5-Hydroxytryptophol: Evidence for its Having Physiological Properties[1]

Norepinephrine, in addition of being a neurotransmitter, has been suggested as functioning as an ergotrophic hormone to produce stimulation of the CNS together with various behavioral manifestations of excitation. 5-Hydroxytryptamine (5-HT) has been postulated as being a tropotrophic hormone, participating in the production of opposite responses of nervous inhibition and behavioral depression[2]. Anomalies have been observed in this simple classification, especially for 5-HT, thus, a more complex classification of biogenic amines has been proposed and 5-HT categorized into a biphasic group[3].

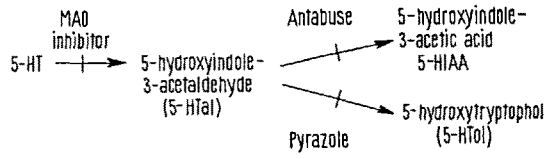
However, another resolution of the dilemma to explain the biphasic actions on CNS states of some biogenic amines as 5-HT is by considering that some of their effects may be due to one of their metabolites having opposite neurohormonal properties. The metabolism by the whole animal and liver[4] and brain[5] of 5-HT has been shown to follow the pathway shown in the Figure, where 3 different products are formed. One of these metabolites, 5-hydroxytryptophol (5-HTol), was chosen as the subject of this study.

The difficulty of assessing actions of CNS active physiological substances is due to the fact that the blood-brain barrier may be an evolutionary adaptation produced specifically to exclude modulators from the brain. However EEG studies with White Leghorn chicks of up to 14 days old indicate that they do not possess a completed blood-brain barrier and many substances, such as 5-HT, which are inactive in other species do possess activity on the CNS in the chick[6]. EEG slow waves and a characteristic sleep posture were shown to correlate so that the latter behavioral manifestation can be used as a reliable indicator of CNS effects[6].

Antabuse, an aldehyde dehydrogenase inhibitor[7], iproniazid, a MAO inhibitor[8] and pyrazole, an alcohol dehydrogenase inhibitor[9], were injected into 1–14-day-old White Leghorn cockerels by the i.p. route. In combinations with them or alone, 5-HT, 5-HTol and 5-methoxytryptophol (5-MTol) were also administered. Unlike the first two compounds, the latter was not considered as being a physiological substance, but was included in the study because it was anticipated that it might be an analog of 5-HTol, more lipophilic than the parent. Dosages used of the enzyme inhibitors were selected according to published data on toxicity or effectiveness in vivo. A group of 8 chicks were used in each experiment. Time of sleep induction was that period until the entire group was in some postural stance of sleep. Duration of sleep was that time until 3 of the animals of the group had awakened spontaneously and were active. Solvents used (up to 0.2 ml of propylene glycol, dimethylsulfoxide or saline) had no observable effects. 5-HTol, m.p. 107–108° was prepared according to a described synthesis[4] and other compounds were obtained from commercial sources. Dosages used and the results obtained are shown in the Table.

When analyzing the data it must be remembered that enzyme inhibitors in vivo do not produce complete enzyme deactivation and observable effects are due to short-term shifts in metabolite ratios. Pyrazole, Antabuse or iproniazid had no sleep producing effects by themselves. 5-HTol by itself produced sleep, but was less potent than 5-HT. When Antabuse was given along with 5-HTol there was no increase in sleeping time. As was shown previously, a MAO inhibitor shortens the sleeping time produced by 5-HT. 5-MTol, in a dosage equivalent to its hydroxyl analog, produced a much longer sleeping time and incidentally a deeper sleep.

Depletion of brain 5-HT by the decarboxylase inhibitor, parachlorophenylalanine produces chronic insomnia[10] and sleep is induced in some mammals by administration of 5-hydroxytryptophan which can cross the blood-brain barrier and elevate 5-HT levels in the CNS. However, when MAO inhibitors are also administered, again insomnia results[11]. Thus sleep is not induced by 5-HT per se but appears to result from one of its metabolites, in fact,



Metabolism of 5-hydroxytryptamine and enzyme inhibitors which block block the different conversions.

