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RELATIONSHIP BETWEEN PHARMACOLOGICAL EFFECTS AND BLOOD AND BRAIN LEVELS OF GAMMA-BUTYROLACTONE AND GAMMA-HYDROXYBUTYRATE

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Abstract—After Roth and Giarman[1] discovered the "anesthetic" activity of gamma-butyrolactone (GBL) numerous pharmacological and clinical studies have been conducted using either GBL or the product of its hydrolytic cleavage gamma-hydroxy-butyrate (GHB). The present study was conducted to clarify whether GBL or GHB is the substance active as a depressant of C.N.S. Using a spectrophotometric method of assay (based on the Hestrin reaction) we found that while it is possible to obtain a correlation between the loss of righting reflex and the concentration of GHB in rat brain, a strict correlation is not obtained with GBL. The study of plasma and cerebral levels of GHB and GBL as a function of the route of administration illustrates the importance of consideration of this factor in the appraisal of the action of these two agents. GBL given per os, i.p. or i.v. is capable of producing higher concentrations of GHB in brain and blood than an equimolecular amount of GHB-Na administered by the same route. This result is particularly evident when GBL and GHB-Na are given orally.

FOLLOWING the observation of Rubin and Giarman[1, 2] that gamma-butyrolactone (GBL) produced depression of CNS in the cat and in the rat, numerous studies have been conducted on the effect of GBL and the product of its alkaline hydrolysis, gamma-hydroxybutyrate (GHB-Na).

The fact that GHB is formed from GABA[3] or succinic semialdehyde metabolism[4, 5] and that it has been found as a naturally occurring substance in brain of mammals[6, 7] together with the observation that a specific enzyme "Lactonase" is capable of converting GBL into GHB and vice versa[8, 9] suggests a physiological role of these two substances on the CNS.

However, there is still some controversy as to which of the products exerts the depressive effect when these compounds are administered in anesthetic doses.

Benda and Perlés[10] and Jouvet[11] in different animal species (rat, mouse, pigeon, rabbit) noted that administration of GHB-Na produced a sedative effect on the CNS inferior, in both intensity and duration, to that obtained with GBL. The later authors suggested that GBL and not GHB was responsible for the resultant CNS depression. This hypothesis was confirmed by Bessman and Skolnik[12] who using a spectrophotometric method of assay found that the onset and duration of anesthesia in the rat was correlated with the presence of the lactone in the brain.

However, an entirely different conclusion was reached by Giarman and Roth[13] who, using a gas-chromatographic method, observed that the anesthetic effects of these

drugs are mediated by the presence of gamma-hydroxybutyrate (GHB) in the brain, either after administration of GBL or GHB-Na.

The present study was undertaken to clarify whether GBL or GHB is the substance active as a sedative.

Using a spectrophotometric method of assay we found in agreement with Giarman and Roth[13] that in rat brain and blood the largest amount of the two drug forms was represented by GHB. It is interesting to note that higher concentrations of GHB in blood and brain are reached after oral and i.p. administration of GBL and not after administration of GHB-Na. This finding is particularly evident when GBL and GHB-Na are given orally.

From these experiments a correlation between loss of righting reflex and concentration of GHB in the brain was also obtained while there was no strict correlation between the loss of righting reflex and the concentration of GBL in the brain.

MATERIALS AND METHODS

(1) *Assay method of standard solution of GBL and GHB*

(a) *Spectrophotometric assay.* The determination of both GBL and GHB (the latter after conversion into lactone with 2 volumes of concentrated HCl for 1 hr at 100°) was performed according to the method of Hestrin.[14]

One ml of alkaline hydroxylamine reagent (2 N hydroxylamine hydrochloride diluted 1:1 with 3·5 N sodium hydroxide) was added to a standard solution of one of the two compounds and shaken for 1 min. Then 2 ml of an ethanolic ferric chloride solution (10% $FeCl_3$ in 4 N HCl mixed in equal volumes with 99% ethanol) was added.

The color produced by the samples was read after 10 min at 520 mμ against a blank, prepared by adding first 2 ml of ethanolic ferric chloride reagent and then 1 ml of alkaline hydroxylamine solution to 1 ml of water containing the GBL or GHB.

