LOCOMOTOR ACTIVITY OF O-METHYLFLAVINANTINE IN MICE

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ABSTRACT

O-methylflavinantine (OMF) an alkaloid with narcotic analgesic effects caused a dose dependent increase in locomotor activity in mice at low dose levels (1-10mg/kg) and a highly significant dose-dependent hypoactivity at higher dose levels (25-100 mg/kg). The hypoactive dose of OMF (100 mg/kg) antagonised amphetamine-(2.5 or 5mg/kg) induced increases in locomotor activity produced in naive mice as well as apomorphine - (2mg/kg) induced locomotor stimulation in α-methyl-p-tyrosine (α-MPT) (150 mg/kg) plus reserpine - pretreated (5 mg/kg) 24 hr.) mice. The results suggest that the predominant effect of O-methylflavinantine on locomotor activity in the mouse is hypoactivity which may involve dopomine receptor blockade similar to that reported for morphine and the opioid peptides.

KEYWORDS: *Rhygiocarya racemifera, O- methylflavinantine, morphinandienone alkaloid, locomotor activity.*

INTRODUCTION

The leaves of Rhigiocarya racemifera, an Apocynaceae plant, are widely used in West Africa as a nasal decongestant and the leaves, root, bark and seeds are used as an aphrodisiac and for treating various forms of stomach pains[13]. In previous reports it has been demonstrated that O-methylflavinantine (OMF, Fig.1), a morphinandienone alkaloid from the root bark of Rhigiocarya racemifera[22] possesses narcotic analgesic activity [2,4], produces tolerance and has the ability to substitute for morphine in morphine-dependent mice[3].

Narcotic analgesic drugs are reported to stimulate locomotor activity in the mouse [12,17]. Like narcotic analgesia, this effect is stereospecific [17]. In this report the effect of OMF and morphine on locomotor activity in naive mice have been compared and its effects on amphetamine or apomorphine-induced locomotor stimulation have also been examined.

METHODS

Locomotor activity studies

The method used was based on that of Dews [8]. Male QS stain mice (18-22 g) were housed in groups of 12 under a 12h light-dark cycle at a temperature of 21° ± 1°C. They had free access to food and water except during experimentation. After suitable pretreatments, groups of three mice were placed in four matched activity cages (length 390 mm, width 265 mm, height 76 mm) constructed from plexiglass. Two light beams shone across the short axis of the cage and focussed on a photoelectric cell which was coupled to a relay and operated a digital counter each time the light beam was interrupted. Cumulative activity counts (from which single counts were obtained) were recorded every 10 or 15 min. immediately after treatment for 90 min to 180 min. depending on the drug being investigated.

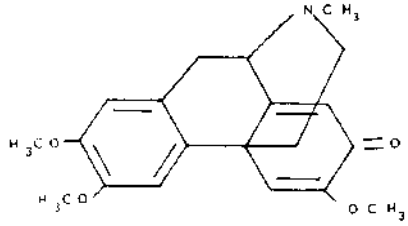

Fig. 1 O-METHYLFLAVINANTINE

Statistical comparisons were made by two-tailed Student's t-tests. Locomotor activity was subjected to a square root transform (x) of cumulative counts prior to analysis [19].

Drugs and Materials

The following drugs and chemicals were used. Reserpine, alpha-methyl-p-tyrosine methyl ester hydrochloride (α-MPT) (Sigma Chemical Company); morphine hydrochloride (McFarland Smith); apomorphine (Sandoz); dexamphetamine sulphate (Smith, Kline & French Laboratories), O-methylflavinantine, (Faculty of Pharmacy, UST, Kumasi, Ghana). O-methylflavinantine was dissolved in a few drops of 1N HCl and the pH was adjusted to 6.5 by

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the addition of 10% NaOH (solution of sodium hydroxide in distilled water) and then diluted with 0.9% w/v solution of sodium chloride (NaCl) in distilled water (Saline) to the required volume. Reserpine was dissolved in a drop of glacial acetic acid and diluted with distilled water. α-MPT and apomorphine were dissolved in 0.05% ascorbic acid solution in distilled water. The solution of apomorphine was protected from light and used within 8h. All other drugs were dissolved in saline. Appropriate vehicle (10 ml/kg) controls were used in all cases.

