Influence of Some Dopaminoceptor Agents on Nitrazepam-Induced Sleep in the Domestic Fowl (*Gallus domesticus*) and Rats

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Abstract—The influence of apomorphine, levodopa and haloperidol was studied on nitrazepam sleep using young chicks and rats. In addition, the influence of dopamine and ADTN was studied in young chicks. Nitrazepam dose-dependently (0.4–51.2 mg/kg, i.p.) induced behavioural sleep in chicks. However, higher doses of nitrazepam (12.8–51.2 mg/kg, i.p.) were required to induce behavioural sleep in rats. Dopamine (12.5–100 mg/kg, i.p.) and ADTN (2.5–80 mg/kg, i.p.) delayed the onset but prolonged nitrazepam sleep in chicks: these effects were statistically significant. Levodopa (12.5–100 mg/kg, s.c.) and apomorphine (0.2–0.8 mg/kg, s.c.) profoundly delayed the onset and shortened the duration of nitrazepam sleep in both chicks and rats. Noradrenaline (20–80 mg/kg, i.p.) shortened the onset and prolonged nitrazepam sleep in chicks. Pimozide (1–8 mg/kg, i.p.) potentiated nitrazepam sleep and antagonized the effects of dopamine, levodopa and ADTN on nitrazepam sleep in chicks. Similarly, haloperidol (0.5–1.0 mg/kg, i.p.) potentiated nitrazepam sleep and antagonized the effects of levodopa and apomorphine on nitrazepam sleep in rats. The EEG synchronization and decreased EMG induced by nitrazepam (1.6 mg/kg, i.p., and 12.8 mg/kg, i.p., for chicks and rats, respectively) were antagonized by levodopa (12.5 mg/kg, s.c.). The behavioural and electroencephalographical results suggest that enhancement of dopaminergic neurotransmission may be involved in the mechanisms of wakefulness in both chicks and rats.

The sedative-hypnotic effect of the benzodiazepines is well known. However, the mechanisms of the actions of the benzodiazepines are still poorly understood (1, 2).

Osuide and Wambebe (3) implicated dopamine in both behavioural alertness and the waking process from pentobarbitone sleep in young chicks. In addition, Wambebe and Osuide (4) reported that FLA-63 (an inhibitor of dopamine-beta hydroxylase enzyme) antagonized pentobarbitone sleep in both chicks and rats. This antagonism was at both behavioural and electroencephalographical levels. Consequently, the authors attributed their observation to an increased endogenous dopamine neurotransmission. To my knowledge, the influence of dopaminergic agents on benzodiazepine sleep has not been documented. It is therefore the objective of this project to investigate the influence of dopamine, levodopa, apomorphine, 2-amino - 6,7 - dihydroxy - 1,2,3,4 - tetrahydro - naphthalene (ADTN—a dopaminoceptor agonist) and pimozide on nitrazepam sleep using the domestic fowl and rats. In view of the immaturity of the blood-brain barrier in young chicks (5), it has been possible to study the effects of intraperitoneally injected dopamine, noradrenaline and ADTN on nitrazepam sleep in chicks in this project.

Materials and Methods

White Ranger cockerels (obtained from Arewa Agricultural Enterprises Nigeria, Ltd.,
5–8 day old and weighing between 35 and 45 g, were used in all the experiments. Male albino rats (local strain, inbred in our Animal House) weighing 200±20 g were used in this project. The rats were about 3 months old.

The criteria for behavioural sleep in chicks were those used by Fugner and Hoefke (6). The procedures of Osuide and Wambebe (3) and Wambebe and Osuide (4, 7) for behavioural sleep experiments, preparation and implantation of EEG (unipolar and bipolar) and EMG electrodes into young chicks and adult rats were followed in this project. Four chicks were used per experiment. Each experiment was repeated three times. Ten rats (5 drug-treated and 5 saline-treated controls) were observed concurrently using specially-designed behavioural cages. The experiments were conducted in a quiet air-conditioned room (ambient temperature was 22±2°C). Control chicks (injected with either 0.4 ml of physiological saline or 1.6 mg/kg i.p. of nitrazepam) were studied concurrently with the test chicks (i.e., chicks pretreated with dopaminergic agents). The recording of the EEG and EMG was made using a Grass polygraph (model 79 D). 24 hr (chicks) and 36 hr (rats) after implantation of electrodes into discrete areas of the brain. Thus, the EEG and EMG recordings were done using conscious and freely moving chicks, while the conscious rat was placed in a restraining cage. In each case, the animal was put inside a screened cage before and during EEG and EMG recordings. A control recording was taken for about 60 min prior to injection of nitrazepam or levodopa after which recording was continued for 120 min. In view of the circadian fluctuation in brain monoamine levels which would consequently influence gross behaviour of the animals, all the experiments were performed during the same period of the day (i.e., between 13:00 and 17:00 hr).

