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Stubies on γ-amino-β-hydroxybutyric acid I. Influences of γ-amino-β-hydroxybutyric acid on the blood preure, respiration and body temperature

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Stubies on γ-amino-β-hydroxybutyric acid I. Influences of γ-amino-β-hydroxybutyric acid on the blood preure, respiration and body temperature*

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Abstract

The biological specificity of GABOB on the blood pressure, respiration and body temperature was observed in dogs. The results show that GABOB has the similar action as GABA on the lowering of blood pressure as shown by subcutaneous, intravenous or intrathecal injection, but loses its action on the respiration. The specificity of GABOB action on the blood pressure is seen in the initiation of the effect at which the transient rise in pressure can be seen, the like of which can never be seen in the case of GABA injection. The lowering mechanism of GABOB on blood pressure should be the central one as the intrathecal injection is most effective comparing with those of intravenous and subcutaneous injection.

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STUDIES ON γ-AMINO-β-HYDROXYBUTYRIC ACID

I. INFLUENCES OF γ-AMINO-β-HYDROXYBUTYRIC ACID ON THE BLOOD PRESSURE, RESPIRATION AND BODY TEMPERATURE*

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Ever since the discovery of γ-aminobutyric acid (GABA) in the brain tissue by AWAPARA *et al.*[1] and ROBERTS *et al.*[2] in 1950, the biological importance of this acid has been reported by many authors. The acid is found to be contained considerably high in the brain tissue, and to be produced from glutamic acid, which is an essential substance for the function of brain. GABA itself also plays an important role for the function of the central nervous system[3,4,5,6,7].

TAKAHASHI *et al.*[8,10,17,18], IINO[9,11], HAMA *et al.*[12,13,19], TAKAYASU[14], SAKAE *et al.*[15] and TERASHI *et al.*[16,20] have reported that GABA acts as to lower the blood pressure and gives some effects on the respiration, when it is introduced into vein, subcutaneous tissue or cerebellomedullary cistern.

γ-Amino-β-hydroxybutyric acid (GABOB) produced by the direct oxidation of GABA in the brain is also known as a substance which acts as to depress the function of the motor center. But it is not yet well established whether or not GABOB could give any effects on the function of autonomic nervous system. In this connection the author intended to examine the influences of GABOB on the blood pressure, respiration and body temperature. In this paper, the observations on these functions after the introduction of GABOB into subcutaneous tissue, vein or cerebrospinal cavity of animals are reported.

MATERIALS AND METHODS

Eighty-five adult dogs of both sexes, weighing 5 to 15 kg were used. These animals were devided into 4 groups, 19 animals including 3 controls in each. The animals belonging to the first group received GABOB intravenously, 10 mg/kg of body weight in 1 per cent solution in 8 animals, 10 to 30 mg/kg of body

* The outline of this paper was presented at the 8th Meeting of the Chugoku-Shikoku Biochemical Society.

weight in 10 per cent solution in 8 animals. Other 3 animals, controls, were introduced with 3 to 5 ml of physiologic saline solution from the same route. Of the animals belonging to the second group, 16 animals were injected with GABOB into subcutaneous tissue, and 3 control animals with physiologic saline solution. The amounts of the introduced GABOB and saline were the same as those in the cases receiving intravenous injections. The animals of the third group received intrathecal injections. The animals of the third group received intrathecal injections, 16 animals received GABOB and 3 animals saline. The amounts of the injected GABOB and saline were the same as in the former 2 groups. The animals belonging to the last group, 24 animals, were used to confirm the minimum dose of GABOB required to cause the fall in blood pressure. These were subdivided into 3 groups, 8 animals in each, one group for the intravenous, the other one for subcutaneous and the last group for intrathecal injections. GABOB used in these groups was of 1 per cent or 0.1 per cent solution. Prior to the injection the animals were anesthetized by injecting 1 g of urethane and 2 mg of morphine/kg of body weight. The blood pressure in the femoral artery was measured by inserting a cannula connected directly to the mercury manometer. The curve of respiration was recorded on a kymographion connected to an accordion bandage set surrounding the chest of the animals. The rectal temperature was measured.

Crude material GABOB was donated by Ono Parmaceutical CO., Ltd. GABOB used for this experiment was obtained from this material by recrystallization from the saturated solution adding pure ethanol till the solution became muddy. This was left standing for 24 hours at 1° to 5°C and supernatant was decanted. The precipitate was washed with ethanol twice and dried at room temperature. Obtained crystals were used as a solution in distilled water and the pH was adjusted to 7.0—7.3 by adding 0.1N NaOH. Intravenous injection was done into the femoral vein, subcutaneous injection into the femoral subcutaneous tissue and intrathecal injection into the cerbellomedullary cistern after exposing dura mater by laminectomy.

