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THE ACTION OF SODIUM 4-HYDROXYBUTYRATE ON SPINAL REFLEXES

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The actions of sodium 4-hydroxybutyrate, γ-aminobutyric acid and meprobamate have been studied in unanaesthetized animals, in local anaesthetic tests, on isolated organ preparations, on convulsions induced by picrotoxin and strychnine, and on monosynaptic (patellar) and polysynaptic (plantar) reflexes of the spinal cord. Sodium 4-hydroxybutyrate induced a sleep-like state with three unusual features: the righting reflex was remarkably persistent, respiration was good throughout and recovery was abrupt. γ-Aminobutyric acid was inactive and meprobamate caused flaccid paralysis with loss of the righting reflex. None of the agents affected the responses of the rat diaphragm either to direct stimulation of the muscle or to indirect stimulation through the phrenic nerve. Only meprobamate reduced the responses of the guinea-pig isolated ileum preparation, showed local anaesthetic action and had an anticonvulsant action. All three compounds were capable, after intravenous or topical application, of blocking plantar reflexes in doses which did not affect the patellar reflex. The spinal animal responded in the same way, to the same dose of sodium 4-hydroxybutyrate, as the decerebrate preparation. Topical application to the motor cortex had no effect on spinal reflexes. We conclude that sodium 4-hydroxybutyrate acts preferentially on the internuncial neurones in the spinal cord but differs from meprobamate in its other actions. The similarity between the actions of sodium 4-hydroxybutyrate and of γ-aminobutyric acid provides further evidence in support of the hypothesis that sodium 4-hydroxybutyrate is involved in the γ-aminobutyric acid metabolic pathways.

Rubin & Giarman (1947) noted that sublethal doses of γ-butyrolactone completely suppressed cortical activity, measured from the electroencephalograph, and reversibly inhibited voluntary movements in mice. Benda & Perlés (1960) obtained similar results in pigeon, rat and rabbit and found that sodium 4-hydroxybutyrate had a similar action. Laborit, Jouany, Gérard & Fabiani (1960) carried out the first extensive pharmacological study of sodium 4-hydroxybutyrate. They found that, in doses of 250 mg/kg or more, it caused a sleep-like state without loss of the righting reflexes in several species. The drug had little effect on respiration, did not alter oxygen consumption, and recovery from it was abrupt. Jouany, Gérard & Laborit (1960) found that sodium 4-hydroxybutyrate did not cause ketosis.

Sodium 4-hydroxybutyrate has been used successfully as an anaesthetic adjuvant in France and in the United States (Laborit, Buchard, Laborit, Kind & Weber, 1960; Blumenfeld, Suntay & Harmel, 1962). It decreased the amount of analgesic required in surgical procedures considerably and has been used mainly in neurosurgery and in manipulations of the respiratory system. Sodium 4-hydroxybutyrate

is closely related chemically to γ-aminobutyric acid, and Dana, Baron & Laborit (1962) have suggested that it may act on the γ-aminobutyric acid metabolic pathways.

In our investigation the action of sodium 4-hydroxybutyrate on spinal reflexes has been studied and compared with the actions of γ-aminobutyric acid and the muscle relaxant, meprobamate.

METHODS

Actions in the unanaesthetized animal. Cats, rats, mice and chicks, fed with normal diets, were used. They were injected intraperitoneally, except the chicks which were injected intravenously. All animals were observed in a quiet room for at least 2 hr after injection.

Intra(cerebro)ventricular injection into unanaesthetized cats. The drugs were injected in a volume of 0.3 ml. into a lateral cerebral ventricle of female cats through a chronically implanted Collison cannula (Feldberg & Sherwood, 1953). The cats were placed in a quiet room and observed for at least 2 hr after injection.

Blood pressure measurements. Experiments were performed in cats anaesthetized with chloralose (80 mg/kg) and rats anaesthetized with urethane (1.75 g/kg). Blood pressure was recorded from the left carotid artery using a Statham P23A pressure transducer, and drugs were administered into an external jugular vein. Movement at the ankle joint due to contraction of the tibialis anterior muscle in response to electrical stimulation of the cut sciatic nerve was recorded using a Grass FT03 force-displacement transducer. Contraction of the nictitating membrane in the cat was similarly recorded and was elicited by electrical stimulation of the cut preganglionic cervical sympathetic nerve.

