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Pharmacological properties of amino-oxyacetic acid in the chicken

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Summary

1. The effects of amino-oxyacetic acid (AOAA) on the central nervous system and on skeletal muscle have been examined in the chicken.

2. AOAA had both anticonvulsant and convulsant effects, depending on the dose, as in other species.

3. The convulsant effect, accompanied by EEG spiking, decreased rapidly with increase in age of young chicks.

4. The convulsant effect was exerted primarily through supraspinal centres.

5. Of control depressants tested, only troxidone and small doses of AOAA afforded significant protection against AOAA seizures.

Introduction

Gamma-aminobutyric acid (GABA) does not penetrate the blood brain barrier in mammals (Van Gelder & Elliott, 1958 ; Purpura, Girado & Grundfest, 1957) but does so in young chicks (Sisken, Sano & Roberts, 1961), although large doses injected intraperitoneally are necessary (Scholes, 1965 ; Kramer & Seifter, 1966 ; Kramer, Sherman & Seifter, 1967). In mammals, Van Gelder (1965, 1966) observed that pretreatment with amino-oxyacetic acid (AOAA) potentiates the depressant action of GABA. In experiments described in this paper, a preliminary study of the effects of AOAA in chicks was made.

Methods

Experiments were performed on male white leghorn chicks aged 1 day to 12 weeks and on 1-day old rats.

Crossed extensor reflexes (CER)

Chicks aged 2–7 days were anaesthetized with chloralose (30–60 mg/kg, i.p.). The chick was laid on its back, the trachea cannulated and artificial ventilation was applied using a Palmer Miniature Ideal Pump. Crossed extensor reflexes were recorded from one limb on a smoked paper using a light spring loaded lever (Bowman, Callingham & Osuide, 1964 ; Bowman & Osuide, 1967) or an Ether transducer on a Devices M4 recorder using DC2C preamplifier on heat sensitive paper. Many chicks were spinalized by sectioning the cord between ligatures at the level of the tenth cervical vertebra. Drugs tested on the CER were always injected intravenously.

Maximal twitches

Twitches of the gastrocnemius or tibialis interior were elicited at a frequency of 0·1 Hz by stimulation of the peripheral portion of the severed ipsilateral sciatic nerve with rectangular shocks of 0·1 ms duration and of about twice the strength required to evoke a maximal twitch (Bowman *et al.*, 1964).

Convulsant and anticonvulsant experiments

Conscious chicks were used in groups of ten in an observation box of dimensions $0{\cdot}6 \times 0{\cdot}45 \times 0{\cdot}23$ m.

Electrocorticogram (ECoG)

Chicks were anaesthetized with halothane and implanted with electrodes made from the pointed end of stainless steel insect pins (No. 00). The electrodes were implanted on to the superficial areas of the striatum (accessory hyperstriatum, hyperstriatum and neostriatum) using the method of Spooner (1964, 1965). Electromyogram (EMG) electrodes were made with stainless steel insect pins (No. 00) bent at the ends. The electrodes were positioned in the proximity of the head 5 mm above the insertion of the semispinalis capitis muscle. Chicks were allowed 24 h for recovery. During recording the chick was placed in a screened cage in a quiet room. The ECoG from the superficial striatal areas was recorded with the comb as reference. Behaviour, ECoG and EMG were observed for at least 1 h before drug administration. The ECoG and EMG were recorded using a Devices M4 four channel recorder with AC7C preamplifiers.

Drugs and solutions

Drugs used were pyridoxal 5′ phosphate (Sigma), pyridoxal hydrochloride (Sigma), amino-oxyacetic acid semihydrochloride (Upjohn), pyridoxamine hydrochloride (Sigma), pyridoxine monohydrochloride (Sigma), nembutal (Abbott's), tridione (Abbott's), diphenylhydantoin (Parke Davis), ethosuximide (Parke Davies), mephenytoin (Sandoz), mephenesin (B.D.H.), styramate (Armour Pharmaceuticals), diazepam Roche), chloralose (Hopkin & Williams), methyldopa (Merck, Sharp & Dohme), sodium acetazolamide (Lederle), reserpine (Ciba), trimipramine (May & Baker), chlorpromazine (May & Baker), leptazol (B.D.H.), picrotoxin (Savory & Moore), strychnine hydrochloride (B.D.H.). Doses refer to the salts except for strychnine, and leptazol which are given in terms of the bases. Amino-oxyacetic acid solutions were adjusted to pH 7·0. Diphenyl-hydantoin, diazepam, methyldopa, mephenytoin, ethosuximide, tridione were administered intraperitoneally after suspension in 5% acacia. The other drugs were administered subcutaneously except where otherwise stated.

Results

Convulsant effects

AOAA produced clonic convulsions with some tonic seizures in chicks. The convulsions were preceded by hyperkinetic activity or 'starting' behaviour. The incidence and severity of the seizures varied with the dose of AOAA, the route of administration and age of the chicks. The ED50 for the convulsant effect on subcutaneous administration was $5{\cdot}5 \pm 0{\cdot}3$ mg/kg in chicks aged 2 days. AOAA pro-

duced the most pronounced effect on intravenous injection, 4 mg/kg evoking mild clonic convulsions lasting 5–15 min in chicks aged 2 days. Similar doses did not produce convulsions on subcutaneous or intraperitoneal injection. In young chicks

