cdot \text{kg}^{-1}$ or Sch-23390

Tab 2. Influence of DA receptor antagonists upon the place preference produced by DHE (0.5 $\mu\text{g}\cdot\text{kg}^{-1}$, sc). $\bar{x} \pm s$. ^a $P < 0.01$ vs systemical injection of NS group. ^b $P > 0.05$ vs systemical injection of vehicle group. ^c $P < 0.01$ vs intra-NAcc injection of NS group. ^d $P > 0.05$ vs intra-NAcc injection of vehicle group.

Pretreatment	Dose/ $\text{mg}\cdot\text{kg}^{-1}$	Systemical injection Time spent in drug- paired side/s	Rats	Dose/ $\mu\text{g}\cdot\text{kg}^{-1}$	Intra-NAcc injection Time spent in drug- paired side/s	Rats
NS		752 \pm 60	8		644 \pm 75	6
Haloperidol	0.2	249 \pm 149 ^c	8	5	319 \pm 182 ⁱ	6
Sch-23390	0.05	338 \pm 102 ^c	8	2	358 \pm 105 ^j	7
Vehicle		721 \pm 75	8		669 \pm 75	6
<i>l</i> -Sulpiride	10	690 \pm 86 ^d	8	20	656 \pm 62 ^j	7
	20	617 \pm 122 ^d	8	40	672 \pm 158 ^j	5
	40	694 \pm 129 ^d	8			
Spiperone	0.1	629 \pm 208 ^d	8	0.5	709 \pm 73 ^j	4
	0.5	641 \pm 159 ^d	8	1.0	721 \pm 105 ^j	3
	1.0	608 \pm 170 ^d	8			

2 $\mu\text{g}\cdot\text{kg}^{-1}$ intra-NAcc, DHE (0.5 $\mu\text{g}\cdot\text{kg}^{-1}$, sc) did not produce significant place preference. In contrast, neither *l*-sulpiride (20 and 40 $\mu\text{g}\cdot\text{kg}^{-1}$) nor spiperone (0.5 and 1.0 $\mu\text{g}\cdot\text{kg}^{-1}$) intra-NAcc modified the place preference of DHE (Tab 2).

DISCUSSION

Haloperidol is a mixed D_1/D_2 DA receptor antagonist. In this study, haloperidol sc or microinjection into NAcc attenuated the place preference of DHE. It indicated the involvement of DA receptors in NAcc in the mediation of the place preference produced by DHE.

Sch-23390 binds with high affinity to the D_1 receptor and is considered a selective antagonist of D_1 receptor subtype^[11]. Although Sch-23390 may function as a serotonin antagonist^[12], the dose of Sch-23390 used in the present study had no effect in blocking serotonin receptors. Studies suggested that rats treated with high dose of Sch-23390 produced place aversion, but low dose was without significant effect^[2]. Therefore, the attenuation of DHE-induced place preference by Sch-23390 can not be attributed to an aversive action by itself or antagonism of serotonin receptors, confirming the important role of D_1 receptors in the establishment of opioids reinforcement. Microinjection of Sch-23390 into NAcc resulted in an inhibition of DHE-induced place preference. It

demonstrates a crucial role for the D_1 receptors in NAcc in the mediation of rewarding effects.

l-Sulpiride and spiperone bind with substantially higher affinity to D_2 receptors than to D_1 receptors and are considered to be D_2 receptor specific antagonists^[13]. These two antagonists produced neither place preference nor place aversion^[2]. Systemically pretreatments with *l*-sulpiride or spiperone could not block DHE-induced place preference. Such findings are similar to the observation of Shippenberg *et al*^[2]. Microinjections of *l*-sulpiride or spiperone into NAcc did not modify the place preference of DHE, suggesting that the place preference of DHE might be independent of NAcc D_2 receptors. The attenuation of place preference of DHE by haloperidol should contribute to the D_1 receptor antagonism.

The role of D_1 receptors in opioids reward has been accepted widely. But the role of D_2 receptors in opioids reward remains controversy. Recently, Maldonado^[14] found that morphine could not produce place preference in mice lacking D_2 receptors. This led to conflicting results with some other studies^[2,4], including the present study, which demonstrated that D_2 receptor antagonists could not block opioids-induced place preference. Bardo^[15] explained that opiate reward might be mediated by a subset of DA receptors other than D_2 sites. The discrepancies between these reports are probably due to the experimental conditions used, the pharmacological specificity of the D_2

antagonists, and/or the dose ranges studied. Further studies are required to examine whether DHE and other opioids could produce place preference in mice lacking D_2 receptors.

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大鼠多巴胺 D_1 受体介导二氢埃托啡位置偏爱效应

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关键词 二氢埃托啡; 多巴胺 D_1 受体; 多巴胺 D_2 受体; 伏隔核; 心理适应; 动物行为; 氟哌啶醇; Sch-23390; 舒必利; 螺哌隆

目的: 研究不同的多巴胺(DA)受体拮抗剂对二氢埃托啡(DHE)奖赏效应的影响。 **方法:** 采用条件性位置偏爱模型评价 DHE 的奖赏效应。 DA 受体拮抗剂采取外周给药或直接到伏隔核内。 **结果:** DHE (0.05、0.5 和 5.0 $\mu\text{g}\cdot\text{kg}^{-1}$, sc) 产生位置偏爱效应。 氟哌啶醇、Sch-23390 sc 或直接注射到伏隔核都能抑制 DHE (0.5 $\mu\text{g}\cdot\text{kg}^{-1}$, sc) 的偏爱效应; 而 *l*-舒必利和螺哌隆在这两种给药途径下都不影响 DHE 的偏爱效应。 **结论:** 伏隔核中 D_1 受体(而不是 D_2 受体)在 DHE 偏爱效应中起关键作用。

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