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Brain Serotonin Metabolism in the Cockroach, *Periplaneta americana*—Effect of Nialamide and Correlation with Behaviour

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Although Serotonin has been implicated as a neurotransmitter in the insect nervous system, not much work has been done so far. Significantly high concentrations of Serotonin are found in the cockroach brain during the present studies. Nialamide is known to block the catabolic step of Serotonin metabolism by inhibiting Monoamine oxidase enzyme, and leading to the alterations in the brain Tryptophan, Serotonin and 5-Hydroxyindoleacetic acid levels. Nialamide treatment leads to an inerease in Serotonin level and a decrease in 5-Hydroxyindoleacetic acid level of the brain. A negative correlation between brain Tryptophan and protein levels is observed after Nialamide treatment.

Key Words: 5-Hydroxytryptamine (Serotonin), Tryptophan, Tryptophan hydroxylase, Monoamine Oxidase, 5-Hydroxyindoleacetic acid

Introduction

Serotonin (5-hydroxytryptamine, 5-HT), an important biogenic amine, has been known as a neurotransmitter in the central nervous system (CNS) of most of the vertebrates and invertebrates (Brodie & Shore 1957). It is synthesized in the nervous system from its precursor tryptophan (Trp), an essential aromatic amino acid, by a two-step process of hydroxylation and decarboxylation. The first enzyme, Tryptophan-hydroxylase (TH), is a rate-limiting enzyme (Moir & Eccleston 1968, Volicer 1969), and is highly specific for Trp. The enzyme involved in decarboxylation step is a nonspecific aromatic amino acid decarboxylase. Mono amine oxidase (MAO) is the only enzyme involved in the catabolism of 5-HT, and 5-Hydroxyindole acetic acid (5-HIAA) is the immediate metabolite (Hiripi & Salanki 1973).

Serotonin is known to play an important role in a number of processes besides its main function of neurotransmission. In the case of higher animals, serotonin is involved in temperature-regulation (Feldberg & Myers 1963, Sheard & Aghajanian 1967, Lin 1978, Lin et al. 1978). Control of prolactin secretion

(Clemens et al. 1977, Clemens et al. 1978, Advis et al. 1979), Thyrotropin release (Jordan et al. 1978), control of food-intake (Samanin et al. 1977, Samanin et al. 1977), pain sensitivity (Harvey & Lints 1971, Messing & Lytle 1977, Schlosperg & Harvey 1978, Telner et al. 1979) and sexual behaviour (Dallo 1977, Everitt & Fuxe 1977). The role of Serotonin has also been investigated in mammalian behaviour (Isaacson et al. 1977), mood (Mandell & Knapp 1979), and mental retardation (Oikawa et al. 1978). Serotonin depletion caused an increase in sexual activity and insomnia (Torda 1967, Jouvet 1969).

However, the information available in the insects is scanty. The presence and distribution of 5-HT have been already established in invertebrate nervous system (Welsh & Moorhead 1960, Colhoun 1963). Significantly high concentrations of 5-HT have been found in the cockroach brain (Pandey & Habibulla 1980). Serotonin has been proposed to act as a neurohumoral agent in the lower animals (Welsh 1954, Welsh 1957). Serotonin has been identified in the molluscan and vertebrate tissues at about the same time and the role of 5-HT as a neurotransmitter has been proposed for molluscan system (Welsh 1953). Serotonin has been known to be involved in rhythmic regulation of heart muscle cells of Gastropoda and insecta (Rozsa et al. 1973). It is also supposed to have a role in the control of periodic activity of freshwater mussel (Salanki 1963), in contraction of molluscan smooth muscle (Twarog 1954, 1960, Hidaka et al. 1967), and in ciliary activity of mytilus gill (Aiello 1957).

Keeping in view the enormous importance of 5-HT as a behaviour-altering drug in the higher organisms, the present work is an attempt to study 5-HT in the cockroach nervous system along with its behavioral effects. The significantly high quantities of 5-HT point towards its importance. The levels of 5-HT and 5-HIAA in the cockroach brain were estimated and the effects of nialamide, an inhibitor of MAO enzyme, on the function and metabolism of 5-HT were investigated.

