J. Exp. Biol. (1963), **40**, 343–350
Printed in Great Britain

THE POSSIBLE INVOLVEMENT OF AN AMINO ACID DECARBOXYLASE IN THE STIMULATION OF THE PERICARDIAL CELLS OF *PERIPLANETA* BY THE CORPUS CARDIACUM

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(*Received* 5 *February* 1963)

INTRODUCTION

The corpus cardiacum of *Periplaneta* contains a material which increases the amplitude and frequency of contraction of the muscles of the heart, gut, and Malpighian tubules (Cameron, 1953). The hormone from the corpus cardiacum does not exert its effect directly on the muscles of the heart, but stimulates the pericardial cells to produce an amine from an inactive precursor (Davey, 1961*a*, *b*). Among vertebrates the various biogenic amines are normally produced from their analogous amino acids by the action of the appropriate decarboxylase. Thus, serotonin is produced by the action of 5-OH tryptophane decarboxylase upon 5-OH tryptophane. The present paper examines the possibility that the mode of action of the hormone from the corpus cardiacum on the pericardial cells might involve a decarboxylase.

MATERIALS AND METHODS

Most of the methods used in this study have been described in earlier papers. All of the chemicals used were obtained from L. Light, Colnebrook, Essex, England, with the exception of the 2-bromolysergic acid diethylamide bitartrate which was obtained from Sandoz Pharmaceuticals, New Jersey, U.S.A.

EXPERIMENTS AND RESULTS

(1) *The effect of inhibitors of amino acid decarboxylases on the response of the heart to the hormone from the corpus cardiacum*

The inhibitors tested were semicarbazide and isonicotinic acid hydrazide which are known to inhibit amino acid decarboxylases by virtue of their ability to interfere with the combination between the amino acid and phosphopyridoxal, the co-factor for the decarboxylase.

Hormone preparations were made by grinding up corpora cardiaca in Ringer solution. The concentration of hormone is expressed in terms of the number of corpora cardiaca present in 100 ml., i.e. unit concentration is 1 corpus cardiacum present in 100 ml. of Ringer in the bath bathing the heart preparation. The percentage increase in the rate of beating of the isolated heart was determined for several concentrations of hormone, and the percentage increase in rate was plotted against the logarithm of the

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concentration of the hormone, giving an approximately linear relation. The preparation was washed free of hormone with Ringer, after which one of the inhibitors was added to the bath and the relation between percentage increase in rate and concentration of hormone was re-determined. From these relations the percentage increases in rate, with and without inhibitor, were calculated for a hormone concentration of 5 units, and are to be found in Tables 1 and 2.

Table 1. *The percentage increase in rate of beating of isolated hearts resulting from a concentration of five pairs of corpora cardiaca per* 100 *ml. applied before and after the addition of semicarbazide hydrochloride*

Heart no.	Concentration of semicarbazide	Percentage increase: Before inhibition	Percentage increase: After inhibition
1	10^{-3}M	35	0
2	10^{-3}M	39	0
3	10^{-4}M	28	18
4	10^{-4}M	40	28

Table 2. *The percentage increase in rate of beating of isolated hearts resulting from a concentration of five pairs of corpora cardiaca per* 100 *ml. applied before and after the addition of isonicotinic acid hydrazide*

Heart no.	Concentration of isonicotinic acid hydrazide	Percentage increase: Before inhibition	Percentage increase: After inhibition
1	10^{-3}M	34	19
2	10^{-3}M	42	33
3	2×10^{-3}M	49	30

Table 3. *The percentage increase in rate of beating of isolated hearts in Ringer containing semicarbazide hydrochloride brought about by the addition of breis of corpora cardiaca followed by the further addition of phosphopyridoxal*

Heart no.	Concentration of semicarbazide	Concentration of hormone (pair/100 ml.)	% increase	Concentration of phosphopyridoxal	% increase
1	5×10^{-4}M	2·0	39	10^{-4}M	56
2	5×10^{-4}M	2·0	27	10^{-4}M	40
3	10^{-3}M	1·5	3	10^{-4}M	12
4	5×10^{-4}M	1·5	2	10^{-4}M	11
5	5×10^{-4}M	1·5	10	10^{-4}M	26
6	5×10^{-4}M	1·5	17	10^{-4}M	27

From these results it is apparent that the presence in the bath of semicarbazide hydrochloride at 10^{-3}M completely inhibits the response of the heart to the corpus cardiacum, and at 10^{-4}M the inhibition is about 30%. Similarly, isonicotinic acid hydrazide at 10^{-3}M partially blocks the increase in rate normally associated with the presence of a brei of corpora cardiaca. Semicarbazide and isonicotinic acid hydrazide are in themselves without effect on the heart rate.

