Inactivation Process of Gamma-Aminobutyric Acid Action on the Isolated Ileum

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Following the discovery of large amounts of gamma-aminobutyric acid (GABA) in mammalian brain (1, 11), a number of studies have contributed to observations on physiological actions of GABA (cf. 4). The mechanism of the action has been analyzed in some simple preparations, such as the crustacean stretch receptor (2, 9) and the isolated mammalian ileum (5, 6, 7, 8). GABA produced a specific action similar to natural events by impulses of inhibitory fibres in the crustacean stretch receptor (9). It was also found that the effect of GABA on this receptor was gradually decreased when the receptor was left immersed in the GABA solution (2, 9). A similar inactivation of GABA action has been observed in the blood pressure of mammals (3, 12), in spinal reflexes (10) and in the isolated ileum (6, 7).

No systematic approach has, however, been made toward this problem.

The present study is concerned with characterization of the inactivation process of GABA action on the isolated ileum. In the course of this study, it was found that there were some discrepancies in the action of GABA on the isolated ileum between the present results and those obtained by previous workers. These differencies are also described.

METHODS

The experiments were carried out mostly on male and female guinea pigs weighing 200–450 g. In some experiments, white rabbits were also used. Pieces of the terminal ileum were removed from the animal and immersed into Tyrode solution at room temperature (12–24°C). In 1 to 12 hr. after the isolation, a piece of the ileum, approximately 1.5 cm long, was suspended in a 60 ml. bath of Tyrode solution. In order to prevent the preparation from spontaneous activity, the bath was usually kept at a relatively low temperature (about 35°C) and constantly aerated. In a number of observations, it was revealed that pH 6.9 was most preferable for the present type of experiment.

Changes in tone of the longitudinal muscle were recorded with a modified Magnus

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method on a kymographion. The organ bath was drained and refilled with fresh, pre-warmed and aerated Tyrode solution after each test of drugs. As the stimulant drugs, acetylcholine chloride and nicotine (base) were used. The antagonistic action of GABA to the stimulant drugs was observed by adding GABA with various timing prior to the application of the stimulant drugs. In every instances the drugs were dissolved in distilled water and added to the organ bath in a volume of 0.6 ml, and the final volume in the bath was kept constant at 60 ml. Successive applications of drugs were made at intervals of 6 to 20 min, unless otherwise specified.

RESULTS

Effect of GABA on the Spontaneous Activity

It has been found that administration of GABA in general reduced the spontaneous activity of the isolated rabbit ileum although there was a considerable variation (6). The same type of observation was repeated mostly on rabbits in the present study. In contrast with the above description, typical reduction in the spontaneous activity by GABA was observed only in 20% of preparations tested. In most cases

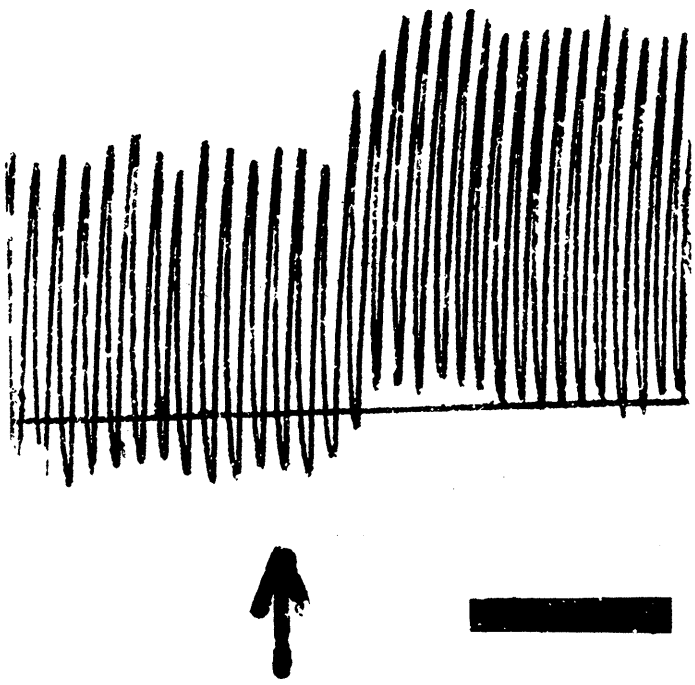

Fig. 1. Effect of GABA, 10 μg/ml, on the spontaneous activity of the isolated rabbit ileum. An arrow indicates the administration of GABA. Time, 60 sec. Tyrode solution, pH. 8.2, 37°C.

