Influence of Dopamine, Levodopa and Apomorphine on Maximal Electroconvulsive Seizure in the Domestic Fowl (Gallus domesticus) *

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Abstract—The influence of dopamine, levodopa and apomorphine on maximal electroconvulsive seizure was studied in young chicks, adult cocks and rats. The susceptibility of chicks to maximal electroshock seizure increased with age between 1 to 7 days. Low to moderate doses of dopamine (12.5–150 mg/kg, i.p.), levodopa (6.25–25 mg/kg, s.c.) and apomorphine (0.25–2.0 mg/kg, s.c.) significantly (P<0.005) protected chicks against electroshock seizure, while high doses (200–400 mg/kg, i.p. of dopamine, 50–200 mg/kg, s.c. of levodopa and 2.5–5 mg/kg, s.c. of apomorphine) enhanced electroshock seizure in 1 to 7 day old chicks. However, when 14 day old chicks were used, these dopaminergic agonists protected the chicks against maximal electroshock seizure. Noradrenaline (1–40 mg/kg, i.p.) had no significant effect on electroshock seizure in chicks. Both pimozide (4 mg/kg, i.p.) and haloperidol (0.4 mg/kg, i.p.) antagonized the effects of levodopa (12.5 and 50.0 mg/kg, i.p.) and apomorphine (0.5–5 mg/kg, s.c.) on maximal electroshock seizure. The seizure susceptibility of both adult rats and fowls to electroshock was not altered by dopamine (12.5–400 mg/kg, i.p.). Central dopamine neurotransmission might be involved in the biphasic dose-dependent effects of dopamine, levodopa and apomorphine on maximal electroshock seizure in young chicks.

Brain monoamines have been implicated in seizure mechanisms in different mammalian species. For example, it was observed that an increase of 5-hydroxytryptamine in the brain did not significantly influence the electroshock threshold in the rabbit, while there was a profound increase in brain dopamine levels (1). On the other hand, other workers have accumulated data to indicate that both noradrenaline and dopamine exerted central effects which limited the spread of electroshock seizure discharge and consequently modulated the intensity of electroshock seizure (2). Similarly, it has been reported that 6-hydroxy-

dopamine injected intraperitoneally into 1–3 day old chicks and rats produced a significant increase in electroshock activity which was still evident 3 months later (3). There are other conflicting reports by many investigators indicating that in the rat, noradrenaline, dopamine and/or 5-hydroxytryptamine might subserve a modulating function in electroshock seizure (4–6). These conflicting reports suggest that it is not yet clear which of the amines are most directly involved in seizure mechanisms. The issue is further complicated by the fact that these workers could not study the direct effects of these amines in mammals since the monoamines do not cross the mammalian blood-brain barrier. However, the young chick (1–28 day old) lacks a functional blood-brain barrier to catecholamines (7). Thus, it has been

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possible to study the direct effects of dopamine, levodopa and apomorphine on maximal electroshock seizure using young chicks in this project.

**Materials and Methods**

One to fourteen day old white cockerels (Ranger strain), weighing between 35 and 55 g, were used. For any experiment, a difference of ±5 g in weight of chicks was allowed. For the rat experiments, adult male albino rats (200±20 g; inbred in our Animal House) were used. Both young and adult cocks (12 week old) were locally obtained from Arewa Agricultural Enterprises, Zaria.

The drugs used were apomorphine hydrochloride (British Drug Houses Ltd.), dopamine hydrochloride (Sigma Chemical Company), levodopa (Sigma Chemical Company), haloperidol (serenace, G.D. Searle & Company), pimozide (Janssen Pharmaceutical), noradrenaline hydrochloride (Sigma Chemical Company) and FLA-63 (Labkemi). Sodium metabisulphite (0.1% w/v) was used as an antioxidant for apomorphine, dopamine and noradrenaline, and recently boiled and cooled distilled water was used to dissolve these drugs. Levodopa and FLA-63 were dissolved in 0.05 M hydrochloric acid solution and diluted appropriately with saline (0.9% w/v NaCl). Haloperidol from the ampoule was diluted with saline, and pimozide was suspended in 3% Tween 80. Dopamine, haloperidol, noradrenaline and pimozide were injected intraperitoneally, while apomorphine, FLA-63 and levodopa were administered subcutaneously. From preliminary studies in our laboratory, these routes of drug administration appeared to produce consistent effects. Fresh drug solutions were prepared for each day's experiments. The doses of dopamine, apomorphine and noradrenaline administered were in the form of their salts mentioned above. The duration of drug pretreatment time prior to electroshock delivery was 10 min for apomorphine, dopamine and noradrenaline; 2 hr for haloperidol, pargyline and pimozide; 30 min for levodopa and 4 hr for FLA-63.