(b) *Gas-chromatography assay.* This was carried out on a standard solution of GBL and of GHB (the latter after regeneration of lactone) in chloroform. Fractions varying from 1 to 3 μl were injected into a gas-chromatograph, Aerograph model 1520 using a 15% D.E.G.S. column on gas-chromo Z solid support 100–104 mesh.

The temperature of both injector and ionization detector was 225° while the column temperature was 175°, N_2 with a flow 50 ml/min was employed as a carrier.

The assay of an unknown solution was carried out by the direct calibration method, each test being repeated at least three times. The peak area was estimated as follows: area $= \Delta h/2$.[15]

(2) *Extraction of GBL and GHB from tissues*

A study of the most favourable conditions for the isolation and extraction of GBL and GHB from tissues was carried out with standard solutions following the spectrophotometric method of assay. On the basis of these results, a standard method for the measurement of GBL and GHB in tissues was obtained.

(a) *Extraction with organic solvents.* GBL standard solution (200 μg/ml H_2O) was extracted with chloroform, benzene and ether (1:4/v:v) shaking vigorously for 3 min.

GBL in the order of 98% (SD 3·2; $n = 8$) was recovered with chloroform extraction and 67·19% (SD 1·8; $n = 8$) with benzene. It was not possible to measure the extract-

ing capacity of ether due to interference with the Hestrin reactive by the ether solvent.

(b) *Protein precipitation.* The most suitable substance for protein precipitation was chosen on the basis of the results obtained when $ZnSO_4$, $ZnSO_4$ and $Ba(OH)_2$ or trichloroacetic acid was added to GBL and GHB standard solutions. When, in a group of seven experiments, solutions of GBL or GHB in 1 ml of H_2O were treated with 6 volumes of 5% solution of $ZnSO_4$, subsequently extracted with chloroform and treated with Hestrin reagent, a linear correlation between final concentrations of 10, 25, 50, 100 and 200 μg of the two substances and optical density was obtained.

Such correlation is statistically represented by the following equations: $\bar{y} = -0{\cdot}003 + 0{\cdot}00303X$ for GBL and $0{\cdot}015 + 0{\cdot}00200X$ for GHB. Recovery after treatment with 5% $ZnSO_4$, if compared with standard solutions in water, was in the order of 91·4% for GBL and 93% for GHB (these values were calculated by the coefficient b of the regression lines).

When 200 μg of GBL was treated first with $ZnSO_4$ and subsequently with $Ba(OH)_2$,[12] at pH varying between 7·5 and 8·0, the yield was 56% (SD 10%; $n = 10$). At pH varying between 8·4 and 11, obtained by adding an excess of $Ba(OH)_2$, the yield of GBL fell to very low values (26 per cent at pH 8·4; $n = 4$; 20 per cent at pH 10; 5 per cent at pH 11; $n = 6$).

The disappearance of GBL was dependent upon the conversion into GHB, but the total yield of acid + lactonic form was no higher than that obtained when treatment with $ZnSO_4$ plus $Ba(OH)_2$ was carried out at a pH of 7·5–8·0.

Treatment of standard solutions of GHB (200 μg) with trichloroacetic acid (TCA) at 20% (1:3; v:v) (13) caused partial conversion of GHB into lactone. Such conversion was produced as a constant percentage (37·9%; SD = 0·89; $n = 10$) for incubation periods between 1–60 min and for temperatures in the range of 0°–22°.

Furthermore, it is worth noting that trichloroacetic acid interferes with Hestrin reagents producing a color of optical density in the range of 0·050 and 0·060. This value was subtracted from the spectrophotometric readings of samples.

(c) *Standard method—Assay of GBL and GHB present in the brain and blood.* The final procedure adopted for GBL and "after conversion into lactone" for GHB assay was as follows: 6 volumes of 5% $ZnSO_4$ were added to standard samples. Two ml of this solution were diluted to 4·5 ml with H_2O and subsequently extracted with 16 ml of chloroform, shaking vigorously for 3 min. Eight ml of chloroform extract were used for the Hestrin colorimetric reaction; the remaining 8 ml were adopted as a blank. GHB and GBL assay of the brain (1·7–2g) and blood (1 ml) was carried out on the supernatant obtained by homogenization of the tissues at a temperature of 0–4° in the presence of $ZnSO_4$ (6 ml) and subsequent centrifugation for 10 min at 1000 g.

