Biochemical Pharmacology, 1960, Vol. 3, pp. 297–304. Pergamon Press Ltd., Printed in Great Britain.

PHARMACOLOGICAL PROPERTIES OF γ-AMINOBUTYRYLCHOLINE A SUPPOSED INHIBITORY NEUTROTRANSMITTER

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(*Received* 22 *February* 1960)

Abstract—The pharmacological properties of γ-aminobutyrylcholine (GABuCh) have been investigated on isolated organs and in the anaesthetized cat. This choline ester was found to be extremely inactive on traditional test organs as compared with acetylcholine. Given in moderate doses to the cat, GABuCh most consistently gave a fall in blood pressure and a neuromuscular block. The effect on heart rate was irregular. Only in extremely high doses was any effect observed on ganglionic transmission; this took the form of a slight ganglionic block. GABuCh in itself had negligible effects on the guinea pig ileum but sometimes exhibited slight antihistaminic and anticholinergic action on this test organ. No anti-5-hydroxytryptamine effect was demonstrable in the rat fundus preparation. GABuCh is hydrolysed at a very low rate by serum cholinesterase but not at all by human red cell cholinesterase. The results are discussed in connexion with other available data of GABuCh.

Ever since the first reports about a brain extract with inhibitory action upon the crayfish stretch receptor (Factor I)[1] there has been an intense search for the active component which could be assumed to be an inhibitory transmitter. The interest was first of all concentrated on γ-aminobutyric acid (GABA) which occurred in the inhibitory extract prepared by Bazemore *et al.*[2] and which since 1950[3] had been known to be a normal constituent of the mammalian brain. However, many but not all of the actions of Factor I could be reproduced by GABA. Further analysis of the inhibitory extract showed that other amino-acids like γ-guanidinobutyric acid might be responsible for some of the effects on the crayfish stretch receptor and other test organs. Another constituent of the active inhibitory extract might be γ-aminobutyrylcholine (GABuCh).[4] The presence of this ester in the brain was first pointed out by Kuriaki *et al.*[5] In an elaborate study, entirely unconcerned with Factor I, Kewitz has recently demonstrated beyond doubt the presence of GABuCh in considerable amounts in pig's brain.[6,7] Investigations of the effects of GABA and GABuCh on the cortical activity by Takahashi *et al.*[8] have shown that both substances are active in suppressing metrazol-induced spikes but that GABuCh is about 1000 times as active as GABA. The present study deals with the peripheral pharmacological effects of synthetic GABuCh.

EXPERIMENTAL

Compounds used

GABuCh as the chloride hydrochloride was obtained from L. Light & Co. Ltd., Colnbrook, England, and Riker Laboratories Inc., Northride, Calif. (the latter

kindly provided by Dr. Georg E. Cronheim). Also used were GABA (Light & Co.), acetylcholine iodide, butyrylcholine iodide, histamine dihydrochloride and 5-hydroxytryptamine creatinine sulphate (5-HT), (Hoffmann-La Roche & Co., Basel), suxamethonium iodide (Celocurin, Vitrum, Stockholm, Sweden) and hexamethonium bromide (Vegolysen, May & Baker Ltd., Dagenham, England). Choline chloride was kindly placed at our disposal by Dr. L. E. Tammelin, Research Institute of National Defence, Sundbyberg 4, Sweden. Nembutal, eserine salicylate and atropine sulphate were as described in the *Swedish Pharmacopoeia*, 11th Ed.

Recording of respiration, blood flow and pressure and muscular contractions

The effect of GABuCh was studied in more than twenty-five anaesthetized cats and a few unanaesthetized rabbits. Anaesthesia was induced with 5 per cent nembutal (0·8 ml/kg) intraperitoneally and maintained with further small doses of nembutal intravenously. Drugs were injected through a plastic cannula in the femoral vein of the cat and the marginal vein of the ear of the rabbit. Respiration, blood pressure and contractions of the gastrocnemius muscle and the nictitating membrane were recorded by passing the output of suitably arranged transducers into a Grass model 5 polygraph.

