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Bicuculline administration into ventromedial hypothalamus: effects on fear and regional brain monoamines and GABA concentrations in rats

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Abstract. The effects of bicuculline methiodide administration into ventromedial hypothalamus (15 ng *per site*, bilaterally) on fear behavior and monoamines (NA, DA, 5-HT) and GABA in structures of the brain defensive system (hypothalamus, midbrain gray matter, amygdala, hippocampus and frontal cortex) were studied. Fear behavior was examined in the modified version of light-dark transition test. The time out from the illuminated compartment of chamber, the time spent there and number of returns to the illuminated compartment was measured. Additionally motor activity, i.e., number of crossings and rearings in dark as well as in the illuminated part of compartment, was registered. Blockade of $GABA_A$ receptors in the ventromedial hypothalamus resulted in increased fear behavior, i.e. decrease of time out from illuminated compartment and decrease of the time spent there. Motor behavior remained unchanged. HPLC analysis showed reduction of GABA concentration in all investigated brain structures. An increase of NA concentration in all examined structures with exception of the hypothalamus without effect on MHPG/NA was observed as well. Dopamine level remained unchanged, but DOPAC/DA ratio increased in all structures, except frontal cortex. Also HVA/DA ratio increased in the hypothalamus and midbrain. 5-HT concentration increased only in midbrain, 5-HIAA increased in midbrain and in frontal cortex, and 5-HIAA/5-HT ratio increased only in frontal cortex. These results indicate that GABA-ergic and monoaminergic systems remain in functional interactions and that these interactions may play an important role in the neurochemical regulation of fear behavior. The possible mechanism of GABA – monoaminergic interactions is discussed.

Key words: light-dark transitions test, bicuculline, fear, brain monoamines, GABA, HPLC, rat

INTRODUCTION

Many studies conducted for years on various animal models allowed to identify the anatomical bases of fear and anxiety. It is now well established that neural substrate of fear is represented by an integrated circuitry known as the brain defense system i.e., a system that detects threatening or stressogenic stimuli and organizes responses to them. The main constituents of this system include amygdala, hypothalamus, hippocampus and periaqueductal gray matter (Blanchard and Blanchard 1988, Graeff 1990, Graeff et al. 1993). Electrical or chemical stimulation of these regions evokes species--specific behavioral patterns of defense. Fear as a natural reaction to threatening events is an emotion particularly important to human and animal existence, and disturbances in its regulation produce severe clinically distinguished affective disorders like panic, phobias, generalized anxiety. In spite of the fact, that contemporary pharmacology knows many anxiolytic drugs used quite effectively to treat human emotional disorders, the neurochemical mechanisms underlying fear and disturbances in its control are not fully understood yet.

The results of various authors indicate that excitatory and inhibitory aminoacids, aminergic transmitters and some peptides, remaining in functional interactions, are involved in different ways and to different extents in the regulation of the brain defensive system (Graeff 1990).

Several lines of evidence implicate the inhibitory role of GABA-ergic mechanisms in the control of fear and defensive behavior (Depaulis and Vergnes 1984, Carrive et al. 1986, Shaikh and Siegel 1990, Corbett et al. 1991, Shekhar 1993). This has been also very recently confirmed by the study on knockouts. Crestani et al. (1999) showed that the mice heterozygotes for the ^{2}subunit of $GABA_A$ receptors exhibit enhanced anxiety in several tests of innate and learned fear.

The hypothalamus has long been implicated as a critical region in the neural regulation of mammalian emotions. It has been shown that $GABA_A$ receptors in dorsomedial hypothalamus in rats regulate fear and its physiological (heart rate, blood pressure and plasma norepinephrine level) concomitance (Shekhar 1993, Shekhar et al. 1993). We were interested in the possible role of ventromedial part of hypothalamus in the mechanisms controlling fear behavior. It is suggested that in the region of medial hypothalamus, irrespectively of its anatomical subdivisions, neurons commanding defense reactions and elaborating aversive states are under tonic GABA-ergic inhibition (Graeff 1990). GABA in high concentration has been described within this structure (Tappaz et al. 1977, Blume et al. 1981, Tokyama and Takatsuji 1998).

The present study was undertaken to test the hypothesis that GABA-ergic mechanisms in ventromedial hypothalamus are involved in the regulation of fear in animals confronted with stressogenic stimulus. In the biochemical analysis we were interested not only in changes at the level of GABA, but also DA, 5-HT, NA and their metabolites in order to determine the possible interactions between GABA and monoaminergic systems activity in correlation with behavioral alterations.

