ACTIONS OF DESOXYNUPHARIDINE HYDROCHLORIDE IN THE CENTRAL NERVOUS SYSTEM OF CATS (II)

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Abstract—Effects of desoxynupharidine hydrochloride (DN) on the central nervous system of cats were studied using electric physiological techniques. With regard to the EEG arousal responses, DN (1-3 mg/kg, i.v.) inhibited the responses induced by midbrain reticular formation and posterior hypothalamus (P-Hypo) stimulation. The threshold voltage of stimulation was elevated. DN decreased the amplitude of the augmenting response and the recruiting response induced by the stimulation of specific and non-specific pathways in the thalamus. As to the evoked potentials in the somatosensory area I, DN decreased the amplitude of the fast and late components. This compound slightly inhibited the monosynaptic and polysynaptic reflexes, in the spinal cats. With regard to autonomic responses on stimulation of the P-Hypo, DN decreased contractions in the nictitating membrane, the hypertensive action and the galvanic skin response. Our findings indicate that the inhibitory effects of DN on the central nervous system may be related to the brainstem reticular formation and the hypothalamus activating system, the non-specific pathway, the specific pathway and the limbic system etc., and may involve the central anti-adrenergic actions.

In a foregoing study (1-3), DN was found to decrease spontaneous movement and prolong hexobarbital-Na sleeping time in mice. In acute and chronic cats, 12-14 Hz waves, slow waves and 12-14 Hz waves superimposed on the high amplitude slow wave, and the so-called light sleep pattern appeared on the all EEGs. These EEG changes seen with DN were inhibited by epinephrine hydrochloride and potentiated by α-adrenergic blocking agents. The behavior in the chronic cats was sedative. Thus, DN had an inhibitory effect on the central nervous system and anti-adrenergic action were assumedly involved.

Cats were used in the present work to examine the EEG arousal responses produced by high frequency stimulation of the midbrain reticular formation and the posterior hypothalamus, the recruiting and augmenting responses, contraction of the nictitating membrane, hypertensive actions and the galvanic skin response produced by the stimulation of the posterior hypothalamus.

MATERIALS AND METHODS

Thirty-seven adult cats of both sexes were used. Animals were fixed on a stereotaxic instrument (Todai Nohken type) following anesthetization with ether. After tracheotomy, the cranial bone was exposed and stainless steel concentric electrodes with a diameter 0.8 mm were placed in midbrain reticular formation (MRF) (F: 2.0, L: 3.0, H: -3.0),
nucl. centrum medianum (CM) (F: 7.0, L: 2.5, H: +1.0), nucl. ventralis postero-lateralis (VPL) (F: 9.0, L: 7.0, H: +1.0), posterior hypothalamus (P-Hypo) (F: 10.0, L: 1.0, H: -5.0), hippocampus dorsalis (D-Hippo) (F: 3.0, L: 11.0, H: +2.5) and amygdala (Amy) (F: 13.0, L: 9.0, H: -5.0), according to the atlas of Jasper and Ajimone-Marsan (4).

Metal screw electrodes were placed on the frontal (F: 26.0, L: 6.0), temporal (F: 8.0, L: 16.0) and occipital (F: -2.0, L: 8.0) cortices. The indifferent electrode was placed on the frontal cranial bone. After the recovery from ether anesthesia, the experiments were conducted with the cats under artificial respiration (the rate: 25 rounds/min, 12.4 ml) in paralysis with gallamine triethiodide (5-10 mg/kg, i.v.). Blood pressure, ECG, heart rate, body temperature, Hb and O₂ saturation were always recorded during the experiments. All 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 /1. The areas of electrode insertion were checked histologically. In the following experiments, the evoked responses were averaged by signal processor 7T07 (San- ei Instrument Co., Ltd.) via electroencephalography (Nihon Kohden) and these recordings were made on an X-Y recorder WX442 (WATANABE). The upper side was expressed as a negative response and the lower side as a positive one.

EEG arousal responses: Effects of DN on the EEG arousal responses produced by high frequency stimulation of MRF (100 Hz, 0.1-0.2 msec, 1.5-2.5 V, 8 sec) and P-Hypo (100 Hz, 1 msec, 3.5-4.0 V, 8 sec) using a digital stimulator (MEC) were recorded on the posterior sigmoid gyrus (PSG). The evoked responses obtained by 48 stimuli were averaged and effects of DN on these responses were examined.