GABA was ineffective on the spontaneous activity or produced an increase of the activity. As shown in Fig. 1, the enhancement of the spontaneous activity by GABA was characterized by increase both in tonus and the amplitude of movement, leaving the frequency unaltered. These results, however, were not confirmed in the isolated guinea pig ileum. In guinea pigs, GABA generally tended to depress the spontaneous activity of the ileum. This result was again contrast with the descriptions by previous workers (6, 8).

Antagonistic Actions of GABA to Stimulant Drugs

It is generally conceded that an increase in tone of the ileum produced by some stimulant agents is partially prevented by a previous addition of GABA (5, 6, 7, 8). The antagonistic action of GABA to acetylcholine chloride (Ach) and nicotine was reinvestigated in the present study. The antagonistic action of GABA showed a considerable variation. About 50% of the preparations tested with Ach, and a slightly smaller percentage of those with nicotine, were not influenced at all by ad-

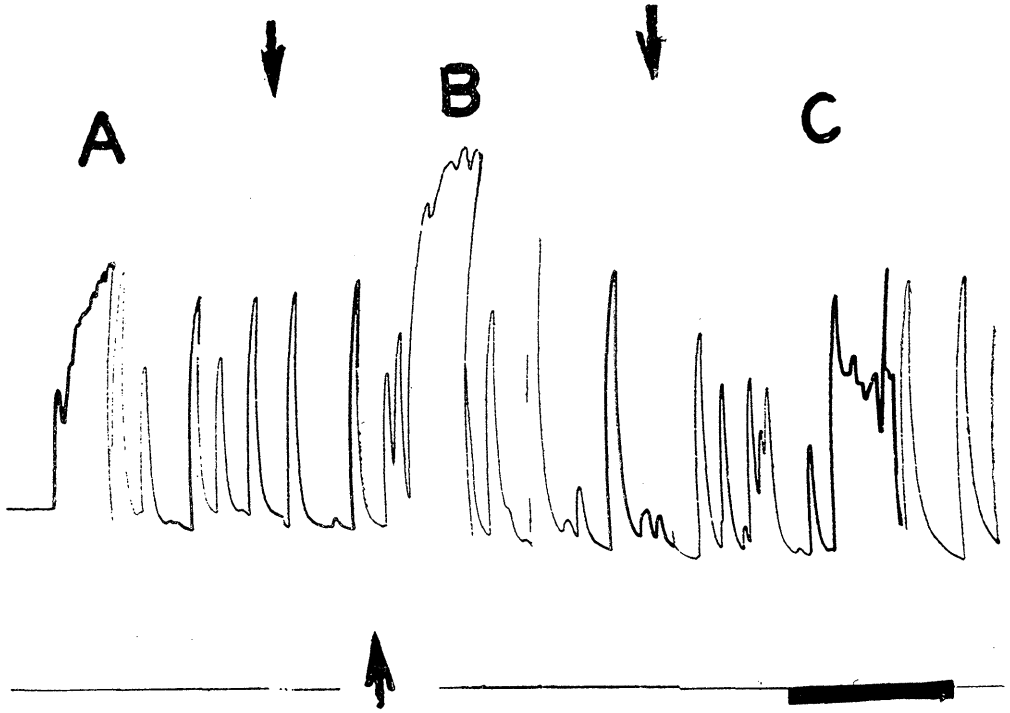

Fig. 2. Effect of GABA, 10 μg/ml, on Ach-induced response of the isolated rabbit ileum. A and C are control responses produced by acetylcholine (0.03μg/ml) alone. Administration of GABA was marked by a up-pointing arrow. Downward arrows indicate administration of Ach. Note an increase in the Ach-induced response by GABA (B). Time, 60 sec. Tyrode solution, pH. 8.2, 37°C.

ministration of GABA. This was the case both in rabbits and guinea pigs. Also the action of GABA on responses induced by stimulant drugs was not always depressant. In some cases, GABA produced potentiation of the responses induced by stimulant drugs. Fig. 2 gives an example of such cases. Fig. 2A and C were control responses induced by Ach alone which were obtained before and after the test

