INDUCTION AND SUPPRESSION OF AMINOSULFONIC ACID SPIKES IN WULST EEG OF ADULT CHICKENS

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In order to learn the effects of a series of aliphatic ω -aminosulfonic acids with varying numbers of carbon chains, changes in the electroencephalogram (EEG) of adult hens were investigated as the criteria. The drug solution ($10\ \mu\text{l}$) was locally injected into the telencephalon of a curarized animal under artificial respiration and Wulst EEG was recorded near the administered region. No influence was seen on EEG by the administration of amino acids with 1–3 carbon chains (short-chain structure) at a concentration of $6 \times 10^{-1}\text{M}$. Characteristic biphasic spikes developed after the administration of amino acids with 4 or more carbon chains (long-chain structure) at a concentration higher than $2 \times 10^{-2}\text{M}$, and their amplitudes gradually increased over 1.5–2.0 mV. Spikes were induced by guanyl compounds of amino acids of long-chain structure even at a concentration of $2 \times 10^{-3}\text{M}$. Convulsant drugs also induced spikes when used in doses similar to the spike-inducing ω -aminosulfonic acids. The development of spikes by ω -aminosulfonic acids was antagonized by aminosulfonic acids with a short-chain structure. On the basis of the above-mentioned results, ω -aminosulfonic acids can be classified into excitants and depressants according to their activities in inducing characteristic spikes with high amplitudes. In the development of spikes, the excitants and depressants are antagonistic to each other. The doses of excitants which induce spikes are comparable to those of convulsants and coincide very well with the earlier reported relationship between convulsion development rates of excitants and dosage administered to the cerebral ventricle of mice.

Keywords— ω -aminosulfonic acids; excitant; depressant; EEG; electroencephalogram; characteristic biphasic spike; adult chicken

INTRODUCTION

We earlier reported our studies on the influence of aliphatic ω -aminosulfonic acids and their guanyl compounds (ω -guanidinosulfonic acids) on the behavior and electroencephalogram (EEG) of animals through intraperitoneal administration to young chickens in which the blood-brain barrier is still incomplete.^{1,2)} The results revealed that a general anesthesia-like inhibition or clonic and tonic convulsions developed in the behavior. In the EEG, one evident effect was the induction of rhythmic spikes with large amplitude before the occurrence of the convulsion. From these results, it was clarified that ω -aminosulfonic acids can be classified into depressants and excitants. However, a high dose

(3 g/kg) of the agents was needed to develop their effects.

In the present study ω -aminosulfonic acids were directly administered into the brain of adult chickens. The relationship between the chemical structures of amino acids and their spike-inducing activities was studied using the characteristically high amplitude-regulating spikes induced by these agents on EEG of neighboring regions as a criterion. The effects of the administration of depressant aminosulfonic acids upon spike development were also studied.

EXPERIMENTAL

Agents Employed—Six kinds of aliphatic ω -aminosulfonic acids were employed for the

experiment, from one with a one-carbon chain length between the sulfonic acid group and the amino group up to one with a six-carbon chain length. These were aminomethanesulfonic acid (C_1 , Aldrich Chemical), taurine (C_2 , Nakarai Chemicals) and others synthesized by our laboratory,^{3,4)} *i.e.*, 3-aminopropanesulfonic acid (C_3), 4-aminobutanesulfonic acid (C_4), 5-aminopentanesulfonic acid (C_5), and 6-aminohexanesulfonic acid (C_6). Their guanyl compounds,⁴⁾ *i.e.*, 3-guanidinopropanesulfonic acid (G- C_3), 5-guanidinopentanesulfonic acid (G- C_5), and 6-guanidinohexanesulfonic acid (G- C_6) were also used.

As central nervous system (CNS) excitants of the convulsant type, strychnine nitrate (Wako Pure Chemical Industries) and pentylenetetrazol (Tokyo Kasei Kogyo) were employed. As a local anesthetic, procaine hydrochloride (Yoshitomi Pharmaceutical Industries) was used, while gallamine triethiodide (2% gallamine injection "Teisan"® Teikoku Kagaku Sangyo) was used as a muscle relaxant.

The above-mentioned amino acids and CNS excitants were dissolved in 0.75% saline⁵⁾ to a final concentration of 2×10^{-3} – 2×10^{-1} M and injected into the brain.

Animals Employed, Recording of EEG and Administration of Test Agents—In the present experiment, 28 adult White Leghorn hens (8 months old and each weighing 1.4–1.8 kg) were employed. All hens were curarized with an intravenous injection of gallamine triethiodide (5 mg/kg). Each hen was placed in a head holder which was part of a stereotaxic apparatus (Takahashi Shoten) and under artificial respiration (animal respirator: model SN-480-7, Shinano Seisakusho, tidal volume: 20 ml, rate: 30 breaths/min) its upper beak and both of the meatus acustics externus were fixed therein. Head feathers were sheared and a 1% procaine solution was infiltrated into the subcutaneous layer. The skin of the head was incised along the median line from the upper side of the occipital bone toward the anastomotic part down to the end of the comb to remove the periosteum and to expose the skull.

