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Short communication

Effects of midazolam and buspirone on *in vivo* concentration of amino acids and monoamine metabolites in the rat hippocampus

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Abstract:
The effects of anxiolytic doses of buspirone and midazolam (established in the conditioned fear test) on extracellular concentrations of glutamate, GABA, serotonin and dopamine metabolites in the hippocampus were examined *in vivo*, in freely moving rats. Buspirone at a dose of 1.5 mg/kg *ip* disinhibited rat behavior in the conditioned fear test (a freezing response) much stronger than 1.0 mg/kg of midazolam. Both drugs enhanced the local concentration of glutamate to the similar extent, and decreased the concentration of 5-hydroxyindole acetic acid (5-HIAA). Buspirone increased also the extracellular levels of dopamine metabolites: homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC). It is suggested that the changes in hippocampal glutamate probably are not directly associated with modification of rat emotional behavior after benzodiazepines and azapirones. The present results provide more arguments for the role of hippocampal 5-HT in the effects of anxiolytic drugs.

Key words:
midazolam, buspirone, freezing, 5-HIAA, glutamate, hippocampus

Abbreviations: CFR – contextual fear reaction, DOPAC – 3,4-dihydroxyphenylacetic acid, 5-HIAA – 5-hydroxyindole acetic acid, HVA – homovanillic acid

Introduction

Different monoaminergic, amino acidergic and peptidergic neurotransmitter systems have been found to process emotional input in the brain. Among them, the GABAergic and serotonergic systems seem to play a pivotal role. Such conclusion is supported by many preclinical findings and clinical data on the widespread use of benzodiazepine and azapirone derivatives for the treatment of anxiety disorders [3, 5, 9, 24]. It was also found that intrahippocampal injections of a benzodiazepine (midazolam), 5-HT_{1A} receptor agonists (buspirone, 8-OHDPAT), and NMDA receptor antagonists (MK-801, AP-7), selectively in-

creased exploratory behavior of naive rats in the open field test, and disinhibited a fear-controlled behavior also in models of a conditioned emotional reaction [10, 15, 28]. Furthermore, as all these ligands have been shown to exert an inhibitory influence on neuronal activity in the hippocampus, causing, *via* different mechanisms, the hyperpolarization of neuronal membranes [17], it has been consequently suggested that the inhibition of hippocampal activity can be an important mechanism of the antianxiety action of anxiolytic drugs [18, 31, 32].

Overall, these data indicate that the GABAergic and serotonergic innervation of the hippocampus may be the target of all known anxiolytic drugs. In the present paper, we aimed to analyze further the role of hippocampal neurotransmitters in the central effects of two representative anxiolytic drugs: midazolam (a benzodiazepine derivative, an agonist of the $GABA_A$ receptor), and buspirone (an azapirone derivative, a partial agonist of the $5\text{-}HT_{1A}$ receptor). To this end, the effects of anxiolytic doses of both drugs, established in the conditioned fear test, on the local concentration of GABA, glutamate, 5-HT, dopamine, and their metabolites, were examined in the hippocampus of freely moving rats using the microdialysis technique. In spite of a large body of experimental evidence documenting the role of the hippocampus in the control of emotions, little is known about changes in local concentrations of monoamines after administration of anxiolytic drugs. Even less is known about changes in the concentration of hippocampal glutamate and GABA. This is an important shortage, given the well recognized anxiolytic effects of the ionotropic and metabotropic glutamate receptor antagonists [6, 7, 19], and the well established role of glutamate in the mediation of cognitive and emotional processes [4, 6]. Examination of the changes in extracellular concentrations of monoamines and amino acids in the hippocampus after anxiolytic drugs could help to better understand the neurochemical background of their effect on emotional reactions.

Materials and Methods

Animals

The experiment was performed on 54 adult male Wistar rats (200 ± 20 g), which were purchased from a licensed breeder. Animals were housed in standard laboratory conditions under 12 h : 12 h light : dark cycle (lights on at 7 a.m.), at a constant temperature (21 ± 2°C) and 70% humidity. The rats were given free access to food and water. The experiments were performed in accordance with the European Communities Council Directive of 24 November 1986 (86/609 EEC). The Local Committee for Animal Care and Use at Medical University of Warsaw, Poland, approved all experimental procedures using animal subjects.

