ON THE SITE OF ACTION OF γ -AMINOBUTYRIC ACID ON BLOOD PRESSURE

HIDEHIKO TAKAHASHI, MASAKO TIBA, TOSHIHITO YAMAZAKI
AND FUMIO NOGUCHI*

Tokyo Medical College, Tokyo

In the preceding paper (1), the authors reported that γ -aminobutyric acid which exists abundantly in the nervous tissue (2, 3), caused fall of blood pressure, when injected intravenously and that its effect was the most powerful among ω -aminoacids. In the present investigation, the mechanism of the fall of blood pressure and the site of action of this acid are the main concern.

METHODS

Rabbits, dogs and cats were used. They were anesthetized with 25% urethane (1-1.5 g. per Kg). given either intramuscularly or intraperitoneally. The carotid arterial pressure was recorded by a mercury manometer. Respiration was recorded by a tambour connected to a tracheal cannula. The heart rate was calculated from the electrocardiogram. The sympathetic preganglionic fibres were stimulated with square wave electric pulses at a distance of 4-5 cm from the superior cervical ganglion. In case of arterial injection of drugs, the retrograde injection method of McDonald and Potter (4) was used. In order to know changes of the peripheral circulation, the plethysmographic or oncometric measurement was made on various organs.

To denervate the carotid sinus, the sinus nerve was severed and the wall of the vessel at the level of the ramification of the common carotid artery was deprived of connective tissues and painted with 5-10% carbolic acid solution. The decerebration was done under ether anesthesia and experiments were started from 1-2 hrs. after the operation.

Topical stimulation in the medulla oblongata was effected with a stereotactically oriented monopolar silver electrode insulated except at the tip and not exceeding 10 μ in tip diameter. Another large silver electrode was placed on the subcutaneous tissue of the neck. A stimulator yielding square-wave pulses of 2-8 v. with an output of 5000 ohms was used. The pulses were applied for 5-10 seconds at the frequency of 50 cps. and the duration of each pulse was 10 msec.

For stimulation in the medulla oblongata, the first cervical vertebra, the atlanto-occipital membrane and the occipital region of the skull were exposed.

Received for publication May 15, 1958.

* 高橋日出彦, 千葉正子, 山崎寿仁, 野口文夫

The atlanto-occipital membrane was opened by a longitudinal section in the midline and the floor of the 4th ventricle was exposed. In most experiments the distal part of the medulla oblongata was exposed by sucking away the caudal portion of the cerebellum. In other experiments the electrode was pushed through the cerebellar tissue and oriented within the medulla by means of the Horsley-Clark instrument alone.

For topical injection of γ -aminobutyric acid in the medulla, a fine glass capillary with the tip of $50\ \mu$ in outside diameter was used. This capillary was connected with a fine vinyl tube to a small glass syringe whose plunger was operated with the spindle of a micrometer. The limit of accuracy of delivery was about 2×10^{-4} ml. Orientation of a capillary was also carried out stereotaxically.

For partial destruction of the medulla oblongata, thermocoagulation was performed with a small thermo-electric cautery. At the conclusion of an experiment the brain was removed, hardened in formalin and examined macroscopically. Then, the brain was sectioned and stained with van Gieson's procedure, or with hematoxylin-eosin method. Transections of the brain stem were performed with the sweep of a scalpel. Care was taken to avoid cutting into the basilar artery. After an experiment the brains were hardened in formalin and examined the exact level and completeness of the transection.

RESULTS

The fall of blood pressure and bradycardia.

Fig. 1 shows the fall of blood pressure and bradycardia following an intravenous injection of γ -aminobutyric acid in a dog. The bradycardia was not very severe (the heart rate never decreased to half) and sometimes failed to occur. When it appeared, its degree and time course were not necessarily parallel with those of the fall of blood pressure.

In order to observe changes of the mechanical activity of the heart, a small balloon was inserted at the apex region into the pericardium and the pressure change in the balloon was led to a tambour and recorded on the kymograph. No change or a slight increase in the heart stroke was found by this method while the arterial pressure and heart rate fell moderately by injection of the acid.