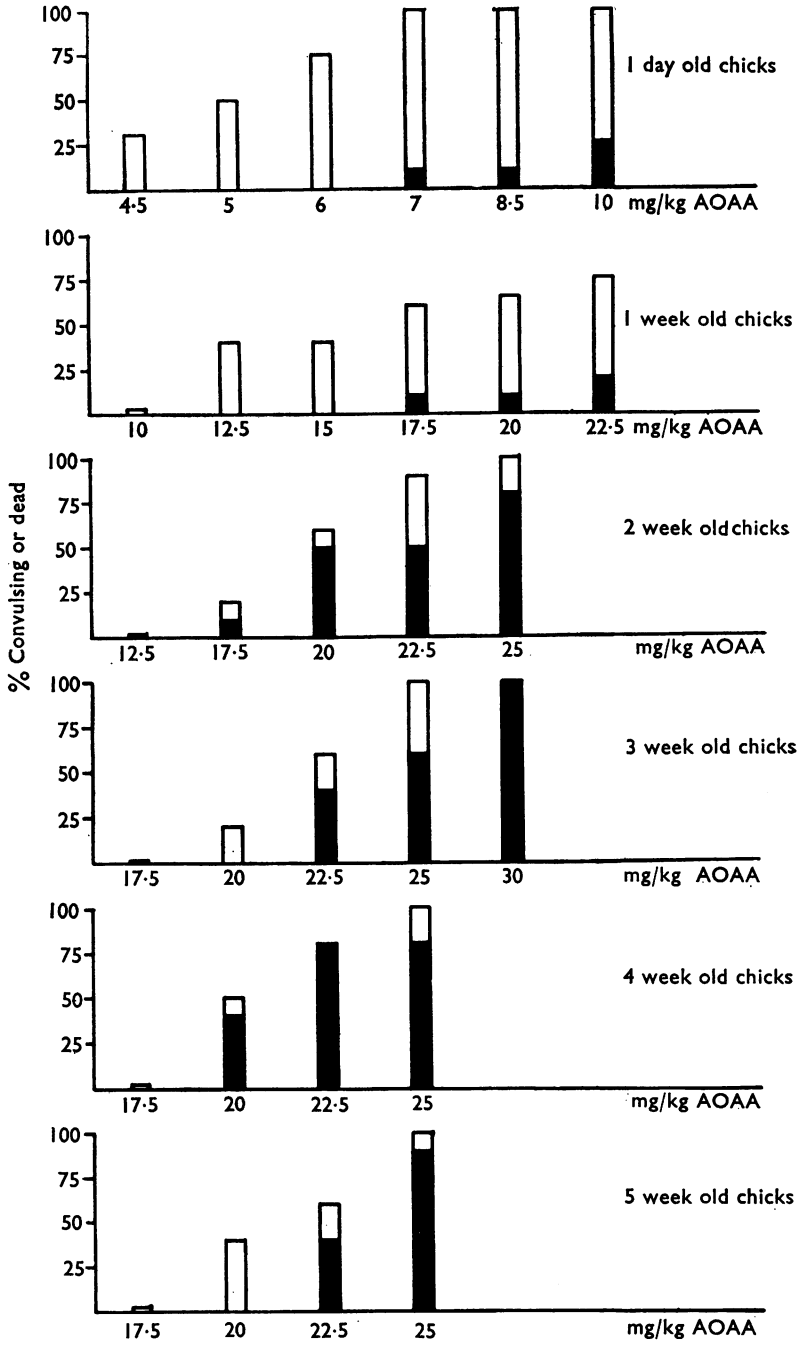

FIG. 1. Histogram showing decrease in convulsant and lethal effects of subcutanous AOAA in chicks aged 1 day–5 weeks. □, % Convulsing; ■, % dead.

non-convulsant doses of AOAA did not produce the marked behavioural depressant effects observed in other species. The convulsant effect of AOAA was most severe in 1-day old chicks and there was a rapid decrease in the convulsant effect with increase in age. For example, whereas 4 mg/kg intravenously produced convulsions in 100% of chicks aged 2 days, 8·5 mg intravenously did not affect chicks aged 3 weeks (Fig. 8); 8·5 mg/kg subcutaneously produced convulsions in 100% of chicks aged 2 days with 10–30% lethality. The only effect observed on intravenous injection of 8·5 mg/kg AOAA in chicks aged 3 weeks was the assumption of catatonic-like postures.