Respiration was recorded as pressure differences in the tracheal cannula by means of a pressure transducer (model PT 5, Grass Instrument Corporation, Quincy, Mass., U.S.A.).

Blood pressure was recorded with a Statham electromanometer from the left or right femoral artery.

The effect of drugs on neuromuscular transmission was determined by recording isometric contractions of the gastrocnemius muscle in response to supramaximal stimulation of the sciatic nerve. A force displacement transducer model FT 10, Grass Instrument Corporation, was used for recording the muscle contractions and the electrical stimuli were applied to the nerve distally to a crushed region by means of a Grass stimulator, model S 4, and shielded silver electrodes, with a duration of 5 msec and a frequency of 30/min.

The neuromuscular blocking effect of the drugs is expressed in terms of the PD_{50}, namely the dose required to produce 50 per cent blockade and the duration of such a block. To determine these quantities, the drugs were injected intra-arterially through a cannula in the femoral artery of the leg opposite to that being stimulated, the tip of the cannula lying proximally to the aortic bifurcation. The percentage diminution of twitch height and duration of block were recorded for at least three doses and the PD_{50} estimated by graphical interpolation.

Contractions of the nictitating membrane were recorded by means of a force displacement transducer FT 03. The cervical sympathetic trunk was dissected free from the vagus. The excitability of the membrane was tested by stimulating the preganglionic trunk for 5–10 sec every 2 min with rectangular pulses of the duration of 5 msec, frequency of from 5 to 10/sec before and after administration of drugs. An automatic timer was used for the stimulation.

Blood flow measurements. Blood flow was recorded by the method of Lindgren[9]. All injections were given in saline intra-arterially in a volume of 0·1 ml. Acetylcholine was used as a standard and saline as repeated controls. An especially designed ordinate writer (unpublished) allowed the recording of the blood flow directly on one of the channels of the Grass instrument.

Other pharmacological preparations

Frog rectus abdominis muscle preparations. The procedure was essentially that of Chang and Gaddum[10]. In this and the following preparation the activity of the esters was expressed as the relative molar potency as compared with acetylcholine.

Guinea-pig ileum preparations. A test bath of 4 ml volume was used. An automatic timer regulated the cycle of operations except for the addition of the compounds which was made manually in a small volume (0·1 ml) into the test bath by means of a microsyringe fitted with plastic tubing. The time of contraction was 30 sec and the different doses of GABA and GABuCh were added 50–60 sec before the stimulating drugs and in the resting intervals. The doses are given as micrograms added to the bottom of the test bath.

Rat fundus preparation. The influence of GABuCh on the 5-HT-induced contractions of the rat fundus was studied by the method described by Vane[11]. The volume of the test bath without fundus strip and oxygenation was 7 ml. The same automatic timer and means of addition as for the guinea pig ileum preparation were used. The cycle was that given by Vane. GABuCh in volumes 0·1—0·2 ml was added to the bottom of the test bath 20–60 sec before submaximal doses of 5-HT. Corresponding volumes of the incubation solution alone were added as controls before 5-HT.

Hydrolysis of esters by cholinesterases

The enzymic hydrolysis was measured electrometrically at 250 °C and pH 8 in barbitone buffer as described by Tammelin[12]. The following enzyme preparations were used: purified human plasma cholinesterase stated to correspond to Cohn's plasma fraction IV-6-3 (AB Kabi, Stockholm, Sweden), human erythrocyte cholinesterase prepared by haemolysing red cells with distilled water (final dilution 1:3) after repeated washing with saline solution, guinea pig brain homogenized in barbitone buffer and containing 0·23 g brain tissue/ml.