The direct action of γ -aminobutyric acid.

a) Intravenous injection of γ -aminobutyric acid in animals, whose spinal cords were sectioned transversely at an upper level, caused neither bradycardia, nor the fall of blood pressure. In these preparations stretch and flexor reflexes disappeared completely and blood pressure was very low, but acetylcholine chloride still caused fall of pressure (fig. 2).

b) The perfusion with γ -aminobutyric acid of isolated hearts of Japanese toads was performed, and it was found that this acid (10^{-6} – 10^{-3} mole/l) did not show any appreciable effect on both the mechanical and electrical activity of the toad's heart directly.

FIG. 1. The effect of γ -aminobutyric acid on respiration, blood pressure and heart rate.

Dog. (10 Kg) Room temperature 22°C. 1 cc. of 10% γ -aminobutyric acid (m.p. 199-201°C). was injected in the external jugular vein.

Resp.-respiration, B.P.-blood pressure.

H.R.-heart beats per minute.

Time mark-6 seconds.

FIG. 2. The effect of γ -aminobutyric acid in an acute spinal dog. Dog. (10 Kg) R.T. 22°C. The spinal cord was transected between 1st and 2nd cervical cord.

1 cc. of 10% γ -aminobutyric acid was injected in the external jugular vein.

As a control 10 γ (in 1 cc.) of acetylcholine chloride was injected subsequently.

The fall of blood pressure and volume changes of various organs.

a) Changes in the volume of the paw. Both the plethysmogram of the paw and blood pressure were recorded at the same time, while γ -aminobutyric acid was injected intravenously. The decrease in the volume of the paw was usually observed in parallel with the fall of pressure in dogs. But in cats, the increase of the volume of the paw was sometimes observed in spite of the fall of pressure, especially when the injection was made into cisterna cerebello-medullaris. The increase of the volume of the paw could be more easily seen

after bilateral severance of the splanchnics, in dogs as well as in cats.

b) Changes in the volume of the spleen. An increase in the volume of spleen was found in intact dogs, starting at the fall of arterial pressure and persisting for a few minutes. But, after denervation of the spleen, its volume decreased in parallel with the fall of pressure. (fig. 3)

FIG. 3. The effect on the blood pressure and the volume of the spleen.

A: Dog. (9 Kg) R.T. 23°C, 1 cc. of 10% γ -aminobutyric acid was injected in the external jugular vein.

Resp.-respiration, B.P.-blood pressure.

S.V.-the oncogram of the spleen.

B: Dog. (11.4 Kg) R.T. 27°C. The spleen was denervated. 1 cc. of 2.5% γ -aminobutyric acid was injected in the external jugular vein.

The effect of γ -aminobutyric acid after severance of various nerves.

From the data described above, it seems quite possible that γ -aminobutyric acid induces the fall of blood pressure through the nervous system. Experiments of division of various nerves were therefore performed.

It was found that the severance of the cervical vagi, cervical sympathetics, and depressor nerves combined with the sinus denervation on both sides caused no appreciable change in the effect of γ -aminobutyric acid on the arterial pressure. But, vagotomy resulted in some changes in the bradycardia induced with this acid, that is, the heart rate sometimes did not change, or the decrement of the heart rate did not occur so much strongly as before the section. The results were similar after atropinization.

The effect of γ -aminobutyric acid and autonomic ganglia.

Subsequently, the relation between the depressant action of γ -aminobutyric acid on blood pressure and the autonomic or especially sympathetic ganglia was investigated. At first, we tested the direct action of this acid on the superior cervical sympathetic ganglion of cats. The contraction of the nictitating membrane induced with electrical stimulation of the preganglionic fibres was used as indicator. γ -Aminobutyric acid caused no changes in the

contraction of the nictitating membrane induced by preganglionic stimulation (fig. 4).

FIG. 4. The effect of γ -aminobutyric acid on the superior cervical ganglion. Cat (3.0 Kg) R.T. 27°C.