Experimental design

The experiment consisted of 2 parts: behavioral (a conditioned freezing reaction, contextual fear reaction – CFR), and biochemical (microdialysis study). Different groups of animals were used in each part. The scheme of the experiment is shown in Figure 1. The behavioral part of the experiment was conducted on 4 groups of animals: control (without shock), conditioned, midazolam- and buspirone-pretreated rats. The first group of animals did not receive aversive stimulation on the 2 day of CFR.

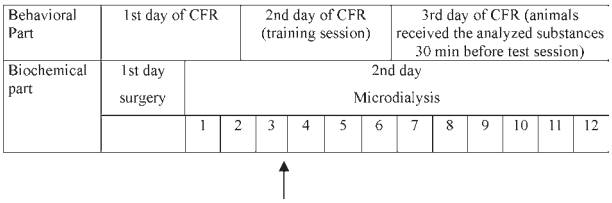

Fig. 1. Scheme of the experiment. An arrow represents the time of administration of saline, midazolam or buspirone (after collection of the 3rd sample of a microdialysate). Numbers represent microdialysate samples: the first 3 (1, 2, 3) were used to establish the baseline values, next 9 samples were collected after administration of saline, midazolam or buspirone. The time of sample collection was 20 min. For more details see Materials and Methods

Drugs

Buspirone (Anpharm, Poland) and midazolam maleate (Hoffmann La Roche, Switzerland) were dissolved in 0.9% NaCl, and administered intraperitoneally (2 ml/kg). Control groups received vehicle in the same volume per body weight. The doses of the drugs: buspirone 1.5 mg/kg and midazolam 1 mg/kg were chosen based on the pilot experiment.

Conditioned freezing reaction

The experiment was performed using a computerized fear-conditioning system (TSE, Bad Homburg, Germany), during three consecutive days in the same testing box (36 × 21 × 20 cm) under constant, white noise (65 dB). The box was cleaned after each trial with 95% ethanol. On the first day, animals were placed separately for 2 min in the box, for adaptation to experimental conditions. The following day, i.e. training day, after a 5-min pause, the animal received three 1 s footshocks (0.7 mA, 150/300 ms, repeated every 59 s) and then remained in the box for additional 3 min after the last footshock had been delivered. On the third day, experimental animals received the drug or saline 30 min before testing, and then the freezing behavior of the animals was analyzed for 10 min in the same box with the use of PC-based Videomot System software (TSE, Bad Homburg, Germany). The freezing was defined as absence of any movements except for those required for respiration. The apparatus and experimental procedure were verified in our previous experiment [12].

Surgery

The rats were anesthetized by intraperitoneal injection of ketamine (100 mg/kg), and fixed on a stereotaxic apparatus (Stoelting & Co., USA). The dialysis probe with an outer diameter of 0.3 mm, and 6 mm long dialysate membrane (U-shaped – 3 mm the tip length), pore diameter 0.8–2.0 μm, 30 kDa cut-off, was implanted into the dorsal part of left hippocampus, according to the coordinates given in the atlas of Paxinos and Watson (2.8 mm posterior to bregma, 2.5 mm lateral to bregma and 3.5 mm ventral to dura) [16]. The dialysis probe was prepared according to Gołembiowska and Dziubina [8], and the recovery for amino acids and monamines was about 15–25%. The dialysis probe was fixed to the skull with jewelry screws and dental acrylic cement. The microdialysis procedure started one day after surgery. The dialysis probe was perfused by artificial cerebral spinal fluid containing (mM): NaCl 140, KCl 2.4, $MgCl_2$ 1.0, $CaCl_2$ 1.2, $NaHCO_3$ 5.0, pH 7.4, at a flow rate of 2 μl/min with a help of micro-syringe pump (BAS, USA) in the conscious, freely moving rats. Starting the following day, after 90 min of perfusion for stabilization, three consecutive samples were collected at 20-min intervals to measure basal levels of amino acids and monoamines and their metabolites before midazolam, buspirone or vehicle administration. Perfusate samples were then collected after administration of the examined substances, at 20-min intervals for the next 180 min (9 collections) into polypropylene microcentrifuge vials and stored at –70°C until analysis. When the experiment was terminated, the brains from each animal were sliced to verify the probe placement. The extracellular concentrations of amino acids (glutamate and GABA), monoamines and their metabolites (5-HT, 5-HIAA; DA, DOPAC and HVA) were determined by a fully automated high performance liquid chromatography system (HPLC) with electrochemical detection. Additionally, the Glu/GABA ratio (a theoretical parameter defining neuronal excitation) was calculated.

