Studies on the Mode of Action of Ambrein as a New Antinociceptive Compound

Sadek A. Taha

Department of Pharmacology, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia

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ABSTRACT—The compound ambrein was isolated from ambergris, which is commonly used as an analgesic in the Saudi folklore medicine. The LD$_{50}$ of ambrein, given intraperitoneally (i.p.) in mice, was found to be high (7.5 g/kg), and ambrein proved to be a safe compound in this species. In the hot-plate test, ambrein was found to possess antinociceptive activity in mice at doses which did not sedate or incapacitate the animals. By the i.p. administration route, ambrein produced antinociception in mice at a dose as low as 10 mg/kg. The antinociceptive activity of ambrein (250 mg/kg i.p.) was inhibited by a noradrenergic neurotoxin (DSP-4) and by naloxone, methysergide or prazosin. It was not influenced by a serotonin depletor, p-chlorophenylalanine. The possible mechanism of ambrein antinociception is discussed.

Keywords: Ambrein, Antinociception, Noradrenaline, 5-HT, Opioid receptor

Ambergris is a fragrant substance of an ash-grey color commonly used in perfumery. It is an internal secretion of the sperm blue whale (Physeter catodon) (1). The substance is found floating on the sea, cast on the seacoast of warm countries, and in the intestines of the whale. The constituents of ambergris include the triterpenoid ambrein, which is the major component, as well as some other sterols. Recently, the chemical structure of ambrein which was isolated from ambergris (purchased from the local market in Riyadh, Saudi Arabia) was confirmed by NMR (2).

Ambergris has been extensively used in Eastern folklore medicine for the treatment of various ailments. For example, ambergris has been claimed to alleviate headache, migraine or the common cold when applied topically. Also, it has a good reputation as a topical antirheumatic compound. I recently performed general pharmacological screenings of ambergris extract and ambrein on some isolated and intact animal preparations (3–5). However, no pharmacological studies have been done on the antinociceptive activity of this substance.

Therefore, the present study was performed in mice to investigate whether ambrein has any antinociceptive activity. For this purpose, an authenticated sample of ambrein obtained from locally purchased ambergris was used (2). The possible mechanism of ambrein antinociceptive activity was also investigated.

MATERIALS AND METHODS

Animals

Male Albino Swiss mice (Animal Care Center, College of Pharmacy, King Saud University) weighing 22–25 g were used. Mice were housed in groups at a temperature of 24 ± 1.0°C. Food and water were made available freely except on the day of the experiment.

Drugs

Ambrein was purchased from the local market in Riyadh, Saudi Arabia. Ambrein was extracted as described previously (2). The yield was 45–80% w/w of dried starting material. The dried ambrein was freshly dissolved in olive oil. Other drugs used were: p-chlorophenylalanine (PCPA, Fluka), N-(2-chloroethyl)-N-ethyl-2-bromo-benzylamine hydrochloride (DSP-4, Fluka), naloxone hydrochloride (Dupont), prazosin hydrochloride (Pfizer) and methysergide hydrogen maleate (Janssen Pharmaceutical). All drugs were dissolved in double distilled water. Doses are expressed as mg salt/kg, and the dose volume was kept constant at 4 ml/kg.
**LD₅₀ determination**

Various doses (0.005–25 g/kg) of ambrein were administered intraperitoneally (i.p.) to 7 groups of male mice. Each group consisted of 10 animals. The animals were observed periodically during 24 hours for gross toxic effects, and mortality was recorded. The LD₅₀ was determined by the method of Litchfield and Wilcoxon (6).

**Hot plate test**

The method employed for testing antinociceptive activity was basically described by Eddy and Leimbach (7). Each animal was placed gently on a hot plate (Columbus Instruments, Ohio, USA), maintained at 55 ± 0.5°C. The instrument is equipped with a timer which is controlled by a foot switch. The foot switch can start, stop and reset the timer, thus the reaction time was measured in seconds and tenths of seconds. The reaction time was defined by the time from placing the animal on the hot plate until it was observed to lick its forepaw or jump off the hot plate. A cut-off time of 30 sec was adopted in order to avoid tissue injury in non-responding animals. Hot plate reaction times were obtained at 15 min prior to any treatment and at 30 min intervals for a period of two hours after treatment. Experiments were performed at the same time (10.00 AM) each day and an animal was used on only one occasion. Unless otherwise stated, groups of 10 animals each were used.