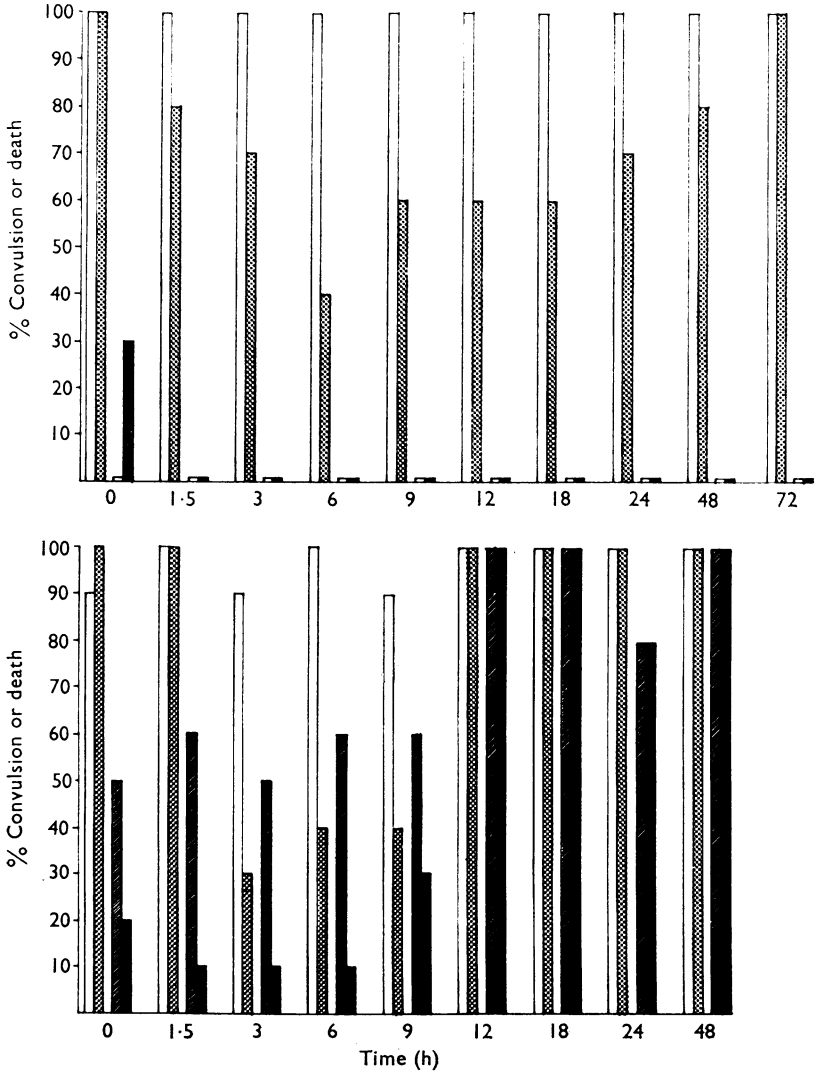

FIG. 2. Histograms showing anticonvulsant effects of AOAA (4 mg/kg, s.c.) against strychnine (1 mg/kg) (upper histogram) and leptazol (90 mg/kg) (lower histogram) administered at various time intervals in 1–3 day old chicks. AOAA exerted most potent anticonvulsant effect 6 h after administration and its anticonvulsant effect on strychnine lasted up to 48 h, but when administered simultaneously with the convulsants it potentiated the lethal effect of strychnine and the convulsant effects of leptazol. The convulsants were injected subcutaneously. Stippled columns, % convulsion AOAA treated; blank columns, % convulsion controls; cross hatched columns, % dead controls; filled columns, % dead AOAA treated.

Figure 1 shows the variation of the convulsant effect of AOAA in 1 day–5 week old chicks on subcutaneous injection. The latency of the convulsant effect of 8·5 mg/kg in chicks aged 2 days was 25–30 min on subcutaneous injection and 5–12 min on intravenous injection. Latency was shorter with larger doses of AOAA. The convulsion after 8·5 mg/kg lasted 1–2 h in young chicks aged 1–2 days, followed by behavioural depression when the chick lay prostrate for about 2 hours. Chicks surviving the convulsion had usually recovered 6 h afterwards. Non-convulsant doses of AOAA potentiated the convulsant effect of strychnine and leptazol when administered simultaneously (Fig. 2). In 1-day-old rats, 15 mg/kg AOAA did not produce any convulsions on subcutaneous injection. This is similar to the observation that strychnine and leptazol do not convulse newly born rats (Pylkko & Woodbury, 1961). AOAA (30 mg/kg) given 8 hourly for 64 h in adult rats does not produce convulsions (Essig, 1968).

Antagonists of convulsant effects of AOAA

Groups of chicks were pretreated with central nervous depressant drugs before administration of convulsant doses of AOAA and the results compared with those of control groups treated with the same dose of AOAA.

Only troxidone (500–900 mg/kg) and small doses of AOAA (Fig. 3) afforded significant protection against AOAA-induced convulsions. The convulsant effect of AOAA was also antagonized if given together with equimolar proportions of a vitamin B_6 derivative (pyridoxine, pyridoxal, pyridoxamine hydrochlorides and

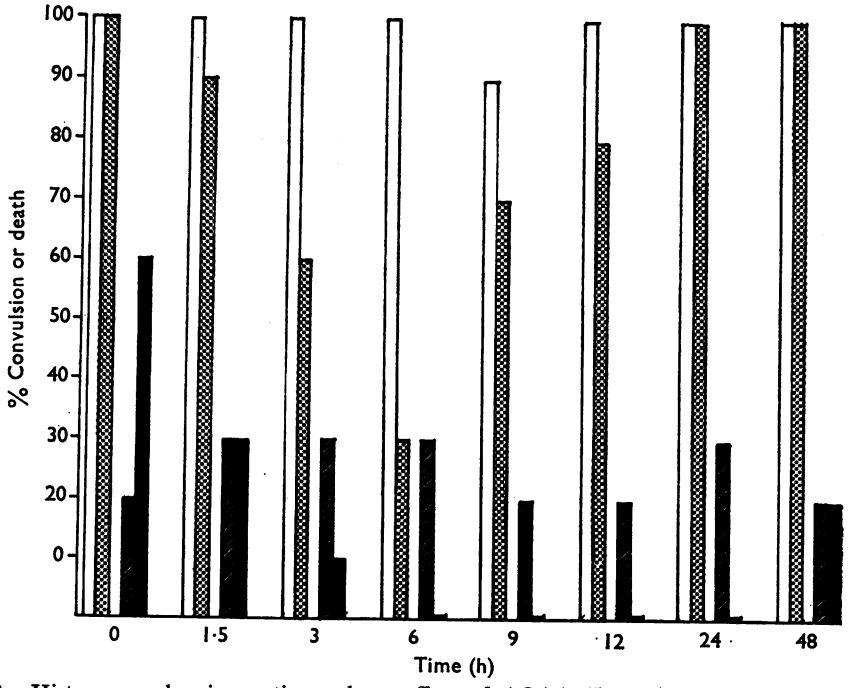

FIG. 3. Histograms showing anticonvulsant effect of AOAA (5 mg/kg) in 1 day old chicks against AOAA (10 mg/kg) administered at various time intervals after AOAA (5 mg/kg). The lower dose of AOAA exerted maximal anticonvulsant effect 6 h after its administration, and its effect lasted up to 24 hours. Both doses of AOAA were injected subcutaneously. Stippled columns, % convulsion AOAA treated; blank columns, convulsion controls; cross hatched columns % dead controls; filled columns, % dead AOAA treated.

pyridoxal 5′ phosphate) and the mixture acidified with 1 drop N/10 HCl (Fig. 4). The following drugs proved ineffective: diphenylhydantoin (40–900 mg/kg), ethosuccimide (500 mg/kg), mephentyoin (200 mg/kg); mephenesin (70 mg/kg), styramate (30 mg/kg), diazepam (10 mg/kg); sodium pentobarbitone (10 mg/kg), chloralose (30 mg/kg); methyldopa (30 mg/kg), acetazolamide (35–150 mg/kg), reserpine (1·3 mg/kg); chlorpromazine (5 mg/kg), trimipramine (10 mg/kg); the hydrochlorides of pyridoxine, pyridoxal, pyridoxamine and pyridoxal 5′ phosphate (up to 100 mg/kg).