Analyses of amino acids

HPLC analysis of amino acids was performed using a Luna C18, 25 cm, 5 μm reversed phase column, the method was described previously by Szyndler et al. [29]. Compounds were eluted isocratically with mobile phase delivered at 0.75 ml/min using a Shimadzu Clas VP LC 10AD pump. Electrochemical detector with a flow-through cell (Intro-Antec Leyden), linked to Shimadzu Class VP Integrator SCL-10 Avp, was used. A high-density glass carbon-working electrode (Antec) operated at + 0.85 V. Rheodyne injection valve with a 20 μl sample loops was used to manually inject the samples. Preparation of the mobile phase and the derivatizing agents were based on the method of Rowley et al. [23] with some modifications. The mobile phase consisted of 45 mM disodium phosphate and 0.15 mM EDTA with 25% methanol (v/v) water adjusted to pH 3.9 with 0.2 M citric acid. It was then filtered through 0.45 μm filters and degassed for 15 min. Stock solutions (0.01 M) of amino acid standards were prepared in double deionized water and kept at 4°C for five days. To prevent adhesion to glass, amino acids (especially GABA) standards were prepared in polyethylene vials. Working solutions were prepared daily by dilutions of the stock solution. To obtain agents for derivatization; OPA (2 mg, Fluka) was dissolved in 0.5 ml of 1 M Na_2SO_3, 0.5 ml of absolute ethanol, and 0.9 ml of sodium tetraborate buffer (0.1 M) adjusted to the pH 10.4 with 5 M sodium hydroxide. The reaction of derivatization was performed at room temperature. Derivatizing agent (20 μl) reacted with 1 ml of an amino acid standard for 5 min in

a polyethylene vial before injection onto the column. For reaction with microdialysis samples (20 µl), the volume of derivatizing agent was reduced to 0.4 µl to eliminate contamination of chromatogram by excessive reagent, which is electroactive. The concentration of amino acids was calculated as µM.

Monoamine analyses

Determination of DA, 5-HT and their metabolites was performed using a modified high-pressure liquid chromatography (HPLC) method reported by Kaneda et al. [11]. The HPLC system consisted of Shimadzu LC-10AP VP pump, electrochemical detector with flow-though cell (Decade – Antec Leyden). A high-density glass carbon-working electrode operated at + 840 mV. The sample was injected manually (Rheodyne 7725 injection valve with a 20-µl sample loop). Separation of monoamines and their metabolites was obtained on the Phenomenex Luna C_{18} column, 150 × 3 mm with Phenomenex KJO-4282 precolumn. The column temperature was 32°C. The mobile phase consisted of 4.1 mM disodium phosphate, 0.027 mM EDTA, 7.95 mM citric acid, 0.175 M sodium chloride, 0.34 mM octane sulfonic acid and 14% methanol. It was filtered through 0.45 µm filters (Millipore). The flow rate was 0.4 ml/min. The mobile phase was degassed with helium. Chromatogram registration and analysis was done using ChromaX 2000 software. The concentration of monoamines and their metabolites was calculated as ng/ml.

Data analysis

Behavioral data were shown as the means ± SEM, and were analyzed by one-way ANOVA followed by Newman-Keuls test (Statistica for Windows, Release 6, Stat-Soft Inc., USA). Dialysate data are shown as the means ± SEM, as percentage changes in relation to baseline levels according to the following formula: the mean of the three samples preceding a challenge injection of substances or vehicle was defined as 100%, and used as a baseline for the following 9 samples from each analyzed animal. Basal levels of neurotransmitters were shown as the means ± SEM, and were analyzed by one-way ANOVA. Dialysate data were evaluated by two-way ANOVA with repeated measures, followed by *post-hoc* NIR Fisher's test. For the total effect, the data are shown as the means ± SEM, as the sum of percentage changes from 9 collections, after administration of drugs. These data were analyzed by one-way ANOVA followed by Newman-Keuls test. A probability value of $p < 0.05$ was considered significant in this study.