The part of skull to receive the surface EEG introduction and the injection of the agent's solution was removed with a round dental burr of 3 mm diameter so as not to damage the dura mater. For recording the EEG, Ag-AgCl wick electrodes were used. Recording was made from the dorsal part of the telencephalon which corresponds to the Wulst, the accessory hyperstriatum. The two recording electrodes were symmetrically placed at a distance of 3 mm from the midline and 4 mm anterior to the bregma (Fig. 1). The recording site thus approximately corresponded to A8.5 in the atlas of Van Tienhoven and Juhász.⁶⁾ An indifferent electrode was placed on the comb and the EEG was recorded by the monopolar leading method. A multipurpose polygraph (RM-45, Nihon Kohden) was used for the recording. The location of injection of the agent was 5 mm outward laterally from the place of EEG introduction on the surface of the right hemisphere at a depth of 1.0–1.5 mm from the surface of the brain. A microsyringe (MS-N50, Terumo) was used for administration of the test agent. The injection was usually made at a rate of 10 μ l of the solution each 30 s.

FIG. 1. A Schema of the Dorsal View of an Adult Hen Brain

The locations of the two recording electrodes are indicated on the Wulst of the telencephalon and the site of administration of the test agent is shown on the right hemisphere. The indifferent electrode for monopolar lead is located on the comb.

RESULTS

Administration of Depressant Aminosulfonic Acids

Despite the administration of C_1 , C_2 , or C_3 in concentrations of $2 \times 10^{-3} - 6 \times 10^{-1} M$, spikes did

FIG. 2. Influences of 3-Aminopropanesulfonic Acid (C_3) upon EEG of Adult Hen curarized by Gallamine (5 mg/kg, i.v.)

The spotted zone represents the time of local injection of the agent's solution ($10 \mu l$) into the brain.

FIG. 3. Status of Development of Spikes induced by 5-Aminopentanesulfonic Acid (C_5) administered to Adult Hen curarized by Gallamine

Spikes are induced on the right hemisphere (R) near the site of administration of the agent and their amplitude gradually increases.

not develop on the EEG by 50 min following administration with any of the amino acids. No other specific change was noted (Fig. 2).

Administration of Excitant Aminosulfonic Acids

Administration was made of C_4 , C_5 , or C_6 at a concentration of $2 \times 10^{-3} M$, but no change was detected on the EEG.

With administered concentrations of $2 \times 10^{-2} M$, all of the amino acids induced spikes on the administered side of the EEG (right hemisphere) during or immediately after injection of the agents. The amplitude was gradually enlarged into a characteristic biphasic status with a fixed high (1.5–2.0 mV) and a frequency

FIG. 4. Status of Development of Spikes induced by 5-Guanidinopentanesulfonic Acid ($G-C_5$) administered to Adult Hen curarized by Gallamine

Spikes are induced on the right hemisphere (R) near the site of administration of the agent, and their amplitudes gradually increase, but disappear at 50 min.

(0.3–0.5 Hz) at 1–5 min, which lasted for more than 2 h (Fig. 3).

In the case of administration at a concentration of $2 \times 10^{-1} \text{M}$, the result was similar: characteristic regular biphasic spikes developed with high amplitude (1.5–2.0 mV).

Administration of ω -Guanidinosulfonic Acids

No change in EEG was seen following administration at a concentration of $2 \times 10^{-3} \text{M}$ of guanyl compound (G-C₃) of C₃ which can be classified as one of the depressants. During its administration at a concentration of $2 \times 10^{-2} \text{M}$, spikes were induced on the administered side (right hemisphere), and the amplitude was gradually enlarged into biphasic and regular spikes with high amplitude (2 mV) at 15 min; however, this gradually diminished to normal, pre-administration EEG at 40 min.

On injection of the solution of excitant guanyl compounds (G-C₅ or G-C₆) of C₅ or C₆ at a concentration of $2 \times 10^{-3} \text{M}$, spikes were induced immediately after administration and the amplitude gradually increased to regular spikes of

FIG. 5. Status of Spikes induced by 5-Guanidinopentanesulfonic Acid (G-C₅) administered to Adult Hen curarized by Gallamine

Spikes are induced on the right hemisphere (R) near the site of the administration of the test agent and their amplitudes are gradually enlarged and become regular.

0.2–1.0 mV at 5–15 min. This diminished by degrees to a level equal to the pre-injection level at 15–50 min (Fig. 4). At a concentration of $2 \times 10^{-2} \text{M}$, spikes developed during the injection and the amplitude gradually increased to regular and biphasic spikes with high amplitude (1.2–2.0 mV) at 5–20 min, which continued for more than 2 h (Fig. 5).

The administration of an agent at a concentration of $2 \times 10^{-1} \text{M}$ resulted in the same findings, showing characteristic and regular spikes with high amplitude (2 mV).

Administration of CNS Excitants

Strychnine at a concentration of $5 \times 10^{-2} \text{M}$ (2%) induced spikes on the administered side

FIG. 6. Status of Spikes induced by Strychnine administered to Adult Hen curarized by Gallamine
The spikes are also spread on the hemisphere of the telencephalon on the opposite side (L) for some time (10 min) after the administration.

