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Metabolism of Serotonin in the Brain of the Cockroach, *Periplaneta americana*: Effect of Reserpine

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Significantly high levels of serotonin were found in the cockroach brain during the present study. Reserpine, a serotonin storage-blocking drug, was found to cause a number of significant alterations in the brain levels of serotonin and its metabolite, 5-hydroxyindole acetic acid. After administration of various doses of reserpine brain serotonin levels were depleted and an increase was observed in 5-hydroxyindole acetic acid levels. The insects became hypoactive after reserpine injection.

Key Words: 5-hydroxytryptamine (serotonin), 5-hydroxyindole acetic acid, Tryptophan

Introduction

An important biogenic monoamine, 5-hydroxytryptamine (5-HT), commonly known as serotonin, has been proposed to act as a neurotransmitter in the central nervous system (CNS) of most of the vertebrates and invertebrates (Brodie & Shore 1957). Serotonin has vasoconstrictor properties (Martins et al. 1978) and may be a growth-promoting or regulatory factor for embryonic brain (Ahmad & Zamenhof 1978). It acts as a mediator in several kinds of behaviour (Brody 1970, Ellison & Bresler 1974 and Lorens 1978), and is known to be involved in mental retardation (Oikawa et al. 1978) and mood changes (Mandell & Knapp 1977).

Serotonin has been recognized as a neurohormone in the insect nervous system (Welsh 1954, 1957 and Colhoun 1963). It has also been known to be involved in the rhythmic regulation of the heart muscle cells of the insects (Rozsa et al. 1973).

P-chlorophenylalanine (PCPA), a Tryptophan hydroxylase-inhibitor, was found to deplete brain serotonin levels in the cockroach by inhibiting specifically this enzyme (Pandey & Habibulla 1980 and Pandey et al. 1981). Also the levels of brain serotonin were found to be closely related with the activity of the insect.

The present investigation is undertaken to study the effect of reserpine, a serotonin-storage blocker, on brain serotonin metabolism along with its behavioural effects.

Materials and Methods

Adult cockroaches, *Periplaneta americana* (body wt. approx. 1 g) were used. In the laboratory these cockroaches were housed in the cages maintained at a constant temperature (25 ± 1°C). Food and water were supplied *ad libitum*.

Stock solution (1.0 mg/ml) of reserpine (Sigma Chemical Co., USA) was injected in dosages of 0.02 ml (single dose) and 0.04 ml (double dose) into the hemolymph of the cockroach with the help of a Hamilton microlitre syringe.

After drug administration at various time intervals the cockroach brains were taken out and used for the experiments. The levels of brain Trp, the precursor amino acid of 5-HT, protein contents, 5-HT and 5-HIAA were estimated quantiatively as described earlier (Pandey & Habibulla 1980, 1981). The brain protein contents were measured by the Folin-phenol method of Lowry et al. (1951). The levels of 5-HT and 5-HIAA were estimated by the improved fluorometric method of Fischer and Aprison (1972). The contents of Trp, 5-HT and 5-HIAA were expressed as mg/g-protein and the protein contents as mg/g wet-weight of brain.

Results

After single dose treatment (20 μg/g) of reserpine, brain Tryptophan and protein levels had shown oppposite quantitative tendencies (Pandey & Habibulla 1980). Similar type of negative correlation between brain Tryptophan and protein contents has been observed after reserpine double dose (40 μg/g) treatment.

