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THE EFFECTS OF FACTOR I AND OF GAMMA-AMINOBUTYRIC ACID ON SMOOTH MUSCLE PREPARATIONS

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In the last few years a number of reports have appeared (Florey, 1954, 1956; Florey & McLennan, 1955*a*, *b*) dealing with the properties of extracts of mammalian brain which contain a principle possessing certain of the characteristics to be expected of an inhibitory transmitter substance. This principle, which has been named Factor I (Florey, 1954), has been demonstrated in some biological preparations to have an anti-acetylcholine action. Thus, for example, application of Factor I solutions can prevent the stimulating action of acetylcholine on the crayfish stretch receptor neurone, the crayfish heart and crayfish intestine; and Factor I can block transmission at the cholinergic synapses of mammalian sympathetic ganglia. By contrast, in other preparations Factor I and acetylcholine may have the same type of action: thus both cause inhibition of the heart beat of cephalopods, and both cause stimulation of the mammalian hypoglossal nucleus.

Recently it has been reported that the whole activity of Factor I preparations, as assayed on the crayfish stretch receptor neurone, could be accounted for by their gamma-aminobutyric acid (GABA) content (Bazemore, Elliott & Florey, 1956, 1957). There is evidence that with other biological preparations GABA does not duplicate the actions of Factor I (McLennan, 1957), and indeed some extracts containing Factor I appear not to have detectable amounts of GABA (McLennan, 1958). Nevertheless, it is true that under some circumstances GABA does have some Factor I-like properties.

The contractions of intestinal smooth muscle induced by various stimulant drugs provide a simple means of testing the inhibitory effects of preparations of Factor I. In the present series of experiments the effects of Factor I on the contractions of the guinea-pig ileum induced by four drugs have been investigated and compared with the effects produced by GABA under the same conditions. Certain experiments have also been performed with rabbit ileum and with the oesophagus of sea-urchins.

The effects of GABA in antagonizing the contractions of guinea-pig and rabbit ileum induced by acetylcholine, nicotine and histamine have been recently investigated by Hobbiger (1958). He has reported that GABA acts to a limited extent as an antagonist of these three stimulant drugs, if they are used in concentrations which give submaximal effects. He has further reported that such inhibition as was produced by GABA was never complete. These observations on the effects of GABA on the guinea-pig ileum have been confirmed in the present series of experiments; however, no inhibitory effect of GABA on induced contractions of rabbit ileum has been detected. Factor I has been found to have effects qualitatively similar to GABA when stimulation of the intestine is produced by acetylcholine or nicotine, but differences are found when contractions are induced with serotonin or with gamma-butyrobetaine. Furthermore, the sea-urchin oesophagus appears to be entirely unaffected by GABA. A preliminary account of some of these results has already been published (Florey, 1953).

METHODS

Male and female guinea-pigs weighing 200–400 g and female white rabbits weighing approximately 2 kg were used. All animals were killed by decapitation. A piece of ileum approximately 4 cm long was suspended in an organ bath of 50 ml. capacity in saline solution. This was Tyrode solution modified by the addition of 0·01 M phosphate buffer to give a final pH of 6·8. This was done since it has been reported that some of the effects of Factor I could only be obtained at a pH below 7 (Florey, 1953, 1954, Florey & McLennan 1955*a*). In the course of the present experiments, however, this dependence on pH has been found only occasionally, and in many preparations identical results were obtained when the bathing solution was buffered as above or when normal Tyrode solution, with a pH of 7·5, was used. The organ bath was surrounded by a water jacket at 38° C, and all solutions added to the bath were pre-warmed to this temperature. Changes in the tone of the longitudinal muscle were recorded by means of an isotonic frontal-writing lever.

Large specimens of the sea-urchin *Strongylocentrotus drobachiensis* were obtained fresh from Vancouver harbour as required. A circular cut was made through the shell around the mouth of the animal, and this portion bearing the lantern and the oesophagus was gently retracted. The internal attachments of the oesophagus were cut, and a 3 cm length of the organ suspended in aerated sea water in a 10 ml. bath. These experiments were performed at room temperature.