Spinal reflex preparations. Cats were anaesthetized with ether and were decerebrated; all records were made on a Grass polygraph. Blood pressure was recorded from the left carotid artery using a Statham P23A pressure transducer. Drugs were administered into an external jugular vein.

The patellar reflex was chosen as a monosynaptic pathway, the stimulus being from a Palmer knee jerk hammer. The polysynaptic reflexes chosen were the plantar (ipselateral-flexor and crossed-extensor) reflexes and these were elicited by electrical stimulation of the foot through electrodes sewn into the footpad. Both hind limbs were secured by drills through the femur. Movements of both legs were recorded, by Grass FTO3 force-displacement transducers attached to the feet, in response to both types of reflex activation. The stimuli were applied to the animal in a predetermined pattern using a uniselector. This pattern was six blows of the knee jerk hammer at 5 sec intervals followed, 20 sec later, by 10 sec of stimulation of the footpad at 10 cycles/sec. This cycle was repeated every 2 min.

In experiments involving topical application of drugs, the spinal cord was exposed by the dorsal approach and the dura was cut. Cotton wool, soaked in the drug solution, was applied to the cord from L5 to S1.

In experiments involving topical application of drugs to the motor cortex, the cortex was exposed in cats anaesthetized with chloralose and the dura was cut. Cotton wool, soaked in the drug solution, was applied to the exposed cortex. In some experiments the cortex was exposed momentarily to a few drops of chloroform-methanol solution (2:1, v/v) to break down the blood-brain barrier (Purpura, Girado, Smith & Gomez, 1958).

Convulsions in mice. Drugs were administered intraperitoneally to male albino mice of 18 to 22 g. The minimal dose of each convulsant which killed all of a group of five mice was determined each day. The ambient temperature was maintained at 24 to 26° C.

Local anaesthetic action. The rabbit eye test (Lesser, 1940), the guinea-pig eye test (Chance & Lobstein, 1944), the guinea-pig weal test (Bülbring & Wajda, 1945) and a modification of the Bianchi test in mice (Bianchi & Franceschini, 1954), in which the compound examined was injected at the base of the tail, were used.

Isolated organ preparations. The effects of drugs were studied on the rat phrenic nerve-diaphragm and the transmurally stimulated guinea-pig ileum preparations by the method of Paton (1957). In all isolated organ preparations contractions were recorded isometrically on a Grass polygraph using a FTO3 force-displacement transducer.

Drugs. All drugs were made up in 0.9% saline with the exception of meprobamate, which was used as a suspension in 0.5% carboxymethylcellulose. The sodium 4-hydroxybutyrate and succinic semialdehyde (succinaldehydic acid) were synthesized by Dr E. G. B. Crundwell. Other drugs used were obtained from the following sources: meprobamate (Wyeth), γ-aminobutyric acid (Light), γ-butyrolactone (Fluka), strychnine hydrochloride (B.D.H.), picrotoxin (B.D.H.), adrenaline hydrochloride (B.D.H.) and acetylcholine chloride (B.D.H.).

RESULTS

Actions of sodium 4-hydroxybutyrate in the unanaesthetized animal. In unanaesthetized cats sodium 4-hydroxybutyrate (250 to 500 mg/kg, intraperitoneally) produced a sleep-like state lasting 1 to 3 hr. There was a delay in onset of action of 15 to 30 min. Recovery was abrupt and the cats appeared normal within 10 min. There was no sign of analgesia and respiration was unchanged throughout. The righting reflex could be evoked readily and it was often accompanied by dilatation of the pupils. Other reflexes were still present but the responses were sluggish. Mydriasis and miosis were seen at various stages and the pupils were sensitive to changes in light intensity. The nictitating membranes were fully contracted throughout. On recovery of consciousness nystagmus and salivation were observed. Similar sedative actions were seen in rats, mice and chicks.

