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Indoline alkaloids modulating the release of growth hormone by rat pituitary

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ABSTRACT The general screening method and theory on rat pituitaries were briefly discussed. Active indoline alkaloids from *Psychotria oleoides* namely psycotridine, oleoidine and caledonine were found to stimulate the release of GH while hodgkinsine acted as antagonist to the SRIF receptor.

ABSTRAK Kaedah penyaringan umum dan teori pituitari tikus dibincangkan dengan ringkas. Alkaloid indolina yang aktif dari *Psychotria oleoides*: saikotrindina, oleodina dan kaledonina mendorong pembebesan GH sementara hodgkinsina bertindak sebagai antagonis pada reseptor SRIF.

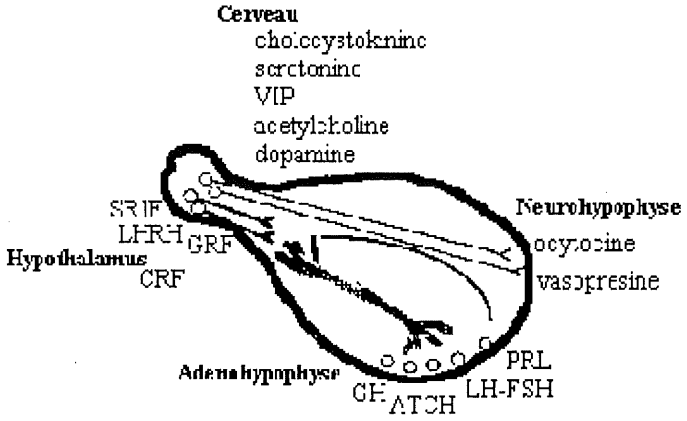
(Indoline alkaloid, growth hormone, *Psychotria oleoides*)

INTRODUCTION

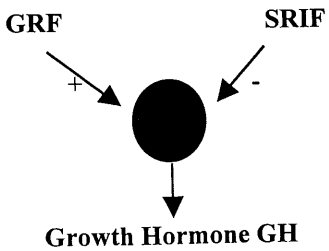
All the scientists working in the field of natural products chemistry know that the procedures applied to the pharmacological screening of crude extracts are global, classical, expensive and the results obtained are sometimes non reproducible.

With this in mind, our group have tried in the early eighties to renew the pharmacological screening by using the modern concepts of neutroendocrinology. In particular, the possible activity of plant extracts on the mammal hypothalamophypophyseal system has been explored and in that way, we have discovered the activity of an alkaloidal plant extract on GH release of rat pituitaries [1,2].

The screening on rat pituitaries



If one examine the mammal pituitary, one observes two parts, the endocrine pituitary and the neutropituitary containing the endings of neurosecretory axones from upstream origin. The endocrine part or adenohypophysis contains cells which release very important hormones such as prolactine, LH-FSH, ACTH and β-endophins, GH, TSH. All of these hormones depend on the corresponding releasing factors in the hypothalamus: CRF for ACTH, LHRH for LH, GHRH or GRF and SRIF for GH.



For instance, the GH cell is under the influence of SRIF or somatostatine, which inhibits the GH release, and GRF or somatoliberine, which stimulates the GH release.

The hypothalamic cells depend themselves from brain factors such as cholecystokinine, VIP, norepinephrin, acetylcholine, 5-HT. So there are 3 levels of possible interaction: downstream, the pituitary, upper, the hypothalamus, and more upstream, the brain factors. It is easy to see that this system with its 3 steps before hormone releasing can serve as a multifactorial system of screening allowing to orientate further pharmacology.

Practically the substance to be tested is injected intraperitoneally to set of rats. Fifteen minutes later, the rats are killed and the main hypophyseal hormones will be evaluated in the rat sera by radioimmunoassay. Any response could be the sign of an action either on pituitary, hypothalamus or brain. These responses could be non specific: all the hormones vary; specific: one hormone only vary.

Thirty natural substances and either pure or a mixture from plant or sea origin have been screened. Among them, the crude alkaloid extract of a Rubiaceae from New Caledonia, *Psychotria oleoides* exhibited interesting properties.