Drugs were added to the organ baths in a volume of 0·5 ml., and the final bath volumes were kept constant. In all cases Factor I and GABA solutions were added to the baths 10–15 sec before addition of the stimulant drugs. Throughout the text the final concentrations in the baths are given. Drugs used were acetylcholine chloride, nicotine (base), serotonin (5-hydroxytryptamine creatinine sulphate), gamma-butyrobetaine (base), and gamma-aminobutyric acid. Factor I was prepared as described by Florey & McLennan (1955*b*), and its potency was checked by means of the crayfish stretch receptor neurone preparation (Florey, 1954). Concentrations of Factor I are expressed in 'crayfish units reference' (c.u.r.), as described by Elliott & Florey (1956).

RESULTS

Guinea-pig ileum

The addition of Factor I to the solution bathing a piece of guinea-pig ileum can cause partial or complete inhibition of the contraction produced by the subsequent addition of ACh. Fig. 1 shows an example of the partial blocks of

an ACh contraction brought about by two different doses of Factor I, and Fig. 2 the almost complete inhibition caused by a larger concentration of the factor in another preparation. In all preparations where an effect of Factor I has been observed it has been found that a suitably high concentration can cause complete inhibition of the acetylcholine contraction, regardless of whether the ACh alone causes a maximal or submaximal response. This is in contrast to the response found with GABA. Hobbiger (1958) showed that a dose of GABA effective in causing, for example, a 60% inhibition of an ACh concentration could be increased ten- or twentyfold without increasing the

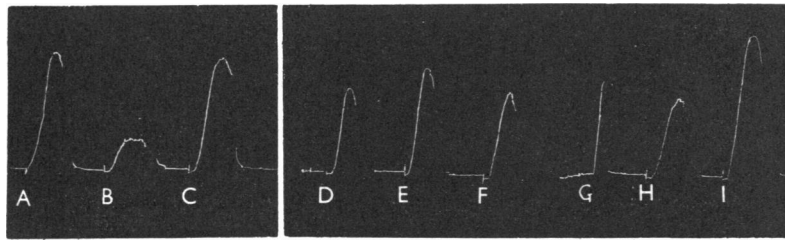

Fig. 1. Effect of Factor I and of GABA on ACh-induced contractions of a portion of guinea-pig ileum. A, ACh 0·02 μg/ml.; B, Factor I 0·85 c.u.r./ml. + ACh 0·02 μg/ml.; C, ACh 0·02 μg/ml.; D, GABA 4 μg/ml. + ACh 0·02 μg/ml.; E, ACh 0·02 μg/ml.; F, GABA 20 μg/ml. + ACh 0·02 μg/ml.; G, ACh 0·02 μg/ml.; H, Factor I 0·4 c.u.r./ml. + ACh 0·02 μg/ml.; I, ACh 0·03 μg/ml.

degree of inhibition. He also reported that if a maximal contraction of the ileum was caused by ACh then GABA no longer produced any inhibition of that contraction. Both these phenomena have been observed in the course of the present work, and it has been found that a dose of GABA as high as 100 μg/ml. is incapable of producing a block similar to that shown in Fig. 2. Fig. 1 also shows the block of the ACh contraction which could be obtained with GABA, and the fact that a fivefold increase in the dose of GABA produced no additional inhibition. The degree of inhibition of ACh contraction produced by GABA has never been found to be greater than about 60%.

In earlier work on the effects of Factor I solutions, Florey (1954) reported that block of the crayfish stretch receptor neurone discharge could only be obtained if the pH of the applied solution was below 7. This pH-dependence for the action of Factor I solutions has occasionally been found also with the guinea-pig ileum. Fig. 3 shows an example of the effect; applied at a pH of 7·5, Factor I solution had no inhibitory action on the ACh contraction, while at a pH of 6·8 it caused an almost complete block of the contraction. This change in pH was found not to have any effect on the contraction produced by ACh alone. The effect is, however, a very elusive one, in that it was by no means found in every experiment. There is no apparent explanation of the variability, and

the same preparation of Factor I may show a pH-dependence on one day, yet upon another ileum, on the following day, it may have identical effects in both acid and alkaline media. Hobbiger (1958) reported that he could find no evidence of a pH-dependence for the effect of GABA, and we also have never observed it.