Materials and Methods

Adult male cockroaches (weight 1 g approximately) were used for the experiments. These cockroaches were housed in a cage, maintained at a temperature (25 ± 1°C). Nialamide (Sigma Chemical Co., USA) was injected into the hemolymph with the help of a Hamilton microlitre syringe. Stock solution (1000 μg/ml) was made and 0.015 ml (15 μg) of this solution was injected into each cockroach in the single-dose experiments. In the double dose experiments 0.030 ml (30 μg) of stock solution was injected into each cockroach.

Following the drug administration at various time intervals the brains were dissected out and used for the experiments.

Tryptophan Determination

Conventional paper chromatographic procedure was used for Trp determination. The brains after dissection were homogenized in acetone. The supernatant was evaporated and the residue was dissolved in small quantity of 0.1N HCl. The sample solution along with standard Trp solution (500 μg/ml) were spotted on a Whatmann No. 1 chromatographic paper and allowed to run in *n*-Butanol : Acetic acid : water (12 : 3 : 5), stained with ninhydrin and eluted in 0.1N NaOH (Pandey & Habibulla 1980). The absorbance of the eluted samples was measured at 405 nm using a spectrophotometer. The mean values were obtained from at least three determinations.

Protein Determination

Protein contents of the brain were determined by the method of Lowry et al. (1951), using Bovine serum albumin as standard. The mean values of brain protein concentrations at each

stage were obtained from three experiments.

5-HT and 5-HIAA Determinations

The improved method of Fischer and Aprison (1972) was adopted for the determination of 5-HT and 5-HIAA using fluorometric technique as described earlier (Pandey & Habibulla 1980). The fluorescence of the samples was measured on Ferrand Spectrofluorometer set at 300 nm for excitation and 545 nm for fluorescence measurement.

Results

Nialamide Effect on Brain Trp and Protein Levels

Single dose effect: The insects treated with single dose of nialamide (15 μg/g) showed a significant ($p < 0.05$) depletion in brain Trp level up to 1 hour of administration, after that Trp level increased significantly ($p < 0.01$) and at 2 hr it reached to a peak value of 61.7 ± 2.04 mg/g protein. After 2 hr, there was a fall in Trp level and lowest level (17.5 ± 1.62 mg/g protein) was found at 7 hr of treatment, after which Trp level increased with fluctuation up to 24 hr but it failed to reach the normal level. Trp level was significantly low ($p < 0.05$) at 24 hr when compared to normal. The protein content of the brain increased at 1 hr, decreased at 2 hr and again started increasing. The highest level of brain protein was found at 4 hr of treatment and lowest at 8 hr. There were not much fluctuations in brain protein contents from