Photic driving responses: The photic driving responses in the posterior lateral gyrus produced by forty strobo flashes with 1 Hz using a photic stimulator (Nihon Kohden) placed at a distance of 20 cm from the eyes of the cats were averaged. The extra-light was blocked by a curtain.

Evoked responses in the somatosensory area i: The stimulating site in the peripheral nerve was the sciatic nerve (SN). SN and the somatosensory area I (SSA-I) were exposed following a standard method and the tissue was covered with liquid paraffin to prevent drying. The monopolar silver ball electrode of 0.5 mm in diameter was used to record the evoked responses. The contralateral nerve against the recording site was stimulated at 10 sec intervals using a bipolar platinum electrode (0.2 m/sec, 4 V, a single rectangular wave). Effects of DN on the evoked responses of an average of 20 stimuli were examined.

Spinal reflex action potentials: Spinal reflex action potentials were assessed in the intact and spinal preparations. In the intact cats, after the spinal cord was exposed by mean of laminectomy the action potentials of the monosynaptic and polysynaptic reflexes in the ipsilateral ventral root were monitored on an oscilloscope (San- ei Instrument Co., Ltd.) by stimulating the dorsal root of L7 or S1, with a single stimulation per 5 sec (0.05-1 msec, supramaximal voltage 2-4 V). The exposed nervous tissue was covered with warmed paraffin and the temperature of its pool was kept between 36-38°C. Spinal cats were prepared by sectioning from T₁ to L₁ and the procedure was the same as above method.
Sympathetic responses: The sympathetic excitatory phenomena produced by high frequency stimulation of P-Hypo (50 Hz, 1 msec, 3–4 V, 10 sec) were recorded as an indicator of the contraction of the nictitating membrane, hypertensive action and the galvanic skin response (GSR) in the cats. The stimulating electrode in P-Hypo was implanted in the area showing evidence of the largest contraction of the nictitating membrane. A force-displacement transducer (Nihon Kohden, SB-1T) was used. Simultaneously, the hypertensive action of the drug was assessed using the femoral artery. For GSR recording, a silver plate electrode with a diameter of 4 mm was placed on the hindlimb pad. The indifferent electrode was mounted on the skin of the lower leg which had been depilated of hair. The difference in electric potentials between two electrodes was recorded via electroencephalography.

Desoxynupharidine HCI was obtained from the Department of Synthetic Organic Chemistry in Showa College of Pharmaceutical Sciences, and the chemical formula is C₁₅H₂₃ON.

RESULTS

EEG arousal responses

We observed the EEG arousal responses which the low voltage fast wave in the cortex, the Amy, and the hippocampal arousal wave in the Hippo produced by the high frequency stimulation of the MRF, the P-Hypo and by auditory stimulation. Effects of DN (1–3 mg/kg, i.v.) on the EEG arousal responses were examined.

After injection of DN, 13–14 Hz waves and the marked high amplitude slow wave appeared in the Amy, MRF and cortex. The hippocampal arousal wave gave way to irregularities in the Hippo. The spindle burst-like waves then spread to all EEGs, and the EEG pattern became the so-called drowsy pattern.

As shown in Fig. 1, the EEG arousal response produced by the high frequency stimulation of the MRF was markedly inhibited at 15–30 min. Moreover, the arousal waves after the stimulation were shortened and almost at the same time after termination of the stimulation, the EEGs returned to the drowsy pattern. These effects were recovered at 90–120 min. The threshold voltage producing the EEG arousal response was
As shown in Fig. 2, the EEG arousal response produced by high frequency stimulation of the P-Hypo was slightly inhibited at 15-30 min and the arousal waves after the stimulation were slightly shortened. Recovery was seen at 90-120 min. The threshold voltage producing the EEG arousal response was elevated to about 50-80%.

As shown in Fig. 3, the EEG arousal response produced by auditory stimulation was inhibited. Here also, recovery was seen at 90-120 min.