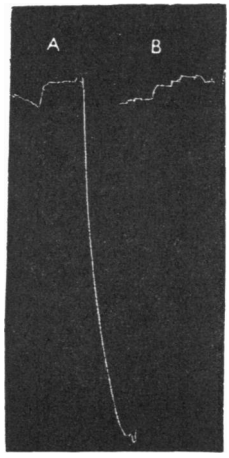

Fig. 2

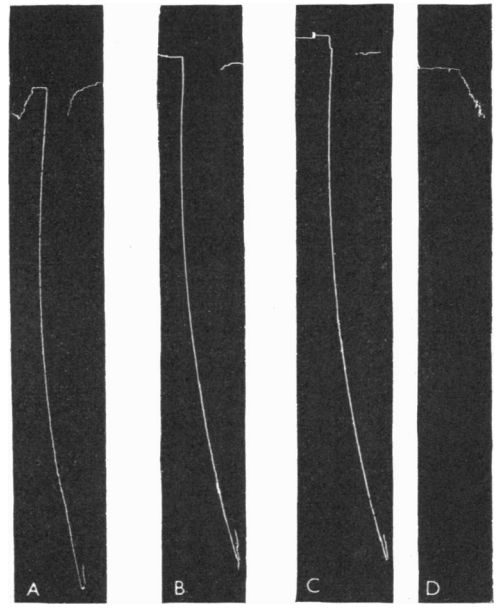

Fig. 3

Fig. 2. Effect of Factor I on the ACh-induced contraction of guinea-pig ileum. A, ACh 0·1 μg/ml.; B, Factor I 1·0 c.u.r./ml. + ACh 0·1 μg/ml. (Contraction downward.)

Fig. 3. The influence of pH on the action of Factor I on the ACh-induced contraction of guinea-pig ileum. A, ACh 0·007 μg/ml., pH 7·5; B, Factor I 1·0 c.u.r./ml. + ACh 0·007 μg/ml., pH 7·5; C, as in A, pH 6·8; D, as in B, pH 6·8. (Contractions downward.)

Another puzzling and unexplained variable found in the course of these experiments was that about 25% of the biological preparations set up were completely unaffected by either Factor I or by GABA. These preparations behaved normally as far as the application of stimulant drugs was concerned, but no effect of addition of even high concentrations of the inhibitory substances could be observed. Again, this was not to be explained as due to some change in the inhibitory solutions, for applied to another preparation upon the same or the following day they could be fully effective. It was found that if one portion of the ileum from a guinea-pig was insensitive to Factor I, then all other portions from the same animal would be similarly insensitive. In the same way, portions of ileum insensitive to Factor I were unaffected also by GABA. The effect could not be attributed to age or sex or feeding of the

animals, to the speed of dissection or setting up of the preparation, or to any obvious technical difference.

Substantially similar results were obtained when nicotine was used in place of ACh as a stimulating drug. Fig. 4 shows the results obtained in a typical experiment: the inhibition produced by GABA was in this case a maximal one, and was not further increased when the dose of the amino acid was increased twentyfold. By contrast, the inhibition produced by the addition of Factor I solutions to the bath is graded, and if a sufficiently large dose of Factor I was applied the inhibition of the nicotine contraction was complete. The result obtained with GABA is again in accord with that found by Hobbiger. As far as stimulation with nicotine or ACh is concerned, then, GABA fails to produce a complete inhibition with either drug, whereas suitable concentrations of Factor I can produce complete inhibition of both.

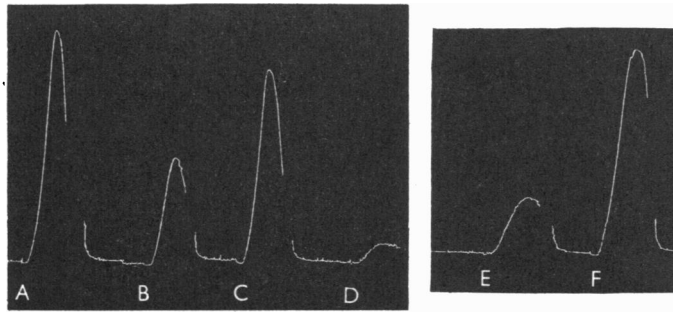

Fig. 4. Effect of Factor I and GABA on nicotine-induced contractions of guinea-pig ileum. A, nicotine 2 μg/ml.; B, GABA 2 μg/ml. + nicotine 2 μg/ml.; C, nicotine 2 μg/ml.; D, Factor I 1·1 c.u.r./ml. + nicotine 2 μg/ml.; E, Factor I 0·55 c.u.r./ml. + nicotine 2 μg/ml.; F, nicotine 2 μg/ml.

