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Effects of gamma-hydroxybutyrate on chick behaviour, electrocortical activity and crossed extensor reflexes

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Summary

1. Convulsant and anticonvulsant effects of gamma-hydroxybutyrate (GHB) have been studied in chicks.

2. When administered alone, GHB produced weak myoclonic seizures accompanied by electrocortical synchrony and spikes.

3. GHB was found to protect chicks against leptazol- and picrotoxin-induced seizures. A slight potentiation of strychnine-induced seizures was evident.

Introduction

Gamma-hydroxybutyrate (GHB) occurs naturally in rat brain (Bessman & Fishbein, 1963). In cats, GHB produces a reduction of crossed extensor reflexes and antagonizes strychnine-induced potentiation of reflexes (Basil, Blair & Holmes, 1964). It has been used as an anaesthetic agent in man (Helrich, McAslan, Skolnik & Bessman, 1964). In cats and rats it has been shown to produce behavioural and EEG seizure activity (Winters & Spooner, 1965a, 1965b ; Marcus, Winters, Mori & Spooner, 1967). The effects of GHB in chicken are described in this paper.

Methods

The experiments were carried out on white Leghorn chicks, 2–10 days after hatching. In conscious chicks, subcutaneous injections were always given at the back of the neck and intravenous injections were given into the right external jugular vein with a number 20 needle. For subcutaneous and intravenous injections drugs were injected in a volume not exceeding 0·2 ml.

Crossed extensor reflexes (CER)

Chicks were anaesthetized with chloralose (30–50 mg/kg, i.p.). The chick was laid on its back, the trachea cannulated, and artificial ventilation applied immediately and throughout each experiment with a Palmer Miniature Ideal Pump. Cross extensor reflexes were elicited once every 20 seconds by stimulating the central portions of the severed right sciatic nerve with rectangular shocks of 1 ms duration and of about twice the strength required to evoke a maximal reflex in the contralateral limb ; they were recorded either on smoked paper by means of a light spring loaded lever, as previously described (Bowman, Callingham & Osuide, 1964 ; Bowman & Osuide, 1967), or by means of an Ether transducer (Dynamometer UF 1) on a Devices M4 recorder using DC2C preamplifier on heat sensitive paper.

Chicks were spinalized during the experiment by sectioning the cord at the level of the 10th cervical vertebra between two ligatures.

Maximal twitches

Twitches of the gastrocnemius or tibialis anterior were elicited once every 10 s by stimulation of the peripheral portion of the severed ipsilateral sciatic nerve with rectangular shocks of 0·1 ms duration and of about twice the strength required to evoke a maximal twitch, according to the method of Bowman *et al.* (1964).

Convulsant and anticonvulsant experiments

For these experiments conscious chicks were used in groups of 10 in an observation box of dimensions $0{\cdot}65 \times 0{\cdot}45 \times 0{\cdot}22$ m.

Implantation of electrocortical and electromyograph electrodes

Chicks were anaesthetized with halothane. Electrodes were made from the pointed ends of stainless steel insect pins No. 00 and were implanted on to the superficial areas of the striatum (accessory hyperstriatum, hyperstriatum and neostriatum) according to the method of Spooner (1964, 1965). Electromyograph (EMG) electrodes were made with stainless steel insect pins No. 00 bent at the ends to make hooks. The electrodes were positioned in the proximity of the head and insertion of the semispinalis capitis muscle of one side of the dorsolateral aspect of the neck. The chicks were allowed 24 h for recovery.

Recording procedures

During recording the chick was placed in a screened cage in a quiet room. The electrocorticograms (ECoG) from the superficial striatal areas were recorded with the comb as reference. The normal behaviour, ECoG and unintegrated EMG were observed for at least one hour before drug administration. The ECoG and EMG were recorded using a Devices M4 four channel recorder with Devices AC7C preamplifiers, and heat writing stylus on heat sensitive paper.

Drugs and solutions

The drugs used were: sodium gamma-hydroxybutyrate (Miles Laboratories), amino-oxyacetic acid semihydrochloride (Upjohn), leptazol (British Drug Houses), picrotoxin (Savory & Moore) and strychnine hydrochloride (British Drug Houses). Amino-oxyacetic acid (AOAA) was always used in a solution adjusted to pH 7. The doses of sodium gamma-hydroxybutyrate, and amino-oxyacetic acid refer to the salt while the doses of strychnine refer to the base.

Results

Conscious chicks

On intravenous injection, 50–150 mg/kg GHB produced sedation; larger doses (200–250 mg/kg) produced loss of the righting reflex for 10–20 minutes. A dose of 340–1,000 mg/kg, injected intraperitoneally, produced loss of the righting reflex within 15 min and some chicks showed myoclonic jerks beginning 25–35 min after injection. Thereafter the chick showed myoclonic jerks about once every 5 min for 20–60 min, after which the seizures became less frequent. The ED50 dose for

