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Fear behavior and regional brain monoamines distribution after R(+)-8-OHDPAT and R(+)-UH-301 injections into the dorsal raphe nucleus in rats

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Abstract. The effects of 8-OHDPAT and UH-301 injection into the dorsal raphe nucleus (DRN) on fear behavior of the light-dark transitions test and regional brain monoamines (NA, DA, 5-HT) and their metabolites (MHPG, DOPAC, 5-HIAA) in the hypothalamus (HPT), midbrain central gray matter (MID), amygdala (AMY), hippocampus (HIP) and pons (PO) were examined. An injection of 8-OHDPAT (300 ng) as well UH-301 (300 ng) into the DRN evoked an increase in the number of head dipping from dark to the illuminated compartment of chamber, an increase of time of motionless in the dark compartment and decrease of time of locomotion activity in the illuminated compartment. HPLC analysis showed reduction of 5-HIAA/5-HT ratio in the HPT, HIP and PO, increase of MHPG/NA ratio in the HIP and PO, and increase of DA content in the HPT, AMY and HIP after 8-OHDPAT injection. But injection of UH-301 reduced 5-HT in the MID and increased in the AMY, reduced 5-HIAA content in the HIP and increased in the MID and decreased MHPG/NA ratio in the PO. These results indicate that both 5-HT_{1A} receptor agonists, acting on the 5HT_{1A} autoreceptors caused the anxiolytic effects, reduced fear behavior on the rat connected with infringement of dynamic balance between the serotonergic and catecholaminergics systems.

Key words: fear, light-dark transitions test, dorsal raphe nucleus, 8-OHDPAT, UH-301, brain monoamines, HPLC, rat

INTRODUCTION

The 5-HT receptor is the best characterized serotonin receptor family. Its stimulation reduces adenylate cyclase activity, activates potassium channels, and inhibits opening of calcium channels, through a pertussis toxin – sensitive G-protein of the G_i/G_o class (De Vivo and Maayani 1986, Albert and Morris 1994, Watson and Arkinstall 1994).

Many reports indicate that the 5-HT_{1A} receptors play an important role in the regulation of emotional and affective behavior, and above all in the fear/anxiety mechanisms (Handley et al. 1993, Andrews et al. 1994, Picazo et al. 1995, File et al. 1996, Lopez-Rubelcava 1996, Remy et al. 1996, Romaniuk et al. 2001).

The role of the 5-HT_{1A} receptors in different fear/anxiety animal models has been studied extensively. Engel et al. (1984) first indicated that the activation of pre- and postsynaptic 5-HT_{1A} receptors can produce different effects in animal tests of fear/anxiety, i.e. anxiolytic and anxiogenic, respectively. It has been indicated that the stimulation of somato-dendritic autoreceptors by selective 5-HT_{1A} agonist 8-Hydroxy-dipropylaminotetralin (8-OHDPAT) hyperpolaryzed and blocked electrical activity of serotonergic neurons in the mesencephalic raphe nuclei in the dose dependent manner and therefore diminished serotonergic neurotransmission (Glaser et al. 1991, Hamon 1997). Moreover, the activation of presynaptic 5-HT_{1A} receptors has been established to produce anxiolytic effects in different animal models of fear/anxiety (Andrews et al. 1994, Hogg et al. 1994, Jolas et al. 1995, Picazo et al. 1995, File 1996, File et al. 1996, Remy et al. 1996, Romaniuk et al. 2001). Only few neurochemical studies have shown the interactions among the 5-HT_{1A} receptors and other neurotransitter systems. In microdialysis studies Gobert et al. (1998) indicated that 8-OHDPAT has increased dialysate levels of noradrenaline and dopamine in the frontal cortex. However, it has been also shown that 8-OHDPAT decreases noradrenaline concentration (Saphier and Welch 1995). The influence of 8-OHDPAT on other neurotransmitter systems is not clear, and arouses controversies. Undoubtedly, neural control of fear/anxiety behavior is controlled by complex neurotransmitter systems of multiple, distributed parallel pathways (Barnes and Sharp 1999).

