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# Hypnotic Effect of Tryptophan Analog in Rats

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FORNAL, C., W. J. WOJCIK, M. RADULOVACKI AND H. G. SCHLOSSBERGER. *Hypnotic effect of tryptophan analog in rats.* PHARMAC. BIOCHEM. BEHAV. 11(3) 319–323, 1979.—The effects of DL 2-amino-3-(1-naphthyl) propanoic acid, a tryptophan analog, on sleep and brain chemistry were investigated in rats. Similar to previous findings with tryptophan, the tryptophan analog (30 mg/kg, IP) reduced slow-wave sleep (SWS) latency. The reduction in SWS latency occurred at a time when 5-hydroxytryptamine (5-HT) concentration was reduced in the cortex, pons-medulla and striatum-thalamus with no change in the concentration of 5-hydroxyindoleacetic acid, a major metabolite of 5-HT. At the same time, norepinephrine concentration was reduced in the cortex, hippocampus and striatum-thalamus with a marked reduction (40%) in cortical dopamine (DA). The reduction of cortical DA coincided with a 53% decrease in homovanillic acid, a major metabolite of DA. The behavioral effect of tryptophan analog for six hours, as monitored by the EEG, was an increase in SWS by 25 min and a decrease in waking by 29 min. These data suggest that the effects of the tryptophan analog on sleep may be due to the attenuation of the activity of brain catecholamines and imply that tryptophan may as well produce its hypnotic effect via a similar mechanism.

Tryptophan analog  Reduced sleep latency  Increased slow wave sleep  Attenuation of catecholamine activity

ADMINISTRATION of the amino acid L-tryptophan to humans has been reported to reduce sleep latency and waking time [6, 7, 9, 18]. Similar results in reduction of sleep latency have also been observed in rats [8]. Since tryptophan is a precursor of 5-hydroxytryptamine (5-HT), which has been implicated in the mediation of sleep [7, 10, 11, 12], it was assumed that tryptophan produces these effects by increasing the availability of 5-HT at sites where serotonin naturally occurs in the brain.

However, in addition to its action on brain 5-HT, it was suggested that tryptophan may produce its hypnotic effects via a non-serotonergic mechanism [9, 18, 19]. Along these lines, we have shown (Wojcik, Fornal and Radulovacki. *Neuropharmacol.*, in press) that the reduction in sleep latency following L-tryptophan administration correlates not only with the expected elevation of 5-hydroxyindoleacetic acid (5-HIAA), a major metabolite of 5-HT, but also with a reduction in the concentration of both dopamine (DA) and norepinephrine (NE) in various brain regions.

In the following study the effects of DL 2-amino-3-(1-naphthyl) propanoic acid, a tryptophan analog, on sleep and brain chemistry were investigated. Although this compound shares several structural similarities with tryptophan, its effects have not been compared with those of tryptophan in a biological system. Therefore, it was of interest to observe whether it would produce similar effects on sleep and brain catecholamines as tryptophan. However, unlike tryptophan, the tryptophan analog would not serve as a 5-HT precursor and therefore would not be expected to increase 5-HT turnover.

## METHOD

### *Synthesis of Tryptophan Analog*

2-Amino-3-(1-naphthyl) propanoic acid, a tryptophan analog, was synthesized according to the method of H. Erlenmeyer and W. Grabenmann [4] with little modifications. This compound contains a naphthalene ring which is substituted for the indole ring found in tryptophan (Fig. 1). The equivalence between the ethylene group and the imino group with regard to the electrons is the basis of the chemical similarity between the indole and the naphthalene nucleus. In both cases there is a 10 pi-electron system. Furthermore, the position of the side chain in tryptophan analog corresponds to the side chain position of tryptophan.

### *Implantation of Electrodes and Polygraphic Recording*

Adult male Sprague-Dawley rats (400–500 g) were implanted with electrodes for EEG and EMG recording to evaluate sleep states during a 6-hour experimental sleep period. The implantation procedure was carried out under pentobarbital anesthesia (40 mg/kg, IP) with supplemental administration of ether as required. Atropine methyl nitrate (2 mg/kg, SC) was also given to eliminate bronchial congestion during surgery. Surgery involved bilateral implantation of two stainless steel screw electrodes (size 0–80×1/8 inch) into the parietal bones and insertion of two paddle-shaped wire electrodes into the dorsal neck muscles. All electrodes were soldered to the appropriate leads of a connector which was fixed to the skull by dental cement. Two additional stainless steel screws were inserted, one into the occipital bone

and the other into the frontal bone, to help anchor the implant. Prior to suturing each animal, Neosporin (each gram contains polymyxin B sulfate, 5,000 units, bicitracin zinc 400 units, and neomycin sulfate 5 mg) ointment was topically applied to the incision site. All animals then received an injection of Bicillin (penicillin G benzathine and penicillin G procaine in aqueous suspension) 150,000 units (SC) and were allowed a minimum of one week recovery.