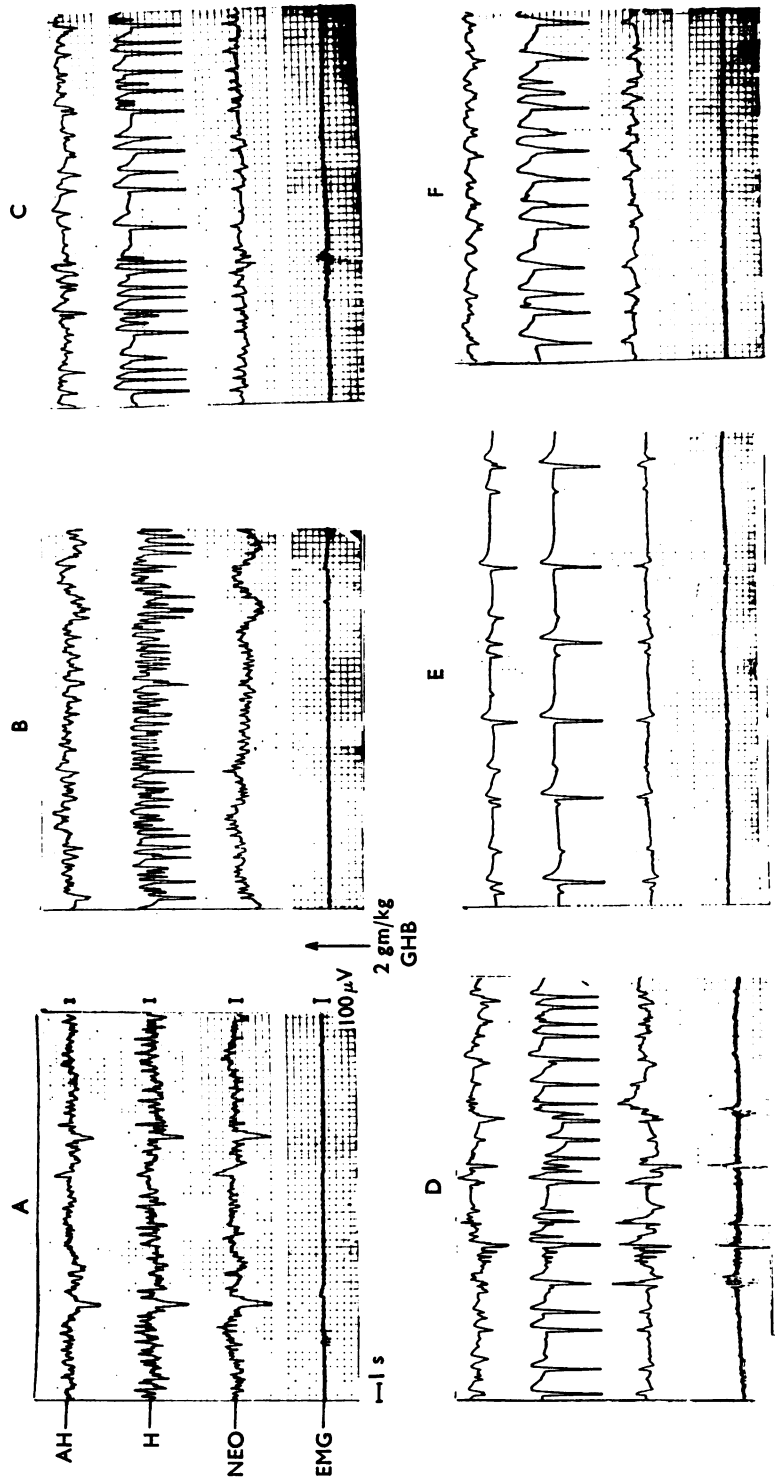

FIG. 1. ECoGs recorded from an 8 day old chick showing effect of GHB 2 g/kg, i.p. Monopolar electrodes were used to record from (AH) the left accessory hyperstriatum, (H) the right hyperstriatum, and (NEO) the right neostriatum. EMG represents electromyogram. In Figs. 3, 4, and 5, AH, H, NEO and EMG are similarly represented. Panel A shows the control, with chick crouched, quiet and with eyes closed. Panels B, C, D, E, F represent 3, 30, 60, 90, and 240 min after GHB respectively. The chick was lying on its side with one myoclonic seizure in Panel C and a series of myoclonic seizures in Panel D. In Panels E and F, the chick was lying on its side with eyes in a catatonic-like posture.

production of myoclonic jerks was 680±22 mg/kg in 2 day old chicks, when injected intraperitoneally. The chicks recovered from the effects of these doses within 2 hours. With doses of 2 g/kg GHB, myoclonic activity was first noticed 10–15 min after injection, and could be induced by sound or tactile stimuli. The chicks developed weak tonic seizures when handled about 60 min after 2 g/kg GHB. With this dose the chicks recovered after about 14 hours. The effects of GHB were accompanied by electrocortical (ECoG) changes. The progression was as follows: synchronous slow waves 2–4 Hz (Fig. 1, B ; Fig. 2, 0·15 h after GHB) sharp waves and spikes 1–3 Hz (Fig. 1, C, D) sharp waves and spikes separated by gradually increasing periods of electrical silence (Fig. 1, D, E ; Fig. 2, 0·75–2·5 h after GHB). The myoclonic seizures were most frequent when the ECoG showed predominantly sharp waves and spike activity. When the ECoG showed sharp wave and spike activity separated by periods of electrical silence, the chick lay on

