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Cannabinoid-induced stimulation of motor activity in planaria through an opioid receptor-mediated mechanism

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Abstract

Planaria, the most primitive example of centralization and cephalization of the nervous system along phylogeny, shows specific stereotyped behavioral patterns following exposure to drugs acting on neural transmission. In this study, the authors investigated the effects of exposure to the synthetic cannabinoid receptor agonist WIN55212.2 on motor activity in planaria. WIN55212.2 produced dose-dependent stimulation of motor behavior. High doses of the drug caused stereotyped activities identical to those seen previously with opioid agonists. These effects were antagonized by coexposure to cannabinoid or opioid receptor antagonists. The results indicate that functional interactions between cannabinoid and opioid systems are highly conserved along phylogeny, at least at the behavioral level.

Keywords: Behavior; Cannabinoid; Opioid; Planaria; WIN55212.2

1. Introduction

There is compelling evidence of similarities and interactions between opioid and cannabinoid system in mammals, with particular reference to analgesia and drug addiction (Manzanares et al., 1999). Endogenous cannabinoid system has been shown very early along phylogeny (De Petrocellis et al., 1999). Hydra, the first organism displaying neuronal cell specialization, synthesizes anandamide and expresses cannabinoid receptors (De Petrocellis et al., 1999). In this species, cannabinoid system plays a role in feeding behavior (De Petrocellis et al., 1999). Planaria, a platyhelminth with bilateral symmetrical central nervous system consisting of a brain and longitudinal medullary nerve cord connected with peripheral nervous plexuses (Pearse et al., 1987), represents the most primitive example of centralization of neuronal cells. A number of transmitter substances and neurosecretory peptides have been identified in planaria, including catecholamines (Gustafsson, 1985), serotonin (Welsh and Williams, 1970), and opioid peptides (Venturini et al., 1983).

Behavioral studies indicate that planaria shows specific motor patterns in response to drugs acting on neural transmission. Thus, stimulation of dopamine system produces hyperkinetic stereotyped activities (Venturini et al., 1989; Palladini et al., 1996), whereas stimulation of acetylcholine transmission causes hypokinesia with fixed posture (Buttarelli et al., 2000). Despite the very simple hierarchy of organization of its nervous system, there is evidence of interactions between distinct neurotransmitter–receptor systems in regulating motor behavior of planaria (Passarelli et al., 1999; Buttarelli et al., 2000). In the present study, the authors investigated the effects of the exposure to the synthetic cannabinoid receptor agonist WIN55212.2 on motor activity in planaria.

2. Methods

2.1. Animals

Planaria (*Dugesia gonocephala*, S.L.) were kept in a volume of 3 ml in Petri dishes into a thermostatic chamber at +18 °C in dim light. Six specimens were used for each single or combined treatment. The number of specimens

Abbreviations: SLH; screw-like hyperkinesia; SLM; snake-like movement

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Table 1
Effects of the single treatments on motor behavior in planaria

Drug	Dose	B	M	Stereotypies
Water		5 ± 2	4 ± 1	–
Ethanol	2–4 μl	4 ± 1	3 ± 2	–
WIN55212.2	20 μg	5 ± 1	4 ± 2	–
	50 μg	53 ± 5*,§	37 ± 6*,§	–
	100 μg	58 ± 16*,§	56 ± 7*,§	SLH+, SLM+
	250 μg	44 ± 6*,§	57 ± 11*,§	SLH+++, SLM++
Naloxone	30 μg	3 ± 1	6 ± 2	–
	60 μg	5 ± 2	4 ± 2	–
	100 μg	3 ± 1	2 ± 1	–
SR141716A	50 μg	5 ± 2	5 ± 2	–
	250 μg	6 ± 2	5 ± 1	–

Data represent means ± S.E. ($n = 6$ for each treatment). B = Number of crosses on the border of the Petri dish. M = Number of crosses in the middle of the Petri dish. See Methods section for (+) as intensity of stereotyped behaviors.

* $P < .05$, different from tap water.

§ $P < .05$, different from WIN55212.2 (20 μg).

was chosen based on the very reproducible responses of planaria to pharmacological treatments (Venturini et al., 1989; Palladini et al., 1996). In all experiments, pH and osmotic pressure were within the range of viability of planaria (Palladini et al., 1976), from a minimum of 7.5 mosM to a maximum of 7.85 mosM. All experiments were performed in dim light.