Reserpine effect on brain 5-HT and 5-HIAA levels

Single dose of reserpine (20 μg/g) caused significant alterations in brain 5-HT and 5-HIAA levels (figure 1). The highest 5-HT levels (0.73 mg/g protein) was

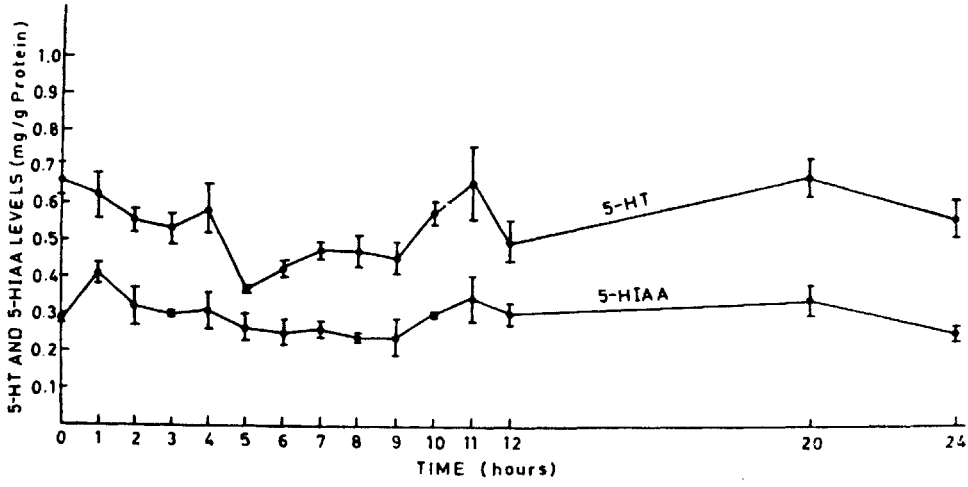

Figure 1 Brain 5-HT and 5-HIAA levels in reserpine-treated cockroaches (dose 20 μg/g). The values are expressed as mean ± S. E. of three determinations. Brain 5-HT levels remained depleted up to 24 hr after reserpine treatment and 5-HIAA levels increased after treatment

found before injection in the normal brain and after reserpine administration brain 5-HT levels started decreasing and at 5 hr of treatment significantly low level of 5-HT ($p < 0.01$) was found when compared to normal level. After 5 hr, it increased but remained at a level lower than the normal up to 24 hr of treatment. The level of 5-HIAA increased in the beginning and highest level was obtained at 1 hr of treatment, after that it decreased and the lowest 5-HIAA levels were found at 8 and 9 hrs.

The insects treated with double dose (40 μg/g) of reserpine showed a slight depletion in 5-HT level immediately after injection. The highest value was obtained at 2 hrs of treatment. Brain 5-HT level depleted significantly ($p < 0.01$) at 3 hr and at this time the lowest level was found. After this the brain 5-HT level again increased with fluctuations but could not reach to the normal level up to 24 hr of injection. Brain 5-HIAA level increased after reserpine injection and the highest level was obtained at 2 hr. After this it fluctuated and remained generally at the level higher than the normal up to 24 hr (figure 2).

Observations on behaviour

After single dose (20 μg/g) treatment of reserpine the insects were found to be hypoactive at 3 hr. The response to external stimuli was missing and they were unable to move. The control of balance was lost. Trp/protein ratio increased at the 3 hr and after this period Trp/protein ratio was either equal to the normal or higher than the normal value up to 24 hr. Immediately after reserpine injection up to 24 hr 5-HT/5-HIAA ratio was found to be lower than the normal. Normal Trp/5-HT ratio was the lowest

