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Journal of Ethnopharmacology 81 (2002) 73–79

Journal of ETHNO-PHARMACOLOGY

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Pseudo-akuammigine, an alkaloid from *Picralima nitida* seeds, has anti-inflammatory and analgesic actions in rats

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Received 8 April 2001; received in revised form 8 February 2002; accepted 21 February 2002

Abstract

Pseudo-akuammigine, an alkaloid from *Picralima nitida* seed extract was investigated for anti-inflammatory and analgesic actions using the carrageenan-induced rat paw oedema and the rat tail flick. The alkaloid, at 1.0, 5.0 and 50 mg kg^{-1} dose-dependently inhibited the mean maximal paw swelling attained during 6 h to 78.2±2.1, 74.7±4.3 and 59.5±2.3% of the mean control value respectively when administered p.o. 1 h before induction of oedema. At the same dose levels, the total paw swelling over the 6-h period was also significantly ($P < 0.05$) reduced to 83.2±9.7, 73.0±5.0 and 55.8±8.3% of the mean control response respectively. When administered after induction of oedema, ψ-akuammigine (5.0 mg kg^{-1}) significantly ($P < 0.05$) reduced established rat paw swelling to 82.8±4.6% of the control response after 5 h. As an analgesic, ψ-akuammigine was 3.5 and 1.6 times less potent than morphine and indomethacin respectively. The ED_{50} values were Morphine (2.9 μM), ψ-akuammigine (10 μM) and indomethacin (6.3 μM). Naloxone (1.0 mg kg^{-1}) significantly ($P < 0.05$) antagonised the analgesic action of the alkaloid by 35.8±6.8%. Pseudo-akuammigine therefore exhibits anti-inflammatory and analgesic actions. The analgesic actions are mediated via interaction with opioid receptors.

Keywords: *Picralima nitida*; Anti-inflammatory; Pseudo-akuammigine; Analgesic

1. Introduction

Extracts from various parts of *Picralima nitida* (Stapf.) Th. & H. Durand (syn. *Picralima klaineana*, Pierre; fam. Apocynaceae) are used in West African traditional medicine for many disease conditions (Abbiw, 1990; Irvine, 1961). The dried powdered seeds of *P. nitida* are encapsulated (250 mg $capsule^{-1}$) and marketed in Ghana by the Noamesi Laboratories, Hohoe, under the brand name 'Picap capsules' for the treatment of pain of various aetiologies and diarrhoea.

Ansa-Asamoah and Ampofo (1986) demonstrated analgesic actions comparable to that of morphine for the aqueous extract of *P. nitida* seeds in rats, thus confirming the ethno-medical uses of the seeds of *P. nitida*. Duwiejua et al. (1995) also reported dose-dependent anti-inflammatory actions for the aqueous ethanolic extract of *P. nitida* seeds in Wistar rats over a dose range of 100–400 mg kg^{-1}, p.o.

As part of our continuing search for analgesic and anti-inflammatory compounds of plant origin, we isolated five indole alkaloids (akuammine, akuammicine, akuammidine, akuammigine and pseudo (ψ)-akuammigine) from *P. nitida* seeds. Using isolated tissue bioassays and radioreceptor binding assays, these alkaloids have been shown to have opioid receptor binding activities (Akuammidine: μ-, δ- and κ-site agonist; Akuammine: antagonist at μ-, δ- and κ-sites; Akuammicine: κ-receptor agonist, ψ-akuammigine: agonist at μ- and δ-sites; Akuammigine showed no selectivity for opioid receptors (Corbett et al., 1996; Menzies et al., 1998).

In this study, we demonstrate in vivo, that pseudo-akuammigine Fig. 1 has analgesic and anti-inflammatory actions in rats using the carrageenan-induced rat paw oedema (Winter et al., 1962)—a model for acute inflammation and the rat tail flick (Ramabadran et al., 1989)—a model for detecting both centrally and peripherally acting analgesics.

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0378-8741/02/$ - see front matter
PII: S0378-8741(02)00058-2

2. Experimental

2.1. Animals

Wistar rats of either sex were used. Clearance for these experiments was obtained from the Food and Drugs Board, the statutory body for regulation of animal experimentation in this country. Animals which lost more than 20% of their starting body weights were euthanised. Animals in which the inflammatory response was severe enough to impede movement which restricted access to food and water were also euthanised. All animals were euthanised at the end of each experiment. Each animal was therefore used only once. The animals were kept at room temperature (25–28 °C). Food and water were provided ad libitum.