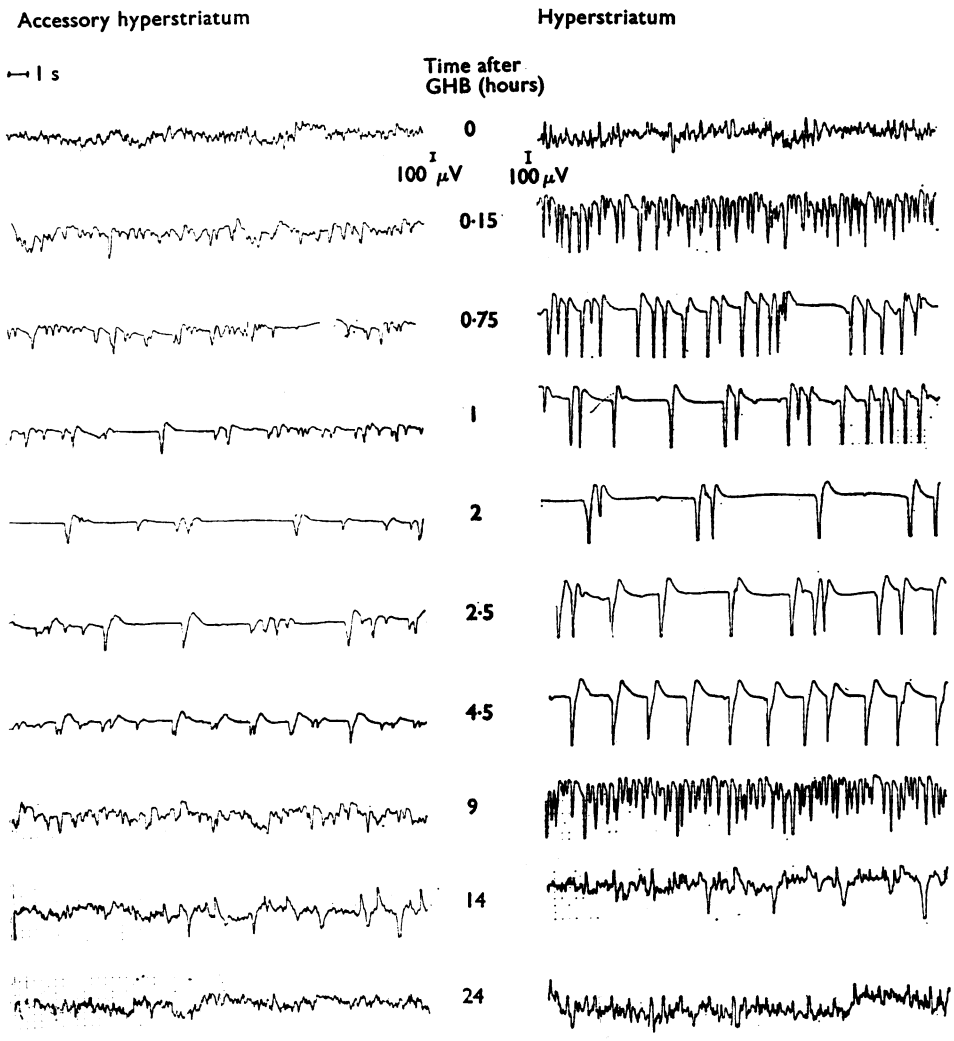

FIG. 2. ECoGs recorded from an 8 day old chick before and after intraperitoneal injection of GHB 2 g/kg. Monopolar electrodes were used to record from the accessory hyperstriatum and hyperstriatum at various time intervals.