METHODS

Subjects

The experiments were performed on 16 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, four per cage, and maintained under controlled environmental conditions of temperature (22 ± 2°C), humidity (55 ± 5%) and on a 12L: 12D cycle (light on at 0800) with food and water ad lib. The behavioral recordings took place between 0900 and 1200 h.

Surgery and microinjections

Animals were implanted bilaterally into the ventromedial hypothalamus (VMH) with guide cannulas under chloral hydrate anesthesia (360 mg/kg IP). Anesthetized rats were positioned in stereotaxic frame (Kopf Instruments, Tujunga, CA). The skull was exposed and the incisor bar adjusted such that bregma and lambda were at the same height. Stereotaxic coordinates of Paxinos and Watson atlas (1982) with reference to bregma for the tip of this cannulas were: F = -2.3, L = 1.0, H = 9.5 at an angle of 13.7° to protected against sinus injury. The guide stainless steel cannulas (0.7 mm external diameter) were fixed to the skull with self polymerizing methacrylate resin (Vertex, Dentimex Manufactures, Holland). Cannulas were shut with a stainless steel stylet (0.5 mm) to prevent clogging. After surgery, rats were allowed to recover for 14 days prior to behavioral testing. During this period animals were handled and familiarized with the injection procedure.

Microinjections were made into each hypothalamus separately by one experimenter, while the other one gently handled the rat. An internal injection cannula (0.3 mm external diameter) was inserted into the guide cannula so that it extended 2.5 mm beyond its 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 bicuculline solution or with the saline by aspiration. A small air gap separated the two solutions, i.e. distilled water and bicuculline or saline. A successful injection was indicated by the movement of the air bubble down the tubing. Solutions were injected manually at a rate of 0.01 l/s. After each infusion the internal cannula was left in place for 30 s before being removed. Zimmermann's microinjector was equipped with the micrometer screw, which allows to deliver of the solutions with accuracy of the 0.01 l.

CHEMICAL COMPOUNDS

Bicuculline (Bicuculline methiodide, Sigma) was dissolved in saline on the day of the test and injected bilaterally into the hypothalamus (15 ng / 0.25 l *per site*). Vehicle alone (0.25 l *per site*) was used as control injection.

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 40 cm each) with an opening allowing the animal to change its location (Fig. 1). The light conditions were changed automatically at selected order – halogen bulbs (2,000 lx) illuminated brightly only the compartment in which the rat was present, leaving the other one dark and safe. The experiment began 1 min after introducing the rat into the experimental chamber. The scheme of the experiment is presented in Fig. 1. Three basic variables were videotaped and measured: (1) time out from the illuminated compartment, i.e. time elapsing from the moment of switching on the aversive light stimulus to the moment of the rat's passing to the dark part of chamber; (2) time

