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Indole Alkaloids from *Sickingia williamsii* Reduce the In-vitro Effects of Morphine Withdrawal in the Guinea-pig

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Abstract

The effect of indole alkaloids from *Sickingia williamsii* Standl. (Rubiaceae) on the effects of morphine withdrawal have been examined in-vitro.

All the indole alkaloids isolated from *S. williamsii* (10^{-4}, 5×10^{-5} and 10^{-5} M) significantly and in a concentration-dependent manner reduced the effects of morphine withdrawal on the guinea-pig ileum.

The results suggest that these alkaloids might be potential anti-addictive agents.

In Peruvian folk medicine the bark of *Sickingia williamsii* Standl. (Rubiaceae), a small tree of South America, is empirically used as remedy against various inflammatory conditions and as an analgesic agent (Soukup 1987); however, the literature contains no references to its pharmacological effects. In a previous paper, the isolation and purification of several glucoindole and β-carboline alkaloids and iridoids from the above plant (Aquino et al 1994) were described.

The alkaloids isolated from *S. williamsii* are characterized by the presence of an indole nucleus; similar compounds are present in plants such as *Tabernanthe iboga* which is known to reduce drug craving in addicted users and to reduce self-administration of both morphine and cocaine (Lotsof 1985; Maisonneuve et al 1991; Glick et al 1992, 1994). On this basis, indole alkaloids are currently being investigated for their potential in treating drug abuse.

Given the above evidence, this paper describes a study of the in-vitro effects of the indole alkaloids from *S. williamsii* on the effects of morphine withdrawal on guinea-pig ileum (Capasso et al 1996).

Materials and Methods

Plant material

The bark of *S. williamsii* Standl. was collected in October 1990 from Cachiya de Huaquisha, District Tochache Nuevo, Prov. Mariscal Càceres, Dep. to San Martin, Perù and identified by Dr O. Lock de Ugaz, Pontificia Universidad Catolica del Peru. A voucher sample is deposited at the Department of Chemistry of this University.

Extraction and isolation

Extraction and isolation of indole alkaloids were performed as described elsewhere (Aquino et al 1994). Indole alkaloids **2–5** (Fig. 1) were identified as 5α-carboxystrictosidine (**2**), ophiorine A (**3**), ophiorine B (**4**) and lyalosidic acid (**5**) by spectral means (Aquino et al 1994).

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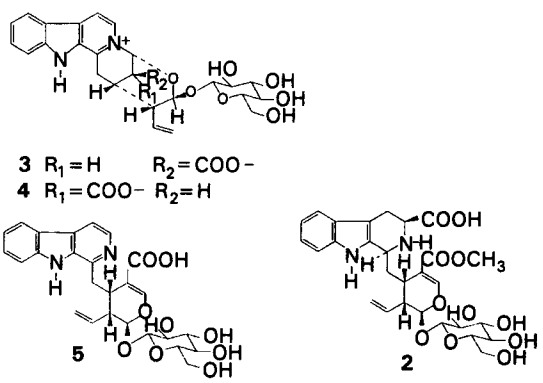

FIG. 1. Indole alkaloids **2–5** isolated from *Sickingia williamsii*.

Animals

Male guinea-pigs, 200–250 g, were supplied by Charles River (Italy). The animals were housed in a colony cage under standard conditions of lighting (lights on from 0700 to 1900 h), temperature (22 ± 1°C) and room humidity (60 ± 10%) for at least 1 week before the experiments. Food and water were freely available.

Drugs

Naloxone hydrochloride was purchased from Sigma (St Louis, MO) and morphine hydrochloride from Carlo Erba (Milan, Italy).

Effect of morphine withdrawal on the guinea-pig ileum

The experimental procedure has been described elsewhere (Capasso et al 1996). The ilea were left to equilibrate for 40–60 min without washing and the response to acetylcholine (10^{-6} M) was determined three times so that responses could be expressed as percentage of acetylcholine maximum. A reproducible acute opiate dependence was obtained by performing the following experimental procedure. A typical trace of contracture responses of the ileum to repeated challenges with opiate and naloxone is shown in Fig. 2. After three similar

