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Short communication

Effect of Δ^9-tetrahydrocannabinol on circling in rats induced by intranigral muscimol administration

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Abstract

Unilateral injection of 25 ng of muscimol into the rat substantia nigra pars reticulata induced contralateral circling. Circling induced by 25 ng of muscimol was increased by simultaneous administration of 1 μg of Δ^9-tetrahydrocannabinol and abolished by 10 μg. Previous experiments have shown that Δ^9-tetrahydrocannabinol enhances muscimol-induced catalepsy in the globus pallidus. These results identify another brain area in which synergism between Δ^9-tetrahydrocannabinol and muscimol can occur and show that Δ^9-tetrahydrocannabinol can enhance an excitatory as well as an inhibitory GABAergic motor effect. The data support the hypothesis that cannabinoids can facilitate transmission along central γ-aminobutyric acid (GABA) releasing pathways.

Keywords: Muscimol; Δ^9-Tetrahydrocannabinol; Substantia nigra, pars reticulata; Circling behavior; Contralateral circling; Drug interaction

1. Introduction

Previous experiments with rats have shown that the $GABA_A$ receptor agonist, muscimol, can interact synergistically with Δ^9-tetrahydrocannabinol in the globus pallidus in the production of catalepsy (Wickens and Pertwee, 1993). The present experiments were designed to investigate whether Δ^9-tetrahydrocannabinol can also interact synergistically with muscimol when injected into the substantia nigra pars reticulata. This was chosen as the injection site for two reasons. First, like the globus pallidus, the substantia nigra pars reticulata is densely populated with cannabinoid receptors (Herkenham et al., 1990). Second, we considered it to be a brain area in which we could establish whether, in addition to its ability to potentiate an inhibitory γ-aminobutyric acid (GABA) receptor mediated effect on motor activity (catalepsy), Δ^9-tetrahydrocannabinol also has the ability to potentiate an excitatory GABAergic behavioural effect. One such effect, produced by unilateral intranigral injection of muscimol, is contralateral circling behaviour (Arnt and Scheel-Krüger, 1979; Olpe et al., 1977; Scheel-Krüger, 1986) and it was this that we chose as our measured response.

2. Materials and methods

2.1. Surgery

Adult Sprague-Dawley rats (300–425 g) were anaesthetized with sodium pentobarbitone (60 mg/kg i.p.) and then injected with scopolamine (1 mg/kg i.p.). The animals were positioned in a stereotaxic frame (Baltimore Instrument Co.) and a hole drilled through the skull 5–6 mm caudal to the bregma and 2 mm to the right or left of the midline. A 22 gauge cannula guide (Plastic Products, Virginia, USA) was inserted through the hole and lowered vertically using stereotaxic coordinates A 2.2, L 2.0 and V 7.5 (König and Klippel, 1963). The cannula guides were fixed to the skull with acrylic dental cement, additional anchorage being pro-

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vided by two 12 BA stainless steel screws. Behavioural experiments were performed at least 48 h after surgery. During surgery, the placement of cannulae was alternated. No more than 3 rats with cannulae implanted on the same side of the skull were assigned to any one experimental treatment.

2.2. Intracerebral administration of drugs

Δ^9-Tetrahydrocannabinol was mixed with 2 parts of Tween 80 by weight and dispersed in saline (Pertwee et al., 1992). Muscimol (Sigma) was dissolved in saline. For experiments in which these drugs were to be coadministered, Δ^9-tetrahydrocannabinol was dispersed (with Tween 80) in a muscimol-containing saline solution. Drug injections were made through 28 gauge injection cannulae (Plastic Products) using the method described by Wickens and Pertwee (1993). These injection cannulae were 1 mm longer than the implanted cannula guides. Each rat received one unilateral injection into the substantia nigra pars reticulata over a period of 2 min. The injection cannula was kept in situ for a further minute to allow time for diffusion and was then withdrawn. The injection volume was 1 μl. Control animals received drug vehicle instead of drug. Animals were returned to their home cage for 15 min and were then placed in the rotometer described below. The positions of injection sites were verified using a histological procedure similar to that outlined by Wickens and Pertwee (1993).

