SPINAL REFLEXES IN CHINOFORM-ADMINISTERED RATS

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Abstracts—We examined the effects of chinoform (CF) on the spinal reflexes and the descending influences on the spinal reflexes from the locus coeruleus (LC) and the nucleus raphe magnus (NRM) in rats. The spinal reflex potential was recorded from the L5 ventral root following stimulation of the L5 dorsal root, and the effects of electrical stimulation of the LC and the NRM were tested in anesthetized rats. CF was suspended in Tween 80 and administered for two days (400 mg/kg, i. p./day) before the measurement of the spinal reflexes. In all rats treated with CF, death or motor incoordinations such as abnormal gait and hindlimb ataxia were observed. However, the control and CF-treated groups are not different in the amplitude and shape of the reflexes and in the influences of the LC and the NRM on the reflexes from the LC and the NRM. These results suggest that segmental spinal reflexes and descending influences from the LC and the NRM are not affected in rats suffered from motor incoordination by acute CF.

Key words: Chinoform, spinal reflex, incoordination, motor system

INTRODUCTION

In dogs (Tateishi et al., 1972; Tateishi et al., 1973), cats (Tateishi et al., 1973) and monkeys (Tateishi et al., 1971), chinoform (CF) causes neurological symptoms and pathological changes similar to those of subacute myelo-optico-neuropathy (SMON). However, in small animals such as rats and mice, neurological and pathological changes have not been observed after oral administration of CF (Hess et al., 1972). Recently, it was shown that repeated intraperitoneal administration of CF can cause ataxia in rats (Kotaki et al., 1983). It is possible that the ataxia rises from functional disorder in motor systems of the spinal cord.

Spinal reflexes, indices of activity of the spinal motor systems, are affected by supraspinal monoaminergic systems. Conditioning stimulation of the locus coeruleus, a
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pontine noradrenergic nucleus, facilitates the monosynaptic reflex (MSR) in cats and the facilitation is blocked by alpha-blockers (Strahlendorf et al., 1980). Conditioning stimulation of the nucleus raphe magnus, a serotonergic nucleus in the medulla oblongata, facilitates the MSR and inhibits the polysynaptic reflex (PSR) in rats and the PSR inhibition is attenuated by serotonin depletors (Kaneko et al., 1983).

In the present study, the spinal reflexes and the effects of conditioning stimulation of the monoaminergic nuclei on the reflexes were examined in CF-administered rats.

MATERIALS AND METHODS

Administration of CF

20 and 4 male Wistar rats (300-400 g) were used for CF-administered and control groups, respectively. CF was suspended in 4% Tween 80 and injected intraperitoneally to rats at the dose of 400 mg/kg/day for 2 days.

Measurement of spinal reflexes

Rats were anesthetized with urethane (1 g/kg, i. p.) and alpha-chloralose (25 mg/kg, i. p.) and fixed on a stereotaxic apparatus. Laminectomy was performed in the lumbo-sacral region and the dorsal and ventral roots below the L5 segment were cut. A skin pouch was formed at the site of the dissection and the exposed tissues were covered with liquid paraffin thermoregulated at 37 ± 1°C. The dorsal and ventral roots of L5 segment were isolated and the L5 dorsal root was placed on a bipolar silver electrode for stimulation (0.05 msec, 0.1 Hz, supramaximal). For recording, the L5 ventral root was placed on a monopolar silver electrode and, as an indiffereent electrode, a steel clip was attached to the clamp which fixed the vertebra. Rats were artificially ventilated and the rectal temperature was maintained at 37 ± 1°C.

Conditioning stimulation

Conditioning stimulation was performed by the method similar to that reported by Kaneko et al. (1984). A concentric stainless steel electrode (1.0 mm diameter, 1.0 mm interpolar distance) was placed into the brain stem at the aimed coordinates, according to the atlas of Pellegrino et al. (1979). Conditioning stimulation (0.1 mA, 0.1 msec, 200 Hz, 4 trains) was delivered between the inner tip of the electrode and the steel clip attached to the head skin. If neccessary, rats were immobilized with d-tubocurarine chloride (2 mg/kg, s. c.).

Drugs

CF (Sigma), urethane (Wako), alpha-chloralose (Tokyo Kasei) and d-tubocurarine chloride (Tokyo Kasei) were used.