When serotonin was used as a stimulant drug, however, a more striking difference between the two appeared. Figure 5 shows the results obtained in a typical experiment. It is clear that addition of Factor I to the bath had in this case no effect upon the height of contraction of the longitudinal muscle, whereas the inhibition produced by GABA was virtually complete. The concentration of Factor I used in this experiment (1·1 c.u.r./ml.) was the same as that which caused a virtually complete block of a nicotine contraction, but with serotonin as the stimulating drug even 10 c.u.r./ml. was ineffective in reducing the height of contraction. Occasionally Factor I in doses of this magnitude itself produced a large, very quick and unsustained contraction of the intestine, quite unlike that seen with any of the other drugs used.

An effect in the opposite direction was observed when the ileum was stimulated with gamma-butyrobetaine. This is a substance which has been known for many years to be present in the tissues of some cold-blooded animals, for

example snakes (Keil, Linneweh & Poller, 1927), and fresh-water eels (Hoppe-Seyler & Schmidt, 1927); it appears however not to be present normally in mammals, but may appear in the urine in phosphorus poisoning (Takeda, 1910) or in pernicious anaemia (Reinwein & Thielmann, 1924). Recently Hosein (unpublished observations) has shown that gamma-butyrobetaine can be isolated from the brains of animals which die during convulsions, and Hosein & McLennan (unpublished observations) have found that in many cases the substance behaves pharmacologically in a very similar manner to acetylcholine, but weight for weight is much less potent.

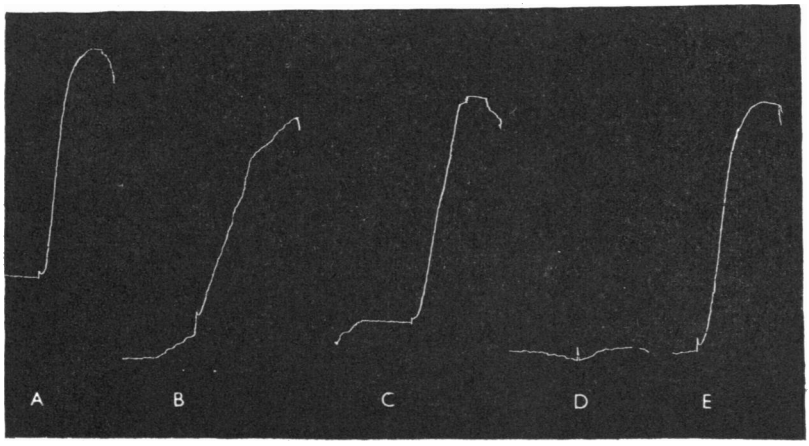

Fig. 5. Effect of Factor I and GABA on serotonin-induced contractions of guinea-pig ileum. A, serotonin 0·2 μg/ml.; B, Factor I 1·0 c.u.r./ml. + serotonin 0·2 μg/ml.; C, serotonin 0·2 μg/ml.; D, GABA 2 μg/ml. + serotonin 0·2 μg/ml.; E, serotonin 0·2 μg/ml.

Addition of gamma-butyrobetaine to a bath containing a guinea-pig ileum causes the muscle to contract (Fig. 6), and this contraction, like that caused by ACh, can be inhibited to the extent of 50–60% by the addition of GABA. However, the addition of small quantities of Factor I to the bath before gamma-butyrobetaine is added invariably causes a potentiation of the contraction due to the latter, and an inhibitory effect is never observed. The potentiating action of Factor I on the contraction caused by gamma-butyrobetaine is independent of pH.

Rabbit ileum

Hobbiger (1958) has reported that the addition of GABA to a bath containing a portion of rabbit ileum reduced the spontaneous activity. The concentration of GABA required to produce this effect varied over wide limits (1–100 μg/ml.), and even in those preparations in which the effect was most pronounced recovery of the spontaneous activity took place in the continuing

presence of the acid. The anti-acetylcholine and anti-nicotine effects of GABA were less marked with rabbit than with guinea-pig ileum, and Hobbiger reported that in many of his preparations no effect of GABA could be obtained.