The present study was undertaken to compare behavioral and neurochemical effects evoked by R(+)-8-OHDPAT and R(+)-5-Fluoro-Hydroxy-dipropylaminotetralin (R(+)-UH-301), two 5-HT_{1A} receptor agonists, administered into the dorsal raphe nucleus in rats. Fear behavior was evaluated in the modified version of light-dark transitions test. Ethological studies provide findings that it is a natural tendency of rodents to avoid brightly lit, potentially dangerous areas, while various anxiolytic drugs attenuate this tendency, and even evoke an increase in exploratory activity in the lit sections of light-dark test chamber (Costall et al. 1989, Kilfoil et al. 1989, Lister 1990, Cole and Rodgers 1993, Anseloni and Brandao 1997). In the biochemical studies we analyzed the distribution of monoamines (NA, DA, 5-HT) and their metabolites (MHPG, DOPAC, 5-HIAA) in the hypothalamus, midbrain central gray matter, hippocampus and pons forming the neural system which detects threatening and stressogenic stimuli and organizes response to them (Adams 1979, Graeff 1990, Blanchard et al. 1993, Graeff et al. 1993) in correlation with behavioral alterations.

We think that the use of two selective 5-HT_{1A} receptor agonists may be one of the ways to show a specific role of these receptors in the central regulation of fear, if behavioral and neurochemical effects are similar, close and comparable. At present in another experiment we examine whether the behavioral and neurochemical effects induced by 8-OHDPAT are counteracted or prevented by selective 5-HT_{1A} receptor antagonist--WAY-100635.

METHODS

Subject

The experiments were performed on 24 male Wistar rats weighing 280 - 320 g bred in the licensed animal husbandry of the Institute of Occupational Medicine in Łódź. The animals were housed in groups, three per cage, and maintained under controlled conditions of temperature (22 ± 2°C), humidity (55 ± 5%) and on a 12L : 12D cycle (light on at 8 h) with food and water available continuously. In animal cages only male rats were kept. Experiments were carried out between 9 and 12 h.

Drugs

R(+)-8-Hydroxy-dipropylaminotetralin hydroxybromide, (R(+)-8-OHDPAT) RBI, Natick, MA and R(+)-5-Fluoro-8-Hydroxy-dipropylaminotetralin hy-

drochloride, (R(+)-UH-301) RBI, Natick, MA were dissolved immediately before use in saline solution (0.9%), which alone served as a vehicle control.

Surgery

Stereotaxic operations were performed under chloral hydrate anaesthesia (360 mg/kg IP). Twenty four rats had single guide cannula implanted into the dorsal raphe nucleus (DRN).

Anaesthetized rats were positioned in stereotaxic frame (Kopf Instruments, Tajunga, CA). The skull was exposed and the incisor bar adjusted such that bregma and lambda were at the same hight. Stereotaxic coordinates of Paxinos and Watson atlas (1982) with reference to bregma for the tip of this cannula was: F = -7.3, L = 1.3, H = 5.0. The cannula was inserted into the DRN at an angle of 12° at the horizontal plane to avoid penetration of the midline sinus and the aqueductus cerebri. The guide stainless steel cannula (0.7 mm external diameter) was fixed to the skull with stainless steel screws and self-polymerizing methacrylate resin (Vertex, Dentimex Manufactures, The Netherlands). The cannula was shut with stainless steel stylet to prevent clogging. After surgery rats were allowed to recovery for 14 days prior to behavioral testing. During this period animals were handled and familiarized with the injection procedure.

Intracerebral microinjections

Microinjections were made in awake rats while gently handled by experimenter. An internal injection cannula (0.3 mm external diameter) was inserted into the guide cannula so that it extended 1.1 mm beyond the tip. The internal cannula was connected to a microinjector (E. Zimmermann, Leipzig) by polyethylene tube filled with the distilled water. The injection cannula was filled with the 8-OHDPAT or with the R(+)-UH-301 solutions or with the saline by aspiration. A small air gap separating the two solutions, i.e. distilled water and 8-OHDPAT or saline or distilled water and R(+)-UH-301 or saline. The movement of the air bubble down the tubing indicated a successful injection. Solutions were injected manually at a rate of 0.01 µl/s. After each infusion the internal cannula was left in place for a 30 s before being removed to allow drug absorption. Zimmermann's microinjector was equipped with the micrometer screw, which allows for delivery of the solutions with accuracy of the 0.01 µl.

Behavioral procedure

In order to measure the reaction of animals to the stressogenic, aversive stimulus the modified version of Light-Dark Transitions Test first described by Crawley and Goodwin (1980) was used. The experimental chamber was divided into two compartments (40 x 40 x 50 cm each) with an opening allowing the animal to change its location. The light conditions were changed automatically at selected order – bulb (900 lx) located 50 cm above the floor of each compartment illuminated brightly only one compartment at the time, leaving the other one dark and safe. The experiment began after 1 min when the rat was introduced into the experimental chamber. One experimental session consisted of 5 trials with light stimulus. The time duration of the light stimulus was 60 s, and intervals between trials (dark time) were irregular: 120 s between trials 1 and 2, and between 3 and 4, 90 s between trials 2 and 3, and between 4 and 5.