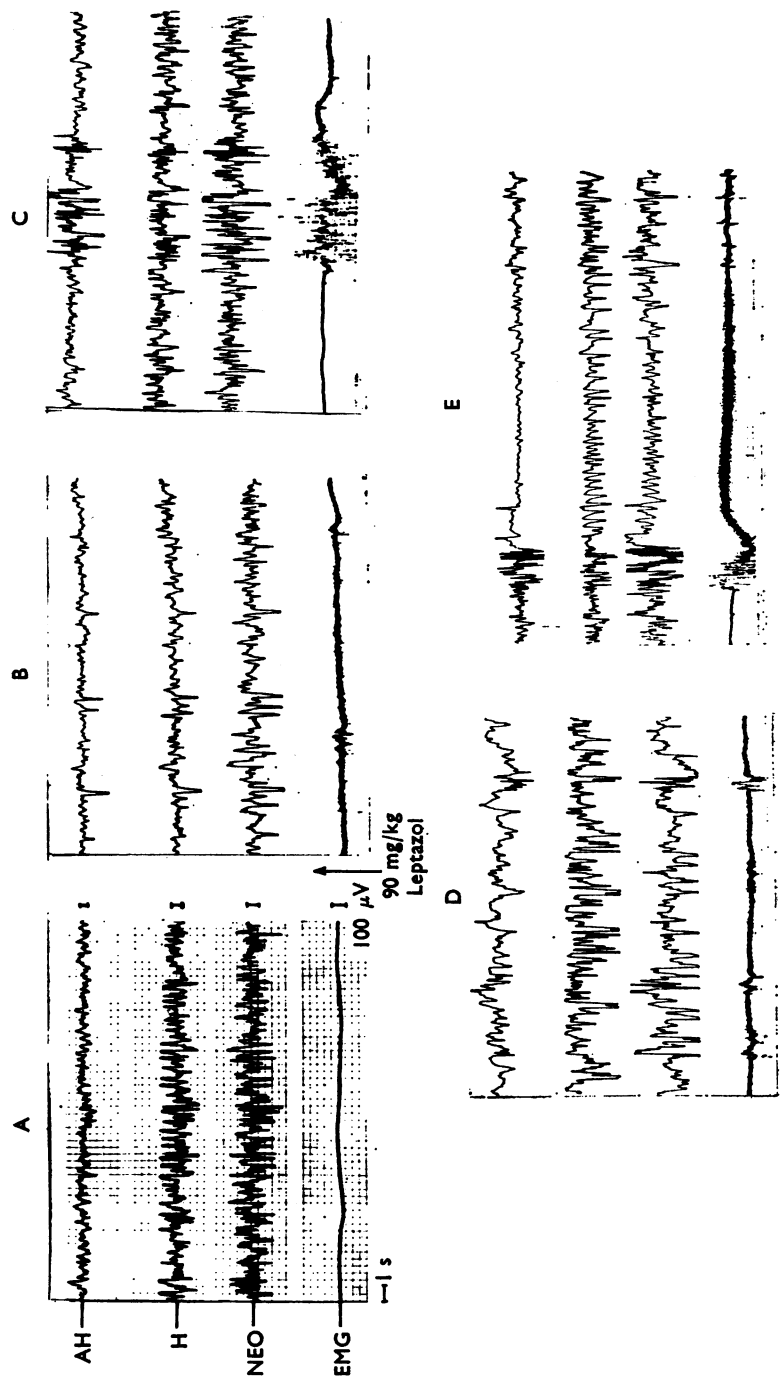

FIG. 3. ECoGs recorded from a 4 day old chick show the convulsant effect of leptazol (90 mg/kg) injected subcutaneously. Panel A shows the control with chick crouched, quiet and with eyes closed. Panels B, C, D and E, represent 2, 5, 9 and 12 min after leptazol. In panel B the chick was standing with eyes wide open. In panel C, the chick had myoclonic seizure, in D it had brief myoclonic seizures and in E it had a brief clonic seizure followed by a more prolonged tonic extensor seizure.