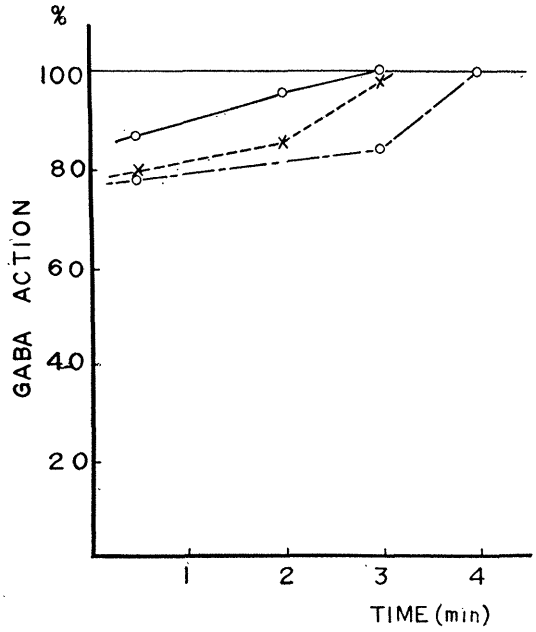

Fig. 3. Inactivation process of GABA action on nicotine-induced responses. Ordinate gives the amplitude of the response under administration of GABA in percentage of the control responses produced by nicotine (0.3μg/ml) alone. Three different preparations. The result presented by crosses was obtained with GABA, 100μg/ml. Other two experiments were made with GABA, 10μg/ml. Abscissa show the interval between administrations of GABA and nicotine. Tyrode solution, pH. 6.9, 28°C.

of the antagonistic action of GABA (B). An addition of GABA, 20 sec. prior to Ach, apparently caused a potentiation of the response. Florey and McLennan (5) pointed out that the variable actions of GABA were dependent upon properties of preparations examined, and for example, they 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 (5). In the present study, however, even in the same preparation, a portion of the ileum was characterized by the antagonistic action of GABA and another portion of the ileum was potentiated by addition of GABA.

Such dissimilarities of GABA action noted in this and the previous sections were

not attributable to the concentration of GABA administered, the pH of Tyrode solution and time elapsed after excision of the ileum. It was not possible to find any particular factor or factors which may control diverse actions of GABA.

Inactivation of GABA Action

It has been observed that if GABA was present in the Tyrode solution for 5 min. or longer, a decrease in effectiveness of GABA with time occurred regularly (6, 7). The following experiments were undertaken to analyze the time course of the inactivation process of GABA action.

In the analysis, two methods were adopted. The first method was to observe changes in the intensity of the antagonistic action of GABA, changing the interval between the administrations of GABA and stimulant drug. In the second method, tone of the longitudinal muscle of the ileum was modified by stimulant drug for a long period, and the effect of previously applied GABA was observed. As a stimulant drug, nicotine was exclusively used.

Typical results in the first method are summarized in Fig. 3. In ordinate, the amplitude of the response to nicotine under action of GABA which was administered in various time before application of nicotine was plotted as percentage of the amplitude of the control response produced by nicotine alone. Abscissa gives the interval between administrations of GABA and nicotine. GABA exerted the maximal antagonistic action immediately after the administration. The action of GABA gradually decayed thereafter and became completely ineffective within approximately 3 to 5 min. This type of experiment was carried out in a number of preparations. Although there was a considerable variation in different preparations, it could reasonably be assessed that the action of GABA is approximately halved every

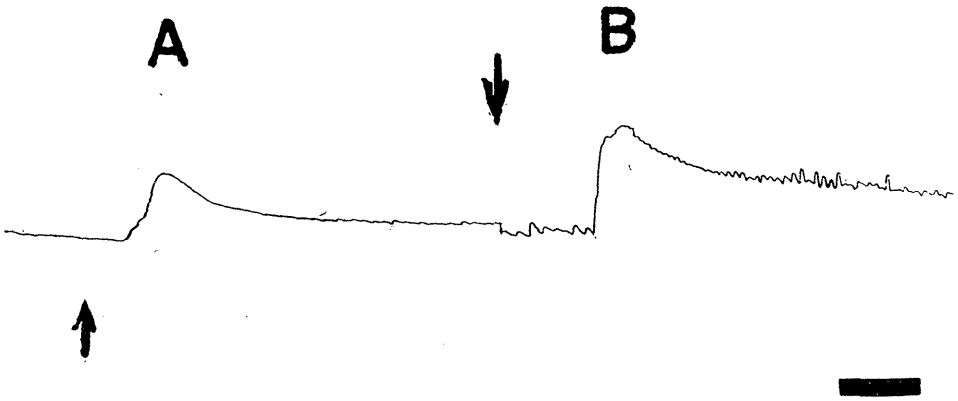

Fig. 4. Responses produced by nicotine, 0.3 μg/ml. B. Control response induced by nicotine alone (downward arrow). A. Same as in B, but GABA, 10μg/ml, was administered at the up-pointing arrow. Tyrode solution, pH. 6.9, 28°C.

60 to 120 sec. and becomes completely ineffective within 2 to 7 min. It was interesting to note that there was little difference in the inactivation process between doses of GABA, 10 μg/ml and 100 μg/ml and that the inactivation was independent upon the pH of Tyrode solution in a range of 6.9 to 8.0.