If semicarbazide is acting as an inhibitor of a phosphopyridoxal-dependent system, then the addition of phosphopyridoxal to an isolated heart which is under both stimula-

tion from the corpus cardiacum and inhibition from semicarbazide should result in an increase in rate. The addition of phosphopyridoxal alone to a concentration of 10^{-3}M has no effect on the isolated heart, nor does it affect the rate when it is present in the bath with either a brei of corpora cardiaca or semicarbazide. On the other hand, when phosphopyridoxal is added to the fluid bathing a heart which is contracting under the influence of both the corpus cardiacum and semicarbazide, a further increase in rate occurs, as is shown in Table 3.

(2) *The effect of semicarbazide on the argentaffin reaction of the pericardial cells*

Pericardial cells which have been exposed to relatively high doses of the hormone from the corpus cardiacum are more markedly positive to the argentaffin staining reaction than are those which have been maintained for some time in the absence of the hormone (Davey, 1962*b*). The argentaffin granules are probably a manifestation of the presence in the cell of the amine (Pearse, 1960). If semicarbazide were acting on the cells to prevent the formation of the amine, cells treated with semicarbazide and hormone should exhibit a smaller proportion of argentaffin-positive cells than those treated with hormone alone.

Table 4. *The percentage of argentaffin-positive cells among pericardial cells from cockroach hearts*

(One portion of each heart has been treated with a brei of corpora cardiaca plus 10^{-3}M semicarbazide, the other with hormone alone.)

	Percentage argentaffin-positive	
Heart no.	Portion treated with hormone alone	Portion treated with hormone + inhibitor
1	24	16
2	16	7
3	44	27
4	50	28
5	45	37
6	60	69

Accordingly, the abdominal portion of the heart was removed from six insects, together with the associated pericardial cells and strip of overlying cuticle. These hearts were divided into halves transversely and the two halves placed in separate dishes containing Ringer. One dish of each pair contained semicarbazide at 10^{-3}M. After 15 min. the same quantity of a brei of corpora cardiaca was added to each dish. Thirty minutes later the cells were fixed in neutral formol and paraffin sections were cut and stained by the argentaffin technique (Pearse, 1960). A total of something over 100 cells was examined from each half heart, and the number of argentaffin cells was expressed as a percentage of this total. As can be seen from Table 4, in five of the six hearts examined the presence of semicarbazide decreased the percentage of argentaffin-positive cells.

(3) *The action of amino acids on the rate of beating of isolated hearts*

If an amino acid decarboxylase capable of producing a biogenic amine is present in the pericardial cells, then the addition of a suitable amino acid might lead to the

production of an amine and an elevated heart rate. Accordingly, various amino acids were added to the Ringer bathing isolated hearts.

Since it has been suggested that the amine produced by the pericardial cells is an indole (Davey, 1961*b*), 5-OH tryptophane, which on decarboxylation yields serotonin, was among the first amino acids to be tested. Only the racemic form was available in this laboratory.

Table 5. *The percentage increase in rate of beating of isolated hearts resulting from a concentration of five pairs of corpora cardiaca per* 100 *ml. of Ringer applied before and after the addition of* 5×10^{-4}M 5-OH DL-*tryptophane*

Heart no.	Percentage increase: Before adding 5-OH tryptophane	Percentage increase: After adding 5-OH tryptophane
1	21	0
2	53	31
3	25	13

Table 6. *The percentage increase in rate of beating of isolated hearts resulting from the addition of serotonin creatine sulphate before and after the addition of* 5-OH DL-*tryptophane at* 5×10^{-4}M

Heart no.	Concentration of serotonin	Percentage increase: Before adding 5-OH tryptophane	Percentage increase: After adding 5-OH tryptophane
1	10^{-6}M	20	10
2	10^{-6}M	20	12
3	5×10^{-7}M	16	0
4	5×10^{-7}M	13	2
5	10^{-6}M	26	6

Table 7. *The percentage increase in rate of beating of isolated hearts resulting from the addition of* L-DOPA *before and after the addition of* 10^{-3}M *semicarbazide*

Heart no.	Concentration of DOPA	Percentage increase: Before semicarbazide	Percentage increase: After semicarbazide
1	10^{-4}M	12	5
2	5×10^{-4}M	18	6
3	10^{-3}M	22	6
4	10^{-4}M	11	0
5	5×10^{-4}M	14	6
6	5×10^{-4}M	32	7

The effect of 5-OH DL-tryptophane on the rate of beating of isolated hearts was somewhat variable. Normally the addition of this amino acid up to a concentration of 10^{-3}M had no effect on the heart, but occasionally concentrations between 10^{-4}M and 10^{-3}M produced some increase in rate. This increase was never greater than 10 or 15%.

On the other hand, the addition of 5-OH DL-tryptophane markedly reduced the effect of added hormone (Table 5), and further investigation revealed that the amino acid also antagonized the response of the heart to serotonin (Table 6).