Effects produced by compounds given to 1-14-day-old chicks

Compound	Dosage mg/kg	Dosage umole/kg	Effects
Pyrazole	25	334	No effect
Antabuse	25	84	No effect
Iproniazid phosphate	125	450	No effect
5-HT creat. sulfate	25	62	Sleep, induction, 3–5 min duration, 62 min
5-Hydroxytryptophol	125	705	Sleep, induction, 2–4 min duration, 17 min
5-Methoxytryptophol	125	655	Sleep, induction, 2–4 min duration, 38 min
Iproniazid phosphate+	125	450	Sleep, induction, 3–5 min
5-HT creat. sulphate	25	62	duration, 32 min
Iproniazid phosphate+	125	450	Sleep, induction, 2–5 min
5-Hydroxytryptophol	125	705	duration, 15 min
Pyrazole+	25	334	Sleep, induction, 2–4 min
5-Hydroxytryptophol	125	705	duration, 68 min
Antabuse+	25	84	Sleep, induction, 2–5 min
5-Hydroxytryptophol	125	705	duration, 16 min

[1] This work was supported by funds from Enzomedic Laboratories, Incorporated.

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the data from sleep studies with MAO inhibitors suggests that 5-HT may be antagonistic to sleep production and cause stimulation[12].

It has been reported that indole-3-acetaldehyde and the 5-hydroxyl analog, when injected i.p. into 1-2-day-old chicks, produce sleep states as from 5-HT or 5-hydroxytryptophan[13]. Whereas effects of the latter compunds could be reduced by pretreatment with the MAO inhibitor, pargyline, it had no effect on the sleep producing properties of the aldehydes. Since enzyme catalysis is reversible, the aldehydes and alcohols are introconvertible, the effects of the alcohol dehydrogenase inhibitor, pyrazole, would be to increase the half-life of administered 5-HTol and to diminish accumulation of the corresponding aldehyde. Since pyrazole in this study increased the sleep effects of the alcohol, it is inferred that increased quantities of the latter in spite of a resultant decrease of the aldehyde correlate with increased sleep production.

The necessity of using a high dosage of 5-HTol compared to 5-HT to produce sleep might be explained by a greater polarity of the former or its inability to form lipophilic complexes. Thus even in the chick it may not cross the blood-brain barrier appreciably. The expectedly more lipophilic 5-MTol was a more potent sleep producing agent. However, even with it in past studies the tagged compound when injected i.p. into rats caused only a maximum of 0.04% of the radioactivity to appear in the brain[14].

It is concluded from these studies and past reported data that it is probable that 5-HTol is the sleep producing neutral metabolite of 5-HT and that the sleep producing effects of indoleacetaldehydes are also due to their conversions to quantities of the tryptophols which are the active agents.

Résumé. Chez le poussins de 1–14 jours l'agent inhibitoire de déhydrogénation d'alcohol et pyrazol prologeait considérablement la période de sommeil produit par 5-hydroxy-tryptophol, les données d'ensemble suggérant que ce dernier composé est le métabolite neutre, somnifère, de 5-hydroxytryptamine.

R. G. TABORSKY

26415 Somerset Lane, Kent (Washington 98031, USA), 5 March 1971.

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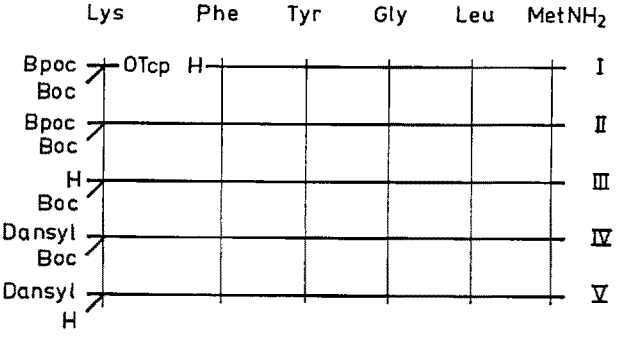
Ein fluoreszenzmarkiertes Physalaemin-Analoges mit hoher biologischer Aktivität