A grid was drawn to divide the surface of a 3-ml Petri dish into squares (0.5 × 0.5 mm). WIN55212.2 (Sigma RBI, Italy) was dissolved in tap water with 0.1% ethanol. The following concentrations of WIN55212.2 were used: 20, 50, 100, and 250 μg/3 ml tap water. Each planaria was kept in tap water in a Petri dish for 20 min, and spontaneous motor activity was measured as the number of crosses along the border of the dish (“B” activity) or in the middle of the dish (“M” activity). Planarias were then removed from tap water and exposed to WIN55212.2 and the same behavioral measurement was repeated for further 20 min. The length of observation period was chosen since the behavioral response of planaria to WIN55212.2 started within 2–5 min.

For the combined treatments, planarias were preexposed for 20 min to either the cannabinoid receptor antagonist SR141716A (gift of Sanofi Research) or the opioid receptor antagonist naloxone (Sigma). The specimens were then washed three times in tap water and coexposed to WIN55212.2 and either antagonist for further 20 min, as summarized in Table 2.

In all experiments, the intensity of stereotyped behaviors observed was rated as “intermittent” (+) if present for less than 2 min, “subcontinuous” (++) if present for 3–10 min, or “continuous” (+++), if present for more than 10 min.

2.2. Data analysis

The results were expressed as means ± S.E.M. The effects of the exposure to each single or combined treatment on motor activity in planaria were analyzed for significance by means of one-way ANOVA, followed by Bonferroni’s *t* test for multiple comparisons.

3. Results

The results of the study are summarized in Tables 1 and 2. The overall ANOVA showed that the various doses of WIN55212.2 produced significantly different effect on motor activity in planaria, $F(5,30) = 38.92$, $P < .0001$.

Planarias exposed to tap water showed weak motor activity, with few crosses in the middle (“M” activity) of the Petri dish to reach the border, followed by few movements along the border (“B” activity) of the dish (Table 1). Normal behavior in planaria is shown in Fig. 1A. Exposure to vehicle (2–4 μl ethanol in 3-ml tap water) did not modify motor activity (Table 1).

The lowest dose of WIN55212.2 (20 μg) did not modify the motor behavior of planaria (Table 1). At the dose of 50 μg, WIN55212.2 significantly increased both “B” and “M” activities ($P < .05$ different from values in tap water), without producing stereotyped behavior (Table 1). At the dose of 100 μg of WIN55212.2, a marked stimulation of motor activity was observed, together with weak stereotyped behaviors such as head swinging and intermittent “snake-like” movements (SLM) (Fig. 1B) or “screw-like”

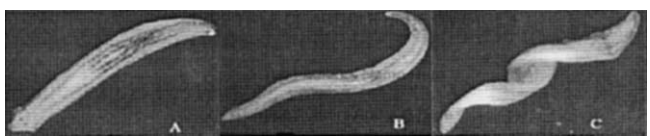

Fig. 1. Behavioral consequences of the exposure to WIN55212.2 in planaria. Shown are normal behavior (A), SLM (B), and SLH (C).

Table 2
Effects of the combined treatments on motor behavior in planaria

Antagonist	WIN55212.2 (μg)	B	M	Stereotypies
Naloxone (μg)				
30	50	$5 \pm 1^{\#}$	$4 \pm 2^{\#}$	–
30	100	$22 \pm 3^{\#}$	43 ± 9	–
30	250	33 ± 5	86 ± 13	SLH+++, SLM++
60	100	$7 \pm 4^{\#}$	$7 \pm 2^{\#}$	–
100	250	$23 \pm 5^{\#}$	$17 \pm 4^{\#}$	–
SR141716A (μg)				
50	100	$19 \pm 6^{\#}$	$17 \pm 3^{\#}$	–
250	250	$29 \pm 5^{\#}$	73 ± 11	–

Data represent means ± S.E. ($n = 6$ for each treatment). B = Number of crosses on the border of the Petri dish. M = Number of crosses in the middle of the Petri dish. See Methods section for (+) as intensity of stereotyped behaviors.

