BRADYCARDIA IN THIAMIN DEFICIENCY
AND THE ROLE OF GLYOXYLATE

C. C. LIANG

Department of Physiology, University of Hong Kong, Hong Kong
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Summary

1. Method of recording the heart rate in rats is described.
2. It was found that during the course of developing thiamin deficiency, the heart rate of rats dropped from 448 to 220 beats/min. (There was no difference between male and female rats.)
3. A number of metabolic products were injected into the normal rats and only glyoxylate was shown to cause a significant drop in heart rate.
4. Graded amounts of Na-glyoxylate were injected peritoneally into the normal rats and blood samples were taken at intervals to test the amount of glyoxylate present. It was found that peak concentration appeared 6 min after injection and thereafter declined or disappeared, depending on the amount of glyoxylate administered.
5. It was found that glyoxylate accumulates in the body of thiamin-deficient rats and in the pair-fed rats reared on restricted diet for a long period.
6. Drop in heart rate depended on the appearance of glyoxylic acid present in the blood rather than on the reduction of body weight.

Bradycardia has been described as a distinctive feature of thiamin deficiency in rats (1, 2) and in other animals, such as dogs, swines, cats and monkeys (3). Although inanition may lead to bradycardia, thiamin deficiency usually causes a greater drop in heart rate (4–7).

The cause of bradycardia, as well as electrocardiographic change and morphological lesions in thiamin-deficient animals is obscure. The accumulation of metabolites has been suspected to be responsible for the changes (8), however, several metabolites, including pyruvic acid, lactic acid, ammonium chloride, methylglyoxal, glyceraldehyde, glucose and adenylate, have been found not related to the changes observed in thiamin-deficient animals (8).

The detection of glyoxylate in urine and blood of thiamin-deficient rats and pair-fed rats on a restricted diet for a long period (9) suggests that the presence of this substance, to a certain extent, is related to bradycardia in thiamin deficiency. STARKENSTEIN (10) stated that glyoxylic acid is more potent than digitalis...
in lowering the heart rate.

The aim of the present study is to re-examine the gradual decline of heart rate during the course of developing thiamin deficiency and to determine the effect of glyoxylate on the heart rate, in comparison with other metabolites.

METHODS

1. **Experimental animals.** One hundred fifty-two Sprague-Dawley rats (250 to 300 g body weight) of either sex were divided into two experimental groups. Eighty-four rats in the first group were fed a thiamin-deficient diet *ad libitum* (9) while the second group of 48 rats were kept on a restricted basal diet (9). The daily intake of the rats in the second group in terms of calories was the same as that of the first group, but sufficient amounts of thiamin-HCl and cellulose powder were added to satisfy the animals' hunger (9). The intake of food, body weight and heart rate were recorded daily.

2. **Heart rate recording.** The upper part of the forelimb of a rat was shaved and rubbed with a jelly made of gelatin (5 parts), glycerine (5 parts), KCl (5 parts), procain (0.5 part) and water (4.5 parts). Heart clips were attached to the outer aspect of two limbs and connected to a DC amplifier and in turn fed into a Sanborn Electrocardiograph. The rat was then placed in a wire gauge cage (4"×4"×8") small enough to limit its movement. Before recording, the rat was stroked gently on the back with a small brush to give a feeling of being treated gently. This treatment was important for obtaining a constant record of heart rate with little stress on the rat and at the same time, for minimizing its muscular movements. After a recording, 5 ml of 0.9% saline was injected peritoneally to rat. After 6 min, the heart rate was determined to see whether this dummy dose of saline would affect the heart rate or not. Then a dose of test substance dissolved in 5 ml. 0.9% saline was injected peritoneally into the rat and the heart rate was recorded 6 min afterwards. The heart rate was obtained by counting the number of beats on the electro-cardiogram.

3. **Test substances.** Graded amounts of the following substances were brought to pH 7.4 by the addition of 1/10 n NaOH and diluted to 5 ml with 0.9% saline.

- Glucose (E. Merck);
- fructose (E. Merck);
- acetyl-CoA (Kock-Light);
- adenosine triphosphate, Na-salt (Sigma);
- flavine-adenine-dinucleotide (Calbiochem);
- L(+) alanine (E. Merck);
- L(−) serine (E. Merck);
- glycine (E. Merck);
- glycerol (E. Merck);
- palmitic acid (E. Merck);
- D,L-glycer-aldehyde (Kock-Light);
- methyl glyoxal (prepared according to the method described by Shroder and Woodward (II));
- glyoxylic acid (Kock-Light);
- oxalo-acetic acid (B.D.H.);
- acetic acid (B.D.H.);
- lactic acid (B.D.H.);
- malic acid (Sigma);
- oxalic acid (B.D.H.);
- citric acid (B.D.H.);
- iso-citric acid (B.D.H.);
- aconitic acid (B.D.H.);
- succinic acid (B.D.H.);
- oxo-glutaric acid (B.D.H.);
- pyruvic acid (E. Merck);
- fumaric acid.
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(Kock-Light); aceto-acetate (Kock-Light).

