L-DOPA DECARBOXYLASE IN THE HAEMOCYTES OF DIPTERA

D.L.WHITEHEAD
Department of Zoology, University of Oxford, Oxford, England

Received 22 January 1970

1. Introduction

L-*p*-Tyrosine decarboxylase (EC.4.1.1.25) has recently been reported [1] in the haemocytes of an orthopteran *Periplaneta americana* (L).

The tanning hormone (bursicon) released from the terminal abdominal ganglion in cockroaches [2] appears to function by increasing the permeability of haemocytes to L-*p*-tyrosine [3] thus allowing the biosynthesis of *p*-tyramine and 3-hydroxytyramine (dopamine) to occur rapidly after ecdysis [1].

Seligman et al. [4] have proposed that in adult Diptera, bursicon promotes sclerotization of the cuticle by somehow potentiating the hydroxylation of tyrosine to form 3,4-dihydroxyphenylalanine (dopa). It was therefore of interest to check this theory in view of the knowledge that cockroach haemocytes contain two of the enzymes required for the biosynthesis of *N*-acetyldopamine from tyrosine.

2. Methods

Cultures of the fleshfly *Sarcophaga peregrina* Robineau-Desvoidy (Diptera:Sarcophagidae) were maintained as described by Ohtaki [5]. Puparia were transferred to a refrigerator (4°C) on the 7th day after pupariation to enable emergence of the imago to be timed to suit the investigation.

Haemolymph was collected in a glass capillary tube after pricking the ptilinum of flies at eclosion, or 30 min after and immediately mixed before weighing with a known volume (ca. 1/5 of total) of 0.4 M sucrose containing 1 mM EDTA (disodium salt). This mixture (called whole blood) was then used to study enzymes in intact haemocytes or it was frozen (called frozen blood) for subsequent experiments on the disrupted cells.

U-^{14}C-L-*p*-Tyrosine (475 mCi/mmole) in 2% ethanol and 2-^{14}C-L-3,4-dihydroxyphenylalanine (50 μCi/2.2 mg) dissolved in 0.08 M ascorbic acid, were supplied by the Radiochemical Centre, Amersham. After incubating these amino acids with blood *in vitro*, ^{14}C-labelled metabolites were separated and activity measured by electrophoresis on Whatman 3 mM paper (600 V/cm, 25 min, 100–150 mA) at pH 6.5 (pyridine/acetic acid/water = 25 : 1 : 225) followed by ascending paper chromatography in butan-1-ol/acetic acid/water, 4 : 1 : 1 (BAW) and ethanol/ammonia (0.880)/water, 18 : 1 : 1 (EAmW) [1]. A Beckman Liquid Scintillation Counter was used to assess the radioactivity present on 1 or 2 cm strips of the paper immersed in Liquifluor (New England Nuclear, Boston) mixed with sulphur-free toluene (48 ml/l).

Diluted whole or frozen blood was incubated with 2 μCi U-^{14}C-tyrosine or 2-^{14}C-dopa et 37°C. At intervals, 20 μl of reaction mixture were removed and added to 50 μl of 95% ethanol and 20 μl 0.1 M ascorbic acid to stop further enzyme action and oxidation.

3. Results

Frozen blood from *S. peregrina* unlike that of *P. americana* rapidly converts tyrosine not to tyramine [1] but to dopa (fig. 1). 30 min after eclosion, whole blood forms dopa from ^{14}C-tyrosine even more rapidly than frozen blood but because the dopa formed is decarboxylated to dopamine, it does not accumulate in the medium (figs. 2 and 3). Decarboxylation of dopa by

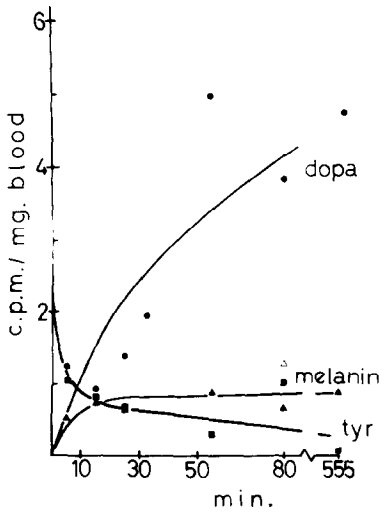

Fig. 1. Formation of dopa (•-cpm X 10^3/mg) and "melanin" (▲-cpm X 10^3/mg) from 0.212 nmoles (0.1 μCi) U-^{14}C-tyrosine (■-cpm X 10^4/mg) in aliquots of frozen *S. peregrina* adult haemolymph (16.4 mg) collected ½ hr after emergence and incubated at 37°C. Also shown is the dopamine (△-cpm X 10^3/mg) (separated by high voltage electrophoresis) formed if pyridoxal phosphate (2 μmoles) was added to the blood (50 mg) before incubating with tyrosine. All cpm values are corrected and expressed per mg of haemolymph.