Weighed quantities of nitrazepam (Mogadon, Roche Products; 1.6–51.2 mg/kg, i.p.), dopamine HCl (12.5–100 mg/kg), (+) ADTN HBr (Research Biochemical, MA, U.S.A.; 2.5–80 mg/kg), noradrenaline HBr (20–80 mg/kg) and amino oxyacetic acid (AOAA, 2.5 mg/kg) were separately dissolved in physiological saline, while pimozide (Janssen Pharmaceutica, 2–8 mg/kg), haloperidol (Serenace, Janssen Pharmaceutica, 0.5–1 mg/kg) and bicuculline (5 mg/kg) were separately suspended in 3% v/v Tween 80 prior to intraperitoneal injection. Apomorphine HCl (0.2–0.8 mg/kg) and levodopa (12.5–100 mg/kg) were injected subcutaneously. Dopamine, noradrenaline, apomorphine, levodopa, AOAA and bicuculline were purchased from Sigma Chemical Company, U.S.A. Levodopa was dissolved in 0.05 M hydrochloric acid with the aid of gentle warming over a water bath. The doses of the drugs used in this project refer to their salts mentioned above. Sodium meta bisulphite (0.1% w/w) was used to retard the hydrolysis of dopamine, apomorphine and noradrenaline solutions. All the drug solutions were freshly prepared on the days of the experiments. The respective pretreatment times (min) for dopamine, apomorphine, ADTN, levodopa, noradrenaline, pimozide, AOAA and bicuculline prior to the administration of nitrazepam were 5, 5, 10, 30, 30, 5, 60, 10 and 10, respectively. The data was statistically analyzed using Student’s t-test.

Results

Influence of dopamine and pimozide on nitrazepam sleep in chicks (Table 1): Dopamine (12.5–100 mg/kg, i.p.) did not induce behavioural sleep, but reduced the proportion of chicks which were hypnotized by nitrazepam (1.6 mg/kg, i.p.). In addition, dopamine significantly (P<0.001) delayed the onset of nitrazepam sleep. Apparently, the duration of nitrazepam sleep was prolonged by dopamine; this effect was statistically non-significant. On the other hand, pimozide (2–8 mg/kg, i.p.) increased the proportion of chicks that slept following the injection of nitrazepam (1.6 mg/kg, i.p.). Similarly, the duration of nitrazepam sleep was profoundly increased (P<0.001) by pimozide. The influence of dopamine on nitrazepam sleep was antagonized by pimozide.

Influence of apomorphine on nitrazepam sleep in chicks (Table 2): Apomorphine (0.2–0.4 mg/kg, s.c.) profoundly reduced the proportion of chicks that slept following the injection of nitrazepam, delayed onset and
shortened the duration of sleep. In addition, 0.8 mg/kg, s.c., of apomorphine completely antagonized nitrazepam sleep. Pimozide (2 mg/kg, i.p.) blocked the antagonistic effect of apomorphine against nitrazepam.

Influence of ADTN on nitrazepam sleep in chicks (Table 3): ADTN (2.5–80 mg/kg, i.p.) reduced the proportion of chicks that slept following the administration of nitrazepam (1.6 mg/kg, i.p.). In addition, the onset of nitrazepam sleep was significantly delayed (P<0.01), while the sleeping time was increased (P<0.01). Apparently, pimozide (2 mg/kg, i.p.) antagonized the effects of ADTN on nitrazepam sleep.

Influence of levodopa on nitrazepam sleep in chicks (Table 4): Levodopa (12.5–100 mg/kg, s.c.) did not induce sleep, but rather excited the chicks behaviourally. Low doses (12.5–25 mg/kg, s.c.) significantly delayed the onset and reduced nitrazepam sleeping time. Higher doses (50–100 mg/kg, s.c.) completely antagonized nitrazepam sleep.