The hydrolysis was measured over a wide range of substrate concentrations using acetylcholine iodide, butyrylcholine iodide and GABuCh chloride hydrochloride. Enzyme concentrations were adjusted to give convenient rates of hydrolysis with the different substrates. Controls with inactivated enzymes were run simultaneously. Spontaneous hydrolysis never exceeded 1 per cent. The rate of hydrolysis is expressed as millimoles acid liberated per milligram enzyme preparation per minute. Hydrolysis of acetylcholine and butyrylcholine was calibrated by introducing known amounts of acetic acid into the reaction vessel instead of substrate by means of an Agla micrometer syringe (the pK's of acetic and butyric acid are sufficiently close for this calibration to be valid for both substrates). Calibration of the hydrolysis of GABuCh chloride hydrochloride was carried out by introducing known amounts of a mixture of GABA (0·2 M) and HCl (0·2 M) in equal amounts.

RESULTS

Unanaesthetized rabbits

At repeated doses of 0·5–1·0 mg GABuCh/kg there were no objective symptoms in the rabbit. At 2 mg there was a distinct decrease in the frequency and amplitude of respiration and the animal appeared to feel ill. All symptoms occurred within 30 sec

after the injection. At 4 mg per kg a further decrease in the rate of respiration occurred which gave a distinct peripheral cyanosis. Some salivation was observed. There was a successive paralysis of the muscles with a head drop within 3 min. Even after 10 min the rabbit was incapable of standing on its feet. At 15 min the respiration was normal again and at 20 min the animal was able to walk around slowly on the floor. On the following days the animals did not show any symptoms of illness. Thus, in the unanaesthetized rabbit at least two objective symptoms are clearly visible, namely depression of respiration and paralysis of the skeletal muscles.

Anaesthetized cats

Effects on respiration. As seen in Figs. 1 and 2, after small doses which did not markedly affect the neuromuscular transmission, there were sometimes single gasps immediately after the injection. Equimolar doses of GABA always stimulated the respiration. At higher doses (2 mg or more) there was a distinct decrease in the amplitude and frequency of respiration. The dose which gave apnoea varied between 3 and 10 mg/kg with big individual fluctuations. The cats always survived after a short period of artificial respiration.

Effects on blood-pressure and heart rate. GABuCh produces a fall in the arterial blood pressure (Figs. 1 and 2) when given intravenously to cats. The depressor effect is first evident at doses of 0·2 mg/kg. At 1 mg/kg the average fall in blood-pressure is about 20 per cent. There could sometimes be observed two phases in the blood-pressure fall, one immediate and short-lasting phase and another longer-lasting phase with a very slight decrease. Atropine in different doses (0·5–4 mg/kg) did not abolish or reverse the depressive effect of GABuCh on the blood pressure. However, before atropine the fall was more pronounced. GABA in equimolar doses sometimes caused a moderate decrease, sometimes a slight increase of the blood pressure.

The effect of GABuCh on the heart rate was more variable. In general there was an immediate decrease in the heart rate after the injection. However, in a few cats an acceleration of the pulse rate was observed and in other cats the effects were negligible. Bradycardia occurring could be inhibited by atropine (Fig. 2).

Effect on nictitating membrane. The effect of GABuCh on ganglionic transmission in the superior cervical ganglion as recorded by the contractions of the nictitating membrane after preganglionic sympathetic stimulation is almost negligible. Systemic intravenous doses from 1 to 10 mg do not produce any demonstrable ganglionic block (Fig. 1). From the figure it can be seen that there is a slight elevation of the base line of the nictitating membrane at the injection of GABuCh. However, this effect persists after hexamethonium and extirpation of the ganglion and gives no simultaneous potentiation of the contractions. This effect is possibly due to the influence of the drug on the external muscles of the eye. At extremely high doses there are effects on the ganglionic transmission. At 1 mg given directly into the artery supplying the ganglion there is a rapidly disappearing ganglionic block (Fig. 3).

