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NEUROCHEMICAL AND PHYSIOLOGICAL ASPECTS OF THE ROLE OF γ-HYDROXYBUTYRIC ACID AS A NATURAL SOPORIFIC

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ABSTRACT

The following aspects of the role of γ-hydroxybutyric acid (GHBA) in the CNS are reviewed in the present paper.

1. The administration of GHBA or its lactone to mammals produces a sleep condition similar to the natural one.

2. Electrophysiological studies have shown that the activity of the reticular formation is probably involved in the soporific effect of GHBA, although this compound does not seem to act as a synaptic transmitter.

3. GHBA is a normal constituent of brain tissue. Among the several possible biosynthetic pathways which could be responsible for its synthesis in this tissue, the γ-aminobutyric-acid-succinic semialdehyde pathway seems to be the most probable.

4. GHBA is rapidly metabolized to CO_2 and H_2O. The biochemical reactions involved in this degradation are not known.

5. Little is known on the biochemical mechanisms underlying the soporific action of GHBA. It seems possible that modifications of dopamine and serotonin metabolism in certain areas of the CNS are involved. The possible participation of other alcohols derived from biogenic amines in sleep mechanisms is also discussed.

RESUMEN

En el presente trabajo se revisan los siguientes aspectos del papel del ácido γ-hidroxibutírico (GHBA) en el sistema nervioso central.

1. La administración de GHBA o de su lactona a mamíferos produce un estado de sueño muy similar al natural.

2. Mediante estudios electrofisiológicos se han obtenido datos que indican que la actividad de la formación reticular participa como sustrato anatómico en el efecto soporífico del GHBA. Sin embargo, este ácido no parece actuar como transmisor sináptico.

3. El GHBA es un constituyente normal del tejido cerebral. Entre las varias vías metabólicas que pudieran ser responsables de su síntesis, la vía ácido γ-aminobutírico-semialdehido succínico parece ser la más probable en el cerebro, *in vivo*.

4. El GHBA se metaboliza rápidamente a CO_2 y H_2O. No se conocen las reacciones bioquímicas involucradas en esta degradación.

5. Se conoce muy poco acerca de los mecanismos bioquímicos subyacentes en la acción soporífera del GHBA. Sin embargo, algunos resultados experimentales sugie-

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ren que el metabolismo de la serotonina y de la dopamina juega un papel en dicho efecto. Asimismo, parece posible que otros alcoholes derivados de aminas biogénicas participen en los mecanismos moleculares del sueño.

INTRODUCTION

Since the discovery that γ-hydroxybutyric acid (GHBA) * can produce a sleep condition similar to the natural one (Laborit *et al.*, 1960), this compound raised considerable interest. This interest was stimulated when it was shown that GHBA was a normal constituent of brain (Wolff, 1960; Bessman and Fishbein, 1963). Due to its small concentration, however, only with the use of sensitive techniques as gas chromatography was possible to ascertain without any doubt its presence in nervous tissue. GHBA has been found in the brain of rat (Bessman and Fishbein, 1963; Fishbein and Bessman, 1964; Roth and Giarman, 1970), cat and guinea-pig (Roth and Giarman, 1970) and man (Bessman and Fishbein, 1963; Fishbein and Bessman, 1964). The GHBA concentration in brain has been reported to be 10^{-6} M to 10^{-3} M, depending on the method used for its determination. The high values were obtained following a colorimetric procedure, based in the formation of the corresponding hydroxamic acid from the lactone derived from free GHBA (Bessman and Fishbein, 1963); the low values were obtained by gas chromatographic techniques coupled with isotope dilution methods (Roth and Giarman, 1970).

When the regional distribution of GHBA in the guinea-pig brain was studied, it was found that the hippocampus, the mesencephalon, the diencephalon and the cerebellum had the highest concentration (Roth, 1970). Postmortem changes, however, could have occurred in these studies. On the other hand, subcellular fractionation studies have shown that GHBA is a soluble component (Roth, 1970), although its possible redistribution during the homogenization and fractionation cannot be discarded.

Concentrations of GHBA smaller than those present in brain have been found in liver (Roth, 1970). Unfortunately, other organs have not been studied in this respect.