4. Estimation of glyoxylate present in the blood. Graded amounts of glyoxylic acid adjusted to pH 7.4 were injected peritoneally into 20 normal rats (10 male and 10 female rats) and 0.2 ml of blood was then taken from the tail vein at intervals. The amount of glyoxylic acid present in the blood was then determined according to the method described by McFADDEN and HOWES (12).

RESULTS

The concentrations of glyoxylic acid present in the blood after injection peritoneally of graded amounts of glyoxylic acid, are listed in Fig. 1. It was shown that the peak concentration of glyoxylic acid present in the blood occurred 6 min after the administration peritoneally. It was shown that other substances showed the same trend of absorption into the blood and reached the peak concentration at the same intervals. The effect of drugs on the heart rate is listed in Table 1.

A rather constant heart rate of 448 ± 18 beats min⁻¹ was observed in the rats reared on thiamin-deficient diet during the first two weeks, then from the 15th day onward, the heart rate declined slowly, and reached a value of 220 ± 15 beats min⁻¹ near the moribound state (Fig. 2). The heart rate of rats reared on restricted diet for a long period showed a rather constant heart rate of 448 ± 18 min⁻¹ for the first 40 day, and then declined gradually to 430 beats min⁻¹ (Fig. 3). On the other hand, the body weight of these rats was reduced from 252 g to 196 g on the 56th day.

The heart rate of rats in both groups coincided with the appearance of
Table 1. Effect of different substances on the heart rate of rats. Different quantities (mg) of substance were brought to pH 7.4 by addition of 1/10 N NaOH and diluted to 5 ml by addition of normal saline. This was injected peritoneally into the rat and the heart rate was determined 6 min afterwards. The values were the mean values ± S.E. from 10 male and 10 female rats.

