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Responses of the Flexor Reflex to LSD, Tryptamine, 5-Hydroxytryptophan, Methoxamine, and *d*-Amphetamine in Acute and Chronic Spinal Rats*

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Abstract. The flexor reflex of acute (40–48 h after mid-thoracic spinal transection) and chronic (at least 2 months after transection) spinal rats was evoked by tetanic electrical stimulation of both hindfeet and recorded on a polygraph using a transducer connected to the left hindfoot. The flexor reflex in the chronic spinal rat was more responsive to electrical stimulation and to the actions of drugs studied than was the flexor reflex in the acute spinal rat. In chronic spinal rats, *d*-amphetamine, methoxamine, LSD, tryptamine, and 5-hydroxytryptophan (5-HTP) facilitated the flexor reflex and induced spontaneous movements. These facilitative effects were seen in acute spinal rats only when much larger i.p. doses of amphetamine, methoxamine, and LSD were used. Small i.v. doses of tryptamine also produced the facilitation. The facilitation caused by LSD and tryptamine, but not 5-HTP, in chronic spinal rats was antagonized by cyproheptadine. These observations suggest that chronic spinal rats were more sensitive to the drugs than acute spinal rats and support the hypothesis that the mode of action of LSD is similar to that of tryptamine but different from that of 5-HTP since cyproheptadine antagonized the facilitative effects of LSD and tryptamine but not those of 5-HTP.

Key words: LSD – Tryptamine – 5-HTP – Flexor reflex – Acute spinal rats – Chronic spinal rats

The existence of descending adrenergic and serotonergic spinal cord pathways is well established (Carlsson et al., 1963; Dahlström and Fuxe, 1965; Andén et al., 1964a) and indirect evidence has been obtained suggesting a descending tryptaminergic pathway in the spinal cord (Martin et al., 1975, 1976). Evidence bearing on the physiologic role of these adrenergic, serotonergic, and tryptaminergic pathways in the spinal cord has been obtained in the cat, dog, and rat, using different procedures and prototypic agonists and antagonists, as well as different preparations (e.g., acute and chronic spinal animals) and different routes for administering drugs. The present study was conducted to study the effects of a group of presumed noradrenergic, tryptaminergic, and serotonergic agonists and an antagonist and their interactions on the flexor reflex of both the acute and chronic spinal rat.

Studies on the mode of the hallucinogenic action of LSD have produced several hypotheses (see review of Brawley and Duffield, 1972). One of these (Andén et al., 1968, 1971) is that LSD acts directly to activate serotonergic receptors. This conclusion was reached because high doses of LSD (4.0 mg/kg), and 5-HTP (10–75 mg/kg), a serotonin precursor, produced tonic extension of the hindlegs in acute spinal rats and the retardation of serotonin turnover by LSD due to negative feed-back mechanisms evoked by direct stimulation of central serotonergic receptors. In contrast to these observations, Martin and Eades (1970) showed that the mode of action of LSD in facilitating the flexor reflex in the chronic spinal dog was similar to that of tryptamine because the facilitative actions of tryptamine (0.5 mg/kg/min) and small doses of LSD (15 µg/kg) but not 5-HTP were blocked by cyproheptadine. Also, cross tolerance developed to tryptamine in LSD tolerant dogs (Martin and Eades, 1972). In order to generate data that could resolve these apparent discrepancies, studies were conducted to compare the actions of various doses of LSD, tryptamine, and 5-HTP in acute and chronic spinal rats.

* A preliminary report of this work was presented in 1976 at the Meeting of the Federation of American Societies for Experimental Biology, Anaheim, California (see Nozaki and Bell, 1976)

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MATERIALS AND METHODS

Subjects. Two hundred seventy-seven female Wistar rats (Laboratory Supply, Indianapolis, Indiana) weighing 180–200 g were spinalized by removal of a segment of the mid-thoracic spinal cord (T-4 to T-6) using suction under ether anesthesia. The experiments were conducted 40–48 h after the spinalization in 130 acute spinal rats (body weight 180–200 g) or 2 months after transection in 147 chronic spinal rats (body weight 250–280 g). The spinal rats required special care during the early postoperative period. Four to five spinal rats were housed in cages containing a layer of sawdust. Because the rats urinated frequently 2–4 days after the transection and bit themselves, their abdomens were cleaned daily by washing with warm water. After 1–2 weeks most spinal rats required little care other than a change of sawdust once every 2 days.