2.2. Anti-inflammatory evaluation

Oedema was induced according to the method of Winter et al., (1962). A 1% carrageenaan suspension in normal saline was injected (0.1 ml, s.c.) into the subplantar tissue of the right hind paw of Wistar rats of either sex (180–200 g). Oedema was then monitored at 1 h intervals over 6 h as percent increase in paw thickness. Total oedema induced during the 6 h was measured as area under the time course curves. Drug effects were evaluated by comparing the maximal and total oedema responses attained during 6 h in drug-treated groups with the corresponding values attained in drug vehicle-treated inflamed control groups. Experimental groups ($n = 5$) consisted of

Group I—Inflamed control receiving drug vehicle (1% Cremorphor El, Sigma, 1.0 ml kg^{-1}, p.o.).
Group II—Positive control receiving indomethacin (2.5 mg kg^{-1}, p.o.).
Group III—Test groups receiving ψ-akuammigine suspension (1.0, 5.0 and 50.0 mg kg^{-1}, p.o.)

Drugs were given prophylactically, 1 h prior to the induction of oedema. The effect of ψ-akuammigine on established oedema was investigated by administering the compound (5 mg kg^{-1}, p.o.) 1 h after injection of carrageenan.

2.3. Analgesic evaluation

A modification of the method of Ramabadran et al. (1989) was used to evaluate ψ-akuammigine for analgesic activity. Noxious stimulation was provided by immersing the tails of Wistar rats of either sex (180–230 g, $n = 60$) in warm water maintained at 58 °C. The pain threshold or baseline latent reaction time of each rat (measured as the time taken for a rat to react to heat stimulus when the tail is immersed in the warm water) was recorded. The critical reaction time (CRT), defined as the theoretical time at which an average rat should react to the heat stimulus (calculated as the mean baseline pain threshold $\pm 2 \times$ (standard deviation)). Rats with baseline pain thresholds greater than the CRT were excluded from the main study.

Rats used in the main study were randomly distributed to experimental groups ($n = 5$) as:

Group I—drug vehicle treated control (1.0 ml kg^{-1}, p.o.)
Group II—indomethacin treated (2.5 mg kg^{-1}, p.o.)
Group III—morphine treated (1.0 mg kg^{-1}, s.c.)
Group IV—ψ-akuammigine treated (5.0, mg kg^{-1}, p.o.)

Before drug administration, the baseline latent reaction time for each rat was measured three times at 2-min intervals. The mean of these times for each rat was recorded as the pre-drug latency time. Following drug administration, pain reaction times were then taken again for each rat at 15, 30 and subsequent 30-min intervals for up to 4 h after drug administration. The mean percentage changes in pain thresholds were plotted against time. The time taken for each drug to attain the peak effect was noted. This was used as the end point for subsequent assays. Positive analgesia was recorded for a rat if the latent reaction time (pain threshold) after drug administration exceeded the CRT.

The influence of naloxone (1.0 mg kg^{-1}, p.o.) on analgesic activities of the test drugs was determined by administering naloxone 30 min prior to administering the test drugs.

The potency of ψ-akuammigine (2.7–136.6 μmol kg^{-1}, p.o.) relative to those of indomethacin (3.5–14.0 μmol kg^{-1}, p.o.) and morphine (1.3–7.9 μmol kg^{-1}, s.c.) was determined by plotting the number of rats showing analgesia (expressed as percent of group total) for each test drug against the log dose.

2.4. Statistics

All results are presented as mean ± s.e.m, $n = 5$. Group means were compared using one-way analysis of variance, followed by the Neuman–Keul's range test where a difference existed. Differences were considered significant at $P \leqslant 0.05$.

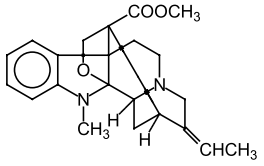

Fig. 1. Pseudo-akuammigine.