RESULTS

Influence of injected GABOB on the blood pressure

In the case of intravenous injection : The introduction of 10 mg to 30 mg of GABOB/kg of body weight at 1 per cent or 10 per cent solutions resulted in a transient rise in blood pressure in 15 cases of 16 animals. The blood pressure raised immediately after the injection by 2 mmHg to 10 mmHg and then fell rapidly reaching the minimum value already 12 to 36 seconds after the injection, with the fall by 22 mmHg to 62 mmHg. Thereafter, it commenced to rise gradually and 13 minutes to 23 minutes after the injection it returned to the original level as shown in Tables 1, 2 and Fig. 1. The minimum dose that brings about the

Table 1. The effect of GABOB on the blood pressure of dogs. The animals received intravenous injection of 1 per cent solution once in each. The values show the maximum and minimum points of the pressure level after the injection.

Case No.	Body weight of dog (kg)	GABOB (mg/kg)	Blood pressure bofore injection (mm/Hg)	Transient rise in blood pressure after injection		Fall in blood pressure after injection		Time required to reach minimum level	Time required for recovery
				elevated pressure (mm/Hg)	increase (mm/Hg)	decreased pressure (mm/Hg)	decrease (mm/Hg)		
1	7	10	148	148	0	124	−24	36″	16′
2	15.7	10	136	146	+10	86	−50	24″	20′25″
3	5	10	142	150	+ 8	98	−44	23″	20′
4	6.6	10	116	124	+ 8	96	−22	12″	13′
5	8.7	10	132	136	+ 4	106	−26	32′	12′30″
6	6.3	10	122	126	+ 4	80	−42	28″	16′25″
7	9.2	10	118	120	+ 2	84	−34	24″	14′20″
8	7.5	10	142	148	+ 6	96	−46	28″	19′10″

Table 2. The effect of GABOB on the blood pressure of dogs. The animals given intravenous injection of 10 per cent solution once. The values show the maximum and minimum points of the pressure level after the injection.

Case No.	Body weight of dog (kg)	GABOB (mg/kg)	Blood pressure before injection (mm/Hg)	Transient rise in blood pressure after injection		Fall in blood pressure after injection		Time required to reach minimum level	Time required for recovery
				elevated pressure (mm/Hg)	increase (mm/Hg)	decreasod pressure (mm/Hg)	decrease (mm/Hg)		
1	7	30	160	162	+ 2	98	−62	29″	20′30″
2	15.7	10	128	132	+ 4	76	−52	29″	17′
3	6.6	30	156	158	+ 2	96	−60	27″	18′10″
4	8.7	10	134	144	+10	86	−48	11″	19′
5	6.6	10	128	136	+ 8	76	−32	21″	20′20″
6	9.4	30	146	152	+ 6	94	−52	26″	22′40″
7	5.7	30	128	136	+ 8	72	−56	29″	23′15″
8	5.5	30	156	166	+10	108	−48	32″	19′20″

fall in blood pressure by the intravenous injection of 0.1 per cent GABOB solution was 200γ to 500γ/kg of body weight (Table 3).

No marked difference in the patterns of fall in blood pressure was recognized between the cases in which GABOB was injected by 10 mg and 30 mg/kg of body weight. The difference in the concentration of GABOB solution gave also no significant change on the patterns of the fall in blood pressure, though the larger the total amount of GABOB given per kilogram of body weight was, the deeper the fall in the pressure.

Fig. 1. The change in blood pressure in a dog (15.7 kg) seen after a single injection of 10 per cent GABOB solution 10 mg/kg intravenously, total amount 15.7 ml. B. P. : Blood pressure, Resp. : Respiration, Time : 3 sec.

Table 3. Minimum dose required to cause the fall in blood pressure of the dog in the case of intravenous injection of GABOB (0.1 per cent).

Case No.	Minimum dose (γ)
1	500
2	300
3	500
4	200
5	300
6	200
7	300
8	500

In the case of subctaneous injection : The blood pressure fell slowly and gradually comparing with those injected intravenously and it reached the lowest level 5 minutes to 15 minutes later, showing the fall by 8 mmHg to 36 mmHg (Tables 4, 5 and Fig. 2). Subsequently it started to rise very slowly and by 36 minutes to 53 minutes after the injection the pressure returned to the original level. The minimum dose bringing about the fall in the blood pressure tested by using 1 per cent GABOB solution was 5 mg to 10 mg/kg of body weight(Table 6). The patterns of the fall hardly varied in the animals treated with the 1 per cent and 10 per cent of GABOB solutions, whereas the depth of the fall was deeper if the total amount was larger, similar as in the cases treated with intravenous

Table 4. The effect of GABOB on the blood pressure of dogs. The animals given subcutaneous injection of 1 per cent solution once. The values show the maximum and minmum points of the pressure level after the injection.