In cats, γ-aminobutyric acid (500 mg/kg, intraperitoneally) had no observable effect. Meprobamate (400 mg/kg, intraperitoneally) caused a flaccid paralysis with complete relaxation of voluntary muscle including the jaw muscles. There was a 15 to 20 min delay in onset of action and recovery occurred after about 5 hr. Respiration was unchanged throughout and the pupils were dilated. The righting reflex was abolished but the pinna and corneal reflexes were present throughout. The nictitating membranes were relaxed so that they almost covered the eyes. At no stage was the animal analgesic. In contrast with the response to sodium 4-hydroxybutyrate, recovery was rather slow, lasting about 1 hr.

Intraventricular injection in unanaesthetized cats. Intraventricular injection of sodium 4-hydroxybutyrate (20 mg) produced the same signs as intraperitoneal injection except that the delay in onset of action was shorter. γ-Aminobutyric acid (50 mg) and meprobamate (2 mg) had no observable effect.

Blood pressure measurements. In the cat anaesthetized with chloralose, sodium 4-hydroxybutyrate (200 mg/kg, intravenously) caused a slight fall in blood pressure which returned to normal in 2 to 3 min (Fig. 1). It had no effect on the response of the tibialis muscle to stimulation of the cut sciatic nerve. The pressor response to occlusion of the right carotid artery for 15 sec was considerably reduced for about 10 min. The responses of the nictitating membrane to stimulation, and of the blood pressure to adrenaline, were unaffected by the drug.

γ-Aminobutyric acid (300 mg/kg, intravenously) caused a small sustained rise in blood pressure. It had no effect on the responses to carotid arterial occlusion,

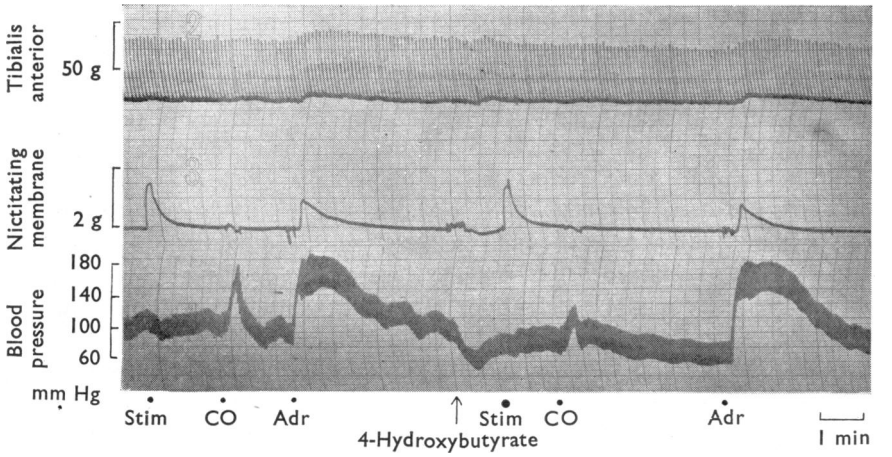


Fig. 1. Cat, 3.1 kg, anaesthetized with chloralose (80 mg/kg). Top trace, tibialis anterior muscle contractions elicited by stimulation of the cut sciatic nerve at 5 V every 5 sec; middle trace, nictitating membrane contractions; bottom trace, arterial blood pressure. Stim, stimulation of the preganglionic cervical sympathetic nerve for 10 sec at 10 cycles/sec and 3 V. CO, occlusion of the right common carotid artery for 15 sec. Adr, intravenous injection of adrenaline (5 μg). Sodium 4-hydroxybutyrate (200 mg/kg) was injected intravenously at the arrow.

intravenous injection of adrenaline, or stimulation of the cut sciatic and cervical sympathetic nerves.

Meprobamate (25 mg/kg, intravenously) caused a considerable fall in blood pressure with slow recovery to the initial level over 20 min. It caused a small transient reduction in the contraction of the nictitating membrane and had no effect on the responses to carotid arterial occlusion, intravenous injection of adrenaline and stimulation of the cut sciatic nerve.

The rat blood pressure experiments confirmed the results obtained in the cat with all three drugs at the same doses, except that the fall in blood pressure caused by meprobamate was not so conspicuous.