Effect on neuromuscular transmission. GABuCh has neuromuscular blocking effects (Fig. 1) in doses above 0·5 mg/kg intravenously. The block is intensified by eserine and preceded by fasciculations, suggesting a depolarizing type of block. The duration of the block at doses of 1 to 2 mg was about 10 to 15 min. The PD_{50} was estimated to 65 μg/kg as compared with 3 μg/kg for suxamethonium (Table 1). The duration of the GABuCh block was a little longer.

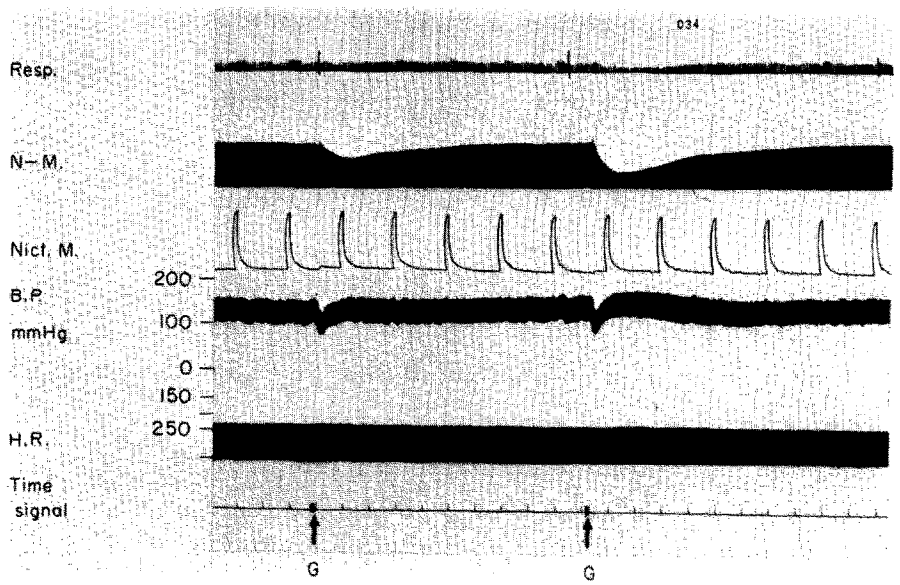

FIG. 1. Effect of GABuCh (G) on respiration (RESP.), neuromuscular transmission (N–M.), nictitating membrane, (NICT.M.), blood pressure (B.P.) and heart rate (H.R.). Abscissa, time in min. Cat 1·7 kg. Anaesthetic, nembutal. Heart rate in beats/min. (scale inverted). First injection, 1·5 mg/kg., second injection 2·0 mg/kg G.

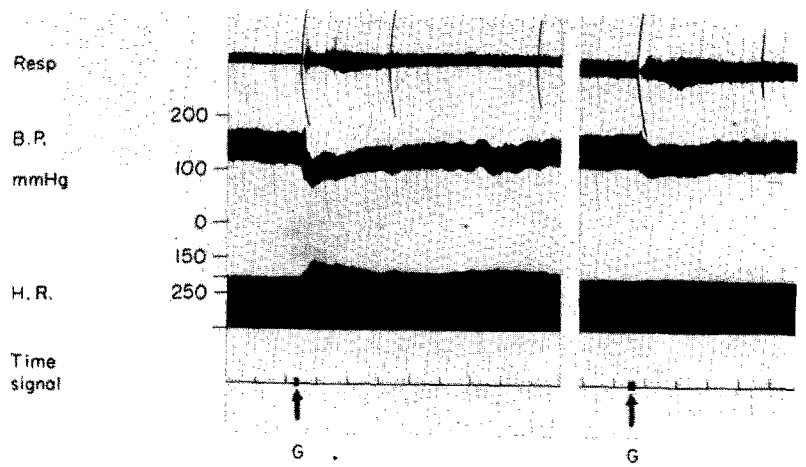

FIG. 2. Effect of GABuCh on respiration, blood pressure and heart rate. Abbreviations, units and anaesthetic as in Fig. 1. Cat 2·1 kg. Dose, 1 mg/kg. First injection before and second injection after atropine (2 doses of 2 mg/kg).