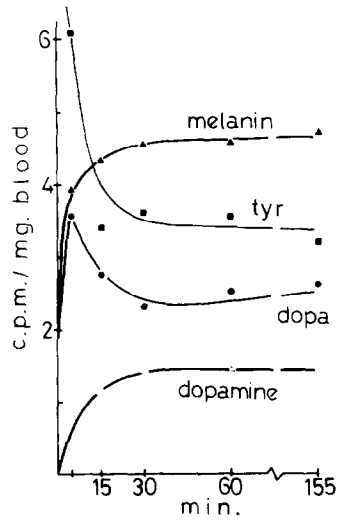

Fig. 2. Conversion of 0.1 μCi U-^{14}C-tyrosine (0.212 nmoles) (■-cpm X 10^3/mg) to dopa (•-cpm X 10^3/mg), dopamine (△-cpm X 10^3/mg) and "melanin" (▲-cpm X 10^3/mg) in min after incubating (37°C) with aliquots containing whole blood (7.25 mg) collected ½ hr after eclosion of the fly. Cpm values are corrected and expressed per mg of blood.

disrupted haemocytes in frozen blood could be limited by the availability of the co-enzyme which presumably diffuses out of the broken cells. When 2 μmoles of pyridoxal phosphate is added to the incubation mixture, ^{14}C-dopamine is formed apparently at the expense of dopa (fig. 1).

2-^{14}C-Dopa is decarboxylated very rapidly by whole blood obtained 30 min after emergence of *S. peregrina* but ^{14}C-dopamine does not accumulate in the medium (fig. 4). In whole blood it appears that dopa and dopamine are converted *in vitro* to an insoluble polymer which could be described broadly as "melanin". The amount of labelled product which is electrically neutral and is not resolved when chromatographed in BAW (see fig. 3) is shown in figs. 2 and 4.

The rate of "melanin" formation is faster in whole blood than in frozen blood when both are incubated with ^{14}C-tyrosine.

The rate of dopamine formation from tyrosine is ten times faster in blood (per mg) taken from a fly 30 min after emergence than in blood which is removed immediately after eclosion (fig. 3c).

4. Discussion

(a) Haemocytes in *in vitro* preparations of whole blood from flies after eclosion can synthesize dopa and dopamine from tyrosine (fig. 2).

(b) These *o*-diphenols do not accumulate *in vitro* (figs. 2 and 4) because they are probably oxidized to an insoluble polymer "melanin" by the same enzyme which hydroxylates tyrosine *in vivo* [6]. Natural wounding or artificial lysis of the cells activates this *o*-diphenol oxidase (EC.1.10.3.1) [7, 8], but in this case (fig. 1) dopamine formation is retarded unless the pyridoxal level is raised.

(c) The presence of dopa decarboxylase (EC 4.1.1. 26) in insect haemocytes has not been reported before.

(d) Although the hydroxylase and decarboxylase are capable of converting tyrosine to dopamine at eclosion, the rate of synthesis is not accelerated (fig. 5) until after the release of bursicon 20–30 min later [9, 10].

(e) The most likely explanation for the effect of bursicon is not that it activates the enzymes [1,4] but that it overcomes a barrier to tyrosine in the appropriate haemocytes [3].

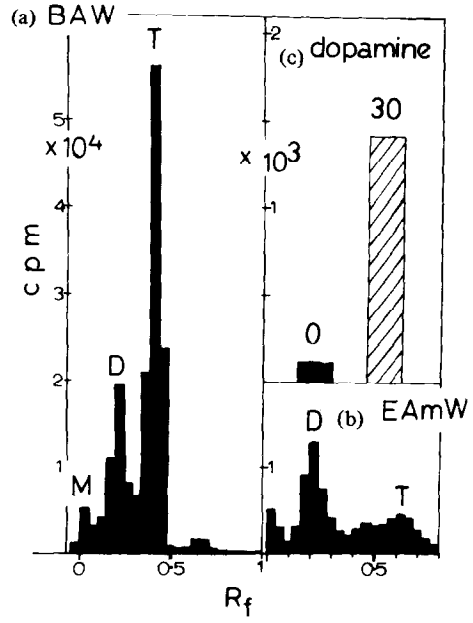

Fig. 3a. Ascending paper chromatographic separation (BAW) of the electrically neutral compounds (pH 6.5) contained in frozen fleshly blood (16.4 mg) incubated with 0.212 nmoles of U-^{14}C-tyrosine for 80 min. The ^{14}C label (cpm X 10^4) on 1 cm strips of the chromatogram was assessed using a liquid scintillation spectrometer.
Authentic R_fs: dopa, 0.25
tyrosine, 0.35
M = "melanin", D = dopa, T = tyrosine.