METABOLISM OF GHBA

Biosynthesis

GHBA and its lactone, γ-butyrolactone (GBL) are rapidly accumulated in brain after their systemic administration to several species, including man (Bessman and Fishbein, 1963; Giarman and Roth, 1964; Bessman and Skolnik, 1964; Roth and Giarman, 1966; Helrich *et al.*, 1964). Under these conditions, GHBA is rapidly and extensively distributed along the CNS, with increasingly higher concentrations from the cervical cord to the cerebral cortex, where the highest levels have been observed, especially in the temporal lobe (Roth and Giarman, 1966).

These results indicate that there is practically no blood-brain barrier for

* Abbreviations used:

1:4BD, 1:4-butanediol; CNS, central nervous system; L-DOPA, L-dihydroxyphenylalanine; EPP, 5-ethyl,5-phenyl,2-pyrrolidinone; GABA, γ-aminobutyric acid; GBL γ-butyrolactone; GHBA, γ-hydroxybutyric acid; LDH, lactic dehydrogenase; SSA, succinic semialdehyde.

GHBA and GBL, a very interesting finding in view of the fact that many physiologically important substances in the CNS, as γ-aminobutyric acid (GABA) (Van Gelder and Elliott, 1958), catecholamines (Whitbey *et al.*, 1961; Marks *et al.*, 1962) and 5-hydroxytryptamine (Axelrod and Inscoe, 1963), do not easily reach the brain. The possibility exists, therefore, that the small amounts of GHBA present in brain are only the result of uptake from the blood and that its formation occurs in periferic tissues. However, there are no available data on the formation of GHBA in extraneural tissues. On the other hand, it has been shown that this compound can be synthesized in the CNS, both *in vivo* (Roth and Giarman, 1969) and *in vitro* (Roth, 1970).

The most probable metabolic precursor of GHBA in the CNS seems to be GABA: Roth and Giarman (1969) have isolated radioactive GHBA after the administration of ^{3}H-GABA, and the same conversion has been reported also in rat brain slices (Roth, 1970). Since it is known that GABA is transaminated in nervous tissue to yield succinic semialdehyde (SSA) (Bessman *et al.*, 1953; Roberts and Bregoff, 1953), it has been postulated that the latter compound is an intermediate metabolite in the conversion of GABA to GHBA. In fact, although the main pathway of SSA metabolism is probably its NAD-dependent oxidation to succinic acid by the corresponding dehydrogenase (Albers and Salvador, 1958), the anaerobic utilization of SSA by rat brain mitochondria (Bessman *et al.*, 1953) and the NADH consumption in the presence of SSA (Bessman *et al.*, 1953; Albers and Salvador, 1958) clearly indicate that some other metabolic pathways for SSA exist. On the other hand, it has been reported (Fishbein and Bessman, 1964) that SSA can be converted *in vitro* to GHBA in the presence of a soluble enzyme from rat brain; this enzyme seems to be identical to lactic dehydrogenase (LDH).

An alternative biosynthetic pathway for GHBA could be through 1:4-butanediol (1:4BD) (see Fig. 1 for structural relationships). It has been reported that the administration of this compound to rats produces both an elevation of GHBA levels in brain and some behavioral effects similar to those observed after GHBA administration (Roth and Giarman, 1968). 1:4BD could be a natural metabolite in the CNS or it could be transported from liver, since in the latter tissue it has been found as a normal constituent (Bergelson *et al.*, 1966).

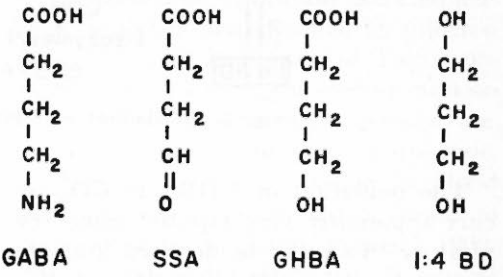

Fig. 1. Structural relationships among GABA, SSA, GHBA and 1:4BD.

The postulated biosynthetic pathways of GHBA are summarized in Fig. 2.

Metabolic degradation

It has been shown that GHBA, when administered to animals, is rapidly transformed to the corresponding γ-butyrolactone (GBL), which has been found in liver (Bessman and Skolnik, 1964), brain (Giarman and Roth, 1964; Bessman and Skolnik, 1964; Roth and Giarman, 1966) and blood (Roth and Giarman, 1966). The inverse reaction has also been reported to occur after the administration of GBL (Roth and Giarman, 1966). This interconversion of GBL and GHBA could be catalyzed by

a lactonase, which has been detected in plasma and liver (Roth and Giarman, 1966; Fishbein and Bessman, 1966a, 1966b). The physiological role of this enzyme, however, is doubtful, since the tissue concentration of GBL and GHBA is very low, whereas the Km of the lactonase for these substrates is very high (Roth and Giarman, 1966; Fishbein and Bessman, 1966).