Case No.	Body weight of dog (kg)	GABOB (mg/kg)	Blood pressure before injection (mm/Hg)	Transient rise in blood pressure after injection (mm/Hg)	Fall in blood pressure after injection		Time required to reach minimum level	Time required for recovery
					decreased pressure (mm/Hg)	decrease (mm/Hg)		
1	7	5	114	(−)	104	−10	5′12″	41′
2	7	10	92	(−)	80	−12	6′18″	35′30″
3	12	10	116	(−)	108	− 8	13′	40′
4	5.6	10	132	(−)	120	−12	15′20″	38′10″
5	8.2	10	146	(−)	126	−20	7′30″	41′
6	5.6	10	118	(−)	106	−12	12′	52′40″
7	6.5	10	124	(−)	108	−16	11′40″	47′
8	7.7	10	136	(−)	124	−12	9′20″	42′20″

Table 5. The effect of GABOB on the blood presure of dogs. The animals given subcutaneous injection of 10 per cent solution once. The values show the maximum and minimum points of the pressure level after the injection.

Case No.	Body weight of dog (kg)	GABOB (mg/kg)	Blood pressure before injection (mm/Hg)	Transient rise in blood pressure after injection (mm/Hg)	Fall in blood pressure after injection		Time required to reach minimum level	Time required for recovery
					decreased pressure (mm/Hg)	decrease (mm/Hg)		
1	7	30	118	(−)	92	−26	5′18″	46′
2	7	100	122	(−)	92	−30	12′	40′30″
3	12	30	134	(−)	98	−36	6′20″	45′
4	5.6	30	152	(−)	134	−18	13′	41′10″
5	7.4	30	124	(−)	96	−28	14′	53′
6	8.5	30	156	(−)	134	−22	5′30″	39′40″
7	9.2	30	142	(−)	118	−24	7′20″	38′20″
8	6.7	30	126	(−)	96	−30	5′20″	49′

introduction.

In the cases of intrathecal injection : A transient rise in the blood prssure by 8 mmHg to 30 mmHg occurred immediately afterwards and then fell rapidly reaching the lowest level within 39 to 100 seconds, at which the fall was 14 mmHg to 44 mmHg. Thereafter, rising gradually it tended to revert to the original level after 9 minutes to 29 minutes (Tables 7, 8 and Fig. 3). In the case of intrathecal injection the minimum dose of GABOB required for the fall in the pressure was 50γ to 100γ/kg of body weight as revealed by using 0.1 per cent

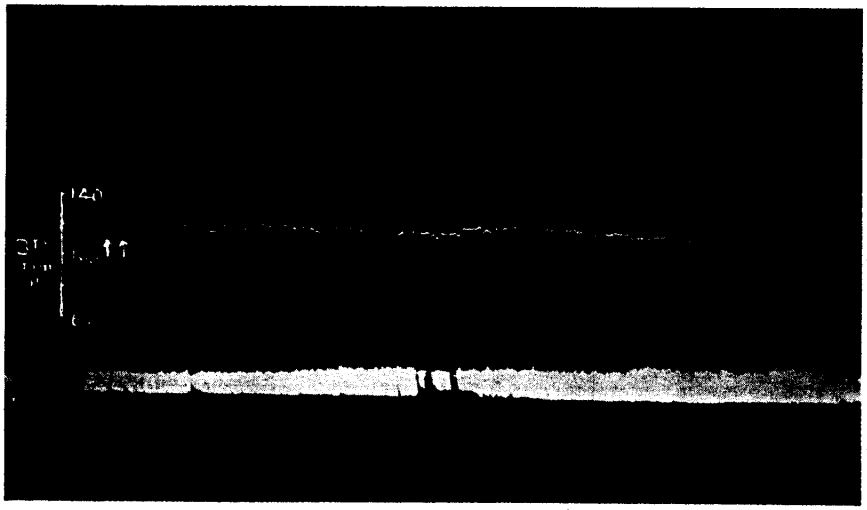

Fig. 2. The change in blood pressure in a dog (7 kg) seen after the single injection of 10 per cent GABOB solution 30 mg/kg subcutaneously, total amount 2.1 ml. B. P. : Blood pressure, Resp. : Respiration, Time : 3 sec.