RESULTS

Locomotor stimulant effect of low doses of OMF

Mice were injected (i.p) with either OMF or morphine (1,5 or 10 mg/kg) and activity counts monitored every 10 min. for 180 min. A significant increase in locomotor activity count was produced by 10 mg/kg OMF, 70-130 min. after injection when compared to the activity of mice treated with saline (Fig.2), but no significant changes ($p > 0.05$) in activity occurred in mice treated with lower doses of OMF (1 or 5 mg/kg). Maximum stimulation occurred 120 min. after 10 mg/kg OMF injection ($p < 0.001$; df = 33). Similarly, significant increases in locomotor stimulation were produced by 5 mg/kg and 10 mg/kg of morphine but not by the lower dose (1 mg/kg). (Fig.3). Maximum stimulation of activity occurred 30 min. and 70 min. after the 5 mg/kg ($p < 0.001$; df = 27) and 10 mg/kg ($p < 0.00001$, df = 29) doses of morphine respectively. Doses of morphine (10 mg/kg) and OMF (10 mg/kg) which produced increases in locomotor activity when given alone caused a greater increase in the number of activity counts over 180 min. when they were co-administered (Fig.4) but the increases were not significant ($p > 0.05$). When higher doses of OMF which were within the analgesic dose (Ansa-Asamoah, 1984) range (20 - 100 mg/kg) were administered (i.p) to mice, a dose-dependent depression of locomotor activity was observed.

The maximum effect was reached after 1 hour. (50 mg/kg, $p < 0.001$; 100 mg/kg, $p < 0.001$).

Fig. 2 Locomotor Stimulant Effect of OMF in the Mouse (☆p< 0.05; ☆☆p< 0.01).

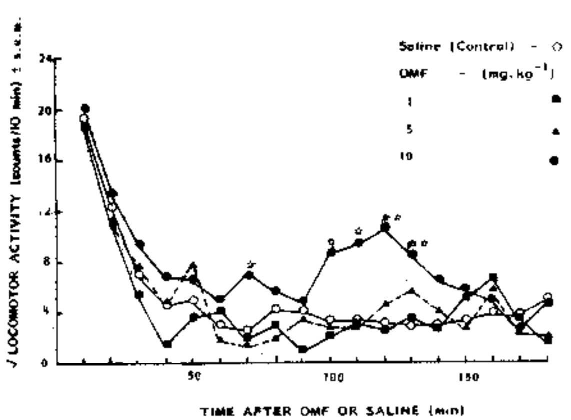


Fig. 3 Locomotor Stimulant Effects of Morphine in the Mouse (Saline ○; 1mg.kg⁻¹ ■ 5 mg.kg⁻¹ ▲, 10mg.kg⁻¹ □ ; ☆ p < 0.05, ☆☆ p < 0.01, ✱ p < 0.001)

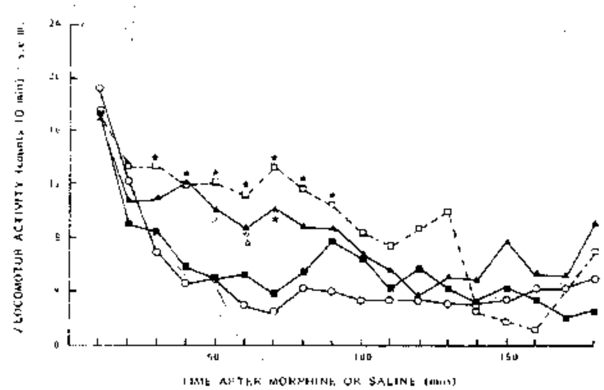
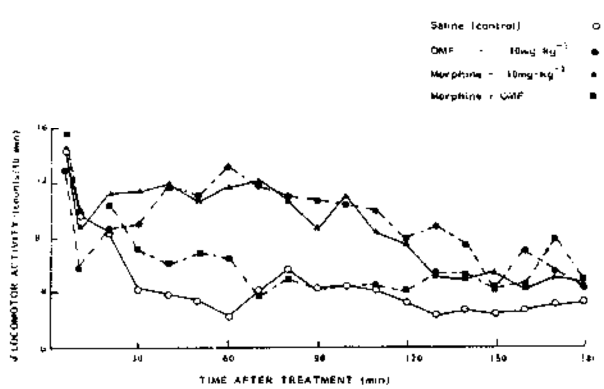


