TRANQUILIZING EFFECTS OF β-PHENYL-γ-AMINOBUTYRIC ACID (PHENYGAM)

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γ-Aminobutyric acid (GABA) has attracted the interest of research workers because of its effect on the processes of central inhibition, and it has been suggested that it plays the part of a mediator of the central nervous system.

The inhibitory effect of GABA may be demonstrated experimentally only after direct injection of the compound into the brain, for it is almost completely unable to pass through the blood-brain barrier. This has been proved by various methods [5, 6, 7, 8, 10], including the administration of C-labeled GABA. In experiments on animals most investigators [1, 3, 5, 9] were unable to detect any influence of GABA, administered parenterally or enterally, on the behavior of the animals or on the action of certain drugs. When the blood-brain barrier was injured (by ethyl chloride or chloroform), parenterally administered GABA reached the brain and produced a depressant effect, as shown by changes in the EEG [2, 6]. In Japan, GABA is marketed commercially under the name "Gammaron" and is given internally for the treatment of epilepsy.

Effect of PGABA and GABA on the Motor Activity of Albino Mice

Substance	Dose (in mg/kg)	No. of experiments	No. of mice	% of depression	P
H_2O	–	6	30	0	–
GABA	400	3	15	0	–
PGABA HCl	35	4	20	52	0.1
PGABA HCl	70	4	20	74	0.02
PGABA HCl	140	3	15	90	0.01
PGABA HCl	210	2	10	97.6	0.001

Since GABA is a metabolic product of the brain and possesses low toxicity (LD_{50} for mice by intraperitoneal injection is 5000 mg/kg), we have attempted to obtain from it a compound possessing a central depressant action, yet able to pass through the blood-brain barrier. With the cooperation of chemists, it was decided to introduce a phenyl radical into the GABA molecule, for this would increase its pharmacological activity and facilitate the penetration of the drug into the brain. Most centrally acting compounds (anticonvulsants, central cholinolytics, antidepressants, and so on) contain this radical in their molecule. The compound β-phenyl-γ-aminobutyric acid (PGABA) was synthesized in the Department of Organic Chemistry of the A. I. Gertsen Pedagogic Institute under the direction of Professor V. V. Perekalin.

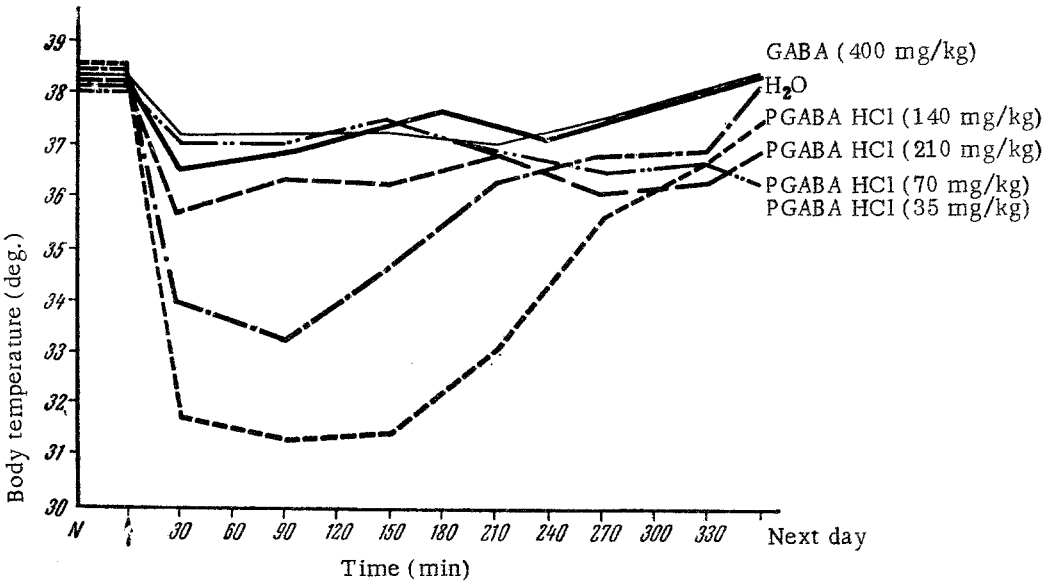
EXPERIMENTAL METHOD

The action of PGABA was investigated on various species of laboratory animals (mice, rats, cats, and rabbits) using different methods of administration (intravenously, intramuscularly, intraperitoneally, orally). Most of the investigations were carried out on mice, which received PGABA intraperitoneally. Each series of investigations was accompanied by a parallel experiment using GABA.

EXPERIMENTAL RESULTS

Effect on motor activity and the orienting reaction. Visual observations on the behavior of the animals receiving PGABA were sufficient to detect appreciable depression of their motor activity: the animals scarcely moved, and if large doses were given they remained motionless. This action of PGABA was shown more demonstratively in experiments in which the motor activity was assessed by an electronic integrator (constructed by the engineer N. E. Zil'berman), providing quantitative recordings of the total kinetic energy of all the animal's movements (with 5 mice in a group). Observations were made over a period of 10-30 min. In a dose as small as 70 mg/kg, PGABA caused a statistically significant depression of motor activity (see the table).

