

Inhibitory regulations of ohmefentanyl and morphine on postsynaptic dopamine receptors

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ABSTRACT Ohmefentanyl, a highly selective agonist for μ opioid receptors, produced a naloxone-reversible inhibition of amphetamine-induced ipsilateral and apomorphine-induced contralateral rotation in rats with 6-hydroxydopamine-induced unilateral lesion of nigro-striatal neurons. Acute treatments of ohmefentanyl and morphine decreased dopamine receptor density and inhibited the enhanced binding of [3 H]spiperone to rat striatal dopamine receptors induced by denervation. It is proposed that these two drugs probably have an indirect inhibitory effect on postsynaptic dopamine receptors in rat striatum.

KEY WORDS ohmefentanyl, animal rotational behavior; apomorphine; amphetamine; substantia nigra; caudate nucleus; supersensitivity; postsynaptic dopamine receptors

The rotational behavior of rat with 6-hydroxydopamine (6-OHDA)-induced unilateral nigro-striatal lesion is a very useful pharmacological model for distinguishing actions for drugs in dopaminergic nigro-striatal system⁽¹⁾. Dopamine receptor agonists such as apomorphine (Apo) cause contralateral rotation, presumably due to the development of the increased receptor sensitivity

on the lesioned side, while amphetamine (Amp), which releases dopamine from the intact nigro-striatal nerve ending, induces ipsilateral rotation.

Ohmefentanyl (Ohm), a new agonist for μ opioid receptors⁽²⁾, induces catalepsy and increases striatal dopamine metabolism in rat⁽³⁾. In this paper, we investigated the effects of Ohm and morphine (Mor) on Amp- and Apo-induced rotational behaviors and on the supersensitivity of dopamine receptors following unilateral lesion of nigrostriatal neurons with 6-OHDA.

MATERIALS AND METHODS

Chemicals Ohm was synthesized in our laboratory. Morphine hydrochloride was obtained from Qinghai Pharmaceutical Factory, naloxone hydrochloride from Shanghai Medical University, apomorphine hydrochloride from Shenyang First Pharmaceutical Factory. 6-OHDA was obtained from Sigma and amphetamine sulfate from The British Drug House LTD. Dopamine hydrochloride was purchased from Fluka AG & Buche SG, [³H]spiperone (629 GBq/mmol) from Amersham. All the reagents are AR.

Lesion of nigro-striatal neurons with 6-OHDA and rotation test Male rats (160 \pm SD 8 g) were anaesthetized with pentobarbital sodium 40 mg/kg ip and secured in a stereotaxic frame. Unilateral 6-OHDA-induced lesions of the substantia nigra were made by injecting 6-OHDA 10 μ g at the coordinates: A 3.0 mm; H 8 mm; L 2 mm⁽⁴⁾. The 6-OHDA was dissolved in 4 μ l of isotonic saline solution containing 0.1% of ascorbic acid. At least 2 wk were allowed for degeneration. Circling was recorded in a flat-bottomed bowl (30 cm diam) and only rats which circled at a rate of at least 5 turns/min in response to Apo 2 mg/kg were used. Repeated sc with Apo caused an increase in the circling rate. Once a consistent response was obtained, the effects of drugs on Apo-induced rotation

were examined.

Dopamine receptor binding assays Binding assays were performed⁽⁵⁾. The lesioned rats were treated with Apo 2 mg/kg repeatedly (q4 d, 3 injections). About 2 months after lesions, the left and right corpora striata were assayed separately in triplicate tubes with 6 different concentrations of [³H]spiperone 0.1–1 nmol/L. Nonspecific binding was determined by adding 1 mmol/L dopamine-HCl. The K_d and B_{max} values were calculated from Scatchard plots of [³H]spiperone binding data and analysed by *t*-test.

RESULTS

Tab 1 shows the effects of drugs on ipsilateral and contralateral rotation induced by Amp and Apo respectively. The peak effects of Amp and Apo were reached respectively 30–50 min and 5–15 min after ip. In Amp-induced rotation, Ohm was ip 20 min and naloxone (Nal) 15 min after Amp. Mor was given (ip) together with Amp. In Apo-induced rotation, Ohm, Nal and Mor were injected ip respectively 5 min, 10 min and 20 min before Apo. Acute ip of Ohm and Mor significantly inhibited Amp-induced ipsilateral rotation. The fact that the effect of Ohm was easily reversed by Nal indicated that Ohm acts on opioid receptors. Rotation produced by Apo 0.5 mg/kg was antagonized by either Ohm or Mor. Only Ohm effectively antagonized rotation induced by Apo 2 mg/kg. The inhibitory effect of Ohm on Apo-induced rotation was reversed by Nal.