RESULTS AND DISCUSSION

Rubiaceae constitute a broad tropical family with about 6,000 species. One can find many biologically active substances such as emetine from *Ipeca*, quinine from *Cinchona*, cafeine from *Coffea* or yohimbine from *Pausinystalia*.

In New Caledonia, the genus *Psychotria* includes about 80 species. Only few of them contain alkaloids. The plant selected, *Psychotria oleoides*, has been extracted for its alkaloids and they were screened on rat pituitary.

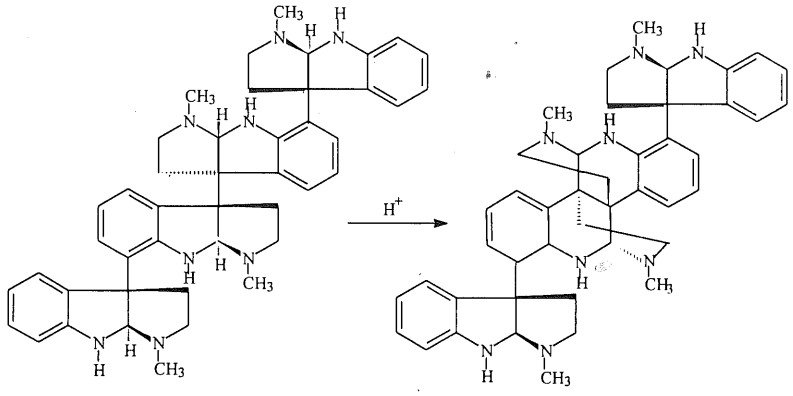
After evaluation of the blood level of the main pituitary hormones, the mixture of alkaloids has been shown to be inhibiting the GH release *in vivo* in a dose-dependent and specific manner. This can be due to an action on the brain, on the hypothalamus or on the pituitary. *In vitro*, on slices of halves pituitaries, the mixture also inhibits the basic level of GH release and it prevents slightly the GRF activation of GH release. This is due to a direct action on the pituitary, maybe by interaction with the SRIF receptor.

In binding studies using ^{135}I-Tyrosine-SRIF, it was demonstrated that there is a substance in the mixture that is binding specifically the SRIF receptor of the GH cells. The affinity is about 5.10^{-6} M.

By using the binding assay, it was possible to monitor the fractionations by reverse silica gel chromatography of the crude mixture, thus led to the isolation of the most active compound [3,4].

The ^{1}H and ^{13}C-NMR spectra show that this alkaloid has a polyindoline skeleton. This is not a quadrigemine structure, as there are no fragments at 344 or 173 in the MS. It is probably a calycanthine derivative, with the only loss of 2 ethanamine fragments. The recording of spectra at low temperature allows to observe the presence of 2 sets of signals, meaning that in solution two conformers occur.

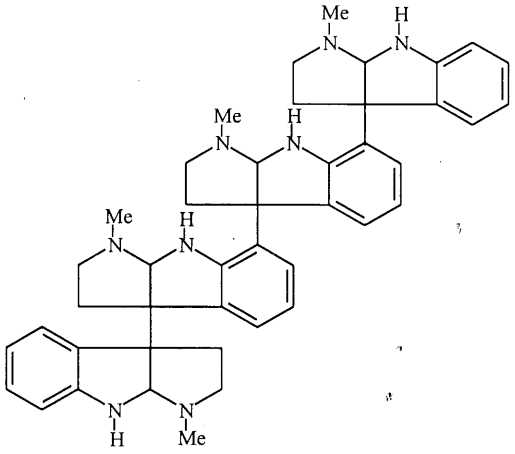
Acid treatment of quadrigemine C, the structure of which having been determined, leads to the formation of psycholeine.



Quadrigemine C

Psycholeine

A further systematic purification has led to the isolation of many pyrrolidinoindolinic alkaloids such as, hodgkinsine (trimer), calycosidine (its acid transformation product), two quadrigemines (the quadrigemine B and C), two psychotridines (5), and finally the oleodine (6 units) and caledonine (7 units).