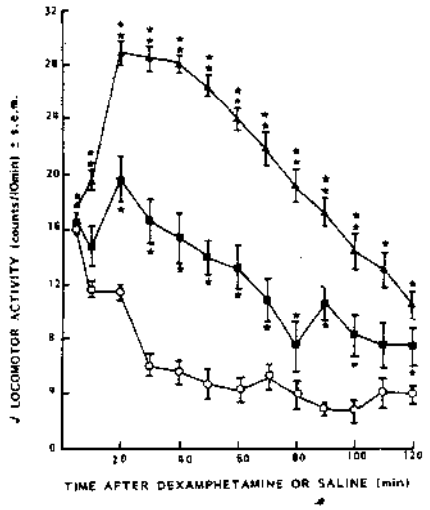
Fig. 4 Locomotor Activity of Mice Pretreated with Morphine and/or OMF



Antagonism of dexamphetamine-induced stimulation of locomotor activity

Mice were pretreated (30 min.) with either saline or OMF (100 mg/kg) followed by an injection of dexamphetamine (2.5 or 5 mg/kg; i.p). Locomotor activity counts were recorded immediately after dexamphetamine injections at 10 min. intervals for 120 min. (Fig.5). A significant dose-dependent increase in locomotor activity counts occurred

Fig. 5 Locomotor Stimulant Effects of Dexamphetamine in the mouse. (Saline ○, Dexamphetamine, ■ 2.5; ▲ 5 mg.kg^{-1}. (* p< 0.001, ** p< 0.0001).



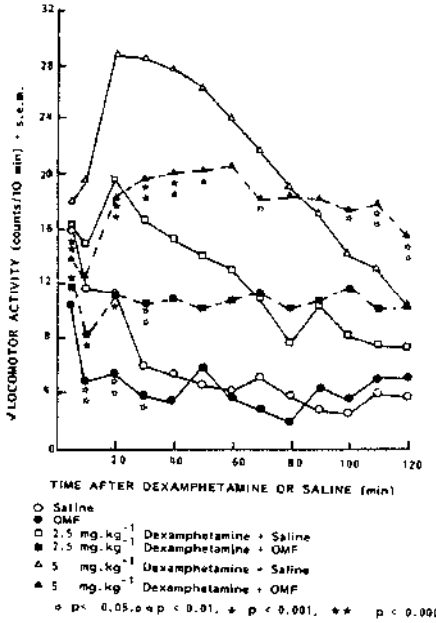
after dexamphetamine alone when compared to the activity of saline-treated mice. The maximum increase in activity occurred (2.5 mg/kg, $p < 0.001$, df = 33; 5 mg/kg, $p < 0.0001$, df = 56) 20 min. after injection. OMF alone (100 mg/kg) produced a significant depression of activity (5 min. $p < 0.001$; 20 min. $p < 0.01$; 30 min. $p < 0.05$; df = 32) when compared to the activity counts of saline-treated mice. OMF (100 mg/kg) pretreatment (30 min.) caused a significant reduction of dexamphetamine-induced (2.5 or 5 mg/kg) stimulation of locomotor activity during the first 30 min. of treatment (Fig.6). A lower dose of OMF (120 mg/kg) given alone, did not depress activity. The activity counts at peak effect (30 min.) for saline (5.93 ± 0.82) and OMF (20 mg/kg) (5.81 ± 2.19) treated mice were not significantly different ($p > 0.05$, df = 29). However, this dose of OMF (20 mg/kg) also reduced significantly the dexamphetamine-induced stimulation 30 min. after its administration. The dexamphetamine (5 mg/kg) control counts at peak effect (30 min.) of 28.52 ± 0.70 were significantly ($p < 0.01$, df = 36) reduced to 22.98 ± 1.83.

Antagonism of apomorphine-induced stimulation of locomotor activity

To investigate whether or not dopamine receptor blockade is involved in the depressant effect of OMF, a dopamine receptor stimulant drug, apomorphine (2 mg/kg, i.p) was used to induce locomotor stimulation.