The experimental room was illuminated with weak red bulb (4 lx). All behavioral experiments were videotaped and next analyzed with the Etho Vision 1.90 software (Noldus, Wageningen, The Netherlands). We measured the following variables of rats' behavior in the experimental chamber: (1) time out from the illuminated to the dark compartment, i.e. time elapsed from the moment of switching on the aversive light stimulus to the moment of the rat's passing to the dark compartment; (2) time of locomotion activity in the illuminated compartment; (3) time of locomotion activity in the dark compartment; (4) time of motionless in the illuminated compartment; (5) time of motionless in the dark compartment; (6) number of returns from the dark to the illuminated compartment; (7) number of head dipping from the dark to the illuminated compartment. As some animals returned to the light part of the experimental chamber, then time out from the illuminated to the dark part of the experimental chamber did not equal the sum of time of locomotion activity and time of motionless behavior in the light of the experimental chamber.

Each animal was individually habituated to the experimental conditions in two consecutive sessions. In such session rats were introduced into the experimental chamber for 5 min in the absence of any external stimuli. Then the basic experiment was performed.

Experimental groups

All rats (n = 24) were tested two times: first, on the 14th days following the surgery, with intra-DRN saline

injection. Then after the next week rats were divided into three groups and tested again.

Group 1 ($n = 8$) saline was injected in a volume of 0.3 ml into the DRN, 3 min prior to behavioral testing.

Group 2 ($n = 8$) R(+)-8-OHDPAT was injected at the dose of 0.3 μg/0.3 ml, into the DRN, 3 min prior to behavioral testing.

Group 3 ($n = 8$) R(+)-UH-301 was injected at the dose of 0.3 μg/0.3 ml into the DRN, 3 min prior to behavioral testing.

R(+)-8-OHDPAT and R(+)-UH-301 concentrations reflect base weight and were equimolar.

Rats injected with saline (Group 1) served as control for biochemical analysis. Three animals, one from each group were submitted for histological verification of the injection sites.

Histology

Histological verification of the injection sites was carried out in three rats that were randomly chosen from three groups, one from each. Three days after completion of behavioral experiments those animals were anaesthetized by chloral hydrate (360 mg/kg IP). Next the rats were killed by decapitation, their brains were removed from the skull and stored in 4% formaldehyde solution. After fixation brains were sectioned into 80 μm slices in coronal plane on a microtome and stained with cresyl violet. The exact sites of injections in the dorsal raphe nucleus were determinated by comparing the sections with the Paxinos and Watson atlas (1982). The example of the injection site is presented in Fig. 1.

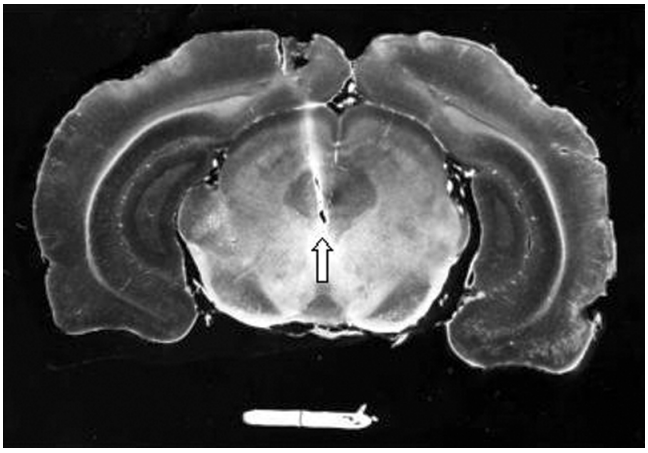

Fig. 1. Photomicrograph of the coronal section through the rat brain showing the injection site in the dorsal raphe nucleus (indicated by arrow). Bar 3 mm.

Biochemical analysis

The concentrations of NA, DA, 5-HT, MHPG, DOPAC and 5-HIAA were determinated in the selected brain regions using high-performance liquid chromatography with electrochemical detection (HPLC-ED).