Apparatus. The following studies employed modifications of methods that have been previously described (Wikler and Frank, 1948; Martin and Eades, 1967, 1970). Spinal rats were taped in the prone position to an animal board. The hindlimb flexor reflex was elicited by a tetanic stimulus consisting of a 200 ms train of 100 Hz, 1 ms in duration square waves through electrodes applied to both hindfeet with electrode paste. The stimulus was given every 10 s throughout the experiment. A mean stimulating voltage was selected which gave a flexor reflex deflection of the hindfoot of 2–2.5 cm that was recorded as a 1 cm deflection on the polygraph (Fig. 1). The polygraph gave a continuous recording of the flexor reflex as well as spontaneous movements that were measured isotonically with a transducer connected to the left hindfoot. A 10-min predrug control was obtained in all studies. Drug effects were then expressed as a percentage of this control.

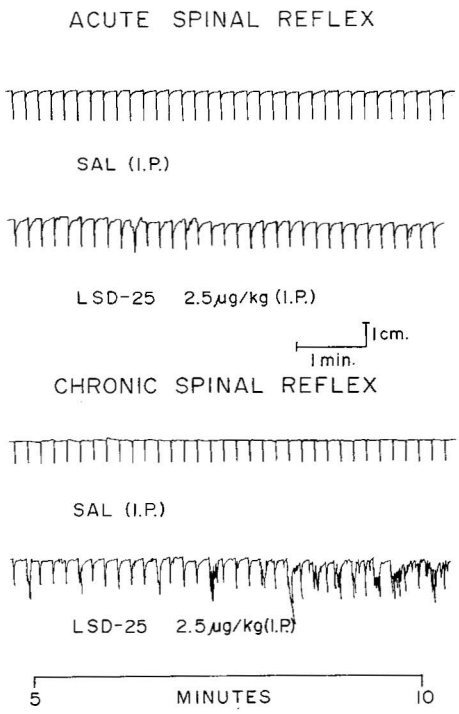

Fig. 1. Effect of saline and LSD on flexor reflex of acute and chronic spinal rats during 5–10 min after drug administration. In acute spinal rats LSD 2.5 µg/kg did not facilitate flexor reflex and did not produce spontaneous movements. However, in chronic spinal rats same dose of LSD enhanced amplitude of flexor reflex and produced spontaneous movements

Procedure. Drugs were administered i.p. (0.5 ml/100 g), except tryptamine and L-tryptophan (1.0 ml/100 g), which were administered i.p. in some experiments and i.v. in others. All drug solutions were made up in normal saline.

Drugs. The doses of drugs and the route of the administration are listed in Table 1. The i.v. dose of tryptamine is described case by case in the text.

Statistics. Areas under the time action curves were calculated during a 60-min experimental period following a 10-min control period or 30-min after the administration of the antagonist.

Because tryptamine had a short duration of action, areas under the time action curves during a 10-min period after its i.p. injection were analyzed. Statistical evaluations for areas were made using the independent *t*-test. The accommodation of the flexor reflexes was analyzed by both independent and paired *t*-tests.

RESULTS

The strength of stimulation necessary to evoke flexor reflexes of the same amplitude was significantly greater ($P < 0.001$) in the acute (36.90 ± 2.54 v) than in the chronic spinal rat (13.64 ± 0.87 v). Although more accommodation of the flexor reflex occurred 30 to 60 min after saline injection in chronic spinal rats ($P < 0.05$) than in acute spinal rats, the difference of the areas under the time action curves during the total 60-min experimental period was not significant (Table 1).

Table 1 summarizes the effects of *d*-amphetamine, methoxamine, LSD, tryptamine, 5-HTP, and L-tryptophan in both acute and chronic spinal rats. The amplitude of the flexor reflex in the acute spinal rat was significantly facilitated by the i.p. injection of *d*-amphetamine (2.0, 4.0, and 5.0 mg/kg), methoxamine (8.75 mg/kg), and LSD (1.25 and 4.0 mg/kg). Small doses of i.p. injected methoxamine (0.875–4.375 mg/kg), LSD (2.5–100 µg/kg), tryptamine (6 mg/kg), 5-HTP (2.5–50 mg/kg), i.v. injection of L-tryptophan (150 and 300 mg/kg), and large doses of i.p. injected tryptamine (50 mg/kg) failed to facilitate the flexor reflex. However, i.v. injected tryptamine (3.0 mg/kg) in acute spinal rats produced tonic extension of the hip and knee on which was superimposed knee flexion produced by the stimulus. The flexor reflex was depressed during tonic extension with low doses of tryptamine and following tonic extension after i.v. injection of higher doses (6.0–25.0 mg/kg) of tryptamine. In chronic spinal rats, small i.p. doses of amphetamine (2.0 mg/kg), methoxamine (0.875 mg/kg), LSD (2.5 µg/kg), tryptamine (6.0 mg/kg), and 5-HTP (3.75 mg/kg) facilitated the flexor reflex and also produced spontaneous movements (Table 1). The duration of the facilitative effects of tryptamine on the flexor reflex was much shorter than those of LSD or