In the first fraction of the supernatant (2 ml) GBL was assayed after chloroform extraction, as reported above, while in the second fraction of 2 ml the spectrophotometric assay of GHB was performed after treatment with 0·5 ml of NaOH 3·5 N (to transform all the GBL present into its anionic form) and subsequent "conversion" of all the GHB into GBL.

The optical density values thus achieved are reported on the standard curve and the actual amount of GHB present in the brain or blood was estimated by subtracting the value obtained in the first fraction from that obtained in the second. The equation of regression line of standard curve of GBL and GHB added to rat brain are as follows: $\bar{y} = 0{\cdot}019 + 0{\cdot}00244X$ for GBL and $\bar{y} = 0{\cdot}044 + 0{\cdot}00181X$ for GHB ($n = 7$).

These equations are obtained with final concentrations of 10, 25, 50, 100 and 200 μg of the two substances.

The variation of optical density of the experimental values from the regression line was in the range of $\pm 0{\cdot}006$ with a confidence limit of 95 per cent.

The amount of GBL recovered was 80·5 per cent compared to a standard in water, and 88·1 per cent compared to standard solutions submitted to $ZnSO_4$ treatment and chloroform extraction.

The yield of GHB was 90·5 per cent compared with a standard solution in water and 97 per cent compared with solutions treated with $ZnSO_4$ and extracted with chloroform. If GHB is added to a rat brain *in vitro* no GBL could be detected in the chloroform extract.

The equations of the regression lines obtained adding GBL or GHB to blood (1 ml) are equal to those obtained with brain.

From the study of the regression line optical density of 0·019 for GBL and 0·044 for GHB (value of coefficient a) was considered equal to 0. The lowest amounts of the substance that could be assayed in brain or blood extracts, taking as confidential limit ($P < 0{\cdot}05$) for each experimental value the optical density of 0·006, were 3 μg for GBL and 3·9 for GHB. These values corrected for dilution became 18 μg for GBL and 24 μg for GHB. However, GHB values, below the sensitivity of the method are obtained from the difference in GBL levels as reported above.

(3) *Protein content of the brain*

Assay was performed following the method of Lowry[16] on brain homogenates diluted 1:1000 with water.

The optical density values are compared with a standard curve obtained with solutions of bovine serum albumin "Sclavo" (Siena).

(4) *Animals*

Male albino Sprague–Dawley rats, weighing between 200–250 g were used. The animals were starved overnight. Blood was removed from the heart, using an intracardiac puncture in one group of animals, while from another the brain was removed as rapidly as possible after decapitation.

(5) *Statistical methods*

The relationship between GBL and GHB concentrations and color produced in the Hestrin reaction was estimated by plotting the regression of the color (expressed as optical density), with the dose (expressed in μg) using the variation analysis method according to Lison.[17]

The variation of experimental values from the regression line with a confidence limit of 95 per cent was calculated with the following formula:

$$Sy_i = \sqrt{s_a^2 + s_b^2(x_i - \bar{x})^2}$$

where: Sy_i = standard error i in every point of the regression line; s_a = standard error of the coefficient a, s_b = standard error of the coefficient b; $(x_i - \bar{x})$ = difference between the mean dose and the dose in the point i.

(6) *Chemicals*

Pure GBL was prepared by distillation from GBL Schuchardt. GHB-Na was

prepared using equimolecular doses of GBL to which NaOH was added until the pH of the solution reached values of 11–12; the pH values were then adjusted under potentiometric control, to 8–8·2 adding HCl drop by drop. GBL was found to be very soluble in chloroform, ethanol, ether, benzene, xylol and water but was practically insoluble in petroleum ether, ground nut oil, vaseline and fatty acids. All the other reagents used were Merck or B.D.H. purified for analysis.

