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The Influence of Serotonin and p-Chlorophenylalanine on Locomotor Activity of *Drosophila melanogaster*

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KAMYSHEV, N. G., G. P. SMIRNOVA, E. V. SAVVATEEVA, A. V. MEDVEDEVA AND V. V. PONOMARENKO. *The influence of serotonin and p-chlorophenylalanine on locomotor activity of* Drosophila melanogaster. PHARMACOL BIOCHEM BEHAV **18**(5) 677–681, 1983.—Two hours after injection of serotonin into 3-day-old virgin females of *Drosophila melanogaster*, a significant dose-dependent increase in locomotor activity was observed. Since this stimulating effect can be produced either by serotonin or by some of its derivatives that might have formed during these two hours, the fate of injected [^{3}H]-serotonin in the organism of *Drosophila* was traced by means of thin layer chromatography. The only metabolite found appeared to be N-acetylserotonin. Its formation was rather intense immediately after injection of [^{3}H]-serotonin, and its excretion was rapid enough to make it undetectable at the end of the second hour, when more than 50% of the injected [^{3}H]-serotonin still remained and was being absorbed by tissues. Thus, the increase in locomotor activity observed two hours after injection should be wholly attributed to serotonin, while the rather long latency might be related to some effect of N-acetylserotonin. p-Chlorophenylalanine, an inhibitor of tryptophan-5-hydroxylase, both injected or administered with food, led to increases in locomotor activity level and to some decreases in serotonin content in the heads of flies. The effect of p-chlorophenylalanine on locomotor activity in *Drosophila* seems to be non-specific in relation to serotoninergic mechanisms of its regulation.

Serotonin p-Chlorophenylalanine *Drosophila* Locomotor activity

THE involvement of the serotoninergic system in the regulation of a level of locomotor activity in *Drosophila* has been repeatedly discussed [8, 19, 21]. However, there is yet no direct evidence about the influence of pharmacologically applied exogenous serotonin or specific drugs, altering its endogenous content, on the fruit fly activity.

The work reported in the present paper was designed to study the effects of injected serotonin and p-chlorophenylalanine (PCPA), either injected or administered with food, on locomotor activity in *Drosophila*. PCPA, an inhibitor of tryptophan-5-hydroxylase [12,14], which is known to reduce the serotonin level in mammalian brain, was reported to reduce the serotonin synthesis in insects as well, namely in the cockroach, although in this case its effect is probably not so pronounced as in mammals [18].

METHOD

Subjects

Virgin females of the Canton-Special (CS) wild type outbred strain was used. Flies were reared on yeast-raisin medium in rooms kept on a cycle of 12 hr of light and 12 hr of dark. The maintenance procedures and all the experiments were undertaken at 25°C.

Injections

All drugs were dissolved in Ephrussi-Beadle solution [6] and were injected into the ventral part of the fly's abdomen using a half-automatic injecting apparatus (to be described elsewhere). The volume of the solution injected was 0.2 μl. Flies injected with Ephrussi-Beadle solution served as a control variant.

For immobilizing a fly two approaches were used. In the initial experiments (injections of serotonin) flies were exposed to carbon dioxide for 30 sec and it took them approximately 1 min to recover from the anesthesia. In the subsequent experiments (injections of PCPA and [^{3}H]-serotonin) cooling was used since it allowed the immobilization for a rather long period without affecting behavioral performance. Cooling was performed in the apparatus described by Trout [20]. After injection, flies were transferred into vials with fresh medium, 10 flies per vial.

Administration of PCPA

Standard yeast-raisin medium was prepared in a double volume of water without agar and centrifuged upon cooling. A portion of supernatant was used to dissolve PCPA (150 μg per ml of supernatant). Aliquotes of the solution were kept

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frozen at −4°C. Immediately before the beginning of feeding on PCPA, 3 paper filters were put into the empty vials, and 0.4 ml of fresh-thawed solution were applied to them. In control vials 0.4 ml of supernatant without PCPA were applied.

Flies that emerged during the 4 hr period after 8 a.m. were collected and after a 2 hr period in empty vials (without medium) were transferred into vials with PCPA; 10 virgin females per vial. After daily measurements of locomotor activity flies were transferred into fresh vials with PCPA.

Determinations of serotonin content in the heads of the flies, which fed on PCPA for 5 days, were performed according to Curzon and Green [3].