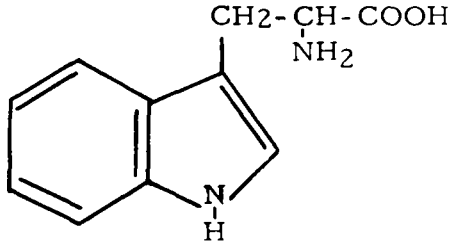
At 10:00 a.m. on the day of the experiment, the animals received either saline (4 ml/kg, IP or DL 2-amino-3-(1-naphthyl) propanoic acid, HCl (30 mg/kg) IP dissolved in saline at a concentration of 7.5 mg/ml, pH 6.0) and were polygraphically recorded until 4:00 p.m. No more than eight animals were recorded at one time on two Grass-VIII channel electroencephalographs. Prior to each recording session, all animals were acclimated to the recording unit and cable for 2 days. Evaluations of the polygraphic records were made using standard techniques where each epoch of record was determined to be either wakefulness (W), slowwave sleep (SWS) or rapid eye movement (REM) sleep. The epochs were one minute long and the speed of the paper drive was two minutes per page (2.5 mm/second). Behavioral observations via closed circuit television were noted on the EEG record during the experiment. The amount of time spent in either W, SWS or REM sleep was calculated at half-hour intervals for the first hour and at one hour intervals for the entire 6-hour recording session. The effects of tryptophan analog on the sleep-wake pattern was assessed using the Student *t*-test in which the amount of time spent in each of the three behavioral states during the various time intervals was compared to the control.

In addition, SWS and REM sleep latencies (time between injection and the appearance of the first two minute SWS or the first one minute REM sleep episode) were determined. The Student *t*-test was used to determine significance between control and experimental latencies.

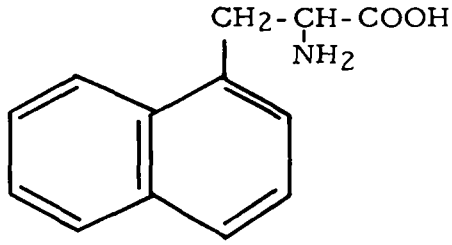
*Determination of Monoamines and Monoamine Metabolites*

Another group of male Sprague-Dawley rats of the same age and weight which were not implanted with electrodes received either saline or tryptophan analog. All injections were given in the same manner as for the sleep study. The animals were then decapitated by guillotine fifteen or forty-five minutes after the injection and their brains were removed and washed with ice-cold saline. The cerebral cortex, hippocampus, pons-medulla and striatum-thalamus, which did not include the head of the caudate nucleus, were quickly dissected on ice. The excised brain regions were then weighed and stored at −20°C for future analysis.

The concentration of 5-HT, 5-HIAA, NE and DA were simultaneously analyzed from the various tissue samples for both time periods by a spectrophotofluorometric assay using solvent extraction methods. Homovanillic acid (HVA), a major metabolite of DA, was also determined fluorometrically in the cortex fifteen minutes after tryptophan analog administration. Each HVA sample consisted of pooled cortical tissue from two animals. The extraction procedure for all of the above compounds was slightly modified from that reported by Welch and Welch [16] and Alpers and Himwich [1]. The method used for developing fluorophores of 5-HT was that of Maickel and Miller [15]. 5-HIAA was determined according to the method of Korf and Valkenburgh-Sikkema [13] and HVA by the method of Gerbode and Bowers [5]. The formation of fluorescent derivatives of the catecholamines was reported by Chang [3]. An extracted standard curve for each compound was generated during each assay from which the unknown concentrations, in the various brain tissues, were determined. The Student *t*-test or the Welch test, when variances were unequal, was used to determine significance between the control and tryptophan analog treated group.




Tryptophan
(2-amino-3-(3-indolyl) propanoic acid)




Tryptophan Analog
(2-amino-3-(1-naphthyl) propanoic acid)

FIG. 1. Structures of tryptophan and tryptophan analog.