Influence of noradrenaline and phentolamine on nitrazepam sleep in chicks (Table 5): Noradrenaline (40–80 mg/kg, i.p.) alone hypnotized young chicks. Similarly, noradrenaline (40 mg/kg, i.p.) increased the number of chicks hypnotized by nitrazepam (1.6 mg/kg, i.p.), shortened the onset of sleep and significantly (P<0.001) prolonged the sleeping time of nitrazepam. On the other hand, phentolamine (2.5–10 mg/kg, i.p.) weakly antagonized both nitrazepam sleep as well as the effects of noradrenaline on nitrazepam sleep.

Influence of levodopa and haloperidol on nitrazepam sleep in rats (Table 6): Nitrazepam

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**Table 1. Influence of dopamine and pimozide on nitrazepam sleep in chicks**

| Doses (mg/kg) | No. asleep/No. used | Onset of sleep (min) | Duration of sleep (min) |
|--------------|---------------------|----------------------|-------------------------|
| Dopamine     | Nitzapem | Pimozide | Mean±S.E.M. | Mean±S.E.M. | Mean±S.E.M. |
| 0            | 1.6       | 0        | 8/12       | 6.8 ± 1.0     | 14.2 ± 1.9     |
| 12.5         | 1.6       | 0        | 8/12       | 13.4* ± 1.4  | 15.5 ± 1.7     |
| 25           | 1.6       | 0        | 6/12       | 15.6* ± 1.7  | 16.5 ± 2.0     |
| 50           | 1.6       | 0        | 4/12       | 19.7**± 1.5  | 18.4 ± 1.8     |
| 100          | 1.6       | 0        | 2/12       | 21.1**±1.6   | 20.3 ± 1.8     |
| 0            | 0         | 2        | 0/12       |               |               |
| 0            | 1.6       | 2        | 12/12      | 4.0 ± 0.5    | 25 ± 1.6       |
| 0            | 1.6       | 4        | 12/12      | 2.1 ± 0.3    | 28.4*±4.4      |
| 0            | 1.6       | 8        | 12/12      | 2.0 ± 0.4    | 36.2*±5.6      |
| 50           | 1.6       | 2        | 8/12       | 10.0 ± 1.0   | 13.0 ± 1.4     |

* and ** are significantly different from the control chicks (same age of chicks and dose of nitrazepam), and represent P<0.01 and P<0.001 by Student’s t-test, respectively.

**Table 2. Influence of apomorphine on nitrazepam sleep in chicks**

| Doses (mg/kg) | No. asleep/No. used | Onset of sleep (min) | Duration of sleep (min) |
|--------------|---------------------|----------------------|-------------------------|
| Apomorphine  | Nitzapem | Pimozide | Mean±S.E.M. | Mean±S.E.M. | Mean±S.E.M. |
| 0            | 1.6       | 0        | 8/12       | 6.8 ± 1.0     | 14.2 ± 1.9     |
| 0.2          | 1.6       | 0        | 7/12       | 15.3* ± 1.3  | 15.5 ± 1.6     |
| 0.4          | 1.6       | 0        | 6/12       | 28.8**±5.6   | 10 ± 1.0       |
| 0.8          | 1.6       | 0        | 3/12       | 38.3**±6.7   | 7.0 ± 1.5      |
| 0            | 0         | 2        | 0/12       |               |               |
| 0            | 1.6       | 2        | 12/12      | 4.0 ± 0.5    | 25 *±1.5       |
| 0.4          | 1.6       | 2        | 10/12      | 15.0* ± 2.4  | 13.4 ± 1.8     |

* and ** are significantly different from the controls (same age of chicks and dose of nitrazepam) and represent P<0.01 and P<0.001 by Student’s t-test, respectively.
Table 3. Influence of ADTN on nitrazepam sleep in chicks

