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Gender specific influence of endogenous glutamate release on stress-induced fear in rats

[1,2]Jain S.K, [2]Zelena D,

[1]*Department of Zoology, Dr.H.S.Gour University, Sagar, India;* [2]*Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary*
e-mail: subjain@gmail.com

Objective. Stress, fear and anxiety are among major public health concerns. The role of glutamate in these processes is becoming more recognized with promising new drug targets. The aim of this study was to establish the gender specificity of a possible treatment of fear by glutamate antagonists in correspondence with changes in stress-hormone release.

Methods. Footshock-induced fear was used as an anxiogenic situation in rats. A combination of two ionotrop receptor antagonists such as MK-801 (dizocilpine; 0.2 mg/kg) for NMDA (N-methyl-D-aspartic acid) and GYKI 52466 (benzodiazepine derivative; 10 mg/kg) for AMPA/kainate receptors were used for 5 days following the hypothesis that they potentiate each other the main action, but at the same time the side effects may be minimized.

Results. Female rats tried to avoid the electrical stimulus more actively than males, as they spent more time with exploration and jumping and less time with freezing or rest. Ionotropic glutamate receptor antagonists have anxiolytic action. MK-801 was more effective in females, as it prevented the footshock-induced freezing per se, while in males it was effective only in combination with GyKI 52466. The locomotor side effect of MK-801 was not visible after repeated administration. The freezing behavior was positively correlated with the changes in prolactin but not with adrenocorticotropin levels.

Conclusions. We proved the involvement of endogenous glutamate neurotransmission in stress-induced fear. Therapeutical usage may involve a combination of different receptor antagonists. Special attention should be paid to the gender, as females seem to be more sensitive, therefore they require smaller doses. During the treatment the prolactin levels should be monitored.

Keywords: MK-801, GyKI 52466, footshock, freezing, prolactin

Nowadays the anxiety, stress and trauma-related disorders are considered major public health concern. Drugs that target gamma-aminobutyric acid (benzodiazepines) or serotonergic system (selective serotonin reuptake inhibitors) are the most widely prescribed treatment for these disorders. However, the role of glutamate in anxiety disorders is becoming more recognized with the belief that the drugs that modulate glutamatergic function have the potential to improve the current treatment of these severe and disabling illnesses (Cortese and Phan 2005).

Glutamate is the major excitatory neurotransmitter in the central nervous system and mediates its actions via the activation of both ionotropic and metabotropic receptor families. Rapid excitatory neurotransmission is mediated by ionotrop glutamate receptors of the N-methyl-D-aspartic acid (NMDA) and non-NMDA (α-amino-3-hydroxy-S-methyl-4-isoxazolepropionic acid [AMPA] and kainate) receptor families (Bigge 1999; Kew and Kemp 2005). NMDA receptors are activated by the presynaptic release of glutamate and instantaneous depolarization of the postsynaptic membrane

Corresponding author: Subodh Kumar Jain, Department of Zoology, Dr.H.S.Gour University, Sagar - 470 003 M.P. India; Phone: +91-7582-237175; Fax: +91-7582-264163; e-mail: subjain@gmail.com

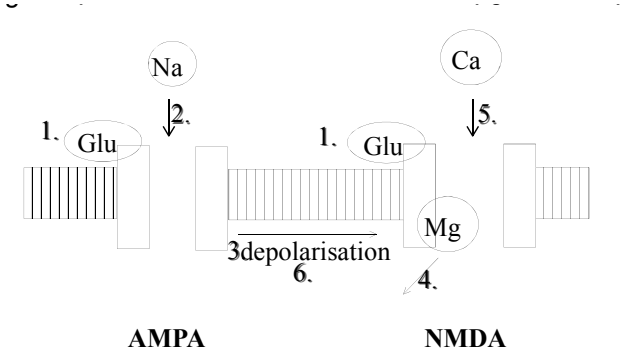

Fig. 1. Sequence of the activation of ionotropic glutamate receptors. 1. binding glutamate; 2. Na-influx through AMPA receptors; 3. depolarization of the membrane; 4. magnesium leaves the NMDA channel; 5. Ca-influx through NMDA receptor; 6. further depolarization

via colocalized AMPA receptors (Fig.1) (Bigge 1999). Several neurones express two or more types of glutamate receptors simultaneously, so that modulation of more than one receptor may be needed to noticeably modify physiological responses. In the process, glutamate fulfil numerous physiological functions, but also plays an important role in the pathophysiology of different neurological and psychiatric diseases, especially when an imbalance in glutamatergic neurotransmission occurs.

