and their another homologue *iso*-butyropyrrothine along with a polyene (heptaene) and an unidentified antibiotic in the same fermentation broth.

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Résumé

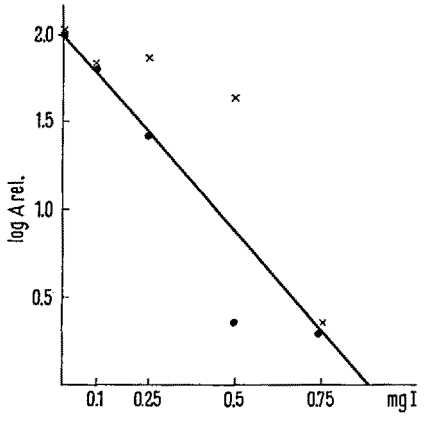
Une nouvelle espèce de Streptomyces (*S. pimprina*) produit l'*iso*-butyropyrrothine avec la thiolutine (l'acétopyrrothine), l'aureothricine (la propriopyrrothine) et un antibiotique antifongique du groupe du polyène (heptaène).

Über die Hemmung von Hurain, einer pflanzlichen Protease, durch Trypsininhibitoren

Hurain ist ein eiweissverdauendes Ferment aus dem Saft von *Hura crepitans* L. (Euphorbiaceae). Es ist von den Papainasen deutlich zu unterscheiden wegen seiner Resistenz gegen Oxydation, weil es von Glutathion und andern Aktivatoren von Papain nicht aktiviert wird und weil es Ascaris und andere Darmparasiten *in vitro* nicht angreift, wie es die Papainasen tun[1].

Aus der letztgenannten Eigenschaft wurde geschlossen, dass Hurain möglicherweise von Trypsininhibitoren in seiner Wirkung gehemmt wird, wie das Trypsin oder Chymotrypsin. In der vorliegenden Arbeit wurde diese Möglichkeit experimentell bewiesen.

Material und Methoden. Hurain wurde aus dem Saft, der durch Einschnitte in den Stamm von *Hura crepitans* gewonnen wird, durch Filtrierung und Lyophilisierung dargestellt. Eine weitere Reinigung wurde in dieser Arbeit nicht vorgenommen. Das verwandte Trypsin war ein kommerzielles Präparat (Biochemical Preparation Inc. 1:150). Der kristallisierte Trypsininhibitor aus Soyabohnen wurde von derselben Firma bezogen, die Inhibitoren aus Ascaris und Bohnen wurden nach den entsprechenden Vorschriften der Literatur hergestellt[2,3]. Die proteolytische Aktivität wurde nach der Methode von Anson[4] mit leichten Modifikationen bestimmt.



Hemmung von Trypsin und Hurain durch Bohnen-Hemmstoff. *Abzisse.* mg des Hemmstoffes. *Ordinate.* Log der Enzymaktivität nach Anson[4]. Jeder Ansatz bestand aus 5.0 ml Substrat, 1.0 ml Ferment (1 mg Trypsin oder 6.66 mg Hurain) und der entsprechenden Menge Hemmstoff. Die Fermentaktivität ohne Hemmstoff wird als 100% bezeichnet. Die Ferment- und Hemmstoffmengen wurden bestimmt als N × 6.25.

Hemmstoff	Hemmung %	
	Trysin	Hurain
—	0	0
Bohnen	26.6	29.5
Ascaris	18.8	18.5
Soya	28.8	34.0

Hemmung von 3 Inhibitoren auf die proteolytische Wirkung von Trypsin und Hurain, ausgedrückt in % der Anfangsaktivität und errechnet pro mg Hemmstoff.

Ergebnisse. In der Fig. wird das Ergebnis der Hemmung von Trypsin (1 mg/Versuch) und Hurain (6,6 mg/Versuch) durch verschiedene Mengen von Bohneninhibitor angegeben. Die Werte beider Fermente fallen unter Berücksichtigung der Streuung auf eine Gerade, wenn die Hemmstoffmengen gegen den Logarithmus der Fermentaktivität aufgetragen werden.

Der Vergleich der 3 geprüften Inhibitoren wird in der Tabelle vorgelegt. Es wurde in jedem Versuch mit 5 verschiedenen Mengen von Ferment und einer festen Menge von Hemmstoff (0.1 mg für Trypsin und 0.25 mg für Hurain) gearbeitet. Die Hemmung ist in % der Anfangsaktivität, die in Trypsineinheiten errechnet wurde, ausgedrückt und auf 1 mg Hemmstoff berechnet. Die Inhibierungswirkung der 3 geprüften Hemmsubstanzen war quantitativ fast gleich stark für beide untersuchte Enzyme.