The results of the binding of [³H]spiperone to rat striatal dopamine receptors are shown in Tab 2. A significant increase in the number of binding sites (B_{max}) was apparent in the lesioned corpus striata, whereas the dissociation constant (K_d) was unaffected by the lesion. After acute treatments of Ohm and Mor, the B_{max} of both lesioned and control side of corpus striatum

Tab 1. Effects of ohmefentanyl (Ohm) and morphine (Mor) on ipsilateral and contralateral rotation rates induced by ip amphetamine (Amp) and apomorphine (Apo) and recorded after 30-50 and 5-15 min, respectively. Nal = Naloxone n = 6-7. $\bar{x} \pm SD$ *p > 0.05, ** p < 0.05, ***p < 0.01 vs saline group(NS).

		Rotation rate (turns/min)		
		Amp	Apo	Apo
		(5 mg/kg)	(2 mg/kg)	(0.5 mg/kg)
NS	10 ml/kg	13±7	22±5	17±3
Ohm	2.5 µg/kg	3±3**	—	6±3**
Ohm	5 µg/kg	—	11±3***	—
Ohm	10 µg/kg	—	5±4***	—
Ohm	2.5 µg/kg	13±3*	—	—
+ Nal	1 mg/kg	—	—	—
Ohm	5 µg/kg	—	20±7*	—
+ Nal	2 mg/kg	—	—	—
Mor	10 mg/kg	4±3**	20±5*	12±4**

Tab 2. Effects of Ohm and Mor on binding of [³H]spiperone in rats with 6-OHDA-induced unilateral nigro-striatal lesion. The rats treated (ip) with saline 10 ml/kg or Ohm 5 µg/kg were decapitated 20 min after ip, and those ip with Mor 10 mg/kg decapitated 40 min after ip. n = 3-4 $\bar{x} \pm SD$ *p > 0.05, *** p < 0.01 vs control side. †p < 0.05, ††p < 0.01 vs saline group.

	Control Side		Lesioned Side	
	K _d	B _{max}	K _d	B _{max}
	(nmol/L)	(pmol/g)	(nmol/L)	(pmol/g)
Saline	0.17 ± 0.05	74 ± 6	0.19 ± 0.05	94 ± 7***
Ohm	0.238 ± 0.02†	61 ± 3††	0.25 ± 0.04	68 ± 4*
Mor	0.29 ± 0.08	51 ± 5†††	0.31 ± 0.08	58 ± 10*

was decreased, but no differences between two sides were seen.

DISCUSSION

Ohm inhibition of Amp-induced rotation which could be reversed by Nal suggests that this drug probably exerts a pre-synaptic inhibitory modulation on dopaminergic system in corpus striatum by acting on μ receptors.

It has been reported that high dose of Mor decreases the number of rat striatal dopamine receptors, which is considered as

an indirect regulation⁽⁵⁾. Our experiments indicated that Ohm and Mor reduced the number of striatal dopamine receptors in rat with 6-OHDA-induced unilateral lesion of nigrostriatal neurons. It seems that supersensitive dopamine receptors were more subject to the influence of opioids. It has been proved that dopamine receptor bindings increase after lesion of the nigro-striatal dopamine neurons in those rats which are behaviorally supersensitive, as reflected by Apo-induced contralateral rotation⁽⁶⁾. Mor inhibition of Apo-induced rotation has been reported⁽⁷⁾. Ohm could antagonize Apo-induced rotation much more effectively than Mor did. This inhibitory effect of Ohm could be easily reversed by Nal. Binding studies also showed that Ohm and Mor inhibited the supersensitivity of dopamine receptors induced by denervation. These results strongly suggest that Ohm and Mor probably have inhibitory effects on post-synaptic dopamine receptors in rat corpus striatum by acting on μ receptors.

Lesion studies have shown that in addition to 26% [³H]Ohm binding sites located on the presynaptic dopamine nerve terminals in corpus striatum, 53% [³H]Ohm binding sites are also present on intrastriatal neurons (to be published). It is therefore proposed that Ohm and Mor probably act at one of those latter sites to have an inhibitory regulation on postsynaptic dopamine receptors and antagonize Apo-induced rotation.

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羟甲基芬太尼和吗啡对突触后多巴胺受体的抑制性调节

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提要 在6-羟多巴胺单侧损毁黑质纹状体神经元的大鼠模型上, μ 阿片受体激动剂羟甲基芬太尼既能抑制苯丙胺引起的同侧旋转, 又能抑制阿扑吗啡引起的对侧旋转。其作用可被纳络酮所拮抗。急性给予羟甲基芬太尼和吗啡能使受损大鼠纹状体多巴胺受体密度降低, 并能抑制去神经引起的多巴胺受体超敏。这些结

果提示羟甲基芬太尼和吗啡对大鼠纹状体突触后多巴胺受体可能有一种抑制性的调节作用。

关键词 羟甲基芬太尼, 动物旋转行为, 阿扑吗啡, 苯丙胺, 黑质, 尾状核, 超敏, 突触后多巴胺受体