Effect of 6-Hydroxydopamine Treatment in the Area Postrema on Morphine-Induced Emesis in Ferrets

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ABSTRACT—To investigate the role of catecholamine release in emesis, we examined the effects of pretreatment with 6-hydroxydopamine (6-OH-DA) administered into the area postrema in morphine-induced emesis in ferrets. In the 6-OH-DA pre-treated animals, the latency to the first emetic response induced by morphine hydrochloride (1.0 mg/kg, s.c.) was significantly prolonged and the number of retches and emetic episodes was markedly reduced. In the medulla oblongata, the levels of dopamine and homovanillic acid were reduced by 6-OH-DA pretreatment. These results suggest that catecholamine release in the medulla oblongata, mainly dopamine release, may play an important role in morphine-induced emesis in ferrets.

Keywords: Morphine, 6-Hydroxydopamine, Emesis

The most troublesome side effects of morphine administration in humans are nausea, vomiting, constipation, drowsiness and respiratory depression (1). Nausea and vomiting occur frequently after administration of morphine not only in humans but also in dogs, cats and ferrets (2). The emetic effect of morphine is due to an action on the chemoreceptor trigger zone (CTZ), since ablation of the area postrema prevents vomiting in ferrets, dogs or cats (3, 4).

It has been reported that naloxone, an opiate µ-receptor antagonist, reduces morphine-induced emesis in animal models and that phenothiazine-type dopamine D₂-receptor antagonists reduce morphine-induced emesis in man (3, 5). In addition, the finding that morphine acting via µ-receptors can induce the release of dopamine from dopaminergic nerves in the substantia nigra, the caudate nucleus and the nucleus accumbens (6, 7) offers an explanation for the effectiveness of dopamine-receptor antagonists. Indeed, autoradiographic studies using µ-receptor ligands revealed the binding sites in the area postrema, the nucleus tractus solitarius and dorsal motor vagal nucleus in cats and ferrets (5). Moreover, immunohistochemical studies using dopamine antisera revealed the presence of dopamine immunoreactive neurons in the area postrema, the nucleus tractus solitarii and the dorsal motor vagal nucleus in cats and rats (8).

These findings indicate that morphine probably acts on opiate receptors in the area postrema, possibly µ-receptors, causing dopamine release into the CTZ including the area postrema. However, it is not clear whether morphine-induced emesis is mediated by dopamine release in the CTZ. Therefore, the aim of the present study was to investigate the effect of pretreatment with 6-hydroxydopamine (6-OH-DA), a dopaminergic neurotoxin, administered into the area postrema on morphine-induced emesis in ferrets.

In this study, male albino ferrets (Marshall Res. Animal, North Rose, NY, USA) weighing 1.0 – 1.3 kg and individually housed in a room kept at 22 – 25°C under a 12-h light/dark cycle were used. They were given a standard cat diet (70 – 80 g/animal, Purina®; Ralston Purina Co., St. Louis, MO, USA) and allowed free access to water. The ferrets were anesthetized with pentobarbital sodium (35 – 40 mg/kg, i.p.) and placed in a stereotaxic apparatus. The skull was exposed and a small hole drilled to allow unilateral stereotactic insertion of the injection cannula (27 gauge, 23-mm length) into the area postrema. The coordinates for the operated region were −17.10-mm anterior to bregma, −4.30-mm lateral to the midline, and −15.00-mm at 20° from the vertical axis from the skull surface. The injection cannula was inserted very slowly (over 1 min) and then fixed for a few minutes. 6-OH-DA (30 µg) or vehicle (0.01% ascorbic acid) was administered in a volume of 5 µl over a period of 5 min. Two minutes after administration, the injection cannula was removed and the small hole was closed with bone wax. At least 10 days after treatment
with 6-OH-DA, morphine (1.0 mg/kg) was administered s.c. in a volume of 2 ml/kg into the ferrets. The animals were then observed for 30 min, and the number of retches and vomits were recorded.

One day after administration of morphine, the ferrets were killed with pentobarbital sodium (100 mg/kg, i.p.) and their brains were removed rapidly from the skull. The medulla oblongata and the frontal cortex were then dissected and placed on dry-ice, and their concentrations of dopamine (DA), homovanillic acid (HVA), noradrenaline (NA), 5-hydroxytryptamine (5-HT), and 5-hydroxyindole acetic acid (5-HIAA) were determined by high-performance liquid chromatography with electrochemical detection.

The drugs used in this study were 6-OH-DA hydrochloride (Sigma, St. Louis, MO, USA) and morphine hydrochloride (Takeda Chemicals Industries, Ltd., Osaka). 6-OH-DA hydrochloride was dissolved in sterile distilled water containing 0.01% ascorbic acid and its dose was expressed in terms of the free base. Morphine hydrochloride was dissolved in sterile distilled water and its dose was calculated on the basis of its base weight. The data presented as the mean ± S.E.M. Wilcoxon rank sum test or parametric Dunnett’s multiple comparison test was used as a measure of significance. The significance level was set at P<0.05.

The effects of pretreatment with 6-OH-DA administered into the area postrema on morphine-induced emesis in the ferret are shown in Fig. 1. In the vehicle (0.01% ascorbic acid) pretreated group, morphine hydrochloride (1.0 mg/kg, s.c.) evoked a marked emetic response. However, in the 6-OH-DA pretreated group, the latency to the first emetic response induced by morphine hydrochloride was prolonged, and the number of retches and emetic episodes was reduced. No emetic episode was evoked in all animals pretreated with 6-OH-DA, although one animal exhibited retches.