Measurements of Locomotor Activity (LA)

A set of vials with flies was placed in a row in front of a white screen, and illumination of a 150 lux was maintained. In experiments with PCPA administration, the number of moving flies in each vial was scored each 5 min during a single 30 min test, and a mean percentage of moving flies was calculated for each vial. The test was performed daily at noontime. During continuous observation of LA (in experiments with serotonin injection) a mean percentage of moving flies was calculated for each vial for each half-hour after injection. The data were analysed with the use of Student's *t* criterion.

Biochemical Tests

Labelled serotonin (^{3}H-[G]-5-hydroxytryptamine creatinine sulfate, Amersham) with specific activity 1 mCi/ml (approximately 3 ng of serotonin-base in 0.2 μl of Ephrussi-Beadle solution) was injected to 3-day-old females, which were frozen in liquid nitrogen at different times after the injection. Flies were homogenized for 1 min at 0°C in a microhomogenizer with a motor-driven teflon pestle (10,000 rpm) in 50 μl of 0.05 M $NaHCO_3$ (pH=10). Homogenate was centrifuged at 8,000 rpm for 15 min at 0°C. 25 μl of supernatant were transferred to a microtube and 25 μl of ice-cold acetone were added. After 30 min at −4°C the mixture was centrifuged for 15 min at 0°C (8,000 rpm), and 1 μl of supernatant was applied to a Silufol TLC plate (Silufol, Kavalier). Authentic serotonin creatinine-sulfate (Reanal), 5-hydroxyindoleacetic acid (Serva), melatonin (Serva) and N-acetylserotonin (serotonin creatinine-sulfate acetylated with acetic anhydride by a method similar to that described by Evans and Fox [7] for acetylation of tryptamine) were applied to the same spot.

Chromatography was performed in an ascending system. Stripes 15 mm wide corresponding to separated spots were cut from the TLC plate and divided into equal parts (Fig. 2). These were placed into scintillation vial and counted in a liquid scintillation counter ISOCAP-300 upon adding a cocktail (p-Terphenyl+POPOP+toluene). To check quenching effects a calibration curve was prepared as follows. An extract of flies not injected with [^{3}H]-serotonin was chromatographed in presense of authentic substances listed above. Before counting, equal amounts of labelled serotonin were applied to each excised part of the TLC plate. A coefficient for correction of quenching for each part of chromatogram was calculated as a quotient from division of the largest cpm (counts per minute) of the calibration curve by cpm of the corresponding part of the chromatogram. To control the radiochemical purity of [^{3}H]-serotonin and the quality of separation in a given chromatographic system the original solution of [^{3}H]-serotonin was subjected to chromatography as well.

