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Studies on γ -amino- β -hydroxybutyric acid II. Oxygen consumption of brain homogenate in the presence of γ -amino- β -hydroxybutyric acid*

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Abstract

With the purpose to see if GABOB is in any way concerned with the mechanism of the epileptic attack observations were carried on the oxygen consumption of the brain homogenates of rabbits, normal and CLA, and of human, epileptic and non-epileptic. The experiment proved that the oxygen consumption is increased in the epileptic brain and in the brain of CLA rabbit. It was raised by adding ATP-Na salt or DPN, but GABOB itself showed only a slight effect. The results suggested that the oxygen consumption of brain is not so closely correlated with GABOB, but there is a possibility that the decrease in GABOB contents in epileptic brain by the accelerated decomposition with its elevated oxygen consumption may be correlated to the epileptic attack, though the final conclusion requires further observations.

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STUDIES ON γ -AMINO- β -HYDROXYBUTYRIC ACID

II. OXYGEN CONSUMPTION OF BRAIN HOMOGENATE IN THE PRESENCE OF γ -AMINO- β -HYDROXYBUTYRIC ACID*

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It has recently be clarified that the brain tissue contains γ -amino- β -hydroxybutyric acid (GABOB), a direct oxidation product of γ -aminobutyric acid (GABA), by the works of KODAMA¹, OHARA *et al*.², and INOUE³. Quantitative analysis carried out by INOUE⁴ showed that the contents is about 1.87 mg per cent in human brain tissue and 1.34 mg per cent in rabbit brain tissue. Concerning the biological significance of GABOB, it has been clarified that it acts as to suppress the excitability of the motor center as revealed by HAYASHI *et al*.^{5,6}, and HEMMI *et al*.⁷, and it lowers the blood pressure as shown in the 1st report⁸. The reports of MURAOKA⁹ and YABUUCHI¹⁰ demonstrated that GABOB acts competitively with the effect of the running fit or convulsion inducing agents. SUGIURA *et al*.¹¹, proposed the hypothesis on the metabolic pathway of GABOB that GABOB is first converted to γ -aminoacetoacetic acid and decomposed into glycine and acetic acid from his observation on brain tissue elucidating that GABOB is oxidized by brain tissue. These will be further oxidized and decomposed finally to CO₂ and water. But it is uncertain whether or not GABOB is led to complete decomposition. If this process actually occurs in the brain tissue, it is reasonably expected that the oxygen consumption of brain tissue will increase in the presence of GABOB. For the purpose to confirm this point the author measured the oxygen consumption of the tissue homogenates of rabbit brain. After these basic observations the author also observed the difference in oxygen consumption between epileptic and non-epileptic human brains and those from normal rabbit brains and brains of rabbit with experimental latent cerebral local anaphylaxis (CLA rabbit brain) by adding GABOB.

MATERIALS AND METHODS

The 47 normal rabbit brains, 8 CLA rabbit brains, 8 epileptic and 8 non-

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epileptic human brains (mainly tumor bearing brains) served as materials.

Thirty-two normal rabbits were used for observing the effect of GABOB on the oxygen consumption under varied pH.

Fifteen normal rabbits, 8 CLA rabbits and all the human materials were used to observe the effects of ATP, DPN, $MgCl_2$, vitamin B_6 , cytochrome C and FAD, added to the media containing GABOB, excepting one control lacking GABOB for the observation of specificity in epileptic states. CLA rabbits were obtained by the repeated intravenous injection of the ox brain phosphatids by the methods of KASAI¹², by which the "preparatory epileptic state" or "experimental epileptic state" can be induced as reported by JINNAI¹³.

The animals were sacrificed by decapitation and the brains were used for the experiment immediately after the removal. The human materials were also used for the experiment immediately after the excision at surgical operation. One g. each of the cerebral cortex of these materials was placed in Potter's homogenizer, containing 10 ml. of 1 per cent KCl solution. Then it was minced at 1°C to 5°C for about 3 to 5 minutes. Each 0.5 ml. of homogenates was put into the manometric rooms of Warburg's apparatus. Six to 18 series of samples were used for one observation from one material. The reaction media were put in the isolated chambers. After setting the apparatus in 37.5°C water bath, the volumes of oxygen consumption were recorded for 90 minutes after adding the reaction medium to the brain homogenates.