Anticonvulsant properties

In most of the studies of the anticonvulsant properties of AOAA, 4 mg/kg was injected subcutaneously, its maximal effect occurring 6 h after administration (Fig. 2). For this reason, when testing the anticonvulsant property of AOAA, it was administered 6 h before the convulsant. AOAA protected chicks against the convulsant effects of strychnine, leptazol, picrotoxin, hydrazine, semicarbazide and against convulsant doses of AOAA itself (Fig. 3). When chicks recovered from AOAA convulsions, the AOAA continued to exert anticonvulsant effects. In experiments with chicks aged 1 day–12 weeks, pretreated with 4 mg/kg AOAA, 6 h before 1 mg/kg strychnine or 90 mg/kg leptazol; maximum anticonvulsant effects were found in 3 week old chicks (Fig. 5). Unlike the rapid decline of the convulsant effect with age (Fig. 1), anticonvulsant effects were maximal in 1–3 week old chicks (Fig. 5). Although the convulsant effect of strychnine increases with age in chicks up to 6 weeks of age (Osuide, 1968), 4 mg/kg AOAA was most potent in antagon-

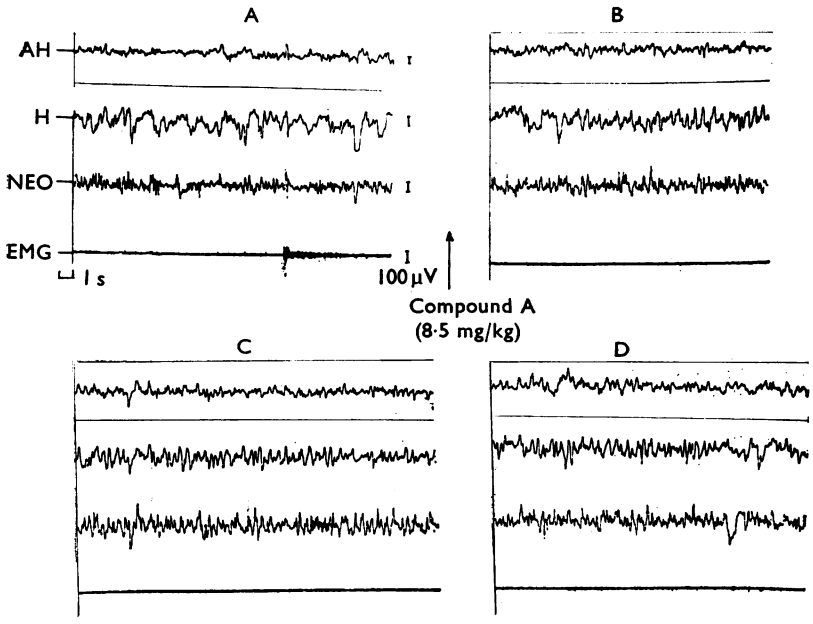

FIG. 4. ECoGs recorded from 2 day old chick while standing quietly with eyes closed before and after injection of AOAA (8·5 mg/kg) mixed with an equimolecular proportion of pyridoxal phosphate subcutaneously, represented by compound A above. Monopolar electrodes recorded responses from left accessory hyperstriatum (AH), left hyperstriatum (H), and right neostriatum (NEO). (EMG) represents the electromyogram. Panel A, control; Panels B, C, and D, 40 min, 2·5 and 6 h after the injection.

izing strychnine-induced seizures in 1–3 weeks old chicks. AOAA (8·5 mg/kg) which produced convulsions in 100% of chicks aged 2 days, did not convulse chicks aged 3 weeks even on intravenous injection (Figs. 1 & 8). This shows that the decrease in the convulsant effect of AOAA with age is not primarily due to blood brain barrier effects.

Crossed extensor reflexes

Chicks aged 2 days were usually used as small doses of AOAA were often ineffective on crossed extensor reflexes of chicks 7 days old. Doses exceeding 8 mg/kg augmented reflex contraction after a latency of 1–20 min, depending on the depth of anaesthesia and dose of AOAA. In some experiments, after initial augmentation of the reflexes, powerful contractions unrelated to electrical stimulation, separated by periods of reflex depression were produced (Fig. 6). AOAA was more potent in augmenting reflex contractions in intact lightly anaesthetized chicks. In deeply anaesthetized chicks high doses of AOAA were required to augment the reflex contractions. When the same dose of AOAA was injected immediately after the contractions had returned to normal following an initial dose, a greatly increased augmentation was observed (Fig. 6). Similar cumulative effects in chicks are produced by tremorine, harmine, picrotoxin and leptazol (Bowman & Osuide, 1967; Osuide, 1968). If, immediately after recovery from the effect of the first dose, the

