ACTIONS OF DESOXYNUPHARIDINE HYDROCHLORIDE IN THE CENTRAL NERVOUS SYSTEM OF EXPERIMENTAL ANIMALS (1)

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Abstract—The effects of desoxynupharidine hydrochloride (DN) as the extractive component of the Nuphar japonicum DC on the central nervous system were studied. In the acute cats, DN (1-3 mg/kg, i.v.) produced 8-10 Hz waves in the hypothalamus lateralis and amygdala, and the hippocampal arousal wave gave way to the irregular high amplitude slow waves. In the midbrain reticular formation, nucl. reticularis and cortex, 12-13 Hz waves and high amplitude slow waves increased. A sleep-like pattern with spindle burst-like waves formed the general pattern of all EEGs. These spontaneous EEG activities were significantly inhibited by epinephrine hydrochloride and were potentiated by tolazoline hydrochloride and dibenamine hydrochloride etc. The above EEG changes were obtained even in the spinal cats. DN depressed the EEG arousal response produced by high frequency stimulation of the sciatic nerve and increased the threshold of stimulation. In the chronic cats, DN (1 mg/kg, i.v.) produced spindle burst-like waves of 12-14 Hz in the cortex and sedation concomitantly. These findings suggest that DN has an inhibitory effect on the central nervous system and that adrenergic neurons are probably involved.

The extractive components of the Nuphar japonicum DC (Nymphaeaceae) were reported to be alkaloids: nupharidine and desoxynupharidine etc (1-5). Regarding the effect of desoxynupharidine hydrochloride, the general pharmacological effects were described (6), however, studies on the central nervous system have not been documented.

We carried out electroencephalographical studies using this compound and our findings are reported herein.

MATERIALS AND METHODS

Chemicals: Desoxynupharidine hydrochloride (DN) was synthesized. The chemical structure of DN is 1,7-dimethyl-4-(3-furyl)-quinolizidine, the molecular formula is C_{18}H_{23}ON, molecular weight 233 and the compound is a white crystal, which freely dissolves in water. The melting point of the hydrochlorate is 262°C.

Animals: ICR strain male mice (18-25 g), male Wistar rats (150-230 g) and both sexes of cats (2.0-3.5 kg) were used. Food and water were provided ad libitum. The animals were used for the experiments after breeding for one week. Breeding conditions were constant temperature (22±1°C) and humidity (52±2.5%).
Spontaneous motor activity: Alterations in the spontaneous motor activity of 45 mice were determined by using a monitor of activity (Animex, Farad Electronics, Sweden). A group of 5 mice was placed in the counting box and the spontaneous motor activity was measured at intervals of 15 min. Five mice were given DN s.c. and the spontaneous motor activity was recorded from 7:00 to 10:00 p.m.

Acetic acid writhing method: Forty mice were used. DN was given s.c. and ten min later each mouse was given acetic acid (0.7%, 0.1 ml/10 g, i.p.). The number of writhings or stretchings was counted for 10 min. Aminopyrine (60 mg/kg, s.c.) was given as a standard drug.

Electrical stimulation method: Twenty mice were given DN s.c. and fifteen min after, each mouse was given a shock at the root of a tail (stimulator MSE-3: Nihon kohden, 1 Hz, 10 msec, 50 V). The reaction time (sec) was expressed as the index of animal's squeak.

Hexobarbital-Na sleeping time: Twenty mice were given DN s.c. and fifteen min after, 100 mg/kg of hexobarbital-Na was given i.p. The sleeping time was expressed as the time of duration when the animals lost their righting reflex.

Anticonvulsive activity 1) strychnine and pentetrazol-induced convulsion: Forty mice were given DN s.c. and fifteen min later strychnine nitrate (1.5 mg/kg, s.c.) and pentetrazol (150 mg/kg, s.c.) were given, respectively. The onset time of convulsion and time of death were recorded.

Anticonvulsive activity 2) electroshock method: Twenty mice were given DN s.c. and fifteen minutes later, the bilateral corneas were given an electroshock (30 mA, 0.2 sec). The duration time of convulsion and the onset time of the righting reflex were measured, respectively.

Hypothermic activity: To ten rats with a rectal temperature of 37.5–38.5°C DN was given s.c. and the rectal temperature was measured at 30 min intervals for 2 hr.