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Facultád de Ciencias, Universidád Central de Venezuela, Caracas, 10. Mai 1960.

Summary

Hurain, a protease from the sap of the tree *Hura crepitans* L., is inhibited by trypsin inhibitors from soya, kidney beans, and ascaris.

Ein ausführlicherer Bericht über die hier mitgeteilten Versuche wird in Acta Cientifica Venezolana erscheinen.

[1] W. G. Jaffé, J. biol. Chem. *149*, 1 (1943).

[2] N. M. Green, Biochem. J. *66*, 416 (1957).

[3] D. E. Bowman, Proc. Soc. exp. Biol. Med., N. Y. *86*, 491 (1954).

[4] M. L. Anson, J. gen. Physiol. *22*, 79 (1938/39).

[5] Z. Zt. Max-Planck-Institut für Eiweiss- und Lederforschung, München 15.

Pharmacological Actions of 4-Hydroxytryptamine and 4-Hydroxytryptophan

4-Hydroxytryptamine (4-HT) and 4-hydroxytryptophan(4-HTP) are of conspicuous biological interest because 4-HTP is the only tryptophan amino acid which so far has been found to be decarboxylated in the mammalian organism[1], and because 4-HT, besides being one of the most active hydroxyindolealkylamines, is strictly re-

The amines and amino acids used in the present investigation were synthetized at the Farmitalia S. p. A. Research Laboratories, Milan, by Dr. C. Pasini and V. Coló.

[1] V. Erspamer, A. Glässer, M. B. Nobili, and C. Pasini, Exper. *16*, 506 (1960).

lated to psilocin, a naturally occurring compound, which is nothing but its N,N-dimethyl derivative[2].

The present communication describes some pharmacological actions of 4-HT and 4-HTP, as compared with those of 5-hydroxytryptamine(5-HT) and 5-hydroxytryptophan(5-HTP). Cats were anaesthetized with chloralose and dogs with Pernocton.

4-Hydroxytryptamine and 5-hydroxytryptamine

Blood pressure. In the intact cat, 4-HT (100 µg/kg, i.v.) produced a rise of blood pressure which was 2–6 times less intense but more sustained than that elicited by the same dose of 5-HT. In the spinal cat, both 5-HT und 4-HT provoked hypertension but, whereas with 5-HT the pressure rise was proportional to the dose administered, this did not occur with 4-HT.

Respiration. In the dog, 5-HT(3-4µg/kg and more, i.v.) produced a transitory stimulation of respiration, eventually followed by a period of hypopnoea. 4-HT required a higher dosage than 5-HT and the result was always respiratory depression.

In the cat both 5-HT and 4-HT produced bronchospasm. The effect of 4-HT(100 µg/kg, i.v.) was 2–4 times less intense but lasted longer than that caused by 5-HT.

Dog urinary bladder in situ. The stimulant action of 4-HT was approximately 2 times less intense than that of 5-HT (minimum active dose: 1–2µg/kg, i.v.), but was more sustained.

Diuresis of hydrated rats. Unlike 5-HT, subcutaneous 4-HT showed only a poor antidiuretic effect. In fact, 6 mg/kg were less effective than 0·04 mg/kg 5-HT.

Nictitating membrane. 5-HT was 2–5 times more active than 4-HT in contracting the nictitating membrane of the cat, but whereas the 5-HT contraction was rapidly followed by relaxation, that caused by 4-HT lasted considerably longer.

4-Hydroxytryotophan and 5-hydroxytryptophan

Blood pressure. Intravenous 5-HTP, in doses of 2·5-5 mg/kg, elicited in the cat a moderate fall of blood pressure which lasted 15–20 min; the same dose of 4-HTP produced a more pronounced rise of blood pressure, lasting 30–50 min.

Bronchial musculature. Both 5-HTP and 4-HTP produced a spasm of the bronchial musculature when given i.v. in the cat. However, bronchospasm elicited by 4-HTP was considerably more intense and lasted longer than that produced by 5-HTP.

Dog urinary bladder in situ. The contraction produced by 2–4 mg/kg 4-HTP given i.v. was 3 to 4 times more intense and considerably more sustained than that caused by the same dose of 5-HTP.

