Effects of Gamma-Aminobutyric Acid and Gamma-Amino-Beta-Hydroxybutyric Acid on the Avoidance Conditioned Reflexes

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INTRODUCTION

According to the biochemical and neurophysiological investigations by many workers\(^1\) there can be little doubt that \(\gamma\)-aminobutyric acid (GABA) or a closely related substance is concerned in some way with the transmission of nerve impulses in the central nervous system and that the mode of its action is inhibitory in nature, although the exact nature and site of its action are not yet clear. Hayashi\(^2\) asserts that \(\gamma\)-amino-\(\beta\)-hydroxybutyric acid (GABOB) is more important than GABA as an inhibitory substance. If GABA or GABOB is closely related with the inhibitory processes within the central nervous system, it may be also connected with the internal inhibition of the conditioned reflex (CR).

It is, therefore, the purpose of the present study to investigate the effects of these substances on the avoidance CR in animals. In this field, it was reported by Suhara\(^3\) that the positive CR of discrimination was always inhibited by oral administration of GABOB in the salivary CR in man.

EXPERIMENTAL

Method

Experiments were performed on adult dogs in which an avoidance CR was established by the so-called instrumental training method.

In this method the occurrence of a noxious stimulus was prevented automatically during occurrence of a CR. For training or testing the animals were gently restrained in a hammock which permitted freedom of movement of all four limbs. All experiments were performed in a sound-proof room. As the conditioned stimulus (CS) a 1000 c/s tone enduring about 1 sec., from an audio-frequency oscillator was used. Its
termination was overlapped with the unconditioned stimulus (US). The US, rhythmic electric shocks (20-40 V) from an electronic stimulator, was delivered to the right hind leg. One pole of the stimulating electrode was a needle inserted into the skin of the right hind leg, and the other a tinplate (30×20 cm) on which the leg was placed. One training session was 20 trials each except for discrimination training in which it was 30 trials each. The intervals between the separate trials varied from 30 sec. to 3 min. at random, in order to avoid temporal conditioning.

Discrimination training was attempted in some animals using alternatively a 1000 c/s tone as the positive stimulus and a 800 c/s tone as the negative one.

GABA or GABOB not only increased the latency of the CR, but also augmented its fluctuations. Therefore the standard deviation (SD) of latencies was utilized for characterizing the effects of the agents. When the latency became short and steady by sufficient reinforcement, the test agent was administered intravenously through a cutaneous vein of the left hind leg or intracisternally.

In the following experiments SDs of latencies obtained in successive 20 trials were measured immediately after, 5 hours after and 24 hours after the administration of the test agent.

Results

1. Latency as an index of the central excitatory level

As a preliminary experiment the latency of CR was investigated to see whether or not it might be used as an index of the central excitatory level.

About three weeks after the beginning of training (one session a day) the CR was established. When tested without reinforcement several days or longer after completion of training, animals were found to be thoroughly conditioned (see Fig. 1). Although the percentage frequency of CR (proportion of positive CRs to the number of trials in each session) remained 100% even without further reinforcement, it was noted that the latencies showed remarkable variations from about 0.5 to 1.5 sec. So further reinforcement with much shortened intervals between the CS and US was attempted to obtain a more rapid and steady response to the CS. After the further reinforcement the latencies became shorter and more regular, ranging from about 0.35 to 0.45 sec. (see Fig. 2).

Inhibition of CR may be caused by different kinds of experimental procedure, such as extinction, discrimination, delay, distraction and so on. Among these various procedures the experimental extinction and discrimination were attempted in the present experiments. In the experimental procedure of acute extinction in which the CS was repeatedly
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Fig. 1. Learning curves by instrumental avoidance training. Abrupt down-ward deflection of curves on the 10th day of training resulted from temporary suspension of reinforcement.

Fig. 2. Changes in standard deviations of latencies during the advanced course of conditioning. Experiments were started when percentage frequency became 100%.

Presented without reinforcement, the latencies became irregular, showed a progressive prolongation and finally the percentage frequency of CR began to decrease (see Fig. 3). Whenever extinceptive inhibition occurred, decrement of percentage frequency was preceded by the prolongation and irregularity of latencies.