Table 1. The effects of *d*-amphetamine, methoxamine, LSD, tryptamine, 5-HTP, and L-tryptophan on the flexor reflex of acute and chronic spinal rats

Drug	Dose	Route	*n*	Area Acute spinal rats	*n*	Area Chronic spinal rats
Saline	5.0 ml/kg	i.p.	8	−23.00 (−2.66)	6	−55.42 (−0.46)
d-Amphetamine	1.0 ml/kg	i.p.	—	—	4	−26.38
	2.0 ml/kg	i.p.	4	60.94*	4	248.81**
	4.0 ml/kg	i.p.	4	63.88**	—	—
	5.0 mg/kg	i.p.	4	70.56**	—	—
Methoxamine	0.875 mg/kg	i.p.	4	−27.56	7	116.54*
	4.375 mg/kg	i.p.	4	25.93	—	—
	8.750 mg/kg	i.p.	8	61.47**	—	—
LSD	2.5 µg/kg	i.p.	4	−29.31	6	215.25***
	100.0 µg/kg	i.p.	4	−5.63	—	—
	1.25 mg/kg	i.p.	7	90.07*	—	—
	4.0 mg/kg	i.p.	4	154.56*	—	—
Tryptamine	6.0 mg/kg	i.p.	7	(1.04)	5	(8.65)*
	12.0 mg/kg	i.p.	—	—	6	(18.43)*
	25.0 mg/kg	i.p.	—	—	4	(44.19)*
	50.0 mg/kg	i.p.	7	(−7.18)	7	(36.46)**
5-HTP	2.5 mg/kg	i.p.	—	—	4	27.06
	3.75 mg/kg	i.p.	—	—	4	79.13*
	5.0 mg/kg	i.p.	4	−37.25	4	177.38*
	50.0 mg/kg	i.p.	5	32.85	—	—
L-Tryptophan	150.0 mg/kg	i.v.	7	0.04	6	−21.17
	300.0 mg/kg	i.v.	8	9.94	—	—

Each value is mean of area under time action curve during 60 min after drug injection. Parentheses show area during 10 min after the injection

* $P < 0.05$ for difference from control (saline)

** $P < 0.01$

*** $P < 0.001$

5-HTP. Facilitation produced by these relatively low doses of drugs in chronic spinal rats was greater than that produced by higher doses in acute spinal rats. Also, spontaneous movements were not seen in acute spinal rats (Fig. 1). L-Tryptophan did not significantly change the flexor reflex in either acute or chronic spinal rats, but it produced a facilitative trend in acute spinal rats (Table 1).

Figure 2 summarizes the effect of cyproheptadine on the facilitative effects of LSD, tryptamine, and 5-HTP in the chronic spinal rat. Cyproheptadine (0.4–5.0 mg/kg) did not change the amplitude of the flexor reflex itself. As can be seen in Figure 2, cyproheptadine completely antagonized the facilitative effects and spontaneous movements produced by LSD (2.5 µg/kg) ($P < 0.0001$) and tryptamine (6.0 mg/kg) ($P < 0.05$). Cyproheptadine (0.4 mg/kg) also antagonized the effect of 12.0 mg/kg of tryptamine by significantly decreasing the increase in reflex amplitude ($P < 0.05$) and by partially preventing the spontaneous movements.

On the other hand, cyproheptadine (0.4 mg/kg) failed to antagonize the production of spontaneous movements and the facilitation of the flexor reflex produced by 5-HTP (3.75 mg/kg) (Fig. 2), a dose that completely antagonized the facilitative effect of LSD and tryptamine in the chronic spinal rat. A larger dose of cyproheptadine (5.0 mg/kg) produced a modest but not statistically significant decrease in 5-HTP-induced facilitation (Fig. 2).