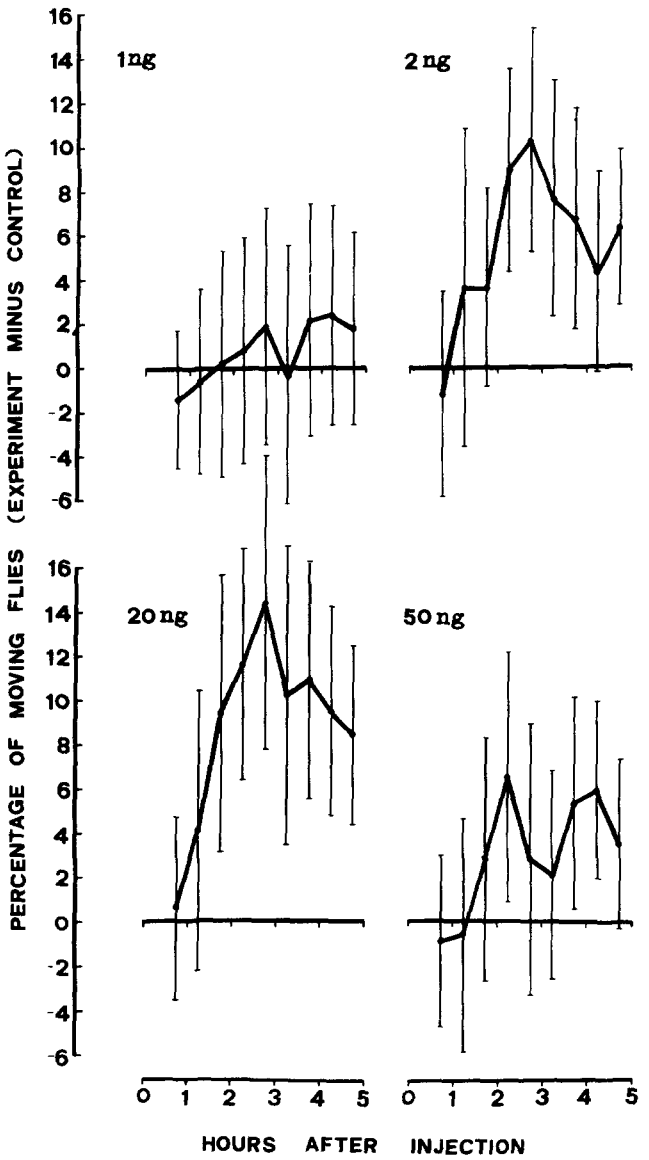

FIG. 1. Change of locomotor activity after injections of various doses of serotonin. Vertical bars: 95% confidence intervals.

RESULTS

The Influence of the Injected Serotonin on LA of Drosophila

The dynamics of LA after injections of serotonin to 3-day-old females is shown in Fig. 1. The data plotted are the differences between the corresponding percentage of moving flies in the experimental (injections of a certain dosage of serotonin) and in the control (injections of Ephrussi-Beadle solution) groups. The level of LA increases by the second hour after injection, and the effect produced by serotonin depends on its dosage (the dosages are given in recalculation for serotonin-base). One ng of serotonin per fly does not

TABLE 1

EFFECT OF INJECTED p-CHLOROPHENYLALANINE (300 ng PER FLY) ON LOCOMOTOR ACTIVITY OF FEMALES OF CS STRAIN

Age (days)	Time after injection (days)	Percentage of moving flies (Mean±SE) Control	PCPA	p_{Ho}*
3	1	20.6 ± 1.68	40.2 ± 1.26	<0.001
4	2	6.7 ± 0.92	11.3 ± 1.48	<0.05
5	3	7.3 ± 0.98	5.4 ± 0.55	>0.05
6	4	10.7 ± 1.20	11.2 ± 1.39	>0.05
7	5	7.5 ± 1.35	4.6 ± 0.61	>0.05

*Student's t criterion

produce any significant alteration of LA. The doses of 2 ng and 20 ng per fly are likely to be in the optimal range, since the effects produced are maximal and do not essentially differ from each other. The subsequent increase of dose up to 50 ng per fly leads to a decline in the stimulating effect of serotonin on LA, probably, due to non-specific toxic effect of large amounts of injected serotonin.

The dynamics of LA in *Drosophila* following injection of serotonin prompts this question: is the increase in LA observed by the second hour after injection a result of the effect of serotonin itself, or is it caused by any of its metabolites, which could be produced in the organism of *Drosophila* during these two hours? To answer the question, a study of possible consequences of in vivo inhibition of serotonin synthesis and of the fate of injected [^{3}H]-serotonin in *Drosophila* was undertaken.

The Influence of Injected or Administered with Food PCPA on LA

The dynamics of LA following the injection of PCPA is shown in Table 1 and, following its administration with food, in Table 2. Injections of PCPA lead to a two-fold increase in LA over the control level the next day. By the second day LA declines and subsequently keeps fluctuating near the control level. The decrease in LA which is observed in both experimental and control groups from the second day on is probably a result of an injection injury.

Feeding on media supplemented with PCPA also leads to the increase in LA. As shown in Table 2, 5-day-long feeding on PCPA results in some decrease in the serotonin content in the heads of the flies.

The Fate of Injected [^{3}H]-Serotonin in Organism of Drosophila

The results of chromatography of serotonin and its derivatives in two systems are shown in Fig. 2. Neither of the chromatographic systems used allowed simultaneous separation of all the substances. However, the radioactivites measured in 5-hydroxyindole-3-acetic acid spot (in the first system) and in melatonin spot (in the second system) were at the background level at any time after injection of [^{3}H]-serotonin. The only measurable radioactivity was associated with serotonin as well as with N-acetylserotonin.

The dynamics of conversion of injected serotonin into N-acetylserotonin and their excretion are shown in Fig. 3 (results of 4 unrelated experiments). It appears that approximately 30% of injected serotonin is converted to N-acetylserotonin almost immediately. Later on, production of N-acetylserotonin is slowed down or stopped altogehter, while the rate of its excretion exceeds that of serotonin. It seems likely, that the latter, being taken up by tissues of the fly, is utilized by them rather slowly.