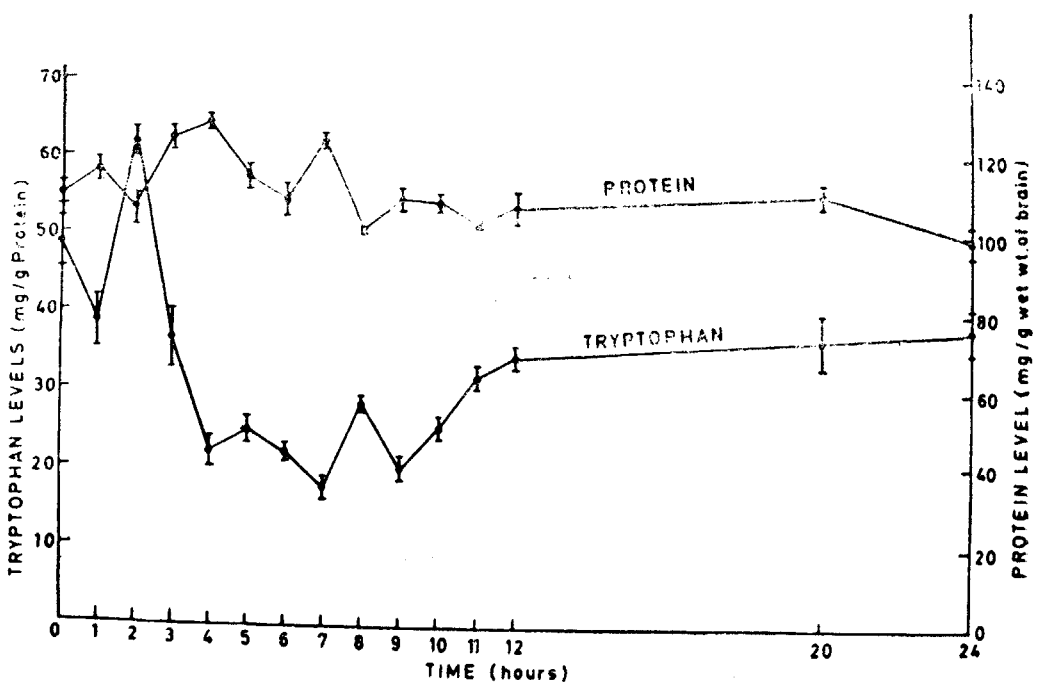


Fig. 1 Brain tryptophan and protein levels in nialamide-treated cockroaches (dose 15 μg/g). The values are expressed as mean ± S.E. of three determinations. The protein and tryptophan levels showed opposite quantitative tendencies. As tryptophan level was increasing, the protein level was decreasing or vice versa

8 hr onwards, and at 24 hr the protein level was almost equal to its normal level (figure 1). It is clear from the graph that brain Trp and protein contents have shown opposite quantitative tendencies. When Trp level was increasing, protein level was decreasing and vice versa.

Double-dose effect: The cockroaches treated with double dose (30 μg/g) of nialamide showed a significant ($p < 0.01$) decrease in brain Trp level (almost 50% than normal) in the beginning just after injection. Again, it decreased significantly ($p < 0.01$) and at 1 hr 5.50 ± 0.54 mg/g protein of Trp was found while the normal level was 46.59 ± 1.49 mg/g protein. The lowest level of Trp (2.60 ± 0.48 mg/g protein) was obtained at 3 hr, which was significantly lower ($p < 0.01$) than the normal level. After 12 hr, Trp level started increasing but at no time did it reach the normal level. Brain protein levels showed a decrease up to 2 hr. The lowest level of protein (61.33 ± 6.18 mg/g wet weight of brain) was found at 2 hr while normal level (112.67 ± 0.94 mg/g wet weight of brain) was the highest. After 2 hr, it started increasing with some variations up to 7 hr. There was a slight fall at 8 hr but again brain protein contents increased significantly ($p < 0.01$) at 9 hr, after that it decreased and remained depleted onwards (figure 2). In the double-dose experiments also brain Trp and Protein levels exhibited opposite quantitative tendencies at most of the places as in the case of single-dose experiments.