Next, experiments of discrimination were carried out. In these experiments, even after training of about 50 days discrimination was not yet
well-established and apt to generalize (see Fig. 4). In the course of training it was found that in unsuccessful discrimination the latency of the positive CR was rather shorter and more regular, whereas it was unstable in successful discrimination (see lower diagram in Fig. 4). It seemed that conditions under which short and rigid latencies of CR were obtained were unfavorable for discrimination. The longer and variable latencies of CR in association with successful discrimination may be due to internal inhibition caused by negative stimuli.

From the above-mentioned experiments it may be considered that the latency and its fluctuation can serve as an index of the central excitatory level.

2. **Effects of GABA and GABOB administered intravenously upon conditioned responses**

No changes in the behavior and percentage frequency of CR were observed with doses of 25 to 100 mg./kg. The animals were as usual friendly or affectionate and showed interest in the environment. Irregularity or prolongation of latency, however, was noted immediately after and 5 hours after the injection. After 24 hours the latency was found to
Fig. 4. Learning curves by discrimination training (upper graph). Filled circle: percentage frequency of CR to positive stimulus. Open circle: that of CR to negative stimulus. Difference between both percentage frequencies indicates degree of discrimination. Both percentage frequency of CR and SD of latency at points indicated by arrows in upper diagram are shown in lower diagram.

Fig. 5. Effects of intravenous administration of GABA in various doses. Filled bar: percentage frequency of CR. White circle: SD of latency. Arrow: immediately after injection of GABA. Exceptional case in which SD of latency was extraordinarily high is shown by broken curve.
have been restored to the initial level. The larger the doses of GABA, the more pronounced the irregularity and prolongation of latency. The effect of GABA was especially marked in cases in which a sign of irregularity of latency was observed before the injection. None of these changes was noted with control injection of 0.9% saline solution (see Fig. 5).

The effects of GABOB was almost identical with that of observed with GABA, but there was some quantitative difference; while no change occurred in percentage frequency of CR after administration of GABA, a slight decrement of percentage frequency was noted 5 hours after or immediately after the injection of 25 to 50 mg./kg. GABOB without any behavioral changes (see Fig. 6). As an exception one of dogs did not show any decrement of percentage frequency by the injection of GABOB with a dose of 50 mg./kg. (see bottom diagram in Fig. 6).

The effect of GABOB upon discrimination was investigated. Following GABOB with a dose of 50 mg./kg. the positive CR in discrimination

![Graphs showing effects of GABA and GABOB](image)

Fig. 6. Effects of intravenous administration of GABOB. Decrement of percentage frequency was observed. At bottom exception is shown in which no decrement of percentage frequency occurred following administration of GABOB.
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was slightly inhibited immediately after the injection, did not occur after 24 hours and was found markedly inhibited even after 48 hours, although any other behavioral changes were not observed (see Fig. 7).

![Diagram of CR frequency over time]

Fig. 7. Effect of intravenous administration of GABOB on discrimination. Positive CR was markedly inhibited (filled bar).

3. Effects of GABA and GABOB administered intracisternally

With intracisternal administration of GABA there were some differences from the effect of the intravenous injection. Following GABA in doses of 10 to 20 mg. some slight ataxia appeared, which recovered completely within about 20 min. With a dose of 10 mg. irregularity or prolongation of latencies could be observed immediately after the administration. With a dose of 20 mg. a slight decrement of percentage frequency and prolongation of latencies were observed. No changes were noted with control administration of 0.9% saline solution (see Fig. 8).

The effect of GABOB was also similar to that observed when GABA was applied intracisternally. After the administration the animals became depressive and was not so affectionate as before the administration. Some ataxia was also observed. The animals were apt to sink to the floor, although they showed interest in the environment. These behavioral changes were outstanding with a dose of 50 mg. Recovery from these abnormal behaviors was found to be complete within about 30 min. With a dose of 25 mg. a slight irregularity of latencies was noted without any decrement of percentage frequency. With a dose of 35 mg. a decrement of percentage frequency was observed immediately after the administration. With a dose of 50 mg. of GABOB no CR occurred immediately.
after and 5 hours after the administration (see Fig. 9).