Fig. 4 shows a typical result in the second method. Nicotine was applied into the bath and left for several min. This resulted in an increase of the tone of the ileum with a slow decay (Fig. 4B). When GABA was given in approximately 30 sec. before the administration of nicotine, the time course of nicotine-induced tone was considerably modified (Fig. 4A). The second method was based on an assumption that a subtraction of the record in Fig. 4A from the control response in Fig. 4B would give an approximate time course of the GABA action. This was done in Fig. 5, and the intensity of the antagonistic action of GABA was plotted as a function of time elapsed after the onset of the response. In this method, there was also a considerable fluctuation in the time course of GABA action. In addition, this had a disadvantage that the time course of the inactivation process of GABA was not completely obtained because of a transitory change in tone of the ileum by nicotine. Nevertheless, the results gave a support for the previous conclusion that GABA action was gradually inactivated with a half-decay time of approximately 60 to 120 sec. and became completely ineffective within several min.

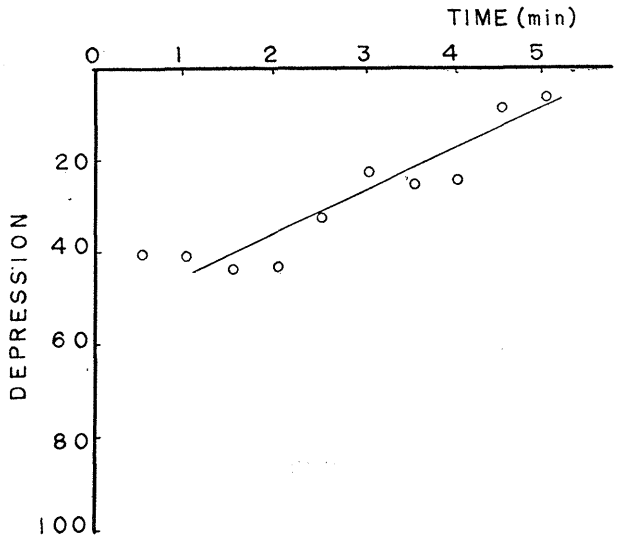

Fig. 5. Plot obtained from the result in Fig. 4. Abscissa, time elapsed after the onset of the nicotine-induced response. Ordinate, subtraction of the response A in Fig. 4 from the control response B in Fig. 4 in percentage of the control response.

Discussion

While the inactivation of GABA action has been observed in a number of preparations (2, 3, 6, 7, 9, 10, 12,), no systematic analysis of the process has been made. A purpose of the present analysis was to obtain information as to the possible way in which GABA action is inactivated in the ileum. The inactivation process of GABA action in the ileum was characterized by; 1) that the inactivation is independent upon the dose of GABA administered and the pH of the solution within a certain limits, 2) that the inactivation occurres immediately after the administration and completes within 2 to 7 min. and 3) that the inactivation is approximately a linear function of time elapsed after the application, GABA action being almost halved every 60 to 120 sec.

Edwards and Kuffler (2) proposed two possible explanations for the inactivation process of GABA in the crustacean stretch receptor; one is chemical alteration of GABA administered and the other is an active uptake of GABA by the cell. The present study did not provide any definite conclusion for these possibilities. However, the results suggest that the second possibility may be more likely. If the inactivation is by chemical alteration, it would be expected that the time course of the inactivation may be prolonged when a dose of GABA administered in increased. This was not the actual case. On the other hand, if the inactivation is by an uptake by cells, the rate of uptake would be accelerated when the dose of GABA outside is increased, giving almost the same time course of the inactivation process as in a small dose of GABA. The inactivation process was also independent upon the pH of the solution in a range of 6.9 to 8.0. If the inactivation is by chemical alteration, the process would be expected to be sensitive to changes in the pH of the solution.

It should, however, be noted that inactivation in the crustacean stretch receptor was abolished when the solution surrounding the receptor was stirred (2). Also the inactivation of GABA action on the receptor was observed with a time lag of about 90 sec (2). These are contrast to the present results. The inactivation in the ileum was obtained immediately after administration of GABA into the Tyrode bath which was continuously stirred with aeration bubble. The basis of these differences is of interest.

Summary

1) The action and inactivation process of GABA were analyzed in the excised ileum of rabbits and guinea pigs.

2) GABA usually produced an increase in the spontaneous activity of the ileum of rabbits, while in guinea pigs the action was in general depressive.

3) GABA action on nicotine-induced responses was gradually inactivated immediately after the application with a half-decay time of approximately 60 to 120 sec.

and became completely ineffective within 2 to 7 min.

4) The inactivation process was independent upon the pH of GABA solution and upon the dose of GABA administered.

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