RESULTS

(1) *Substances interfering with the Hestrin reaction; comparison between colorimetric and gas-chromatographic assay*

As can be seen from the regression lines, brain and blood extracts interfere, if only to a small degree, with the Hestrin reactives. In the first group of experiments therefore it was ascertained that such interference was not related to glucose and acetylcholine which increased significantly in the brain on treatment with GBL or GHB.[18, 19]

Chloroform extraction under the present experimental conditions guaranteed that the interference of these substances was insignificant up to concentrations of 200 μg of ACh and 1 mg of glucose.

In a second group of experiments, the results obtained with the spectrophotometric method were compared with those found with gas-chromatography, in order to exclude the possibility that substances extractable with chloroform could cause an incorrect evaluation of the GBL and GHB concentrations.

In this group, chloroform extraction was performed with a single volume of solvent for a period of 30 min in order to obtain higher concentrations of GBL.

Such variation was necessary as chloroform extract evaporation even if carried out using a cold air stream, causes a marked loss of the GBL present in the solution.

As can be seen from Fig. 1, there is no appreciable difference in the symmetry of the peaks obtained with the gas-chromatograph between three different solutions of GBL: (a) A standard solution in chloroform; (b) A GBL solution extracted from the brain

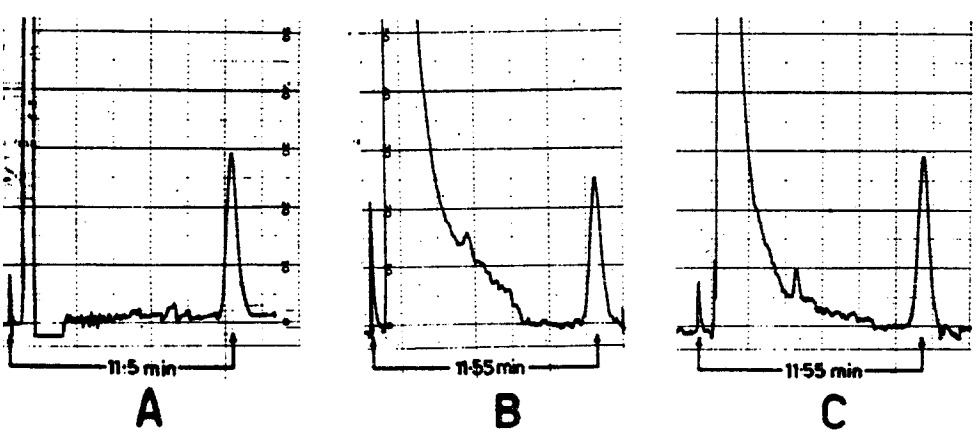

FIG. 1. Gas chromatograms of (A) Standard solution of GBL in chloroform (B) Standard solution of GBL added to the rat brain *in vitro* (extraction according to the method described in the paper) (C) GBL solution extracted from rat brain treated with GBL i.p.

which was added to a known quantity of GBL *in vitro*; (c) A GBL solution extracted from the brain of a rat treated with GBL i.p.

The retention time of the three peaks was found to be characteristic and identical.

Quantitative gas-chromatographic assay gave similar results to those obtained with the spectrophotometric method (see Table 1).

TABLE 1. LEVELS OF GBL* IN BRAIN EXTRACT OF RATS TREATED WITH GBL† i.p.—COMPARISON BETWEEN SPECTROPHOTOMETRIC AND GAS-CHROMATOGRAPHIC ASSAY

Exp. No.	Spectrophotometric assay‡ (μg/ml of brain extract)	Gas-chromatographic assay§ (μg/ml of brain extract)
1	532	590
2	516	430–510–466–520
3	552	540–615–483

* The levels of GBL were determined after conversion of GHB into GBL by heating the brain extract to 100° in a water bath for 1 hr.

† The animals received 1 g/kg of GBL 30 min before decapitation.

‡ Spectrophotometric assay was carried out on 0·25 ml of chloroform extract (D.O. ~ 0·300).

§ Gas-chromatographic assay was carried out on volumes of 1, 2, 3 μl of the chloroform extract; the values obtained are reported for each determination.

(2) *Behavioural effects and cerebral and blood concentrations of GBL and GHB as a function of the route of administration*

The purpose of this group of experiments was to study the influence of different routes of administration on the concentration and behavioural effects of GBL and GHB-Na.