Nialamide Effect of Brain 5-HT and 5-HIAA Levels

Single dose effect: Normal 5-HT level, i.e., prior to treatment, was the highest

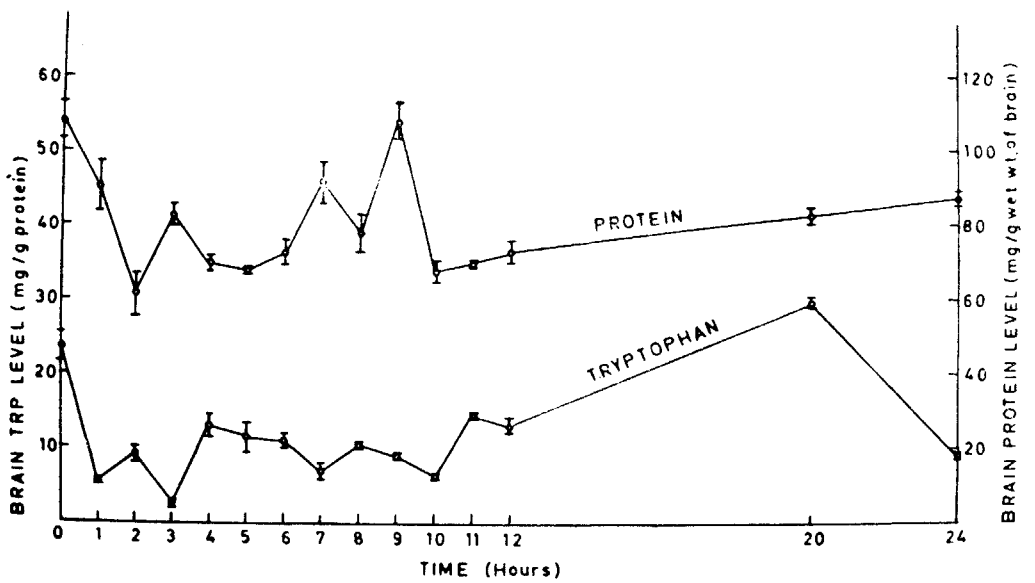


Figure 2 Brain tryptophan and protein levels in nialamide-treated cockroaches (dose 30 μg/g). The values are expressed as mean ± S.E. of three experiments. The levels of both tryptophan and protein were depleted after treatment, and at most of the places brain tryptophan and protein levels exhibited opposite quantitative tendencies

(0.73 ± 0.0 mg/g protein). Just after injection, up to 7 hr of single-dose treatment, brain 5-HT level was depleted gradually and at 7 hr lowest 5-HT level was obtained which was significantly lower ($p < 0.01$) than the normal level. After 7 hr again 5-HT level increased with small insignificant variations but could not regain the normal level. Brain 5-HIAA level was not changed much up to 2 hr of treatment. Then, there was a fall up to 4 hr and at 6 hr lowest 5-HIAA level was obtained. At 11 hr highest 5-HIAA level was found. After 11 hr it depleted and up to 20 hr 5-HIAA level was approximately normal, but hr at 24 hr 5-HIAA level was slightly higher than the normal (figure 3).

Double dose effect: Nialamide effect on brain 5-HT level was more clear and pronounced after double dose (30 μg) treatment. The 5-HT level slightly decreased just after injection and then increased significantly up to 2 hr where highest 5-HT level was obtained which was significantly ($p < 0.01$) higher than the normal level and remained at a higher value up to 6 hr, then it started decreasing and at 9 hr lowest level of 5-HT was obtained. After 9 hr, 5-HT level started increasing and from 12 hr onwards it maintained almost a constant value which was higher than the normal. Brain 5-HIAA level elevated up to 2 hr of nialamide double-dose treatment, but at 3 hr it was reduced to the normal level. After 3 hr, it increased and exceeded the normal value and highest 5-HIAA level was found at 10 hr which was significantly ($p < 0.01$) higher than the normal 5-HIAA level. After 10 hr, it showed a depletion but did not come back to the normal value (figure 4).

