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Short communication

Effect of (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide on spinal motor systems in anesthetized intact and spinalized rats

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Abstract

In the present study, we examined the effect of the 5-HT_{1A} receptor agonist (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide (8-OH-DPAT), on the mono- and polysynaptic reflexes in intact and spinalized rats. 8-OH-DPAT (10 μg/kg i.v.) significantly potentiated the amplitude of the monosynaptic reflex in intact rats. In contrast, 8-OH-DPAT (30 and 100 μg/kg i.v.) produced a significant dose-related inhibition of the amplitude of the monosynaptic reflex in spinalized rats. These results suggest that 8-OH-DPAT predominantly excites spinal motor systems at the supraspinal site, and inhibits such systems at a spinal cord site.

Keywords: Spinal reflex; 5-HT_{1A} receptor; 8-OH-DPAT ((±)-8-hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide); Motor function; Spinal cord

1. Introduction

Serotonin (5-hydroxytryptamine, 5-HT) can be found in the nucleus raphe pontis, the nucleus raphe obscurus and the nucleus raphe pallidus (Dahlström and Fuxe, 1964; Wiklund et al., 1981), and 5-HTergic neurons project to the spinal ventral horn (Nygren et al., 1974; Martin et al., 1982; Basbaum et al., 1987). Several studies have examined the spinal motor systems, but 5-HT has various functional roles, and conflicting evidence has been reported. In the cat, the monosynaptic reflex is potentiated by D,L-5-hydroxytryptophan (5-HTP), a precursor of 5-HT (Anderson and Shibuya, 1966), and 5-HT directly depolarizes spinal motoneurons (Connell and Wallis, 1988) and enhances their excitability (Barasi and Roberts, 1974; Roberts et al., 1988). On the other hand, the monosynaptic reflex is inhibited by 5-HT in isolated frog spinal cord (Carels, 1962) and isolated neonatal rat spinal cord (Saito et al., 1982; Wu et al., 1991). In our previous studies, 5-methoxy-*N*,*N*-dimethyltryptamine (5-MeODMT) and 5-HTP inhibited the monosynaptic reflex in spinalized rats (Nagano et al., 1988). Thus, the effect of 5-HT seems to vary between intact animals and spinalized animals, or between in vivo and in vitro studies. In the present study, we examined the effect of (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide (8-OH-DPAT), a 5-HT_{1A} receptor agonist, on the spinal reflex in intact and spinalized rats to determine the action of 5-HT at sites including supraspinal sites and spinal cord sites.

2. Materials and methods

2.1. Measurement of mono- and polysynaptic reflexes

Male Wistar rats (8–9 weeks old) were anesthetized with α-chloralose (25 mg/kg i.p.) and urethane (1000 mg/kg i.p.) and a cannula was inserted into the trachea and the tail vein for drug administration. Spinalized rats were obtained by C1 transection, and the brainstem was crushed by stuffing with cotton. A dorsal laminectomy was performed in the lumbo-sacral region, and both the ventral and dorsal roots below L4 were cut distally at their points of exit from the vertebral column. All exposed surgical areas were covered with liquid paraffin kept at 36.5 ± 0.5°C by radiant heat. Rectal temperature was maintained at 37 ± 0.5°C.

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Bipolar platinum electrodes were used for stimulation and recording. An L5 dorsal root was stimulated with 0.2 Hz rectangular pulses 0.05 ms in duration and with supramaximal voltages which were approximately twice the intensity required to cause a maximal reflex response. Mono- and polysynaptic reflexes were recorded from the L5 ventral root and displayed on an oscilloscope, and eight consecutive responses were averaged by an averager.

2.2. Drug

8-OH-DPAT HBr (Research Biochemicals International, USA) was dissolved in 0.9% physiological saline and administered intravenously at 1 ml/kg of body weight.

2.3. Statistical analysis

Mono- and polysynaptic reflexes were calculated as percentages of the amplitude of the predrug values. All data are shown as the means ± standard error. The statistical significance of differences was evaluated by one-way analysis of variance (ANOVA) followed by Bonferroni's multiple *t*-test.