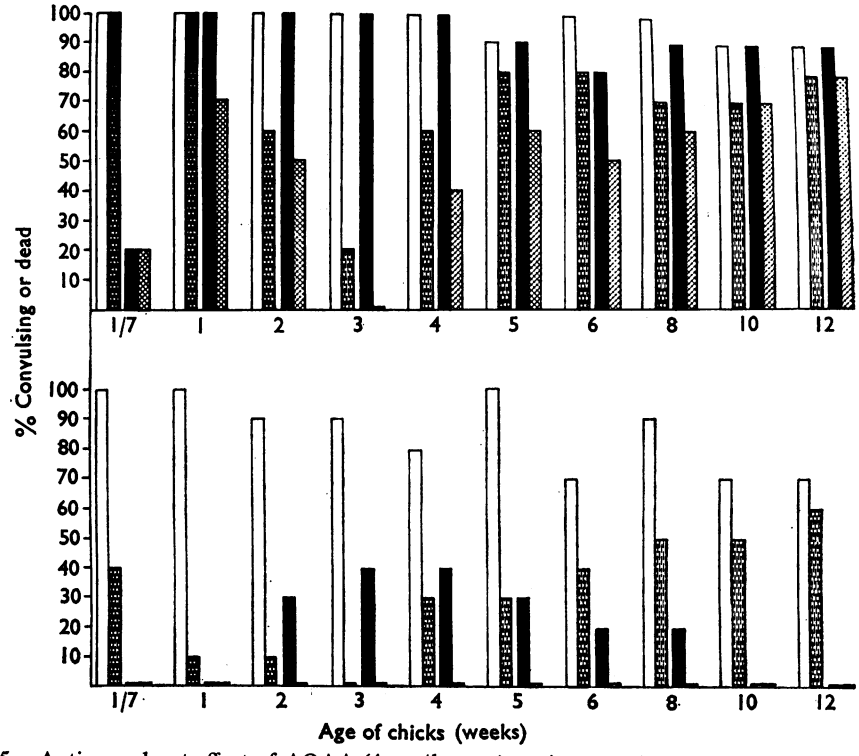

FIG. 5. Anticonvulsant effect of AOAA (4 mg/kg, s.c.) against strychnine (1 mg/kg) in 1 day to 12 week old chicks (lower histogram) and leptazol (90 mg/kg) in 1 day old to 12 week old chicks (upper histogram). At the doses used AOAA exerted maximum anticonvulsant effect in 3 week old chicks. Blank columns, % convulsing controls; striped columns, % convulsing AOAA treated; filled columns, % dead controls; cross hatched columns, % dead AOAA treated.

same dose of AOAA was injected mixed with an equimolecular proportion of pyridoxal phosphate, no potentiation was observed. This is similar to the observation in conscious chicks, that convulsant doses of AOAA, mixed with an equimolecular proportion of a vitamin B_6 derivative produced no convulsions. When an intact chick was spinalized during augmentation of reflex contractions, potentiation was reduced. If the same dose of AOAA was quickly injected again there was a reduced potentiation, or no effect on the CER of the spinal chick, when small doses of AOAA were used. In some experiments no AOAA was administered before spinalization. In these spinal chicks, as in intact chicks, AOAA did not produce a depression of the reflex contraction. This is in contrast to spinal cats (Bell & Anderson, 1968) in which AOAA depressed mono and polysynaptic reflexes. Only a potentiation was observed when doses above 20 mg/kg were used, the magnitude of the effect being much less than in intact chicks. The potentiation of crossed extensor reflexes in spinalized chicks shows that the stimulant effects of AOAA are exerted also on the spinal cord of chicks. Only a few drugs antagonized the potentiation of the CER produced in intact and spinal chicks. Although complexing convulsant doses of AOAA with their equimolecular proportions of vitamin B_6 abolished the effect, even 10 times the equimolecular proportions of the doses of AOAA producing a potentiation of CER produced only a transient reduction of CER when injected intravenously during a potentiation of CER produced by convulsant doses of AOAA. This suggests that vitamin B_6 derivatives do not affect the convulsant processes produced by AOAA, but only antagonize the effects of AOAA by chemical combination. The drugs found to antagonize the potentiation of CER produced by AOAA were chloralose (30 mg/kg), sodium pentobarbitone (15 mg/kg) and mephenesin (70 mg/kg). These drugs did not antagonize the AOAA-induced convulsions in conscious chicks. Their effects in antagonizing AOAA effects on the

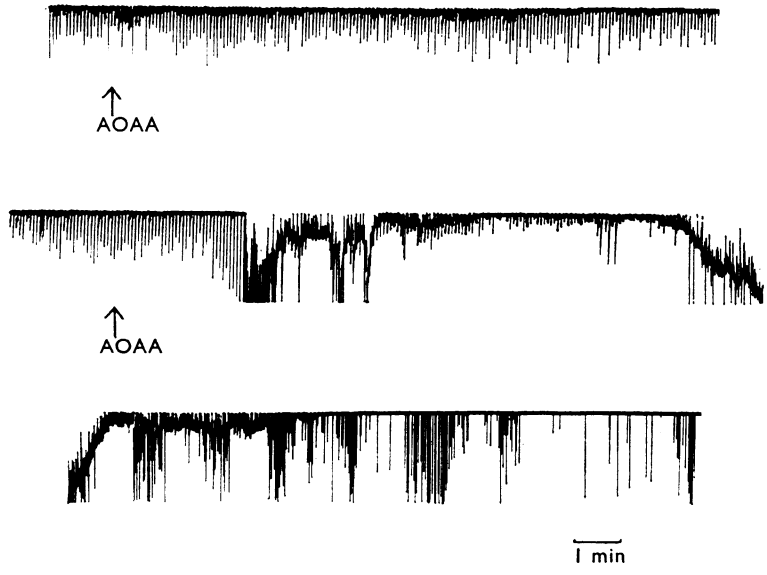

FIG. 6. Crossed extensor reflexes in an intact 3 days old chick anaesthetized with chloralose (40 mg/kg). In the top panel AOAA (8·5 mg/kg) was injected intravenously with no observed effect. In the middle panel the same dose of AOAA was injected and a cumulative effect was observed. Interval between top trace and middle trace was 15 minutes. The lowest trace was a direct continuation of the middle trace.

reflex contractions may be due to non-specific central nervous systems depression produced on intravenous injection in the anaesthetized chick.

Neuromuscular effects

AOAA (4–30 mg/kg) lacked effect on twitches of the chicks gastrocnemius elicited by stimulation of the sciatic nerve.

Effect on ECoG

AOAA (4 mg/kg) injected subcutaneously in 2 day chicks produced high voltage synchronous waves with sharp waves especially over the hyperstriatum (Fig. 7). These waves first appeared 30 min after AOAA injection and were most prominent 3 h after injection. ECoG activation, on auditory stimulation produced by clapping, was diminished, compared to the control (Fig. 7B & D). Similar block of arousal response produced by non-convulsant doses of AOAA has been observed in cats (Da Vanzo, Matthews & Stafford, 1964). In chicks aged 3 weeks, AOAA (4 mg/kg) produced similar but less pronounced effects on the ECoG.