Observations on Behaviour and Correlation with Serotonin

Single-dose effect: After 2 hr of nialamide

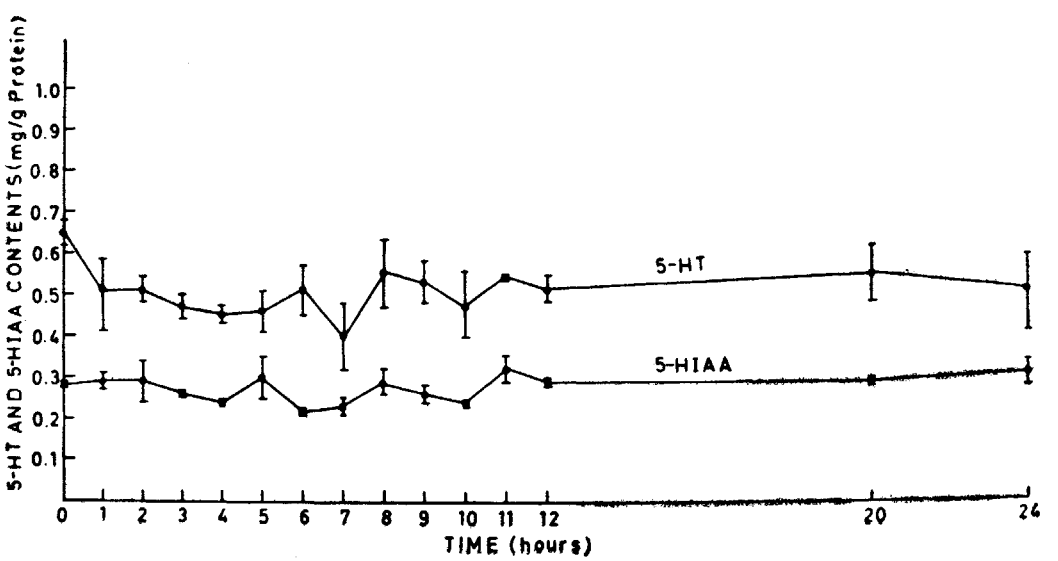


Figure 3 Brain 5-HT and 5-HIAA leve's in nialamide-treated cockroaches (dose 15 μg/g). The values are expressed as mean ± S.E. of three determinations. At 7 hr of treatment lowest 5-HT level was obtained while normal 5-HT level was the highest. Brain 5-HIAA level shows no much variations

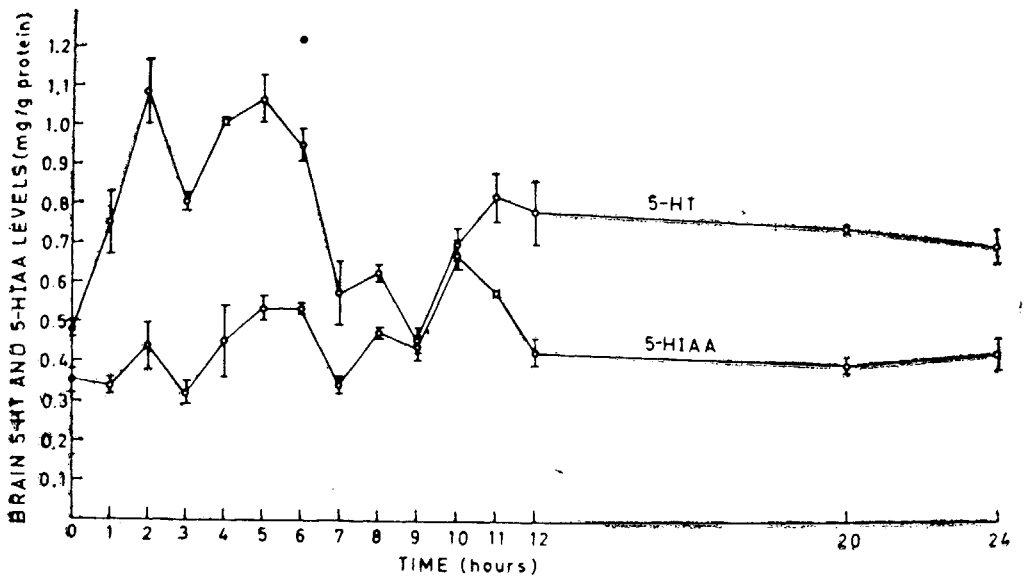


Figure 4 Brain 5-HT and 5-HIAA levels in nialamide-treated cockroaches (dose 30 μg/g). The values are expressed as mean ± S.E. of three determinations. The level of 5-HT increased after nialamide injection and at 2 hr highest level was obtained which was significantly higher than the normal 5-HT level. Brain 5-HIAA level remained slightly higher than the normal at most of the time periods after treatment.

single-dose treatment, the insects became hypoactive and lost their normal activity. They lost the response to light and darkness. At this time, they were unable to respond to external stimuli like touch. Up to 6 hr, they were sluggish in movements and were not trying to regain their normal posture if turned turtle. Trp/protein, Trp/5-HT and Trp/5-HIAA ratios were found to be highest at 2 hr (table 1). Trp/protein ratio was increased significantly ($p < 0.01$) up to 0.58 at 2 hr from the normal value of 0.42. After 2 hr, Trp/protein ratio decreased and remained low up to 24 hr. Trp/5-HT ratio was increased significantly ($p < 0.01$) at 2 hr. A significant rise ($p < 0.01$) was reported in Trp/5-HIAA ratio also. 5-HT/5-HIAA ratio was depleted at 2 hr and it was 1.7 at this time while the normal ratio was 2.6. Up to 12 hr the insects were hypoactive, after that the activity was improved a bit, and they started moving slightly on the floor of the cage. After nialamide single-dose injection of 5-HT/5-HIAA ratio was always found to be below normal at all durations tested.