3. Results

3.1. Effect of 8-OH-DPAT in spinalized rats

The monosynaptic reflex was significantly inhibited in a dose-dependent manner. At 30 and 100 μg/kg, the amplitude of the monosynaptic reflex was reduced to 65.5 ± 4.3% ($n = 3$) and 30.7 ± 7.5% ($n = 5$) of the control, respectively (Fig. 1A, Table 1). On the other hand, although 8-OH-DPAT at a dose of 100 μg/kg appeared to potentiate the polysynaptic reflex by 37.9 ± 9.2% ($n = 4$) in spinalized rats (Table 1), this increase was not significant. These responses peaked at 3 min after drug administration and the amplitudes returned to their respective control levels within 60 min.

3.2. Effect of 8-OH-DPAT in intact rats

8-OH-DPAT at a dose of 10 μg/kg significantly potentiated the amplitude of the monosynaptic reflex by 66.4 ± 24.7% ($n = 4$) in intact rats (Fig. 1B, Table 1), but did not affect the polysynaptic reflex. In intact rats, 8-OH-DPAT did not inhibit the amplitude of the monosynaptic reflex when administered at a dose of 100 μg/kg (data not shown). The effect of 8-OH-DPAT on the monosynaptic reflex in intact rats peaked at 3 min after administration and the amplitude returned to its control level within 30 min.

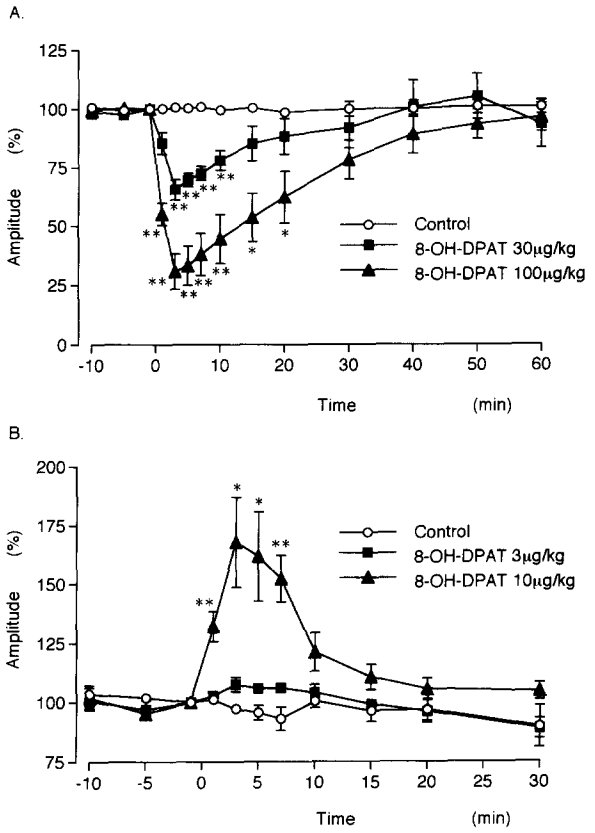

Fig. 1. Effect of 8-OH-DPAT on the amplitude of the monosynaptic reflex in spinalized (A) and intact (B) rats. The monosynaptic reflex was evoked by electrical stimulation (0.05 ms, 0.2 Hz, supramaximal voltage) of the L5 dorsal root, recorded from the L5 ventral root, and displayed on an oscilloscope. Eight consecutive responses were averaged by an averager. Each point represents the mean ± S.E. for 3–5 rats in each group. The statistical significance of differences was determined by one-way analysis of variance (ANOVA) followed by Bonferroni's multiple *t*-test. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. Control.