It should be noted that when observations were conducted on mice in a chamber, depression of the orienting reaction also was observed. In animals receiving a large dose of PGABA, no sign of the "what is it?" reflex appeared when they were placed on the experimental area. They immediately hid themselves in a corner, where they sat throughout the period of observation. The depression of the orienting reaction, possibly resulting from the depression of their motor activity, was also observed in experiments with a "flap-box" used in our laboratory to investigate motor activity and the orienting reaction in albino mice. This box is made of wood and is divided longitudinally into two halves and crosswise by means of movable flaps into several compartments (in our examples 5). Measurements are made of the latent period (the time the mouse spends in the first compartment), the time of its run, and the number of flaps through which it passes, there and back, in a period of 5 min. After administration of PGABA, in doses starting with 70 mg/kg, most mice did not shift at all, and those which did run passed through far fewer flaps. The latent period in the mice which ran was almost unchanged from its control value.



Effect of GABA and PGABA on body temperature of albino mice.

Effect on conditioned food reflexes. The method of conditioned food reflexes in a maze was used. The time taken for 8 consecutive runs was measured, during which the mouse ran the distance from the first to the last compartment, before and after receiving an intraperitoneal injection of PGABA in doses of between 20 and 35 mg/kg. Altogether 88 experiments were performed (including controls) on 8 mice. A dose of 20 mg/kg in 8 or 16 experiments ($P = 0.002$) caused a marked increase in the total time of running. It must be realized that an increase in the total time of running could not always be attributed to a disturbance of motor activity, for of the 8 runs some were very fast and others were slow, or the mice remained in the first compartment. These experiments demonstrated the depressant action of PGABA on higher nervous activity. The time taken to eat the food reinforcement was unchanged. Doses greater than 20 mg/kg caused an appreciable disturbance of motor activity, which complicated the evaluation of the results.

Effect on muscle tone and movement coordination. The revolving rod method was used. Starting with doses of 60-70 mg/kg, PGABA caused a disturbance of coordination in some of the mice: the mice could not stay on the shaft for 1 h after the injection. With large doses the effect increased in both strength and duration. For instance,

injection of PGABA in a dose of 140 mg/kg, none of the mice stayed on the shaft and the effect lasted for 2-3 h. GABA did not cause these changes in doses of 200-400 mg/kg. The revolving rod method was also used to assess the muscle-relaxant properties of the compounds. In this case the inability of the mice to stay on the shaft after the injection of PGABA could be attributed to the central relaxant action of the drug. PGABA evidently had no direct action on the muscles or myoneural junctions, for the excitability of the mucles in response to direct and indirect electrical stimulation (experiments on frogs) was preserved. The response to nociceptive stimulation was also preserved in mice (withdrawal of the limb and generalized motor reaction to a pinprick).

Analgesic action was not exhibited by PGABA when the following tests were used: speed of jumping from a can placed in hot water or from a chamber through the floor of which an electric current was passed; a squeak and motor reaction in response to squeezing the base of the tail with a Dieffenbach's clamp.

The hypothermic action of PGABA was marked. An obvious effect was observed after injection of the compound in a dose of 70 mg/kg (see the figure). No effect on the body temperature of albino mice was caused by GABA.

General action and toxicity. After injection of PGABA into rabbits (intravenously) and cats (intramuscularly) in doses of 50-100 mg/kg, a generalized depressant action developed in 20-30 min: the animal ceased to make spontaneous movements, its limbs were sprawled, its head hung down, and its reaction to external stimulation and food was considerably depressed. Respiration became slower, but the blood pressure in the cats receiving the acid intravenously remained almost unchanged. The LD_{50} for mice by intraperitoneal injection was 900 mg/kg, and for rats 700 mg/kg.

Effect of PGABA on healthy human subjects. Investigations were carried out on 6 volunteers who were given the acid by mouth in doses of 5-15 mg/kg. Approximately 1-1½ h after taking the compound, the subjects noticed some slight drowsiness, lethargy, heaviness in the head, and a loss of sharpness of perception of surrounding objects (but without deterioration of vision). After taking a dose of 15 mg/kg one subject noticed a sensation of weakness, impairment of hearing, and difficulty in thinking clearly. This state lasted for 2-6 h. When psychological tests were carried out 1½-2 h after administration of PGABA, in 4 of the 6 subjects some increase in the latent and motor periods of a simple motor reaction and slowing of the response in an association test were observed. In all the subjects nocturnal sleep was deeper and more prolonged. No changes were found in the blood pressure, pulse, respiration, and body temperature. Next day all the subjects felt fit and were able to do their normal work.

SUMMARY

In experiments on cats, rabbits, rats and mice a study was made of the action produced by γ-aminobutyric (GABA) and β-phenyl-γ-aminobutyric acids (PGABA) administered by different routes. While GABA had no effect on the animal behavior after parenteral administration, PGABA caused inhibition of the CNS, manifested by a depression of the motor activity, orienting reaction, conditioned reflexes, disturbances of motor coordination, and of central myorelaxation, and hypothermic effect, PGABA produced no analgesic effect. LD_{50} of PGABA when administered intraperitoneally to mice is 900 mg/kg, to rats—700 mg/kg.

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