## RESULTS

*Effects of Tryptophan Analog on Sleep Latencies*

Table 1 shows the effects of tryptophan analog on latencies to the first SWS and first REM sleep episodes. It can be seen that the administration of this agent reduced SWS latency from 40 ± 5 min in the control group to 25 ± 5 min in the experimental group ($p<0.02$). This represents a 37% decrease from control. However, no change occurred in the latency to the first REM sleep episode following tryptophan analog administration.

TABLE 1

EFFECTS OF TRYPTOPHAN ANALOG (TA), 30 mg/kg, IP, ON LATENCIES TO FIRST SWS AND FIRST REM SLEEP EPISODES IN RATS

| | Control | TA |
|---|---|---|
| SWS Latency | 40 ± 4 | 25 ± 2* |
| REM Sleep Latency | 70 ± 5 | 58 ± 7 |

The results are expressed as means ± SE (minutes) for 14 control and 7 TA animals *$p<0.02$ by Student *t*-test.

TABLE 2

EFFECTS OF TRYPTOPHAN ANALOG (TA), 30mg/kg, IP, ON W, SWS AND REM SLEEP IN RATS

| Time Interval (Hr) | W | | SWS | | REM | |
|---|---|---|---|---|---|---|
| | Control | TA | Control | TA | Control | TA |
| 0.0–0.5 | 29 ± 1 | 25 ± 1§ | 1 ± 1 | 5 ± 1§ | 0 | 0 |
| 0.5–1.0 | 17 ± 2 | 12 ± 4 | 12 ± 2 | 17 ± 4 | 1 ± 0 | 1 ± 1 |
| 0–1 | 46 ± 3 | 36 ± 5 | 13 ± 2 | 22 ± 4* | 1 ± 0 | 1 ± 1 |
| 1–2 | 11 ± 1 | 20 ± 3‡ | 42 ± 1 | 35 ± 3+ | 7 ± 1 | 5 ± 1 |
| 2–3 | 17 ± 4 | 9 ± 2 | 37 ± 4 | 43 ± 2 | 6 ± 1 | 8 ± 1 |
| 3–4 | 28 ± 4 | 17 ± 3 | 29 ± 3 | 38 ± 3 | 3 ± 1 | 5 ± 1 |
| 4–5 | 22 ± 4 | 20 ± 5 | 33 ± 4 | 33 ± 4 | 5 ± 1 | 6 ± 2 |
| 5–6 | 17 ± 5 | 10 ± 4 | 37 ± 4 | 43 ± 4 | 7 ± 1 | 7 ± 1 |
| 0–6 | 141 ± 8 | 112 ± 8* | 190 ± 6 | 215 ± 2* | 28 ± 4 | 33 ± 4 |

The results are expressed as means ± SE (minutes) for 14 control and 7 TA animals.

*$p<0.05$, +$p<0.02$, ‡$p<0.005$, §$p<0.002$ by Student $t$-test

TABLE 3

CONCENTRATIONS OF 5HT AND 5HIAA (ng/g wet weight) IN SPECIFIC BRAIN REGIONS IN RATS, 15 AND 45 MIN AFTER THE ADMINISTRATION OF TRYPTOPHAN ANALOG (30 mg/kg, IP)

| Time | Brain Region | 5 HT | | 5 HIAA | |
|---|---|---|---|---|---|
| | | Control | TA | Control | TA |
| 15 Min After Injection | Cortex | 595 ± 21 (7) | 533 ± 4 (5)† | 180 ± 10 (7) | 207 ± 20 (5) |
| | Hippocampus | 529 ± 23 (7) | 476 ± 16 (4) | 174 ± 12 (7) | 172 ± 13 (4) |
| | Pons/Medulla | 857 ± 24 (7) | 772 ± 29 (5)* | 323 ± 30 (7) | 332 ± 13 (5) |
| | Striatum/Thalamus | 800 ± 18 (7) | 732 ± 20 (5)* | 293 ± 28 (7) | 291 ± 33 (5) |
| 45 Min After Injection | Cortex | 449 ± 20 (6) | 444 ± 32 (6) | 89 ± 10 (6) | 95 ± 11 (6) |
| | Hippocampus | 474 ± 32 (6) | 412 ± 7 (6) | 140 ± 14 (6) | 143 ± 11 (5) |
| | Pons/Medulla | 600 ± 24 (6) | 542 ± 22 (6) | 564 ± 42 (6) | 555 ± 70 (6) |
| | Straitum/Thalamus | 704 ± 36 (6) | 593 ± 6 (5)† | 412 ± 45 (6) | 441 ± 29 (6) |

The results are expressed as means ± SE

The number of animals in each group is indicated in parentheses.