The time course of concentration of the two substances in the brain of the adult rat after oral and i.p. treatment with GBL (500 mg/kg) or GHB-Na (732 mg/kg) is shown in Table 2.

The cerebral concentration of gamma-hydroxybutyrate tends to be significantly higher than that of GBL, except in the value found 3 min after the i.p. injection of GBL At this time, the GBL cerebral concentrations are higher than those of GHB ($P < 0{\cdot}001$).

The highest cerebral concentrations of gamma-hydroxybutyrate were observed 15 and 30 min after i.p. administration of GBL and GHB-Na. Lower concentrations were obtained after oral administration of GBL, while the lowest concentrations were found after administration of GHB-Na by the oral route.

In blood as well, the levels of the acidic compound were definitely higher than those of GBL (Table 3). In fact, the latter were so low as to be often below the sensitivity limits of the method employed.

As in the case of the cerebral levels, GHB-Na administered orally produced in the blood GHB concentration notably lower than those observed after oral administration of GBL (Fig. 2). A study of the GHB concentrations, conducted separately on plasma and red cells by removing the blood 30 min after the i.p. administration of GBL (500 mg/kg) showed a prevalence of the substance in the plasma fraction (56·7 and 68·7%; $n = 2$) over the globular fraction (31·3 and 43%; $n = 2$).

TABLE 2. CONCENTRATIONS* OF GBL AND GHB IN RAT BRAIN† AFTER ORAL AND INTRAPERITONEAL ADMINISTRATION OF GBL AND GHB-Na IN EQUIMOLAR DOSES

Treatment	Administration route	GBL (μg/g/w.v.) after: 3 min	15 min	30 min	60 min	GHB (μg/g/w.v.) after: 3 min	15 min	30 min	60 min
GBL 500 mg/kg	os	18·5 ± 2·3	18·7 ± 4·2	37·0 ± 9·9‡	29·1 ± 4·5	4·3 ± 2·9	21·3 ± 4·3	94·4 ± 12·6	98·9 ± 29
	i.p.	170·0 ± 3	29·9 ± 4·6	14·8 ± 4·4§	29·1 ± 6·2	8·3 ± 4·1	145·8 ± 20·3	191·6 ± 6·2	129·1 ± 12
GHB-Na 732 mg/kg	os	—	16·7 ± 5·4	20·8 ± 4·5	25·5 ± 6·1§	—	60·4 ± 25·0	26·6 ± 3·8	47·0 ± 6·1
	i.p.	21·2 ± 3·3	33·3 ± 10·4	41·7 ± 1·2	23·4 ± 4·6	20·9 ± 7·3	147·5 ± 6·2	111·1 ± 40·6	47·5 ± 12·5

* The figures represent the mean values of at least six determinations ± S.E.M.; if not otherwise specified:
† Each determination was carried out on one rat brain.
‡ = Three out six experiments were below the sensitivity of the method and were not utilized in the mean;
§ = Two out six experiments were below the sensitivity of the method.

TABLE 3. CONCENTRATIONS* OF GBL AND GHB IN RAT BLOOD† AFTER ORAL, INTRAPERITONEAL AND INTRA VENOUS ADMINISTRATION OF GBL OR GHB-Na IN EQUIMOLAR DOSES

Treatment	Administration route	GBL (μg/ml) after: 5 min	15 min	30 min	60 min	GHB (μg/ml) after: 5 min	15 min	30 min	60 min
GBL 500 mg/kg	os	82·5§	33·8 ± 8·9‖	22·5§	37·5§	562 ± 72	611 ± 64	462 ± 112	466 ± 70
	i.p.	63 ± 16·5	32·6 ± 6·2	33·8 ± 8·9‖	22·5¶	694 ± 35	544 ± 32	665 ± 34	521 ± 55
	i.v.	85 ± 12	n.m.‡ →			513 ± 62	550 ± 78	514 ± 39	430 ± 48
GHB-Na 732 mg/kg	os	n.m. →				32 ± 7	39·2 ± 12	57·8 ± 9·5	95 ± 15
	i.p.	n.m. →				581 ± 22	561 ± 75	578 ± 37	557 ± 49
	i.v.	n.m. →				920 ± 70	876 ± 68	617 ± 28	773 ± 30

* The figures represent the mean values of at least six determinations ± S.E.M.; if not otherwise specified:
† Assay was conducted on 1 ml of blood withdrawn by intracardiac injection and immediately submitted to extraction procedure.
‡ n.m. = GBL not measurable—(values below 18 μg/ml).
§ = Five out six experiments were below the sensitivity of the method and were not utilized in the mean;
‖ = Three out six experiments were below the sensitivity of the method;
¶ = Four out six experiments were below the sensitivity of the method.