DN inhibited the EEG arousal responses produced by high frequency stimulation of the MRF and the P-Hypo and by the auditory stimulation. In particular inhibition was seen with stimulation of the MRF.

Recruiting response

The recruiting response produced by the low frequency stimulation of CM (6 Hz, 0.5 msec, 4 V, 8 sec) was observed in the cortex. The averaged responses in the posterior sigmoid gyrus had positive and negative components and the peak was seen at about 14 and 38 msec, respectively. Effects of DN on the amplitude from the basic line to the peak were also examined.

When DN was given i.v. in a dose of 1 mg/kg, no marked alteration occurred. As shown in Fig. 4, in a dose of 2 mg/kg, the amplitude decreased to about 25% at 30 min. Recovery occurred in about 90 min. With a dose of 3 mg/kg, the amplitude decreased to about 40% at 30 min and here the recovery was evident at about 120 min. DN (1-3 mg/kg, i.v.) had little influence on the positive component.

Augmenting response

The augmenting response produced by the low frequency stimulation of the VPL (6 Hz, 0.5 msec, 4 V, 8 sec) was observed exclusively in the somatosensory area of the neocortex. The averaged responses in the posterior sigmoid gyrus had positive and negative components.
negative components with the time to peak of about 6 and 26 msec, respectively. Effects of DN on the amplitude from the basic line to the peak were then examined.

When DN was given i.v. in a dose of 1 mg/kg, no marked alteration occurred. As shown in Fig. 5, with a dose of 2 mg/kg, the amplitude decreased to about 20% at 30 min and recovery was seen after 90 min. In a dose of 3 mg/kg, the amplitude decreased to about 35% at 30 min and these effects tended to recover from 120 min. DN (1–3 mg/kg) had little influence on the positive component.

**Photic driving response**

The photic driving response in posterior lateral gyrus was produced by strobo flash stimulation. The averaged responses had a positive 1, negative and positive 2 components with the time to peak of about 24, 42 and 100 msec, respectively. Effects of DN on the peak to peak amplitude of positive 1-negative component were then examined.

DN (1–3 mg/kg, i.v.) had little influence on the photic driving response.

**Evoked potentials in the somatosensory area I**

The evoked potentials in SSA-I were produced by SN stimulation. The averaged responses had fast and late components with the time to peak of about 11 and 40 msec. Effects of DN on the amplitude from the basic line to the peak were also examined.

DN (1 mg/kg, i.v.) influenced little the fast and late components. As shown in Fig. 6, injection of 2 mg/kg decreased the amplitude of the late component by about 20% at 30 min. Injection of 3 mg/kg decreased the amplitude of the fast component and the late one about 15% and 40%, respectively. Control levels were reverted to at 150–180 min.

**Spinal reflex action potentials**

The effects on the ventral root potentials in the spinal reflex were examined with the amplitude of the monosynaptic reflex (MSR) and the contents of the polysynaptic reflex (PSR).

1) **Intact cats**: Injection of DN (1 mg/kg) produced a decrease in MSR and PSR from 10 min, and there was a decrease by 25–35% and 30–40%, respectively, at 20–30 min. Recovery was seen at 90–120 min. As shown in Fig. 7A, with the dose of 2 mg/kg, MSR and PSR were decreased by 30–40% and 35–45% at 20–30 min, and recovery occurred at 120–150 min. With the dose of 3 mg/kg, MSR and PSR began to decrease.

![Fig. 6. Effect of DN 2 mg/kg, i.v. on the evoked potentials in the somatosensory area I produced by the sciatic nerve stimulation in a cat (0.2 msec, 4 V, a single rectangular wave). Potentials illustrated are averages of 20 responses. The timing of the stimulus is indicated by dots.](image)

![Fig. 7. Effect of DN 2 mg/kg, i.v. on the spinal reflex action potentials control, 10 min, 20 min, 30 min, 60 min and 90 min after injection of DN. A=intact cat, B=spinal cat. Potentials illustrated are averages of 6 responses.](image)
from 5 min and were decreased by 35-45% and 45-50% at 20-30 min. The recovery was seen at 150-180 min.