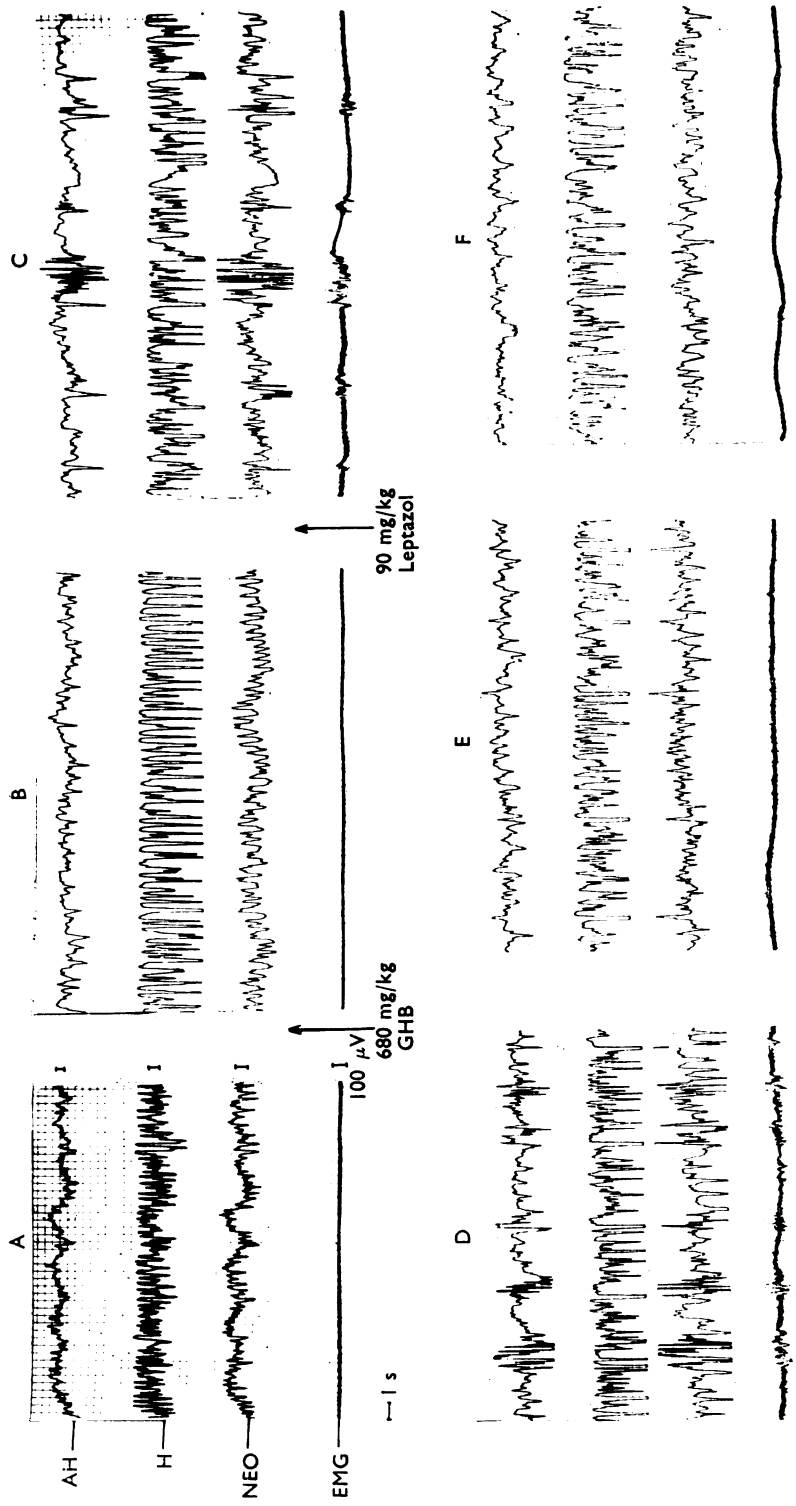

FIG. 4. ECoGs recorded from a 4 day old chick to show the antagonism of leptazol effects by GHB. Panel A represents the control record with the chick crouched, quiet and with eyes closed. Panel B represents 30 min after GHB with the chick lying on its side. Panels C, D, E, and F, represent 12, 20, 30 and 60 min respectively after injection of leptazol. The chick showed only myoclonic seizures in panels C and D. The chick was lying on its side in panels B, C, D, and E. In panel F, the chick was crouched with eyes closed. No tonic seizures were produced.

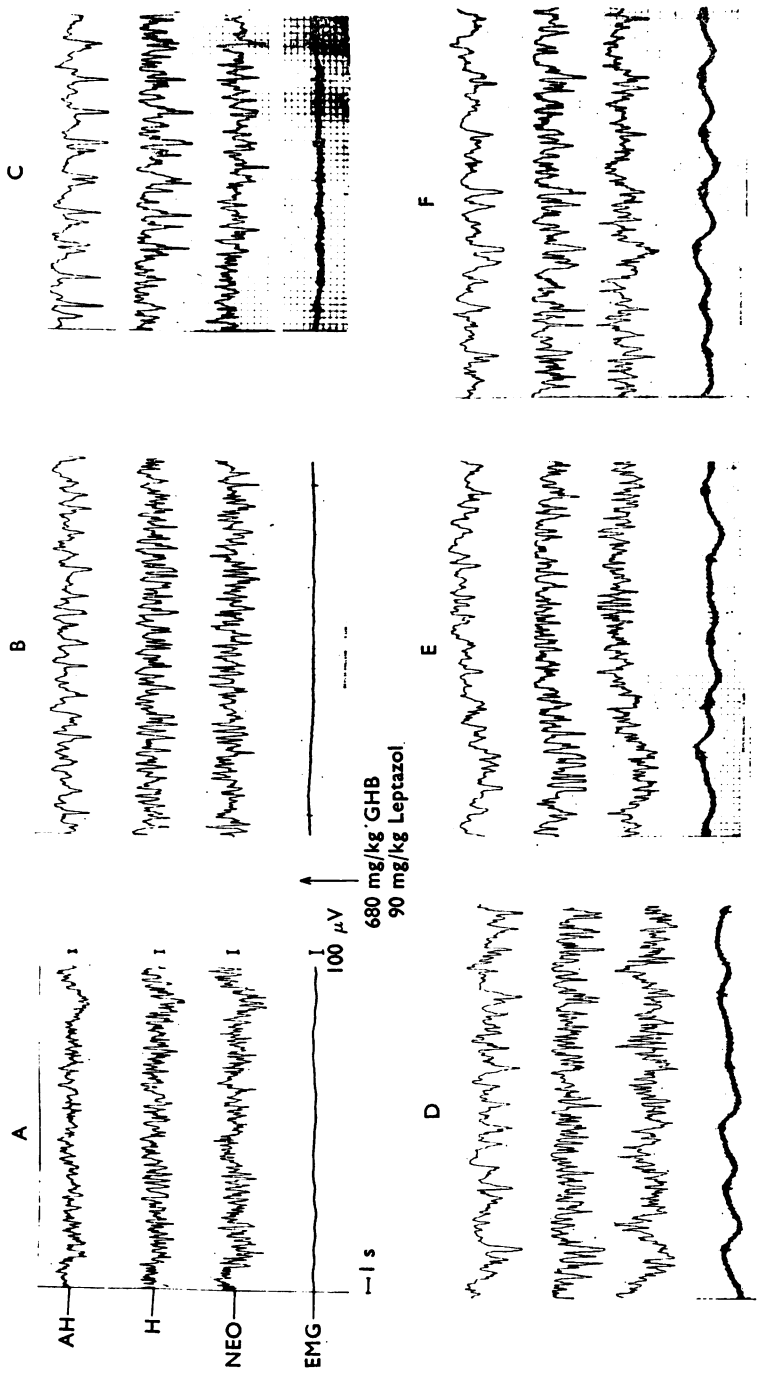

FIG. 5. ECoGs recorded from a 2 day old chick showing the effect of simultaneous administration of leptazol (90 mg/kg, s.c.) and GHB (680 mg/kg, i.p.). Panel A represents the control with chick crouched, and eyes closed. Panels B, C, D, E and F represent 12, 20, 30, 45 and 60 min respectively after injection of both drugs. The chick was lying on its side in panels B to E, and crouched in panel F prior to full recovery. In panels D, E and F the chick showed head movements.