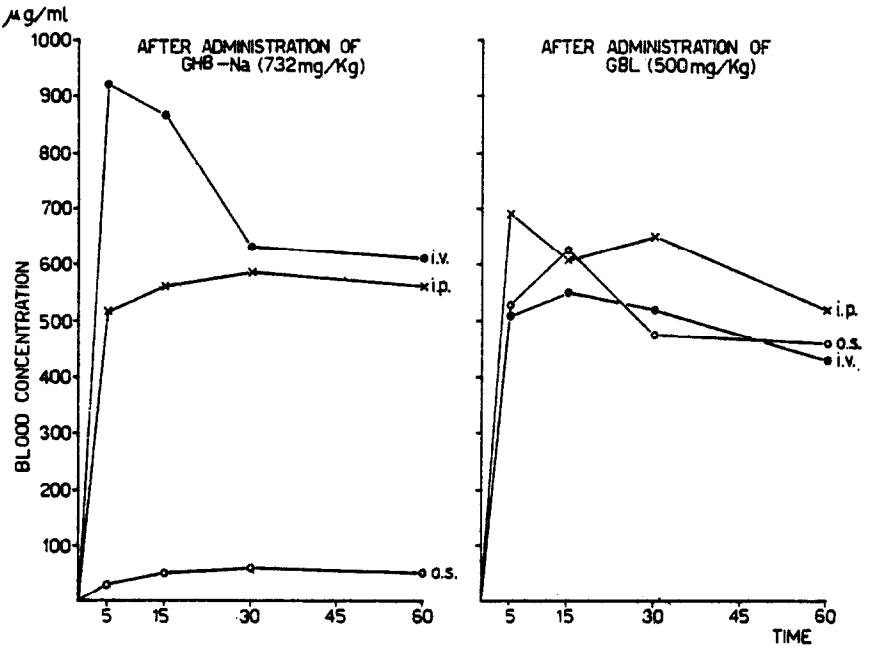

FIG 2. Concentration of GHB in the blood as a function of the route of administration and of the compound employed (time in min).

The pharmacological effects of the administration of GBL and its sodium salt appear as a depression of the C.N.S. and loss of the animal's righting reflex.

The disappearance of this reflex starts about 15 min after i.p. injection of GBL (500 mg/kg) and GHB-Na (732 mg/kg) and 30 min after oral administration of GBL.

No loss of righting reflex was noted for GHB-Na administered orally (twelve observations).

The righting reflex normally returns 60 min after i.p. injection of GHB-Na, and between 90–110 min after the oral and i.p. administration of GBL.

On the basis of the present findings it was possible to obtain a correlation between GHB levels in the brain and the time course of the pharmacological effects measured by observation of the loss of righting reflex; a cerebral concentration of GHB greater than 100 μg/g of fresh tissue was associated with a loss of this reflex in all the animals observed (Fig. 3).

In a further series of experiments, the effects on the behaviour and the cerebral concentrations of GHB and GBL after oral administration of GBL (500 mg/kg) were studied in a group of rat weighing 20 g (SD = 2·6; $n = 6$). Five minutes after administration the rats were seen to be asleep and both loss of righting reflex and eyelid ptosis were observed.

Table 4 demonstrates that concentrations of GHB in the brain are about 2·5 times higher than those obtained with adult animals treated by the same route. The levels of GBL was as low as in adults.

From examination of the protein content in rat brain it is evident that the different cerebral concentrations of GHB are not due to the difference in water content in the animals of the two ages.