| ADTN | Nitrazepam | Pimozide | No. asleep/ No. used | Onset of sleep (min) | Duration of sleep (min) |
|------|------------|----------|---------------------|----------------------|------------------------|
| 0    | 1.6        | 0        | 8/12                | 6.8 ± 1.0            | 14.2 ± 1.9             |
| 2.5  | 1.6        | 0        | 7/12                | 6.5 ± 0.8            | 20.0 ± 3.2             |
| 5    | 1.6        | 0        | 6/12                | 7.2 ± 1.2            | 28.0*±4.0              |
| 10   | 1.6        | 0        | 6/12                | 10.0 ± 1.6           | 30.0*±5.5              |
| 20   | 1.6        | 0        | 5/12                | 13.0*±2.5            | 32.0*±4.8              |
| 40   | 1.6        | 0        | 6/12                | 18.0*±2.6            | 30.0*±4.5              |
| 80   | 1.6        | 0        | 6/12                | 15.0*±3.6            | 35.0*±4.4              |
| 0    | 0          | 2        | 0/12                |                      |                        |
| 0    | 1.6        | 2        | 12/12               | 4.0 ± 0.5            | 25.0*±1.5              |
| 5    | 1.6        | 2        | 9/12                | 7.0 ± 1.2            | 14.0 ± 2.2             |
| 10   | 1.6        | 2        | 8/12                | 9.0 ± 2.1            | 22.0 ± 1.8             |

* Significantly different from the controls (same age of chicks and dose of nitrazepam). P<0.01 by Student’s t-test.

Table 4. Influence of levodopa on nitrazepam sleep in chicks

| Levodopa | Nitrazepam | Pimozide | No. asleep/ No. used | Onset of sleep (min) | Duration of sleep (min) |
|----------|------------|----------|---------------------|----------------------|------------------------|
| 0        | 1.6        | 0        | 8/12                | 6.8 ± 1.0            | 14.2 ± 1.7             |
| 12.5     | 1.6        | 0        | 6/12                | 7.2 ± 1.8            | 10.2 ± 1.3             |
| 25.0     | 1.6        | 0        | 3/12                | 18.2*±2.1            | 8.4 ± 1.0              |
| 50.0     | 1.6        | 0        | 0/12                |                      |                        |
| 100.0    | 1.6        | 0        | 0/12                |                      |                        |
| 0        | 0          | 2.0      | 0/12                |                      |                        |
| 0        | 1.6        | 2.0      | 12/12               | 4.0 ± 0.5            | 25.0*±2.2              |
| 25.0     | 1.6        | 2.0      | 7/12                | 13.0*±1.2            | 18.0 ± 2.2             |

* Significantly different from the controls (same age of chicks and dose of nitrazepam). P<0.01 by Student’s t-test.

Table 5. Influence of noradrenaline and phentolamine on nitrazepam sleep in chicks

| Noradrenaline | Doses (mg/kg) | Nitrazepam | Phentolamine | No. asleep/ No. used | Onset of sleep (min) | Duration of sleep (min) |
|---------------|---------------|------------|--------------|---------------------|----------------------|------------------------|
| 20.0          | 0             | 0          | 0            | 0/12                |                      |                        |
| 40.0          | 0             | 0          | 0            | 8/12                | 4.5±0.5              | 13.5 ± 1.0             |
| 80.0          | 0             | 0          | 0            | 12/12               | 3.5±1.0              | 19.5 ± 2.1             |
| 0             | 1.6           | 0          | 0            | 8/12                | 6.8±1.0              | 14.2 ± 1.9             |
| 20.0          | 1.6           | 0          | 0            | 12/12               | 2.0±1.2              | 60.2***±3.4            |
| 40.0          | 1.6           | 0          | 0            | 12/12               | 1.1±0.7              | 100.1***±7.2           |
| 80.0          | 1.6           | 0          | 0            | 12/12               | 0.5±0.5              | 150.1***±15.0          |
| 0             | 1.6           | 2.5        | 0            | 8/12                | 7.0±0.6              | 13.5 ± 1.0             |
| 0             | 1.6           | 5.0        | 0            | 6/12                | 8.0±0.5              | 13.0 ± 1.6             |
| 0             | 1.6           | 10.0       | 0            | 5/12                | 8.8±0.5              | 10.2 ± 1.4             |
| 20            | 1.6           | 5.0        | 0            | 10/12               | 6.5±1.2              | 38.3 ± 2.2             |

** Significantly different from the controls (same age of chicks and dose of nitrazepam). P<0.001 by Student’s t-test.
(12.8 mg/kg, i.p.) induced sleep in all the rats used. Levodopa did not induce sleep in rats. Levodopa at 6.25–50 mg/kg, s.c., reduced the proportion of rats that slept, significantly delayed the onset and profoundly shortened nitrazepam sleeping time. These antagonistic effects of levodopa against nitrazepam appear to be dose-dependent. In fact, 100 mg/kg, s.c., of levodopa completely antagonized nitrazepam sleep. On the other hand, haloperidol (0.5–1.0 mg/kg, i.p.) reduced the latency and prolonged nitrazepam sleeping time. Similarly, haloperidol (1.0 mg/kg, i.p.) effectively antagonized the effects of levodopa (25.0 mg/kg, s.c.) on nitrazepam sleep.