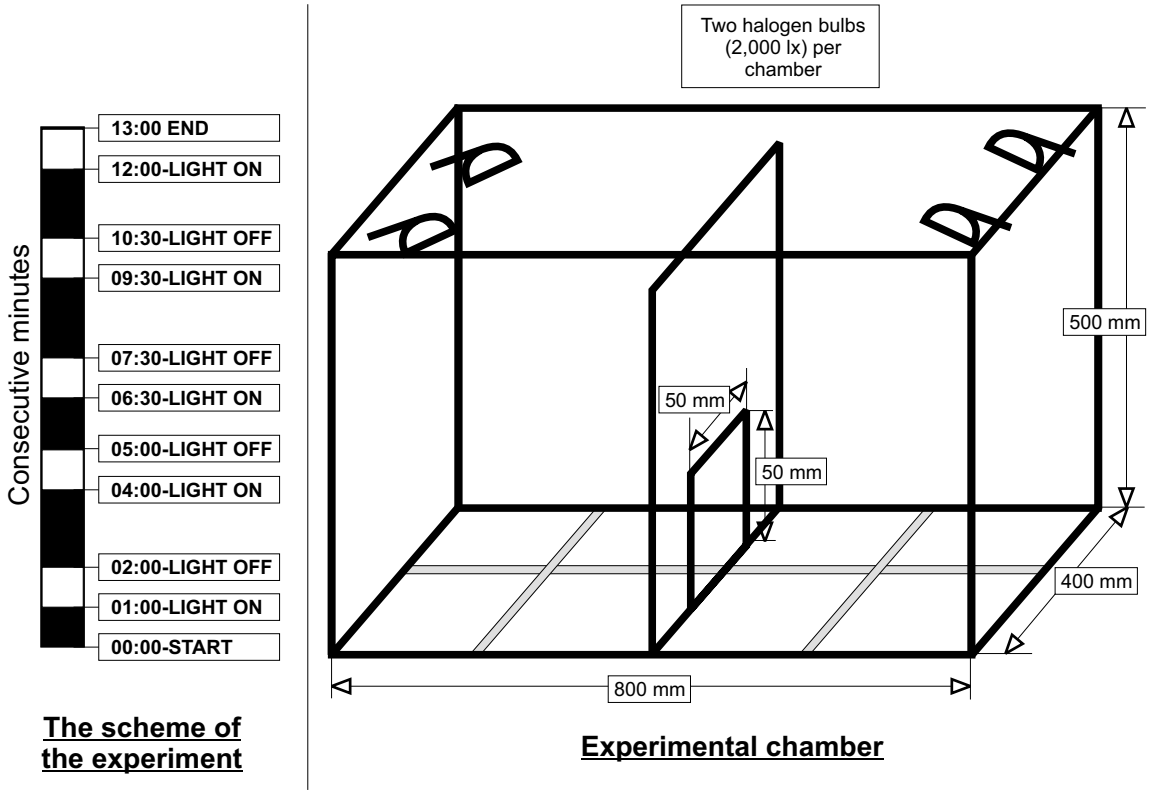

Fig. 1. The scheme of the experiment and experimental chamber.

spent in the illuminated compartment; (3) number of returns to the illuminated compartment after first escape. Additionally, ambulations in both, dark and light part of the chamber as well as number of rearings were registered.

All rats ($n = 16$) were tested two times – First after two weeks of recovery, following intrahypothalamic saline injection. Then 72 hours later rats were divided into the two groups and tested again. The Control (CNT) group ($n = 8$) was injected with saline into the VMH, and the experimental (BMI) group ($n = 8$) was injected with bicuculline into the VHM. Behavioral tests in each case were performed 2 min after intrahypothalamic injections.

Histology

Histological verification of the cannulas placements and injection sites in the VHM were carried out in the four animals that were randomly chosen from both groups: two rats from control one and two from BMI group. Three days after the completion of behavioral experiments these animals obtained under chloral hydrate anesthesia an infusion of the dye (fatty hrizoidin), of the same amount as BMI or saline. Next their brains were removed from the skull and cut on the 80 m slices with the freezing microtom. Next, the slices were photographed and used for the verification of the injection sites in the VHM. The typical example of the tip of injection cannulas placement in the VHM is presented (Fig. 2.)

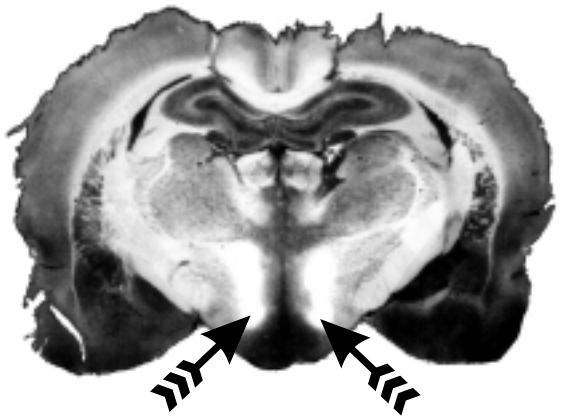

Fig. 2. Histological verification of the cannulas placement.

Biochemical analysis

The concentrations of NA, DA, 5-HT, DOPAC, HVA, 5-HIAA and GABA 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, rats from both groups, i. e., control (CNT) and experimental (BMI), were injected with saline or BMI respectively and killed after 2 min by decapitation. Their brains were rapidly removed and kept frozen at -70°C. Next day after the brains were frozen, selected regions were dissected under the visual control as following: the first cut was made at the level of optic chiasm and the second one at the level of the mammillary body. From this slick the following areas were dissected: the hypothalamus (HPT), the amygdala (AMY), the hippocampus (HIP) and the part of frontal cortex (CTX). Next, we isolated the midbrain, and from this area we dissected the midbrain central gray matter (MID). Then the dissected brain regions were 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 l 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 filtered through a 0.22 m filter (Sigma) and frozen at -70°C until analysis. Next 5 l of filtrates was injected into the HPLC system.