The addition of 3,4-dihydroxy-L-phenylalanine (DOPA) consistently resulted in an elevated heart rate. The increase developed more slowly than after the addition of corpus cardiacum, and required 7–8 min. to reach its maximum value. The maximum response varied with the concentration of DOPA and with individual heart preparations, but normally a concentration of 5×10^{-4}M increased the rate of beating by 15–20%. The threshold concentration was about 10^{-5}M.

This increase in rate was inhibited by the presence in the bath of semicarbazide. Table 7 sets out the increase in rate resulting from the addition of DOPA before and after the addition of 10^{-3}M semicarbazide. Furthermore, the inhibition resulting from added semicarbazide can be partially reversed by the addition of phosphopyridoxal. Table 8 gives the increase in rate for six hearts after the addition of 10^{-4}M DOPA, and after the further addition of 10^{-4}M phosphopyridoxal before and after inhibition by 5×10^{-4}M semicarbazide hydrochloride.

Table 8. *The percentage increase in rate of beating of isolated hearts after the addition of* L-DOPA *at* 10^{-4}M, *and after the further addition of* 10^{-4}M *phosphopyridoxal, before and after inhibition by* 5×10^{-4}M *semicarbazide*

(The preparation was rinsed with Ringer before the semicarbazide was added.)

	Percentage increase without semicarbazide		Percentage increase with semicarbazide	
Heart no.	After DOPA	After phosphopyridoxal	After DOPA	After phosphopyridoxal
1	52	52	21	47
2	27	28	22	38
3	14	14	0	18
4	20	20	0	23
5	15	15	4	11
6	50	51	23	47

Table 9. *The effect of adding dopamine to isolated hearts before and after exposure of the heart to 2-bromolysergic acid diethylamide bitartrate at* $2{\cdot}5 \times 10^{-5}$M

Heart no.	Concentration of dopamine	Percentage increase	
		Before bromo-LSD	After bromo-LSD
1	5×10^{-8}M	22	6
2	5×10^{-8}M	7	0
3	10^{-7}M	30	6
4	2×10^{-7}M	35	8
5	5×10^{-7}M	41	14

Evidently, then, the response of the heart to added DOPA is mediated by a phosphopyridoxal-dependent system. If this system were DOPA decarboxylase, the resulting amine would of course be 3-hydroxytyramine or dopamine. Dopamine will stimulate the isolated heart of *Periplaneta*; the threshold appears to be in the region of 10^{-8}M. This stimulation is not inhibited by semicarbazide, but 2-bromolysergic acid diethylamide bitartrate antagonizes the effects of dopamine (Table 9). In this respect, the action of dopamine resembles the action of serotonin.

The preparations of isolated hearts contain a variety of tissues, and the possibility

exists that the excitation of the heart by DOPA is not mediated by the pericardial cells. However, blocking the pericardial cells of isolated hearts by exposure to ammonia carmine (Davey, 1961*a*) eliminated or markedly reduced the response of the heart to DOPA (Table 10). Furthermore, DOPA stimulates the isolated hind gut of *Periplaneta*, a preparation which, while it contains cells analogous to the pericardial cells (Davey, 1962*a*) contains fewer other tissue elements than the isolated heart.

Among the other amino acids which were assayed were L-tyrosine and L-tryptophane. The behaviour of both of these amino acids with respect to their heart-stimulating properties was inconsistent. Tyrosine sometimes stimulated the isolated hearts at concentrations up to about 5×10^{-5}M, but at higher concentrations it depressed the rate. The excitatory effects appeared to be unaffected by semicarbazide. On other occasions, however, tyrosine had no effect on the heart. Tryptophane usually, but not always, stimulated the heart by a small amount. This effect was not inhibited by semicarbazide.

Table 10. *The percentage increase in rate of beating of isolated hearts brought about by adding* 10^{-4}M DOPA *before and after staining the pericardial cells with ammonia carmine*

Heart no.	Percentage increase before staining	Percentage increase after staining
1	24	12
2	10	6
3	22	19
4	29	11
5	35	9
6	19	4
7	20	6
8	21	0

DISCUSSION

It is clear from the results presented here that a phosphopyridoxal-dependent system in the pericardial cells is involved in the chain of events leading to stimulation of the isolated heart by breis of the corpora cardiaca. Semicarbazide, an inhibitor of phosphopyridoxal-dependent enzymes, reduces the proportion of argentaffin-positive cells among pericardial cells which have been exposed to the hormone. Since the presence of argentaffin granules usually indicates the presence of a biogenic amine (Pearse, 1960), it is likely that semicarbazide is interfering with the production of the amine. Of the various enzyme systems which require the co-factor phosphopyridoxal, the amino acid decarboxylases are the only ones likely to give rise to an amine.

