ω-AMINOSULFONIC ACIDS AS DEPRESSANT OR EXCITANT COMPOUNDS: THEIR EFFECT ON THE BEHAVIOR AND ELECTROENCEPHALOGRAM OF YOUNG CHICKENS

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Young chickens with inefficient blood-brain barrier were employed to study the effects of aliphatic ω-aminosulfonic acids with varying numbers of carbon chains on the central nervous system. Each of the amino acids was intraperitoneally administered to unrestrained and unanesthetized animals, to investigate its effects upon their behavior and electroencephalogram (EEG). ω-Amino acid with a short-chain structure (3-aminopropanesulfonic acid) had a depressing effect similar to that of γ-aminobutyric acid which is known as a depressant amino acid. On the other hand, the ω-amino acids with long-chain structure (5-aminopentanesulfonic acid and 6-guanidinohexanesulfonic acid) acted as excitants, induced convulsions and developed typical biphasic spikes with high amplitudes in the EEG. The spikes appeared early, and could be detected at the excitant stage before convulsion. In contrast, with the administration of convulsant drugs, the spikes appeared only after the occurrence of convulsion. However, a high dose (1–9 g/kg) of the ω-amino acids was needed to develop their effects. These results suggest that young chickens are valuable in clarifying the characteristic behavior and the brain electrical activity of ω-amino acids. But the ω-amino acids cannot easily pass through the blood-brain barrier in young chicken.

Keywords — ω-aminosulfonic acid; 3-aminopropanesulfonic acid; 5-aminopentanesulfonic acid; 6-guanidinohexanesulfonic acid; γ-aminobutyric acid; electroencephalogram; young chicken; depressant; excitant

INTRODUCTION

In previous studies, we examined changes in the behavioral patterns of rats and mice following intracerebroventricular administration of aliphatic ω-aminosulfonic acids having 1–6 carbon chains, and we classified these amino acids into depressants and excitants.1,2) Our classifications coincided with those of the studies made by Curtis et al.3) with the isolated and sagittally hemisected spinal cord of toads.

The effects of drugs upon the central nervous system (CNS) are very much dependent on whether or not they pass through the blood-brain barrier. Therefore, in studies on the CNS effects of drugs which do not pass through this barrier, the drugs must be administered directly into the brain.

Since the blood-brain barrier in a chicken is known to be inefficient up to at least 3–4 weeks after hatching,4) the drugs systemically administered are relatively easily transferred into the CNS where their effects are manifested. Chicks immediately after hatching are fully mature neurologically, and the variations in their behavior and electroencephalogram (EEG) are identical with those which can be observed in mature poultry.5,6) In spite of the anatomical differences, the majority of neuropharmacological responses of the chick are similar to those of higher vertebrates.7,8) The price of cockerels is less than 1/5 that of mice. Because of the above-mentioned advantages, the chicken can be used as a neuropharmacological preparation for the study of agents which do not pass the mature barrier.9–13)

The present study focused on the influence of ω-aminosulfonic acids intraperitoneally admin-
istered on the behavior and EEG of chickens.

MATERIALS AND METHODS

Agents Employed — 3-Aminopropanesulfonic acid (C₃, synthesized by our laboratory¹⁴) and γ-aminobutyric acid (GABA, Daiichi Seiyaku) as the depressant amino acids. The excitant amino acids employed were 5-amino-pentanesulfonic acid and 6-guanidinohexanesulfonic acid (C₅ and G-C₆, both synthesized by our laboratory¹⁸). Pentylentetrazol (Tokyo Kasei Kogyo), strychnine nitrate (Wako Pure Chemical Industries) and picrotoxin (Wako Pure Chemical Industries) as CNS excitants of the convulsant type. As a local anesthetic, procaine hydrochloride (Hoei Yakko) was used, while d-tubocurarine chloride (Amerizol®, Yoshitomi Pharmaceutical Industries) was used as a muscle relaxant.

Animals — In the present experiment, approximately 100 White Leghorn cockerels (4—7 d after hatching and each weighing 45—60 g) were employed.