CHROMATOGRAPHIC AND DETECTION CONDITIONS

The HPLC system consisted of a quaternary gradient 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 m (Hewlett-Packard) protected by guard column (Lichnospher 100 RP-18, 4 X 4 mm), particle size 5 m (Hewlett-Packard). The electrochemical detector model HP 1049 A (Hewlett-Packard) with glassy carbon working electrode was used at a voltage setting of +0.65 V for monoamines and their metabolites, and +0.50 V for GABA, vs. an Ag/AgCl reference electrode. The detector response was plotted and measured using a chro-

matointegrator ver. 1.2 (Esoft, Łódź). The concentration of monoamines and their related metabolites in each sample were calculated from the integrated chromatographic peak area and expressed as ng/g wet tissue. GABA concentrations were calculated in the same way as the monoamine ones, but expressed in g/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 phosphoric acid, filtered through 0.22 m filter (Sigma) and degassed with helium. A column temperature of 32°C and a flow rate of 1.4 ml/min were used.

GABA DETERMINATION

The mobile phase for GABA determination was 0.1 mM sodium acetate buffer with 0.1 mM EDTA and 5 mM lithium chloride in 25 % (v/v) methanol. The mobile phase was adjusted to pH 5.5 with acetic acid, filtered through 0.22 m filter (Sigma) and degassed with helium. The examined amino acid was eluted with the linear methanol gradient from 25% to 75% in 15 min, 75% in next 2 min and from 75% to 25% in following 6 min. Just before the injection into the HPLC system, GABA was derivatized with o-phtalaldehyde-thiol (OPT-thiol) reagent for 2 min. A column temperature of 34°C and a flow rate of 1.3 ml/min were used.

CHEMICALS

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

Statistical analysis

The behavioral results were analyzed by the Wilcoxon test for paired data, and biochemical results by the two-way ANOVA. In the case of significant differences between the groups, the planned contrast analysis was carried out to find the significant differences in the particular brain area.

RESULTS

Behavioral reaction to the aversive stimulus

Behavioral results in control (CNT) and in experimental group (BMI) are presented in Table I. As it is seen, bicuculline injection resulted in decrease of the time out from the illuminated compartment and decrease of the time spent in the light. Wilcoxon test showed significant decrease of the time out from the illuminated compartment after BMI vs. saline ($P<0.01$). Wilcoxon test showed also decrease of the time spent in the illuminated compartment after BMI injection vs. saline

Table I

Behavioral events registered during light-dark transitions test in control and in experimental groups

Behavioral events	Control		Experimental	
	NaCl - 1	NaCl - 2	NaCl	BMI
Time out from light (s)	15.07 ± 1.04	15.57 ± 0.77	19.63 ± 5.16	7.25 ± 1.51[a]
Time spent in light (s)	15.55 ± 1.15	17.12 ± 0.84	28.25 ± 4.93	17.00 ± 3.40[b]
Number of returns to the light	0.07 ± 0.05	0.10 ± 0.07	0.38 ± 0.18	1.00 ± 0.46
Number of crossings in light	0.92 ± 0.16	1.27 ± 0.31	2.75 ± 0.53	4.38 ± 1.12
Number of rearings in light	0.08 ± 0.05	0.07 ± 0.05	0.50 ± 0.38	0.00 ± 0.00
Number of crossings in dark	3.37 ± 0.21	3.30 ± 0.48	2.63 ± 0.80	4.50 ± 0.94
Number of rearings in dark	0.10 ± 0.05	0.02 ± 0.02	0.00 ± 0.00	0.25 ± 0.16

Values are mean SEM, $n = 8$; Statistical significance: Wilcoxon test; a, $P<0.01$ as compared BMI vs. NaCl injected; b, $P<0.02$ as compared BMI vs. NaCl injected.