Die kovalente Fluoreszenz-Markierung von Molekeln zur Gewinnung von Informationen über deren Verteilung, Konformation und Wechselwirkung mit anderen Molekülen hat sich in der Protein-Chemie allgemein bewährt[1]. Als besonders günstig erwies sich die Dansyl-Gruppe[2,3], die in einigen Fällen auch zur Markierung von Peptid-Wirkstoffen herangezogen wurde[4–6].

Im Rahmen unserer Untersuchungen über Physalaemin-Peptide haben wir ein kurzkettiges fluoreszenzmarkiertes Peptid synthetisiert, das am α-Stickstoff des N-terminalen Lysins eine Dansyl-Gruppe trägt (Figur, Peptid V). Für die Substitution wählten wir die α-Position, weil bereits von LÜBKE et al.[7] an einer Serie von Eledoisin-Heptapeptiden und von uns[8] bei analogen Hexapeptiden des Physalaemins festgestellt wurde, dass die α-Aminogruppe des Lysins für die biologische Aktivität dieser Peptide von weitaus geringerer Bedeutung ist als die ε-Aminogruppe. Das Pentapeptid I[9] wurde von uns durch stufenweisen Aufbau mit Hilfe von Boc-Aminosäure-2,4,5-trichlor-phenylestern ohne Schutz des phenolischen Hydroxyls des Tyrosins dargestellt[8]. Umsatz dieses Pentapeptids I mit N^{α} Bpoc-N^{ε} Boc-Lysin-2,4,5.-trichlorphenylester [$C_{33}H_{37}Cl_3N_6O_6$, (664,01) ber. 59,69%C, 5,62%H, 4,22%N; gef. 59,48%C, 5,61%H, 4,37%N; Schmp. 67–71°; $[\alpha]_D$–18,4° (c = 1; DMF)] lieferte das geschützte Hexapaptid II. Selektive Abspaltung der Bpoc-Schutzgruppe mit Ameisensäure/Essigsäure-Gemisch[10] führte zu dem Hexapeptid III.

Durch Dansylierung der freien α-Aminogruppe des Lysyl-Restes von III erhielten wir das geschützte Dansyl-Peptid IV, das wir auf mit 30 g Kieselgel PF_{254} (Merck) beschichteten Platten von 20 × 20 cm im Gemisch Chloroform/Methanol (9:1) reinigten. Dabei isolierten wir ein Nebenprodukt mit höherem Rf-Wert als IV, das bei der sauren Hydrolyse ausser Phenylalanin, Glycin, Leucin, Methionin und N^{α}-Dansyl-Lysin noch *O*-Dansyl-Tyrosin lieferte, wie durch Papierelektrophorese mit authentischen Vergleichssubstanzen gezeigt werden konnte. Wir schreiben dieser Verbindung daher die Struktur N^{α}-Dansyl-N^{ε}-Boc-Lys-Phe-Tyr (*O*-Dansyl)-Gly-Leu-MetNH_2 zu.



Syntheseschema für das fluoreszenzmarkierte Physalaemin-Hexapeptid.

Wirkung des fluoreszenzmarkierten Peptides im Vergleich zu dem entsprechenden unsubstitutierten Peptid

	Meerschweinchen-Ileum	Meerschweinchen-Blutdruck
ED_{50} bzw. ED_{30} von Dansyl-Hexapeptid V	12,4 ng/ml	290 ng/kg
ED_{50} bzw. ED_{30} von Hexapeptid VI	8,7 ng/ml	130 ng/kg
Relative Aktivität VI = 100%	84	45