Table 1 shows the levels of amines and their metabolites in the medulla oblongata and the frontal cortex in ferrets pretreated with vehicle or 6-OH-DA administered into the area postrema. In the medulla oblongata, the levels of NA, DA and HVA were significantly reduced by 6-OH-DA

| Regions         | Treatment | n  | NA     | DA     | HVA   | 5-HIAA | 5-HT   |
|-----------------|-----------|----|--------|--------|-------|--------|--------|
| Medulla oblongata | vehicle   | 5  | 635 ± 46 | 49.4 ± 6.4 | 79.5 ± 7.5 | 148 ± 38 | 774 ± 97 |
| 6-OH-DA         |           | 5  | 412 ± 44** | 33.5 ± 1.8* | 58.8 ± 4.4* | 141 ± 14 | 677 ± 52 |
| Frontal cortex  | vehicle   | 5  | 430 ± 29 | 59.6 ± 5.1 | 118 ± 24 | 30.0 ± 4.4 | 128 ± 12 |
| 6-OH-DA         |           | 5  | 487 ± 72 | 63.1 ± 7.6 | 87.4 ± 11.4 | 30.8 ± 2.2 | 124 ± 9  |

Ferrets were killed with pentobarbital sodium (100 mg/kg, i.p.) 1 day after morphine hydrochloride treatment. Each value represents the mean ± S.E.M. Statistically significant difference from the vehicle (0.01% ascorbic acid) treated group is indicated by *P<0.05, **P<0.01, parametric Dunnett’s multiple comparison test.
treatment. However, in the frontal cortex, the levels of amines and their metabolites were not affected.

This study demonstrates that DA release in the medulla oblongata plays an important role in morphine-induced emesis in ferrets. Namely, pretreatment with 6-OH-DA, a dopaminergic neurotoxin, administered into the area postrema inhibited morphine-induced emesis and reduced NA, DA, and HVA levels in the medulla oblongata of ferrets.

The area postrema, a circumventricular organ, is located outside the blood-brain barrier and has been implicated in the mediation of emesis by many centrally acting emetic agents, including opiates (2). Indeed, administration of morphine evokes nausea and vomiting in dogs, cats and ferrets; and this is mediated via opiate receptors located in the area postrema because ablation of the area postrema dramatically reduced this emetic response (4, 9, 10). However, the neuronal pathway of morphine-induced emetic response in the central nervous system including the area postrema is not clear. It is well known that morphine can induce the release of dopamine from dopaminergic nerves in the substantia nigra, the caudate nucleus and the nucleus accumbens (6, 7). However, it is not clear whether dopamine release occurs in the area postrema when animals show the emetic response induced by morphine. Furthermore, it has been reported that neurons within the area postrema contain not only neuropeptides but also catecholamines and catecholamine-synthesizing enzymes (11, 12). We, therefore, considered it necessary to examine the effects of pretreatment with 6-OH-DA in the area postrema on morphine-induced emesis in ferrets. 6-OH-DA is known to deplete not only dopamine but also NA in the central nervous system and to destroy noradrenergic and dopaminergic neurons in the brain (13). In this study, the emetic responses induced by morphine were significantly reduced in ferrets pretreated with 6-OH-DA. In addition, our results confirmed that pretreatment with 6-OH-DA partially destroys noradrenergic and dopaminergic neurons in the medulla oblongata, because this pretreatment significantly reduced the contents of NA, DA and HVA in the medulla oblongata. In contrast, noradrenergic and dopaminergic neurons in the frontal cortex, and serotonergic neurons in the medulla oblongata and the frontal cortex, were not affected by pretreatment with 6-OH-DA. Although the noradrenergic neuronal pathway, which is mediated via α-adrenoceptors in the area postrema is important in emetic responses in dogs and cats, α1 and α2 adrenoceptors antagonists did not inhibit emesis induced by morphine (14). These findings indicate that DA neurons in the medulla oblongata including the area postrema play a major role in morphine-induced emesis.

In humans and in animal models, phenothiazine- and butyrophenone-type dopamine D2 receptor antagonists are known to reduce nausea and vomiting induced by morphine (3, 15). However, another peripheral dopamine D2 receptor antagonist, domperidone, which cannot penetrate into the brain across the blood brain-barrier, did not reduce emetic responses induced by morphine, although it perfectly reduced apomorphine-induced emesis, which is mediated via dopamine receptors in the area postrema (15). These findings suggest that dopamine release after D2-receptors activation by morphine in the area postrema induces emetic responses mediated via dopamine D2 receptors located inside the blood-brain barrier. However, more direct studies are necessary to clarify this hypothesis: e.g., microdialysis study. In addition, the role of DA within the emetic reflex pathway in another emetogens-induced emesis must also be clarified in further studies.

In conclusion, this study shows that pretreatment with 6-OH-DA in the area postrema inhibits emetic responses induced by morphine and significantly reduces the levels of dopamine and its metabolite, HVA, in the medulla oblongata in ferrets. These findings indicate that dopaminergic mechanisms in the area postrema may be involved in the regulation of morphine-induced emesis in ferrets, although the precise site of its action is not clear. From the results of this study, it is assumed that morphine acts on opiate μ-receptors, thereby stimulating dopamine release in the medulla oblongata.

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