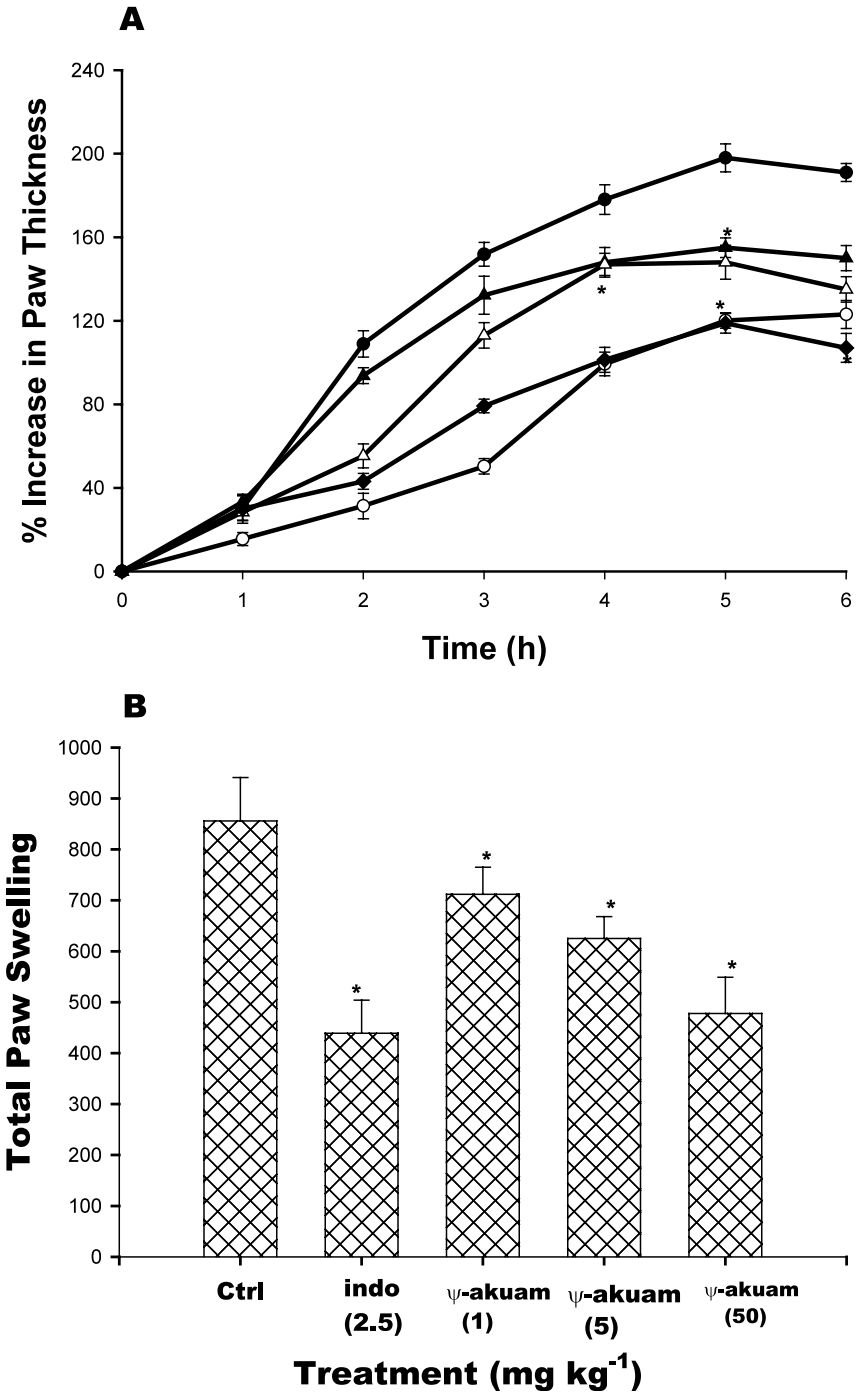

Fig. 2. Effect of ψ-akuammigine (1.0 (▲), 5.0 (△) and 50 (◆) mg kg^{-1}, p.o.; vehicle-treated control (●)and indomethacin (○) 2.5 mg kg^{-1} p.o.) on: (A) The time-course of carrageenan-induced rat paw oedema and (B) The total oedema response attained during 6 h. Drugs were administered 1 h prior to induction of oedema. * = significant ($P < 0.05$) vs vehicle-treated control.