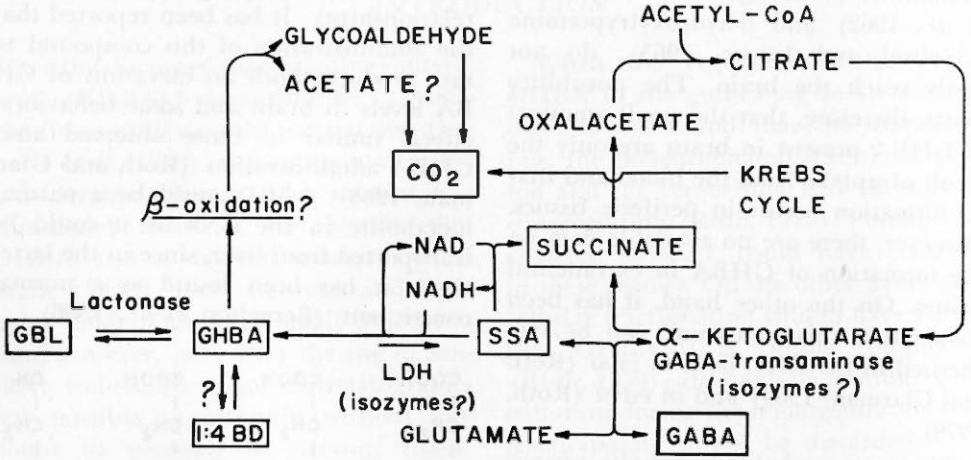

Fig. 2. Metabolism of GHBA. Description in the text.

The oxidation of GHBA to CO_2 occurs apparently very rapidly: some respiratory $^{14}CO_2$ can be detected four minutes after the administration of ^{14}C-GHBA to rats, and 2.5 hr later 60% of the injected radioactivity has been eliminated as $^{14}CO_2$ (Roth and Giarman, 1965, 1966). The metabolic pathways involved in this oxidation are unknown. GHBA can be converted to GABA (Mitoma and Neubauer, 1968; De Feudis and Collier, 1970), although the levels of the latter amino acid are not increased after GHBA administration (Mitoma and Neubauer, 1968; Godin *et al.*, 1968; Margolis, 1969; see, however, Pietra *et al.*, 1966). This conversion of GHBA to GABA does not seem to occur through glutamic acid, since the specific activity of GABA is greater than that of glutamic acid after the administration of labelled GHBA (Mitoma and Neubauer, 1968; De Feudis and Collier, 1970); the possibility that a small compartmentalized pool of glutamic acid (Berl and Clarke, 1969) is the responsible for the latter finding seems to be ruled out by the observation that thiosemicarbazide, a potent inhibitor of glutamic decarboxylase *in vivo* (Baxter and Roberts, 1960; Maynert and Kaji, 1962; Tapia *et al.*, 1967), did not affect the conversion of ^{14}C-GHBA to ^{14}C-GABA (Mitoma and Neubauer, 1968). A direct pathway for GHBA metabolism could be its oxidation to SSA, followed by transamination of the latter compound. As mentioned above, lactic dehydrogenase could catalyze the oxidation step (Fishbein and Bessman, 1964) (specific GHBA dehydrogenases have been found only in microorganisms (Nirenberg and Jakoby, 1960; Hardman, 1962)). If SSA were formed from GHBA, however, it would be expected that succinic acid should be also formed, because of the activity of SSA dehydrogenase (Albers and Salvador, 1958). Surprisingly, this conversion of GHBA to succinic acid does not seem to occur