In chicks aged 2 days, AOAA (8·5 mg/kg, s.c.) produced synchrony within 10 min (Fig. 9) with sharp waves gradually developing. After 25–30 min, clonic convulsions occurred accompanied by sharp waves and spikes in all leads, most prominent over the neostriatum. These spikes were at a frequency of 2–3 Hz and amplitude 150–550 μV. The convulsions continued for about 2 h interposed with periods when the chicks lay prostrate. After this, and until the chicks gained righting

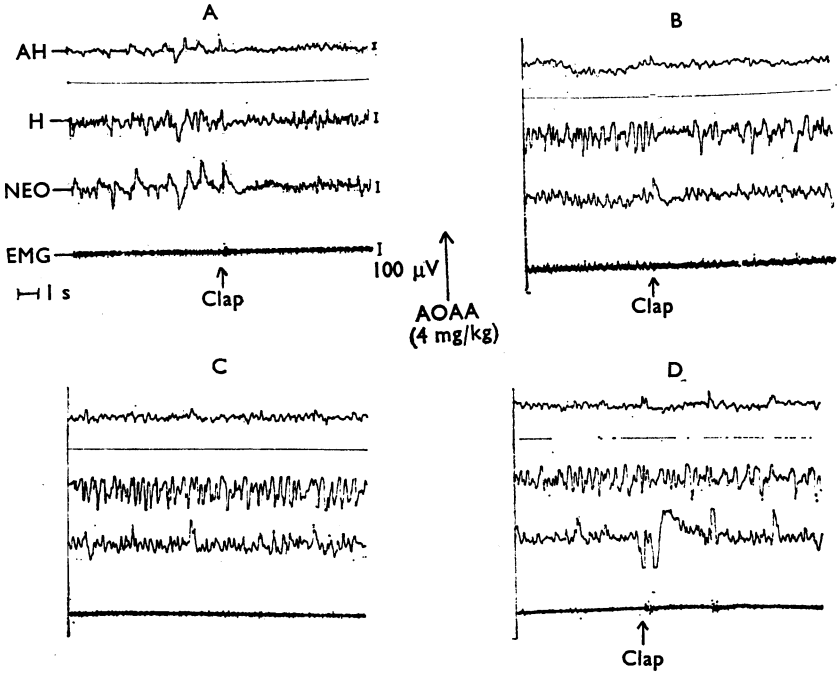

FIG. 7. ECoGs recorded from a 3 day old chick while standing quietly with eyes closed before and after injection of AOAA (4 mg/kg, s.c.). Monopolar electrodes recorded responses from left accessory hyperstriatum (AH), left hyperstriatum (H) and right neostriatum (NEO). (EMG) represents the electromyogram. Panel A, controls ; Panels B, C and D, 40 min, 2·5 h and 6 h respectively after injection.

reflexes the ECoG contained large voltage synchronous sharp waves particularly prominent in the hyperstriatum (Fig. 9). In 2 day old chicks, AOAA (8·5 mg/kg)

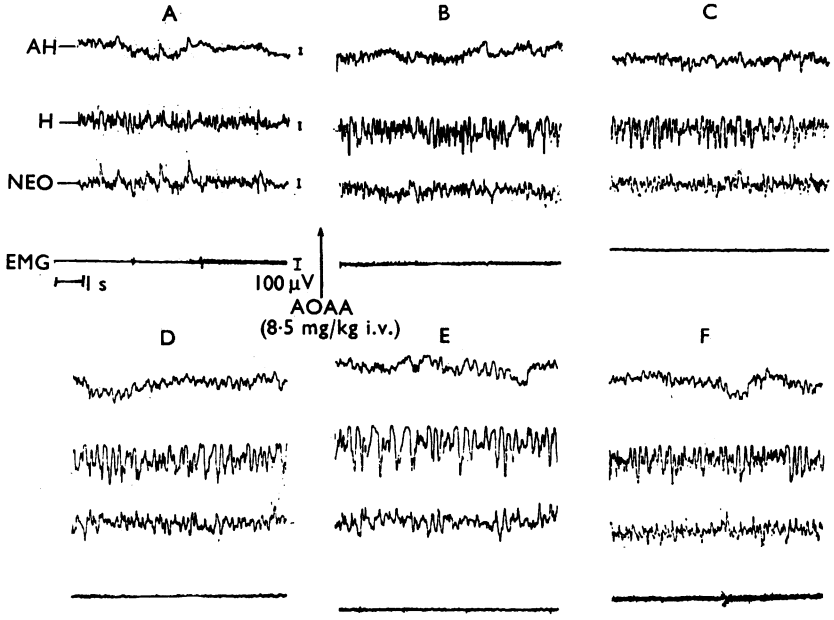

FIG. 8. ECoG recorded from 3 week old chick while crouched quiet with eyes closed before and after injection of AOAA (8·5 mg/kg, i.v.). Monopolar electrodes recorded from left accessory hyperstriatum (AH), right hyperstriatum (H), and right neostriatum (NEO). (EMG) represents the electromyogram. Panel A, control; chick suddenly alerted in second half of trace with increase EMG activity. Panels B–F, 10 min, 1 h, 3 h and 5 h respectively after AOAA. In panel F chick was standing with eyes closed. Note AOAA did not produce convulsions. It produced catatonic like behaviour accompanied by high amplitude slow waves.