3. Results

3.1. Effect of ψ-akuammigine on carrageenan-induced rat paw oedema

When administered at 1.0, 5.0 and 50 mg kg^{-1}, p.o. before the induction of oedema, ψ-akuammigine significantly ($P < 0.05$) reduced the maximal oedema response attained during 6 h to 78.2±2.1, 74.7±4.3 and 59.5±2.3% of the inflamed control response respectively (Fig. 2A). The total paw swellings induced over the 6 h (measured as area under the time-course curves) were also dose-dependently and significantly ($P < 0.05$) reduced at the dose levels to 83.2±9.7, 73.0±5.0 and 55.8±8.3% of the control response respectively (Fig. 2B).

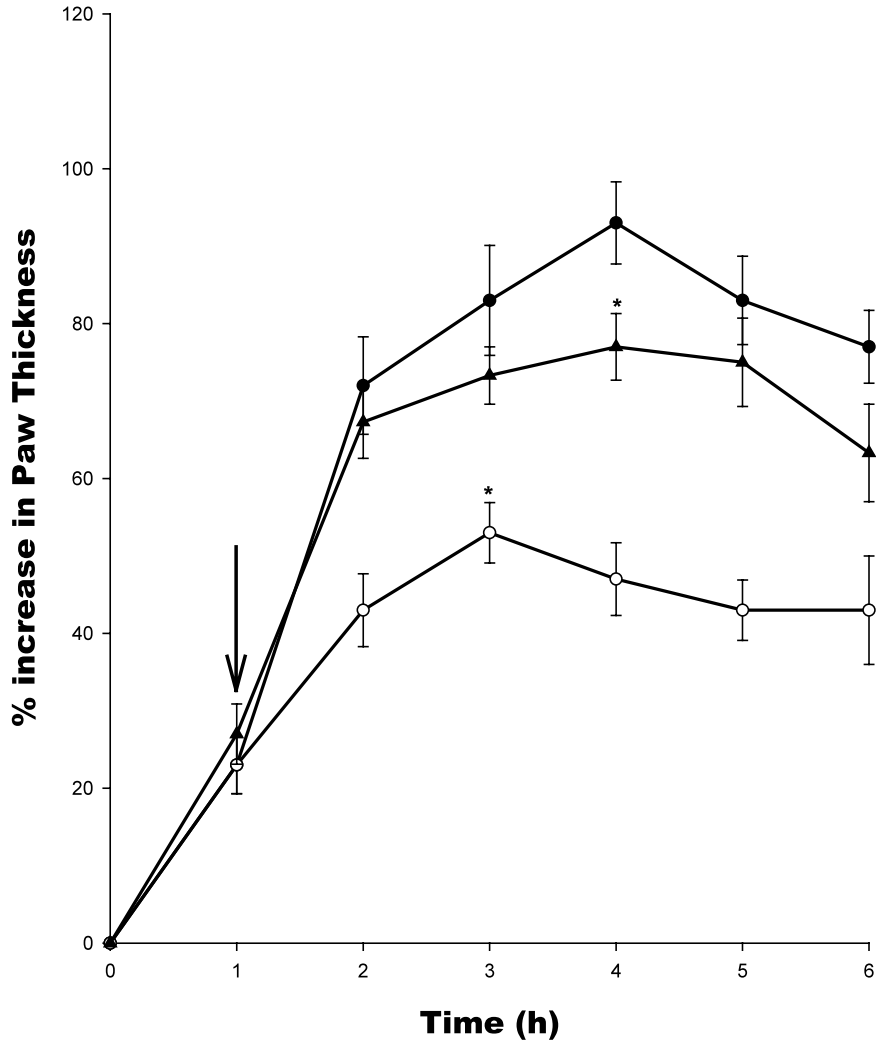

Fig. 3. Influence of ψ-akuammigine (5.0 (▲) mg kg^{-1}, p.o.) on the time-course of carrageenan-induced rat paw oedema when administered 1 h after induction of the oedema. Arrow indicates point of drug administration.

Administration of ψ-akuammigine (5.0 mg kg^{-1}, p.o.) 1 h after induction of oedema caused a significant ($P < 0.05$) suppression of the mean maximal paw swelling attained during 5 h to 82.8 ± 4.6% of the control response (Fig. 3).

3.2. Analgesic effect of ψ-akuammigine

The CRT was calculated as 4 s. The analgesic effect of morphine peaked after 30 min whilst those of ψ-akuammigine and indomethacin peaked at 180 and 60 min respectively (Fig. 4). The drug vehicle had no analgesic effect.