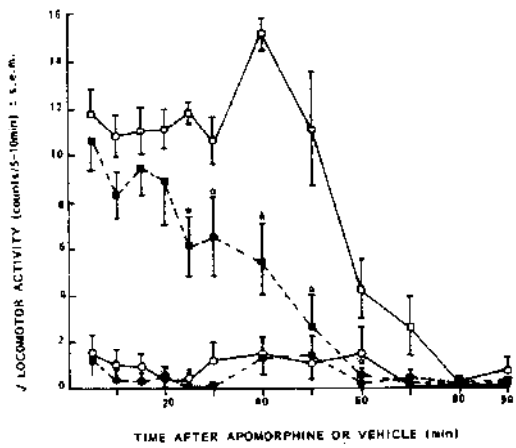
Mice were injected with reserpine (5 mg/kg) plus α -MPT (150 mg/kg) intraperitoneally 24h prior to experimentation in order to eliminate endogenous stores of noradrenaline, dopamine and 5-hydroxytryptamine as well as to block the synthesis of noradrenaline and dopamine. Mice were then pretreated with either OMF (100 mg/kg) or saline for 30 min. followed by an injection of either apomorphine (2 mg/kg) or vehicle (0.05% ascorbic acid in saline). The number of activity counts were recorded at 5 min. intervals for a further period of 90 min. No significant change in activity counts occurred in mice pre-treated with OMF (100 mg/kg) compared to those of mice which received saline when both groups were injected with the vehicle. A significant increase in locomotor activity was produced by apomorphine (2 mg/kg) when compared to that of mice injected with the vehicle (Fig.7) but this was significantly reduced after OMF-pretreatment (25 min., $p < 0.001$; 30 min $p < 0.05$; 40 min., $p < 0.001$; 50 min., $p < 0.02$; 60 min.; $p < 0.02$; df = 13).

Fig. 6 Antagonism by OMF (100 mg.kg^{-1};) of the Locomotor Stimulant Effect of Dexamphetamine in the Mouse.



o p< 0.05, oo p < 0.01, * p < 0.001, ** p < 0.0001

Fig. 7 Antagonism of Apomorphine-Induced Locomotor Stimulation in α-MPT- and Reserpine-Pretreated Mice by OMF (Ascorbic Acid Vehicle, ○ ; OMF, 100 mg.Kg^{-1} ● ; Apomorphine, 2 mg.kg^{-1} □ ; Apomorphine + OMF, ■ ; * p< 0.05, * p< 0.001).



DISCUSSION

We have previously demonstrated that OMF possesses morphine-like analgesic properties in the mouse [2, 4] and a common receptor site of action for the two drugs has been proposed. Narcotic analgesics cause an increase in locomotor activity in mice similar to that reported for morphine [7] and the opioid peptides [16] in the rat; hyperactivity at low dose levels (10 mg/kg) and a highly significant dose-dependent hypoactivity at analgesic dose levels (25 -100 mg/kg). In a parallel experiment with morphine (1-10 mg/kg) a significant dose dependent hyperactivity was observed in mice which confirms previous reports in this species [12, 17]. On the other hand, the intracerebral administation of an enkephalin analogue, (D-Met[2], Pro[5]) -enkephalinamide and morphine is reported to produce a biphasic response on locomotor activity in the mouse, [21] suggesting that both the narcotics and the opioid peptides may affect locomotor activity in mice and rats in a similar manner.

Amphetamine-induced hyperactivity which may be due to an increase in the availability of dopamine at the post-synaptic dopamine receptor site [15] is specifically blocked by neuroleptic drugs as well as by marcotic analgesics [10]. The dose-dependent antagonism by OMF of amphetamine-induced increases in locomotor activity appears to be similar to that previously reported for narcotic analgesics [10]. This observation supports our previous observation that both morphine and OMF may share a common central receptor site with probably a similar mechanism of analgesic action [2, 4].

Apomorphine is considered a relatively pure DA-receptor agonist [1, 9]. Since higher doses of morphine have been shown to antagonise the apomorphine-induced hyperactivity [14], the blockade by OMF of apomorphine-induced increases in locomotor activity in mice pretreated with α-MPT plus reserpine (24 hr.) may indicate that the hypoactivity observed after OMF may involve post-synaptic dopaminergic receptor blockade in a similar manner as previously reported for the analgesic drugs morphine [14] and methadone [18, 11]. The observation that reticuline, an alkaloid structurally related to the morphinandionones [20] and which is involved in the biogenesis of morphine [6] is capable of blocking central dopamine receptors [5] further supports the present findings.

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