A-control, B-the inhibition of the contraction of the nictitating membrane following the intravenous injection of hexamethonium, C-no change in the contraction following the intravenous injection of γ -aminobutyric acid.

The contraction of the nictitating membrane was elicited by the electrical stimulation of the preganglionic fibres with square wave pulses at the freq. of 10 per second. The injections were made in the femoral vein during the electrical stimulation.

Int. 5-35 mv. Int. 6-37 mv. Int. 10-60 mv.

FIG. 5. The disappearance of the effect of γ -aminobutyric acid following the administration of tetraethylammonium bromide. Dog (5 Kg) R.T. 12°C.

1 cc. of 2% γ -aminobutyric acid was injected in the saphenous vein, after the intravenous administration of TEAB (25 mg).

γ -Aminobutyric acid had no inhibiting effect on the superior cervical sympathetic ganglion, but following the intravenous administration of autonomic ganglionic blocking agents, such as tetraethylammonium bromide or hexamethonium bromide, it affected no longer blood pressure. After the autonomic ganglia had recovered from the action of such agents, the effect of this acid on blood pressure reappeared (fig. 5).

Observation on the latency of the effects.

With presumption that changes in the latent period for the outbreak of the effect of γ -aminobutyric acid due to differences in the routes of its administration may reveal, though roughly, the site of its action, observations as specified in table 1 were performed. As can be seen from this table, the latent period was the longest when the acid was injected into arteries. To our surprise, the latency with the internal carotid injection was of the same order as that of the injection into the subaxillary artery. The latency was much shorter on intravenous injection, but shortest when injected into the vertebral artery. This seems to suggest that the site of action of this acid lies in the stream bed of the vertebral arteries.

Experiments with intracisternal injections.

γ -Aminobutyric acid when injected into the cerebello-medullar cisterna, caused also bradycardia and fall of blood pressure. The fall of blood pressure lasted for much longer time (sometimes for 1-2 hrs.) than in the intravenous injection and the inhibition of respiration was also much stronger. As a rule, bradycardia was more or less induced even after the section of the cervical vagi. Fig. 6 presents one example representative of the intracisternal injections.

FIG. 6. The effect of γ -aminobutyric acid injected intracisternally. Rabbit (2.1 Kg) without anesthesia.

0.5 cc. of 1% γ -aminobutyric acid were injected into the cerebellomedullar cisterna.

TABLE 1.

Experi- mental animals	Absences of innervations	The route of injection
Dog	The denervation of carotid sini. otherwise intact.	The internal carotid artery (sinist). The subaxillary. artery (dext.) The external jugular. vein (dext.)
	The denervation of carotid sini. and the severance of vago-sympa-thetic nerves on both sides.	The external jugular vein (dext.) The subaxillary artery (dext.) The internal carotid artery (sinist.)
Dog	The denervation of carotid sini. otherwise intact.	The vertebral artery (sinist.) The vertebral artery (sinist.) The external jugular vein. (dext.)
	The denervation of carotid sini. and the severance of vago-sympathetic nerves on both sides.	The vertebral artery (sinist.) The external jugular. vein (dext.)
Dog	Intact	The vertebral artery (dext.) The external jugular vein (sinis.)
Dog	The denervation of carotid sini. otherwise intact.	The vertebral artery (dext.) The internal carotid (sinist.) The vertebral artery (dext.) The external jugular vein. (sinist.) The subaxillary artery (dext.) The subaxillary artery (dext.)
Dog	The severance of vago-sympa-thetic nerves.	The external jugular vein. (sinist.) The vertebral artery (sinist.) The vertebral artery (sinist.) The vertebral artery (sinist.) The external jugular vein. (sinist.)

The fall of blood pressure induced with γ -aminobutyric acid and the medulla oblongata.

(1) The level of transections of the brain stem and the effect of γ -aminobutyric acid.