DISCUSSION

The present studies indicate that denervation hypersensitivity is observed in chronic spinal rats to electrical stimulation as well as to drugs and that the mode of action of LSD is similar to that of tryptamine but different from that of 5-HTP. The phenomenon of posttransectional hyperflexia is well known, but its mechanism is not well understood. Cannon and Rosenblueth (1949) believed it to be due to denervation hypersensitivity, but McCouch et al. (1958) have suggested that increased reflex responsiveness following chronic spinal cord transection could be due to increased innervation of spinal neurons that occurs

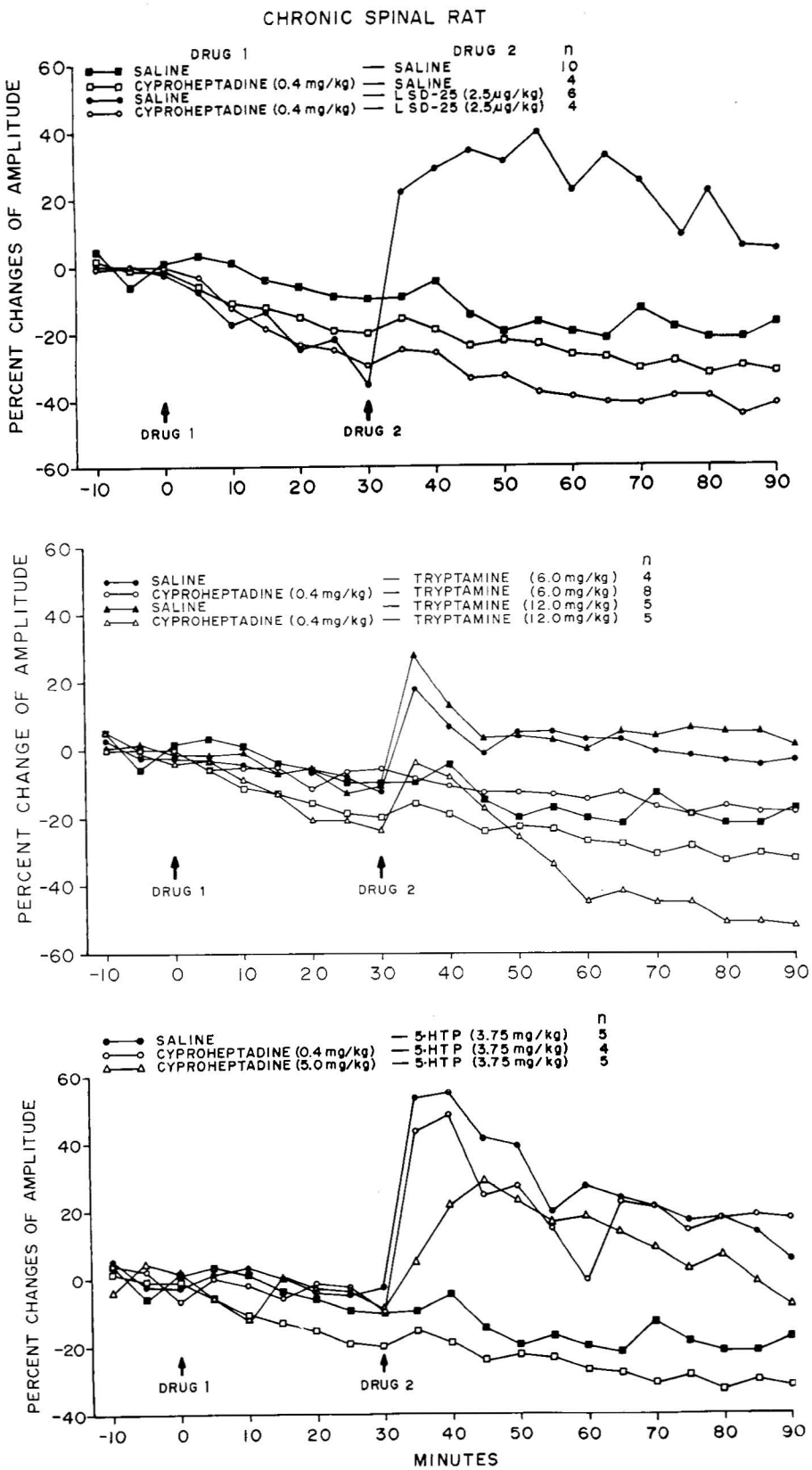

Fig. 2. Facilitation of flexor reflex by i.p. administration of LSD, tryptamine, or 5-HTP and its blockade by i.p. administration of cyproheptadine in chronic spinal rat. Each point represents mean of determinations made in 4–10 rats

following a sprouting of primary afferents, which they observed in chronic spinal animals.