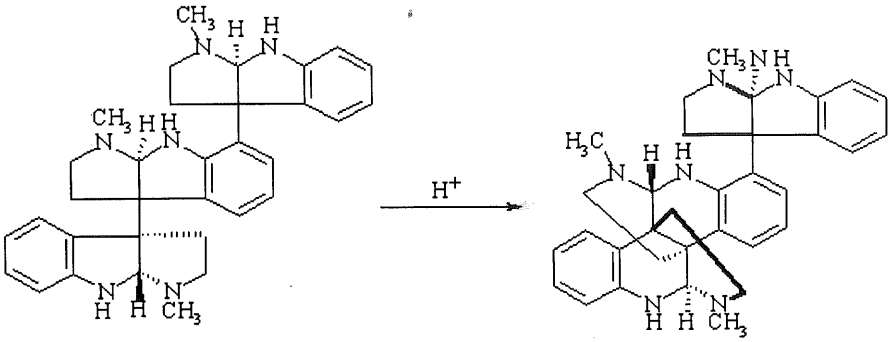


Quadrigemine B

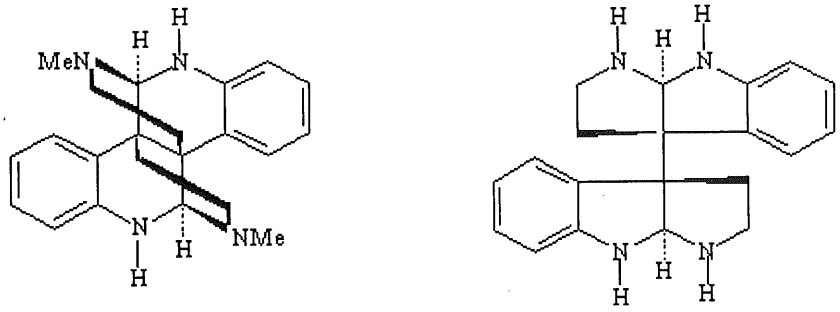
Similarly, hodgkinsine, in acid medium leads to calycosidine, an alkaloid isolated from *P. oleoides*, previously described in *Calycosia milnei* and in *Psychotria rostrata.* Finally, an example has been provided by the acid transformation of chimonanthine into calycanthine, by hydrolysis, ring opening and ring closing in quinoline system.

The availability of GH cells cultures has allowed to screen carefully the activity of each alkaloid on the basal secretion of GH and on this secretion inhibited by addition of somatostatine at 10^{-9} M. Isopsychotridine, quadrigemine B, hogkinsine, calycosidine and psycholeine do not possess any activity on the basal secretion, while psychotridine, oleoidine and above all caledonine, are strongly stimulating the release of GH, mimicking the action of GRF.

When somatostatine is added in the cell culture medium, one observes a significant lowering of GH release. This lowering is antagonized by hodgkinsine, but not by calycosidine, neither by mesochimonanthine or mesocalycanthine. This lowering is strongly significant at 10^{-7} M, meaning that hodgkinsine is able to bind specifically the SRIF receptor, by antagonizing its inhibiting activity.



Hodgkinsine Calycosidine



Chimonanthine Calycanthine

Finally, the alkaloid mixture of *Psychotria oleoides* has been selected because it provokes *in vivo* a lowering of GH release in the rat blood. Many alkaloids belonging to the same series have been isolated some as psychotridine, caledonine or oleoidine stimulating GH release, some others antagonizing the SRIF inhibition. It is probable that there are other factors involved in the *in vivo* action.

MECHANISM OF ACTION

Somatostatine is a tetradecapeptide possessing an active site with a sequence including many aromatic amino acids, and a disulfide bridge. It is possible, but we have not succeed in modelling, that hodgkinsine has a structure close to that of somatostatine, and that the others are different and cannot be bound to the receptor.

Bioassay-guided fractionation allows the isolation of many alkaloids possessing a pyrrolidinoindolinic structure. In GH cell culture, some of these alkaloids stimulate the GH, some others like hodgkinsine binds to the SRIF receptor of the GH cell.

The activity is possibly due to the similarity of the molecule with the aromatic amino acid sequence of the active site of somatostatine. Hence, we have discovered the first natural non-peptidic substance able to bind to the SRIF receptor.

CONCLUSION

Pharmacological screening of several plant extracts has led to the selection of *Psychotria oleoides* because of its chemical content inhibits the GH release.

Such a research is a good example of serendipity, where an antagonist of SRIF has been discovered, while some inhibitors of GH release were expected.

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