Table 1
Maximal changes in the amplitudes of the mono- and polysynaptic reflexes

			Response (%) (*n*)	
			Spinalized rat	Intact rat
MSR	Control		100.6 ± 1.5 (3)	97.2 ± 2.0 (4)
	8-OH-DPAT	3 μg/kg		107.5 ± 3.0 (3)
		10 μg/kg		166.4 ± 24.7 (4) [a]
		30 μg/kg	65.5 ± 4.3 (3) [a]	
		100 μg/kg	30.7 ± 7.5 (5) [b]	
PSR	Control		104.6 ± 3.3 (3)	100.7 ± 10.0 (4)
	8-OH-DPAT	3 μg/kg		89.0 ± 11.5 (3)
		10 μg/kg		108.4 ± 18.6 (4)
		30 μg/kg	116.5 ± 18.2 (3)	
		100 μg/kg	137.9 ± 9.2 (4)	

The monosynaptic reflex (MSR) and polysynaptic reflex (PSR) were calculated as a percentage of the amplitude of the predrug values. Data represent the means ± S.E. The significance of differences was determined by one-way analysis of variance (ANOVA) followed by Bonferroni's multiple *t*-test. [a] $P < 0.05$ and [b] $P < 0.001$ vs. Control.

4. Discussion

In the present study, the monosynaptic reflex was significantly potentiated by 10 μg/kg of 8-OH-DPAT in the intact rat. In the spinalized rat, while the monosynaptic reflex was significantly inhibited by 30 and 100 μg/kg of 8-OH-DPAT, 10 μg/kg of 8-OH-DPAT had only a negligible effect (data not shown). Thus, it is most likely that the enhancing effect of 8-OH-DPAT is produced at a supraspinal site, while the inhibitory effect is produced at a spinal site. Furthermore, the inhibitory effect of 8-OH-DPAT in the spinal cord must be masked by a descending excitatory pathway in the monosynaptic reflex.

In spinalized rats, the monosynaptic reflex was dose dependently inhibited by 8-OH-DPAT, although a dose 10-fold higher than that used in intact rats was required to evoke an inhibitory effect on the monosynaptic reflex. This inhibitory effect is antagonized by haloperidol, a non-selective dopamine/5-HT receptor antagonist, but not by spiroxatrine, a selective 5-HT_{1A} receptor antagonist (unpublished observations). Recently, it has been shown that 5-HT_{1A} receptors in the dorsal raphe and the hippocampus have different properties, since spiperone antagonizes 8-OH-DPAT-induced firing in the dorsal raphe, but not in the hippocampus (Blier et al., 1993). Therefore, it may be possible that the inhibitory effect of 8-OH-DPAT on the monosynaptic reflex in spinalized rats is mediated via unclassified 5-HT receptors. Recently, it has been considered that 8-OH-DPAT is a ligand of the 5-HT_7 receptor (Tsou et al., 1994). Therefore, the inhibitory effect of 8-OH-DPAT in the spinal cord may involve the 5-HT_7 receptor. It has been reported that 5-HT increases motoneuron excitability in decerebrated cats in which the central nervous system is intact between the brainstem and the spinal cord (White and Fung, 1989). Electrical stimulation of the nucleus raphe medianus (White and Fung, 1989) or the nucleus raphe obscurus (Roberts et al., 1988) also increases the amplitude of the monosynaptic reflex and evokes excitation of spinal motoneurons in anesthetized intact rats. However, 8-OH-DPAT applied iontophoretically in the ventral horn was without effect on the monosynaptic reflex in halothane-anesthetized rats (Roberts et al., 1988). On the other hand, in a study on isolated rat spinal cord, it was shown that 8-OH-DPAT and other 5-HT_{1A} receptor ligands inhibit the monosynaptic reflex (Crick and Wallis, 1991; Wallis et al., 1993). 5-HTP and 5-methoxy-*N*,*N*-dimethyltryptamine also inhibit the monosynaptic reflex in spinalized animals (Hall, 1981; Yamazaki et al., 1991). Thus, 5-HT most likely excites spinal motor systems by acting at supraspinal sites in intact animals. However, in spinalized animals or isolated spinal cord, 5-HT or 5-HT_{1A} receptor ligands have an overriding inhibitory effect against spinal motor pathways which masks any excitatory, depolarizing action on motoneurones.

In summary and conclusion, 8-OH-DPAT has complex effects on motor systems in the rat, since it acts at both supraspinal and spinal sites. In the intact rat, 8-OH-DPAT predominantly acts at supraspinal sites and excites motor systems in the spinal cord. On the other hand, 8-OH-DPAT inhibits spinal motor systems at the spinal cord level, but this effect is likely to be masked by supraspinal responses in intact rats.

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