We have not been able, in sixteen experiments, to observe a consistent effect either of GABA or of Factor I on the spontaneous activity or on ACh-induced contractions of rabbit ileum. Occasionally a change in the rhythm of the spontaneous contractions was noted with both substances, in that they became

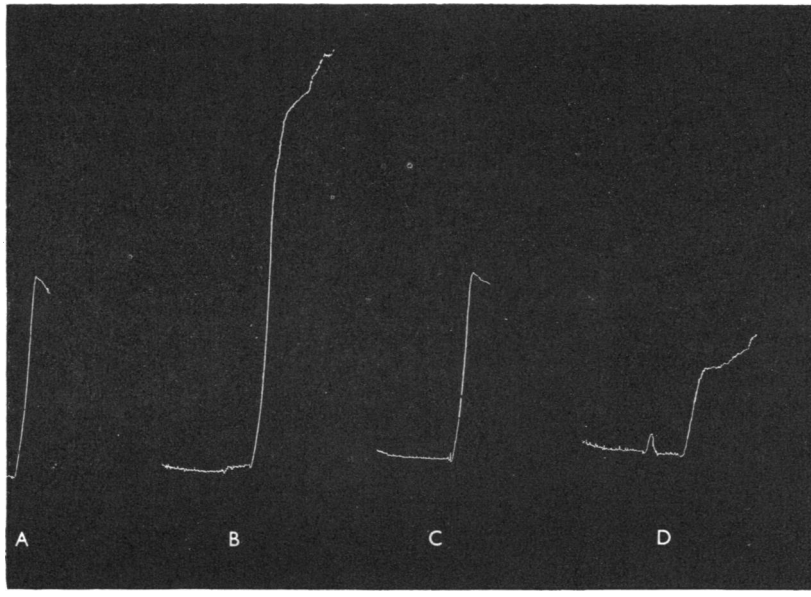

Fig. 6. Effect of Factor I and GABA on gamma-butyrobetaine (γBB)-induced contractions of guinea-pig ileum. A, γBB 0·15 μg/ml.; B, Factor I 1·0 c.u.r./ml. + γBB 0·15 μg/ml.; C, γBB 0·15 μg/ml.; D, GABA 2 μg/ml. + γBB 0·15 μg/ml.

smaller and faster. The impression gained is that the contractions of the ileum have become 'desynchronized'. The concentrations used were high (100 μg/ml. and 10 c.u.r./ml. respectively). Our results therefore do not agree with those of Hobbiger; but a possible explanation may be that insufficient experiments have been performed. Thus Hobbiger reported successful antagonism of ACh contractions by GABA in only two out of eight experiments.

Sea-urchin oesophagus

A difference between GABA and Factor I is noticed when their effects upon the ACh-induced contractions of the sea-urchin oesophagus are compared. Fig. 7 shows the effect of addition to the bath of Factor I solution, which in this instance caused an almost complete inhibition of the ACh contraction in

a concentration of 0·1 c.u.r./ml. It should be noted that although rather high doses of ACh are required to produce a contraction of the oesophagus, the concentration of Factor I needed to inhibit it is small, and this preparation in fact rivals or surpasses the crayfish stretch receptor neurone in its sensitivity to Factor I (Florey, 1954; Elliott & Florey, 1956). In Fig. 7 also the result obtained when GABA was added to the bath with ACh is shown. It is clear that GABA has no inhibitory effect on this preparation, although the concentration used here (100 μg/ml.) is ten times higher than that producing a considerable effect on the guinea-pig ileum. In other experiments even higher concentrations of GABA have been employed with a similar lack of inhibitory effect. It is concluded that the sea-urchin oesophagus is almost entirely insensitive to this substance, although it is very sensitive to Factor I.

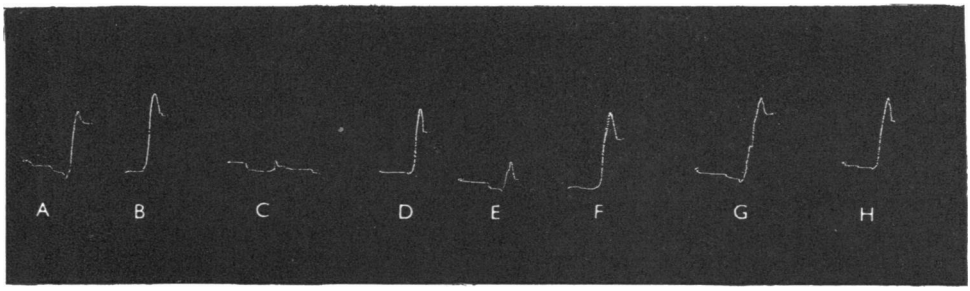

Fig. 7. Effect of Factor I and GABA on ACh-induced contractions of sea-urchin oesophagus. A, ACh 0·2 μg/ml.; B, ACh 0·2 μg/ml.; C, Factor I 0·1 c.u.r./ml. + ACh 0·2 μg/ml.; D, ACh 0·2 μg/ml.; E, Factor I 0·01 c.u.r./ml. + ACh 0·2 μg/ml.; F, ACh 0·2 μg/ml.; G, GABA 100 μg/ml. + ACh 0·2 μg/ml.; H, ACh 0·2 μg/ml.