EEG activities in acute cats: Thirty cats of both sexes were fixed on a stereotaxic instrument (Todai Nohken type) after anesthesia with ether. After tracheotomy the cranial bone was exposed and stainless steel concentric electrodes with a diameter 0.8 mm were placed in nucl. reticularis (R) (A: 13.0, L: 3.0, H: +1.5), midbrain reticular formation (MRF) (A: 2.0, L: 3.0, H: −3.0), nucl. ventralis postero-lateralis (VPL) (A: 9.0, L: 7.0, H: +1.0), hippocampus dorsalis (D-Hippo) (A: 3.0, L: 11.0, H: +2.5), hippocampus ventralis (V-Hippo) (A: 6.0, L: 12.0, H: −4.0), amygdala (Amy) (A: 13.0, L: 9.0, H: −5.0) and hypothalamus lateralis (Hypo) (A: 11.0, L: 2.5, H: −3.5) according to the atlas of Jasper and Ajimone-Marsan (7). The metal screw electrodes were placed on the frontal (A: 26.0, L: 6.0), temporal (A: 8.0, L: 16.0) and occipital (A: −2.0, L: 8.0) cortices. The indifferent electrode was placed on the frontal cranial bone.

The EEG arousal response following high frequency stimulation (100 Hz, 0.1 msec, 2 V, 6 sec) of the sciatic nerve was examined. After the recovery from ether anesthesia, the spontaneous EEG activities and EEG arousal response were recorded under artificial respiration (the rate: 25 rounds/min, 12.4 ml) in cats immobilized with gallamine triethiodide (5–10 mg/kg, i.v.). Blood pressure, ECG, heart rate and body temperature were simultaneously recorded. The drugs were administered into the femoral vein.

At the end of experiment, the brain was fixed in 10% formalin solution and then sectioned serially at 50 μ. The areas of electrode insertion were checked histologically.

Spontaneous EEG activities in chronic cats: Five cats of both sexes were used. The animals were fixed on a stereotaxic instrument (Todai Nohken type) after anes-
thetization with pentobarbital-Na (30 mg/kg, i.p.). After exposure of the cranial bone, the metal screw electrodes were placed on the frontal, temporal and occipital cortices. Stainless steel bipolar electrodes with a diameter 0.25 mm and insulated with enamel were implanted in Hypo, D-Hippo, MRF, VPL and Amy. The stereotaxic position of the cortical and subcortical electrodes and the indifferent electrode was respectively shown in the acute experiment of the EEG activities. Each electrode was fixed on the cranial bone with dental cement and was soldered to the connector sockets. After these procedures, the open wound edges were disinfected with 0.2% acrinol for one week, and procaine penicillin G 300,000 units were given i.m. once daily x3. The experiment was carried out when the operative wounds had recovered completely and stable EEG activities were recorded. EMG from the platysma was recorded simultaneously with the EEG (Electroencephalograph : Nihon kohden).

**Test on significant difference:** Student's t-test was used.

**RESULT**

**Spontaneous motor activity:** As shown in Fig. 1, DN (2 mg/kg) did not alter the spontaneous motor activity up to 60 min. From 90 min, the activity began to decrease significantly and was inhibited by 31% at 180 min. With a dose of 4 mg/kg, the activity began to decrease significantly from 60 min and was inhibited by 54% at 180 min.

**Acetic acid writhing method:** As shown in Table 1, DN (2 and 4 mg/kg) showed 53.5 and 68.0% inhibition of the writhing, in comparison with the control group.

**Electrical stimulation method:** DN (2 and 4 mg/kg) produced no significant alteration, as compared with the control group.

**Hexobarbital-Na sleeping time:** DN (2 and 4 mg/kg) produced a shortening of hexobarbital-Na sleeping onset time for 2.4 and 2.5 min respectively, and a prolongation of the sleeping lasting time for 7.2 and 9.7 min. Therefore, DN produced a prolongation

| Table 1. Analgesic effect of DN in mice using the acetic acid writhing method |
|-----------------------------------------------|
| Test substances | Dose (mg/kg, s.c.) | Number of writhings | Inhibition (%) |
|-----------------|---------------------|---------------------|----------------|
| Saline          | 20.0±1.4            | 20.0±1.4            | 53.5           |
| DN              | 9.3±1.0*            | 9.3±1.0*            | 68.0           |
| Aminopyrine     | 6.4±1.9*            | 6.4±1.9*            | 55.5           |
|                 | 8.9±1.6*            | 8.9±1.6*            | 55.5           |

Each value represents the mean±standard deviation.

*significantly different from control, p<0.01 (N=10)
of hexobarbital-Na sleeping time for 9.6 and 12.2 min respectively.

**Anticonvulsive activity:** As shown in Table 2, DN (2 and 4 mg/kg) showed no effect on the onset time of strychnine nitrate-induced convulsion and significantly prolonged the time of death. Regarding pentetrazol-induced convulsion, DN (4 mg/kg) showed a significant prolongation of the onset time and the time of death. DN (2 and 4 mg/kg), however, did not alter the duration time of convulsions induced by electroshock (30 mA, 0.2 sec).