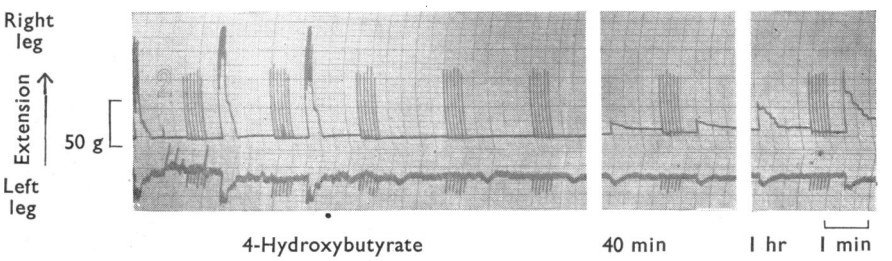


Fig. 2. Decerebrate cat, 2.6 kg. Top trace, movement of the right leg; bottom trace, movement of the left leg. The patellar reflex was elicited on the right leg by six blows of the knee jerk hammer, at 5 sec intervals, every 2 min. The plantar reflexes were elicited by 10 sec, stimulation of the left footpad at 10 shocks/sec and 20 V. Sodium 4-hydroxybutyrate (50 mg/kg, intravenously), completely blocked the plantar reflexes without affecting the patellar reflex. The plantar reflexes began to recover after 40 min. Recovery was almost complete in 1 hr.

Spinal reflex preparations. In the decerebrate cat, sodium 4-hydroxybutyrate (50 to 100 mg/kg, intravenously) reduced or completely blocked the plantar reflexes within 1 to 3 min while the patellar reflex was unaffected (Fig. 2). Recovery from this block was usually seen in 1 to 3 hr. A general reduction in resting muscle tone was often seen. Doses from 100 to 200 mg/kg caused complete block of the plantar reflexes with some reduction of the patellar reflex. Doses of 250 mg/kg or more caused complete block of both reflexes. When, after obtaining a block of the plantar reflexes and recovery with sodium 4-hydroxybutyrate, the spinal cord was sectioned at C2 and the dose repeated, the same response was obtained as in the decerebrate preparation. The block caused by sodium 4-hydroxybutyrate could be reversed by strychnine (60 μg/kg) and facilitation of the reflexes caused by an infusion of strychnine could be blocked by sodium 4-hydroxybutyrate. γ-Butyrolactone had the same action as sodium 4-hydroxybutyrate when administered in equimolar doses.

In the decerebrate cat, γ-aminobutyric acid (100 mg/kg or more, intravenously) caused facilitation of the reflexes after 1 min which lasted about 1 min and was immediately followed by a reduction or complete block of the plantar reflexes with the knee jerk unaffected (Fig. 3). Recovery occurred in 5 to 90 min. There

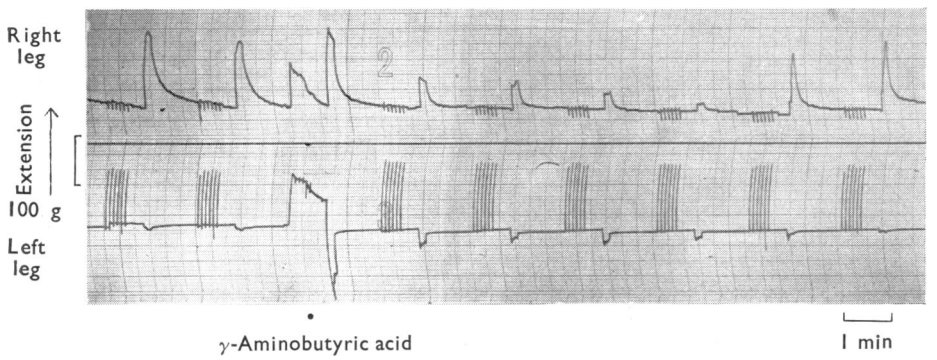


Fig. 3. Decerebrate cat, 3.6 kg. Top trace, movement of the right leg; bottom trace, movement of the left leg. The patellar reflex was elicited on the left leg by six blows of the knee jerk hammer, at 5 sec intervals, every 2 min. The plantar reflexes were elicited by 10 sec stimulation of the left footpad at 10 shocks/sec and 6 V. γ-Aminobutyric acid (100 mg/kg, intravenously) caused an initial increase in the reflexes followed by suppression of the plantar reflexes with the patellar reflex unaffected. The plantar reflexes recovered after about 10 min.

was a considerable variation in the sensitivity of the preparations to lower doses of γ-aminobutyric acid, and sometimes only facilitation was seen. To ensure consistent block of the plantar reflexes it was necessary to use a dose of 300 mg/kg. In many of the experiments there was a reduction in general muscle tone which led to a potentiation of the knee-jerk.