Animal models for fear and anxiety have provided a method to study the role of glutamate in anxiety. Tail or footshock have been extensively used in order to alter behavioural responses. Animals confronting threatening stimuli respond with a coordinated set of autonomic, neuroendocrine, neurochemical and behavioral changes that constitute the stress response. Stress seems to play an important role in the etiology of mood disorders (Frank and Landgraf 2008). It is well established that acute exposure of experimental animals to stressful stimuli can alter behavioural responses in a variety of behavioural paradigms as measured up to 1 month post-stress (Mikics et al. 2008). The father of the stress concept, Hans Selye, established that the hypothalamo-pituitary-adrenal (HPA) axis is the main system, which is activated during different stressful events (Selye 1973). The series of events lead to an elevation of the corticotropin (ACTH) and glucocorticoid (mainly corticosterone in rodents) elevation in the plasma of stressed subjects. Besides the plasma prolactin (PRL) level is also elevated probably with an immunomodulatory role (Lahat et al. 1993).

The aim of this study was to establish the gender specific effect of endogenous glutamate release on footshock-induced fear simultaneously with its role in stress-hormone release. A combination of two ionotrop receptor antagonists (MK-801 for NMDA and GYKI 52466 for AMPA/kainate receptors) was used with the hypothesis that they potentiate each other for the main action but at the same time the side effects may be minimized.(Fig. 1).

Materials and Methods

Animals. Naive male and female Wistar rats (Charles River, Budapest) weighing 200-300 g were used. The animals were individually housed in a temperature (23±1 °C) and humidity (60±10 %) controlled room at 12:12 h light:dark cycle with lights on at 0700h. Food and water were freely available. The rats were allowed to acclimatize for 2 weeks prior to the start of the experiments which were performed in accordance with regulations set by the European Communities Council Directive of 24 November 1986 (86/609/EEC) and were supervised by the Institutional Animal Care and Use Committee.

Experiments. Rats were treated intraperitoneally (i.p.) with 0.2 mg/kg dizocilpine (in saline) and/or 10 mg/kg GYKI 52466 once per day for 5 days. On the fifth day the drug pretreatment was followed with 20 and 10 min by a stressful stimulation, respectively. This pretreatment was necessary, since MK-801 activates ACTH and corticosterone release, which disappears after repeated administration (Pechnick et al. 1987, 1989). Two days before exposure to footshock, the rats were anaesthetized with pentobarbital sodium (Serva, 40 mg/2 ml/kg in saline, i.p.) and implanted with a silicon rubber jugular cannula (Medical grade silicone tubing, ID 0.635, OD 1.1938; Irigny, France) for repeated blood sampling. The cannulae were tunneled under the skin of the back, closed and connected with a longer polyethylene tube only early in the test morning. Stress exposure was a psychogenic one and consisted of electrical footshock (10 ms pulses of 0.8mA, 50 Hz, 1 s, repeated every 30 s for 5 min) applied through a grid floor (Yagi and Onaka 1996). Rats were placed in a semi-transparent plexiglass box (32 x 32 x 44 cm) with a cover. The shocks were generated by the impulse generator and delivered via a floor of stainless steel rods (0.4 cm diameter, 1.0 cm apart). Control rats were placed in the shock apparatus for 5 min without receiving shocks. After each shock session the box was cleaned. The behaviour of animals was

recorded via a video camera for 5 min. Blood samples (0.5 ml/sample) were collected before and at the end of the stress session (5 min) and were immediately replaced by saline to avoid volume loss. The blood was collected into ice cold tubes with 50 l 20 % K-EDTA, centrifuged and plasma was stored at -20 °C until assayed for ACTH, corticosterone and PRL.