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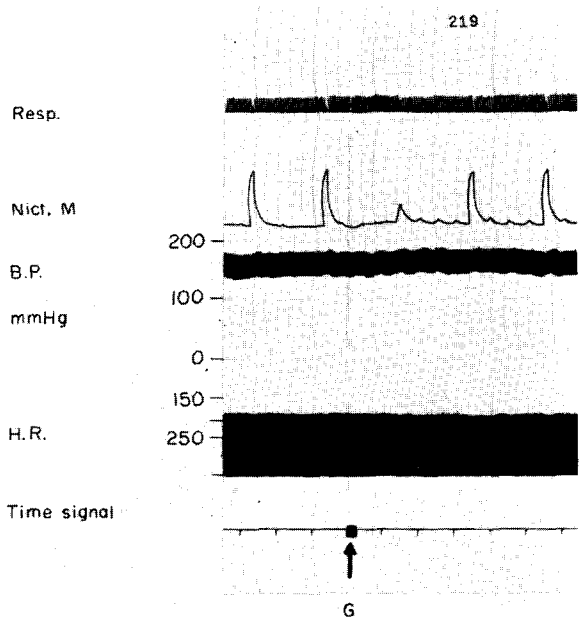

FIG. 3. The influence of GABuCh on the ganglionic transmission. Abbreviations, units and anaesthetic as in Fig. 1. Cat 2·2 kg. Dose, 1·0 mg injected into the external carotid artery, the branches of which except those to the superior cervical ganglion had been ligated. Stimulation of the isolated central stump of the sympathetic trunk for 10 sec every 2 min. with rectangular pulses of 5 msec. duration, 3 V amplitude and at a frequency of 5/sec.

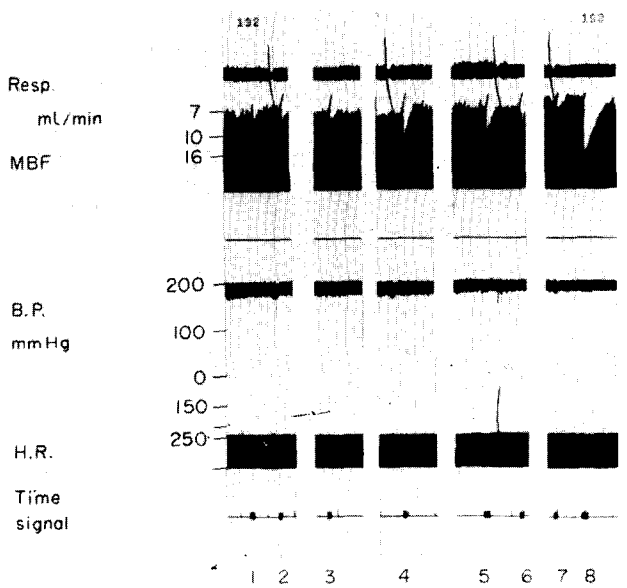

FIG. 4. Effect of GABuCh compared with that of acetylcholine on the muscular blood flow (MBF) in the left hind limb recorded in the femoral artery. Other abbreviations, units and anaesthetic as in Fig.1. Cat 3·3 kg. The following injections were made intraarterially in 0·1 ml saline: (1) saline alone; (2) to (4) GABuCh, 1, 100 and 1000 μg; (5) saline immediately after (4); (6, 7) saline; (8), acetylcholine 0·01 μg.