Attachment of Electrodes — The animals were anesthetized with ether using a face mask. The feathers on head and neck were sheared, and 1% procaine solution was infiltrated into the subcutaneous layer. A median incision of 1 cm was made on the skin of the head from above the occipital bone down to the rostrum; the periorbital was removed and the skull was exposed. The intersection of a line 3 mm lateral to the midline of the frontal bone with a line 3 mm anterior to the bregma was selected as the site to record the EEG and the smallest possible hole was made with great care using a dental burr (Fig. 1). Through this hole, a recording electrode was inserted vertically to a depth of 0.5 mm from the skull surface, and was bent at a right angle for 5 mm along the surface of the skull. The electrode was fixed to the skull using an adhesive agent (Aron Alpha®), and further tightly fixed by covering it with skin. The recording electrode employed was a stainless steel wire with a diameter of 0.2 mm; this was coated and insulated with an enamel paint except for 0.2—0.3 mm of its tip. An indifferent electrode was placed on the comb and the EEG was recorded by the monopolar leading method.¹⁶

Recording of EEG and Administration of Test Agents — A multipurpose polygraph (RM-45, Nihon Kohden Kogyo) was used for the recording. EEG recording was carried out immediately after the animal was free from ether anesthesia. The animal was put unrestrained into an electrically shielded chamber (21 × 34 × 25 cm). When the animal exhibited a convulsion, 6 mg/kg of d-tubocurarine was intraperitoneally injected so as to prevent the possible artifact contamination associated with convulsive movements on the EEG, and artificial respiration (animal respirator: model SN-480-7, Shinano Seisakusho, tidal volume: 0.4 ml, rate: 70 breaths/min) was applied via a cannula inserted into the trachea. Drugs were dissolved in 0.75% saline and injected intraperitoneally.

RESULTS

Administration of Depressant Amino Acids

1) Effects of C₃ — 0.5 g/kg: No effects on the behavior and EEG of animals were observed.
1 g/kg: Slight behavioral depressions were ob-

FIG. 1. Dorsal View of the Head of a 6-day-old Chick

Broken lines illustrate the projected outline of the brain. Fine dot on the forebrain represents the position of the recording electrode. The indifferent electrode for monopolar lead is located on the comb.
served; they became drowsy and closed their eyes 18 min after administration; after 25 min they stood immobile and nonvocal with open eyes. At all times the EEG readings remained almost the same as those taken in the resting state before administration.

3 g/kg: Depressant action was increased; after 60 min they sat folding legs under the trunk, nonvocal with closed eyes, and the response to the auditory stimulus reduced. EEG at this time showed high amplitude slow waves.

9 g/kg: Sleep was followed by anesthesia-like states; after 7 min they took sleep-like posture, closing the eyes, sitting with the legs folded under the trunk; after 10 min they put the head on the floor, abolishing the righting reflex; after 14 min depression increased and death occurred after 20 min.

In the early stage of depression, high amplitude and serial spiky slow waves were observed in the EEG as seen under the ether anesthesia. As the severity of the depression became stronger, the appearance of the spiky and high amplitude waves became clearer, showing bursts of biphasic spikes separated by isoelectric segments (burst suppression).

2) Effects of Large Dose (9 g/kg) of GABA — Approximately similar marked behavioral depression and EEG changes as those with large dose administration of C₃ were detected.

Administration of Excitant Amino Acids

1) Effects of C₅ — 1 g/kg: They became drowsy, nonvocal, often closing the eyes and remaining still. The EEG activities were not significantly altered.

2 g/kg: A little while after administration they showed a slight depression but after 30 min they became excited, sitting and twittering fervently with wings spread; then clonic and tonic convolution occurred, leading to death after 80 min.

3 g/kg: A slight sleep-like posture was observed for some time and then gradually shifted to the excited state. We correlated the findings on EEG with these changing patterns of behavior. After 5 min, they stood with closed eyes,
beak touching the floor. After 8 min they sat with eyes closed. In this depressive state high amplitude slow waves were observed on the EEG but low amplitude fast waves recovered when they sat with eyes open, attentive to the surroundings. After 12 min, excitement was observed with eyes open and twittering. At this stage a biphasic spike of about 300 $\mu$V appeared on the EEG, which increased its frequency and amplitude gradually to 600 $\mu$V or more.