**Hypothermic activity:** DN (2 and 4 mg/kg) showed no significant alteration in comparison with the control group.

**Spontaneous EEG activity in acute cats:** As shown in Figs. 2–7, after injection of DN (1 mg/kg, i.v.), 8–10 Hz (50 nV) waves, 12–14 Hz (150–250 nV) waves and the high amplitude slow waves began to appear in the Amy. In the R high amplitude slow waves appeared. Then in the MRF the high amplitude slow waves and 13–14 Hz waves increased in number. In the Hypo, 13–14 Hz waves increased and the amplitude also enhanced. In the Hippo, hippocampal arousal wave seen with 4–6 Hz changed to be a irregular wave and from about 5 min, it transferred to the high amplitude slow waves. The high amplitude slow waves and 12–14 Hz waves which had appeared in the Amy, R, MRF, Hypo and Hippo spread to the cortex from about 5 min markedly. Afterwards spindle burst-like waves spread to all EEGs, and the EEG pattern transferred to be so-called drowsy pattern. These EEG changes continued up to about 30 min and a tendency toward recovery was seen at 60–80 min. These EEG changes were evident even in spinal cats. Immediately following after injection of DN (2 mg/kg, i.v.), 8–10 Hz waves began to appear in the Amy and the Hypo, and then the high amplitude slow waves appeared in the MRF and the VPL. At about 5 min, hippocampal arousal wave changed to an irregular wave and then transferred to the high amplitude slow waves. The high amplitude slow waves (0.5–1 Hz) which had appeared in the MRF and VPL spread and spindle burst-like waves sometimes intermixed in the cortex. At about 15 min, spindle burst-like waves spread to all EEGs. These changes were marked at about 30 min and a tendency toward recovery was seen at 90–120 min. Immediately after injection of DN (3 mg/kg, i.v.), 12–14 Hz waves which superimposed on the irregular high amplitude slow waves appeared in all EEGs, significantly. The EEG patterns were marked at about 15–30 min and then, all EEGs transferred to the light sleep patterns. A tendency toward recovery was seen at 120–150 min. These EEG changes after

| Table 2. Anticonvulsive effect of DN in mice |
|---------------------------------------------|
| **Convolants** | **Drugs** | **Dose (mg/kg, s.c.)** | **OC (min)** | **Time of death (min)** |
|----------------|----------|----------------------|--------------|------------------------|
| Strychnine nitrate (1.0 mg/kg, s.c.) | Saline | | 7.7±0.1 | 7.9±0.1 |
| DN | 2.0 | 7.8±0.2 | 8.1±0.2 |
| 4.0 | 7.8±0.2 | 8.3±0.2 |
| Pentetrazol (150 mg/kg, s.c.) | Saline | | 3.4±0.3 | 9.1±0.7 |
| DN | 2.0 | 3.4±0.4 | 9.3±0.1 |
| 4.0 | 3.8±0.5* | 11.1±0.7* |

OC=Onset of convulsion. Each value represents the mean±standard deviation.

* significantly different from control, p<0.01 (N=10).
injection of DN (2–3 mg/kg) were seen even in the spinal cats.

Two minutes after the preliminary treatment with atropine sulfate (0.25 mg/kg, i.v.), injection of DN (1 mg/kg) inhibited changes in EEGs, as compared to effects seen with DN alone until about 40 min later. Two minutes after the preliminary treatment with DN, injection of epinephrine hydrochloride (1 μg/kg, i.v.) inhibited EEGs induced by DN until about 8 min later and the EEG pattern produced by dosing with DN appeared at about 15 min. Even in spinal cats, the similar results as seen epinephrine hydrochloride treatment in intact cats were obtained. Two minutes after the preliminary treatment with DN, injection of propranolol hydrochloride (1 mg/kg, i.v.) had little influence on the EEG changes produced by DN alone. Two minutes after the preliminary treatment with tolazoline hydrochloride (0.01 mg/kg, i.v.), injection of DN significantly potentiated changes in the EEGs produced by DN alone, and these changes continued

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![Fig. 2. Effect of DN on spontaneous EEG activities in an acute cat at 3 min after administration.](image)

_Hypo=hypothalamus lateralis, VPL=nucl. ventralis postero-lateralis, Amy=amygdala, D-Hippo=hippocampus dorsalis, V-Hippo=hippocampus ventralis, MRF=midbrain reticular formation, R=nucl. reticularis, Fr=frontal cortex, T=temporal cortex, O=occipital cortex, r=right, l=left, EGG=electrocardiogram, time marker=0.1 sec. Abbreviations are the same for all successive figures.

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![Fig. 3. Effect of DN on spontaneous EEG activities in an "acute cat" at 30 min after administration.](image)

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These changes in EEGs tended to recover in about 120 min. The combined use of phentolamine mesylate (0.01 mg/kg, i.v.) and of dibenamine hydrochloride (0.01 mg/kg, i.v.) with DN produced results similar to those seen with tolazoline hydrochloride treatment.