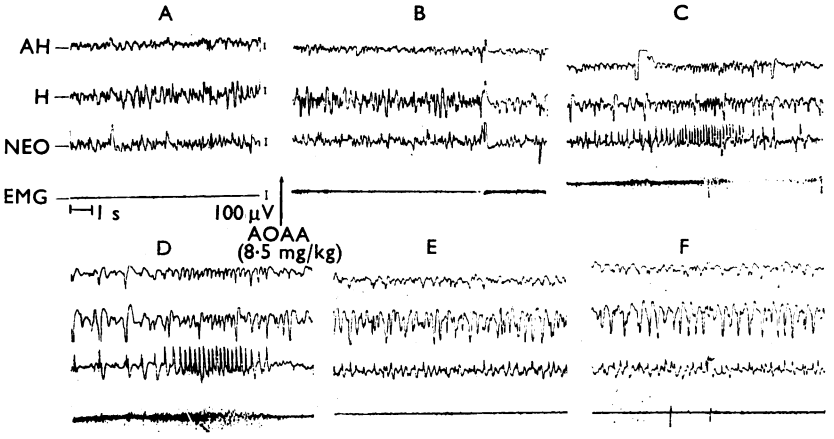

FIG. 9. ECoG recorded from 2 day old chick before and after subcutaneous injection of AOAA (8·5 mg/kg). Monopolar electrodes recorded from right accessory hyperstriatum (AH), hyperstriatum (H), right neostriatum (NEO). (EMG) represents the electromyogram. Panel A, control, chick crouched, quiet with eyes closed. Panel B, 20 min after injection chick standing with eyes open showing 'starting' behaviour at the end of the trace. In panels C (27 min after injection) and D (1 h after injection) the chick developed tonic seizure accompanied by spikes and sharp waves in all the leads. In panel E, 3 h after injection, chick lay prostrate and quiet with eyes closed. In panel F, the chick was standing, quietly with eyes closed; ECoG still shows synchronous epileptoid slow waves.

mixed with an equimolecular equivalent of pyridoxal phosphate, and injected subcutaneously or intravenously, did not elicit seizure activity even after 6 hours. The ECoG activity induced, consisted of decreased frequency and increased amplitude in all leads, with some sharp waves over the neostriatum (Fig. 4). In 3 weeks chicks AOAA (8·5 mg/kg, i.v.) did not produce ECoG spike potentials. Within 10 min of intravenous injection, the ECoG had synchrony with sharp waves most prominent over the hyperstriatum (Fig. 8). As the time progressed the frequency of the synchronous waves was reduced and the amplitude increased ; the maximal effect on the ECoG was obtained 3 h after injection. The ECoG effects of AOAA (8·5 mg/kg) injected intravenously in chicks aged 3 weeks resembled the postseizure depression period of ECoG of chicks aged 2 days and given convulsant doses of AOAA (Fig. 9).

Strychnine (1·4 mg/kg, s.c.) produced tonic convulsions in 6–7 min in chicks aged 2 days or 3 weeks ; seizures were more severe in chicks aged 3 weeks. The tonic seizures were accompanied by high voltage high frequency spikes in all leads, but

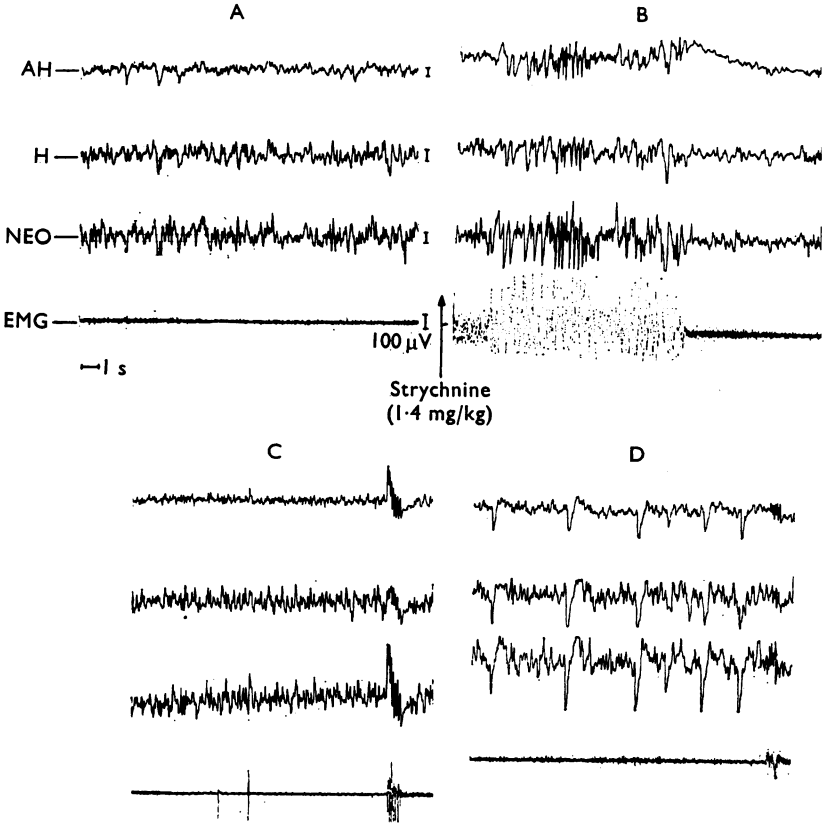

FIG. 10. ECoGs recorded from 2 day old chick before and after subcutaneous injection of strychnine (1·4 mg/kg). Monopolar electrodes recorded from left accessory hyperstriatum (AH), right hyperstriatum (H) and right neostriatum (NEO). (EMG) represents the electromyogram. Panel A, control, chick crouched with eyes closed. Panels B, C and D, 6 min, 10 min, and 30 min respectively after injection. Six minutes after injection, the chick showed tonic convulsion accompanied by spiking in all the leads. Subsequently the chick lay prostrate with low voltage high frequency waves (C) alternating with slow waves with high voltage (D).

most pronounced over the accessory hyperstriatum and hyperstriatum (Fig. 10). The strychnine seizures diminished until, about 30 min after injection, the chick began to recover. In contrast, strychnine (1·4 mg/kg) given to chicks aged 2 days or 3 weeks, 6 h after injection of 4 or 8·5 mg/kg AOAA, did not elicit tonic seizures, nor prominent spikes in the ECoG (Fig. 11). The only effect of strychnine in chicks aged 2 days given AOAA (8·5 mg/kg) 6 h previously, was intense tremor.