Behavioural variables. Videorecorded behaviour was analysed by means of a computer based event recorder (Mikics et al. 2008). Five behaviour types were recorded: 1. defensive behaviour (footshock induced freezing with immobility) as a main parameter of stress-induced fear; 2. locomotion/exploration (moving within the box and/or sniffing movements directed towards the environment) to monitor the locomotory side-effect of the drugs; 3. escape (jumping movement); 4. self-care (grooming with forepaws and scratching with hindlegs); 5. resting (no obvious action).

Drugs. MK-801 (dizocilpine, (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohep-ten-5,10-imine maleate), a non-competitive NMDA antagonist, was obtained from RBI (Natick MA USA) and dissolved in saline (0.9% NaCl). We used the (+) enantiomer, because it is more potent in increasing plasma levels of ACTH and corticosterone suggesting that this drug is more effective on the HPA axis (Pechnick et al. 1989). GYKI 52466 (1-(4-amino-phenyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine), a non-competitive non-NMDA (AMPA/kainate) receptor antagonist, was kindly supplied by Dr. I. Tarnawa (Institute for Drug Research, Hungary) and suspended in Tween 80 and distilled water.

Table 1

A. Male rats, time percent

		Control	GYKI 52466	MK-801	GYKI+MK
	n (C/F)	7/13	8/10	8/12	8/13
Exploration	C	33.2±5.4	43±10.6	72±9.7*	62.1±11.6**
	F	18.6±3.3	29.3±6.75	67.93±7.47**	80.05±6.2**
Grooming	C	16.3±3	16.2±3.6	4.2±1.7**	5.36±1.97*
	F	0.06±0.06(**)	1.31±1.26(**)	0.2±0.2	0.22±0.19
Rest	C	37.1±3.4	33.03±7.6	16.3±5.3	18.16±6.63
	F	31.6±4.5	28.6±5.54	22.03±5.6	13.38±3.7

B. Female rats, time percent

		Control	GYKI 52466	MK-801	GYKI+MK
	n (C/F)	9/9	9/13	9/13	8/13
Exploration	C	51.07±8.6##	42.68±9.6	94±2.4**##	85.8±4.5*#
	F	37.92±3.16##	36.15±4.8	89.2±5.2**##	86±5.9**
Grooming	C	18.18±2.86	13.66±3.5	4.4±2.1**	6±2.5**
	F	0 (**)	0.04±0.04(**)	1.08±0.78	0.34±0.3
Rest	C	30.16±6.96	42.54±9.8	1.59±1.59*##	8.06±4##
	F	20.96±2.3##	40.9±3.9##	8.15±3.8##	10.2±4.6

C = control, unshocked, underwent shame procedure; F = footshock for 5 min;
* = p<0.05; ** = p<0.01 vs. control (saline treated animals); (*) = p<0.05; (**) = p<0.01 vs. unshocked group; # = p<0.05; ## ´ p<0.01 vs. male rats in the same group

The time percent of exploration was significantly affected by footshock and MK-801, and was markedly different in the two genders. The effect GYKI 52466 on exploration was different in the two genders and MK-801 had different effect in shocked and control animals. Footshock significantly reduced thime spent with grooming. In MK-801 trested animals this movement was significantly reduced especially in shocked animals.MK-801 caused a significant reduction in the duration of resting. Females were more active in this respect and the reducing effect of MK-801 was also more expressed in this gender.

Hormone measurements. Hormones were determined in unextracted plasma samples by a specific radioimmunoassay as described earlier (Zelena et al. 1999, 2008). The ACTH antibody (No. 8514) was raised in rabbit in the Institute of Experimental Medicine, Hungarian Academy of Sciences (Budapest, Hungary). PRL levels were measured by a specific radioimmunoassay using material kindly provided by NIDDK. All samples were run in the same assay.

Statistical evaluation was done by four way analysis (gender, GyKI 52466, MK-801, shock) or by repeated measures (hormones) ANOVA/MANOVA followed by Tukey's HSD test for multiple comparison, using the STATISTICA software (StatSoft, Tulsa, OK). Data has been expressed as mean±SEM and statistical significance was set to $p<0.05$.