its side with eyes wide open in a catatonic-like state and did not respond to sound stimuli. During this period the seizures were less frequent. Unlike the effect of GHB in cats (Winters & Spooner, 1965a), generalized tonic-clonic seizures were not observed after GHB ; also the duration of the loss of righting reflex produced by similar doses was shorter in chicks compared with the effects in cats and rats (Marcus *et al.*, 1967). Peters, Vonderahe & Schmid (1965), Ookawa & Gotoh (1965) and Spooner & Winters (1966) have shown that chicks, like higher vertebrates, show episodes of the rhombencephalic phase of sleep (RPS). However, as in the rat (Marcus *et al.*, 1967), there was no evidence that GHB induced this type of sleep in chicks at the doses which induce it in cats (Matsuzaki, Takagi & Takizane, 1964). The electrocortical changes waned in the reverse order of their development.

Because of the seizure activity developed in chicks, two groups of experiments were designed in which graded doses of chemical convulsants were given subcutaneously, (a) 30 min after 680 mg/kg GHB given intraperitoneally, (b) simultaneously with 680 mg/kg GHB given intraperitoneally. This was done in an

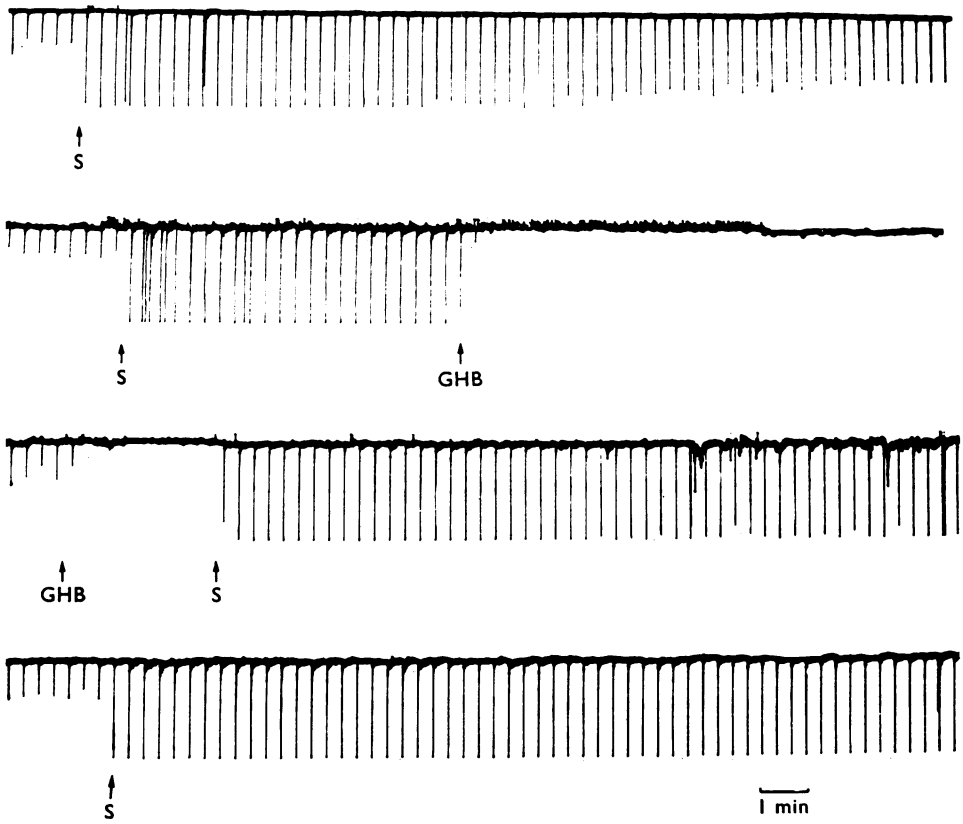

FIG. 6. Intact chick, 2 days old and weighing 39 g, anaesthetized with chloralose 50 mg/kg. Crossed extensor reflexes. At S, 4 μg strychnine, i.v. was injected, and at GHB, 5 mg GHB, i.v. The top trace shows the effect of the first dose of strychnine. Subsequent traces were recorded when the reflex had recovered from the effects of the drug illustrated in the trace immediately above it. GHB abolished the effect of strychnine when it was injected during the potentiation of the reflex but when GHB was injected before strychnine, no antagonism of strychnine was evident.