DISCUSSION

Acetylation of Serotonin

The absence of any measurable conversion of [^{3}H]-serotonin into 5-hydroxyindole-3-acetic acid is in good agreement with the findings that in many insects the enzyme

TABLE 2

EFFECT OF FED PCPA (50 μg PER ml OF MEDIA) ON LOCOMOTOR ACTIVITY OF FEMALES OF CS STRAIN

Days from the beginning of feeding	Percentage of moving flies (Mean±SE) First replication Control	Experiment	Second replication Control	Experiment
1	32.0 ± 2.45*	40.7 ± 1.81	30.7 ± 2.62	36.3 ± 2.60
2	45.4 ± 2.15	45.6 ± 1.90	33.6 ± 1.96†	44.9 ± 2.15
3	27.1 ± 1.66*	34.3 ± 1.61	31.3 ± 1.88†	45.2 ± 2.48
4	25.9 ± 1.63†	36.4 ± 1.04	28.9 ± 1.98	34.6 ± 2.81
5	24.2 ± 2.16†	37.4 ± 1.95	24.2 ± 1.29†	38.8 ± 3.00
Serotonin content in heads of flies (μg/g)	0.310	0.224	0.301	0.244

The differences between experiment and control are significant: *p_{Ho}<0.01, †p_{Ho}<0.001 (Student's t criterion).

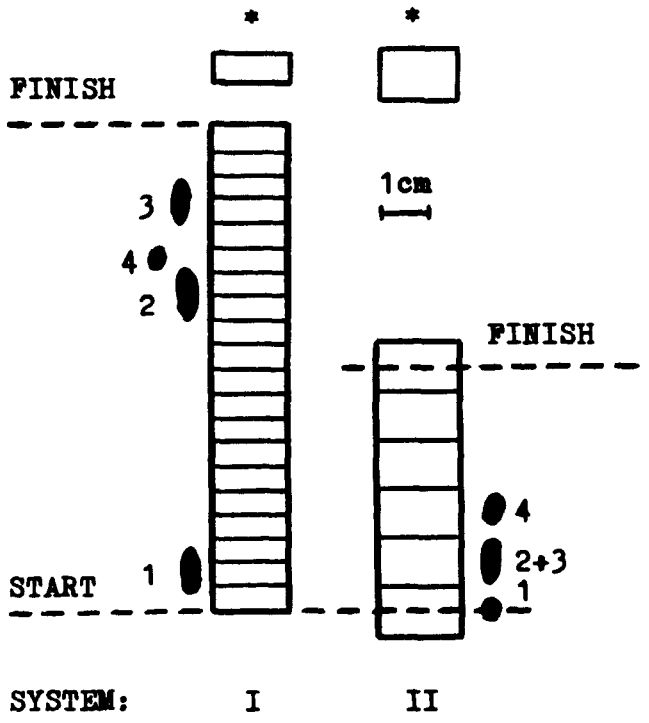

FIG. 2. Results of separation of serotonin and its metabolites in two chromatographic systems. Designations. Chromatographic system: I—n-butanol: chloroform: acetic acid (12:3:5); II—chloroform: ethanol: acetic acid (80:15:5). Spots: 1—serotonin; 2—N-acetylserotonin; 3—5-hydroxyindole-3-acetic acid; 4—melatonin. *—Excised parts of chromatographic plate.