Results

The unshocked males spent more time with freezing than the females (Fig.2). The footshock induced a significant rise in control and GyKI 52466 pretreated rats (5-times in males and 20-times in females). The protecting effect of MK-801 was present in both genders, but the effect of GyKI 52466 showed a remarkable gender difference: in females diminished the effect of footshock already per se, while in male in combination with MK-801 only. The frequency of the freezing behaviour was influenced by the shock procedure, MK-801 treatment being different in the two genders (Fig. 3). In male rats there was a tendency for elevated freezing frequency in shocked animals and the drug treatment diminished its level only weakly, but not significantly. The sexual differ-

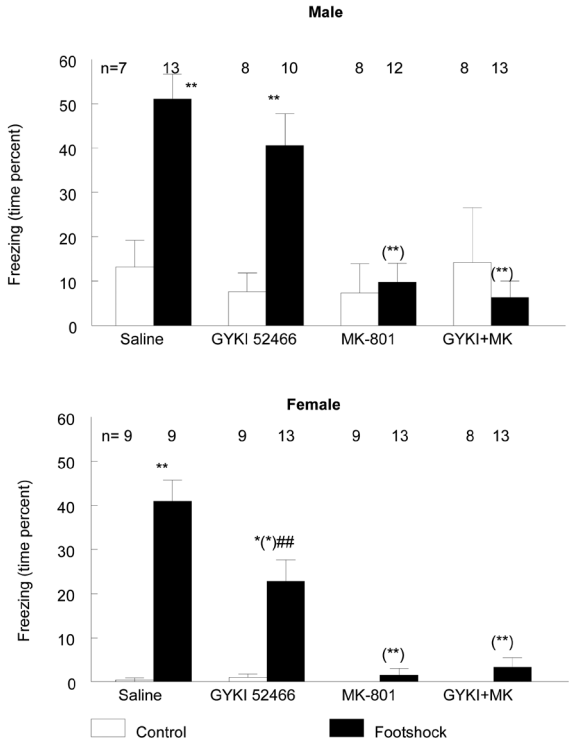

Fig. 2. Effect of repeated (5 times daily, intraperitoneal) treatment with MK-801 (0.2 mg/kg, 20 min after the last) and GyKI 52466 (10 mg/kg; 10 min after the last) on the time percent of freezing behaviour during 5 min footshock. The effect of gender, GYKI 52466, MK-801, footshock and the interaction of MK-801 x = footshock and gender; x = MK-801 were significant; * = p<0.05; ** = p<0.01 vs. unshocked; (*) = p<0.05; (**) = p<0.01 vs. saline treated; ## = p<0.01 vs. male

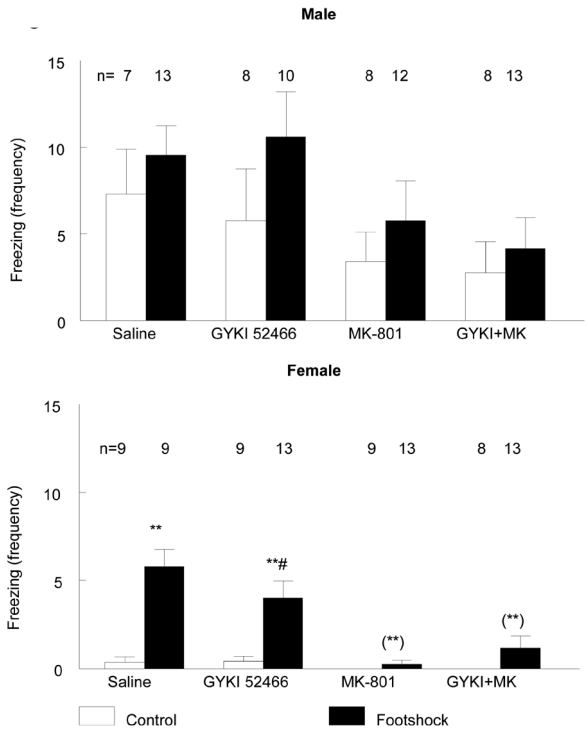

Fig. 3. Effect of repeated (5 times daily, intraperitoneal) treatment with MK-801 (0.2 mg/kg, 20 min after the last) and GYKI 52466 (10 mg/kg; 10 min after the last) on the frequency of freezing behavior during 5 min footshock. The effect of gender, MK-801 and footshock were significant. ** = p<0.01 vs. unshocked; (**) = p<0.01 vs. saline treated; # = p<0.05 vs. male