The shock duration, frequency and pulse width were respectively maintained at 0.8 sec, 100 pulses/sec and 0.4 msec for young chicks and 1.5 sec, 150 pulses/sec and 0.8 msec for 12 week old cocks. For the rat experiments, the shock duration, frequency and pulse width were maintained at 0.2 sec, 150 pulses/sec and 0.4 msec, respectively. The current chosen for testing the anti-convulsant effect produced 70–90% tonic seizure in the controls. On the other hand, when testing for the seizure-enhancing effect, the chosen current produced 10–30% tonic seizure in the controls. Generally, the current used varied between 40 and 60 mA for chicks and between 70 and 100 mA for rats and adult cocks. An Ugo Basile constant current electroshock treatment machine (model 7800) was used. The electrodes were applied to the upper eyelids of the chicks, while in the case of rats, the electrodes were applied to the ears. Maximal electroshock seizure was recorded when tonic extension of the forelimbs was produced. Each experiment was repeated four times using five animals per experiment. The results were analyzed using the Chi-squared test with Yates's correction for continuity.

**Results**

Influence of age of chicks on electroshock seizures: Table 1 shows that the incidence of

| Age of chicks (days) | Current level (mA) | No. convulsed/No. used | % Incidence of tonic seizure |
|----------------------|--------------------|------------------------|-----------------------------|
| 1                    | 40                 | 0/40                   | 0                           |
| 2                    | 40                 | 8/40                   | 20                          |
| 5                    | 40                 | 24/40                  | 60                          |
| 7                    | 40                 | 32/40                  | 80                          |
| 14                   | 40                 | 32/40                  | 80                          |
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Tonic seizure resulting from the delivery of electroshock to chicks depended on the age of the chicks. All the electroshock parameters were maintained at the same level for all the chicks of various ages. All the experiments were carried out during the same period of the day so as to overcome the influence of circadian changes on the susceptibility of the young chicks to electroshock. The results shown in Table 1 indicate that the incidence of tonic seizure was 0% at the age of 1 day, 20% at the age of 2 days and attained the peak effect of 80% at the age of 7 days. The results also show that there was no further increase in the incidence of electroshock seizure when 14 day old chicks were used.

**Influence of dopamine, levodopa and apomorphine on electroshock seizure using 5 and 14 day old chicks:** According to the results shown in Table 2, the protective effect of dopamine was only dose-dependent between 12.5 and 100 mg/kg. Although 200–400 mg/kg of dopamine protected 14 day old chicks against electroshock seizure, this effect was neither significant nor dose-related. Similarly, the protective effect of levodopa was statistically significant (P<0.05) between the doses of 6.25 and 25 mg/kg, while higher doses of levodopa (50–100 mg/kg) protected 14 day old chicks non-significantly. However, only 5 mg/kg of apomorphine significantly protected the 14 day old chicks against electroshock seizure. The lower doses of apomorphine (i.e., 0.25–2.5 mg/kg) protected the chicks non-significantly. None of the doses of noradrenaline (5–40 mg/kg) used in this project exhibited any significant change on the susceptibility of 14 day old chicks to electroshock. The electroshock parameters were kept constant throughout all the experiments.

Relatively low doses of dopamine (100 mg/kg), levodopa (12.5 mg/kg) and apomorphine (0.5 mg/kg) profoundly protected both 5 and 14 day old chicks against electroshock seizure. On the other hand,

Table 2. Influence of dopamine, levodopa (DOPA), apomorphine (APO) and noradrenaline (NA) on seizure susceptibility of 14 day old chicks

| Dopamine | DOPA | APO | NA | No. convulsed/No. used | % Incidence of tonic seizure |
|----------|------|-----|----|-----------------------|----------------------------|
| —        | —    | —   | —  | 16/20                 | 80                         |
| 12.5     | —    | —   | —  | 16/20                 | 80                         |
| 25.0     | —    | —   | —  | 4/20**                | 20                         |
| 50.0     | —    | —   | —  | 4/20**                | 20                         |
| 100.0    | —    | —   | —  | 2/20**                | 10                         |
| 200.0    | —    | —   | —  | 12/20                 | 60                         |
| 400.0    | —    | —   | —  | 14/20                 | 70                         |
| —        | 6.25 | —   | —  | 8/20+                 | 40                         |
| —        | 12.5 | —   | —  | 6/20*                 | 30                         |
| —        | 25   | —   | —  | 8/20+                 | 40                         |
| —        | 50   | —   | —  | 14/20                 | 70                         |
| —        | 100  | —   | —  | 15/20                 | 75                         |
| —        | 200  | —   | —  | 16/20                 | 80                         |
| —        | —    | 0.25| —  | 14/20                 | 70                         |
| —        | —    | 0.5 | —  | 12/20                 | 60                         |
| —        | —    | 1.25| —  | 12/20                 | 60                         |
| —        | —    | 2.5 | —  | 10/20                 | 50                         |
| —        | —    | 5.0 | —  | 6/20*                 | 30                         |
| —        | —    | —   | 5  | 18/20                 | 90                         |
| —        | —    | —   | 10 | 16/20                 | 80                         |
| —        | —    | —   | 20 | 16/20                 | 80                         |
| —        | —    | —   | 40 | 16/20                 | 80                         |