As the reaction media (A) the following solutions were used for the brains from 32 normal rabbits: 0.5 ml. each of M/10 tris-buffer, M/15 tris-buffer or M/15 phosphate buffer (pH 6.5, 6.7, 7.0, 7.2, 7.4, 7.6, 8.0, 8.5 or 9.0) with 0.5 ml. of 10 per cent brain homogenate in main room, 0.3 ml. of 10 per cent KOH in well room and 0.5 ml. of 0.1 M GABOB in side room. Adequate amounts of distilled water is added to the solution into the main room so that the total volume of reaction system may be 2 ml.

For the brains of 15 normal and 8 CLA rabbits and 16 human materials the following reaction media (B) were used. 0.5 ml. of M/10 tris-buffer pH 6.8 with 0.5 ml. of M/10 GABOB, 0.1 ml. of 0.2 per cent FAD, 0.5 ml. of 0.01 M ATP-Na salt, 0.5 ml. of 0.01 M ATP-K salt, 0.5 ml. of 10^{-5} M cytochrome C, 0.2 ml. of 0.2 M $MgCl_2$, 0.5 ml. of 100 γ vitamin B_6 or 0.1 ml. of 0.01 M DPN in main room, 0.3 ml. of 10 per cent KOH in well room, and 0.5 ml. of 10 per cent brain homogenate in side room. The total volume of the reaction system was adjusted to 3 ml. by adding adequate amount of distilled water to each main room.

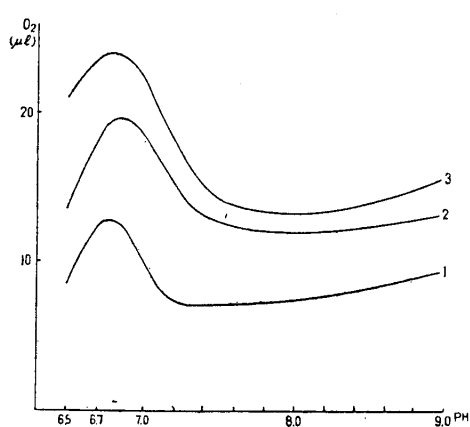
Crude material GABOB was donated by Ono Pharmaceutical Co., LTD. GABOB used for this experiment was obtained from this material by recrystallization from the saturated solution adding pure ethanol till this solution became muddy. This was left standing for 24 hours at 1° to 5°C and the supernatant

was decanted. The precipitate was washed with ethanol twice and dried at room temperature. ATP-Na salt, ATP-K salt, cytochrome C and DPN were of Wako Pure Chemical Industries Ltd., FAD was of Wakamoto Pharmaceutical Co., Ltd., and vitamin B₆ of Torii & Co., Ltd.

RESULTS

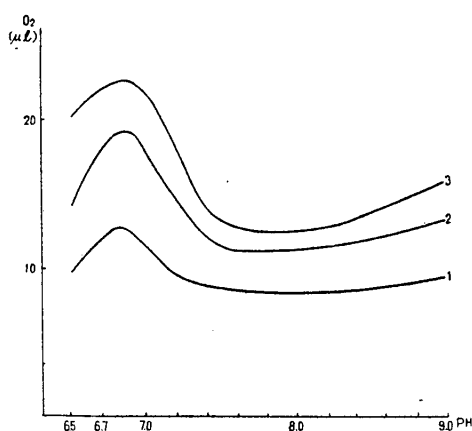
In the attempts to find out the optimal pH for the reaction media (A) to observe the O₂ consumption of brain tissues with GABOB, M/10 tris-buffer, M/15 tris-buffer and M/15 phosphate buffer in varied pHs were used as the component of the media (A) described above.