Sample preparation

Five days after the second behavioral test, the rats from Group 1 were injected with saline, from Group 2 with R(+)-8-OHDPAT and from Group 3 with R(+)-UH-301 in the same way at it was described in section "Experimental groups" and killed after 3 min by decapitation. Their brains were rapidly removed and kept frozen at -70°C. Next day the selected brain regions, i.e., hypothalamus (HPT), midbrain central gray matter (MID), amygdala (AMY), hippocampus (HIP) and the pons (PO) were dissected according to the stereotaxic atlas of Paxinos and Watson (1982), placed into the Eppendorf tubes and weighed. Afterwards each brain tissue was homogenized with an ultrasonic cell disrupter (Vibracell 72434, Bioblock, Illkrich-Cedex) in 150 μl 0.1 M perchloric acid containing 0.4 mM sodium metabisulphite. The homogenates were then centrifuged at 10,000 x g for 25 min at 4°C and the supernatants were filtrated through a 0.22 μm filter (Sigma) and frozen at -70°C until analysis. Next 5 ml of filtrates was injected into the HPLC system.

Chromatographic and detection conditions

The HPLC system consisted of a quaternary delivery pump Model HP 1050 (Hewlett-Packard), a sample injector Model 7125 (Rheodyne, Berkeley), and an analytical column ODS 2 C18, 4.6 x 250 mm, particle size 5 μm (Hewlett-Packard) protected by guard column (Lichrospher 100 RP-18, 4 x 4 mm), particle size 5 μm (Hewlett-Packard). The electrochemical detector HP 1049A (Hewlett-Packard) with glassy carbon working electrode was used at a voltage setting of +0.65 V for monoamines and their metabolites vs. an Ag/AgCl reference electrode. The detector response was plotted and measured using a chromatointegrator (Esoft, Łódź). The concentration of monoamines and their metabolites in each sample were calculated from the integrated chromatographic peak area and expressed as ng/g wet tissue.

Monoamines and their metabolites determination

The mobile phase comprised a 0.15 M sodium dihydrogen phosphate, 0.1 mM EDTA, 0.5 Mm sodium octanesulphonic acid, 10-12% methanol (v/v) and 5 mM lithium chloride. The mobile phase was adjusted to pH 3.4 with ortophosphoric acid, filtrated through 0.22 μm filter (Sigma) and continuously degassed with helium during analysis. A column temperature of 32°C and flow rate 1.4 ml/min were used.

Chemicals

Methanol was purchased from Merck. Other chemicals for HPLC were purchased from Sigma Chemical Co. (St. Louis, MO).

Statistics

The behavioral results were analyzed by the Wilcoxon test for paired data, and biochemical results by the two-way ANOVA followed by the planned contrast test.

RESULTS

The effects of R(+)-8-OHDPAT injection into the dorsal raphe nucleus

BEHAVIORAL DATA

The Wilcoxon test showed an increase by 177.8% ($P<0.01$) in the number of head dipping from the dark to the illuminated part of the experimental chamber and an increase by 58.5% ($P<0.02$) in the time of motionless in the dark part of chamber after R(+)-8-OHDPAT injection as compared to the same rats saline-injected. Simultaneously, a decrease by 22.3% ($P<0.02$) in the time of locomotion activity in the illuminated part of the experimental chamber after R(+)-8-OHDPAT injection as compared to the same rats saline-injected was observed. No significant change was observed in the time out from the illuminated to the dark part of chamber, in the time of locomotion activity in the dark part of chamber, in the time of motionless behavior in the illuminated part of chamber and in the number of returns from the dark to the illuminated part of chamber (Table I).

The same statistical procedure did not show any significant differences in the measured factors in Group 1-control (Table I).

Comparison of the control animals, saline-injected (Group 1) and 8-OHDPAT-treated (Group 2) indicates that change of measured variables of behavior in the 8-OHDPAT-treated rats does not depended on repeated exposition to the aversive stimulus.

BIOCHEMICAL DATA

Regional brain concentrations of monoamines, metabolites and the ratio of metabolites to their parent amines are presented in Table II and Table III.

ANOVA demonstrated statistically significant differences between the groups in the content of DA ($F_{1,60}=102.43$, $P<0.001$), of DOPAC ($F_{1,60}=25.98$, $P<0.001$) of MHPG ($F_{1,60}=6.12$, $P<0.01$), of MHPG/NA

Table I

Behavioral events registered during light-dark transitions test after R(+)-8-OHDPAT injections into the dorsal raphe nucleus