TABLE 1. ACTIVITY OF GABuCh ON FROG RECTUS ABDOMINIS MUSCLE, GUINEA-PIG ILEUM AND CAT SCIATIC NERVE-GASTROCNEMIUS MUSCLE PREPARATION

Ester	Relative molar potency		
	Frog rectus	Guinea-pig ileum	PD_{50} Cat sciatic-gastrocnemius preparation
Acetylcholine	100	100	—
GABuCh	0·1	0·05	2·7
Succinylcholine	—	—	100

Effects on peripheral blood flow. When compared with acetylcholine, which has a well-known peripheral vasodilator effect, GABuCh shows an extremely weak and nonspecific one (Fig. 4). At doses of 1 mg intra-arterially there is a demonstrable increase in the muscular blood flow of the leg. Even this high dose had no effect whatsoever on the blood flow in the superior mesenteric artery.

Activity on frog rectus and guinea pig ileum

The nicotinic action was stronger than the muscarinic action. However, both were extremely low (Table 1). A typical response curve of the frog rectus is seen in Fig. 5.

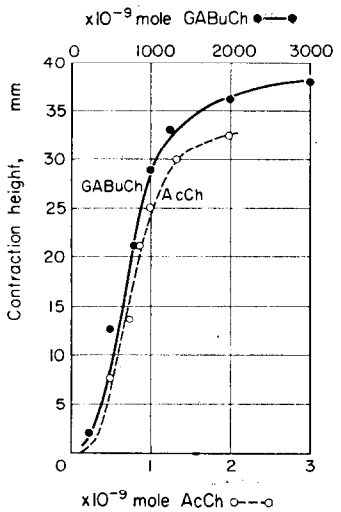

FIG. 5. The effect of GABuCh on the frog rectus preparation compared with acetylcholine. Abscissa doses in mμ moles.

Investigations concerning possible inhibitory effects on the action of smooth muscle stimulating drugs

The possible inhibitory effect of GABuCh was studied on the guinea pig ileum (histamine and acetylcholine) and on the rat fundus strip (5-HT). In highly-sensitive intestines (reacting to from 0·001 to 0·01 μg histamine base and 0·2 μg acetylcholine) the addition of from 0·1 to 10 μg GABuCh *sometimes* achieved an inhibitory effect

amounting to about 50 per cent of the contraction height. Generally this effect could not be repeated on the same intestine. Doses above 10 μg of either had no effect at all or gave a slight potentiation of the contraction.

The 5-HT contraction of the fundus strip was not significantly modified by doses of GABuCh from 1 to 100 μg. In higher doses the 5-HT contraction was slightly potentiated (Figs. 6–7), however,not more than by equimolar doses of choline. Nor were the contractions of GABuCh alone greater than those of choline. GABA itself had no inhibiting effect on this test preparation.

Enzymic hydrolysis

The results of the studies of enzymic hydrolysis are evident from Fig. 8. It will be seen, that a highly-purified preparation of butyro-cholinesterase hydrolyses GABuCh very slowly compared with butyrylcholine and acetylcholine. Erythrocytes did not hydrolyse GABuCh at all. Neither did the fairly-concentrated homogenate from rabbit brain produce any significant degree of hydrolysis of the GABuCh, the rate relative to acetylcholine being about 1 per cent.

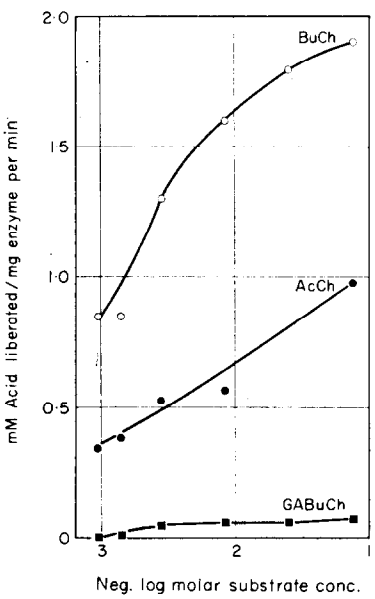

FIG. 8. Hydrolysis of butyrylcholine (BuCh), acetylcholine, (AcCh) and GABuCh by a purified preparation of butyrylcholinesterase.