*$p<0.05$ by Student $t$-test

†$p<0.05$ by Welch's nonparametric method.

*Effects of Tryptophan Analog on Sleep States*

Table 2 shows the effects of tryptophan analog on W, SWS, and REM sleep. The data are presented in one-hour intervals for the entire 6-hour recording session. In addition, the first hour of the recording is subdivided into two consecutive half-hour periods in order to correlate the initial effects of the drug on sleep with its effects on brain chemistry. It can be seen that the administration of tryptophan analog reciprocally enhanced SWS ($p<0.002$) and reduced waking time ($p<0.002$) by 4 min during the first half-hour period of EEG recording. This increase in SWS time coincided with the reduction found in SWS latency. During the second half-hour period, there was a tendency toward an increase in SWS and a decrease in W. However, this was not statistically significant. Statistical analysis performed at one-hour intervals showed that tryptophan analog increased SWS by 9 min ($p<0.05$) during the first hour with no change in W or REM sleep. However, during the second hour of EEG recording, SWS time was reduced by 7 min ($p<0.02$) and W was increased by 9 min ($p<0.005$). There were no changes in either W, SWS or REM sleep for the remaining four hours of the recording session.

The overall behavioral effect of tryptophan analog as monitored by the EEG for 6 hours was an increase in SWS by 25 min, decrease in W by 29 min and no change in REM sleep.

*Effects of Tryptophan Analog on 5-HT and 5-HIAA Concentrations*

As shown in Table 3, fifteen minutes after the administration of tryptophan analog there was a significant reduction in the content of 5-HT by 10% in both the cortex and pons-medulla and by 8% in the striatum-thalamus. The fourth brain area that was examined, the hippocampus,

TABLE 4

CONCENTRATION OF DA AND NE (ng/g wet weight) IN SPECIFIC BRAIN REGIONS IN RATS, 15 AND 45 MIN AFTER THE ADMINISTRATION OF TRYPTOPHAN ANALOG (30 mg/kg IP)

| Time | Brain Region | DA | | NE | |
|---|---|---|---|---|---|
| | | Control | TA | Control | TA |
| 15 Min After Injection | Cortex | 1039 ± 56 (7) | 626 ± 25 (5)‡ | 709 ± 36 (7) | 597 ± 28 (5)* |
| | Hippocampus | 733 ± 29 (7) | 636 ± 82 (4) | 555 ± 20 (7) | 466 ± 16 (4)† |
| | Pons/Medulla | 757 ± 35 (7) | 712 ± 40 (5) | 861 ± 21 (7) | 762 ± 32 (5)* |
| | Striatum/Thalamus | 869 ± 81 (7) | 913 ± 68 (5) | 939 ± 34 (7) | 853 ± 52 (5) |
| 45 Min After Injection | Cortex | 1076 ± 104 (6) | 971 ± 5 (6) | 442 ± 14 (6) | 450 ± 21 (6) |
| | Hippocampus | 805 ± 43 (6) | 693 ± 32 (6) | 561 ± 33 (6) | 499 ± 24 (6) |
| | Pons/Medulla | 846 ± 41 (6) | 828 ± 44 (6) | 739 ± 29 (6) | 736 ± 44 (6) |
| | Striatum/Thalamus | 1258 ± 54 (6) | 1005 ± 75 (6)* | 807 ± 26 (6) | 882 ± 28 (6) |

The results are expressed as means ± SE
The number of animals in each group is indicated in parentheses.
*$p<0.05$, †$p<0.02$, ‡$p<0.0005$.

showed no change in the level of 5-HT. Forty-five minutes after drug administration the concentration of 5-HT was reduced by 16% only in the striatum thalamus. We observed no change in the levels of 5-HIAA in any of the studied regions during the two time periods.

*Effect of Tryptophan Analog on NE, DA and HVA Concentrations*

Analysis of the catecholamines NE and DA showed that the level of these amines were also reduced in various brain areas following tryptophan analog administration. As shown in Table 4, fifteen minutes after drug administration the concentration of DA was reduced by 40% in the cortex. The reduction in cortical DA coincided with a 53% decrease in cortical HVA (Table 5). NE concentrations were also reduced by 16% in both the cortex and hippocampus and by 11% in the pons-medulla. Forty-five minutes after administration of tryptophan analog the concentration of DA was reduced by 20% only in the striatum-thalamus. There were no changes in the level of NE in any of the studied brain regions during this time.