Behavioral events	Group 1- control		Group 2- experimental	
	1- saline	2- saline	1- saline	2- 8-OHDPAT
Time out from light to dark (s)	19.5 ± 2.4	22.0 ± 2.4	26.3 ± 1.9	22.1 ± 3.1
Time of locomotion in light (s)	9.0 ± 0.7	10.2 ± 0.7	13.8 ± 1.3	9.9 ± 0.6*
Time of locomotion in dark (s)	19.8 ± 1.2	17.1±1.7	18.2 ± 1.3	17.7 ± 1.4
Time of motionless in light (s)	10.6 ± 1.9	11.9 ± 2.3	15.7 ± 2.3	12.7 ± 3.1
Time of motionless in dark (s)	20.6 ± 1.4	20.8 ± 1.4	12.3 ± 1.2	19.5 ± 2.1*
Number of returns from dark to light	0	0	0.05 ± 0.03	0.1 ± 0.06
Number of head dipping from dark to light	0.9 ± 0.2	0.7 ± 0.2	0.9 ± 0.1	2.5 ± 0.3**

Values are mean ± SEM, $n = 8$ for each group. Statistical significance Wilcoxon test. *, $P<0.02$; **, $P<0.01$ as compared to saline-injected in Group 2-experimental.

Table II

Regional brain concentrations of monoamines and their metabolites after R(+)-8-OHDPAT injections into the dorsal raphe nucleus

Group	Brain region	Monoamine and metabolite content in ng/g wet tissue					
		NA	DA	5-HT	MHPG	DOPAC	5-HIAA
1. Control	HPT	877.7 ±127.5	279.1 ± 81.3	1056.9 ± 162.8	79.0 ± 12.8	54.9 ± 17.5	613.8 ± 73.3
2. 8-OHDPAT		1298.5 ± 79.2	1003.3 ± 116.5	1066.4 ± 69.9	69.8 ± 8.1	146.7 ± 9.1	459.1 ± 31.2
		$P<0.01$	$P<0.01$				
1. Control	MID	1159.5 ± 180.3	190.2 ± 46.4	2455.7 ± 340.0	185.5 ± 23.4	62.9 ± 15.4	1024.1 ± 164.3
2. 8-OHDPAT		1014.9 ± 122.6	411.5 ± 45.7	1386.7 ± 160.4	149.0 ± 15.2	75.1 ± 20.8	1097.2 ± 128.7
				$P<0.001$			
1. Control	AMY	232.2 ± 31.2	120.6 ± 70.7	820.5 ± 147.5	70.5 ± 4.8	119.6 ± 51.7	305.1 ± 52.2
2. 8-OHDPAT		810.8 ± 78.3	1175.8 ± 130.7	1213.6 ± 114.4	87.5 ± 14.8	173.4 ± 10.0	519.9 ± 45.2
		$P<0.001$	$P<0.001$				
1. Control	HIP	777.4 ± 234.7	920.5 ± 335.5	1268.4 ± 227.4	107.6 ± 19.5	216.4 ± 34.7	779.2 ± 130.2
2. 8-OHDPAT		506.2 ± 70.6	3578.8 ± 250.5	1303.2 ± 98.6	302.3 ± 60.5	1106.3 ± 196.7	500.7 ± 40.3
			$P<0.001$		$P<0.001$	$P<0.001$	
1. Control	PO	389.1 ± 114.8	36.4 ± 7.7	609.0 ± 168.9	92.6 ± 11.8	17.6 ± 4.4	570.5 ± 84.9
2. 8-OHDPAT		454.5 ± 55.6	256.9 ± 102.2	880.4 ± 68.6	121.7 ± 27.3	36.8 ± 6.1	462.4 ± 42.7

Values are mean ± SEM; $n = 7$ for each group. Statistical significance: planned contrast test.

Table III

Ratio of metabolites to their parent amines in regional brain areas after R (+)-8OHDPAT injections into the dorsal raphe nucleus

Group	Brain region	MHPG/NA	DOPAC/DA	5-HIAA/5-HT
1. Control	HPT	0.089 ± 0.007	0.224 ± 0,030	0.552 ± 0.023
2. 8-OHDPAT		0.065 ± 0.010	0.174 ± 0,006	0.457 ± 0.016
				$P<0.02$
1. Control	MID	0.151 ± 0.011	0.275 ± 0,024	0.511 ± 0.017
2. 8-OHDPAT		0.183 ± 0.023	0.207 ± 0,033	0.545 ± 0.015
1. Control	AMY	0.333 ± 0.044	0.281 ± 0.059	0.423 ± 0.020
2. 8-OHDPAT		0.202 ± 0.029	0.211 ± 0.005	0.379 ± 0.016
1. Control	HIP	0.132 ± 0.016	0.099 ± 0.012	0.549 ± 0,036
2. 8-OHDPAT		0.396 ± 0.095	0.192 ± 0.028	0.372 ± 0.017
		$P<0.001$		$P<0.001$
1. Control	PO	0.295 ± 0.056	0.461 ± 0.095	0.735 ± 0.067
2. 8-OHDPAT		0.512 ± 0.121	0.412 ± 0.032	0.555 ± 0.010
		$P<0.01$		$P<0.001$