4. Intracisternal application of GABOB in experimental neurosis

One of the most interesting effects was that obtained by the intracisternal application of GABOB in experimental neurosis. Some animals showed chronic abnormal behavior, such as struggling or flinching, during the course of conditioning or after establishment of CR. The most prominent abnormal behavior was frequent tic-like flexions and sustained ones of the conditioned right hind leg without the CS. A slight autonomic discharge (vomiting, salivation and pupillary dilatation) was also observed. These abnormal behaviors were observed only in the experimental situation; the animals appeared normal outside the experimental room. Intracisternal administration of GABOB with a dose of 100 mg. ceased all such abnormal behaviors immediately after the administration.

Particularly the frequent spontaneous flexion of the right hind leg disappeared; the animals responded only to the CS and became relaxed and gentle. But on the following day the animals showed the same neurotic
Fig. 9. Effects of intracisternal administration of GABOB.

signs as observed before the administration (see Fig. 10).

**DISCUSSION**

As mentioned above, a prolongation of latency of CR observed during the course of acute extinction or discrimination may be considered as an expression of developing internal inhibition. As far as conditioned flexion responses in dogs, conditioned eyelid reactions in man, and conditioned knee jerks in man are concerned, the percentage frequency of response and amplitude has been said to have appreciably higher reliabilities than the latency, but the present experiments have shown that the latency, especially its fluctuation, is a more sensitive index than the percentage frequency. It has been, however, reported by Shimizu that in conditioning experiments on the rats the latency could remain unchanged in spite of a slight change of the central excitatory level, when the latter was extremely high.

From the results obtained by the administration of GABA or GABOB, it may be said that these substances contribute to the development of
internal inhibition. Between the inhibitory actions of GABA and GABOB no essential difference was noted. Judging from the results that the percentage frequency of CR decreased following both intravenous and intracisternal administrations of GABOB, but not following intravenous administration of GABA, the inhibitory action of GABOB might be slightly stronger than that of GABA. Hayashi\cite{2} reported that the inhibitory action of GABOB was about 10 times as strong as that of GABA, judging from the critical concentration to stop a generalized seizure in dogs.

In the present experiments the effective intracisternal dose was about one tenth the effective intravenous dose. In contrast to topical application of GABA, intravenous administration is said to produce neither central synaptic actions nor any detectable increase in brain concentration of the amino acid; in other words under ordinary circumstances GABA does not effectively penetrate the blood-brain barrier.\cite{6} However, the effectiveness of intravenously administered GABA observed in the present experiment suggests that this agent penetrated the blood-brain barrier to some extent. After an intracarotid injection of GABA or GABOB Marrazzi\cite{7} et al. and Hayashi\cite{2} were able to observe the effects of these substances.

However, it is a question whether GABA or GABOB is a natural inhibitory factor in the brain, because the effective doses of these substances are too large in comparison with those of the known natural pro-
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According to Pavlov, experimental neuroses develop only in animals of extreme types, either the extremely inhibitable or the extremely excitable. The animals observed in the present experiment appeared to belong to the latter type. It is of interest that the abnormal behavior observed in the experimental situation disappeared, though temporarily, following the intracisternal administration of GABOB.

Although there is no conclusive evidence to believe that GABA or GABOB is a natural inhibitory factor in the brain, it is of great interest that they are normal products of brain metabolism and show certain inhibitory effects upon the central mechanism of CR.

SUMMARY

The effects of γ-aminobutyric acid (GABA) and γ-amino-β-hydroxy butyric acid (GABOB) on the avoidance conditioned reflexes (CR) in dogs were investigated. The results obtained as follows:

1. A prolongation of latency and decrement of percentage frequency of CR were observed during the course of acute extinction and discrimination.

2. Similar changes of latency and percentage frequency were observed following intravenous and intracisternal administration of GABA or GABOB. There was no marked difference between effective doses of GABA and GABOB.

3. The positive conditioned reflexes of discrimination were inhibited following the intravenous administration of GABOB.

4. The abnormal behavior observed in the experimental situation (experimental neurosis) disappeared, though temporarily, following the intracisternal administration of GABOB.

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References

3) Suhara, R., Personal communication.