acetylcholine responses, the preparation was electrically stimulated for 10–20 min (0·5 ms pulse delivered transmurally, at a frequency of 10 s at supramaximum voltage, 25 V). Before addition of the morphine to the bath the electrical stimulation was switched off. Under these conditions initial contact with the opioid agonist then, after 4 min, exposure to naloxone, induced strong contracture (about 60% of the acetylcholine maximum). However, after wash-out, another acetylcholine response was performed (to verify whether the ileum responsiveness was modified after withdrawal contracture; Fig. 2a) and, after a 30-min resting period under stimulation, a further 4-min exposure of the ileum (without electrical stimulation) to the opiate and naloxone elicited a reproducible response. After wash-out, measurement of acetylcholine response (Fig. 2b) and another 30-min resting period under stimulation, the ileum responded again to the morphine and naloxone with the same intensity (Fig. 2c). In our experiments, to avoid possible tolerance of repeat morphine injection, each preparation was submitted to three challenges only with morphine and naloxone. Naloxone alone had no effect on 'fresh' preparations or on those washed after opiate contact.

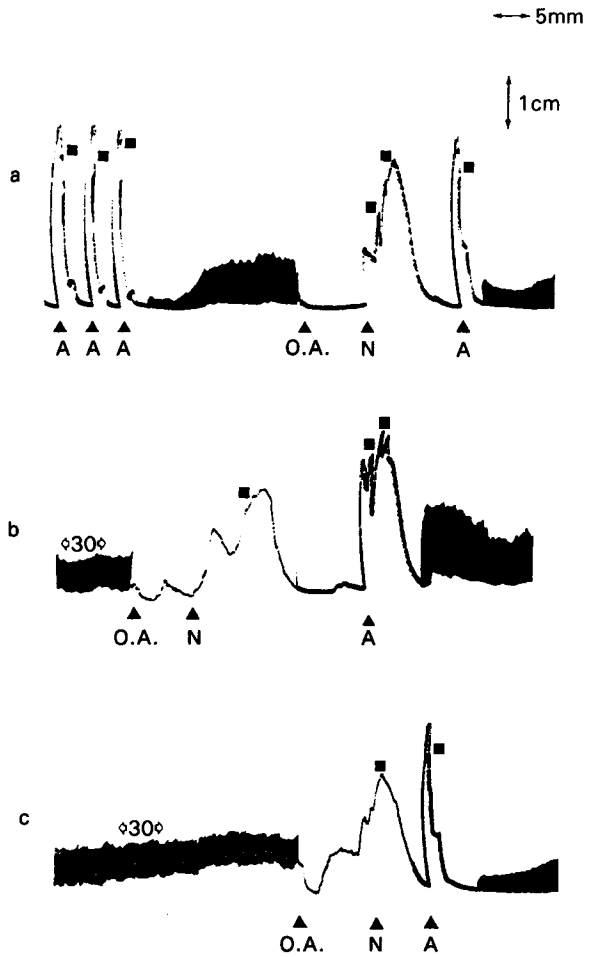

FIG. 2. Typical traces of the effects of morphine withdrawal on guinea-pig ileum. a. Three similar responses to acetylcholine (A), electrical stimulation, injection of the opioid agonist (O.A.) then 4 min later addition of naloxone (N) which induces contraction (primary opioid withdrawal); after wash-out (■) response to acetylcholine was measured again. b. After a 30-min resting period under electrical stimulation, a further 4 min exposure of the ileum to the O.A. and then naloxone elicited a reproducible response (secondary opioid withdrawal). c. After another 30-min resting period under electrical stimulation, the ileum again responded to the O.A. and naloxone with the same intensity (tertiary opioid withdrawal).

The effects of alkaloids **2–5** from *S. williamsii* were determined according to the following schedule. After measurement of three responses to acetylcholine and electrical stimulation for 10–20 min, morphine (10^{-5} M) was injected in the absence of electrical stimulation and then, 4 min later, naloxone (10^{-5} M) was added with subsequent contraction (primary opioid withdrawal). After wash-out, response to acetylcholine was again measured. After electrical stimulation for 30 min alkaloids of *S. williamsii* (10^{-4}, 5×10^{-5} and 10^{-5} M) were injected without electrical stimulation. Morphine was added 10 min later, then naloxone (secondary opioid withdrawal). After wash-out, response to acetylcholine was again measured. Electrical stimulation for 30 min was followed by final control opiate withdrawal (tertiary opioid withdrawal).

Each experiment was performed on at least six to nine isolated preparations from different animals.