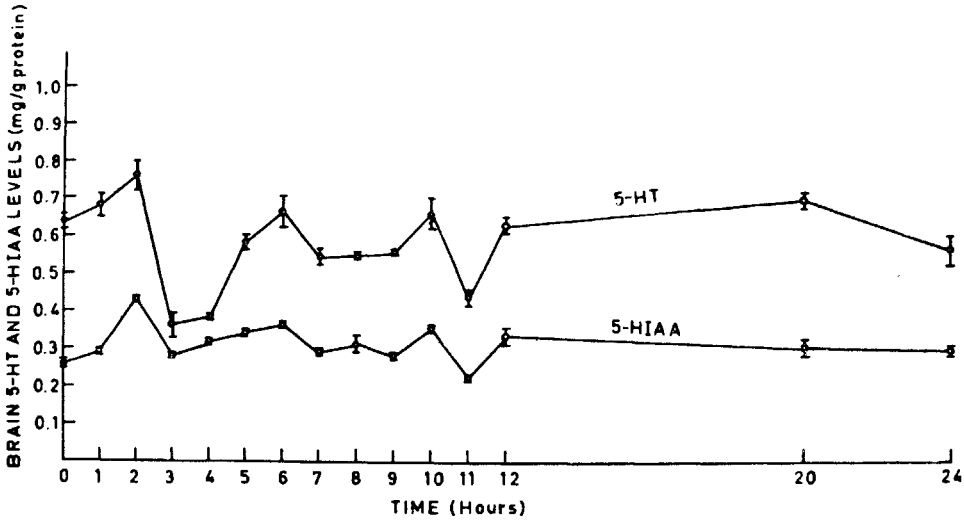

Figure 2 Brain 5-HT and 5-HIAA levels in reserpine treated cockroach (dose 40 μg/g). The values are expressed as mean $\pm$ S. E. of three experiments. The levels of both brain 5-HT and 5-HIAA increased up to 2 hours and after that 5-HT levels depleted to a value lower than the normal and remained depleted onwards while 5-HIAA levels remained above the normal level at most of the time periods studied

Table 1 *Changes in Trp/Protein, 5-HT/5-HIAA, Trp/5-HT and Trp/5-HIAA ratios at different time intervals after reserpine single dose (20μg/g) administration*

Time duration post injection (hr)	Trp/protein* ratio	5-HT/5-HIAA ratio	Trp/5-HT ratio	Trp/5-HIAA ratio
Normal	0.34	2.53	68.31	173.21
0	0.42	2.35	70.45	166.07
1	0.86	1.51	106.45	160.47
2	0.52	1.72	85.63	147.18
3	0.50	1.77	95.66	169.00
4	0.65	1.87	105.86	198.06
5	0.40	1.42	134.05	190.77
6	0.41	1.68	115.24	193.60
7	0.45	1.81	106.81	193.07
8	0.40	1.96	97.02	190.00
9	0.60	1.87	149.55	280.42
10	0.76	1.90	136.66	259.67
11	1.02	1.91	128.31	245.29
12	1.84	1.63	167.75	274.00
20	1.26	1.97	163.88	322.94
24	1.72	2.24	151.61	339.60

* The values of Trp/protein ratio have been taken from our published data (Pandey & Habibulla 1980)

and at all time intervals studied it was always found to be higher than the normal (table 1). Up to 24 hr of single dose treatment the insect remained dull and after 48 hr they died. Trp/-5-HIAA ratio also increased after treatment.

In double dose (40 μg/g) experiments the insects became hypoactive after 2 hr of injection. Up to 24 hr they remained sluggish and were not responding to external stimuli, like touch. Highest Trp/protein ratio was obtained at 2 hr. Normal 5-HT/5-HIAA ratio was the highest and after reserpine double dose treatment, at all time periods tested, the ratio was found to be below normal (table 2). Trp/5-HT ratio was found to be the highest at 2 hours. Normal Trp/5-HIAA ratio was the highest and after treatment it decreased.

These results indicate that the insects became hypoactive after reserpine administration and during this hypoactive stage Trp/protein, Trp/5-HT and Trp/5-HIAA ratios increased and 5-HT/5-HIAA ratio decreased. The above mentioned ratios showed when tryptophan level in the brain was high and 5-HT content was low, the insects became hypoactive.

