EFFECT OF SUCCINIC SEMIALDEHYDE ON SEIZURE CAUSED BY VITAMIN B₆ ANTAGONISTS

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Summary Since γ-hydroxybutyric acid (GHB) is known to be an effective anesthetic adjuvant in animal and a natural metabolite of succinic semialdehyde (SSA), the effect of SSA and GHB on the convulsive action of vitamin B₆ antagonists was studied using the onset of seizure as a parameter. The administration of SSA and GHB to mice protected them against the convulsive action of B₆ antagonist, such as castrix (2-chloro-4-dimethyl-6-methylpyrimidine), thiosemicarbazide, or penicillamine. The anticonvulsant effect of SSA seems to be due to an increase in brain levels of GHB converted in vivo from SSA.

GHB has been detected in small amounts in the normal nervous system and it seems to be the only physiological compound which has anesthetic activity (1–3). Moreover, GHB has been known to be formed from SSA by action of an enzyme that is indistinguishable from lactic dehydrogenase (4).

On the other hand, recent studies on the biochemical mechanism involved in the production of seizures induced by vitamin B₆ antagonists indicated that changes in GABA metabolism in the brain might be involved in the etiology of the seizures (5, 6).

Since both SSA and GHB are compounds related metabolically to GABA (7, 8), it seems reasonable to investigate the actions of SSA and GHB on the seizures caused by vitamin B₆ antagonists, and this was studied in the present paper. The B₆ antagonists used in this experiment are castrix, thiosemicarbazide and penicillamine.

MATERIALS

SSA was prepared by the method of Bessman et al. (8). GHB was purchased from Sigma Chemical Company. Castrix was kindly supplied by Mr.

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Abbreviations used: GHB, γ-hydroxybutyric acid; SSA, succinic semialdehyde; GABA, γ-aminobutyric acid.
Umeda of Nihon Noyaku Co., Ltd. Thiosemicarbazide and DL-penicillamine were purchased from Nakarai Chemicals.

Male DDY mice weighing 18 to 20 g were used throughout the experiments and allowed free access to food and water.

RESULTS

Some effects of SSA on mouse

To observe the effect of SSA alone, an aqueous solution was injected intraperitoneally. SSA was dissolved in 0.9% saline and neutralized to pH 7.0 with NaOH. A single intraperitoneal dose of 0.01 ml/g body weight was used in the present study.

SSA given at doses of 1 to 5 mg/g consistently produced within 10 to 15 min effects of decreased motor activity, muscle weakness, ataxia, and generalized decrease in response to sensory stimulation. One milligram per gram was considered a threshold dose. The duration of effects was dose-dependent; 5 mg/g were effective for 90 to 120 min. Recovery was complete and no aftereffects could be observed.

Effect of SSA on seizure produced by castrix

In the experiment in our laboratory (9), 2.5 μg/g of castrix was injected subcutaneously in the back of the mice. At 30 min after the injection, 5 mg/g of SSA or the same volume of saline (control group) was injected intraperitoneally. In the control group, "running fit" and convulsion took place after about 40 min, followed by repeated seizures and finally death on cessation of the last seizure (Fig. 1).

On the contrary, all animals receiving an injection of SSA did not experience seizures and did not die. Furthermore, injection of SSA just after the first seizure was also effective enough to prevent animals from subsequent seizures and death

![Graph showing effect of SSA on the onset of seizures induced by castrix.](image-url)
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(Fig. 1). Saline alone had no such significant protective effect on subsequent seizures.

**Effect of SSA on seizure produced by penicillamine**

According to MATSUDA and MAKINO (10), animals were injected subcutaneously with 0.35 mg/g DL-penicillamine. At 160 min after the injection, SSA or saline as control was injected intraperitoneally as described in the castrix experiment. When the penicillamine-treated animals were subsequently given SSA, the seizure was completely prevented and the animals were saved from death (Fig. 2). The preventive action of SSA was effective even after the first occurrence of the seizure.

![Diagram of Fig. 2](image)

**Fig. 2.** Effect of SSA on the onset of seizures induced by penicillamine. ○, onset of seizure; ●, death with seizure; PeA, penicillamine. At arrow DL-penicillamine (0.35 mg/g) was subcutaneously and SSA (5 mg/g) intraperitoneally. In the control group, 0.9% saline was injected intraperitoneally in the place of SSA at the same time as that of SSA injection.