DISCUSSION

Hobbiger (1958) has discussed the question of whether or not the effects of GABA which he observed on the intestine are to be regarded as of physiological significance. It appears that the same question must apply to any consideration of the effects of Factor I in these structures, since no Factor I activity has been detected in any mammalian organ outside the central nervous system (Florey, 1953; Florey & McLennan, 1955*a*). GABA is present in high concentration in the central nervous system, but its presence has been shown in peripheral nerve (May & Thillard, 1951), in ocular tissue (Kojima, Mizuno & Miyazaki, 1958), and in gastric juice (Gilligan, Moor & Warren, 1951). On these grounds it is not intrinsically likely that Factor I has a physiological role in the function of the intestine.

Nevertheless, the results reported here are of interest in that they provide another example of an interaction between Factor I and ACh. There are now, therefore, a number of preparations in which it has been possible to show either

an antagonism or a parallelism between ACh and Factor I (see Florey, 1954, 1956; Florey & McLennan, 1955*a*, *b*; McLennan, 1957). It is tempting to speculate that there may be some structural similarity between the two substances, and that their interaction depends upon activity at identical receptor sites.

Effects of brain extracts which are probably similar in composition to the Factor I preparation used in the present experiments have been described by Lissák & Endröczi (1956). These authors reported that their extracts inhibited the contractions of cat ileum induced by ACh, which is in accordance with the results reported here. That there are differences between the extracts of Lissák & Endröczi and ours is indicated in that they reported a loss of activity in the blood, whereas Factor I activity can be recovered in full from blood following intravenous administration (Florey & McLennan, 1955*a*).

The present experiments provide another example, in addition to those already reported (McLennan, 1957), of the failure of GABA adequately to explain all the effects produced by Factor I. Superficially there are qualitative resemblances between the two as far as their anti-acetylcholine and anti-nicotine effects upon guinea-pig ileum are concerned, just as there are certain similarities between the two on some crustacean structures. However, even with guinea-pig ileum there are differences in action; thus the contraction produced by serotonin is strongly inhibited by GABA (an effect noted also in passing by Hobbiger (1958)), whereas the height of the serotonin contraction is unaffected by Factor I. The synergistic action between gamma-butyrobetaine and Factor I is also in contrast to the inhibitory effect of GABA upon the gamma-butyrobetaine contractions.

The fact that GABA is unable to inhibit the ACh-induced contractions of the sea-urchin oesophagus, whereas Factor I is able to do so, again illustrates the differences between these two substances. A similar difference has also been noted in another structure in these animals, for the movements of the pedicellaria are strongly inhibited by application of dilute solutions of Factor I, whereas high concentrations of GABA (up to 100 μg/ml.) are totally ineffective (McLennan, unpublished observations).

SUMMARY

1. The effects of gamma-aminobutyric acid and of Factor I on the contractions of various smooth muscle preparations induced by stimulant drugs have been compared.

2. With the ileum of the guinea-pig both these substances can inhibit the contractions produced by acetylcholine and by nicotine, although complete inhibition of the contraction by GABA is never observed as it is with Factor I. Serotonin contractions of the guinea-pig ileum are almost totally inhibited by

suitable concentrations of GABA, but the height of the contraction is unaffected by Factor I. Gamma-butyrobetaine contractions of the guinea-pig ileum are partially inhibited by GABA, but potentiated by Factor I. The acetylcholine-stimulated sea-urchin oesophagus is inhibited by Factor I, but unaffected by GABA.

3. The spontaneous activity, and acetylcholine-induced contractions, of rabbit ileum are little affected either by GABA or by Factor I.

4. Two effects with guinea-pig ileum are occasionally noted, but no explanation of their variable occurrence is at present available. These are (*a*) that the ileum is insensitive to applied Factor I or GABA, while retaining its sensitivity to stimulant drugs; and (*b*) that Factor I shows pH-dependence, in that it is only active when added to a bathing solution on the acid side of neutrality.

5. The conclusion from earlier work that the activity of Factor I-containing brain extracts cannot adequately be explained in terms of their GABA content is substantiated.

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