Values are mean ± SEM, $n = 7$ for each group. Statistical significance: planned contrast test.

ratio ($F_{1,60}$=9.24, $P<0.01$), of 5-HIAA/5-HT ratio ($F_{1,60}$=15.83, $P<0.001$) and significant interactions between the brain regions and the groups for NA ($F_{4,60}$=4.28, $P<0.004$), for DA ($F_{4,60}$=21.72, $P<0.001$), for 5-HT ($F_{4,60}$=5.56, $P<0.001$), for MHPG ($F_{4,60}$=6.61, $P<0.001$) for DOPAC ($F_{4,60}$=16.43, $P<0.001$) and for 5-HIAA ($F_{4,60}$=13.01, $P<0.001$). Further analysis by means of planned contrast test showed that the level of NA was higher in HPT and AMY in Group 2 vs. Group 1, the DA level was higher in HPT, AMY and HIP in Group 2 vs. Group 1, the 5-HT level was lower only in MID in Group 2 vs. Group 1, the MHPG level was higher only in HIP in Group 2 vs. Group 1, the DOPAC level was higher only in HIP in Group 2 vs. Group 1, the MHPG/NA ratio was higher in HIP and PO in Group 2 vs. Group 1 and the 5-HIAA/5-HT ratio was lower in HPT, HIP and PO in Group 2 vs. Group 1.

The effects of R(+)-UH-301 injection into the dorsal raphe nucleus

BEHAVIORAL DATA

The Wilcoxon test showed an increase by 58.8% ($P<0.04$) in the number of head dipping from the dark to the illuminated part of the experimental chamber and an increase by 22.7% ($P<0.01$) in the time of motionless in the dark part of chamber after R(+)-UH-301 injection as compared to the same rats saline-injected. Simultaneously, an decrease by 35.2% ($P<0.01$) in the time of locomotion activity in the illuminated part of the experimental chamber after R(+)-UH-301 injection as compared to the same rats saline-injected was observed. No significant change was observed in the time out from the illuminated to the dark part of chamber, in the time of locomotion activity in the dark part of chamber, in the time of motionless behavior in the illuminated part of chamber and in the number of returns from the dark to the illuminated part of chamber (Table IV).

The same statistical procedure did not show any significant differences in the measured factors in Group 1-control (Table IV).

Comparison of the control animals, saline-injected (Group 1) and R(+)-UH-301-treated (Group 3) indicates that change of measured variables of behavior in the R(+)-UH-301-treated rats does not depended on repeated exposition to the aversive stimulus.

Table IV

Behavioral events registered during light-dark transitions test after R(+)-UH-301 injections into the dorsal raphe nucleus

Behavioral events	Group 1- control		Group 3- experimental	
	1- saline	2- saline	1- saline	2- UH-301
Time out from light to dark (s)	19.5 ± 2.4	22.0 ± 2.4	15.2 ± 2.29	11.0 ± 1.8
Time of locomotion in light (s)	9.0 ± 0.7	10.2 ± 0.7	12.5 ± 0.1	8.1 ± 0.2**
Time of locomotion in dark (s)	19.8 ± 1.2	17.1±1.7	27.9 ± 1.7	28.2 ± 1.5
Time of motionless in light (s)	10.6 ± 1.9	11.9 ± 2.3	3.0 ± 0.5	3.6 ± 0.5
Time of motionless in dark (s)	20.6 ± 1.4	20.8 ± 1.4	16.3 ± 1.3	20.0 ± 1.1**
Number of returns from dark to light	0	0	0	0.15 ± 0.2
Number of head dipping from dark to light	0.9 ± 0.2	0.7 ± 0.2	1.7 ± 0.07	2.7 ± 0.2*

Values are mean ± SEM, $n = 8$ for each group. Statistical significance Wilcoxon test. *, $P<0.04$; **, $P<0.01$ as compared to saline-injected in Group 3-experimental.

BIOCHEMICAL DATA

Regional brain concentrations of monoamines, metabolites and the ratio of metabolites to their parent amines are presented in Table V and Table VI.