DISCUSSION

As mentioned in the introduction, GABA has by some investigators been proposed as an inhibitory transmitter. Others do not agree with this (Curtis *et al.*[13]). From investigations made on the crayfish stretch receptor and brain of mammals are to be distinguished experiments on peripheral organs for which no inhibitory nerve supply is known (Hobbiger[14,15]).

From some recently-published investigations it would seem as though the choline ester GABuCh is much more active as an inhibiting compound than GABA[4,8] GABA is known to occur in large amounts in the central nervous system. The

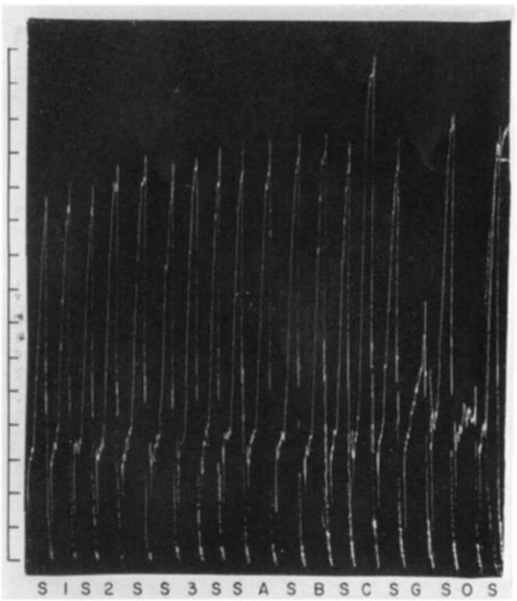

FIG. 6. The effect of GABA and GABuCh on 5-HT contractions of the rat fundus preparation.
(S) 2 mμg 5-HT (mμg = 10^{-9} g).
(1, 2, 3) 10,100 and 1000 μg GABA followed by 2 mμg 5-HT.
(A, B, C) 10,100 and 1000 μg GABuCh followed by 2 mμg 5-HT.
(G) 1000 μg GABuCh alone.
(O)blank = background contraction of Vane preparation with no drug added.

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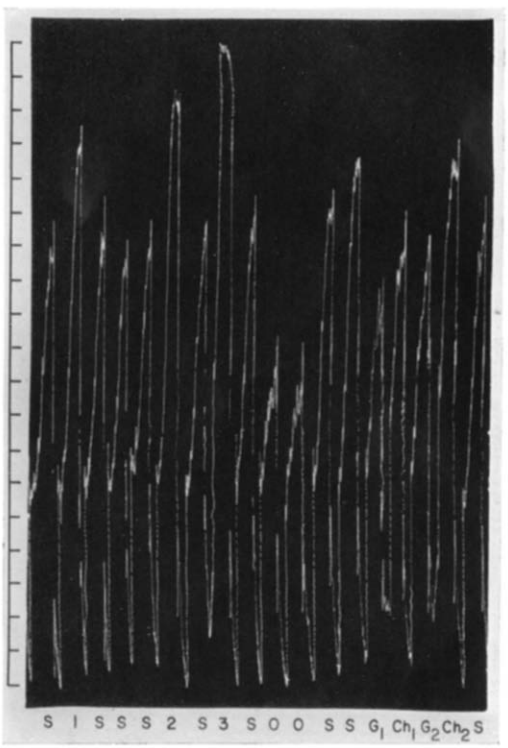

FIG. 7. The effect of GABuCh and choline in equimolar concentrations on 5-HT contractions of the rat fundus preparation.

(S): 0·5 mμg 5-HT.

(1): 0·2 ml 10^{-3}M choline. (2) 0·1 ml 10^{-2}M GABuCh. (3) 0·1 ml 10^{-2}M choline, all followed by 0·5 mμg 5-HT.