2.3. Measurement of circling behaviour

A harness was secured around the upper body of a rat, just behind its front legs. The animal was now placed in a circular open arena of diameter 27 cm and wall height 20 cm and the number of times it circled through 360° recorded using an automated rotometer (Linton Instruments, UK). This consisted of a lead running between the rat harness and a microswitch that was located 60 cm above the animal and swivelled whenever the rat circled. Circling behaviour was monitored by computer (Acer, Model 970L). This was programmed to count the number of circles completed in each of 12 successive 5 min periods.

2.4. Dose levels

Muscimol was administered at a submaximal dose (25 ng) for the production of contralateral circling behaviour (Olpe et al., 1977) and Δ^9-tetrahydrocannabinol at a dose within (10 μg) or below (1 μg) a range known to enhance the cataleptic response to intrapallidal injections of muscimol (Wickens and Pertwee, 1993).

2.5. Analysis of data

Values have been expressed as means and limits of error as standard errors. Comparison of sets of data with significantly different variances ($P < 0.05$) has been achieved using Student's t-test for paired data or the Mann-Whitney U-test to estimate the significance of differences between means or medians ($P \leq 0.05$). For other data, Student's t-test for unpaired data has been used to evaluate the significance of differences between means ($P \leq 0.05$).

3. Results

When 25 ng of muscimol was coadministered unilaterally with Tween 80 into the substantia nigra, it induced significant asymmetric circling (Fig. 1). The number of circles made in the direction opposite to the side of the brain into which muscimol had been injected (contralateral circles) significantly exceeded the number of circles made towards the injection site (ipsilateral circles). Muscimol-induced contralateral circling

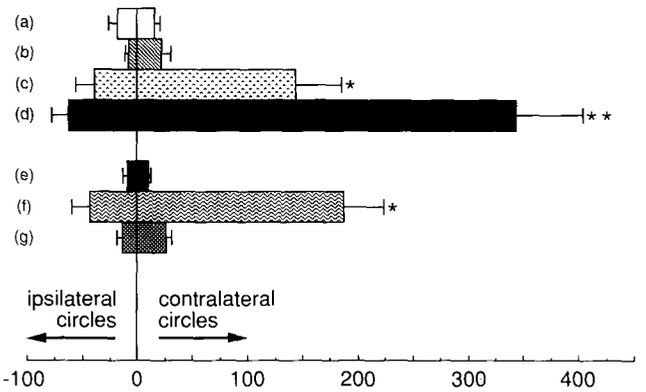

Fig. 1. Circling behaviour after unilateral injection into the substantia nigra pars reticulata of (a) 2 μg Tween 80, (b) 1 μg Δ^9-tetrahydrocannabinol, (c) 25 ng muscimol with 2 μg Tween 80, (d) 25 ng muscimol with 1 μg Δ^9-tetrahydrocannabinol, (e) 10 μg Δ^9-tetrahydrocannabinol, (f) 25 ng muscimol with 20 μg Tween 80, or (g) 25 ng muscimol with 10 μg Δ^9-tetrahydrocannabinol. Circling scores have been plotted using positive and negative values for contralateral and ipsilateral circling respectively. The bars represent mean values ±S.E. for the number of circles completed in a 60 min period, beginning 15 min after injection ($n = 5$ or 6). The significance of differences between mean ipsilateral and contralateral circling scores recorded over the same 60 min period has been calculated by Student's t-test for paired data (* $P \leq 0.05$; ** $P < 0.01$). The mean contralateral circling score following injection of 1 μg Δ^9-tetrahydrocannabinol (b) was not significantly different from that following injection of 2 μg Tween 80 (a) ($P > 0.2$; Student's t-test for unpaired data). Muscimol gave rise to a significantly greater mean contralateral circling score in the presence of 1 μg Δ^9-tetrahydrocannabinol (d) than in the presence of 2 μg Tween 80 (c) ($P < 0.05$; Student's t-test for unpaired data) and to a significantly lower score ($P < 0.01$; Mann-Whitney U-test) in the presence of 10 μg Δ^9-tetrahydrocannabinol (g) than in the presence of 20 μg Tween 80 (f).

was increased by 1 μg of Δ^9-tetrahydrocannabinol and abolished by 10 μg (Fig. 1). The interaction between muscimol and 1 μg of Δ^9-tetrahydrocannabinol was greater than additive as, in the absence of muscimol, unilateral injections of Δ^9-tetrahydrocannabinol or Tween 80 did not induce asymmetric circling behaviour (Fig. 1).