Table II

Regional brain concentration of NA, DA, 5-HT, GABA, MHPG, DOPAC, HVA AND 5-HIAA

Brain region	Group	NA	DA	5-HT	GABA	MHPG	DOPAC	HVA	5-HIAA
HPT	CNT	997.14 ± 237.71	83.72 ± 14.36	631.03 ± 92.12	108.87 ± 5.28	198.66 ± 31.90	161.14 ± 8.03	41.36 ± 2.84	804.72 ± 88.11
	BMI	843.18 ± 122.59	40.01 ± 15.91	864.37 ± 134.22	61.30 ± 7.27	290.92 ± 63.29	105.58 ± 21.70	39.05 ± 3.82	975.78 ± 121.26
					P<0.001				
MID	CNT	441.53 ± 60.72	45.65 ± 6.46	695.53 ± 64.89	101.11 ± 6.04	138.55 ± 21.60	56.22 ± 11.49	19.47 ± 6.61	870.72 ± 44.32
	BMI	833.13 ± 181.2	60.35 ± 17.35	1393.09 ± 522.74	73.94 ± 7.60	188.95 ± 51.10	88.30 ± 15.29	36.79 ± 5.69	1222.01 ± 123.60
		P<0.02		*P*<0.01	*P*<0.001				*P*<0.03
AMY	CNT	93.16 ± 19.11	92.48 ± 28.03	562.69 ± 83.50	87.94 ± 2.40	59.49 ± 2.54	136.35 ± 24.54	51.01 ± 8.23	414.16 ± 31.30
	BMI	407.71 ± 39.77	97.91 ± 24.59	635.16 ± 75.20	45.70 ± 3.90	142.14 ± 46.69	145.69 ± 23.44	56.85 ± 13.82	450.93 ± 57.31
		P<0.05			*P*<0.001				
HIP	CNT	115.05 ± 15.90	161.05 ± 32.87	204.40 ± 36.89	87.09 ± 3.15	63.16 ± 10.23	146.96 ± 27.51	49.89 ± 14.64	304.54 ± 28.92
	BMI	499.88 ± 31.55	197.46 ± 81.86	500.02 ± 76.64	53.82 ± 3.00	84.95 ± 24.96	223.38 ± 50.33	82.48 ± 14.80	461.69 ± 67.35
		P<0.02			*P*<0.001				
CTX	CNT	131.08 ± 34.50	19.70 ± 2.19	391.30 ± 64.61	117.47 ± 6.79	159.48 ± 15.92	36.14 ± 5.18	12.11 ± 3.51	660.19 ± 42.20
	BMI	1721.43 ± 168.49	41.19 ± 18.25	768.83 ± 321.22	95.67 ± 4.96	351.13 ± 66.76	46.94 ± 10.02	7.01 ± 1.73	1079.48 ± 274.84
		P<0.001			*P*<0.005	*P*<0.001			*P*<0.01

Values are mean ± SEM, $n = 6$ for each group; statistical significance planned contrast. Monoamines and metabolites in ng/g and GABA in g/g wet tissue.

Table III

Ratio of monoamines and their metabolites

Brain region	Group	MHPG/NA	DOPAC/DA	HVA/DA	5-HIAA/5-HT
HPT	CNT	0.143 ± 0.06	1.449 ± 0.05	0.410 ± 0.04	0.977 ± 0.16
	BMI	0.601 ± 0.14	4.450 ± 0.84	2.928 ± 0.62	1.921 ± 0.47
			<0.001	<0.001	
MID	CNT	0.299 ± 0.04	0.830 ± 0.10	0.256 ± 0.05	1.044 ± 0.15
	BMI	0.283 ± 0.04	4.308 ± 1.01	1.721 ± 0.89	1.845 ± 0.33
			<0,001	0.01	
AMV	CNT	0.468 ± 0.09	1.063 ± 0.22	0.384 ± 0.07	0.591 ± 0.09
	BMI	0.397 ± 0.12	2.714 ± 0.62	1.058 ± 0.17	0.879 ± 0.18
			<0.05		
HIP	CNT	0.396 ± 0.07	0.808 ± 0.11	0.259 ± 0.06	1.222 ± 0.07
	BMI	0.436 ± 0.10	3.011 ± 0.82	1.335 ± 0.42	1.703 ± 0.33
			<0.01		
CTX	CNT	0.831 ± 0.21	1.649 ± 0.25	0.456 ± 0.12	1.577 ± 0.3
	BMI	0.325 ± 0.05	2.896 ± 0.72	0.693 ± 0.39	3.293 ± 0.82
					<0.001

Values are mean ± SEM, $n = 6$ for each group; statistical significance planned contrast.