*+, **Significantly different from the saline controls (same age of chicks and electroshock parameters) at P<0.05, P<0.005 and P<0.001, respectively.
higher doses of dopamine (400 mg/kg), levodopa (50 mg/kg) and apomorphine (5 mg/kg) failed to protect 5 day old chicks against electroshock seizure, but rather significantly enhanced the susceptibility of 5 day old chicks to electroshock seizure. Interestingly, however, these same high doses of dopamine, levodopa and apomorphine (i.e., 400, 50 and 5 mg/kg, respectively) protected 14 day old chicks against electroshock seizure. It is therefore apparent from the present data that the effects of the dopaminceptor agonists used in this study are age-dependent. These results are presented in Table 3.

| Age of chicks (days) | Doses (mg/kg) | % Protection against electroshock seizure |
|----------------------|---------------|-----------------------------------------|
|                      | DA | DOPA | APO |                               |
| 5                    | 100 |   |   | +55.6* |
| 14                   | 100 |   |   | +87.5** |
| 5                    | 400 | 12.5 |   | -88.9** |
| 14                   | 400 | 12.5 |   | +13.0 |
| 5                    |   | 50  |   | +44.0* |
| 14                   |   | 50  |   | +63.0* |
| 5                    |   | 50  | 0.5 | -60.0* |
| 14                   |   | 50  | 0.5 | +13.0 |
| 5                    |   | 50  | 5.0 | +60.0* |
| 14                   |   | 50  | 5.0 | +25.0 |
| 5                    |   | 50  | 5.0 | -27.1 |
| 14                   |   | 50  | 5.0 | +63.0* |

* and ** are significantly different from the saline controls (same age of chicks and electroshock parameters) at P<0.005 and P<0.001, respectively, Chi-squared test with Yate's correction for continuity, n=20 per dose.

Table 4. Effect of dopamine on electroshock seizure using adult male albino rats and fowls (Warren strain)

| Animal species | Dopamine (mg/kg, i.p.) | No. convulsed/No. used | % Incidence of tonic seizure |
|----------------|------------------------|------------------------|----------------------------|
| Adult          | 0                      | 24/30                  | 80                         |
| Albino rats    | 12.5                   | 16/20                  | 80                         |
|                | 25.0                   | 18/20                  | 90                         |
|                | 50.0                   | 14/20                  | 70                         |
|                | 100.0                  | 16/20                  | 80                         |
|                | 200.0                  | 17/20                  | 85                         |
| Adult cocks (Warren strain) | 0                      | 2/10                   | 20                         |
|                | 100.0                  | 2/10                   | 20                         |
|                | 400.0                  | 2/10                   | 20                         |

Influence of dopamine on seizure susceptibility of adult cocks and rats: Dopamine (12.5–200 mg/kg, i.p.) did not alter the seizure susceptibility of rats to electroshock in any significant manner (Table 4). Similarly, 100–200 mg/kg i.p. of dopamine did not change the susceptibility of adult cocks to electroshock seizure (Table 4).

Influence of FLA-63 on the effect of dopamine on seizure susceptibility using 5 day old chicks: FLA-63 (12.5 mg/kg, s.c.) protected chicks against electroshock seizure. The level of protection of the chicks by FLA-63 was 21%. Pretreatment of the chicks with FLA-63 prior to administration of dopamine significantly enhanced the susceptibility of 5 day old chicks to electroshock seizure.
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Dopamine (50 mg/kg) increased the protection of the chicks against electroshock seizure from 57% to 85%. On the other hand, the protective effect of 100 mg/kg of dopamine in FLA-63-pretreated chicks was decreased from 64.3% to 41.7%. However, the pretreatment of chicks with FLA-63 decreased the seizure-enhancing effect of 400 mg/kg of dopamine from 37.0% to 18.2%. These results are shown in Fig. 1.

Influence of haloperidol and pimozide on the effects of dopamine, levodopa and apomorphine on seizure susceptibility using 5 day old chicks: Both haloperidol (0.4 mg/kg, i.p.) and pimozide (4 mg/kg, i.p.) effectively antagonized the effects of dopamine (100 and 400 mg/kg), levodopa (12.5 and 50 mg/kg) and apomorphine (0.5 and 5 mg/kg) on seizure susceptibility (Figs. 2–4).