TABLE 1. *The influence of GHB on seizures produced by some convulsant drugs*

Convulsant drugs	Doses mg/kg given subcutaneously	Tonic convulsion latency (range in min)			No. convulsing out of 10			No. dead out of 10		
		Control chicks	Chicks given GHB and convulsant simultaneously	Chicks given GHB 30 min before convulsant	Control chicks	Chicks given GHB and convulsant simultaneously	Chicks given GHB 30 min before convulsant	Control chicks	Chicks given GHB and convulsant simultaneously	Chicks given GHB 30 min before convulsant
Strychnine	0·85	6–13	3–10	5–10	8	10	10	0	0	0
	1	3– 9	4–10	5–10	10	10	10	0	0	0
	1·25	5– 7	6–13	7–11	10	10	10	0	0	0
	1·5	4– 7	2– 5	3– 6	10	10	10	7	10	10
	1·75	3– 5	1– 4	3– 6	10	10	10	10	10	10
Picrotoxin	3·5	19–22	40	62–73	2	1	2	0	0	0
	4	14–26	26–40	46–55	5	4	4	0	0	0
	4·5	10–20	25–40	25–44	6	4	5	2	2	2
	5	12–16	25–50	15–55	6	5	6	2	2	2
	5·5	13–17	14–50	19–27	8	8	8	2	2	2
Leptazol	70	10–24	50	34–40	6	1	2	0	0	0
	75	6–23	29	36–42	8	1	2	0	0	0
	80	6–30	27	29–35	8	1	4	1	0	2
	85	6–10	45–50	20–50	10	4	6	5	0	4
	90	4–10	15–16	15–50	10	4	6	5	0	4

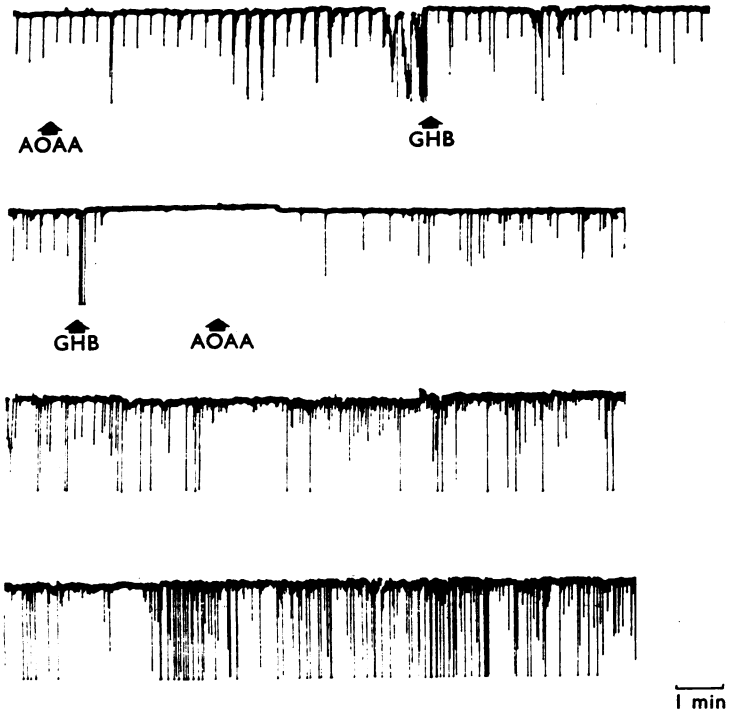

FIG. 7. Intact chick, 2 days old and weighing 42 g anaesthetized with chloralose 30 mg/kg. Crossed extensor reflexes. Each trace is a direct continuation of the trace immediately above it. The top trace shows the effect of AOAA (1·6 mg, i.v.) and antagonized by 6 mg GHB. The next trace shows block of the crossed extensor reflexes produced by GHB (6 mg) after an initial potentiation, and the subsequent administration of AOAA 1·6 gm. Prior administration of GHB only prolonged the latency of the AOAA potentiation.

attempt to see if the convulsant effect of GHB would potentiate the effects of the convulsants, but no significant potentiation was seen in these studies (Table 1). There was, however, a significant increase in the latency of the convulsions produced by picrotoxin and leptazol. The most significant effect was the protection produced by GHB from the convulsant and lethal effects of leptazol, especially when it was administered simultaneously with GHB (Figs. 3, 4 and 5). GHB (500 mg/kg) has also been found to prolong the period of latency before the convulsions in day-old chicks induced by 15 mg/kg amino-oxyacetic acid (AOAA), from 7–15 min to 20–25 min, and also to protect some of the chicks from the lethal effects of AOAA (Osuide, unpublished observations).

Crossed extensor reflexes (CER)

GHB depressed the reflex contractions in chicks with intact CNS in doses of 20–25 mg/kg. The effects lasted 1–30 minutes. After spinalization, a similar