Double-dose effect: The insects were hyperactive at 1 hr of treatment. At 1 hr Trp/protein ratio was depleted significantly ($p < 0.01$), but 5-HT/5-HIAA ratio was increased from normal 1.81 to 2.21. Trp/5-HT and Trp/5-HIAA ratios were also depleted significantly ($p < 0.01, p < 0.01$, respectively) when compared to their normal values (table 2). The cockroaches were very active

Table 1 *Changes in Trp/Protein, 5-HT/5-HIAA, Trp/5-HT and Trp/5-HIAA ratios at different time intervals after nialamide single dose administration (15 μg/g)*

Time duration post injection (hr)	Trp/Protein ratio	5-HT/5-HIAA ratio	Trp/5-HT ratio	Trp/5-HIAA ratio
Normal	0.42	2.6	64.25	167.50
0	0.44	2.3	74.61	173.21
1	0.33	1.7	75.68	133.10
2	0.58	1.7	120.98	212.75
3	0.29	1.8	77.44	140.00
4	0.17	1.8	48.89	91.67
5	0.22	1.5	53.91	82.67
6	0.20	2.3	43.53	100.91
7	0.14	1.7	43.75	76.08
8	0.28	1.9	51.09	96.89
9	0.18	2.0	37.73	76.92
10	0.23	1.8	52.76	103.33
11	0.31	1.7	58.52	98.75
12	0.32	1.7	67.25	118.27
20	0.33	1.9	66.18	125.52
24	0.35	1.6	74.90	123.22

Table 2 *Changes in Trp/Protein, 5-HT/5-HIAA, Trp/5-HT and Trp/5-HIAA ratio at different time intervals after Nialamide dauble dose administration (30 μg/g)*

Time during post injection (hr)	Trp/Protein ratio	5-HT/5-HIAA ratio	Trp/5-HT ratio	Trp/5-HIAA ratio
Normal	0.41	1.81	80.33	145.59
0	0.22	1.37	49.73	68.20
1	0.06	2.21	7.33	16.18
2	0.15	2.45	8.72	24.41
3	0.03	2.50	3.25	8.13
4	0.19	2.22	13.30	29.56
5	0.17	2.00	11.09	22.19
6	0.16	1.77	12.14	21.53
7	0.08	1.68	12.86	21.56
8	0.14	1.32	17.32	22.85
9	0.09	1.05	20.76	21.72
10	0.10	1.05	9.67	10.11
11	0.21	1.42	17.89	25.42
12	0.18	1.83	17.14	31.43
20	0.36	1.87	40.34	75.51
24	0.10	1.64	13.14	21.59

up to 5 hr of treatment. At this stage 5-HT/5-HIAA ratio was higher than normal and Trp/protein, Trp/5-HT and Trp/5-HIAA ratios were found to be lower than their respective normal values. After 5 hr the insects became dull and remained hypoactive up to 10 hr. After 10 hr again they were found to be active comparatively. 5-HT/5-HIAA ratio was depleted after 5 hr and at 9 and 10 hr lowest value was obtained.

Thus results indicated that the activity of the insect was related closely to 5-HT and 5-HIAA levels of the brain. By depleting brain 5-HT levels the activity of the insect was also reduced generally. Also when the activity of the insect was reduced, Trp/protein, Trp/5-HT and Trp/5-HIAA ratios were increased, and 5-HT/5-HIAA ratio was decreased.

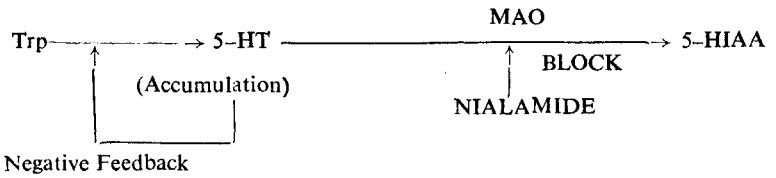
Discussion

Most of the information available is in the higher animals and very little is known in the case of insects. Based on the information available from the mammalian studies, the present results are discussed.