RESULTS

After the first CF administration, the rats showed incoordination: hip-raising awkward gait, hindlimb ataxia. The incoordination was often observed within 60 min after the first CF administration. Two days after the first CF administration, 14 out of 20 rats died and the rest 6 rats maintained the incoordination. The survived rats
Chinoform and spinal reflexes were used for measurement of the spinal reflexes.

In the shape of the MSR and PSR, no difference was observed between in the CF-administered rats and in the control rats. There was no notable difference in the MSR and PSR amplitude between in the two groups (Fig. 1.).

![Graph](image)

**Fig. 1.** Reflex amplitudes in chinoform-administered and control rats. Closed and open columns represent the reflex amplitudes in chinoform-administered rats and control rats, respectively. MSR and PSR amplitudes are shown on the left and right, respectively, of the figure. Each column represents the mean ± SEM of 4 experiments. Chinoform was administered at the dose of 400 mg/kg, i.p./day for 2 days.

Conditioning stimulation of the locus coeruleus facilitated the MSR, when the interval between the conditioning stimulation and the dorsal root stimulation (C-T interval) was set at 20-100 msec. The maximal facilitation was obtained at 20 msec C-T interval. The MSR facilitation evoked by the locus coeruleus stimulation was obtained in the CF-administered rats as well as in the control rats, and there was no notable difference in the extent of the MSR facilitation in the two groups (Fig. 2.).

Conditioning stimulation of the nucleus raphe magnus facilitated the MSR and inhibited the PSR. The maximal effects of the MSR facilitation and the PSR inhibition were observed at 20 msec and 30-40 msec C-T interval, respectively. The MSR facilitation and the PSR inhibition were obtained in the CF-administered rats as well as in the control rats, and there was no notable difference in the extent of the MSR facilitation and the PSR inhibition in the two groups (Fig. 3.).
Fig. 2. MSR facilitation by conditioning stimulation of the locus coeruleus in chinoform-administered and control rats. Each point represents the conditioned MSR (mean±SEM) calculated as a percentage of the unconditioned MSR amplitude. Closed and open circles represent the conditioned MSR in chinoform-administered rats (n = 3) and control rats (n = 4), respectively. Chinoform was administered at the dose of 400 mg/kg, i. p./day for 2 days and the spinal reflexes were measured 2 days after the first administration.

Fig. 3. Effects of conditioning stimulation of the nucleus raphe magnus in chinoform-administered and control rats. Closed and open symbols represent the conditioned reflexes in chinoform-administered rats and control rats, respectively. Circle and square symbols represent the conditioned MSR and PSR, respectively. Each point represents the conditioned reflex amplitude (mean±SEM of 4 experiments) calculated as a percentage of the unconditioned reflex amplitude. Chinoform was administered at the dose of 400 mg/kg, i. p./day for 2 days, and the spinal reflexes were measured 2 days after the first administration.
Chinoform and spinal reflexes

Since the incoordination was often observed within 60 min after the first CF administration, the change of the spinal reflexes were examined after a single administration of CF (400 mg/kg, i. p.). No notable change in the MSR and PSR was observed after CF injection (Fig. 4.).

![Graph showing effects of chinoform on spinal reflexes](image)

**Fig. 4.** Effects of chinoform on spinal reflexes. Chinoform was administered intraperitoneally at the arrow. Each point represents reflex amplitude (mean ± SEM of 4 experiments) calculated at a percentage of the value just before chinoform injection.

**DISCUSSION**

The incoordination observed in CF-administered rats in this study was similar to that described by Kotaki et al. (1983), but the onset of the incoordination was earlier. This difference might be mainly due to the preparation of CF suspension: The diameter of CF particles in the suspension was about 2 μm in this paper, whereas it was 5 μm in the paper by Kotaki et al. (1983).

In this study, there was no difference in the shape and the amplitude of the spinal reflexes between CF-administered and control rats. In addition, the function of the descending pathways from the brain stem monoaminergic nuclei to the spinal cord was normal in the CF-administered rats. Therefore, it is not likely that the incoordination arises from the dysfunction in the spinal reflex systems and the descending monoaminergic systems. The incoordination was possibly caused by functional disorder in the upper central nervous systems or peripheral nervous systems.

The incoordination observed in this study is hardly thought to be due to the pathological changes, because the incoordination occurred within 60 min after the first administration of CF. Therefore, these symptoms may differ from SMON's, which are
accompanied by the pathological changes, and may be related only to the process in developing SMON.

In conclusion, it is suggested that the incoordination which occurred with acute CF in the early days does not arise from functional disorder in the central spinal reflex systems and the descending monoaminergic systems.

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REFERENCES