The observation that methoxamine facilitates the flexor reflex in both the acute and chronic spinal rat extends the observations of Andén et al. (1966) and Andén and Magnusson (1967), suggesting that there are noradrenergic facilitative systems in the spinal cord of the rat, as there are in the acute spinal cat (Vaupel and Martin, 1976) and the chronic spinal dog (Martin and Eades, 1967). The fact that methoxamine produced greater facilitation of the flexor reflex and spontaneous movement in the chronic spinal rat than in the acute spinal rat suggests that methoxamine is acting postsynaptically at receptors where the presynaptic element has degenerated with a resulting degeneration supersensitivity. Amphetamine (2 mg/kg) produced a greater degree of facilitation of the flexor reflex in the chronic than in the acute spinal rat. This observation is difficult to explain in terms of a peripheral action of amphetamine, which releases norepinephrine, since Carlsson et al. (1964) and Dahlström and Fuxe (1965) found that norepinephrine levels in the cords of chronic spinal rats were markedly lower below the level of transection. Low levels of catecholamines are still present in the chronic spinal rat (Häggendal and Dahlström, 1973) and chronic spinal dog (J. W. Sloan and W. R. Martin, unpublished observations) below the level of transection. The liberation of small amounts of these catecholamines by amphetamine in the presence of denervation sensitization may allow this facilitative action to become manifest. In keeping with this suggestion are the observations of Martin and Eades (1967), who found that chronic reserpine treatment reduced the facilitative effect of amphetamine on the hindlimb flexor reflex of the chronic spinal dog.

Facilitation of the flexor reflex by large doses of LSD in acute spinal rats confirms the observations of Andén et al. (1968). 5-HTP (2.5–50 mg/kg) failed to facilitate the flexor reflex of the acute spinal rat using our procedure. However, we found that LSD, tryptamine, and 5-HTP were more potent and effective in facilitating the flexor reflex in the chronic spinal rat. This finding suggests that these drugs act at receptors normally activated by descending monoaminergic axons but are sensitized by denervation produced by spinal cord transection. Although L-aromatic amino acid decarboxylase is associated with descending monoaminergic neurons (Andén et al., 1964b) and is thus considerably reduced after denervation, enough decarboxylase activity remains to convert 5-HTP to serotonin in the chronic spinal cat (Taber and Anderson, 1973). Following pCPA pretreatment, 5-HTP did not facilitate the monosynaptic spike in acute spinal cats, whereas 5-HTP facilitated this reflex in chronic spinal cats pretreated with pCPA (Taber and Anderson, 1973). We found a similar result using the flexor reflex in acute and chronic spinal pCPA-pretreated rats. Therefore, the facilitation caused by 5-HTP in chronic spinal rats might be due to small amouts of serotonin formed or to 5-HTP acting directly on denervation sensitized receptors.

The facilitative trend produced by L-tryptophan may be caused by an increase in tryptamine release from still viable tryptaminergic nerve endings below the level of transection that have been separated from cell bodies in more rostral areas (Martin et al., 1975, 1976; Bell et al., 1976). L-Tryptophan failed to facilitate the flexor reflex in the chronic spinal cat (Shibuya and Anderson, 1968) and in the chronic spinal dog (Martin and Eades, 1970), as well as the C-fiber reflex in the chronic spinal cat (Bell and Martin, 1977). Therefore, it appears possible that 5-HTP can be converted to serotonin at lower levels of decarboxylase activity than can L-tryptophan to tryptamine, or that the decarboxylation of 5-HTP and tryptophan occurs in close proximity to serotonergic but not tryptaminergic receptive neurons.

In acute spinal rats small i.v. doses of tryptamine (3.0–25.0 mg/kg) facilitated the flexor reflex, whereas large doses (50 mg/kg) injected i.p. failed to do so or were very weak (Andén et al., 1971). This failure may be due to the rapid metabolism of tryptamine after i.p. injections since the drug is slowly absorbed and rapidly metabolized by monoamine oxidase.

Andén et al. (1968, 1971) observed that high doses of LSD (4 mg/kg) and 5-HTP (10–75 mg/kg) facilitated the flexor reflex in the acute spinal rat and speculated that LSD and 5-HTP were acting on the same receptor because 5-HTP elevated brain serotonin levels, and tryptamine was only weakly active in their hands. In contrast, the experiments herein reported demonstrate that tryptamine does facilitate the flexor reflex in both the acute and chronic spinal rat. Further, the facilitative effects of both LSD and tryptamine were antagonized by cyproheptadine in the chronic spinal rat while the effects of 5-HTP were not. These data are consistent with the human studies in which tryptamine caused hallucinations (Martin and Sloan, 1970) and in which serotonin antagonists failed to inhibit the hallucinations caused by LSD (Isbell et al., 1959). These observations are similar to those made on the flexor reflex of the chronic spinal dog (Martin and Eades, 1970) and on the C-fiber reflex of the acute spinal cat (Bell and Martin, 1974), and indicate that the spinal cord facilitative effects of small doses of LSD involve a different mechanism than the facilitative actions of 5-HTP and serotonin but are similar to tryptamine. Larger doses of LSD may involve a serotonergic mechanism.

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