In decerebrate animals (cats, dogs and rabbits), γ -aminobutyric acid caused the same degree of the fall of blood pressure and bradycardia as in intact animals. Changes in the effect of this acid were not observed, unless the rhombencephalon was transected at some suitable level. Thus, serial transections of the rhombencephalon of rabbits were carried out.

Fig. 7 shows a typical example of such experiments. At first, a transection was performed at a level of the brachium pontis (Section I). The level of blood pressure was decreased transiently with the sections, but soon recovered to the initial value. After this transection, no change in the effect of γ -aminobutyric acid was observed.

Subsequently, the brain stem was transected at the level slightly rostral to the lower edge of the tuberculum acusticum (Section II). After this section, the level of blood pressure was decreased considerably, but the effect of γ -aminobutyric acid on blood pressure remained unchanged or increased more or

The concentration of γ -aminobutyric acid injected (%)	The velocity of injection	Latency in seconds	The fall of Blood pressure as the percentage of the initial value	Remarks
0.2	0.5 cc/ 6"	27	31	
0.2	0.5 cc/ 6"	24	35	
0.2	0.5 cc/4-5"	11	47	
0.2	0.5 cc/ 3"	11		
0.2	0.5 cc/ 5"	24	29.5	
0.2	0.5 cc/ 8"	34	29.5	
1.0	0.5 cc/ 10"	9	28	*The latency of the bradycardia 11sec.
1.0	0.5 cc/ 4"	5*	40	
1.0	0.5 cc/ 4.5"	11	32	
1.0	0.5 cc/ 6.5"	12	40	
1.0	0.5 cc/ 6"	16	36	
10	1 cc/ 10"	8	35	
10	1 cc/ 7"	12	26	
10	0.5 cc/ 6"	5	46	
10	0.5 cc/ 6"	20	39	
10	0.5 cc/ 6"	6	46	
10	0.5 cc/ 6"	14.4	21	
	0.5 cc/ 4.5"	21	7	
	1.0 cc/ 13"	25	15	
10	0.5 cc/ 5"	11	24	No change in the heart rate
10	0.5 cc/ 6"	6	48	
10	0.5 cc/ 6"	7	54	
10	0.5 cc/ 8.5"	8	41	
10	0.5 cc/ 7.5"	12	40	

less.

At last, the third section at the level of the obex produced a maximal fall in the level of blood pressure together with an almost complete disappearance of the effect of this acid.

(2) Effect of medullary cauterization on the action of γ -aminobutyric acid.

In figure 8 are shown the alterations in the action of γ -aminobutyric acid on blood pressure resulting from cauterization of the medulla. The area which was ablated extended between the obex and 3 mm rostral to the obex and completely across the floor of the ventricle from side to side. After cauterization to a depth of 1.5 mm below the floor of the fourth ventricle, blood pressure remained at the same level as before the operation. But, the depressant effect of γ -aminobutyric acid diminished remarkably and its initial transient pressor action became rather evident.

Both in other preparations where cauterizations were circumscribed within the area postrema and in preparations in which lesions were placed on the caudal region of the floor of the fourth ventricle exclusive of the area postrema the reduction of the depressant action of γ -aminobutyric acid was observed as well.

FIG. 7. Influence of brain stem transections on the effect of γ -aminobutyric acid. Rabbit (2.5 Kg) R.T. 21°C.

1.0 cc. of 0.5% γ -aminobutyric acid was injected intravenously. After the section II, respiration was arrested and artificial respiration was started at once.

FIG. 8. Partial elimination of the medulla and the effect of γ -aminobutyric acid. Rabbit (2.2 Kg) R.T. 26°C. 2 cc. of 0.1% γ -aminobutyric acid were injected intravenously. A-the effect of γ -aminobutyric acid before the elimination.

B-the effect observed 1 hr. afterwards from the elimination.

C-a photograph of the brain stem. The eliminated region was covered with graphite powder for photographing.

D-a section through the obex (van Gieson's stain).

(S-electrical stimulation of the depressor nerve).

Topical application of γ -aminobutyric acid in the medulla oblongata.