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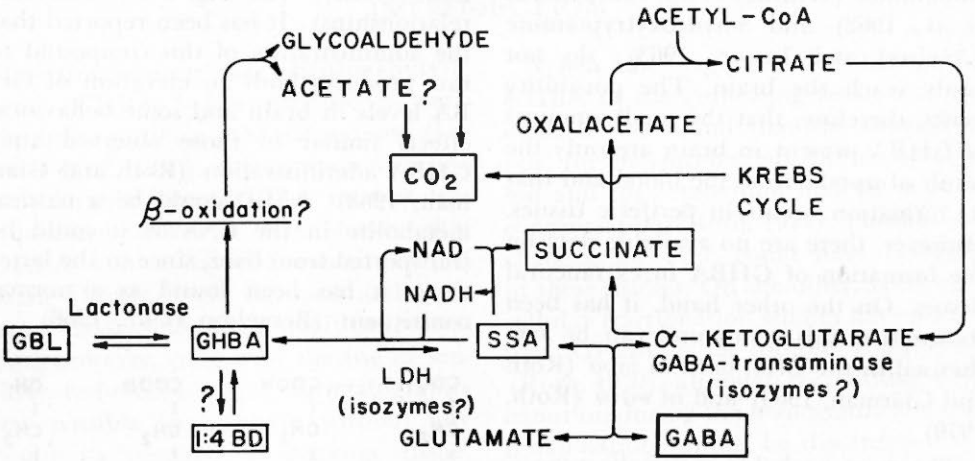

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significantly in the CNS (Roth and Giarman, 1965, 1966; Walkenstein *et al.*, 1964). It is interesting that in rabbits 1:4BD can be converted to succinate in a proportion similar to that of GHBA (Gressner *et al.*, 1960).

The metabolic degradation of GHBA to acetate and glycoaldehyde through a β-oxidation mechanism has been also postulated (Walkenstein *et al.*, 1964). According to this idea, the CO_2 produced from GHBA is the result of the oxidation of acetate and glycolaldehyde (Fig. 2).

Isozymes of GABA transaminase (Waksman and Bloch, 1968) and LDH (Cahn *et al.*, 1962) have been found in brain tissue. It can be speculated, therefore, that some isozymes can be selectively involved in the interconversion between GHBA and GABA, which would mean that compartmentalization of GHBA metabolism might exist. It is interesting that under conditions favorable to intracellular reduction, as after the ingestion of ethanol, the synthesis of GHBA is stimulated (Roth, 1970; McCabe *et al.*, 1971). The possible implications of these factors in the regulation of GHBA metabolism will have to be considered when the reactions and enzymes involved are better known.

EFFECTS OF GHBA ON BEHAVIOR

Sleep

Since the initial studies of Laborit *et al.* (1960), it is known that GHBA administration induces a sleep condition with characteristics similar to those of the natural sleep, as well as an anesthetic effect (Blumenfeld *et al.*, 1962). This soporific effect has been demonstrated in rats (Bessman and Fishbein, 1963; Giarman and Schmidt, 1963; Giarman and Roth, 1964; Godin *et al.*, 1968; Ban *et al.*, 1967), mice (Drakontides *et al.*, 1962; Bessman and Fishbein, 1963; Giarman and Schmidt, 1963; Ban *et al.*, 1967), rabbits (Ban *et al.*, 1967), cats (Drakontides *et al.*, 1962) and men (Laborit *et al.*, 1960, 1961; Bessman and Fishbein, 1963; Giarman and Roth, 1964; Metcalf *et al.*, 1966). It has been observed that some time after the administration of GHBA to mice and cats, the animals show decreased spontaneous muscular activity, weakness, ataxy, loss of the righting reflex and a diminished response to sensorial stimuli. This sleep condition in the cat differed from the natural one in the lack of both a characteristic posture and the closing of the eyelids (Drakontides *et al.*, 1962). In mice, even low doses of GHBA induce a catatonic-like condition in characteristic postures, accompanied by piloerection (Pérez de la Mora and Tapia, unpublished). In men, the sleep condition occurs with a decreased respiratory rate, and increased respiratory amplitude, bradicardia, a slight increase in blood pressure and a diminished response to sensorial stimuli (Laborit *et al.*, 1960; Blumenfeld *et al.*, 1962). In all cases it was observed a sudden wakening of the individuals but no late effects (Laborit *et al.*, 1960; Blumenfeld *et al.*, 1962; Metcalf *et al.*, 1966; Ban *et al.*, 1967). Treatment with insulin (Mitoma and Neubauer, 1968), or pretreatment with L-DOPA (Rizzoli *et al.*, 1969), increased the sleeping time; in contrast, previous treatment with β-hydroxybutyric acid (Roth and Giarman, 1966), pyruvate (Sprince *et al.*, 1966) or amphetamine (Roth and Suhr, 1970) decreased it. In this regard it is noteworthy that the administration of ethanol potentiated the soporific effect of GHBA to a much greater extent than that expected by the sum of the effects of each substance (McCabe *et al.*, 1971). The already mentioned effect of ethanol on brain GHBA levels (Roth, 1970) could be involved in this potentiation. In view of these