Without $d$-tubocurarine and artificial respiration, clonic and tonic convulsions were repeated, leading to death after 50 min.

2) Effects of G-C$_6$ --- 0.5 g/kg: No effect was observed during 80 min of observation.

1 and 2 g/kg: The changes of behavioral and EEG patterns were almost similar to those observed when given 2 and 3 g/kg of C$_6$. They died after 90 and 60 min respectively.

3 g/kg: A slight depression was observed for a while, followed by the excitement of the central nervous system and finally convulsions occurred. After 9 min they flapped the wings in surprise and twittered hard. After 15 min they sat twittering, when a biphasic spike of about 400 $\mu$V appeared which increased gradually to 800 $\mu$V or more.

Without $d$-tubocurarine and artificial respiration, clonic and tonic convulsions were repeated till death after 40 min. Figure 5 shows the spike by G-C$_6$ (3 g/kg, i.p.) under artificial respiration on a curarized animal with $d$-tubocurarine. After 15 min spikes occurred, increasing gradually as is clearly shown.

Administration of CNS Excitants of the Convulsant Type

1) Effects of Pentylentetrazol (100 mg/kg) --- The chickens were sitting on the floor about

![Fig. 4. Effect of 6-Guanidinoheanesulfonic Acid (G-C$_6$) on the EEG](image)

The EEG was continuously recorded under artificial respiration following administration of $d$-tubocurarine (6 mg/kg, i.p.) after convulsion, which was induced 25 min after the administration of the amino acid. Each vertical bar represents 200 $\mu$V.

![Fig. 5. Typical Biphasic Spikes induced by the Administration of 6-Guanidinoheanesulfonic Acid (3 g/kg, i.p.)](image)

The amino acid was administered at 20 min to chickens curarized with $d$-tubocurarine and under artificial respiration. Each vertical bar represents 500 $\mu$V.
1 min after the administration with their eyes wide open frequently twittering in a frightened manner and flapping their wings, showing the so-called pre-convulsive excitant symptoms. Subsequently, tonic convulsion was induced with opisthotonus. Two min after the administration, the chickens repeated running movements of their legs (clonic convulsion), eventually leading to death at 1 h.

The stage where characteristic biphasic spikes (300 \( \mu V \)) could be noted in the EEG was approximately 5–10 min after the administration, if recorded in curarized animals under artificial respiration.

2) **Effects of Strychnine (5 mg/kg)** — The animals began to twitter violently about 1 min following administration and tonic convulsion with extension was simultaneously induced so that they fell down backward with opisthotonus. The animals died with tremors of the body trunk at 13–14 min.

The stage where typical spikes (400 \( \mu V \)) with high amplitudes could be observed in the EEG was 7–10 min after administration in curarized animals under artificial respiration.

3) **Effects of Picrotoxin (15 mg/kg)** — The chickens sat down with their legs folded under them about 2 min after administration with their eyes wide open and with violent twitterings and flappings of wings as if frightened. At about 3 min, tonic convulsion was induced and the animals fell into opisthotonus. With repeated flexions and extensions of the trunk, flapping of wings, and convulsive running movements of the legs, the animals died at 1 h.

The stage where the typical biphasic spikes (500 \( \mu V \)) could be observed in the EEG was 7–10 min if recorded in curarized animals under artificial respiration.

**Effects of \( d \)-Tubocurarine (6 mg/kg)**

In the above experiments, the animals were curarized in order to prevent artifacts into the EEG resulting from the convulsive movements. However, in chickens in which the blood-brain barrier is inefficient, the effect of \( d \)-tubocurarine upon CNS may be manifested. Therefore, the influence of intraperitoneal administration of \( d \)-tubocurarine upon the behavior and EEG of animals was investigated.
Five min after the administration of \( d \)-tubocurarine, the animals sat down with their eyes closed and their legs folded under their bodies. After they began to lie in a recumbent position, artificial respiration was commenced and the EEG was recorded for 85 min; no spike was observed showing any appreciable difference from the findings before administration of \( d \)-tubocurarine.