The main pathway of serotonin inactivation is oxidative deamination by monoamine oxidase (MAO) enzyme (Blaschko & Levine 1966). Nialamide is known to inhibit MAO enzyme, thus inhibiting the catabolism of 5-HT (Hiripi & Salanki 1973). Much less is known about the inactivation of monoamines in insect nervous system. Low MAO activity is found in nervous tissues of cockroaches (Colhoun 1967, Blaschko 1974).

The cockroaches treated with nialamide showed a depletion in brain Trp level. In both the cases of single dose and double dose treatments brain Trp level remained depleted up to 24 hr of treatment. In both the cases brain Trp and protein levels have shown a negative correlation. The treatment of MAO inhibitor has been reported to cause alterations in brain Trp and 5-HT metabolism and also in behaviour of the animal (Grahame-Smith 1971). Because of the presence of nialamide, 5-HT could not be catabolised. In the absence of catabolism, the rate of 5-HT synthesis may be reduced, as high concentration of 5-HT may cause a negative feedback action. Due to reduction in synthesis rate, Trp will not be utilised in 5-HT synthesis. Due to accumulation of 5-HT in the absence of deamination, high 5-HT levels may also affect Trp-uptake process. Trp-uptake is an important process which is known to be involved in the control of 5-HT synthesis from Trp. In the mammais high affinity uptake of Trp into serotonergic neurons regulates 5-HT biosynthesis (Mandell & Knapp 1977). Trp is a main constituent of most of the proteins and its concentration may regulate protein synthesis. When Trp was not utilized in 5-HT synthesis, it appeared that during this duration it was used in protein synthesis. So escalation of protein synthesis in brain tissues may be possible because of the availability of more Trp. These may be possible reasons for negative correlation shown by brain Trp and protein contents.

It is known that intracellular concentration of 5-HT can be increased with injection of MAO inhibitors (Fuller 1972). During the present studies in the double dose experiments, 5-HT levels were increased after injection of nialamide. Brain 5-HIAA levels were also increased slightly after treatment. This increase in 5-HIAA level may be because of incomplete inhibition of MAO. The high concentration of 5-HT itself has a feedback control on its synthesis (Hamon et al. 1973) by acting at first step of biosynthetic path after MAO inhibition. It has been known that 5-HT synthesis is reduced when intraneural concentration of 5-HT reaches 2.5 times normal level following MAO inhibition and it is due to feedback control of 5-HT (Macon et al. 1971).



Small doses of MAO inhibitors may have no effect on brain levels of endogenesus 5-HT. In the case of nialamide single-dose treatment, brain 5-HT levels were reduced and remained at a lower level. Brain 5-HIAA levels also have not shown significant changes. It appears here that MAO inhibition is not complete and in addition to incomplete inhibition of MAO some other factors may be involved in this process and as a result brain 5-HT levels are depleted.

Serotonin and behaviour: In the case of higher animals, the effects of the drugs which either deplete or cause an increase in brain 5-HT levels, on the animal's behaviour have been studied up to some extent but in the insects very little information is available.

The insects treated with single dose of nialamide became dull after treatment. Brain Trp level was found to be increased while 5-HT level was decreased. After double-dose treatment the insects were hyperactive up to 5 hr and during this duration brain 5-HT level was increased and Trp level was depleted. The increase in 5-HT is associated with characteristic behavioural changes, one of which is hyperactivity. There is a positive correlation between rate of accumulation of 5-HT in the brain and rate of development of hyperactivity which suggest that the syndrome is produced by 5-HT (Green & Grahame-Smith 1976).

These results show that the brain 5-HT is closely related to the activity of the insect. When 5-HT level was low, the insects were usually hypoactive. Brain Trp was also found to play a significant role in behavioural expression, and this involvement of Trp might be due to its direct involvement in 5-HT synthesis. So the drugs, which interfere with brain 5-HT metabolism, are found to affect the behaviour of the insects.

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