data, and since GHBA is a constituent of some wines (Webb *et al.*, 1967; Webb *et al.*, 1969; McCabe *et al.*, 1971), it is tempting to speculate that there is possibly a relationship between the well known soporific effect of wine and its effect on the GHBA system.

The possible participation of 1:4BD in the metabolism of GHBA has been already mentioned. It is of considerable interest, therefore, that 1:4BD induces a sleep condition very similar to that produced by GHBA, both behaviorally and electroencephalographically (Sprince *et al.*, 1963; Roth and Giarman, 1968).

Convulsions

It has been observed that GHBA can produce convulsions in some species, as rabbits and mice (Ban *et al.*, 1967). On the other hand, it has been shown that GHBA and GBL can protect against the convulsions induced by strychnine (Laborit *et al.*, 1960; Ban *et al.*, 1967), isoniazide (Laborit *et al.*, 1960), high oxygen pressure (Bertharion and Brue, 1967) and electroshock (Ban *et al.*, 1967). Contradictory results have been obtained when the anticonvulsive action of GHBA or GBL against metrazol or picrotoxin was tested (Laborit *et al.*, 1960; Basil *et al.*, 1964; Ban *et al.*, 1967). In view of these discrepancies, and since it had been shown in our laboratory that 5-ethyl,5-phenyl,2-pyrrolidinone (EPP), a compond structurally similar to GBL (Fig. 3), is a potent anticonvulsant against metrazol and electroshock (Carvajal *et al.*, 1964), we have carried out some experiments to compare the anticonvulsant properties of EPP and GBL. Preliminary results indicate that some differences exist: EPP protects against the convulsions induced by pyridoxal phosphate-γ-glutamyl hydrazone, mercaptopropionic acid and metrazol, while GBL only modifies the tonic phase of the convulsions, but does not show a clear anticonvulsant action. It is interesting that EPP at low doses induces a sleep condition deeper (muscular relaxation) and longer than that induced by equimolar doses of GBL, but without the catatonic-like condition which is observed with GBL or GHBA (Pérez de la Mora and Tapia, unpublished).

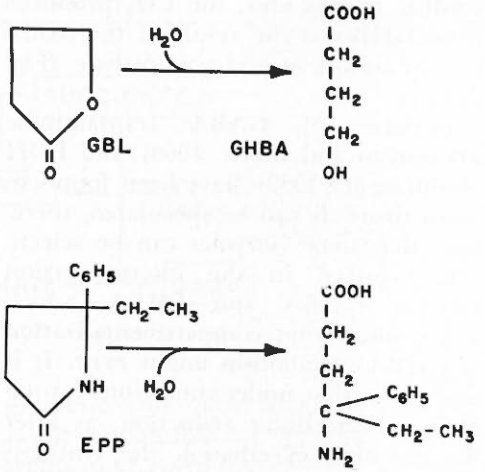

Fig. 3. Structural relationships among GBL, GHBA and EPP.

It is also of interest that GHBA and its lactone abolish the plantar reflex but not the patelar reflex in the descerebrated cat. Since the plantar reflex is polysynaptic, and since the afferent and efferent pathways were intact, it has been suggested that GHBA has an effect at the level of interneurons in the spinal cord (Basil *et al.*, 1964).