On molar basis, ψ-akaummigine was 3.5 times and 1.6 times less potent than morphine and indomethacin respectively as an analgesic. The ED_{50} values were 2.9, 10 and 6.3 μM for morphine, ψ-akaummigine and indomethacin respectively (Fig. 5).

Naloxone (1.0 mg kg^{-1}, p.o.) significantly ($P < 0.05$) antagonised the analgesic actions of both morphine (2.0 mg kg^{-1}, p.o.) and ψ-akuammigine (5 mg kg^{-1}, p.o.) by 46.0 ± 3.2 and 35.8 ± 6.8% respectively (Fig. 6).

4. Discussion

We have investigated ψ-akuammigine for anti-inflammatory and analgesic actions in rats. On carrageegeenan-induced rat paw oedema, a dose-dependent inhibitory activity was revealed over a dose range of 1–50 mg kg^{-1}, p.o. Carrageenan-induced oedema is primarily a vascular event initiated by dilatation of arterioles and an eventual increase in permeability of postcapillary venules resulting in exudation of inflammatory cells and fluids at the site of injury (Vinegar et al., 1987). The process is caused by release of a number of inflammatory mediators. These events parallel the early exudative phase of the inflammatory response (Vinegar et al., 1976). Inhibition of this acute phase of inflammation will therefore ultimately abort the inflam-