**DISCUSSION**

We previously studied the effects of aliphatic \( \omega \)-amino sulfonic acids with varying numbers of carbon chains upon CNS of rats and mice after intracerebroventricular injections.\(^1\) Results revealed that taurine and \( C_3 \) with short carbon chains showed sedative effects and that 4-amino butanesulfonic acid, \( C_9 \), 6-amino hexanesulfonic acid, and their guanyl-compounds with longer carbon chains showed strong excitant effects and induced convulsion in a dose-related manner. The above-mentioned characters of \( \omega \)-amino sulfonic acid, *i.e.*, the phenomenon of a switch from depressant into excitant effects by a reverse effect once the number of methylene groups constituting the carbon chains exceeded 4, coincided with the results of studies by Curtis *et al.*\(^3\) who investigated this character with spinal cord of isolated and sagittally hemisectioned toads.

In the present experiment, the amino acids with a stronger activity in these two groups were respectively selected for intraperitoneal administration to chickens. The behavior and EEG following administration were investigated to explore the characters of the amino acids. The large dose of \( C_3 \) (9 g/kg), which has a chemical structure similar to that of GABA, induced the same changes in behavior as the general anesthetics, showing appearance of high-amplitude slow waves with spikes in the EEG. The slow waves were in a pattern similar to those noted following GABA administration. The convulsive symptoms of chickens observed following administration of the excitant amino acids (3 g/kg) were similar to those of convulsions produced by pentylenetetrazol and picrotoxin, but the characteristic biphasic spikes were developed at an early stage. These typical spikes were apparent at the stage where excitation was observed before occurrence of convulsion. In contrast, the appearance of spikes was seen only after the occurrence of convulsion in the case of administration of convulsants. In the present experiment, spikes due to convulsants could be recorded in the cranized animals after development of convulsion.

As the blood-brain barrier of young chickens is inefficient, biogenic amines and amino acids given systemically, relatively easily attain the CNS and take effect. For example, epinephrine (5 mg/kg), norepinephrine (0.05—4.0 mg/kg) and serotonin (0.1—20 mg/kg) cause the same change in behavior and EEG as natural sleep.\(^10,11,13\) An increased dosage (epinephrine, 25 mg/kg) causes depression of behavior and EEG change which is similar to those observed when given ether or pentobarbital for anesthesia. Just after dopamine (75—100 mg/kg) was given, an awakening reaction occurred, followed by an anesthesia-like state.\(^10\) GABA (4 g/kg) caused the same change of behavior and EEG as natural sleep; the increased dosage (8 g/kg) caused remarkable depression of behavior and an EEG change similar to those in ether anesthesia.\(^11\)

We compared the effectiveness of excitant amino acids and convulsive drugs (strychnine, pentylenetetrazol) injected directly into the brain of fowls, using as a parameter a characteristic high amplitude regular spike produced on the EEG in the vicinity of the injected site.\(^17\) Any drug for which the same dose level was available could cause spikes.

From the above-mentioned results it was confirmed that quite a large dose of amino acids was necessary compared with biogenic amines to cause a remarkable change in behavior and EEG of chickens.

An injection of 30—300 \( \mu \)g of \( d \)-tubocurarine into the lateral cerebroventricle of a cat could induce convulsion similar to that of epilep-
Aminosulfonic Acids and Chicken EEG

sia major ending up in a tonic phase. On the other hand, infusion of a small dose (500 mg) of d-tubocurarine into the cerebral ventricle of a dog induced a typical sleep pattern in its EEG. On the basis of these findings, it may be claimed that the injection of d-tubocurarine could show effects upon CNS in chickens in which the blood-brain barrier has not yet been completed. Therefore, examination continued until 85 min after the administration of d-tubocurarine (6 mg/kg, i.p.), but no spike was induced in the EEG and no other appreciable change could be observed.

The mechanism of the depressant or excitant effect induced by ω-aminosulfonic acid has not yet been clarified and more studies are needed.

REFERENCES