ELECTROPHYSIOLOGICAL EFFECTS OF GHBA

As it could have been anticipated, concomitantly with its effects on behavior GHBA induces remarkable electrophysiological changes in the CNS of mammals, as well as in the nervous ganglia of invertebrates. Since the early finding by Rubin and Giarman (1947) that

GHBA has a depressant action on the electrical activity of the cerebral cortex, and the observation of Wolff (1960) that GHBA was present in the so-called factor I of Florey and that this compound inhibited the stretch receptor in the crayfish, many publications have appeared on this subject. Practically all the electrophysiological studies agree in the observation that GHBA administration produces a depression of spontaneous cerebral cortex activity in many species of mammals, including man (Drakontides *et al.*, 1962; Metcalf *et al.*, 1966; Ban *et al.*, 1967; Godin *et al.*, 1968; Liberson *et al.*, 1969). In the latter species it has been possible to establish different well defined phases of electrical activity during the effect of GHBA, such as the presence of hypersyncrony and alpha rhythm followed by theta and delta rhythms (Metcalf *et al.*, 1966). In the same study some interesting paradoxical changes were recorded, as the presence of alpha frequencies in sleeping subjects, and theta and delta rhythms in waken subjects. Other reported changes are a diminished response of the activating reticular formation to electrical stimuli (Drakontides *et al.*, 1962; Ban *et al.*, 1967), an increase in the thalamo-cortical recruitment (Drakontides *et al.*, 1962), an inhibition of globus pallidus activity (Bertharion and Brue, 1967), and the presence of paradoxical sleep (Winters and Spooner, 1965).

On the basis of these and other neurophysiological studies, and since the reticular formation is considered to play an important role in the mechanisms of sleeping and waking (Oswald, 1962), it has been tentatively concluded that this subcortical structure is the primary site of action of GHBA (Roth and Giarman, 1965; Metcalf *et al.*, 1966; Ban *et al.*, 1967). Other authors, however, consider that GHBA is an epileptogenic drug, rather than an anesthetic drug, because of the observed difference between the effects of GHBA and those of pentobarbital on the spontaneous electrical activity and on the responses evoked by "clicks" (Winters and Spooner, 1965). On the other hand, the effects of GHBA on these parameters are similar to those induced by α-chloralose, which, according to the dose used, is either and anesthetic or a convulsant (Winters and Spooner, 1966).

BIOCHEMICAL-PHYSIOLOGICAL CORRELATION OF THE EFFECTS OF GHBA

It has been reported that GBL induces a sleep condition more rapidly and for longer time than free GHBA (Bessman and Skolnik, 1964; Giarman and Roth, 1964; Roth and Giarman, 1966). In view of the possibility that GBL may be sinthesized from GHBA (Fig. 2), this finding could be interpreted to mean that GBL is the active form of GHBA. Considerable controversy exists on this point, because while some authors have reported a good correlation between the sleep condition and the concentration of GHBA in brain after its administration (Giarman and Roth, 1964; Roth and Giarman, 1966), others have found a similar correlation with the lactone (Bessman and Skolnik, 1964). It has been shown that administered GHBA and GBL are not equally distributed in muscle and brain (Roth and Giarman, 1966); these differences, as well as some methodological ones, could be involved in this discrepancy. Differential measurements of GHBA and GBL in the reticular formation or other areas related to sleep could be useful to solve this point. On the other hand, although 1:4BD shows soporific effects similar to those of GHBA (Sprince *et al.*, 1966; Roth and Giarman, 1968), there is evidence that GHBA or GBL are involved in its mechanism of action (Roth and Giarman, 1968).

With regard to the molecular mechanisms involved in the soporific effect of GHBA, little is known, in spite of some experiments in which some changes in the biochemical pattern in brain tissue have been found after the administration of GHBA. The interpretation of this type of experiments is usually difficult, because, when a biochemical modification is found, it is necessary to prove that it was produced by GHBA itself, and not as a consequence of its physiological effect. If the biochemical change is really produced by GHBA, there is still the question of the relationship between this change and the soporific effect, which could be only coincidental. In spite of these limitations, these studies can give useful information on the biochemical correlates of the soporific action of GHBA. We now will review the pertinent literature on this subject.

GHBA administration induces important modifications in the intermediary metabolism, which are reflected in the whole animal by hyperglycemia (Fleming and La Court, 1965; Mitoma and Neubauer, 1968); in some instances, however, this effect has not been observed (Bessman and Skolnik, 1964; Godin *et al.*, 1968). In the CNS it seems that the Krebs cycle rate is decreased during the GHBA or GBL-induced sleep. In these conditions the brain glucose levels are increased (Fleming and La Court, 1965; Godin *et al.*, 1968; Leonard and Walkinson, 1971) and the percentage radioactivity in free amino acids after labeled glucose administration is decreased (Godin *et al.*, 1968); these findings indicate that glucose utilization is decreased. However, $^{14}CO_2$ production from ^{14}C-U-glucose by rat brain slices is normal after GHBA administration (Roth and Giarman, 1966). On the other hand, it is interesting that GHBA produced an increase in the levels of glucose 6-phosphate and a decrease in the concentration of fructose-1,6-diphosphate in mouse brain (Leonard and Walkinson, 1971). This finding suggests an effect of GHBA on the limiting step in glycolysis, the phosphofructokinase reaction.