2) Spinal cats: With the injection of DN (1 mg/kg), at 10 min MSR and PSR were decreased by 10-15% and 15-20%, and recovery was seen at 45-60 min. As shown in Fig. 7B, with the dose of 2 mg/kg, MSR and PSR were decreased by 15-25% and 25-30% at 10 min, and these effects recovered at 60-90 min. With the dose of 3 mg/kg, at 5 min MSR and PSR were decreased by 25-30% and 30-40% respectively, and these effects recovered at 120-150 min.

Sympathetic responses

As shown in Table 1, DN (1 mg/kg) had no influence on the contraction of the nictitating membrane, the hypertensive action and GSR produced by the P-Hypo stimulation. Injection of 2 mg/kg decreased the contraction of the nictitating membrane by 15%, the hypertensive action by 14%, and GSR by 11%, at 10 min. Control levels were reverted to within 60 min. Injection of 3 mg/kg decreased the contraction of the nictitating membrane by 19%, the hypertensive action by 23%, and GSR by 12%, at 10 min.

**DISCUSSION**

Effects of DN on the central nervous system were mainly examined using the EEG arousal responses produced by the high frequency stimulation of the MRF and the P-Hypo, the augmenting response and the recruiting response, contraction of the nictitating membrane, hypertensive action and the galvanic skin response (GSR) by the P-Hypo stimulation.

In the case of the EEG arousal responses, DN (1-3 mg/kg) inhibited the response induced by MRF stimulation, and in particular, the injection of 3 mg/kg elevated the threshold voltage of stimulation by 50-80%. The response induced by the P-Hypo stimulation was elevated to 30-40%. The activating system consists of the brainstem reticular activating system (5) and the hypothalamic activating system (6). In the spontaneous EEGs of the acute and chronic cats (1-3), DN produced 12-14 Hz waves followed. The above waves transferred to the Hypo, MRF and cortices; in particular in the cortices, the above waves spread from the frontal cortex to all the cortices. In the Hippo, the hippocampal arousal wave gave way to irregular waves. Thus, DN apparently

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**Table 1. Effect of DN on the contraction of the nictitating membrane (NM), the hypertensive action (BP) and the galvanic skin response (GSR) induced by P-Hypo stimulation**

| Drugs | Doses (mg/kg, i.v.) | NM (mm) mean±S.D. | MN Inhibitory % | BP (mmHg) mean±S.D. | MN Inhibitory % | GSR (mV) mean±S.D. | MN Inhibitory % |
|-------|---------------------|------------------|----------------|--------------------|----------------|------------------|----------------|
| Saline | 1.0                 | 29.9±3.4         |                | 54.0±5.3           |                | 4.8±0.2          |                |
| DN     | 2.0                 | 26.3±1.0         | 12.0           | 46.7±4.5           | 8.0            | 4.5±0.1          | 5.4            |
|        | 3.0                 | 24.9±1.0*        | 16.7           | 47.6±2.2*          | 11.9           | 4.4±0.1**        | 7.5            |

The data (N=5) are the mean±S.D. at 10 min following injection. * and ** indicate the significant level at P<0.05 and P<0.01 respectively.
affects not only two activating systems but also the limbic system.

Since DN produced 20–30% inhibition of the amplitude on the recruiting response and the augmenting response induced by the stimulation of the non-specific and specific pathways, respectively, DN may have an inhibitory effect on the thalamo-cortico reverberating circuit and the thalamic specific sensory system.

Regarding the evoked responses in the somatosensory area I, DN had a greater inhibition the late than the fast component; and the PSR more than the MSR on the ventral root potentials of the spinal reflex. In addition, inhibitory effects of DN on MSR and PSR in the spinal cats were weaker than those in the intact cats. Therefore, effect of DN on the spinal reflex may be due to an influence of a higher centers.

The electrical stimulation of the Hypo produces various autonomic responses (7–9). When DN was given i.v., contraction of the nictitating membrane, the hypertensive action and GSR induced by the P-Hypo stimulation were inhibited by 19%, 23% and 12%, respectively. In a previous study (1–3), we found that the EEG changes by DN were inhibited by epinephrine HCl and potentiated by α-adrenergic blocking agents. Thus, the inhibitory effects of DN on the central nervous system apparently involve a central anti-adrenergic action.

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