$^{\#}$ $P < .05$, different from values measured after exposure to WIN55212.2 alone.

hyperkinesia (SLH) (Fig. 1C). The "M" activity score was significantly higher as compared to the 50-μg dose ($P < .05$) (Table 1). Finally, exposure to the highest dose of WIN55212.2 (250 μg) produced continuous stereotyped activities (SLM and SLH) (Table 1). The opioid antagonist naloxone (30, 60, and 100 μg) and the cannabinoid antagonist SR141716A (50 and 250 μg) per se did not modify the motor activity of planaria (Table 1).

Coexposure to the cannabinoid receptor antagonist SR141716A (50 μg) blocked the effects of 100 μg WIN55212.2 (Table 2). At a dose of 250 (μg, SR141716A reduced the effects of WIN55212.2 (Table 2). Under these conditions, stereotyped activities were not observed and the motor behavior was less rapid. However, the number of crosses was not reduced compared to 250 μg WIN55212.2 alone due to the lack of stereotyped activity.

Coexposure to the opioid receptor antagonist naloxone (30 μg) completely abolished the motor stimulatory effects of 50 μg WIN55212.2 and significantly reduced those of 100 μg WIN55212.2, but did not modify the effects of 250 μg WIN55212.2 (Table 2). Coexposure to 60 μg of naloxone completely blocked the effects of 100 μg WIN55212.2 (Table 2). Finally, coexposure to 100 μg of naloxone significantly reduced the effects of 250 μg WIN55212.2 (Table 2).

4. Discussion

The presence of endogenous cannabinoid system in planaria has been suggested previously (Paris and Lenicque, 1975). The results of the present study demonstrate dose-dependent effects of the synthetic cannabinoid receptor agonist WIN55212.2 on motor activity in the planaria. These data provide, to our knowledge, the first report of the effects of cannabinoid drugs on motor behavior in flatworms.

Lower doses of WIN55212.2 (50–100 μg) produced dose-dependent increases of motor activity, in particular in the middle of the Petri dish ("M" activity) throughout all the observation period. This finding, not seen in untreated planarias, suggests that WIN55212.2 stimulates exploratory activity of the specimens. Conversely, the highest dose of WIN55212.2 used (250 μg) produced a pattern of stereotyped, targetless behavior, which closely resembles stereotyped activities in mammals. The stimulation of motor activity by WIN55212.2 in planaria is in agreement with a very recent study focused on the activational role of cannabinoids in rats (Sañudo-Peña et al., 2000). Moreover, the effects of WIN55212.2 on exploratory behavior in planaria, which is in accordance with data obtained in mammals (Sañudo-Peña et al., 2000), suggest the early evolutionary onset of the control of exploratory behavior/movement by the cannabinoid system.

The effects of WIN55212.2 were counteracted by coexposure to the cannabinoid receptor antagonist SR141716A. Specifically, the motor stimulatory and stereotyped effects of 100 μg of WIN55212.2 were significantly reduced by 50 μg of SR141716A (Table 2). Conversely, 250 μg of SR141716A completely abolished the stereotyped activities produced by 250 μg of WIN55212.2 (Table 2), thus allowing animals to express exploratory activity. Because of this latter event, the number of crosses was not modified.

Although there is no direct evidence of the presence of cannabinoid receptors in planaria, the dose-dependent effects of WIN55212.2 and the dose-related antagonism by SR141716A, together with the presence of specific cannabinoid receptor sites in phylogenetically lower animals (De Petrocellis et al., 1999), make it likely that the behavioral changes measured herein depend upon specific interactions of these drugs with cannabinoid receptors. However, the present knowledge of the anatomofunctional organization of signal transmission in planaria does not allow to conclude whether the effects of WIN55212.2 are directly mediated at synaptic level or reflect hormonal-like cellular interactions.

The pattern of stereotypies produced by the highest dose of WIN55212.2 (SLM and SLH) were identical to those seen previously following exposure of planaria to opioid agonist drugs (Passarelli et al., 1999). These behavioral homologies suggested that the effects of WIN55212.2 on motor activity in planaria might depend upon indirect stimulation of endogenous opioid system of the flatworm.

Indeed, the motor-enhancing and stereotyped activities of WIN55212.2 were dose-dependently antagonized by the coexposure to naloxone (Table 2).

5. Conclusions

In recent years, evidence has accumulated on the interactions between central opioid and cannabinoid system in mammals (Bloom and Dewey, 1978; Chen et al., 1990; Smith et al., 1994; Tanda et al., 1997; Vela et al., 1995). The results of the present study indicate that interactions between these two systems occur very early along phylogeny, although they may have evolved from very fundamental behaviors, such as motor activity in planaria, to more complex and integrated functions in vertebrates.