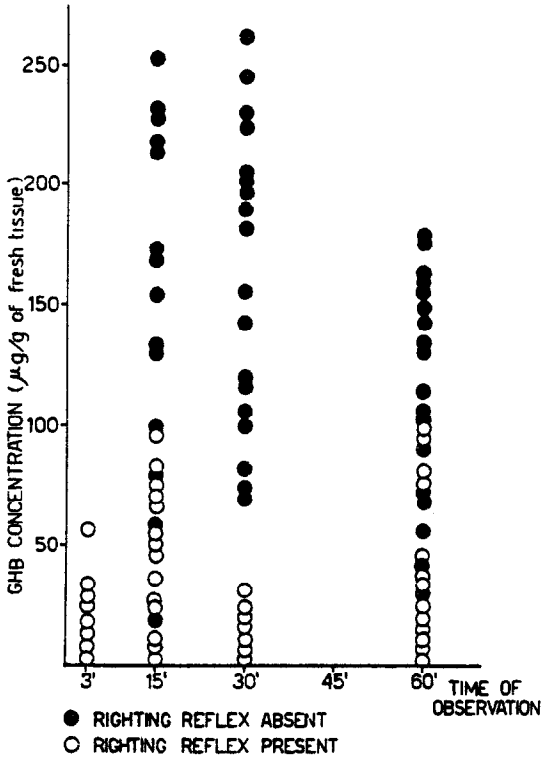

FIG. 3. Correlation between GHB levels in rat brain and righting reflex.

TABLE 4. CONCENTRATIONS* OF GBL AND GHB IN BRAIN† OF NEWBORN‡ RAT AFTER ORAL ADMINISTRATION OF GBL (500 mg/kg)

GBL				GHB			
(μg/g/w.v.) after:				(μg/g/w.v.) after:			
3 min	15 min	30 min	60 min	3 min	15 min	30 min	60 min
36§	40 ± 8	59·4§	59·3§	20‖	127 ± 38	262 ± 34	250 ± 10

* The figures represent the mean values of at least six determinations ± S.E.M.; if not otherwise specified.
† Each determination was carried out on one rat brain.
‡ Rats about 10 days old were used; average weight 20 g.
§ Five out of six experiments were below the sensitivity of the method and were not utilized in the mean.
‖ Four out of six experiments were below the sensitivity of the method.

The protein content is 9·1 mg/100 g of fresh tissue (SD = 0·36; $n = 6$) for adult rats and 8·2 mg/100 g of fresh tissue (SD = 0·29; $n = 6$) for newborn rats. Such difference is not statistically significant.

DISCUSSION

The difficulty in measuring the amount of GBL and GHB actually present in the tissues lies in the capacity of the two substances to convert one into the other as a function of pH.

Protein precipitation with $ZnSO_4$ and $Ba(OH)_2$ according to Bessman and Skolnik[12] not only hinders a high recovery of GBL in solution (50 per cent of the total is lost) but can also favour conversion of GBL into the Ba salt of GHB if the addition of a slight excess of $Ba(OH)_2$ moves the pH above 8.

Protein precipitation carried out with TCA according to Giarman and Roth[13] producing a low pH of the medium favours a partial conversion of GHB into GBL.

In view of these results, only $ZnSO_4$ solution was used to deproteinize the tissue. Such solution, without inducing pH variations, permitted the extraction of the compounds with good recovery.

The present method also differs from that employed by the other two authors in the use of chloroform extraction. Chloroform in fact, while it permits a quantitative extraction of GBL with a higher yield than that obtained with benzene, excludes the extraction of water-soluble substances such as glucose and acetylcholine that interfere with the Hestrin reaction.

The substance (or substances) extracted from the brain or blood with our method that interfere with the Hestrin reaction without modifying the linearity of the regression line is (are) to date chemically unknown. Their concentration, however, does not appear to be substantially modified when the animal is treated with anesthetic doses of GBL or GHB-Na, as a comparison between the spectrophotometric and gas-chromatographic methods shows.

The high GHB concentrations present in the brain or blood of rats and the low concentrations of GBL observed after administration of both GBL and GHB-Na are in contrast with the findings of Bessman and Skolnik[12] while they agree with the results of Giarman and Roth.[13] Such diversity could be explained by different levels of lactonase activity in different animal strain more probably by the accuracy of the method employed, and particularly by the fact that Bessman and Skolnik[12] performed the Hestrin reaction on a supernatant not previously subjected to any extraction process and in which the glucose (that increases on treatment with the two compounds[12]) mimics the presence of GBL.