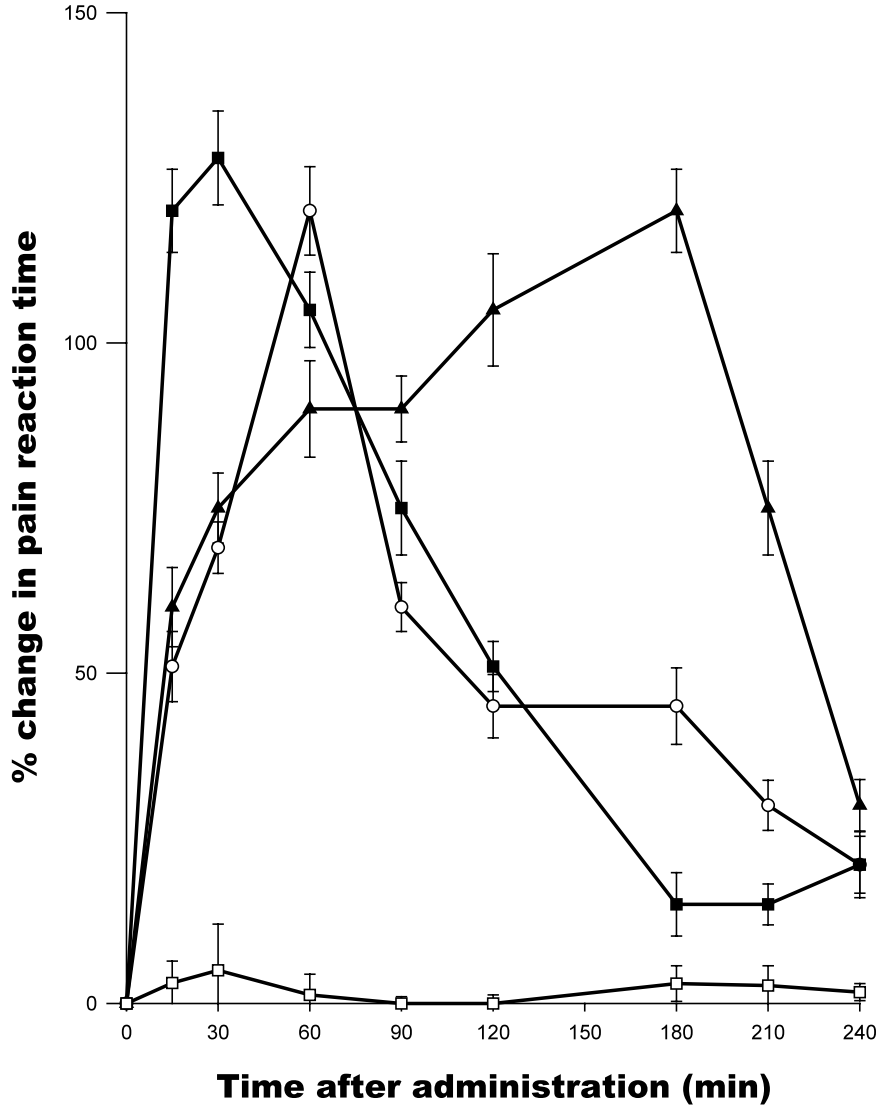

Fig. 4. Time-course of analgesic effect of ψ-akuammigine (5.0 mg kg^{-1} p.o.; ▲), morphine (1.0 mg kg^{-1} s.c.; ■) and indomethacin (2.5 mg kg^{-1} p.o.; ○)

matory process. Though the mechanism of action of ψ-akuammigine is not known at this stage our results indicate that ψ-akuammigine is a potentially effective anti-inflammatory agent, at least in the early exudative phase. Pseudo-akuammigine may therefore be partly responsible for the anti-inflammatory actions reported by Duwiejua et al. (1995) in the aqueous ethanolic extracts of *P. nitida.* We have established in this study a direct correlation between the dose and anti-inflammatory effect of ψ-akuammigine in Wistar rats. The maximal inhibitory effect attained at 50 mg kg^{-1}, p.o. was 44%. The full anti-inflammatory potential of ψ-akuammigine and its ID_{50} are yet to be determined.

Attenuation of the analgesic action of ψ-akuammigine by naloxone indicated an interaction with opioid receptors in vivo. In vitro evidence from our earlier studies (Menzies et al., 1998) supports this finding. In that study we showed in the mouse vas deferens that ψ-akaummigine binds with equal affinity to μ- and d-receptors as an agonist. The lack of specifity for opioid receptor subtypes is a property shared by other picralima alkaloids. Akuammidine, akuammine and akuammicine have been shown by us and other workers to bind with low affinity to μ-, ?- and d- receptors. Akuammigine a more closely related alkaloid to ψ-akaummigine however showed no binding to opioid receptors (Menzies et al., 1988 Lord et al., 1977). Although in vitro studies support opioid receptor occupation by ψ-akaummigine, in vivo actions as observed in this study are more complicated. It is therefore, yet to be determined whether the antinociceptive actions of ψ-akaummigine are due to a metabolite or the parent compound. The analgesic actions of mitragynine, an indole alkaloid with structural similarity to alkaloids from *P. nitida* is attributed to its metabolite (Macko et al., 1972; Matsumoto et al., 1996). Menzies et al. (1998) proposed a

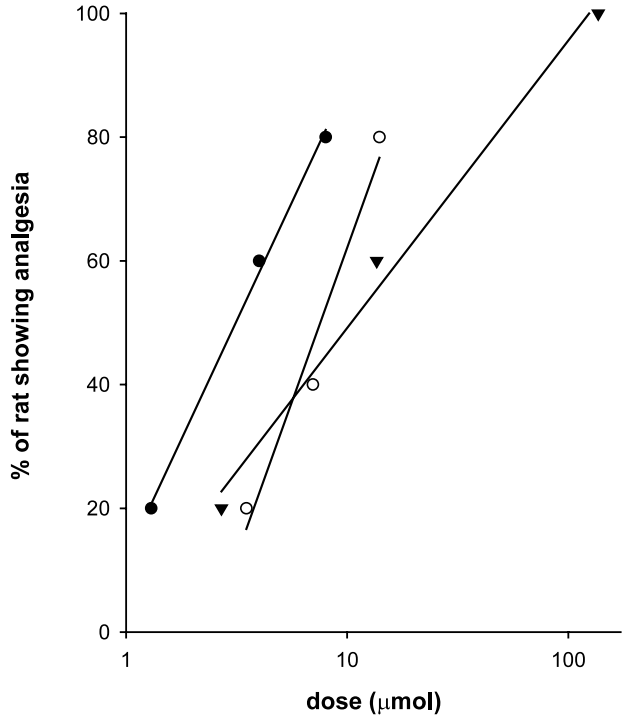

Fig. 5. Potency of ψ-akuammigine (▲) relative to that of morphine (●) and indomethacin (○).

similar explanation for the antinociceptive action of akuammine.