Influence of apomorphine on nitrazepam sleep in rats (Table 7): Apomorphine (0.1–0.4 mg/kg, s.c.) dose-dependently antagonized nitrazepam sleep. Thus, the proportion of rats that slept following the injection of nitrazepam was reduced. Sleep latency was significantly delayed while the sleeping time was drastically shortened. In fact, 0.8 mg/kg, s.c., of apomorphine completely abolished nitrazepam sleep. Haloperidol (1.0 mg/kg, i.p.) effectively antagonized the effects of apomorphine on nitrazepam sleep in rats. AOAA (2.5 mg/kg, i.p.) shortened the

### Table 6. Influence of levodopa and haloperidol on nitrazepam sleep in rats

| Doses (mg/kg) | Levodopa | Nitrazepam | Haloperidol | No. asleep/No. used | Onset of sleep (min) Mean±S.E.M. | Duration of sleep (min) Mean±S.E.M. |
|---------------|----------|------------|-------------|---------------------|----------------------------------|-----------------------------------|
| 0             | 12.8     | 0          | 0           | 20/20               | 63.8±7.1                         | 70.3±3.4                          |
| 6.25          | 12.8     | 0          | 0           | 10/10               | 68.2±4.5                          | 64.7±2.5                          |
| 12.5          | 12.8     | 0          | 0           | 8/10                | 98.7±4.7                          | 43.9±1.9                          |
| 25.0          | 12.8     | 0          | 0           | 5/10                | 109.5±3.3                         | 38.8±2.5                          |
| 50.0          | 12.8     | 0          | 0           | 2/10                | 167.3±1.0                         | 36.0±0.5                          |
| 100.0         | 12.8     | 0          | 1           | 0/10                |                                   |                                   |
| 0             | 12.8     | 0.5        | 0           | 10/10               | 60.6±7.7                          | 97.1±1.6                          |
| 0             | 12.8     | 1.0        | 0           | 10/10               | 38.7±2.0                          | 115.1±2.0                         |
| 25            | 12.8     | 1.0        | 0           | 10/10               | 56.9±2.1                          | 70.4±2.3                          |

* is significantly different from the controls (same dose of nitrazepam). P<0.001 by Student’s t-test.

### Table 7. Influence of apomorphine on nitrazepam sleep in rats

| Doses (mg/kg) | Apo | Nitrazepam | Hal | AOAA | Bicuculline | No. asleep/No. used | Onset of sleep (min) Mean±S.E.M. | Duration of sleep (min) Mean±S.E.M. |
|---------------|-----|------------|-----|------|-------------|---------------------|----------------------------------|-----------------------------------|
| 0             | 12.8| 0          | 0   | 0    | 0           | 20/20               | 63.8±7.1                         | 70.3±3.4                          |
| 0.1           | 12.8| 0          | 0   | 0    | 0           | 10/10               | 84.5±3.6                          | 65.3±1.3                          |
| 0.2           | 12.8| 0          | 0   | 0    | 0           | 8/10                | 119.0±4.2                         | 41.7±2.1                          |
| 0.4           | 12.8| 0          | 0   | 0    | 0           | 6/10                | 153.3±1.8                         | 24.8±1.5                          |
| 0.8           | 12.8| 0          | 0   | 0    | 0           | 0/10                |                                   |                                   |
| 0             | 12.8| 0.1        | 0   | 0    | 0           | 0/10                |                                   |                                   |
| 0.8           | 12.8| 0          | 2.5 | 0    | 0           | 10/10               | 40.0±6.5                          | 100.8±12.6                        |
| 0.8           | 12.8| 0          | 2.5 | 0    | 0           | 4/10                | 70.3±8.2                          | 31.4±3.6                          |
| 0             | 12.8| 0          | 0   | 5.0  | 0           | 6/10                | 84.2±5.5                          | 18.9±3.2                          |
| 0.8           | 12.8| 0          | 0   | 5.0  | 0           | 8/10                | 100.2±10.4                        | 36.4±4.8                          |
| 0             | 12.8| 0.5        | 0   | 0    | 0           | 10/10               | 60.6±7.7                          | 97.1±1.6                          |
| 0             | 12.8| 1.0        | 0   | 0    | 0           | 10/10               | 38.7±3.8                          | 165.2±3.4                         |
| 0.4           | 12.8| 1.0        | 0   | 0    | 0           | 10/10               | 63.7±4.3                          | 84.4±3.1                          |