The notion that an amino acid decarboxylase is active in the pericardial cells receives some support from the experiments involving DOPA. While there is no direct evidence that dopamine is formed from added DOPA, the fact that a pharmacologically active material arises via some phosphopyridoxal-dependent system in the pericardial cells is highly suggestive. It may of course be true that the DOPA is first converted to some other substance by the pericardial cells, and that this substance is utilized by an amino acid decarboxylase to produce the amine.

As a working hypothesis, then, there is some evidence for suggesting that the action of the hormone from the corpus cardicum upon the pericardial cells involves the pro-

duction of an amine from an inert precursor, presumably an amino acid, by the action of an amino acid decarboxylase. In such a mechanism, the hormone could intervene at any one of four points:

(*a*) By stimulating the production of more phosphopyridoxal which would activate the decarboxylase. This hypothesis requires that the pericardial cells be devoid of phosphopyridoxal in the absence of the hormone; added phosphopyridoxal should then mimic the action of the hormone. This, as the results demonstrate, is not the case. A further objection lies in the fact that there appears to be sufficient active decarboxylase present in cells to produce an active material from DOPA.

(*b*) By stimulating the production of the decarboxylase itself. This hypothesis is also open to the objection that cells which are not exposed to the hormone contain active decarboxylase, as evidenced by the conversion of DOPA to a pharmacologically potent material.

(*c*) By stimulating the production of more substrate. This hypothesis cannot be eliminated by the evidence presented here. Such a hypothesis pictures a substance, perhaps an amino acid, closely related to the substrate, but sufficiently different from it so as not to be utilized by the decarboxylase. The presence of the hormone would set in motion events which would lead to the conversion of the closely related material to the substrate, which would then be utilized by the decarboxylase to produce the amine. Since amino acid decarboxylases are known to be fairly specific in their substrate preferences, a system of this sort is at least plausible. However, the action of the hormone is fairly rapid; although the maximum increase in rate is not achieved until 4–5 min. after the addition of the brei to the Ringer bathing an isolated heart, the first indications of excitation appear instantaneously. On this basis, the fourth hypothesis might be more likely.

(*d*) By removing a 'barrier' between the decarboxylase and its substrate. Since the enzyme appears to be present in the pericardial cells in an available form, this hypothesis pictures the substrate as being protected from the action of the enzyme. The effect of the hormone then becomes one of exposing the substrate to the enzyme. The differentiation between the third and fourth hypothesis will have to await the precise identification of the amine and its inert precursor.

Because of the well-known specificity of the amino acid decarboxylases, those amino acids which are decarboxylated by the pericardial cells might provide some clue to the identity of the amine. However, the fact that added DOPA results in an elevated heart rate whereas added 5-OH DL-tryptophane does not may not be of very great significance. The results indicate that 5-OH DL-tryptophane interferes with the action of both the hormone from the corpus cardiacum and serotonin. It is therefore possible that 5-OH DL-tryptophane acts by occupying the same sites on the muscles of the heart as would be occupied by the amine, thereby preventing its action. If this were true, then the failure of 5-OH DL-tryptophane to excite the heart would not necessarily be a result of its failure to be utilized by the decarboxylase. It has been suggested that mammalian DOPA decarboxylase is identical with 5-OH tryptophane decarboxylase (Blaschko, 1961). This suggests that 5-OH tryptophane might be utilized by the pericardial cells, but that the serotonin which would be produced by the action of the decarboxylase is prevented from accelerating the heart by the presence of the amino acid. The fact that DOPA is utilized by the pericardial cells may indicate that the

natural amine of the pericardial cells is derived from a phenolic amino acid; on the other hand, the fact that 5-OH tryptophane is able to compete with the natural amine for the active sites on the heart is perhaps strong evidence for an amine with an indole base.

SUMMARY

1. Semicarbazide hydrochloride and isonicotinic acid hydrazide, which are both inhibitors of phosphopyridoxal-dependent systems, inhibit the increase in the rate of beating of hearts which is brought about by breis of corpora cardiaca. This inhibition is partially reversed by phosphopyridoxal.

2. Semicarbazide decreases the percentage of argentaffin-positive cells among pericardial cells exposed to breis of corpora cardiaca.

3. DOPA increases the rate of beating of the isolated heart. This increase is mediated by the pericardial cells and is inhibited by semicarbazide. The inhibition by semicarbazide is reversed by phosphopyridoxal.

4. It is suggested, as a working hypothesis, that the hormone from the corpus cardiacum stimulates the pericardial cells to produce an amine from an amino acid by the action of the appropriate amino acid decarboxylase. The hormone intervenes either by causing the cells to produce more of the amino acid, or by unmasking the amino acid which is pictured as being protected from the enzyme in the unstimulated cell.

I am indebted to Prof. V. B. Wigglesworth for his unfailing interest. The author is a Research Fellow of Gonville and Caius College.

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