(right hemisphere) during the injection of the agent. The amplitude gradually increased showing regular spikes at 5 min with high amplitude (2 mV) which continued for more than 2 h (Fig. 6).

Administration of 20 μ l of pentylenetetrazol at a concentration of 7×10^{-1} M (10%) also resulted in spikes induced during injection of the test agent. The amplitude gradually increased and, on reaching maximum of 0.5 mV, the spikes became regular and continued.

Effects of Depressant Aminosulfonic Acids upon Spike Occurrence

After confirming that the spikes induced by the administration of aminosulfonic acids or guanidinosulfonic acids showed stable amplitude and frequency at 15 min after their administration, 10–20 μ l of physiological saline was locally administered, but the development of regular spikes was not affected (Fig. 7).

However, when a concentration with 10 μ l of

FIG. 7. Influence of Saline Administration upon Spikes induced by 6-Guanidinoheptanesulfonic Acid (G-C₆)

After confirming the regularity of spike development at 15 min after administration of G-C₆, saline was administered into the brain. This administration did not influence development of the spikes.

2×10^{-1} – 6×10^{-1} M of C₂ or C₃ was administered, the development of spikes was completely inhibited. Figure 8 illustrates the immediate suppression of the regular development of spikes at 20 min following administration of G-C₅ at a concentration of 2×10^{-2} M by the administration of C₂ into the brain at a concentration of 2×10^{-1} M. In this case, spikes were seen appearing at 45 min.

DISCUSSION

We²⁾ earlier reported that intraperitoneal administration of ω -aminosulfonic acids to young

FIG. 8. Influence of Administration of Taurine (C₂) upon Spikes induced by Administration of 5-Guanidinopentanesulfonic Acid (G-C₅)

After confirming the regularity of development of spikes at 20 min after injection of G-C₅, C₂ was administered. For 40 min following this, spike development was suppressed, but they again developed at 45 min.

chickens at the age of 4–7 d without anesthesia developed characteristic and biphasic spikes on the EEG of forebrain which were similar to those of strychnine spikes^{7,8)} and that convulsion was subsequently induced. In young chickens the blood-brain barrier is incomplete, and therefore amino acids and amines systemically administered can reach CNS relatively easily and show their effects.^{9–13)} However, a large amount (3 g/kg) of ω -aminosulfonic acid was found necessary to induce the above-mentioned effects.

In the present experiment, curarized adult chickens were employed, to which aminosulfonic acids were applied into the brain under artificial respiration in order to study the relationship between the dose to induce spikes in the neighboring Wulst EEG and the chemical structures of amino acids. As a result, administration of depressant aminosulfonic acids (C_2 and C_3) at a concentration of $6 \times 10^{-1} M$ showed no influence upon EEG, whereas excitant aminosulfonic acids (C_4 , C_5 , and C_6) at a concentration of $2 \times 10^{-2} M$ and guanidinosulfonic acids (G- C_5 and G- C_6) at even lower concentration ($2 \times 10^{-3} M$) could develop characteristic and biphasic spikes with high amplitudes. Additionally, the doses of these amino acids to induce spikes were found to be comparable to those of convulsants in inducing spikes. On these grounds, it was assumed that the effects of excitant aminosulfonic acids upon the telencephalon of chickens and the counterparts of strychnine were similar to each other. These findings and the doses of test agents for intraperitoneal administration to young chickens to induce spikes (3 g/kg of ω -aminosulfonic acids²⁾ and 5–7.5 mg/kg of strychnine^{2,8)}), suggest that ω -aminosulfonic acid can not easily pass through the blood-brain barrier in young chickens.

We¹⁴⁾ earlier studied the activity of amino acids to develop convulsion through administration of aliphatic ω -aminosulfonic acids into the cerebral ventricle of mice, and classified aliphatic ω -aminosulfonic acids into depressants of short-chain structure and excitants of long-chain structure. The dose of each amino acid for developing

convulsion in mice coincided with the dose required to induce spikes in the present experiment. Curtis *et al.*¹⁵⁾ reported that amino acids of short-chain structure such as C_2 and C_3 show depressant actions, while C_4 with long-chain structure demonstrates excitant actions, on the basis of their effect upon the isolated and sagittally hemisected toad spinal cord. The results of the present experiment revealed that amino acids can be clearly classified into depressants and excitants by checking the appearance of characteristic spikes with high amplitudes on the EEG of adult chickens. Also, in the experiment with intraperitoneal administration with young chickens,^{1,2)} it was indicated that the classification was adequate on the basis of the animal behavior and the changes in EEG.

In this experiment, the spikes with high amplitudes induced by excitant aminosulfonic acids were antagonized by relatively high doses of depressant aminosulfonic acids. The blood pressure lowering effect of γ -aminobutyric acid was reported in conjunction with the antagonism between ω -aminocarboxylic acids with varying numbers of carbon chains; however, the mechanism of the antagonism has not yet been clarified.¹⁶⁾ The mechanism of the depressant or excitant effect induced by ω -aminosulfonic acids and their mutual antagonisms have not yet been clarified and more studies on them are needed.

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