ANOVA demonstrated statistically significant differences between the groups in the content of NA ($F_{1,60}$=11.96, $P<0.001$), of DOPAC ($F_{1,60}$=5.64, $P<0.02$), of MHPG/NA ratio ($F_{1,60}$=4.14, $P<0.04$) and significant interactions between the brain regions and the groups for NA ($F_{4,60}$=12.57, $P<0.001$), for 5-HT ($F_{4,60}$=3.42, $P<0.01$), for DOPAC ($F_{4,60}$=5.46, $P<0.001$) and for 5-HIAA ($F_{4,60}$=8.25, $P<0.001$). Further analysis by means of planned contrast test showed that the level of NA was higher in HPT and lower in HIP in Group 3 vs. Group 1, the 5-HT level was higher in AMY and lower in MID in Group 3 vs. Group 1, the 5-HIAA level was higher in MID and lower in HIP in Group 3 vs. Group 1 and the MHPG/NA ratio was lower in PO in Group 3 vs. Group 1.

DISCUSSION

In our present experiments administration of R(+)-8-OHDPAT as well as R(+)-UH-301, the 5-HT_{1A} receptor agonists, into the dorsal raphe nucleus of rats examined in the modified version of light-dark transitions test resulted in a statistically significant increase of the number of head dipping from dark to the illuminated compartment of experimental chamber, an increase of time of motionless in the dark compartment and decrease of time of locomotion activity in the illuminated compartment of chamber. These behavioral changes and particularly increased number of head dipping from dark to the illuminated compartment indicate that both used 5-HT_{1A} receptor agonists decreases fear behavior. The fact that both R(+)-8-OHDPAT and R(+)-UH-301 evoke identical behavioral changes speaks for the effects being specific, with anxiolytic profile and resulting from stimulation of 5-HT_{1A} autoreceptors in DRN. The results of our studies are significant in complementing the existing ones (Andrews et al. 1994, Hogg et al. 1994, Jolas et al. 1995, Picazo et al. 1995, File et al. 1996, Lopez-Rubelcava 1996, Remy et al. 1996, Romaniuk et al. 2001) and above all constitute a proof indicating that the 5-HT_{1A} receptors play a fundamental, key role in the central mechanisms of the fear/anxiety behavior. Indeed, there are also data indicating the fact 5-HT_2 and 5-HT_3 receptors participate in the regulation of fear/anxiety but they have not been widely documented so far. It should be also noted that 5-HT_{1A} receptors are involved not only in the fear/anxiety mechanisms but also in the regulation of feeding (Hutson et al. 1986), body temperature (Hjorth 1985), sexual behavior (Alhenius et al. 1986, Johansson et al. 1991), locomotion and motor activity (Björk et al. 1992) and various cardiovascular effects (Björk et al. 1991).

The present results make us conclude that R(+)-8-OHDPAT and R(+)-UH-301 might be used interchangeably as selective agonists of 5-HT_{1A} receptors. However, in a great majority of studies racemic

Table V

Regional brain concentrations of monoamines and their metabolites after R(+)-UH-301 injections into the dorsal raphe nucleus

Group	Brain region	Monoamine and metabolite content in ng/g wet tissue					
		NA	DA	5-HT	MHPG	DOPAC	5-HIAA
1. Control	HPT	877.7 ±127.5	279.1 ± 81.3	1056.9 ± 162.8	79.0 ± 12.8	54.9 ± 17.5	613.8 ± 73.3
2. UH-301		2144.2 ± 144.9	363.2 ± 50.2	1322.9 ± 58.6	107.0 ± 10.5	77.5 ± 8.4	718.7 ± 46.0
		$P<0.001$					
1. Control	MID	1159.5 ± 180.3	190.2 ± 46.4	2455.7 ± 340.0	185.5 ± 23.4	62.9 ± 15.4	1024.1 ± 164.3
2. UH-301		133.3 ± 126.4	176.4 ± 6.9	1968.5 ± 138.7	150.6 ± 27.9	48.5 ± 4.3	1551.6 ± 84.2
				$P<0.04$			$P<0.001$
1. Control	AMY	232.2 ± 31.2	120.6 ± 70.7	820.5 ± 147.5	70.5 ± 4.8	119.6 ± 51.7	305.1 ± 52.2
2. UH-301		365.5 ± 33.3	468.8 ± 88.8	1397.5 ± 29.9	72.4 ± 11.1	90.0 ± 16.4	456.1 ± 25.3
				$P<0.02$			
1. Control	HIP	777.4 ± 234.7	920.5 ± 335.5	1268.4 ± 227.4	107.6 ± 19.5	216.4 ± 34.7	779.2 ± 130.2
2. UH-301		279.8 ± 36.8	797.5 ± 219.5	1002.0 ± 101.8	79.8 ± 12.1	56.4 ± 12.4	314.3 ± 21.9
		$P<0.01$				$P<0.001$	$P<0.001$
1. Control	PO	389.1 ± 114.8	36.4 ± 7.7	609.0 ± 168.9	92.6 ± 11.8	17.6 ± 4.4	570.5 ± 84.9
2. UH-301		690.6 ± 49.8	49.3 ± 2.4	931.6 ± 84.4	79.9 ± 7.1	31.4 ± 3.0	603.3 ± 81.9