Experiments were carried out 24 normal animals dividing into 3 groups, 8 animals in each. The brain homogenates from the animals of the 1st group were incubated with the reaction media (A) prepared by using M/10 tris-buffer, and those in 2nd group with reaction media (A) used M/15 tris-buffer, and those in 3rd group with the same reaction media with M/15 phosphate buffer. The experiment proved that the optimal pH for the oxygen consumption of brain tissue was 6.8 for all three buffers, as could be seen in Figs. 1, 2, 3. Another 8 animals were used to determine whether tris-buffer or phosphate buffer would be more suitable one. The brain homogenates were put in the media (A) con-



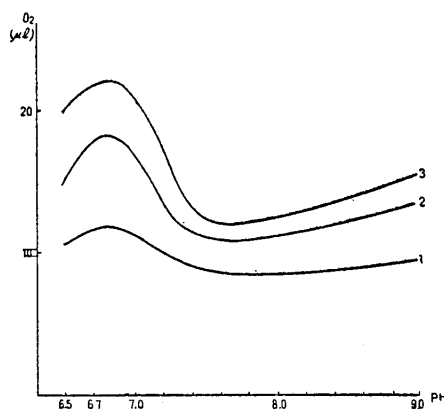
1. O₂ consumption in 30 min.
2. O₂ consumption in 60 min.
3. O₂ consumption in 90 min.

Fig. 1. O₂ consumption of the homogenate of normal rabbit brain (Mean value from the brains of 8 animals) Buffer: M/10 tris-buffer.



1. O₂ consumption in 30 min.
2. O₂ consumption in 60 min.
3. O₂ consumption in 90 min.

Fig. 2. O₂ consumption of the homogenate of normal rabbit brain (Mean value from the brains of 8 animals) Buffer: M/15 tris-buffer.



1. O₂ consumption in 30 min.
2. O₂ consumption in 60 min.
3. O₂ consumption in 90 min.

Fig. 3. O₂ consumption of homogenate of normal rabbit brain (Mean value from the brains of 8 animals) Buffer: M/15 phosphate buffer.

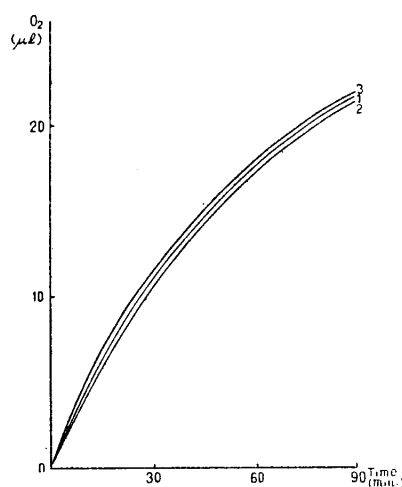


Fig. 4. O₂ consumption of the homogenate of normal rabbit brain (Mean value from the brains of 8 animals).

1. pH 6.8 N/10 tris-buffer
2. pH 6.8 M/15 tris-buffer
3. pH 6.8 M/15 phosphate buffer

taining M/10, M/15 tris-buffer or M/15 phosphate buffer, all at pH 6.8 and placed in WARBURG's manometer at the same time and observed for 90 minutes. Observations revealed hardly any difference with any buffer, as illustrated in Fig. 4. Therefore, for subsequent experiments M/10 tris-buffer at pH 6.8 was used.

With each brain from another 15 normal animals, 10 series of experiments were carried out by using the reaction media (B) with the different substances. The 1st samples are of brain homogenate and M/10 tris-buffer, as the control. Second samples are the media supplemented with GABOB as in the 1st group. Third samples were mixed with GABOB and FAD, fourth samples with GABOB and ATP-Na salt, 5th samples with GABOB and ATP-K salt, 6th samples with GABOB and cytochrome C, 7th samples with GABOB and MgCl₂, 8th samples with GABOB and MgCl₂, 8th samples with GABOB and vitamin B₆, 9th samples with GABOB and DPN and 10th with GABOB, FAD, ATP-Na salt, ATP-K salt, cytochrome C, MgCl₂, vitamin B₆ and DPN.