(O): blank.

(G_1 and Ch_1): 0·05 ml 10^{-2}M GABuCh and choline, respectively.

(G_2 and Ch_2): 0·1 ml 10^{-2}M GABuCh and choline, respectively.

identification of GABuCh in comparatively large amounts in brain arises many questions. It is the third choline ester positively to have been identified in mammalian tissue and the second one to have been found in brain.[16] It might constitute the naturally-occurring substrate for butyro-cholinesterase, the role of which is still unknown;[17] however, the slow rate of hydrolysis of GABuCh in comparison with butyryl- and acetylcholine by both purified butyro-cholinesterase and brain tissue presented in this paper, makes this hypothesis less likely.

Previous publications on the action of GABuCh on pharmacological test organs are few. Our own results disclose surprisingly few peripheral effects of this choline ester when given in reasonable doses. In summary, the cardiovascular effects consist of a blood pressure fall and a slight bradycardia. The fall in blood pressure cannot be explained by peripheral vasodilatation since the doses used did not effect the peripheral blood flow of the leg muscles (Fig. 4) or intestinal canal; furthermore, it cannot be explained by a ganglion-blocking action of the ester, since this was seen only at very high doses (Fig. 3) and the fall in blood pressure also occurs after ganglion-blocking doses of hexamethonium.

Compared to GABA[18] the cardiovascular effects of its choline ester are more pronounced and more consistent. GABA produced either a slight rise or a fall in blood pressure, whereas GABuCh invariably gave a fall in blood pressure, resistant to atropinization. Bradycardia accompanying this fall was, however, susceptible to atropine. That the cardiovascular effects produced were due to contaminants of the substances used is unlikely because compounds from two different sources produced the same effects. Besides having cardiovascular effects, GABuCh is a neuromuscular blocking agent with a potency of about 1/50 of that of suxamethonium. Kuriaki *et al.*[5] have reported a complete block by GABuCh of the histamine-induced contraction of the guinea pig ileum. The antihistaminic effect was evident only over a very narrow dose range. The present writers, however, could never find such a strong antihistaminic potency of the choline ester. Even in intestines reacting to doses as small as 0·001 μg histamine never more than a 50 per cent inhibition was demonstrated and even this effect was very irregular. The weak antihistaminic effect often appeared and disappeared successively.

GABA has been reported to have antihistaminic, antiacetylcholine and anti-5-HT effects on the guinea pig intestine[14,15] in doses from 10 to 100 μg/ml. At submaximal doses of histamine and acetylcholine the inhibiting effect of GABA amounted to 35–75 per cent. The anti-5-HT effect, however, was more pronounced (75–95 per cent.). On other test organs, such as the rat duodenum, Hobbiger could not find as convincing results as with the guinea pig ileum. This species variation is of interest when compared with our completely negative results of both GABA and GABuCh on the rat fundus preparation, an extremely sensitive test organ for 5-HT.

In the guinea pig ileum Whittaker[19] has observed an anti-5-HT effect of GABuCh similar to the antihistamine and antiacetylcholine effects reported here. These irregular and evanescent inhibitions remain to be further investigated; however, it is already clear that they are unlikely to provide a basis for the pharmacological assay of GABuCh.

With reference to the antagonism between GABuCh and histamine it can be noted that Dale[20] demonstrated an inhibitory effect of acetylcholine on the response of the guinea pig ileum to histamine when the two drugs were repeatedly added to the test bath. When the response to small doses of histamine had disappeared the contraction

could be elicited by larger doses. This is similar to our findings and to the investigations of Kuriaki *et al.*[5].

The relative inactivity of GABuCh on all the peripheral test organs studied is somewhat distressing. Two things, however, must be born in mind, firstly, that the possible action in the central nervous system is not imitated by these experiments and, secondly, that peripheral inhibitory nervous pathways up till now have not been found in mammals.

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