Values are mean ± SEM; $n = 7$ for each group. Statistical significance: planned contrast test.

Table VI

Ratio of metabolites to their parent amines in regional brain areas after R (+)-UH-301 injections into the dorsal raphe nucleus

Group	Brain region	MHPG/NA	DOPAC/DA	5-HIAA/5-HT
1. Control	HPT	0.089 ± 0.007	0.224 ± 0,030	0.552 ± 0.023
2. UH-301		0.048 ± 0.004	0.243 ± 0.018	0.448 ± 0.018
1. Control	MID	0.151 ± 0.011	0.275 ± 0,024	0.511 ± 0.017
2. UH-301		0.180 ± 0.014	0.316 ± 0.040	0.683 ± 0.044
1. Control	AMY	0.333 ± 0.044	0.281 ± 0.059	0.423 ± 0.020
2. UH-301		0.135 ± 0.051	0.172 ± 0.013	0.345 ± 0.013
1. Control	HIP	0.132 ± 0.016	0.099 ± 0.012	0.549 ± 0,036
2. UH-301		0.219 ± 0.035	0.127 ± 0.015	0.504 ± 0.010
1. Control	PO	0.295 ± 0.056	0.461 ± 0.095	0.735 ± 0.067
2. UH-301		0.114 ± 0.007	0.561 ± 0.031	0.514 ± 0.032
		$P<0.01$		

Values are mean ± SEM, $n = 7$ for each group. Statistical significance: planned contrast test.

8-OHDPAT was used which is probably due to the fact that this agent was introduced to research a few years earlier than UH-301 and its properties were better recognized. In the recent years R(+)-enantiomer was used because R(+)-8-OHDPAT behaves as a full agonist in the assay of forskolin-stimulated adenylate cyclase activity (Hacksell et al. 1990).

With full compatibility of behavioral effects no such compatibility in neurochemical effects occurred despite the fact that the two agents are selective 5-HT_{1A} receptor agonists. Intra-DRN-injection of R(+)-8-OHDPAT evoked a distinct attenuation of serotonergic system activity, for which an decrease in 5-HIAA/5-HT ratio speaks, in most examined structures – in the hypothalamus, hippocampus and pons. Simultaneously, an increase in noradrenergic system activity occurred, which was manifested by an increase in MHPG/NA ratio in the hippocampus and pons and an increase in NA concentration in the hypothalamus and amygdala. Moreover, an increase in dopaminergic system activity occurred, which was manifested by an increase in DA concentration in the hypothalamus, amygdala and hippocampus. These data indicate that an attenuation of the serotonergic system activity caused by R(+)-8-OHDPAT injection into the DRN infringes a dynamic balance between 5-HT-NA-DA systems. While R(+)-UH-301 injections into the DRN do not cause any distinct and clearly oriented changes in the activity of the serotonergic system, as well as noradrenergic and dopaminergic ones. This lack of conformity in neurochemical effects must result from the fact that R(+)-UH-301 is a 5-HT_{1A} receptor agonist with a pharmacological profile similar to those of R(+)-8-OHDPAT, although of somewhat lower potency (Hillver et al. 1990, Björk et al. 1991,1992, Johanson et al. 1991). Perhaps that is why neurochemical effects after injections of R(+)-UH-301 in a dose equimolar to R(+)-8-OHDPAT did not become evident enough.

CONCLUSIONS

The results of our experiments with use of two 5-HT_{1A} receptor agonists provided one more new piece of evidence on specific and key role of the 5-HT_{1A} receptors in central regulation of the fear behavior. Injections of R (+)-8-OHDPAT and R (+)-UH-301 into the DRN resulted in identical behavioral effects of anxiolytic profile. Simultaneously, especially after 8-OHDPAT a decrease in serotonergic system activity and an increase in noradrenergic and dopaminergic systems activity measured as a ratio of metabolites to their parent

amines in the structures, which form a brain emotional-defensive system, were established.

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