The results are summarized in Fig. 5. The addition of GABOB and FAD, cytochrome C or ATP-K salt gave no actual effect on the oxygen consumption. But MgCl₂ and vitamin B₆ showed a slightly increasing effect on the oxygen consumption. The noticeable effects were seen in the cases added with ATP-Na salt and DPN, showing about the same level as seen in the cases supplemented

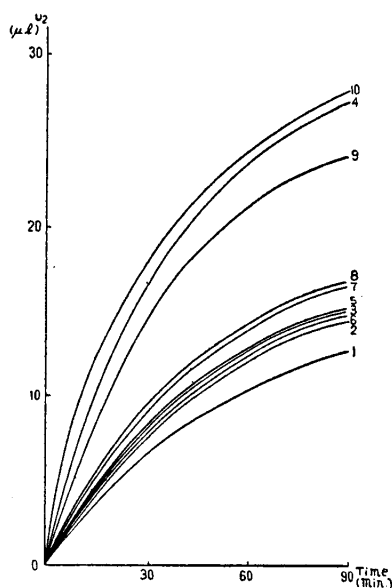


Fig. 5. O_2 consumption of the homogenate of normal rabbit brain (Mean value from the brains of 15 animals) Buffer: M/10 tris-buffer, pH 6.8.

1. GABOB (-)
2. GABOB only
3. GABOB + FAD
4. GABOB + ATP -Na salt
5. GABOB + ATP -K salt
6. GABOB + Cytochrome C
7. GABOB + MgCl_2
8. GABOB + B_6
9. GABOB + DPN
10. Complete system

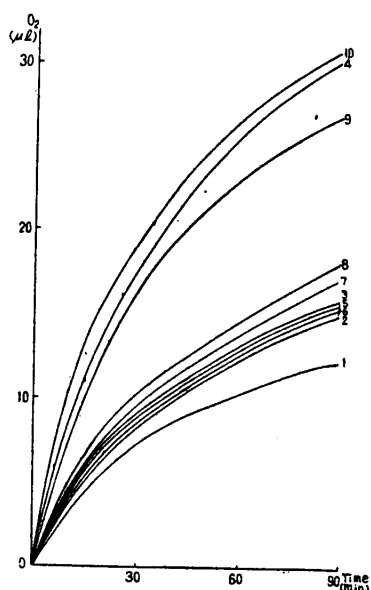


Fig. 6. O_2 consumption of the homogenate of CLA rabbit brain (Mean value from the brains of 8 animals)

Buffer: M/10 tris-buffer, pH 6.8.

1. GABOB (-)
2. GABOB only
3. GABOB + FAD
4. GABOB + ATP-Na salt
5. GABOB + ATP-K salt
6. GABOB + Cytochrome C
7. GABOB + MgCl_2
8. GABOB + B_6
9. GABOB + DPN
10. Complete system

with all the components described.

Almost the same results as just described have been obtained on the brains from CLA rabbits and patient of epileptic and non-epileptic brains. The data are demonstrated in Figs. 6, 7 and 8 respectively.

However, the oxygen consumption itself was higher in the cases of epileptic

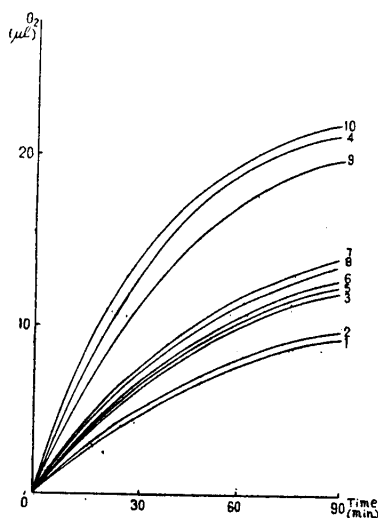


Fig. 7. O_2 consumption of the homogenate of non-epileptic human brain (Mean value from the brains of 8 animals) Buffer: M/10 tris-buffer, pH 6.8.