Succinic semialdehyde (50 mg/kg, intravenously), slightly reduced the plantar reflexes without affecting the patellar reflex. The drug was toxic and death occurred soon after injection.

Meprobamate (25 mg/kg, intravenously) completely blocked the plantar reflexes without affecting the patellar reflex (Fig. 4). The onset of block was immediate, and so persistent that recovery was never seen within 3 hr. There was a slight reduction in general muscular tone.

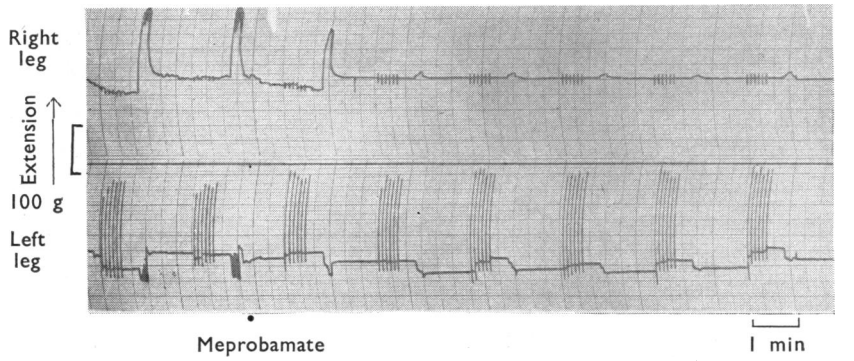


Fig. 4. Decerebrate cat, 3.6 kg. Top trace, movement of the right leg ; bottom trace, movement of the left leg. The patellar reflex was elicited on the left leg by six blows of the knee jerk hammer, at 5 sec intervals, every 2 min. The plantar reflexes were elicited by 10 sec stimulation of the left footpad at 10 shocks/sec and 7 V. Meprobamate (25 mg/kg, intravenously) practically abolished the plantar reflexes without affecting the patellar reflex. This block was so persistent that recovery was not observed.

In decerebrate cats the topical application of sodium 4-hydroxybutyrate to the spinal cord in concentrations from 25 to 100 mg/ml. for 10 min caused complete block of the plantar reflexes with the knee jerk unaffected (Fig. 5). Recovery from this block was rarely seen within 3 hr. The topical application of γ-amino-

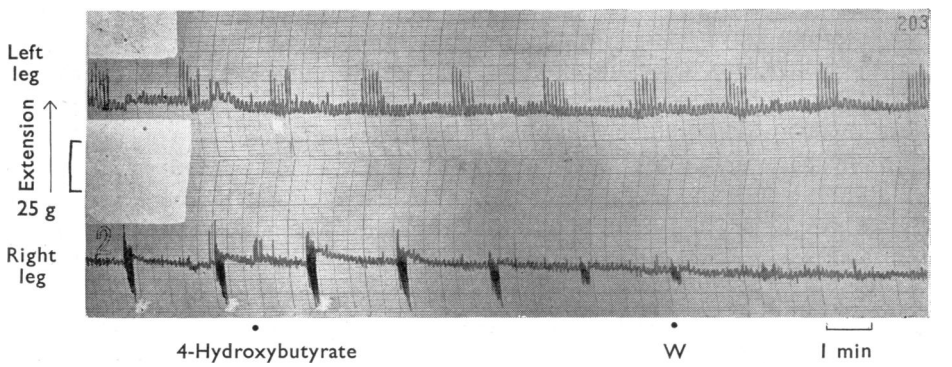


Fig. 5. Decerebrate cat, 2.3 kg, with the spinal cord exposed from the dorsal approach. Top trace, movement of the left leg; bottom trace, movement of the right leg. The patellar reflex was elicited on the left leg by six blows of the knee jerk hammer, at 5 sec intervals, every 2 min. The plantar reflexes were elicited by 10 sec stimulation of the right footpad at 10 shocks/sec and 10 V. The topical application of 4-hydroxybutyrate (30 mg/ml.) to the exposed cord for 10 min blocked the plantar reflexes without affecting the patellar reflex. The exposed spinal cord was washed at W.

butyric acid in concentrations from 30 to 300 mg/ml. for 10 min to the cord had a similar effect (Fig. 6). Over the whole range of concentrations for both drugs the delay in onset of action was about 5 min.