Table 6. Minimum dose required to cause the fall in blood pressure of the dog in the case of hypodermic injection of GABOB (1 per cent).

Case No.	Minimum dose (mg)
1	5
2	10
3	10
4	8
5	5
6	10
7	7
8	10

GABOB solutions (Table 9). Hardly any difference in the patterns of the fall was recognized between those receiving 1 per cent and 10 per cent solutions, but the larger the dose of GABOB given per kg of body weight the more marked was the fall in the pressure.

Influence of GABOB injection on the respiration

Almost no effect could be recognized on the respiration, regardless of the routes of GABOB introduction, subcutaneous, intravenous or intrathecal.

Influence of injected GABOB on the body temperature

The body temperature showed only a slight decrease by 0.2°C to 0.3°C within 2 to 10 minutes after the injection, recovering to the original level by ten

Table 7. The effect of GABOB on the blood pressure of dogs. The animals given intrathecal injection of 1 per cent solution once. The values show the maximum and minimum pressure levels after the injection.

Case No.	Body weight of dog (kg)	GABOB (mg/kg)	Blood pressure before injection (mm/Hg)	Transient rise in blood pressure after injection		Fall in blood pressure after injection		Time required to reach minimum level	Time required for recovery
				elevated pressure (mm/Hg)	increase (mm/Hg)	decreased pressure (mm/Hg)	decrease (mm/Hg)		
1	7	10	98	106	+ 8	78	−20	57″	24′
2	15.7	5	108	118	+10	86	−22	46″	8′30″
3	9	10	116	128	+12	92	−24	1′10″	24′10″
4	8.6	10	126	134	+12	104	−22	58″	23′20″
5	6.5	5	102	132	+30	86	−16	1′10″	13′20″
6	5	5	102	116	+14	78	−24	1′30″	9′30″
7	8.8	10	146	158	+12	124	−20	1′20″	27′15″
8	7	10	138	148	+10	102	−26	1′15″	19′20″

Table 8. The effecct of GABOB on the blood pressure of dogs. The animals given intrathecal injection of 10 per cent solution once. The values show the maximum and minimum pressure levels after the injection.

Case No.	Body weight of dog (kg)	GABOB (mg/kg)	Blood pressure before injection (mm/Hg)	Transient rise in blood pressure after injection		Fall in blood pressure after injection		Time required to reach minimum level	Time required for recovery
				elevated pressure (mm/Hg)	increase (mm/Hg)	decreased pressure (mm/Hg)	decrease (mm/Hg)		
1	7	30	102	120	+18	88	−14	1′30″	25′10″
2	15.7	10	116	138	+22	96	−30	39″	17′20″
3	9	10	124	134	+20	98	−26	1′20″	26′18″
4	8.6	10	132	146	+14	102	−30	59″	29′20″
5	6.5	10	118	132	+14	96	−22	1′10″	11′20″
6	5	10	124	136	+12	84	−40	1′15″	23′30″
7	7.5	10	136	150	+14	92	−44	46″	20′10″
8	13.3	10	108	124	+12	70	−38	1′40″	24′

odd minutes to several hours. In all the three routes of injection both with the 1 per cent and the 10 per cent solution in the doses of 5 mg to 10 mg/kg of body weight, the results were almost the same, showing no marked change in body temperature.

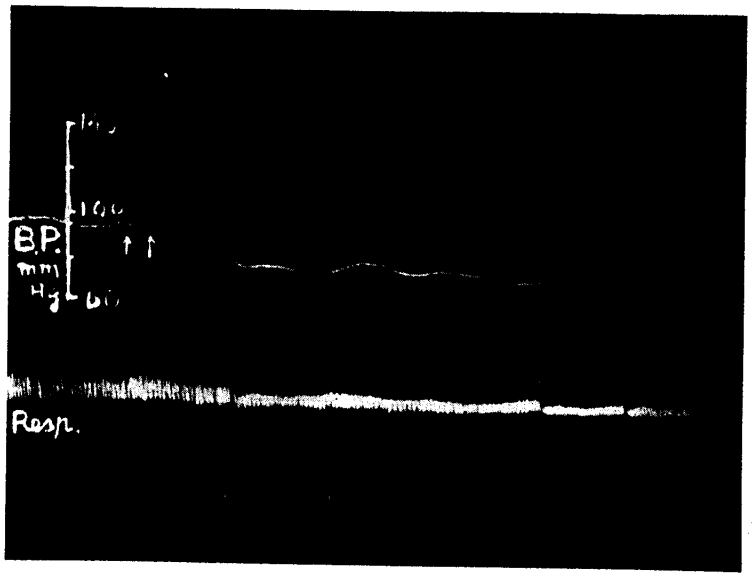

Fig. 3. The change in blood pressure in a dog (7 kg) seen after the single injection of 1 per cent GABOB solution 10 mg/kg intrathecaly, total amount 7 ml. B. P. : Blood pressure, Resp. : Respiration, Time : 3 sec.