1. GABOB (—)
2. GABOB only
3. GABOB + FAD
4. GABOB + ATP-Na salt
5. GABOA + ATP-K salt
6. GABOB + Cytochrome C
7. GABOB + $MgCl_2$
8. GABOB + B_6
9. GABOB + DPN
10. Complete system

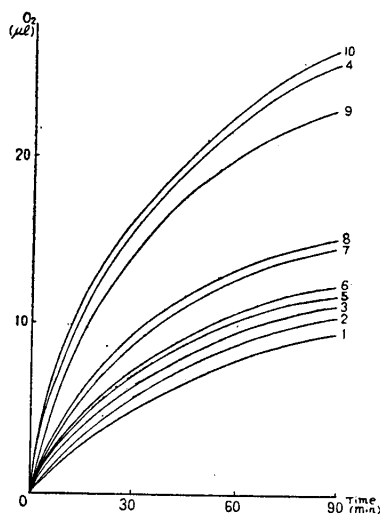


Fig. 8. O_2 consumption of the homogenate of epileptic human brain (Mean value from the brains of 8 animals) Buffer: M/10 tris-buffer, pH 6.8.

1. GABOB (—)
2. GABOB only
3. GABOB + FAD
4. GABOB + ATP-Na salt
5. GABOB + ATP-K salt
6. GABOB + Cytochrome C
7. GABOB + $MgCl_2$
8. GABOB + B_6
9. GABOB + DPN
10. Complete system

human brain and CLA rabbit brain comparing with the cases of non-epileptic human brain and normal rabbits brain.

DISCUSSION

SUGIURA's results showed that the optimal pH for the degradation of GABOB in brain tissue was around 9.0 and at pH 7.0 there could be recognized no activity. And yet he asserted that an inorganic phosphate is required for the GABOB decomposition, showing that the decomposition of GABOB does not occur when

glycyl glycine or veronal buffer is used. In the present experiment the oxygen consumption of brain was most markedly increased at pH 6.8 and the increment was observed equally in both cases where the tris-buffer and phosphate buffer were used, showing no reference to the presence of inorganic P.

As demonstrated in the experiments, ATP-Na salt and DPN showed the effect to increase markedly the oxygen consumption of brain tissue both of human and rabbits but GABOB showed only a slight effect in accelerating the O_2 consumption. This result seems to mean that the oxygen consumption of brain may not be so closely correlated with oxidative decomposition of GABOB, but there is a possibility that the oxidation of GABOB by brain tissue may require the presence of ATP-Na salt or DPN.

However, the fact that GABOB has an effect to suppress excitability of the motor center, is still an attractive problem for the clinics of epilepsy, and it is reasonably deduced that the decreased contents of GABOB in the brain may be responsible for the epileptic attack. The author's experiments showed a higher oxygen consumption in the epileptic human brain as well as in CLA rabbits brain than in non-epileptic human and normal rabbit brains. This fact suggests that GABOB content may decrease in epileptic human brain and in CLA rabbit brain by the accelerated oxidation and by subsequent decomposition, though there are still many problems to be solved to attain definitive result.

CONCLUSION

With the purpose to see if GABOB is in any way concerned with the mechanism of the epileptic attack observations were carried on the oxygen consumption of the brain homogenates of rabbits, normal and CLA, and of human, epileptic and non-epileptic. The experiment proved that the oxygen consumption is increased in the epileptic brain and in the brain of CLA rabbit. It was raised by adding ATP-Na salt or DPN, but GABOB itself showed only a slight effect. The results suggested that the oxygen consumption of brain is not so closely correlated with GABOB, but there is a possibility that the decrease in GABOB contents in epileptic brain by the accelerated decomposition with its elevated oxygen consumption may be correlated to the epileptic attack, though the final conclusion requires further observations.

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