Discussion

In chicks, as in other species (Wallach, 1961 ; Da Vanzo, Greig & Cronin, 1961 ; Da Vanzo, Matthews, Young & Wingerson, 1964 ; Roa, Tews & Stone, 1964 ; Kuriyama, Roberts & Rubinstein, 1966), AOAA had convulsant and at lower doses anticonvulsant properties. AOAA also controls some seizures in man (Carter, 1964). The convulsant effect was most marked in newly hatched chicks (3–6 h after hatching) and fell off rapidly with increase in age. This was unlikely to be related to blood brain barrier maturation for two reasons: (1) doses less than half the convulsant doses in 1-day-old chicks, exert their maximum anticonvulsant effect against strychnine in 3 week old chicks. At the latter age strychnine is a more potent convulsant (Osuide, 1968). (2) AOAA exerts central nervous system effects in adult dogs, cats, mice, rats and rabbits in which the blood brain barrier would be mature (Wallach, 1961 ; Van Gelder, 1965, 1966 ; Da Vanzo *et al.*, 1964).

AOAA is a potent inhibitor of gamma-aminobutyric acid ketoglutaric acid transaminase (GABAT) and consequently increases brain GABA concentrations

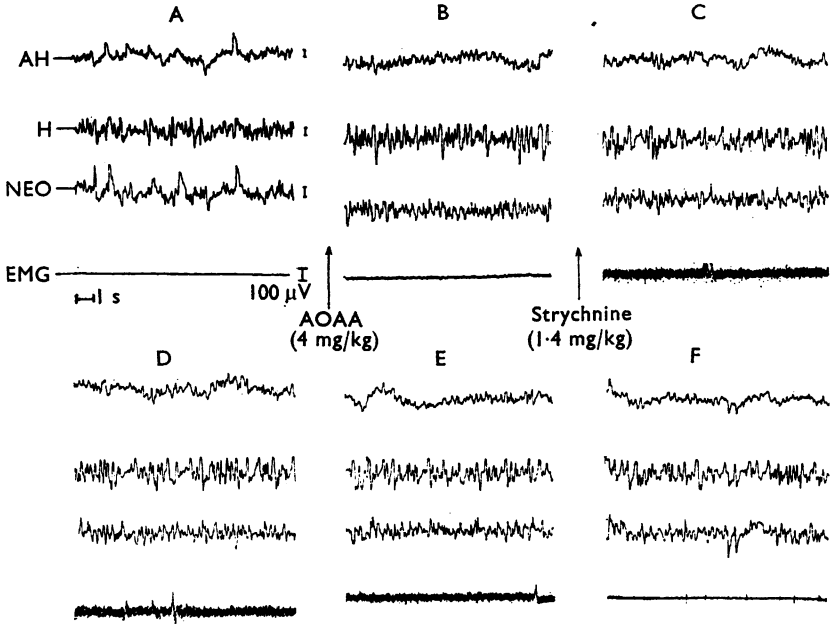

FIG. 11. ECoGs recorded from 3 week old chick to show the antagonism of strychnine effects by AOAA (4 mg/kg s.c.) injected subcutaneously. Monopolar electrodes recorded from left accessory hyperstriatum (AH), left hyperstriatum (H) and right neostriatum (NEO). (EMG) represents electromyogram. Panels A and B, controls with chick crouched, quiet and with eyes closed before injection (panel A) and 6 h after AOAA injection (panel B). Panels C, D, E and F, 6·5, 10, 15 and 30 min respectively after strychnine injection. In panels C, D and E the chick was standing with eyes open with occasional 'starting' behaviour. In panel F the chick was crouched with eyes closed, with signs of recovery from the strychnine effect.

(Wallach, 1961). The increase in brain GABA levels is at least partly responsible for the anticonvulsant property (Kuriyama *et al.*, 1966). Sisken *et al.* (1961) have shown that inhibition of GABAT does not increase brain GABA concentrations in young chicks 3–6 h after hatching. Consequently, injection of AOAA in newly hatched chicks would not increase brain GABA concentrations with consequent lack of antagonism to AOAA. As the chick ages, the increased activity of GABAT would increase brain GABA concentrations and consequently give an impression of decrease in convulsant potency of AOAA. This may not be the complete explanation for the decrease in convulsant effect of AOAA with age. Although its anticonvulsant effect is noticeable 20–30 min after injection, the maximal effect occurs after 6 hours. By this time, the surviving chicks have fully recovered behaviourally from the effects of a convulsant dose. Tapia, Passantes, Mora, Ortega & Massieu (1967) have shown that some drugs which are not anticonvulsant, elevate brain GABA concentrations. Also brain GABA is compartmented and its concentration may not reflect the amounts at physiologically active sites (Kuriyama *et al.*, 1966). It may be there is a change of central nervous system excitability unrelated to GABA metabolism to account for the decrease in convulsant and increase in anticonvulsant action of AOAA as the chick ages.

The convulsant action is probably due to a direct action of AOAA increasing brain excitability. This possibility is supported by the short latency for convulsions with large doses and the almost immediate potentiation of crossed extensor reflexes on intravenous injection of very large doses. Also the central nervous system excitability produced by non-convulsant doses of AOAA potentiates the convulsant effects of strychnine and leptazol when they are administered simultaneously. The anticonvulsant effect may be due to an effect on a brain enzyme system or due to accumulation of some brain transmitter or modulator, since it develops gradually, reaching a maximum in 6 hours. AOAA inhibits pyridoxal dependent enzyme systems such as GABAT (Wallach, 1961) glutamic and 5-HTP decarboxylases (Greig, 1962, personal communication quoted by Da Vanzo, Matthews, Young & Wingerson, 1964), glutamic pyruvic transaminase (Hopper & Segal, 1962) and pyridoxal kinase (Da Vanzo, Kang, Ruchkart & Dougherty, 1966). It also increases glutamine and decreases succinic semialdehyde concentrations in brain (Wallach, 1959 ; Wallach, 1968 ; Roa, Tews & Stone, 1964). Baxter & Roberts (1961) have, however, shown that AOAA does not inhibit glutamic decarboxylase activity *in vivo*.

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