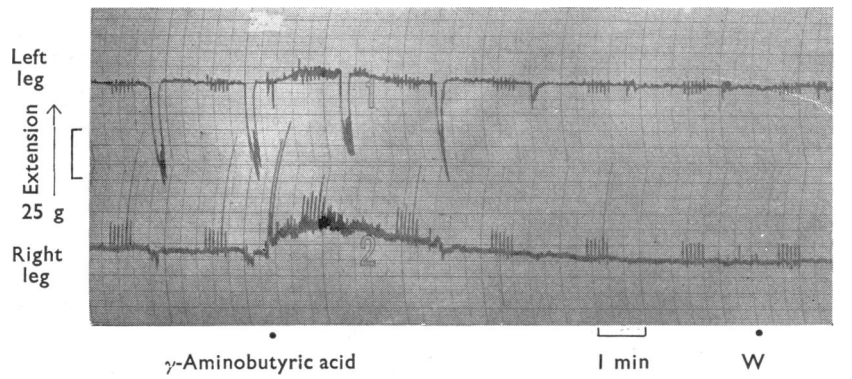


Fig. 6. Decerebrate cat, 1.9 kg, with the spinal cord exposed from the dorsal approach. Top trace, movement of the left leg; bottom trace, movement of the right leg. The patellar reflex was elicited on the right leg by six blows of the knee jerk hammer, at 5 sec intervals, every 2 min. The plantar reflexes were elicited by 10 sec stimulation of the left footpad at 10 shocks/sec and 15 V. The topical application of γ-aminobutyric acid (100 mg/ml.) for 10 min to the exposed spinal cord blocked the plantar reflexes without affecting the patellar reflex. The exposed spinal cord was washed at W.

The topical application of sodium 4-hydroxybutyrate (200 mg/ml.) and γ-aminobutyric acid (500 mg/ml.) for 15 min to the exposed motor cortex in cats anaesthetized with chloralose had no effect on either the plantar or patellar reflexes, even after treatment with chloroform-methanol solution (see Methods).

Convulsions in mice. The results of these tests are summarized in Table 1. Neither sodium 4-hydroxybutyrate nor γ-aminobutyric acid in doses of 500 mg/kg were effective in protecting mice against convulsions caused by either picrotoxin (15 to 25 mg/kg) or strychnine (1 to 5 mg/kg). Sodium 4-hydroxybutyrate did appear to delay onset of convulsions and afford some protection against death induced by picrotoxin. Sodium 4-hydroxybutyrate and γ-aminobutyric acid were given 10 min before the convulsants so that their maximal activity was due at about the same time as the first convulsion. Meprobamate (400 mg/kg) did not delay the onset of strychnine-induced convulsions, but greatly increased, by up to 2 hr, the interval between the onset of convulsions and death. Only slight protection was afforded against death. Meprobamate did protect mice against picrotoxin (5 to 20 mg/kg) convulsions and significantly decreased the number of resultant deaths. The meprobamate was given 15 min before the convulsant.

Local anaesthetic action. Procaine (2%) was a potent local anaesthetic in all four tests and meprobamate (2.5%) had some action. γ-Aminobutyric acid (30%) showed slight activity in the guinea-pig wheal test but was inactive in the other tests, and sodium 4-hydroxybutyrate (10%) had no local anaesthetic activity. Control experiments confirmed that the vehicles used had no activity.