* is significantly different from the controls (same dose of nitrazepam). P<0.001 by Student’s t-test.
sleep latency and prolonged the sleeping time, while bicuculline (5 mg/kg, i.p.) reduced the proportion of rats that slept, delayed the onset and shortened the duration of nitrazepam sleep. AOAA (2.5 mg/kg, i.p.) blocked apomorphine (0.8 mg/
kg, s.c.)-induced antagonism of nitrazepam sleep, while bicuculline failed to potentiate the antagonism.

Influence of levodopa on the effects of nitrazepam on the EEG and EMG of the chick: Nitrazepam (1.6 mg/kg, i.p.) reduced EMG activity, while the EEG of the hyperstriatum (HS), optic tectum (OT), and pontine reticular formation (RF) was synchronized. On the other hand, levodopa (12.5 mg/kg, s.c.) enhanced the EMG activity and desynchronized the EEG of the HS, OT and RF. In addition, the EEG and EMG effects of nitrazepam (1.6 mg/kg, i.p.) were antagonized by levodopa (12.5 mg/kg, s.c., Fig. 1).

Influence of levodopa on the effects of nitrazepam on the EEG and EMG of the rat: Nitrazepam (12.8 mg/kg, i.p.) increased the amplitudes of the EEG of the frontal cortex (FC) and the optic lobe (OL), while the frequencies were reduced. Similarly, EMG activity was reduced by nitrazepam (12.8 mg/kg, i.p.). On the other hand, levodopa (12.5 mg/kg, s.c.) activated the EMG and increased the amplitude of the EEG of the OL. In addition, levodopa (12.8 mg/kg, s.c.) apparently antagonized the EEG and EMG effects of nitrazepam in the rat (Fig. 2).

Discussion

Both dopamine and ADTN (a dopaminergic agonist, ref. 8) reduced the proportion of chicks that slept upon the injection of nitrazepam. Similarly, the two dopaminergic agonists significantly delayed the onset of nitrazepam sleep. On the other hand, both dopamine and ADTN prolonged nitrazepam sleeping time. These results are generally similar to those reported by Osuide and Wambebe (3). In that study, dopamine exhibited a bi-phasic dose-dependent effect on pentobarbitalin sleep. However, there was no biphasic dose-dependent effect in the present study. Obviously, the reduction in percentage hypnosis and the delay in nitrazepam sleep induced by both dopamine and ADTN suggest behavioural antagonism. Such an observation agrees with the report of Wambebe and Osuide (4) that FLA-63 (an inhibitor of dopamine beta-hydroxylase) antagonized pentobarbital sleep both in chicks and rats.

It is however significant to note that nitrazepam sleeping time was prolonged by dopamine and ADTN. Biochemical, physiological, anatomical and pharmacological data indicate that multiple dopamine receptors exist in the brains of both vertebrates and invertebrates (9). Similarly, computer analysis of radio-ligand studies indicate that 3H-dopamine labels two recognition sites having nanomolar and micromolar affinities for spiroperidol (9). It is therefore possible that two types of dopamine receptors subserving excitatory and inhibitory functions exist in the avian brain. Thus, the proposed dopamine excitatory receptors might be more sensitive to dopaminergic agonists possibly due to their structural disposition, preferential affinity or superior functional sensitivity than the inhibitory dopamine receptors. It is also possible that both types of dopamine receptors were activated simultaneously but due to the dynamics of drug-receptor interactions, the effect on the dopamine excitatory receptors was predominant initially but short-lasting. The possibility of the existence of only one type of dopamine receptors subserving two behavioural functions which are dependent on their allosteric changes cannot be ignored. Since both the excitatory (i.e., reduction in proportion of chicks hypnotized and the delay in nitrazepam sleeping time) and inhibitory (i.e., prolongation of nitrazepam sleeping time) behavioural effects of dopamine and ADTN were antagonized by pimozide (a dopaminergic antagonist), dopamine receptors might be involved in both cases.