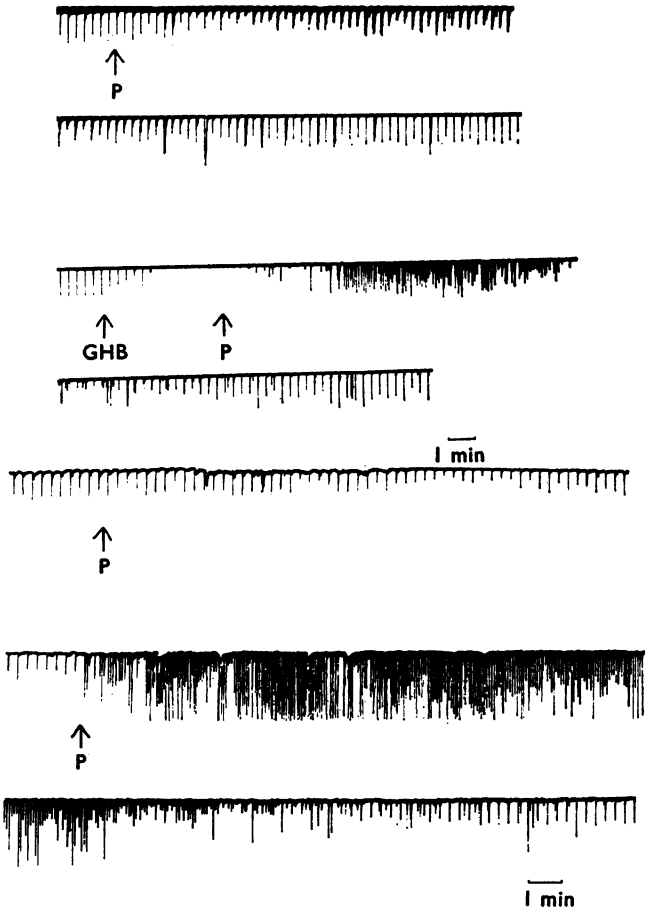

FIG. 8. Intact chicks 1 day old and weighing 35 g (upper four traces) and 40 g (lower three traces) anaesthetized with chloralose 40 mg/kg. At P, 45 μg picrotoxin, i.v. was injected, and at GHB, 3 mg GHB, i.v. In the lower experiment the top-most trace shows the effect of the first dose of picrotoxin and the two succeeding traces show the cumulative effect of the second dose. The upper experiment shows the reduced cumulative effect of a second dose of picrotoxin (3rd and 4th traces from top) after prior injection of GHB. In each experiment the traces are continuations of the ones immediately above.

depressant effect was produced but larger doses of GHB (50–100 mg/kg) were required. In doses above 50–100 mg/kg, GHB abolished the potentiating effect of strychnine, picrotoxin, leptazol and AOAA when administered during the potentiation of CER produced by these drugs (Figs. 6 and 7). When administered before the convulsant drugs the only significant effect of GHB was a prolongation of the latency to the potentiation of the CER produced by the convulsants (Figs. 7 and 8).

Muscle twitches

The doses of GHB (50–2,000 mg/kg) used in this study did not affect maximal twitches of the gastrocnemius or tibialis anterior muscles elicited by stimulation of the peripheral stump of the ipsilateral sciatic nerve.

Discussion

The results of this study show that GHB produces a loss of righting reflex and a catatonic-like, non-responsive state similar to the state it induces in rats, cats and man (see **Introduction** for references), showing that the action of GHB in chickens is essentially similar to its action in higher vertebrates. GHB in the chick produced myoclonic seizures, and sharp waves and spikes in the ECoG. Unlike the cat, however, there was no generalized motor seizure activity. The weaker convulsant effect of GHB in the chick may be related to the absence of a pyramidal motor system in the avian CNS (Bremer, Dow & Moruzzi, 1939). Marcus *et al.* (1967) have attributed the lack of a convulsant action of chloralose in chicks to this factor. Chloralose and GHB have a similar convulsant action in cats (Winters & Spooner, 1966).

It was thought that GHB might potentiate the seizures produced by some convulsants, since when given alone it produces myoclonic seizures in chicks. However, there was only slight evidence of potentiation of the effect of strychnine in conscious chicks and on crossed extensor reflexes. Surprisingly, GHB prolonged the latency period before picrotoxin and leptazol-induced seizures, reduced the incidence of leptazol-induced seizures, and gave complete protection from the lethal effects of leptazol, when both drugs were administered to the chicks simultaneously. Basil *et al.* (1964) have shown that GHB, like centrally acting skeletal muscle relaxants, selectively abolished crossed extensor reflexes without affecting the knee jerk reflex in cats. Centrally acting skeletal muscle relaxants, such as mephenesin, protect conscious chicks against the convulsant effects of leptazol and picrotoxin, but do not protect against strychnine-induced seizures, although they antagonize strychnine potentiation of crossed extensor reflexes (Osuide, 1968). These findings suggest that GHB may have a dual action: (1) a short latency depression of crossed extensor reflexes similar to central skeletal muscle relaxants, and (2) a longer latency convulsant action.

It has been suggested that GHB probably acts by formation of γ-aminobutyric acid (GABA) (Dana, Baron & Laborit, 1962; Basil *et al.*, 1964; Fishbein & Bessman, 1964; Pietra, Illiano, Capana & Rava, 1966). Bessman & Fishbein (1963) have demonstrated that GABA undergoes transamination in the brain to succinic semi-aldehyde and is then converted to GHB by a specific dehydrogenase. Intraperitoneal injection of GABA leads to increased brain levels of GABA in the chick (Sisken, Sano & Roberts, 1961). It might therefore be expected that there

would be some similarity between the action of GHB and GABA in the chick. However, even 8 g/kg GABA injected intraperitoneally, did not produce seizures, and the ECoG, CER and other effects of GABA in the chick do not resemble the effects of GHB (Osuide, unpublished observations). This suggests that the effects of GHB in the chick are not likely to be related to an action on GABA metabolism. A similar conclusion has been reached by Marcus *et al.* (1967), and is supported by the finding that GHB does not increase brain levels of GABA (Baxter & Roberts, 1961 ; Giarman & Schmidt, 1963 ; Roth & Giarman, 1965).

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