TABLE 1

THE EFFECT OF SODIUM 4-HYDOXYBUTYRATE, γ-AMINOBUTYRIC ACID AND MEPROBAMATE ON THE TOXICITY OF STRYCHNINE AND PICROTOXIN GIVEN INTRAPERITONEALLY TO MALE MICE (18 TO 22 G)

The toxicity of the convulsants was judged by their lethal effects. Drugs were given intraperitoneally in 0.2 ml. volume. The figures in parentheses are the mean times to first convulsions in minutes for each group of four to six mice

Toxicity of strychnine				Toxicity of picrotoxin			
Dose of strychnine (mg/kg)	Control mortality (%)	Test antagonist with strychnine (mg/kg)	Mortality after treatment (%)	Dose of picrotoxin (mg/kg)	Control mortality (%)	Test antagonist with picrotoxin (mg/kg)	Mortality after treatment (%)
2	30 (∞)	γ-Aminobutyric acid, 500	30 (∞)	10	30 (∞)	γ-Aminobutyric acid, 500	30 (∞)
3	100 (4)		100 (3)	15	100 (8)		100 (10)
4	100 (2·5)		100 (2·5)	20	100 (7)		100 (10)
5	100 (6)		100 (2)	25	100 (6)		100 (7)
2	100 (5)	4-Hydroxybutyrate, 500	100 (5)	10	0 (∞)	4-Hydroxybutyrate, 500	0 (∞)
3	100 (2)		100 (5)	15	60 (16)		0 (21)
4	100 (2·5)		100 (4)	20	100 (8·5)		100 (15)
5	100 (2)		100 (2)	25	100 (5)		100 (12)
1	75 (∞)	Meprobamate, 400	0 (6)	5	0 (∞)	Meprobamate, 400	0 (∞)
2	100 (3·5)		75 (3·5)	10	75 (13)		0 (∞)
3	100 (3)		75 (3)	15	100 (6·5)		0 (∞)
4	100 (2·5)		100 (3)	20	100 (6)		0 (18·5)

Isolated organ preparations. Sodium 4-hydroxybutyrate (10 mg/ml.), γ-aminobutyric acid (30 mg/ml.) and meprobamate (10 mg/ml.) had no effect on the response of the rat phrenic nerve-diaphragm preparation either to direct stimulation of the muscle or to indirect stimulation through the phrenic nerve.

On the transmurally stimulated guinea-pig ileum, sodium 4-hydroxybutyrate and γ-aminobutyric acid in concentrations of up to 1 mg/ml. did not affect the contractions due to electrical stimulation or to exogenous acetylcholine. Meprobamate (100 μg/ml.) caused an appreciable reduction in the responses both to electrical stimulation and to exogenous acetylcholine. Concentrations of 1 mg/ml. abolished both responses with gradual recovery over 15 min.

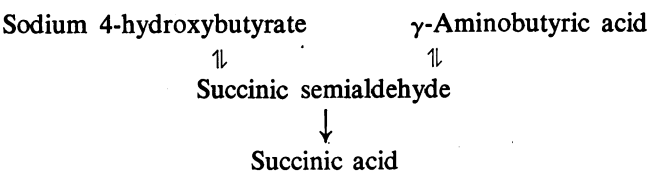
DISCUSSION

Sodium 4-hydroxybutyrate, γ-aminobutyric acid and meprobamate could each block the plantar reflexes in doses which did not affect the patellar reflex. However, the drugs had completely different actions in the unanaesthetized animal: sodium 4-hydroxybutyrate caused a sleep-like state, γ-aminobutyric acid was inactive, and meprobamate caused flaccid paralysis. On the other preparations sodium 4-hydroxybutyrate and γ-aminobutyric acid were very similar; neither had any substantial peripheral action. Meprobamate and sodium 4-hydroxybutyrate differed in their responses on several preparations. Meprobamate had some local anaesthetic action, caused a considerable fall in blood pressure, protected mice against picrotoxin convulsions and in high concentrations antagonized the responses to transmural stimulation and to acetylcholine on the guinea-pig ileum. Jouany *et al.* (1960) reported that sodium 4-hydroxybutyrate protected mice against convulsions due to strychnine but we could find no evidence to support this observation. McLennan

(1957) reported that γ-aminobutyric acid administered subcutaneously protected mice against convulsions due to strychnine but no such action was observed in the present work or by Elliott & Hobbiger (1959) or by Brockman & Burson (1957). From these comparisons it appears probable that sodium 4-hydroxybutyrate and γ-aminobutyric acid have similar modes of action on spinal reflexes and that these are different from that of meprobamate.