**Drug administration**

Ambrein was given i.p. Naloxone, methysergide and prazosin (opioid, serotonin (5-HT) and α-receptor antagonists, respectively) were also given i.p., usually 30 min after ambrein administration. These antagonists were given after ambrein because it was noted that the onset of action of ambrein was delayed (about 60 min post injection).

The 5-HT synthesis inhibitor PCPA was given i.p. at a dose of 150 mg/kg/day for 5 days. The animals were exposed to the hot plate 24 hr after the last administration. The noradrenergic neurotoxin DSP-4 was given i.p. at a dose of 100 mg/kg, and the animals were used in the experiments 4 weeks later.

**Statistics**

Dunnett’s t statistic was used when multiple means were compared with a control, but when two means were compared, Student’s t-test was used. P values less than 0.05 were considered to indicate a significant difference.

**RESULTS**

**LD₅₀ of ambrein**

The acute LD₅₀ value for ambrein given i.p. in male mice was found to be 7.5 g/kg (fiducial limits at the 95% level were 6.9–8.1). Microscopical examination of dead mice failed to reveal the cause of death. Animals which received large doses of ambrein (> 5 g/kg) showed no movement and tended to be lethargic when disturbed.

**Antinociceptive effect of ambrein**

Table 1 shows the antinociceptive effect of ambrein in mice using the hot plate test at doses of 10, 50 and 250 mg/kg, i.p. The effect of ambrein was significantly different (P < 0.05) from the effect of vehicle at 30, 60, 90 and 120 min post injection (F values (df: 3, 36) were 3.54, 21.9, 10.47 and 8, 14, respectively). No incapacitating effect of the drug on the movement of animals was observed. The duration of action of ambrein (10 mg/kg) was from 30–90 min, whereas that of ambrein (50 or 250 mg/kg) was from 60–120 min post injection. Compared to the control, the effect of ambrein (250 mg/kg) was highly significant (P < 0.01). The other

| Test drug (i.p.) | Control | Reaction time in seconds |
|-----------------|---------|-------------------------|
| Olive oil (10 mg/kg) | Time (min) 0 | + 30 | + 60 | + 90 | + 120 |
| 6.0 ± 0.5 | 6.0 ± 0.7 | 6.0 ± 0.7 | 6.8 ± 0.9 | 6.7 ± 1.0 |
| Ambrein | 10 mg/kg | 6.8 ± 0.6 | 8.5 ± 0.6* | 10.9 ± 1.0* | 12.0 ± 0.8* | 10.0 ± 0.9 |
| 50 mg/kg | 6.1 ± 0.6 | 7.8 ± 0.5 | 10.9 ± 0.9* | 11.0 ± 1.0* | 12.0 ± 0.8* |
| 250 mg/kg | 6.3 ± 0.4 | 8.0 ± 0.5 | 16.4 ± 1.0** | 13.4 ± 0.8** | 12.8 ± 1.1** |

Ambrein (or vehicle) was administered i.p. at 0 min. Each value represents the mean ± S.E.M. (n = 10). *Significance of difference from the vehicle control, P < 0.05. **Significance of difference from the vehicle control, P < 0.01.
smaller doses produced effects of smaller magnitude, but their effects were also significant (P < 0.05). It appeared therefore that ambrein (250 mg/kg) was more potent than the other two doses, thus indicating a likely dose-dependent antinociceptive effect of ambrein. The highest dose of ambrein was used in the subsequent experiments.

**Effect of depletion of central serotonin and noradrenaline on the antinociceptive effect of ambrein**

The data in Table 2 show the effects of PCPA or DSP-4 pretreatments alone or in combination with ambrein (250 mg/kg, i.p.) on the hot plate reaction times in mice. The 5-HT depletor PCPA seemed to cause increases in the hot plate baseline latencies, but it did not inhibit the antinociceptive effect of ambrein (except at 120 min after ambrein injection). DSP-4, a neurotoxin that specifically denervates noradrenergic neurons without affecting dopaminergic or serotonergic neurons (8, 9), caused increases in hot plate latencies, but the increases were not as marked as those seen after PCPA. DSP-4 effectively inhibited the antinociceptive effect of ambrein, except at 120 min after ambrein injection.