Parameter evaluation

Naloxone contracture. The size of the contracture produced by the naloxone challenge was expressed as a fraction of the maximum contraction obtained with the subsequent addition of acetylcholine in the same piece of tissue according to a modification of the method of Collier et al (1981): Tension ratio = [(Response to naloxone)/(Maximum response to acetylcholine)] × 100.

Acetylcholine responses before and after treatment. Reduction or increase of response to acetylcholine after administration of the drug was expressed as a percentage of the response to acetylcholine before administration of the drug.

Electrically stimulated contraction before and after treatment. Reduction or increase of the electrical stimulation contraction after administration of the drug was expressed as a percentage of the electrical stimulation contraction before administration of the drug.

Naloxone contraction before and after treatment. Reduction or increase of the naloxone contraction after administration of the drug was expressed as a percentage of the naloxone contraction before administration of the drug.

Statistical analysis

Differences between results obtained before and after treatment of the same preparation were tested for statistical significance by use of Student's *t*-test for paired data.

Results and Discussion

In experiments to determine the effects of the indole alkaloids of *S. williamsii*, the alkaloids were administered 10 min before injection of morphine, i.e. morphine was added 10 min later than when the alkaloids were not being tested. To investigate any possible influence of the different contact periods we performed a series of preliminary experiments to verify whether a contact period longer than 4 min affected naloxone contracture. No differences were observed when the contact period before exposure of morphine was 4 or 10 min (data not shown).

Table 1. The effect on morphine withdrawal of indole alkaloids **2–5** from *S. williamsii*.

Concn	Alkaloid **2**	Alkaloid **3**	Alkaloid **4**	Alkaloid **5**
1×10^{-5} M	72·3 ± 68	67·5 ± 5·7	65·3 ± 7·1	70·3 ± 6·9
5×10^{-5} M	37·4 ± 3·9*	30·1 ± 2·7*	33·1 ± 3·3*	40·5 ± 2·9*
1×10^{-4} M	25·6 ± 2·7*	23·8 ± 2·1*	25·4 ± 1·9*	31·6 ± 2·7*

Each indole alkaloid was injected 10 min before morphine. *$P < 0.01$. Results are expressed as mean ± s.e.m. percent of naloxone contraction.

S. williamsii indole alkaloids (**2–5**) injected 10 min before morphine were able to reduce morphine withdrawal dose-dependently (Table 1). After wash-out, both acetylcholine response and electrical stimulation were not affected by treatment with the alkaloids whereas the effect of final opiate withdrawal was still reduced.

The results of this study indicate that the indole alkaloids of *S. williamsii* significantly reduced the effects of morphine withdrawal in-vitro. Although there are no data in literature on the effects of indole alkaloids from *S. williamsii* on morphine withdrawal, several data are reported for *Tabernanthe iboga*, a species known to contain indole alkaloids (Lotsof 1985; Maisonneuve et al 1991; Glick et al 1992, 1994).

The alkaloids **2–5** of *S. williamsii* have an indole nucleus (Aquino et al 1994), as do ibogain and other alkaloids isolated from *Tabernanthe iboga* (Lotsof 1985; Maisonneuve et al 1991; Glick et al 1992, 1994). The indole alkaloids from *Tabernanthe iboga* are reported to interrupt both physiological and psychological effects of opiate withdrawal in man (Lotsof 1985). In the current study, the indole alkaloids of *S. williamsii* seem to have pharmacological properties very close to those of ibogain as they are able to induce a significant reduction of the effects of morphine withdrawal.

Considering the molecular structures reported in Fig. 1, we note that alkaloids **2–5** contain an indole nucleus linked to a monoterpenoid (iridoid) group. The main structural differences between compounds **2–5** are: an unusual N_b-C17 linkage in compounds **3** and **4**; the C16 stereochemistry of compounds **3** and **4**; a saturated carbon ring and a carboxyl group at C5 in compound **2**; and an unsaturated carbon ring in the β-carbolin glucoindole alkaloid **5**. Despite the above differences, alkaloids **2–5** induce similar effects on morphine withdrawal, showing similar potency in inhibiting naloxone contractions at the concentrations tested (Table 1).

Given the above evidence, we suggest that activity of the *S. williamsii* alkaloids at reducing the effects of morphine withdrawal might be related to the presence of the indole nucleus, in the same way as it is for alkaloids from *Tabernanthe iboga*.

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