It has been found that GHBA decreases the $^{14}CO_2$ production from ^{14}C-2-pyruvate during the potassium-stimulated respiration of rat brain slices (Roth and Giarman, 1966); lactate "steady state" levels were increased (Godin *et al.*, 1968). Measurements of the concentration of citrate, isocitrate, α-ketoglutarate and malate in brain showed that only malate was decreased during the effect of GHBA (Fleming and La Court, 1965). However, the radioactivity in some intermediates of glucose metabolism after labeled-glucose administration was greater in GHBA-treated animals than in the controls, suggesting an impairment in the activity of the Krebs cycle as related to other metabolites. This idea is supported by the finding that the incorporation of radioactivity from glucose to amino acids, which is due to a rapid exchange of the carbon skeleton of amino acids with the Krebs cycle intermediates (Van den Berg *et al.*, 1969), is decreased during GHBA-induced sleep, although no changes of amino acid levels were observed (Godin *et al.*, 1968; Margolis, 1969). In this regard it is of interest that the brain oxygen consuption is decreased during sleep (Kety, 1961) and during phenobarbital or pentobarbital-induced anesthesia (Fleming and La Court, 1965; Bachelard and Lindsay, 1966).

The effects of GHBA coud be mediated by some modifications of synaptic transmission in certain neurones. Therefore, the metabolism of some synaptic transmitters has also been studied in animals treated with GHBA or its lactone. Acetylcholine levels were increa-

sed in rat and mouse brain after GBL administration, and this increase was well correlated with the hypnotic condition; these changes were most notable in the cerebral cortex, whereas in the brain stem they were observed only in the culliculi and the adjacent reticular formation (Giarman and Schmidt, 1963). In this regard it is interesting that cholinergic mechanisms seem to be present in the latter structure (Exley *et al.*, 1958).

A participation of dopamine in the mechanism of GHBA-induced sleep is strongly suggested by several findings: GHBA and GBL produce an increase of dopamine turnover (Spano *et al.*, 1970), of its formation from labeled tyrosine (Roth and Suhr, 1970), and of its concentration in rat (Gessa *et al.*, 1966, 1968; Roth and Suhr, 1970) and rabbit (Gessa *et al.*, 1966) brain. The latter change was correlated with the soporific effect of GHBA. Furthermore, this increase in dopamine levels was more notable when the animals were pretreated with DOPA and less notable after pretreatment with amphetamine (Gessa *et al.*, 1968), a compound that decreases cerebral dopamine concentration (Roth and Suhr, 1970). In general, the duration of GHBA-induced sleep is well correlated with the concentration of dopamine in brain (Rizzoli *et al.*, 1969). Although all these results suggest that dopamine is involved in the soporific effect of GHBA, some others do not agree with this postulate. For instance, it has been reported that when the increase in dopamine levels is prevented by the administration of α-methyl-tyrosine, the sleep condition is not modified (Gessa *et al.*, 1968).

With regard to the mechanism of the elevation of dopamine concentration induced by GHBA, both an inhibition of its release and a compensatory increase in its synthesis have been postulated (Roth and Suhr, 1970). The findings which support the blocking of the release are that homovanillic acid-the product of degradation of dopamine-is decreased in subcortical structures after GHBA administration (Roth and Suhr, 1970), while neither the activity of monoaminooxidase nor that of catechol-O-methyl transferase are affected in these conditions (Gessa *et al.*, 1968). That dopamine synthesis is increased by GHBA is indicated by the finding that the elevation of dopamine levels is blocked by α-methyl-tyrosine (Gessa *et al.*, 1968) and that GHBA induces a selective increase in the transformation of ^{14}C-tyrosine to ^{14}C-dopamine (Roth and Suhr, 1970).