Table 9. Minimum dose required to cause the fall in blood pressuse of the dog by the injection of GABOB (0.1 per cent) into cerebellomedullary cistern.

Case No.	Minimum dose (γ)
1	50
2	100
3	100
4	50
5	100
6	50
7	80
8	100

DISCUSSION

As has been briefly described, the works of TAKAHASHI, IINO and others revealed that GABA acts as to lower the blood pressure and to disturb the respiration. These findings are especially interesting in the point whether the oxidation product of GABA, GABOB, still has such effects or lose the biological specificity of GABA on the blood pressure and respiration. The results showed that GABOB has an action to lower the blood pressure but no effect at all on the respiration with some lowering effect on the body temperature. The fall in

the blood pressure was marked and rapid when the injection was made into the vein or cerebrospinal space comparing with the cases of subcutaneous injection, which required a larger dose of GABOB/kg of body weight in order to attain the similar depth of the pressure fall as in one former case and it took considerably longer time to reach the minimum level, though the pressure lowering effect of GABOB persisted for a longer period of time in the cases of intravenous and intrathecal injections. These data show that GABOB formed by oxidation of GABA still retains the lowering action on the blood pressure as in GABA, though it loses its action on the respiration.

However, the falling pattern of the blood pressure is different from that seen in the animals with GABA, in the point that lowering of the blood pressure by GABOB injection is always preceded by a transient rise in pressure in the cases of intravenous and intrathecal injections (Fig. 4). This suggests that GABOB has a physiologic action different from GABA.

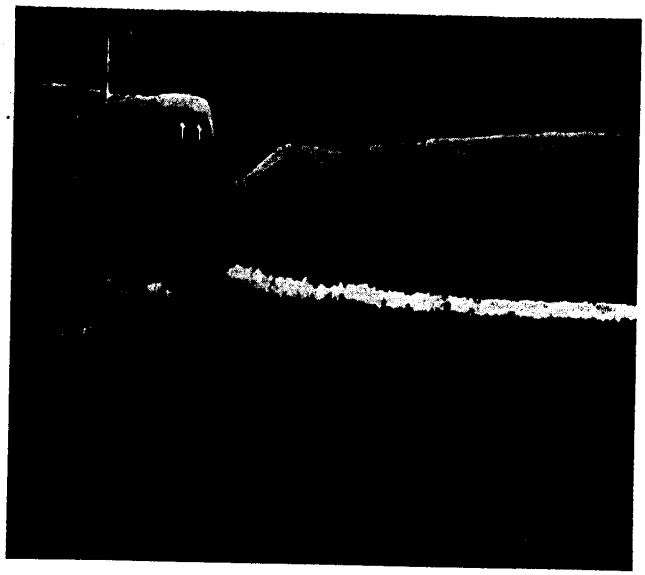

Fig. 4. The change in blood pressure in a dog (8.7 kg) seen after the single injection of 10 per cent GABA solution 10 mg/kg intravenously, total amount 8.7 ml. B. P. : Blood pressure, Resp. : Respiration, Time : 3 sec.

As for the mechanism of the blood lowering effect of GABOB, it is supposed that this substance acts primarily on the vasomotor center. The reason lies in that the minimum dose required to induce a fall in blood pressure was far smaller in the case of intrathecal injection than other two routes of injection and when 10 mg to 100 mg of 1 per cent and 10 per cent GABOB solution/kg of

body weight was injected into cerebellomedullary cistern, several animals died, and the fall in blood pressure persisted much longer than intravenous injection.

The slight lowering of body temperature seen after the GABOB injection will probably be due to the fall in blood pressure but not due to the primary action of GABOB.

CONCLUSION

The biological specificity of GABOB on the blood pressure, respiration and body temperature was observed in dogs.

The results show that GABOB has the similar action as GABA on the lowering of blood pressure as shown by subcutaneous, intravenous or intrathecal injection, but loses its action on the respiration. The specificity of GABOB action on the blood pressure is seen in the initiation of the effect at which the transient rise in pressure can be seen, the like of which can never be seen in the case of GABA injection. The lowering mechanism of GABOB on blood pressure should be the central one as the intrathecal injection is most effective comparing with those of intravenous and subcutaneous injection.

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