On the basis of these considerations and of the results reported in this paper it is suggested that the pharmacologically active compound is GHB. In fact there was no modification in animal behaviour, 3 min after oral or i.p. administration of GBL, when the cerebral levels of lactone were respectively four or twenty times higher than those of the acidic form. On the contrary, C.N.S. depression became evident 15 min after i.p. and 30 min after oral administration of GBL or GHB-Na when the GHB cerebral concentration was from three to twenty times greater than the GBL concentration (see Table 2).

These findings are in agreement with the observations of Giarman and Roth on the monkey[20] and Gessa *et al.* on the rabbit.[21] These authors found that GBL when injected endocisternally or in given areas of the brain produced no central effect while C.N.S. effects appeared after injection of GHB. These results exclude a possible correlation between lactone levels and modification in animal behaviour.

The blood and cerebral levels of GHB change dramatically as a function of the route of administration and the substance employed.

Of particular interest is the observation that GBL administered i.p. and orally, produced cerebral and plasma concentrations of GHB higher than those encountered after administration of GHB-Na. Such a difference was particularly noticeable when

the two substances were given orally. In fact 30 min after oral administration of GBL, GHB concentrations were respectively 3·5 and 7·5 times higher in the brain and plasma than those obtained after administration of GHB-Na by the same route.

The different speed with which the GHB and the lactone traverse the gastric and blood–brain barrier in adult and even more so in newborn animals (Table 1 and Fig. 2) could be explained by the fact that GHB penetrates the cells as a weak electrolyte ($pK_a = 4·73$), while GBL, an uncharged cyclic molecule with a low molecular weight (Mol. wt. = 86), may easily cross the cell membrane through the pores.

When GBL and GHB-Na are given i.v. GHB concentrations in the blood are higher after administration of GHB-Na than GBL. It was noted, however, that blood concentrations fell more rapidly after GHB-Na than GBL (see Fig. 2).

Such findings are in agreement with results of Roth and Giarman.[9]

These authors suggested that lactone could be taken up from the blood into lean body mass more rapidly and efficiently than GHB. This depot pool of drug appears to be metabolized more slowly because it is not readily accessible to enzyme involved in biotransformation and its slow release back into the blood is capable of maintaining blood levels of drug at a higher plateau.

Another interesting point is the rapid disappearance of GBL from the brain (15 min after i.p. administration of GBL the concentration falls from 170 μg/g to 29 μg/g; see Table 2). This is in contrast with the absence in rat brain of an enzyme capable of producing hydrolytic cleavage of lactone.[8, 9] This enzyme is, however, present in the blood where it may explain the rapid disappearance of GBL and its conversion into GHB.

In spite of this rapid fall, GBL levels are always detectable in the brain not only after administration of GBL, but also surprisingly enough after that of GHB-Na; while they are below limits of detection in the blood after administration of GHB-Na and even 15 min after i.v. injection of GBL (see Table 3).

The possibility that the concentration of GBL found in rat brain after administration of GHB-Na might be an expression of a reaction between the Hestrin reagent and substances other than GBL, or of transformation of GHB into GBL during the extraction process, must be excluded on the basis of the following considerations:

(a) the results obtained with the gas-chromatograph as discussed above, reveal an amount of GBL very close to that found for the sample assayed with the spectrophotometric method;

(b) when GHB was added to rat brain *in vitro* and immediately subjected to extraction process no lactone formation was detected;

(c) the optical density of 0·025 was always chosen as the lowest limit of detection of the spectrophotometric method; such a value seems to exclude the interference of substances other than GBL (see Methods).

As can be seen from the above data, the question arises of interpreting the discrepancies between the GBL levels in the blood and those in the brain. This problem is at the present difficult to resolve; however, we may assume that the brain is capable of maintaining a constant GBL concentration by converting GHB to the lactone form. The lactone is removed from the brain through the blood, where, owing to the presence of lactonase activity and the effect of dilution, the GBL concentration is below the sensitivity of the method employed.

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