![Diagram of Fig. 3](image)

**Fig. 3.** Effect of SSA on the onset of seizures induced by thiosemicarbazide. ○, onset of seizure; ●, death with seizure; TSC, thiosemicarbazide. At arrow thiosemicarbazide (20 μg/g) was injected subcutaneously and SSA (5 mg/g) intraperitoneally. In the control group, 0.9% saline was injected intraperitoneally in the place of SSA at the same time as that of SSA injection.
Effect of SSA on seizure produced by thiosemicarbazide

In the experiment in our laboratory (II), animals were injected subcutaneously with 0.02 mg/g thiosemicarbazide. At that time, as shown in Fig. 3, SSA or saline was injected intraperitoneally. Injection of SSA completely prevented all the test animals receiving thiosemicarbazide from seizures for about 150 min after this injection, though after 150 min the seizures took place; the occurrence rate of seizures was 3/4 and mortality 2/4 (Fig. 3). Even after the first occurrence of the seizure the injection of SSA prevented all animals from the subsequent

![Graph showing effect of SSA on seizure induced by thiosemicarbazide.](image)

Fig. 4. Effect of GHB on the onset of seizures induced by castrix. ○, onset of seizure; ●, death with seizure. At arrow castrix (2.5 μg/g) was injected subcutaneously and GHB (0.3 mg/g) intraperitoneally. In the control group, 0.9% saline was injected intraperitoneally in the place of SSA at the same time as that of SSA injection.

![Graph showing effect of GHB on seizure induced by thiosemicarbazide.](image)

Fig. 5. Effect of GHB on the onset of seizures induced by thiosemicarbazide. ○, onset of seizure; ●, death with seizure; TSC, thiosemicarbazide. At arrow thiosemicarbazide (20 μg/g) was injected subcutaneously and SSA (5 mg/g) intraperitoneally. In the control group, 0.9% saline was injected intraperitoneally in the place of SSA at the same time as that of SSA injection.
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seizures and kept them alive for about 200 min after the injection, though some of these animals died following the seizures.

*Effect of GHB on seizure produced by castrix and thiosemicarbazide*

It has recently been demonstrated that administration of GHB causes behavioral “sleep” (3). Varying doses of GHB were injected intraperitoneally and the effect was observed. When 0.30 mg/g was used, the symptoms induced by it appeared within 10 min and was quite similar to those of SSA though the duration of effects was only 50-70 min.

Animals were injected subcutaneously with 2.5 μg/g castrix or 0.02 mg/g thiosemicarbazide. At the time as shown in Figs. 4 and 5, 0.30 mg/g GHB or saline was injected intraperitoneally. Injection of GHB prevented all the test animals receiving castrix or thiosemicarbazide from the seizures and kept them alive for about 60 min after the injection, though these animals mostly died following subsequent seizures.

**DISCUSSION**

In this paper the effect of SSA and GHB on the convulsive action of B6 antagonists, such as castrix, thiosemicarbazide and penicillamine was studied, using the onset of seizure (running fit and convulsion) as a parameter. And it was found that the administration of GHB is able to delay the onset of the convulsions produced by the B6 antagonists, and SSA in many cases completely prevents the convulsions. The anticonvulsant effect of SSA seems to be due to an increase in brain levels of GHB converted from SSA, because the action of GHB appears at lower dose and earlier than with SSA.

The seizures induced by the B6 antagonists have been well known to be completely suppressed by the administration of vitamin B6 (10, 12, 13). Both glutamic decarboxylase and GABA transaminase involving in GABA metabolism are vitamin B6 dependent enzymes, and the glutamic decarboxylase activity is particularly affected by vitamin B6 deficiency (6). Therefore, to the extent that the seizures induced by B6 antagonists do lead to a lowering of the levels of GABA metabolism, it seems reasonable that the levels of SSA and consequently GHB, two possible metabolites of GABA, may also be lowered.

If it can be postulated that a decrease in the brain levels of GHB is an important factor in the production of convolution, the above evidences would also seem logical that the administration of GHB or SSA is able to delay the onset of the convulsions, or in some cases completely prevents the convulsions.

However, no evidence has been demonstrated yet that SSA or GHB play a role in normal neuronal functioning and that there is actually a decrease in amounts of SSA or GHB during convulsions, because the level of endogenous GHB is order of magnitude lower than effective concentrations in the brain following...
intraperitoneal administration (3) and the concentration of endogenous SSA is below the limits of known method of analysis. Therefore, the fact that GHB or SSA may alter the course of the convulsions may not be taken as evidence that the convulsions are caused by depleted endogenous levels of these compounds.

It seems possible that the administration of SSA or GHB might do more than refill the depleted endogenous stores; the drugs may be exerting a "pharmacological" effect other than "biochemical" effect on neuronal excitability.

REFERENCES