At first, the distribution of the points which responded with a fall in arterial pressure to electrical stimulation and that of the points which showed an increase in arterial pressure were observed in the 17 rabbits' rhombencephalon. We confirmed that the distribution of pressor and depressor points in the rabbit's rhombencephalon was in good agreement with that observed by Alexander (7) in the cat.

FIG. 9. Topical application of γ -aminobutyric acid.

Black circles represent the sites which respond to γ -aminobutyric acid with the fall of blood pressure.

Black triangles represent the points which show no response. Quanta injected were 1–10 γ in 10^{-3} ml.

Pressor areas indicated by cross hatching, depressor regions by horizontal ruling.

Legend: A.C.: Ala cinerea; E.N. Trg.: Eminentia nuclei triangularis; Tub. a.c.: Tuberculum acusticum; Br. P. + Con.: Brachium pontis et Brachium conjunctivum.

FIG. 10. The microinjection of γ -aminobutyric acid in the area postrema.

Rabbit (2.2 Kg). R.T. 25°C. 5 μ g of γ -aminobutyric acid were injected. The stick point was macroscopically determined post mortem, after the area postrema was stained with 1% trypan blue injected into the carotid artery.

Sign 1 Penetration of a microtube in the area postrema on the left side.

Sign 2 Injection.

Sign 3 Withdrawal of the microtube.

Subsequent to such observations, a few $\mu\text{g.}$ of γ -aminobutyric acid was injected into various points of the medullar substance through a fine glass capillary of $50\ \mu$ in diameter by the stereotaxic technique. Thus, the points that responded with a fall of blood pressure to γ -aminobutyric acid were explored thoroughly in the medulla oblongata, using 20 rabbits. The distribution of the points that responded to this acid and of the silent points which showed no response is mapped out in fig. 9.

The great majority of the points sensitive to this acid were found between the level of the lower edges of the auditory tubercles and 2 mm caudal to the obex, medial from the lateral edges of the floor of the fourth ventricle.

Although the depressant effect of this acid varied with the depth of narcosis, the microinjection of $0.5\text{--}1.0\ \mu\text{g.}$ in 10^{-3} ml induced usually a transient fall of blood pressure. Fig. 10 shows the depressant effect with topical application of this acid in the area postrema. In the medulla of the rabbits, no point was found that responded with an increase in arterial pressure to γ -aminobutyric acid.

DISCUSSION

It was shown by the present experiments that the fall of arterial pressure by γ -aminobutyric acid is not due to its peripheral action on the blood vessels or autonomic ganglia, or to sino-aortic reflexes, but due to the action on the brain. The injection of this acid into the vertebral artery caused a prompt decrease of arterial pressure and a more striking decrease was resulted by the injection into the cisterna magna. It was finally demonstrated by injections into the medulla oblongata that γ -aminobutyric acid would act on depressor points in the medial reticular formation and the points situated in the area postrema. The experiments with partial elimination or sections of the medulla showed the similar results. The presence of depressor points in the medulla has been reported by many investigators (5, 7, 8, 9). Ranson *et al.* (10) showed that electrical stimulation of the area postrema caused a fall of arterial pressure. The data obtained by us present an important fact that the depressor points in the medial reticular formation and the area postrema are so sensitive as to react to changes in the concentration of γ -aminobutyric acid within the physiological extents. Further, an intimate relation between γ -aminobutyric acid and the ascending reticular activating system (Magoun) has been recognized by various investigators (11, 12, 13).

Oncometric observations on the spleen presented some evidence for active vasodilation occurring concomitantly with the fall of arterial pressure, while little or occasional volume increase was seen in the paw. It seems therefore certain that the vasodilation in the splanchnic area induced from the medullary centers is at least one of the essential factors causing the fall of arterial pressure.

However, active vasodilation in the paw occurred constantly after the splanchnic section. Therefore, the authors think that it should be true that vasodilation in the skin is also present in the intact animals, but owing to

severe vasodilation in the splanchnic region, this vasodilation is not accompanied with volume increase in the skin.