The injection sites were located throughout the anterior and medial parts of the substantia nigra pars reticulata (co-ordinates A2580 to A1610). Of these, 51% lay between the narrower confines of co-ordinates A2420 and A1950.

4. Discussion

Further experiments are required to discover the mechanisms for the interactions between Δ^9-tetrahydrocannabinol and muscimol observed in this investigation. One possibility is that Δ^9-tetrahydrocannabinol can interact directly with the $GABA_A$ receptors that seem to mediate the asymmetric circling induced by unilateral injection of muscimol into the substantia nigra pars reticulata (Arnt and Scheel-Krüger, 1979; Scheel-Krüger, 1986). However, there is already evidence that cannabinoids do not alter the affinity of GABA for its receptors (Leader et al., 1981) and there are no reports that cannabinoids affect $GABA_A$ receptor signal transduction. Another possible explanation, that applies only to the synergism that occurs between Δ^9-tetrahydrocannabinol and muscimol, is that Δ^9-tetrahydrocannabinol somehow increases free extracellular concentrations of GABA and that it is GABA which interacts synergistically with muscimol. [Because the relationship between the concentration GABA receptor agonists and the size of the effects they produce is probably a logarithmic one, interactions between muscimol and GABA may well be synergistic in nature when expressed on an arithmetic scale (Wickens and Pertwee, 1993).] There are at least two mechanisms by which Δ^9-tetrahydrocannabinol might elevate extracellular concentrations of GABA in the substantia nigra pars reticulata. One is through its known ability to inhibit the neuronal uptake of GABA (Banerjee et al., 1975; Hershkowitz et al., 1977). The other is by facilitating the neuronal release of GABA. This Δ^9-tetrahydrocannabinol might do by acting through cannabinoid receptors that are present on the terminals of neurons projecting from the striatum to the substantia nigra pars reticulata (Herkenham et al., 1991). Interestingly, this is a brain area in which cannabinoid receptors may be co-localized with presynaptic dopamine D_1 receptors (Herkenham et al., 1991), a receptor type through which the neuronal release of GABA can apparently be facilitated (Reubi et al., 1977; Starr, 1987).

We have found previously that the cataleptic response to 25 ng of muscimol, injected bilaterally into the globus pallidus, can be enhanced by coadministration of Δ^9-tetrahydrocannabinol at doses of either 3 μg or 30 μg (Wickens and Pertwee, 1993). Our present finding that a dose of Δ^9-tetrahydrocannabinol within this range (10 μg) will attenuate an effect of 25 ng of muscimol, albeit by unilateral injection into a different brain area, is therefore difficult to explain. This is especially so since the globus pallidus, like the substantia nigra pars reticulata, contains both cannabinoid and $GABA_A$ receptors (Herkenham et al., 1990; Scheel-Krüger, 1986). One possibility, that the higher dose of Δ^9-tetrahydrocannabinol enhanced the ability of intranigral muscimol to induce stereotyped behaviour (Scheel-Krüger, 1986) and that this behaviour competed with the motor act of circling, is unlikely as there was no sign of such stereotypy in these experiments.

Further research is required to establish whether Δ^9-tetrahydrocannabinol can facilitate the neuronal release of GABA or, indeed, whether it can elevate extracellular nigral concentrations of GABA. Also still to be determined is whether potentiation of muscimol by Δ^9-tetrahydrocannabinol in the substantia nigra is cannabinoid receptor mediated and if so, whether anandamide or some other endogenous cannabinoid receptor ligand (see Pertwee, 1995) undergoes similar interactions with GABA under physiological conditions, perhaps serving as a modulator of GABAergic neurotransmission in this region of the brain.

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