Fig. 1. Influence of FLA-63 on the effects of dopamine on maximal electroshock seizure using 5 day old chicks. The electroshock parameters were kept constant for both FLA-63-pretreated chicks and the untreated controls. * = P<0.005, Chi-squared test, n=20 per histogram, vertical bars represent the S.E.M. of 4 experiments.

Fig. 2. Influence of pimozide on the effects of dopamine on maximal electroshock seizure using 5 day old chicks. The electroshock parameters were the same for both the controls and those pretreated with pimozide. * = P<0.005, Chi-squared test, n=20 per histogram, vertical bars represent the S.E.M. of 4 experiments.

Fig. 3. Influence of haloperidol on the effects of levodopa on maximal electroshock seizure using 5 day old chicks. The electroshock parameters were the same for both the controls and those pretreated with haloperidol. * = P<0.005, Chi-squared test, n=20 per histogram, vertical bars represent the S.E.M. of 4 experiments.
Both the control and test animals were subjected to the same seizure parameters.

**Discussion**

Since the tonic pattern is a more intense seizure than the clonic pattern (8), an increase in the incidence of tonus was regarded as an enhancement of seizure activity and vice versa. The increased susceptibility of chicks to electroshock seizure with age might be related to neuronal maturational factors. Since the young chicks are neuronally immature (7) and neuronal mechanisms have been implicated in electroshock seizure (3, 8-10), it is proposed that the development of the neurones might contribute to the increased susceptibility of chicks to electroshock with age.

Our results suggest that dopamine is an important modulator of electroshock seizure in the chick, while noradrenaline had no significant effect on seizure susceptibility of chicks within the dose range used in this study. Our data is compatible with other workers who proposed that activation of dopamine receptors can lead to a reduction in myoclonic responses to photic stimulation in papio (9). Such a compatibility indicates that the modulating role of dopamine in both photogenic and electroshock seizures may be quite similar. The results with rats and adult cocks, using an intraperitoneally-injected dopamine suggest that the effect of dopamine on electroshock seizure was mediated centrally. Our results also agree with those of other workers who explained the anticonvulsant effect of levodopa in the rat on the basis of levodopa-induced enhancement on central dopamine mechanisms (10).

It is also indicated from our data that the effects of dopaminergic agents on electroshock seizure in 14 day old chicks and some mammals might be quite similar. Such an observation is in conformity with the idea of Spooner (11) that the chick responds to neuropharmacological agents in a manner similar to most mammals.

FLA-63 inhibits dopamine-beta-hydroxylase enzyme thereby elevating endogenous levels of brain dopamine while noradrenaline levels are drastically reduced (12). The anticonvulsant effect of FLA-63 after 4 hr pretreatment might be attributed to elevated brain dopamine. It is possible, however, that the anticonvulsant effect of FLA-63 might be partly related to the decrease of noradrenaline in noradrenergic neurones. This possibility is supported by the slight seizure-enhancing effect of injected noradrenaline in this study. When FLA-63-pretreated chicks were used, high doses of dopamine still enhanced seizure activity. These results suggest that the biphasic effects of dopamine on electroshock seizure might not involve noradrenaline.
formed from the injected dopamine. It is therefore possible that dopamine acted on two functional types of dopamine receptors (13) mediating seizure-protecting and seizure-enhancing effects in a dose-related fashion. It can also be proposed that the effects observed with high doses of dopamine might be partly due to the direct stimulant effect of dopamine on noradrenergic receptors. Such a proposal is compatible with the seizure-enhancing effect of nor-adrenaline observed in this project.

The effects of dopamine, levodopa and apomorphine on electroshock seizure was significantly influenced by the age of the chicks. Thus, using 1–7 day old chicks, these dopaminoceptor agents produced a dose-dependent biphasic effect on electroshock seizure. On the other hand, low to high doses of dopamine (12, 5–400 mg/kg), levodopa (6.25–100 mg/kg) and apomorphine (0.25–5 mg/kg) protected 14 day old chicks against electroshock seizures (Table 2). Thus, the seizure-enhancing influence of dopamine, levodopa and apomorphine decreased with age within the first week, while the anti-convulsant influence increased with age during the same period. Similar decrease in the effectiveness of some convulsant agents in chicks has also been reported during this period (14–16). It can be speculated from the present data that the protective influence of dopamine against electroshock seizure depends partly or wholly on certain neuronal systems which were immature at the time of hatching. As the chick grew older, such neuronal systems became more developed and thereby permitted the mediation of the anticonvulsant effect of dopaminoceptor agonists.

Both haloperidol and pimozide are conventional antagonists at dopamine receptor sites (17, 18). Our data show a clear antagonism of the effects of dopamine, levodopa and apomorphine on electroshock seizure by haloperidol and pimozide. Such findings specifically implicate the involvement of dopamine receptors in the modulation of electroshock seizure by dopamine, levodopa and apomorphine.

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