Pseudo-akuammigine could also be exerting its pain relieving actions by blocking the action of a pronociceptive/hyperalgesic endogenous substance. Meunier et al. (1995) and Renscheid et al. (1995) proposed nociceptin/orphanin FQ acting at the ORL_1-receptor (an orphan opioid receptor, Mollereau et al., 1994) as such a hyperalgesic substance.

In spite of the evidence provided in this study that ψ-akuammigine has marked analgesic actions mediated via interaction with opioid receptors, there is insufficient evidence to exclude the involvement of analgesic effect mediated via interactions with peripheral non-opioid receptors.

The greater potency of morphine in comparison with ψ-akuammigine can be attributed to the difference in routes of administration. Though the plasma levels of the two drugs are not known, it is reasonable to expect that the bioavailability of morphine, administered subcutaneously, will be higher than that of ψ-akuammigine administered orally because of the greater hazards that orally administered drugs may encounter in the gastrointestinal tract before absorption.

Pseudo-akuammigine exhibits anti-inflammatory and analgesic actions. Its mechanism of action and toxicity should be investigated. Being an opioid, the potential

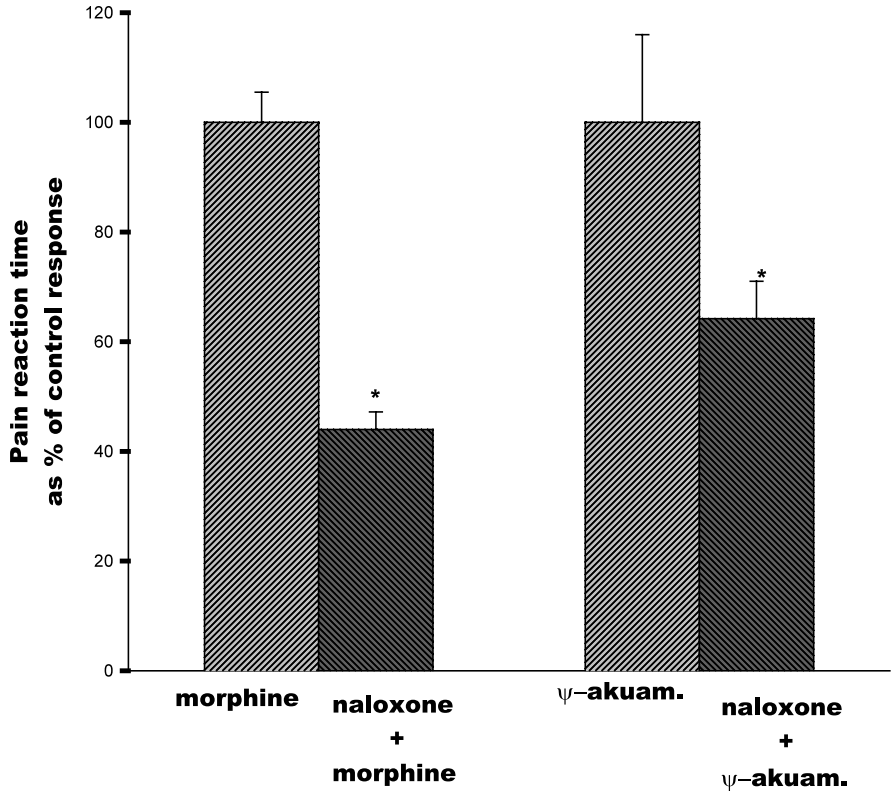

Fig. 6. Influence of naloxone on the analgesic effects of morphine and ψ-akuammigine in rats. Hatched bars indicate control responses due to morphine and pseudo-akuammigine and darker hatched bars indicate responses after naloxone treatment. Naloxone was administered 30 min before administration of morphine and ψ-akuammigine.

for causing addiction and dependence should also be investigated.

An effect of ψ-akuammigine noted in this study worth reporting though unrelated to our present objective is a remarkable transient CNS depressant activity. Rats immediately lost their righting reflexes after drug administration for a few minutes. No rat however died in the process at the doses used. This dramatic effect will be investigated further.

We conclude that pseudo-akuammigine is a potential anti-inflammatory and analgesic compound with possible hypnotic actions.

Acknowledgements

This study was sponsored by International Foundation Science research grant No. F2277-1. We are grateful to the IFS for the support. We acknowledge the assistance of Professor P.G. Waterman in confirming the structures of the compounds.

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