Results

Effect of midazolam and buspirone on animal behavior in the conditioned freezing test

One way ANOVA revealed significant differences in the first 5 min of the test [$F(2, 19) = 8.59$; $p < 0.01$], and in total time of freezing [$F(2, 19) = 3.72$; $p < 0.05$]. Newman-Keuls *post-hoc* test showed a statistically significant decrease in freezing time in the first 5 min of the test in midazolam ($p < 0.05$) and buspirone ($p < 0.01$) group, and a shorter total freezing time in buspirone group ($p < 0.05$). Students' *t*-test showed significant increase in the first 5 min [$t = 3.94$; $df = 13$; $p < 0.01$], and in total freezing time [$t = 2.99$; $df = 13$; $p < 0.05$] in the conditioned animals versus nonconditioned, naive group (Tab. 1).

Tab. 1. The effect of midazolam and buspirone on rat behavior in the conditioned freezing test. Results are shown as the means ± SEM. N – number of animals. * $p < 0.05$; ** $p < 0.01$ – *vs.* control without shock; # $p < 0.05$; ## $p < 0.01$ – *vs.* conditioned/saline group

Group	N	Freezing time 0-5' (s)	Freezing time 6-10' (s)	Total freezing time (s)
Control – naive	8	28.62 ± 12.29	75.25 ± 23.47	103.87 ± 30.63
Conditioned/saline	7	157.14 ± 31.99**	130 ± 26.77	287.14 ± 55.44*
Conditioned/Midazolam 1.0	8	93.62 ± 12.96#	103 ± 21.93	196.62 ± 32.27
Conditioned/Buspirone 1.5	7	35.57 ± 10.67##	89.28 ± 28.26	124.85 ± 34.15#

Biochemistry

Effect of acute midazolam or buspirone treatment on amino acid concentrations (Fig. 2)

Glutamate (Glu)

The basal level of glutamate was 1.54 ± 0.47 μM in the control group, 1.67 ± 0.45 μM in the midazolam group, and 1.47 ± 0.34 μM in the buspirone group. One-way ANOVA did not reveal significant differences between groups in basal glutamate level [$F(2, 19) = 0.17$; $p > 0.05$]

ANOVA with repeated measures showed significant differences between experimental groups: group effect [$F(2, 12) = 6.46$; $p < 0.05$], group × time interaction [$F(16, 96) = 1.8$; $p < 0.05$]. *Post-hoc* LSD test showed increased glutamate release at 120 min after buspirone administration ($p < 0.05$). One-way ANOVA showed also significant differences between groups