Fig. 3b. Separation of the dopa-containing zone D (R_f 0.2–0.3) shown in fig. 3a in EAmW (ascending paper chromatography run twice [1]) to measure and verify the presence of ^{14}C-dopa.
Authentic R_fs: dopa, 0.25
tyrosine, 0.60
D = dopa, T = tyrosine.

Fig. 3c. Histogram showing the amount of ^{14}C-dopamine (cpm X 10^3/mg blood) synthesized from 0.212 nmoles U-^{14}C-tyrosine (0.1 μCi) in 20 min at 37°C when incubated with blood collected either at emergence (B) of the fly or ½ hr after this (A).
A = after release of bursicon.
B = before release of bursicon.

The decarboxylase in fly haemocytes, unlike the cockroach enzyme [1], is not very active towards phenylalanine and tyrosine. This corroborates the findings of Sekeris [11] who prepared the enzyme from

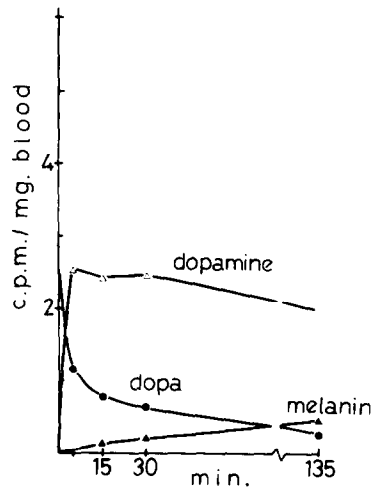

Fig. 4. Decarboxylation of 22.4 nmoles (0.1 μCi) of 2-^{14}C-dopa (•-cpm X 10^4/mg) to form dopamine (△-cpm X 10^3/mg) and their oxidation to give "melanin" (▲-cpm X 10^4/mg) by aliquots (10.7 mg) of whole *S. peregrina* imago haemolymph withdrawn ½ hr after emergence and incubated (37°C) for up to 135 min.

whole blowfly (*Calliphora erythrocephala* Meigen) larvae.

The enzyme was then thought to occur only in epidermal cells [12]. Confusion could arise because the epidermis cannot easily be freed from adhering haemocytes. These cells, which are known to hydroxylate tyrosine and oxidize dopa when lysed [7, 13] are thought to play an important but as yet unspecific role in the tanning of cuticle. Perhaps these are the cells, adhering to the epidermis soon after ecdysis, which synthesize dopamine once bursicon is released.

Crossley [7] believes that ecdysone controls the development of some haemocytes (the phagocytes) in *C. erythrocephala* larvae. It is therefore tempting, even before more evidence is available, to suggest that the development of the haemocytes containing tyrosine hydroxylase and/or dopa decarboxylase is also stimulated by this morphogenic steroid hormone. Moreover, this would fit the data obtained by Karlson [14] which he interpreted to mean that synthesis of dopa decarboxylase in epidermal cells is induced by ecdysone.

Acknowledgements

I thank Drs. R.Offord and E.A.Newsholme for the use of their equipment and Drs. P.C.J.Brunet, R.R. Mills and Professor J.W.S.Pringle, F.R.S., for their encouragement.

References

[1] D.L.Whitehead, Nature 224 (1969) 721.
[2] R.R.Mills, R.B.Marthur and A.A.Guerra, J. Insect Physiol. 11 (1965) 1047.
[3] R.R.Mills and D.L.Whitehead, J. Insect Physiol. 16 (1970) in press.
[4] I.M.Seligman, S.Friedman and G.S.Fraenkel, J. Insect Physiol. 15 (1969) 553.
[5] T.Ohtaki, Japan. J. Med. Sci. Biol. 19 (1966) 97.
[6] I.M.Seligman, S.Friedman and G.S.Fraenkel, J. Insect Physiol. 15 (1969) 1085.
[7] A.C.Crossley, J. Exptl. Zool. 157 (1964) 375.
[8] J.Lai-Fook, J. Insect Physiol. 12 (1966) 195.
[9] C.B.Cottrell, J. Exptl. Biol. 39 (1962) 395.
[10] G.S.Fraenkel and C.Hsiao, J. Insect Physiol. 11 (1965) 513.
[11] C.E.Sekeris, Z. Physiol. Chem. 332 (1963) 70.
[12] C.E.Sekeris, Mechanisms of hormone action, ed. P. Karlson (Georg Thieme, Stuttgart, 1965) 149.
[13] J.C.Jones, Am. Zoologist 2 (1962) 209.
[14] P.Karlson, J. Cellular Comp. Physiol. 66 (1965) 69.