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References

Bloom, A.S., Dewey, W.L., 1978. A comparison of some pharmacological actions of morphine and delta 9-tetrahydrocannabinol in the mouse. Psychopharmacology 57, 243–248.

Buttarelli, F.R., Pontieri, F.E., Margotta, V., Palladini, G., 2000. Acetylcholine/dopamine interactions in planaria. Comp. Biochem. Physiol., C: Comp. Pharmacol. 125, 225–231.

Chen, J.P., Paredes, W., Li, J., Smith, D., Lowinson, J., Gardner, E.L., 1990. Delta 9-tetrahydrocannabinol produces naloxone-blockable enhancement of presynaptic basal dopamine efflux in nucleus accumbens of conscious, freely-moving rats as measured by intracerebral microdialysis. Psychopharmacology 102, 156–162.

De Petrocellis, L., Melck, J., Bisogno, T., Milone, A., Di Marzo, V., 1999. Finding of the endocannabinoid signalling system in hydra, a very primitive organism: possible role in the feeding response. Neuroscience 92, 377–387.

Gustafsson, M.K.S., 1985. Cestode neurotransmitters. Parasitol. Today 1, 72–75.

Manzanares, J., Corchero, J., Romero, J., Fernandez-Ruin, J.J., Ramos, J.A., Fuentes, J.A., 1999. Pharmacological and biochemical interactions between opioids and cannabinoids. Trends Pharmacol. Sci. 20, 287–294.

Palladini, G., Valcomonica, C., Cappiello, A., Giolitti, G., 1976. Effects of some environmental pollutants on the behavior of motility of *Dugesia gonocephala*, S.L. Note I. Riv. Biol. 69, 155–167.

Palladini, G., Ruggieri, S., Stocchi, F., De Pandis, M.F., Venturini, G., Margotta, V., 1996. A pharmacological study of cocaine activity in planaria. Comp. Biochem. Physiol., C: Comp. Pharmacol. 115, 41–45.

Paris, M., Lenicque, P.M., 1975. Effect of tetrahydrocannabinol and of cannabidiol on wound healing and regeneration of the planaria *Dugesia tigrina*. Therapie 30, 97–102.

Passarelli, F., Merante, A., Pontieri, F.E., Margotta, V., Venturini, G., Palladini, G., 1999. Opioid–dopamine interaction in planaria: a behavioral study. Comp. Biochem. Physiol., C: Comp. Pharmacol. 124, 51–55.

Pearse, V., Pearse, J., Buchsbaum, M., Buchsbaum, R., 1987. Living Invertebrates. Boxwood Press, Pacific Grove, CA.

Sañudo-Peña, M.C., Romero, J., Seale, G.E., Fernandez-Ruiz, J.J., Walker, J.M., 2000. Activational role of cannabinoids on movement. Eur. J. Pharmacol. 391, 269–274.

Smith, P.B., Welch, S.P., Martin, B.R., 1994. Interactions between delta 9-tetrahydrocannabinol and kappa opioids in mice. J. Pharmacol. Exp. Ther. 268, 1381–1387.

Tanda, G., Pontieri, F.E., Di Chiara, G., 1997. Cannabinoid and heroin stimulation of mesolimbic dopamine transmission by a common μl opioid receptor mechanism. Science 276, 2048–2050.

Vela, G., Ruiz-Gayo, M., Fuentes, J.A., 1995. Anandamide decreases naloxone-precipitated withdrawal signs in mice chronically treated with morphine. Neuropharmacology 34, 665–668.

Venturini, G., Carolei, A., Palladini, G., Margotta, V., Lauro, G.M., 1983. Radioimmunological and immunocytochemical demonstration of met-enkephalin in planaria. Comp. Biochem. Physiol., C: Comp. Pharmacol. 74, 23–25.

Venturini, G., Stocchi, F., Margotta, V., Ruggieri, S., Bravi, D., Bellantuono, P., Palladini, G., 1989. A pharmacological study of planaria’s dopaminergic receptors. Neuropharmacology 28, 1377–1382.

Welsh, J.H., Williams, L.D., 1970. Monoamine-containing neurons in planaria. J. Comp. Neurol. 138, 103–115.