($P<0.02$). No statistically significant differences occurred in the number of returns to the illuminated compartment. Changes in the motor activity, i.e. ambulations and rearings were not statistically significant after BMI injections vs. saline neither in light nor in dark compartment. The same statistical procedure did not show any significant differences in measured factors in control group (CNT) i.e. first saline injection vs. second saline injection.

Biochemical results

Tables II and III summarize the neurochemical data obtained after intrahypothalamic injection of bicuculline.

ANOVA demonstrated statistically significant differences between the groups in the content of NA ($F_{1,50} = 45.35$, $P<0.001$), of MHPG ($F_{1,50} = 12.23$, $P<0.001$), of 5-HT ($F_{1,50} = 7.05$, $P<0.01$), of 5-HIAA ($F_{1,50} = 10,18$, $P<0.002$), of GABA ($F_{1,50} = 103.45$, $P<0.001$), of DOPAC/DA ratio ($F_{1,50} = 38.31$, $P<0.001$), of HVA/DA ratio ($F_{1,50} = 22.35$, $P<0.001$) and of 5-HIAA/5-HT ratio ($F_{1,50} = 13.37$, $P<0.001$). Further analysis by means of planned contrasts test showed that the level of NA was higher in MID, AMY, HIP and in CTX in BMI Group vs. CNT Group, the MHPG level was higher only in CTX in BMI Group vs. CNT Group, the 5-HT level was higher only in MID in BMI Group vs. CNT Group, the 5-HIAA level was higher in MID and in CTX in BMI Group vs. CNT Group, the level of GABA was lower in all investigated regions, i.e. in the HPT, MID, AMY, HIP and CTX in BMI Group vs. CNT Group. The DOPAC/DA ratio was higher in HPT, MID, AMY and HIP in BMI Group vs. CNT Group, the HVA/DA ratio was higher in HPT and MID in BMI Group vs. CNT Group, the 5-HIAA/5-HT ratio was higher only in CTX in BMI Group vs. CNT Group.

No significant differences occurred between the groups in the contents of DA, DOPAC, HVA and in the MHPG/NA ratio.

DISCUSSION

$GABA_A$ antagonist bicuculline injected into VHM of rats exposed to stressogenic stimulus resulted in statistically significant decrease of time out from the illuminated compartment and statistically significant decrease of the time spent there as compared to vehicle injected animals. Since motor activity in BMI injected rats was not increased, that might indicate the increase of fear response. The effect of increased fear behavior is in agreement with other studies demonstrating that inhibition of GABA-ergic transmission evokes anxiogenic effects (Di Scala et al. 1984, Schmitt et al. 1985, Carrive et al. 1986, Shekhar et al. 1993, Strzelczuk and Romaniuk 1996, Crestani et al. 1999).

Our results showed also that the GABA dependent mechanisms involved in the regulation of fear behavior are not restricted only to dorsomedial part of the hypothalamus, as it has been suggested by the work of Shekhar et al. (1993)

Biochemical analysis showed that BMI injection significantly reduced GABA concentration in all of the investigated structures (hypothalamus, amygdala, hippocampus, cortex and midbrain). Moreover, there occurred statistically significant increase of NA concentration in amygdala, hippocampus, midbrain and cortex. Similar behavioral and biochemical results in cats were described by Strzelczuk and Romaniuk (1996) i.e. intrahypothalamic injections of BMI evoked increased fear response and elevated NA turnover in the key emotional structures of the brain. Authors concluded that fear response occurs only when an inhibitory action of GABA--ergic system is suppressed and therefore tonic inhibition of noradrenergic system became abolished.

Data concerning the role of locus coeruleus (LC) noradrenergic system in fear and anxiety are contradictory (Crow et al. 1978, File et al. 1979, Foote et al. 1991, Wieczorek and Romaniuk 1994, Zagrodzka 1995). It has been proposed however that LC NA system functions as the novelty or danger detector (Redmond and Huang 1979). The destruction of LC in monkeys produced fear reduction (Huang et al. 1975, Mason et al. 1978, Redmond and Huang 1979). In our experiment (Zagrodzka et al. 1994) the noradrenergic depletion after neurotoxic destruction of NA system resulted in significant decrease of fear measured in the photo-phobic open field and increase of offensive postures in previously submissive rats. It was also demonstrated previously that stress or threat evoking stimuli result in the increase of NA metabolism in the hypothalamus, hippocampus, thalamus, midbrain, amygdala and basal ganglia (Glavin et al. 1983, Kantak et al. 1984, Finlay et al. 1995, Krotewicz and Romaniuk 1995). It was also shown that yohimbine, $_2$-adrenoreceptor antagonist, elicits anxiety behavior in nonhuman primates (Coplan et al. 1992) or potentiates defensive responses to threatening stimuli in mice (Blanchard et al. 1993).