Nictitating membrane. Single i.v. injections of 2–5mg/kg 5-HTP did not contract the membrane; the same doses of 4-HTP produced a contraction lasting 30–50 min.

Gastrointestinal tract. In the diarrhoea test in mice, the ED50 of 5-HTP by i.p. route was 25µg/mouse, the ED 100, 300 µg/mouse. For 4-HTP the ED50 could not be exactly established, but it was not less than 400 µg/mouse.

Diuresis of hydrated rats. Like 5-HTP, also 4-HTP caused in hydrated rats an evident antidiuretic effect. 4-HTP was approximately half as potent as 5-HTP, the minimum active i.p. dose being 20 mg/kg. At high dose levels, 100 mg/kg and more, 4-HTP produced renal lesions identical to those seen after 5-HTP.

Body temperature. In mice pretreated with 100 mg/kg iproniazid, 30–60 mg/kg 4-HTP, given i.p., produced, like 5-HTP, a remarkable increase of the rectal temperature (1·5° for 60 mg/kg 4-HTP) accompanied by tremors, lasting 60–90 min. No signs of excitement appeared, but the animals were rather depressed.

In rabbits, similarly pretreated with iproniazid, i.v. doses of 5 mg/kg 4-HTP produced a rise of rectal temperature (1·5–2°) similar to that caused by 15 mg/kg 5-HTP, lasting more than 4 h.

Intraspecific aggressive behaviour of mice. Like 5-HTP, also 4-HTP reduced the aggressive behaviour in male mice. At the i.p. dose of 120 mg/kg, 4-HTP abolished fighting in 50% of the treated couples. The same effect was obtained with 100 mg/kg 5-HTP.

Spontaneous electrical cortical activity of the «midpontine pretrigeminal cat». The slow intracarotid injection of 25–40 mg 4-HTP brought about a synchronization of the EEG, similar in every respect to that produced by 8–20mg 5-HTP or 1–2 mg thiopental.

It may be seen from the preceding data that 4-HT and 4-HTP display in the organism effects which are similar to those possessed by 5-HT and 5-HTP. Both amino acids act presumably after having been decarboxylated to the corresponding amines. However, whereas 5-HT and 5-HTP produce pharmacological effects which, with some important exceptions, are more intense than those caused by 4-HT and 4-HTP, the two 4-hydroxyindoles, and especially 4-HTP, cause more prolonged effects than the corresponding 5-hydroxyindoles. This may in part depend on the fact that 4-HT is a substrate for amine oxidase worse than 5-HT[3].

The failure of 4-HT, in sharp contrast to 4-HTP, to cause antidiuresis is puzzling, but it may depend upon the great lability of the phenolic hydroxy group of 4-HT towards oxidizing enzymes, which could prevent 4-HT from reaching the renal receptors in sufficient amounts or, more likely, for a sufficiently long time, to produce afferent glomerular vasoconstriction.

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Laboratori Ricerche Farmitalia, Milano and Istituto di Farmacologia dell'Università di Parma (Italy), May 2, 1960.

Riassunto

5-HT e 5-HTP da una parte e 4-HT e 4-HTP dall'altra posseggono su tutti i numerosi reattivi saggiati effetti farmacologici similari i che in generale sono meno intensi ma più duraturi per i derivati 4-idrossiindolici.

[2] A. HOFMANN and F. TROXLER, Exper. *15*, 101 (1959).

[3] V. ERSPAMER, R. FERRINI, and A. GLÄSSER, to be published.

The Fate of 4-Hydroxytryptophan in the Rat Organism[1]

Among the many tryptophans so far studied in this Laboratory, DL-4-hydroxytryptophan (4-HTP) was one of the few which were found to be decarboxylated *in vitro* by mammalian decarboxylase[2]. It is probable that 4-HTP is the precursor amino acid of psilocin (N,N-dimethyl-4-hydroxytryptamine) and psilocybin (the O-phosphate ester of psilocin) and, hence, that 4-HTP is an amino acid occurring, together with 5-HTP, in nature. These consid-

A full report on the methods used in this study, as well as on the biochemical and pharmacological results will be published elsewhere, together with the discussion of the collected data.

[1] Supported by a grant from the Rockefeller Foundation, New York.

[2] V. ERSPAMER, A. GLÄSSER, C. PASINI, and G. STOPPANI, Nature, in press.