The fall of pressure induced with this acid was usually accompanied with a slight bradycardia. But, after the section of the cervical vagi, sometimes occurred solely the fall of blood pressure without changes in the heart rate. And in the case of vertebral injection, bradycardia sometimes occurred considerably late after the fall of pressure (ref. Table 1). As the consequence, the fall of blood pressure seems not to be due to bradycardia alone. The finding that complete disappearance of the bradycardia by the section of the cervical vagi was comparatively rare, enables us to think that the bradycardia is due to the inhibition of tonic firings of the sympathetic cardiac nerve as well.

SUMMARY

Experiments were performed on rabbits, dogs and cats and the following results were obtained.

1. γ -Aminobutyric acid caused fall of blood pressure accompanied with a slight bradycardia.

2. This acid had no direct action on the heart and the vessel following the administration of the dose effectual sufficiently on blood pressure.

3. The blood vessel of the spleen actively dilated.

But, this dilatation of the vessel in the spleen did not take place after the denervation.

4. The severance of the cervical vagi, the cervical sympathetics, the depressor nerves and the denervation of the carotid sinu on both sides had no effect on the action of this acid on blood pressure. Bradycardia was more or less lessened, but the complete disappearance hardly occurred by the section of the vagi.

5. γ -Aminobutyric acid showed no inhibiting effect on the superior cervical sympathetic ganglion of the cat. But, after the almost complete blockage of ganglia caused by tetraethyl-ammonium bromide or hexamethonium bromide, this acid affected no longer blood pressure.

6. In decerebrate animals, this acid showed the same effect as in the intact animal.

7. The latency of the fall of blood pressure was the shortest, when γ -aminobutyric acid was injected in the vertebral artery. The latency in the internal carotid injection was much longer than in the intravenous injection. The latency in the internal carotid injection was of the same order as in the injection into the subaxillary artery.

8. The intracisternal injection of γ -aminobutyric acid caused more marked and sustained fall of pressure than the intravenous injection. The inhibition of respiration became more evident. Bradycardia also took place.

9. The region of the central nervous system responsible for the effect of this acid was determined by means of transection of the brain stem and partial elimination of the medulla. The results revealed that the caudal part of the medulla was the concerned region.

10. The distribution of the points that responded with a fall in arterial pressure to topical application of this acid accorded with that of the depressor points stimulated electrically in the medulla.

11. The microinjection of γ -aminobutyric acid in the medial reticular formation of the medulla caused fall of pressure. The microinjection in the area postrema caused also fall of pressure. The minimal effective dose in microinjection was $0.5 \mu\text{g}$ in 10^{-3} ml.

12. It is inferred that γ -aminobutyric acid acts on the depressor points in the medulla and causes fall of blood pressure.

REFERENCE

1. TAKAHASHI, H. ET AL. *Jap. J. Physiol.* 5: 334, 1955.
2. AWAPARA, J. *Texas Report on Biol. and Medic.* 8: 443, 1950.
3. ROBERT, E. AND S. FRANKEL. *J. Biol. Chem.* 187: 267, 1950.
4. McDONALD, D. A. AND J. M. POTTER. *J. Physiol.* 114: 356, 1951.
5. WINGO, W. J. AND J. AWAPARA. *J. Biol. Chem.* 187: 267, 1950.
6. MONNIER, M. *Rev. Neurol.* 70: 521, 1938.
7. ALEXANDER, R. S. *J. Neurophysiol.* 9: 205, 1946.
8. BACH, L. M. N. *Amer. J. Physiol.* 171: 427, 1952.
9. WANG, S. C. AND S. W. RANSON. *J. Comp. Neurol.* 71: 85, 1939.
10. RANSON, S. W. AND P. R. BILLINGSLEY. *Amer. J. Physiol.* 41: 85, 1916.
11. TAKAHASHI, H. Reported in the general congress of the Japanese Physiological Society, 1957.
12. YAMAGUCHI, M. AND S. OMATA. *Diagnosis and therapy.* 43: 1017, 1955 (in Japanese).
13. EZOE, B. ET AL. Personal communication.