| Substance                           | Nothing | Normal saline only | 1      | 5      | 10     | 50     | 100    | 200    | 500    |
|-------------------------------------|---------|--------------------|--------|--------|--------|--------|--------|--------|--------|
| Glucose                             | 440 ±18 | 446 ±18            | 448 ±19| 449 ±18| 450 ±17| 450 ±18| 450 ±18| 450 ±18| 450 ±18|
| Fructose                            | 440 ±18 | 447 ±19            | 447 ±18| 447 ±18| 447 ±18| 448 ±18| 449 ±18| 450 ±18| 450 ±18|
| Acetyl-CoA                           | 447 ±18 | 446 ±17            | 447 ±18| 447 ±18| 447 ±18| 448 ±18| 450 ±17| 450 ±18| 450 ±19|
| Adenosine triphosphate              | 446 ±18 | 446 ±18            | 447 ±18| 447 ±18| 447 ±18| 448 ±18| 448 ±18| 449 ±18| 450 ±18|
| Flavine-adenine dinucleotide        | 446 ±18 | 446 ±18            | 447 ±18| 447 ±18| 447 ±18| 448 ±18| 448 ±18| 449 ±18| 450 ±18|
| Alanine                             | 446 ±18 | 446 ±17            | 447 ±18| 448 ±19| 447 ±19| 447 ±19| 449 ±19| 449 ±19| 450 ±17|
| Serine                              | 448 ±19 | 447 ±18            | 448 ±17| 448 ±19| 448 ±19| 449 ±19| 449 ±19| 449 ±19| 450 ±18|
| Glycine                             | 448 ±18 | 447 ±18            | 448 ±18| 449 ±19| 449 ±19| 449 ±19| 449 ±19| 449 ±19| 450 ±19|
| Glycerol                            | 448 ±19 | 447 ±18            | 448 ±18| 448 ±19| 449 ±19| 449 ±19| 449 ±19| 449 ±19| 450 ±18|
| Palmitic acid                       | 449 ±19 | 447 ±18            | 448 ±18| 449 ±19| 449 ±19| 449 ±19| 449 ±19| 449 ±19| 450 ±18|
| D,L-Glyceraldehyde                  | 448 ±19 | 446 ±19            | 448 ±18| 448 ±19| 449 ±19| 449 ±19| 449 ±19| 449 ±19| 450 ±18|
| Methyl glyoxal                      | 448 ±18 | 446 ±19            | 448 ±18| died   | died   | died   | died   | died   | died   |
| Glyoxylic acid                      | 448 ±18 | 446 ±19            | 440 ±17| 432 ±16| 420 ±13| 403 ±10| 323 ±9 | 260 ±5 | died   |
| Oxalic acid                         | 448 ±19 | 446 ±18            | 445 ±16| died   | died   | died   | died   | died   | died   |
| Aceto-acetic acid                   | 448 ±18 | 446 ±19            | 447 ±19| 447 ±19| 447 ±19| 447 ±19| 448 ±19| 448 ±18| 449 ±19|
| Lactic acid                         | 448 ±18 | 446 ±19            | 449 ±19| 450 ±19| 451 ±19| 452 ±20| 452 ±20| 453 ±21| 454 ±24|
| oxalo-Acetic acid                   | 448 ±19 | 446 ±18            | 449 ±19| 449 ±19| 449 ±19| 449 ±19| 450 ±20| 450 ±20| 450 ±21|
| Acetic acid                         | 448 ±18 | 446 ±19            | 449 ±19| 450 ±20| 450 ±21| 450 ±22| 452 ±23| 453 ±24| 454 ±24|
| Pyruvic acid                        | 449 ±18 | 446 ±19            | 450 ±19| 502 ±20| 508 ±23| 518 ±25| 520 ±28| 525 ±27| 528 ±29|
| Citric acid                         | 448 ±18 | 447 ±19            | 449 ±19| 449 ±21| 452 ±22| 452 ±24| 454 ±25| 455 ±28| 458 ±28|
| iso-Citric acid                     | 448 ±19 | 446 ±19            | 449 ±20| 450 ±21| 451 ±21| 452 ±23| 453 ±24| 455 ±25| 455 ±25|
| Aconitic acid                       | 448 ±19 | 448 ±19            | 449 ±20| 450 ±21| 451 ±21| 452 ±22| 452 ±23| 454 ±23| 454 ±25|
| Isoaconitic acid                    | 449 ±19 | 447 ±19            | 449 ±20| 450 ±21| 451 ±21| 451 ±22| 452 ±22| 452 ±22| 453 ±25|
| oxo-Gluaric acid                    | 447 ±19 | 447 ±19            | 448 ±20| 448 ±20| 448 ±20| 450 ±20| 450 ±22| 451 ±21| 451 ±21|
| Succinic acid                       | 447 ±19 | 447 ±19            | 448 ±19| 448 ±20| 449 ±20| 450 ±20| 450 ±20| 450 ±21| 450 ±20|
| Fumaric acid                        | 447 ±18 | 448 ±19            | 448 ±19| 448 ±20| 448 ±21| 449 ±20| 449 ±20| 449 ±21| 450 ±19|
| Malic acid                          | 448 ±18 | 448 ±18            | 448 ±19| 449 ±19| 449 ±18| 449 ±18| 449 ±18| 449 ±18| 450 ±18|
glyoxylate in the blood, but not with the reduction of body weight. Near the moribound state, the concentration of glyoxylate declined slightly in rats reared on a thiamin-deficient diet, but the heart rate continued to decrease, this paradoxal effect might be due to other possible effects (13).

Fig. 2. Time course of the changes of body weight (●—●), heart rate (○—○) and blood glyoxylic acid concentration (×—×) of rats reared on a thiamin-deficient diet.

Fig. 3. Time course of changes of body weight (●—●), heart rate (○—○) and blood glyoxylic acid concentration (×—×) of rats reared on a restricted diet with added thiamin-HCl.

DISCUSSION

It was observed that all tested substances showed little effect on the heart rate except glyoxylate, methyl glyoxal and oxalic acid (Table 1). Methyl glyoxal and oxalic acid were very toxic based on observations of the effect of drugs. Besides, methyl glyoxal was not found in thiamin-deficient rats (9). Although DRURY
et al. (1) suggested that the decline of heart rate coincided with the decrease in body weight, rats reared on a restricted diet for a long period did not show any changes in heart rate from the 15th to 40th day when their body weight was decreasing, except when glyoxylate began to appear in the body (9). (Also in Fig. 3 in this study.) Since in this study glyoxylate in small amounts affected the heart rate significantly, the role of glyoxylate may be of greater significance in the pathophysiology of bradycardia in the thiamin-deficient animal.

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