The evidence for the site of action of sodium 4-hydroxybutyrate on spinal reflexes can be summarized as follows: (1) The afferent nerves were not blocked ; this was shown by the lack of local anaesthetic action. (2) The efferent nerves were not blocked, as was shown by the lack of activity of the drug tested on the cut sciatic nerve-tibialis muscle and rat phrenic nerve-diaphragm preparations. (3) The spinal animal responded in the same way, to the same dose, as the decerebrate preparation and topical application to the motor cortex was without effect, which indicated that sodium 4-hydroxybutyrate had a specific action on the spinal cord. (4) Topical application of the drug to the exposed spinal cord produced a similar effect to that after systemic administration, namely preferential block of the plantar reflexes. (5) A higher dose of sodium 4-hydroxybutyrate was required to block the patellar reflex than was required to block the plantar reflexes. It is suggested on the basis of these results that sodium 4-hydroxybutyrate acts preferentially on the internuncial neurones in the spinal cord.

The similarity in the actions of sodium 4-hydroxybutyrate and γ-aminobutyric acid tends to support the suggestion that sodium 4-hydroxybutyrate acts on the γ-aminobutyric acid metabolic pathways (Dana *et al.*, 1962). A tentative metabolic pathway for the two drugs could be as follows:



Bessman & Fishbein (1963) found that sodium 4-hydroxybutyrate was present in rat brain in 1 to 2 mmole quantities. Nirenberg & Jakoby (1960) found that rat brain extracts contained small amounts of an enzyme which changed sodium 4-hydroxybutyrate into succinic semialdehyde. They also isolated a 4-hydroxybutyrate dehydrogenase in quantity from a species of *Pseudomonas* grown with sodium 4-hydroxybutyrate as its sole carbon source. Bessman, Rossen & Layne (1953) have isolated a γ-aminobutyric acid—α-ketoglutarate transaminase from mammalian brain which changed γ-aminobutyric acid into succinic semialdehyde. Thus enzymes have been identified in mammalian brain which will catalyse all the reactions of the above scheme. Attempts to test succinic semialdehyde on the spinal reflexes have so far met with little success ; this failure is probably because the compound is very unstable, dimerizes quickly, and as an aldehyde reacts with amino groups. Consequently it probably never reaches the central nervous system when given intravenously.

Krnjević & Phillis (1963) showed that the iontophoretic application of L-glutamic acid to the cortical neurones of cats caused immediate excitation. They also found that γ-aminobutyric acid could depress both glutamic excitation and the spontaneous discharge rate of the neurones. Crawford & Curtis (1964) have demonstrated that sodium 4-hydroxybutyrate had no effect either on the rate of firing induced by amino acids or on the spontaneous discharge rate of the neurones of the pericruciate cortex in cats. This work suggests that sodium 4-hydroxybutyrate does not itself have a direct action. It may act by interfering with extracellular levels of γ-aminobutyric acid.

Purpura *et al.* (1958) have shown that systemic γ-aminobutyric acid produced no significant electrophysiological effect in the presence of a normally functioning blood-brain barrier. Hower, γ-aminobutyric acid in our experiments did block a polysynaptic reflex after the intravenous administration of 300 mg/kg to the decerebrate cat. This action may have been due to a small amount of γ-aminobutyric acid passing the blood-brain barrier and/or to partial circumvention of the barrier due to decerebation. Marrazzi, Hart & Rodriguez (1958) gave γ-aminobutyric acid as a relatively close-arterial injection into a common carotid artery, which served effectively to bring relatively small doses across the blood-brain barrier.

Kuno (1960) also obtained an effect after intravenous injection of γ-aminobutyric acid (0.6 mg/kg) into decapitated cats, but in this instance depression of the extensor (monosynaptic) reflexes was observed. McLennan (1957) and Honour & McLennan (1960) have found that even application of strong solutions of γ-aminobutyric acid to the exposed spinal cord did not inhibit stretch (monosynaptic) reflexes. This observation has also been made in the present study.

The work here presented provides further evidence in support of the hypothesis that sodium 4-hydroxybutyrate is involved in the γ-aminobutyric acid metabolic pathways.

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