According to Scheel-Kruger and Randrup (10), most of the pharmacological effects of levodopa might be due to dopamine formed from it in vivo. Similarly, the effects of apomorphine have been attributed to the activation of dopamine receptors in the brain (11). In this study, both levodopa and apomorphine reduced the proportion of chicks and rats that slept following the injection of nitrazepam. In addition, these drugs prolonged the latency of sleep, while nitrazepam sleeping time was reduced. In fact, 100 mg/kg, s.c., of levodopa completely abolished nitrazepam sleep in both chicks and rats. These behavioural effects of
levodopa and apomorphine were antagonized by pimozide, thereby implicating the activation of dopaminoceptors in the genesis of the observed effects.

It is noteworthy that pimozide profoundly potentiated nitrazepam sleep. Since pimozide is a potent dopaminoceptor antagonist (12), the behavioural potentiation of nitrazepam sleep might be due to the blockade of dopamine receptors. Thus, the functional behavioural role of dopamine receptors involved might be excitatory.

AOAA (increases brain GABA levels by inhibiting GABA-T enzyme, ref. 18) profoundly potentiated nitrazepam sleep. On the other hand, bicuculline (a GABA receptor antagonist) antagonized nitrazepam sleep in rats. Such results agree with those of Wambebe (7) using young chicks. Similarly, AOAA blocked apomorphine-induced antagonism of nitrazepam sleep in rats. Such an observation may be due to physiological antagonism of apomorphine's effect by the enhanced brain levels of GABA by AOAA.

According to Lloyd and his colleagues (13), GABA neurons tonically inhibit the nigro-striatal dopamine pathway as evidenced by decreased dopamine synthesis, turnover and release. In addition, the same authors proposed that GABA neurons can modify dopamine receptor number \( \left( B_{\text{max}} \right) \) in the striatum as well as modulate the expression of dopamine receptor activation at sites distal to the dopaminergic synapse. Thus, the present data essentially agree with the above proposal that dopaminergic and GABAergic mechanisms in the striatum are functionally antagonistic to each other.

The present data also shows that intraperitoneally-injected noradrenaline induced behavioural sleep. Similarly, noradrenaline profoundly potentiated nitrazepam sleep. These effects were weakly antagonized by phenylamine (an alpha-adrenoceptor blocking agent). Thus, activation of alpha adrenoceptors might partly underly the behavioural effects of noradrenaline in this project. The behavioural antagonism of nitrazepam sleep by phenylamine suggests the possible involvement of noradrenergic mechanisms in behavioural depression in the chick.

In both chicks and rats, the EEG synchronization of the frontal cortex and optic lobe or hyperstriatum and optic tectum induced by nitrazepam were reversed by levodopa. Similarly, nitrazepam-induced depression of the EMG activity was antagonized by levodopa. In fact, levodopa (12.5 mg/kg, s.c.) activated the EEG of the frontal cortex, optic lobe and pontine reticular formation, while the EMG activity was increased in rats. Similarly, the EEG of the hyperstriatum and pontine reticular formation was desynchronized, while the EMG was activated by levodopa (12.5 mg/kg, s.c.) in chicks. Thus, the behavioural antagonism of nitrazepam by levodopa was supported by electroencephalographical data.

The results reported in this paper agree essentially with other studies using dopaminoceptor agents against barbiturate sleep in chicks and rats (3, 4). Such a similarity conforms with the hypothesis that both benzodiazepines and barbiturates may increase chloride channels at GABA-\( \alpha \) receptor sites (14). It is therefore possible that the influx of chloride ions might underlie the sedative-hypnotic effect of both benzodiazepines and barbiturates. The reported clinical deterioration of levodopa-treated Parkinsonian patients in the presence of diazepam (14, 15) may be due to antagonism between dopamine and benzodiazepine. The observed antagonism between dopaminoceptor agonists and nitrazepam may be related to the modulatory effect of dopamine on GABAergic neurons in the striatum. It may also be due to the possibility that endogenous dopamine may subserve behavioural alertness. Thus, an additional behavioural and electroencephalographical evidence has been accumulated to support the hypothesis that endogenous dopamine may subserve excitatory behavioural role.

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