TABLE 5

CONCENTRATION OF CORTICAL HVA (ng/g wet weight) IN RATS 15 MIN AFTER THE ADMINISTRATION OF TRYPTOPHAN ANALOG (TA), 30 mg/kg, IP

| Control | TA | % of Control |
|---|---|---|
| 275 ± 17 (4) | 130 ± 34 (4)* | 47 ± 12 |

The results are means ± SE
The number of samples in each group is indicated in parentheses. Each sample consists of tissue pooled from two animals.
*$p<0.01$ by Student $t$-test.

## DISCUSSION

We have previously shown (Wojcik, Fornal and Radulovacki, *Neuropharmacol.*, in press) in an identical experimental design that the reduction in SWS latency seen after the administration of tryptophan to rats correlates with an increase in brain 5-HIAA and a decrease in brain catecholamines. Since increased 5-HT metabolism and decreased DA metabolism have been observed in man [20] and in the cat [2,14] during physiological SWS, we attributed the sleep latency-reducing effect of tryptophan to the interaction between brain serotonin and catecholamines. Present results with the tryptophan analog show that the administration of this agent also produced a significant reduction in the latency to SWS as well as a significant increase in SWS time during the first half-hour period of EEG recording (Tables 1 and 2). These effects of tryptophan analog on sleep occurred at a time when 5-HT levels were reduced in various brain regions while 5-HIAA concentrations remained unchanged. Since no increase in 5-HT metabolism was observed, we could not conclude that the effects of tryptophan analog on sleep involve a serotonergic mechanism. However, there is the possibility that tryptophan analog or a metabolite of tryptophan analog may be a 5-HT agonist. If stimulation of 5-HT receptors initiates sleep, one would predict that such an agent would also reduce sleep latency. Therefore, we are currently conducting experiments in our laboratory to determine if the tryptophan analog has any 5-HT agonistic actions.

Since a decrease in catecholamine utilization may be expected to reduce arousal and thus facilitate sleeping behavior, we were interested in possible effects of the tryptophan analog on endogenous catecholamine levels. The obtained reduction of DA and NE concentrations seen after the injection of tryptophan analog was similar to what we have reported following the administration of L-tryptophan. Since tryptophan analog has produced such a profound effect only on cortical DA levels, HVA was analyzed in this brain region to determine if the decrease in DA represents a decrease in the activity of dopaminergic neurons. Table 5 shows that administration of tryptophan analog significantly reduced the cortical HVA concentration by more than 50% suggesting that the obtained reduction in cortical DA reflects a decrease in the availability of DA at the synapse. Therefore, these results imply that tryptophan analog may have affected DA synthesis in this brain area. The mechanism by which tryptophan analog reduces NE is less clear because the NE metabolite was not determined in this study. However, indi-

cations that tryptophan analog may inhibit DA synthesis could also be applied to NE since both DA and NE are synthesized along the same tyrosine metabolic pathway and a possible inhibition of NE synthesis may account for the reduced NE concentration.

The effects of tryptophan analog on the levels of catecholamines and 5-HT may be due to the drug interfering with the synthesis of these amines. A possible mechanism for this involves competition of amino acids for transport across the blood brain barrier. Wurtman *et al.* [17] have suggested that administration of any neutral amino acid other than the catecholamine precursors, tyrosine and dopa, may be expected to inhibit catecholamine synthesis by lowering brain levels of these amino acids, just as the administration of any neutral amino acid other than tryptophan or 5-hydroxytryptophan should inhibit 5-HT synthesis. Since tryptophan analog is a synthetic neutral amino acid it would be expected to compete with both tyrosine and tryptophan for uptake into the brain. Thus, by decreasing the availability of these amino acids in the brain, synthesis of the monoamines is reduced. Although it is conceivable that such a mechanism may be involved, it seems unlikely that this alone could account for the rapid reduction of the monoamines seen after tryptophan analog administration. Another mechanism by which tryptophan analog may bring about its effect is to interfere with the various enzymes involved in the synthesis of these brain monoamines.

In conclusion, our data suggest that the decrease in SWS latency as well as increase in SWS time seen after the administration of tryptophan analog may be due to the attenuation of the activity of brain catecholamines and imply that tryptophan may as well produce its hypnotic effect via a similar mechanism.

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