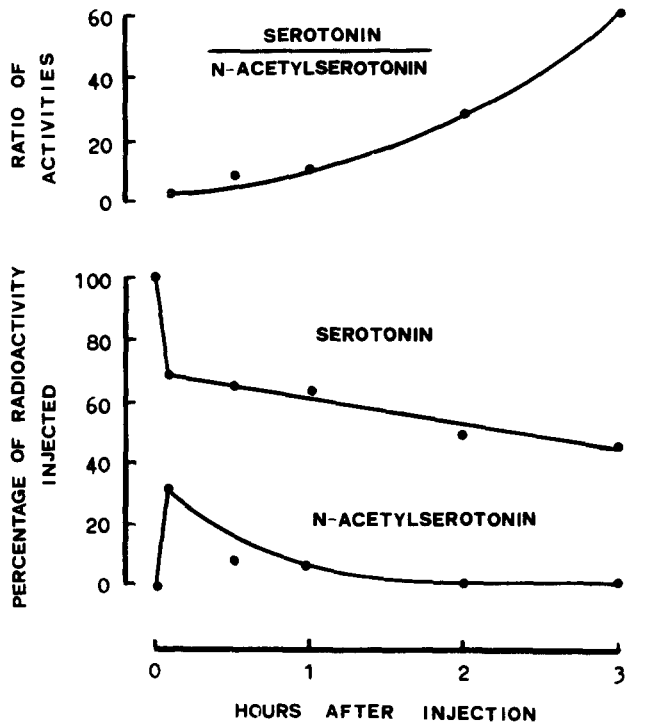

FIG. 3. Dynamics of conversion of injected serotonin to N-acetylserotonin and of their excretion.

monoaminoxidase has an extremely low activity, if any [1, 2, 5, 7, 18]. The data obtained on acetylation of injected serotonin in the organism of *Drosophila* are also in line with the results of Dewhurst and co-workers [5]. The authors have demonstrated that in brain and thoracic ganglion of *Drosophila* N-acetyltransferase has a marked activity, though it is somewhat lower than in extracts of whole flies. The presence of the enzyme in tissues other than nervous is considered by the authors to be necessary for producing N-acetyldopamine, a sclerotizing substance of cuticle [13], while in the nervous system of *Drosophila* the enzyme is likely to be involved in inactivation of biogenic amines by means of their acetylation.

The outburst of N-acetylserotonin formation observed in our experiments immediately after injection of [^{3}H]-serotonin and the subsequent sharp drop of the reaction indicate that the portion of serotonin that has been absorbed by tissues is less subject to acetylation than that of hemolymph. Thus, the conversion of approximately 30% of the serotonin shortly after its injection into N-acetylserotonin is probably performed by a peripheral N-acetyltransferase and, therefore, does not contribute to the nervous system functions.

The Influence of Injected Serotonin on LA

By the second hour after injection almost half of the administered amount of serotonin still remains in the tissues of a fly, while the produced N-acetylserotonin has already been excreted. Since other serotonin metabolites are not detectable, there is no doubt that the increase in LA observed at this time results from an increased level of serotonin.

The similarity of time-dependent alterations both of LA and of level of produced N-acetylserotonin allows us to suggest a causal relation between them. It is possible that an excess of N-acetylserotonin at the first 1.5–2 hr after injection prevents the immediate manifestation of the stimulating effect of serotonin on LA. The excess of N-acetylserotonin could either lead to a toxicosis, which in its turn could depress LA, or exert a more specific effect on LA.

The stimulating effect of serotonin on motor activity has been demonstrated in other insects, namely in crickets [4] and in night moths [11]. In ants, however, injected serotonin causes a decrease of motor activity [16]. Our results are in agreement with those of Fowler and co-workers [8], who have demonstrated positive correlation between LA and serotonin content in the circadian rhythm of *Drosophila*.

The Influence of PCPA on Locomotor Activity

The results of experiments on administration of PCPA and on injections of serotonin are in obvious contradiction. The tracing of the fate of injected serotonin, however, left no doubt that increases in LA following the injection is brought about by increased serotonin content due to the portion being absorbed by the fly's tissues. Thus, administration of PCPA, causing a drop in serotonin content, should lead to a decline in LA level. However, the observed effect of PCPA is opposite to the expected one. This might have been a result of its non-specific action on serotoninergic mechanisms of regulation of LA in *Drosophila*. Indeed, in mam-

mals [9] PCPA inhibits not only tryptophan-5-hydroxylase but phenylalanine hydroxylase as well [14]. Besides, it leads to short-term changes in content of brain catecholamines [15] and blocks amino acid transport across the blood-brain barrier [10]. Though PCPA exerts a minimal effect on catecholamine content in mammals [12, 14, 15], this might not be the case in insects. The fact that catecholamines are involved in regulation of LA in *Drosophila* has been demonstrated [19,21]. Furthermore, reserpine administered with food increases spontaneous LA in *Drosophila* measured by a time-sampling method [17].

The data reported here, thus, provide evidence regarding the stimulating effect of serotonin on LA in *Drosophila* and the non-specific effect of PCPA on LA in relation to serotoninergic mechanisms of activity regulation.

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