$GABA_A$ receptors blockade in our rats resulted also in some changes in other monoaminergic systems, which might contribute to the control of fear and anxiety mechanism. Statistically significant increase of 5-HT occurred in the midbrain (the site of origin of many serotoninergic pathways). 5-HIAA increased in midbrain and frontal cortex, and 5-HIAA/5-HT ratio increased only in the cortex.

The serotoninergic system has long been postulated to have an important role in the neurobiology of anxiety disorders. The 5-HT hypothesis of anxiety proposes that decreasing 5-HT function leads to an anxiolytic effect, whereas increasing 5-HT function, on the opposite, promotes fear and anxiety (Chopin and Briley 1987, File 1990, Graeff 1990, Joffe and Swinson 1990). There are evidences that GABA-ergic and serotoninergic systems are functionally interrelated (Klitenick and Wirtshafter 1989, Soderpalm and Engel 1991, Nazar et al. 1999). File et al. (1993) have found that exposure to the some tests of anxiety cortical GABA function in rats, simultaneously increasing hippocampal 5-HT function.

Most of the research on fear and anxiety is focused on GABA-ergic, adrenergic and serotoninergic systems. Many data, however, speak also for the role of dopamine in the regulation of aversive emotions. Dopamine is known to mediate motor, endocrine and cognitive responses to stressful or aversive stimuli (Le Moal and Simon 1991, Salamone 1994). Studies of many authors have found stress-dependent induction of the mesocortical DA activity and the corresponding augmentation of anxiety (Claustre et al. 1986, Roth et al. 1988, Biggio et al. 1990, Finlay et al. 1990, Coco et al. 1992, Hegarty and Vogel 1995). According to Biggio et al. (1990), activation of DA neurons in response to stressogenic stimuli "is related to the perception of anxiety and fear accompanying stress rather than pain or movement associated with stress paradigms". In our experiment the concentration of DA and its metabolites remained unchanged after BMI injection, but the DOPAC/DA ratio increased in all investigated structures, except frontal cortex, and HVA/DA ratio increased in the hypothalamus and midbrain, indicating the increased activity of the

dopaminergic system. BMI injected animals were not more active in the open field in terms of number of crossings and rearings. Therefore it might be assumed that increase of DA turnover is associated with the augmentation of fear, not with changes in motor activity.

In summary, the results of our experiment show a complex picture of biochemical alterations that may all together underlie the animal's response to stressogenic stimuli and, in consequence, fear regulation. Blockade of $GABA_A$ receptors, on the behavioral level, produced increase of fear in response to aversive stimulus (brightly lit area), which is in agreement with many authors studying GABA involvement in the fear and anxiety control. Considering biochemical alterations, it should be stressed that significant reduction in GABA concentration and increase in monoaminergic systems activity occurred not only in hypothalamus, but also in other investigated structures of the brain defense system. It might be suggested that blockade of $GABA_A$ transmission in VMH abolished the tonic GABA-ergic inhibition on NA, 5HT and DA neurons, affecting therefore the neurochemical balance in one of the important links of the defense circuitry. This process that started in VMH, acted as a triggering mechanism and put in motion a cascade of reactions in other parts of the integrated defense system. There occurred an increase in synthesis and/or release of NA and 5-HT (as indicated by the increase of NA concentration in MID, AMY, HIP and CTX and increase of 5HT concentration in MID and 5HIAA concentration in MID and CTX as well as increase of 5HIAA/5HT ratio in CTX). Additionally an increase of DA turnover was observed (as indicated by the increase of DOPAC/DA ratio in HPT, MID, AMY and HIP and increase of HVA/DA ratio in HPT and MID). Consistently, a decrease of GABA concentration in the other structures involved in defense circuitry might occur as a result of increased GABA utilization, necessary to reestablish the neurochemical balance and inhibit increased activity of the monoaminergic systems. Each of the monoaminergic systems has been proved to modulate fear and anxiety. The character and the extent of this contribution as well as the exact GABA-monoaminergic functional interactions in the central regulation of fear behavior need however further investigations.

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