**Effects of naloxone, methysergide and prazosin on the antinociceptive effect of ambrein**

These antagonists were administered s.c. at the doses shown in Table 3. They were administered 30 min after ambrein administration because the peak effect of

| Test drug (i.p.) | Control Time (min) | Reaction time in seconds |
|-----------------|--------------------|-------------------------|
| + Vehicle (10 mg/kg) | 11.8 ± 0.4 | 11.2 ± 0.7 | 11.0 ± 0.9 | 9.0 ± 0.7 | 12.5 ± 1.5 |
| WC | 12.6 ± 0.5 | 13.5 ± 0.7 | 15.2 ± 0.7*** | 16.5 ± 0.3*** | 13.7 ± 0.8 |
| + Ambrein (250 mg/kg) | 7.8 ± 0.2 | 9.2 ± 0.6 | 9.0 ± 0.6 | 10.0 ± 1.3 | 13.6 ± 1.0*** |

Each value represents the mean ± S.E.M. (n = 12 in PCPA treated groups and n = 10 in DSP-4 treated groups). *P < 0.05, ***P < 0.001.

| Test drug (i.p.) | Time (min) | Control 0 | + 30 | + 60 | + 90 | + 120 |
|-----------------|------------|-----------|-----|-----|-----|-----|
| Vehicle         | 8.4 ± 1.3 | 4.8 ± 0.6 | 5.3 ± 0.4 | 7.2 ± 0.4 |
| + Naloxone (1 mg/kg) | 7.2 ± 1.2 | 4.4 ± 0.5 | 5.7 ± 0.5 | 7.8 ± 0.3 |
| Ambrein         | 7.3 ± 0.2 | 5.8 ± 0.3 | 5.1 ± 0.3 | 7.4 ± 0.2 |
| + Methysergide (4 mg/kg) | 7.4 ± 0.2 | 8.1 ± 0.6*** | 8.6 ± 0.6*** | 8.9 ± 0.6* |
| Vehicle         | 9.0 ± 0.8 | 10.8 ± 1.3 | 13.5 ± 1.2 | 11.2 ± 2.0 |
| + Prazosin (2.5 mg/kg) | 7.8 ± 0.5 | 9.7 ± 0.7 | 9.4 ± 0.6 | 9.8 ± 0.8 |

Ambrein (or vehicle) was administered i.p. at 0 min. The antagonist was given i.p. at 30 min after ambrein. Each value represents the mean ± S.E.M. (n = 10). *P < 0.05, **P < 0.01, ***P < 0.001. Comparisons were made between vehicle + antagonist vs. ambrein + antagonist.
The results presented in this study showed that ambrein, the major component of ambergris, possesses antinociceptive activity in mice as measured by the hot plate test. This activity was dose-dependent with a peak effect occurring at about 60-90 min post administration. Therefore, it seems that its use as an analgesic in folklore medicine is justified. Ambrein did not induce reduction in the animal’s activity nor did it cause sedation in animals at antinociceptive doses.

Interestingly, ambrein antinociception was susceptible to antagonism by naloxone. The likelihood of involvement of endogenous opioid peptides in the action of ambrein is thus suggested.

There is now much evidence to suggest that central 5-HT may play a crucial role in nociception (10, 11). Indeed a role of 5-HT in morphine analgesia was implicated (12-14). It was pertinent, therefore, to consider the role played by 5-HT in the antinociceptive activity of ambrein. PCPA, an irreversible tryptophan hydroxylase inhibitor, which decreases 5-HT concentration in the brain (15), did not inhibit the effect of ambrein on hot plate reaction time. This suggests that presynaptic events in serotonergic transmission do not modify ambrein antinociception. On the other hand, the non-selective 5-HT antagonist methysergide partially inhibited ambrein (refer to Table 1). The a1-antagonist prazosin was also able to reduce the effect of ambrein in the hot-plate test.

DISCUSSION

In summary, the antinociceptive activity of ambrein is likely to involve activation of opioid receptors and, like the opioids, the involvement of both central serotonergic and noradrenergic systems in this activity of ambrein cannot be ruled out.

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