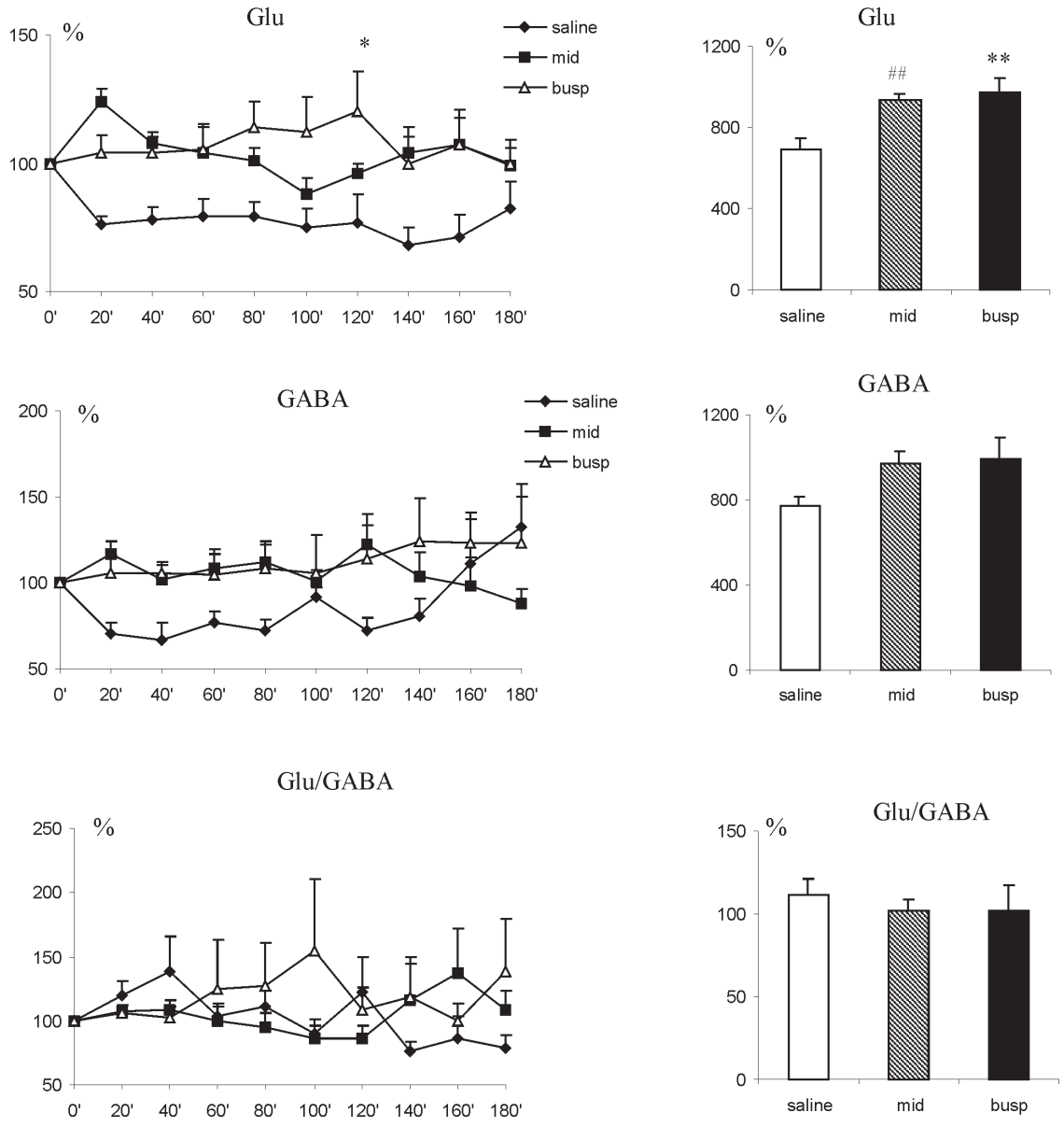

Fig. 2. The effect of midazolam (mid) and buspirone (busp) on amino acids levels in the hippocampus. # $p < 0.05$, midazolam *vs.* saline; * $p < 0.05$; ** $p < 0.01$, buspirone *vs.* saline. Data are shown as the means ± SEM

in total effect [F(2, 18) = 7.21; p < 0.01]. Glutamate release in the midazolam (p < 0.01), and buspirone (p < 0.01) group was increased.

Gamma-aminobutyric acid (GABA)

The basal level of GABA was 0.035 ± 0.01 μM in the control group, 0.038 ± 0.011 μM in the midazolam group, and 0.035 ± 0.025 μM in the buspirone group. One-way ANOVA did not reveal significant differences between groups in basal GABA level [F(2, 19) = 0.03; p > 0.05].

ANOVA with repeated measures did not reveal significant differences between groups in GABA levels: group effect [F(2, 13) = 2.52; p = 0.12], time effect [F(8, 104) = 0.22; p = 0.98], and group × time interaction [F(16, 104) = 1.05; p = 0.4]. One-way ANOVA also did not reveal differences in the total effect between groups [F(2, 19) = 2.79; p = 0.08].