It is noteworthy that neither the levels of norepinephrine (Gessa *et al.*, 1966, 1968) nor those of serotonin (Giarman and Schmidt, 1963; Gessa *et al.*, 1966, 1968), were modified by GHBA administration. However, the turnover of serotonin was increased by GHBA administration (Spano *et al.*, 1970), a very important finding in view of the possible physiological role of this amine in the sleep mechanisms (Jouvet, 1969).

Before finishing this section, we would like to mention some very interesting observations on the soporific effect of other alcohols derived from amines. It has been reported that a series of alcoholic derivatives of tryptophan, such as tryptophol, 5-hydroxytryptophol and 5-methoxytryptophol (Feldstein *et al.*, 1970) and some of the corresponding aldehydic derivatives (Sabelli *et al.*, 1969) can induce a sleep condition similar to that produced by GHBA. These observations seem to be important because of the similar metabolic relationships in the systems GABA-GHBA, tryptamine-tryptophol and serotonin-5-hydroxytryptophol: in all cases there is a deamination of the amine to the aldehyde, followed by a reduction to the alcohol. Since GABA and serotonin are probably synaptic transmitters, it is interesting to speculate on the possibi-

lity that, as a general phenomenon, alcohols derived from biogenic amines with a physiological role in the CNS are involved in the biochemical mechanisms of sleep. This seems not unreasonable in view of the fact that the indole-alcohols are normal constituents of brain (McIsaac *et al.*, 1965), and they can be synthesized in nervous tissue (McIsaac *et al.*, 1965; Eccleston *et al.*, 1966; Feldstein and Williamson, 1968). Moreover, as in the case of GHBA, the administration of ethanol facilitates the formation of 5-hydroxytryptophol (Davis *et al.*, 1967); this could be also a factor in the soporific effect of ethanol.

With regard to the possibility that GHBA acts as a synaptic transmitter in the CNS, the few available data are negative: iontophoretic application of GHBA to cerebral cortex neurons and to interneurons of the spinal cord did not affect either the spontaneous activity or the discharges evoked by the application of excitatory amino acids (Crawford and Curtis, 1964). This type of studies on different neuronal nuclei are most desirable.

MEDICAL USE OF GHBA

Because of the speed at which GHBA and its lactone reach the CNS, and because of their neuropharmacological effects, the sodium salt of GHBA has been used as an anesthetic in several types of surgery, including neurological, gastrointestinal and obstetrical operations (Laborit *et al.*, 1960, 1961; Blumenfeld *et al.*, 1962; Bertoletti *et al.*, 1969; Inzirillo *et al.*, 1969). In all cases GHBA was very effective when the patient had been adequately premedicated. Some advantages of the use of GHBA are a notable relaxation of the jaw, which facilitates the endotracheal intubation, its cardiotonic and regulatory effects on ventilation, a rapid regain of consciousness, its lack of toxicity and the lack of postoperatory problems (Laborit *et al.*, 1960, 1961; Blumenfeld *et al.*, 1962). The main disadvantages are the necessity of a good anesthetic premedication to avoid the waking of the individual by the surgical incision (Blumenfeld *et al.*, 1962), and the long latency period to reach the maximal anesthetic effect (approx. 30 min) (Laborit *et al.*, 1960, 1961; Blumenfeld *et al.*, 1962; Helrich *et al.*, 1964). On the other hand, GHBA is a potentially useful agent as a soporific drug, alone or in combination with other substances (Laborit *et al.*, 1960).

It is now recognized that good results are obtained in the treatment of Parkinson's disease with L-DOPA (Godwin-Austen *et al.*, 1969), most probably because it increases dopamine levels in the striatum. For this reason, and since GHBA administration induces an elevation of dopamine in the caudate nucleus (Gessa *et al.*, 1966), which is potentiated by L-DOPA (Gessa *et al.*, 1968) and occurs at the nerve endings (Aghajanian and Roth, 1970), GHBA is also a potential drug for the treatment of parkinsonism.

NOTE ADDED IN PROOF

While this article was in press, a publication appeared (Bustos, G. and R. H. Roth, 1972. Effect of gamma-hydroxybutyrate on the release of monoamines from the rat striatum. *Br. J. Pharmac.* *44*: 817-820) indicating that GHBA blocks the K^+-induced release of newly formed dopamine from rat striatum. If this effect occurs in man, the increase of dopamine levels after GHBA administration does not imply a better function of the dopamine neurons, and consequently the suggestion of the therapeutic use of GHBA in Parkinson's disease is probably wrong.

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