EEG arousal responses: Effects of DN on the EEG arousal response following high frequency stimulation of the sciatic nerve were also examined. When the sciatic nerve was stimulated at 100 Hz, 0.1 msec, 2 V, 6 sec, low voltage fast waves were produced in the cortex and in the Amy, and hippocampal arousal waves with 4–6 Hz appeared in the Hippo. As shown in Fig. 8, DN (1–2 mg/kg, i.v.) inhibited the EEG arousal responses from 10–60 min, and the effects disappeared at about 90 min. The threshold of the

Fig. 4. Effect of epinephrine hydrochloride on spontaneous EEG activities induced by DN in an "acute cat" at 8 and 15 min after administration.

Fig. 5. Effect of DN on spontaneous EEG activities induced by atropine sulfate pretreatment in an "acute cat" at 7 and 20 min after administration.
stimulation for these changes was increased with DN.

Spontaneous EEG activities in chronic cats: As shown in Figs. 9–10, about 5 min after administration of DN (1 mg/kg, i.v.), high voltage slow waves appeared in the MRF and in the Hippo. In the cortex, spindle burst-like waves with 12–14 Hz increased. These changes spread to all EEGs, and EEG patterns transferred to so-called drowsy pattern. At 90–120 min, these changes tended to recover. On the other hand, when these EEG patterns were observed, the cat was in a sedated condition. Muscle relaxation and ataxia were absent.

**DISCUSSION**

Regarding the spontaneous EEG activity
Fig. 8. Effect of DN on the EEGs arousal response induced by stimulation of the sciatic nerve in an acute cat at 10, 30 and 90 min after administration. The horizontal line indicates the period of electrical stimulation.

Fig. 9. Effect of DN on spontaneous EEG activities in a "chronic cat" at 5 and 15 min after administration.

Fig. 10. Effect of DN on spontaneous EEG activities in a "chronic cat" at 30 min after administration.
in the acute cats, DN (1–3 mg/kg, i.v.) increased the high amplitude slow waves in the Amy, Hypo and Hippo. In the MRF, R and cortex, 12–14 Hz waves and the high amplitude slow waves were intermixed. The spindle burst-like waves spread to all EEGs. Even if the EEG patterns were changed, DN had no effect on the blood pressure. In the case of spontaneous EEG activity in the spinal cats, DN produced similar EEG patterns as seen in the acute cats. These EEG patterns resembled those of the light sleep stage demonstrated by Tokizane et al. (8) in curare immobilized cats on acute experiments. In the chronic cats which were sedated, the high voltage slow waves and spindle burst-like waves appeared on the spontaneous EEG activity. It was assumed that these EEG patterns corresponded to drowsiness or to the D-stage according to Lindsley et al. (9) and Yamamoto (10). DN probably does not influence the peripheral motor system because muscle relaxation and ataxia never occurred. Moreover, DN significantly decreased the spontaneous movement and potentiated hypnosis of hexobarbital-Na in mice. The time of death against strychnine nitrate and pentetrazol-induced convulsion was prolonged. It was considered that the disagreement of the onset time between the spontaneous movement of mice and the EEG changes induced by DN was due to species differences and the method of administration etc. These findings indicate that DN had an inhibitory effect on the central nervous system. On the other hand, DN induced a depression of the EEG arousal responses by high frequency stimulation of the sciatic nerve and increase in the threshold of stimulation in the acute cats. The light sleep pattern on EEGs, the sedation and the depression of EEG arousal response are probably related to the inhibitory effect on the ascending activating system. There are numerous reports concerning the function of the central adrenergic (11) and the cholinergic neurons (12) related to the brainstem activating system. Thus, the combined use of various drugs was examined by assessing activities of the EEGs in order to determine the mechanism involved in the inhibitory effect of DN on the central nervous system. EEG activities induced by DN were significantly inhibited by epinephrine hydrochloride. Even in spinal cats, similar EEG changes were seen, and the duration of EEG changes was long-lasting. Therefore, the EEG changes of DN induced by epinephrine hydrochloride did not appear to be secondary alterations in the peripheral nerve. In addition, the EEG activities of DN were potentiated by tolazoline hydrochloride, phen tolamine mesylate and dibenamine hydrochloride as α-adrenergic blocking agents. This result indicates that DN affected the α-adrenergic neurons. Keane (13) reported that noradrenaline receptor blockers produced the appearance of slow waves and spindle burst-like waves on the spontaneous EEG activity and that the appearance of these EEG patterns was related to the adrenergic neurons. Thus, it might be considered that the inhibitory effect of DN on the central nervous system involved an inhibitory effect on adrenergic neurons. On the other hand, we have reported that the EEG patterns induced by DN were inhibited by atropine sulfate, and that the hypotensive effect in dogs was antagonized by pretreatment with atropine sulfate (14, 15). Thus, cholinergic neurons are probably involved with the actions of DN.

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