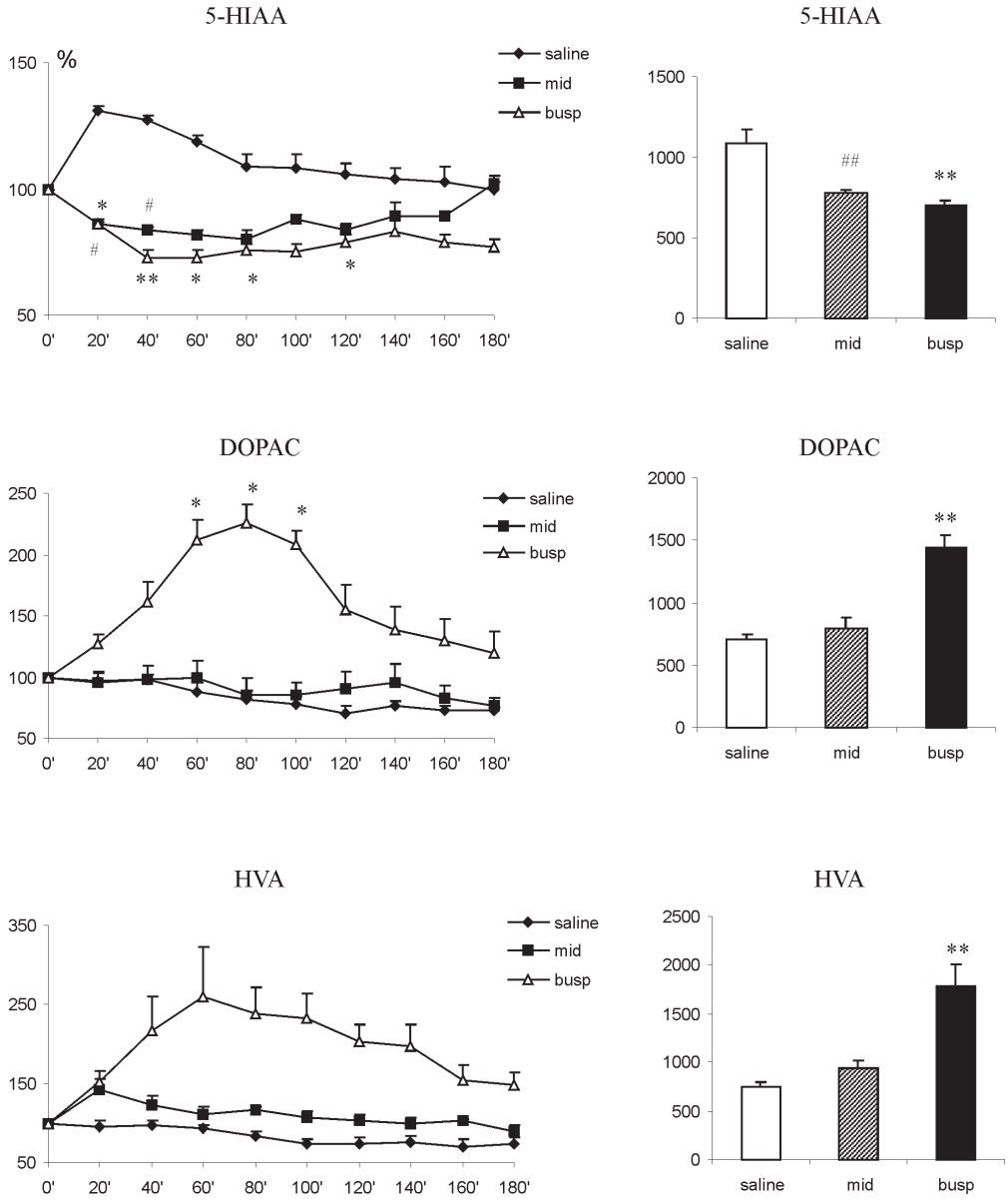

Fig. 3. The effect of midazolam (mid) and buspirone (busp) on monoamine metabolites. # p < 0.05; ## p < 0.01, midazolam *vs.* saline; * p < 0.05; ** p < 0.01 – buspirone *vs.* saline. Data are shown as the means ± SEM

Glu/GABA ratio

ANOVA with repeated measures did not show significant differences between groups: group effect [F (2, 10) = 0.81; p = 0.46], time effect [F(8, 80) = 0.22; p = 0.49], group × time interaction [F(16, 80) = 1.49; p = 0.12]. One-way ANOVA also did not reveal differences between groups in Glu/GABA ratio [F(2, 18) = 0.25; p = 0.78].

Monoamines and their metabolites

The concentrations of 5-HT and DA in the analyzed samples were below the limits of detection (0.01 ng/ml for 5-HT and 0.02 ng/ml for DA).

5-Hydroxyindole acetic acid (5-HIAA)

The basal level of 5-HIAA was 24.00 ± 4.83 ng/ml in the control group, 26.50 ± 5.14 ng/ml in the midazolam group, and 23.42 ± 3.73 ng/ml in the buspirone group. One-way ANOVA did not reveal significant differences between groups in basal 5-HIAA level [F(2, 19) = 0.12; p > 0.05] (Fig. 3).