Table 2 *Changes in Trp/protein, 5-HT/5-HIAA, Trp/5-HT and Trp/5-HIAA ratios at different time intervals after reserpine double dose (40μg/g) administration*

Time duration post injection (hr)	Trp/protein* ratio	5-HT/5-HIAA ratio	Trp/5-HT ratio	Trp/5-HIAA ratio
Normal	0.39	2.56	63.62	162.59
0	0.12	2.46	20.59	50.59
1	0.18	2.34	27.32	64.07
2	0.78	1.77	72.92	128.88
3	0.22	1.29	65.61	84.36
4	0.15	1.19	40.34	47.91
5	0.27	1.71	40.14	68.47
6	0.34	1.83	41.62	76.31
7	0.25	1.86	47.54	88.47
8	0.34	1.74	57.61	100.35
9	0.15	1.96	27.49	54.00
10	0.37	1.86	47.68	88.54
11	0.14	1.95	42.65	83.36
12	0.32	1.88	39.66	74.63
20	0.28	2.30	34.62	79.63
24	0.25	1.93	41.18	79.52

* The values of Trp/protein ratios have been taken from our unpublished data

Discussion

The biogenic monoamines are known to occur in the CNS of the vertebrates and act as neurotransmitters (Carlsson 1969, Hebb 1970 and Krnjevic 1974). In the insects also biogenic monoamines are mainly accumulated in CNS (Welsh & Moorhead 1960, Klemm 1968, 1971, Klemm & Axelsson 1973 and Osborne & Neuhoff 1974) and act as neurohormones Biogenic monoamines in the insect nervous system are located intraneuronally, and normally concentrated in the terminal regions. There is evidence that as in the case of vertebrate neurons, these are stored in the vasicles in the insects also (Frontali & Mancini 1970 and Gearsch et al. 1974).

Reserpine is known to be a 5-HT storage inhibitor in the mammals and it affects intracellular storage mechanisms (Brodie et al. 1957 and Uzan et al. 1979). Reserpine blocks vesicle amine uptake pump thus depleting 5-HT stores in mammalian nervous system (Hiripi & Salanki 1973 and Weiner 1974). It is also reported that reserpine treatment leads to a decrease in 5-HT levels and an increase in 5-HIAA levels (Brodie et al. 1957, Bulat 1974 and Gessa & Tagliamonte 1974). Similar type of reserpine effect was observed during the present studies.

After single dose treatment of reserpine brain 5-HT levels were depleted. The level of 5-HT in the normal untreated brain was the highest and after reserpine injection up to 24 hours 5-HT levels remained depleted. Brain 5-HIAA levels increased after treatment. After 2 hours of reserpine DD treatment up to 24 hours brain 5-HT levels remained lower than the normal and 5-HIAA levels increased and at most of the time periods studied after injection brain 5-HIAA level was found to be either equal to the normal or higher than the normal. Due to presence of reserpine, 5-HT could not be stored and most of it was deaminated by monoamine oxidase (MAO) enzyme, thus increasing brain 5-HIAA levels. The inhibition of 5-HT storage acts as a positive feedback for 5-HT biosynthesis and there may be a rapid shift of Trp to 5 HT synthesis (Pandey & Habibulla 1980). Increased activity of tryptophan-hydroxylase enzyme has been demonstrated in the rat after reserpine administration (Zivkovic et al. 1973). Thus 5-HT synthesis is increased after reserpine treatment but simultaneously degradation is also enhanced due to storage inhibition, leading to elevated brain 5-HIAA levels.

Reserpine is known to produce behavioural effects in the mammals (Warboff et al. 1961, Warboff & Dembicki 1962 and Jewe & Norton 1966). In the case of the insects not much is known about the behavioural effects of reserpine. A reduction in locomotory activity in the house cricket *Acheta-domesticus*, follows after injection of reserpine and lysergic acid diethyl-amide (LSD) into the hemocoel. After 5-HT injection, however, the activity increases (Cymborowski 1970). During the present studies reserpine treated cockroaches became hypoactive and least responsive to the external stimuli. During the hypoactive stage brain 5-HT levels were depleted. After 48 hr of reserpine injection the cockroaches died. Reserpine is known to reduce the activity of the insects after administration and after repeated applications the insects die (Klemm 1976). The present results also show that depleted brain 5-HT levels lead to the reduced activity in the insects.

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