ANOVA with repeated measures showed significant differences between groups: group effect [F(2, 9) = 20.3; p < 0.01], time effect [F(8, 72) = 3.27; p < 0.01], group × time interaction [F(16, 72) = 4.98; p < 0.01]. There was a significant decrease in 5-HIAA in the samples collected 20 and 40 min after midazolam administration, and in the samples collected 20, 40, 60, 80, 120 min after buspirone injection. One-way ANOVA revealed statistically significant differences between groups in total 5-HIAA concentration [F(2, 19) = 17.06; p < 0.01)]. Newman-Keuls *post-hoc* test showed a decrease in total 5-HIAA level in midazolam (p < 0.01), and buspirone (p < 0.01) pretreated rats.

3,4-Dihydroxyphenylacetic acid (DOPAC)

The basal level of DOPAC was 9.00 ± 2.3 ng/ml in the control group, 9.87 ± 2.62 ng/ml in the midazolam group, and 9.7 ± 2.51 ng/ml in the buspirone group. One-way ANOVA did not reveal significant differences between groups in basal DOPAC level [F(2, 19) = 0.03; p > 0.05] (Fig. 3).

ANOVA with repeated measures showed significant differences between groups: group effect [F(2, 12) = 17.68; p < 0.01]; time effect [F(8, 96) = 4.12; p < 0.01], and group × time interaction [F(16, 96) = 4.65; p < 0.01]. *Post-hoc* LSD revealed an increased DOPAC concentration in the samples collected 60, 80 and 100 min after buspirone injection (p < 0.05). One-way ANOVA revealed also statistically significant differences between groups in total effect on DOPAC levels [F(2, 18) = 25.73; p < 0.01]. Newman-Keuls *post-hoc* test showed an increase in total DOPAC concentration in the buspirone group (p < 0.01).

Homovanillic acid (HVA)

The basal level of HVA was 9.42 ± 2.51 ng/ml in the control group, 8.37 ± 1.55 ng/ml in the midazolam group, and 8.42 ± 2.06 ng/ml in the buspirone group. One-way ANOVA did not reveal significant differences between groups in basal HVA level [F(2, 19) = 0.08; p > 0.05] (Fig. 3).

ANOVA with repeated measures showed significant differences between groups: group effect [F(2, 8) = 5.21; p < 0.05]. LSD *post-hoc* test revealed the increased HVA concentration in buspirone group, however, this effect did not reach the level of statistical significance due to high data variability. One-way ANOVA revealed also a significant difference in total homovanillic acid concentration [F(2, 18) = 16.12; p < 0.01]. *Post-hoc* Newman-Keuls test showed the increased HVA concentration after buspirone administration (p < 0.01).

Discussion

It was found that pretreatment of rats with effective behaviorally doses of midazolam and buspirone produced significant changes in the hippocampal concentration of glutamate and metabolites of serotonin and dopamine. Both drugs enhanced the local glutamate concentration and decreased the concentration of 5-HIAA. Buspirone caused also an increase in the extracellular levels of dopamine metabolites: HVA and DOPAC. The most unexpected finding relates to changes in glutamate concentration. The magnitude of this effect was similar in spite of the fact that buspirone at a dose of 1.5 mg/kg disinhibited rats' behavior in the conditioned fear test much stronger than 1 mg/kg dose of midazolam. This indicates that this biochemical effect may not be directly related to the

changes in rats' fearful reaction. Such corollary is also supported by the absence of changes in the Glu/GABA ratio, a hypothetical index of excitatory processes in the brain.

The review of scientific literature (PubMed/Medline) revealed the scarcity of appropriate data on the effects of benzodiazepines and azapirones on the baseline glutamate concentration in the brain. Diazepam (3 mg/kg) given before stress session abolished increase in extracellular levels of glutamate in the prefrontal cortex and hippocampus [2]. Diazepam markedly inhibited also the release of glutamate evoked either by high $K(^{+})$ or veratridine from preloaded hippocampal slices [1]. Conversely, GABA and muscimol (a $GABA_A$ receptor agonist) potentiated the $K(^{+})$-evoked overflow of endogenous glutamate (a superfused synaptosomal preparation), in the cerebellum [26]. Similarly, a potent and selective 5-HT_{1A}-receptor antagonist, lecozotan, significantly potentiated the potassium chloride-stimulated glutamate release in the dentate gyrus of the hippocampus, indicating an inhibitory role of 5-HT_{1A} receptors in the regulation of glutamate release [25]. The majority of data point to the inhibition of hippocampal glutamate release by benzodiazepines and 5-HT_{1A} receptor ligands. The differences between some of the abovementioned data and present results are difficult to explain. In any case, however, it seems that the observed changes in hippocampal glutamate levels do not directly correlate with alteration in rats' fearful behavior.

Midazolam and buspirone did not affect the baseline concentration of GABA in the rat hippocampus. The available data on this topic are equivocal. For example, the release of GABA in guinea-pig dentate gyrus was increased by NAN-190, a 5-HT_{1A} receptor antagonist, but was not affected by 8-OH-DPAT, a 5-HT_{1A} receptor agonist [13]. *In vivo* administration of 8-OH-DPAT and diazepam that have been reported to produce anxiolytic-like effects in the elevated plus-maze, enhanced GABA-stimulated ^{36}Cl-influx in corticohippocampal synaptoneurosomes [27]. The release of GABA was increased in a concentration-dependent fashion by NMDA [13]. The majority of results indicate that the NMDA receptor and the 5-HT_{1A} receptor, which are both located on GABAergic neurons in the dentate gyrus, exert stimulatory and inhibitory impact on neuronal GABA release, respectively. On the other hand, serotonergic innervation increases spontaneous GABA release from inhibitory interneurons in the rat dentate gyrus neurons (an *in vitro* study), *via* the activation of 5-HT_3 and/or 5-HT_2 receptors [17]. It appears, therefore, that the hippocampal GABA release is under a complex control of serotonergic system, and the lack of effect of buspirone, reported in the present paper, could be due to its partial receptor agonist profile, and interaction with central dopaminergic neurons (see below).

More univocally, both drugs, midazolam and buspirone, inhibited the metabolism of serotonin in the hippocampus, as indicated by a decrease in the concentration of its metabolite, 5-HIAA. Unfortunately, it was not possible to measure serotonin and dopamine concentration in the hippocampal dialysate samples because of their very low local levels and a quick process of monoamine catabolism. However, the changes in the amount of their metabolites were univocal, and they are considered to properly reflect the rate of a presynaptic release of monoamines [21].

It has been previously postulated that decrease in serotonin activity in the frontal cortex and hippocampus after selective serotonin depletion with neurotoxins (p-CPA, 5,7-DHT), administration of 5-HT_{1A} receptor ligands, attenuating of the raphe cell body firing *via* stimulation of inhibitory 5-HT_{1A} autoreceptors, or a decrease in 5-HT turnover rate by benzodiazepines (at the raphe nuclei and presynaptic terminal levels, *via* indirect stimulation of inhibitory $GABA_A$ receptors), is an important element of anti-anxiety action of benzodiazepines and 5-HT_{1A} receptor agonists [19, 20]. The present results, i.e. a strong decrease in hippocampal 5-HIAA concentration, provide more arguments for the mediation of rat fearful reactions by limbic 5-HT.

The increase in HVA and DOPAC concentrations after buspirone may be explained by the fact that buspirone is a partial agonist at the 5-HT_{1A} receptors, and interacts also with central dopaminergic system [14, 30]. The drug at the dose of 2.0 mg/kg blocked the apomorphine cue in the apomorphine-trained rats, and acted also as a full D_2 dopaminergic receptor antagonist in the drug discrimination test [22]. It is possible, therefore, that the dopaminergic component of action of the higher dose of buspirone interfered with a 5-HT_{1A} receptor-related behavioral effects and induced antagonist-like stimulation of dopamine release and catabolism. This antidopaminergic effect of buspirone could add to the strong anxiolytic influence of the drug in the conditioned fear test, as it is known from clinical practice that small doses of neuroleptics are

effectively used as sedatives and anxiolytics in the states of strong emotional hyperarousal.

Overall, the present data on the changes in hippocampal concentrations of glutamate and 5-HIAA suggest that hippocampal glutamate does not directly modulate rats' fearful behavior, and indicate the specific involvement of limbic serotonin in the antiemotional effects of anxiolytic drugs.

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