ence was very remarkable, since in unshocked females there was no remarkable freezing, and the NMDA antagonist MK-801 pretreatment prevented the footshock induced very remarkable rise (more then 10 times). The AMPA\kainate antagonist GyKI 52466 was effective alone, but could not diminish further the already minimal levels visible after the MK-801 treatment.

The time percent of locomotion/exploration was significantly affected by footshock and MK-801, and was markedly different in the two genders (Table 1.). During footshock the animals spent less time with exploration. In both genders MK-801 elevated the time of locomotion and GyKI 52466 did not influence its effect. Locomotion/exploration frequency was also very different in males and females, showing smaller levels in females (Table 2.). The effect of MK-801 was detectable only in females.

In unshocked animals only one jumping was observed (Table 2.). Footshock significantly increased the escape movement. The effect of drugs was not coherent, as MK-801 alone tended to elevate in males but lowered in females the number of jumping. Female rats represented a more active escape from the shock showing more jumping behaviour.

Footshock significantly reduced the frequency and time spent with grooming, both almost disappeared during electrical stimulation (Tables 1, 2). The animals treated with MK-801 started the movements, but the duration of grooming was significantly reduced. GyKI

Table 2

A. Male rats, frequency

		Control	GYKI 52466	MK-801	GYKI+MK
	n (C/F)	7/13	8/10	8/12	8/13
Exploration	C	6.57±2	5.1±1.1	9.5±3.3	8.5±3.04
	F	5.8±0.98	8.3±1.3	10.3±2.5	10.4±2.5
Jumping	C	0	0	0	0
	F	0.77±0.57	0.6±0.5	3.75±1.7	0.5±0.38
Grooming	C	2±0.4	3±0.9	2.25±1.3	2±0.94
	F	0.08±0.08(**)	0.6±0.5(**)	0.16±0.16	0.46±0.3(*)
Rest	C	11.6±3	10.25±3.6	8.5±2.98	9±3.7
	F	11.2±1.9	11.7±2.9	10.6±2.9	10.2±2.6

B. Female rats, frequency

		Control	GYKI 52466	MK-801	GYKI+MK
	n (C/F)	9/9	9/13	9/13	8/13
Exploration	C	3.6±0.53	4.3±0.6	2.78±1.19#	5.4±2
	F	7.44±0.8	6.3±0.8	3.08±0.8*#	3.7±0.94#
Jumping	C	0	0	0.22±0.22	0
	F	4.3±1.18#	5.61±2.5#	1.6±0.84	4.62±2#
Grooming	C	3±0.65	3.1±0.7	1.78±1.2	2.25±1.3
	F	0(*)	0.077±0.077(*)	0.46±0.3	0.23±0.166
Rest	C	2.78±0.9##	4.1±0.8##	0.11±0.11##	3±2##
	F	5.67±0.7##	6.8±0.9##	1.92±0.75##	2.9±1##

C = control, unshocked, underwent shame procedure; F = footshock for 5 min;;
* = p<0.05; ** = p<0.01 vs. saline treated group; (*) = p<0.05; (**) = p<0.01 vs. unshocked rats; # = p<0.05 vs. male rats

Exploration frequency was significantly different in males and females. The effect of MK-801 was detectable only in females. In unshocked animals only one jumping was observed. Footshock significantly increased the escape movement. The effect of drugs was not coherent. Female rats represented a more active escape from the shock showing more jumping behaviour. Footshock significantly reduced the frequency with grooming. MK-801 caused a significant reduction in the frequency of resting. Females were more active also in this respect.

52466 per se was ineffective and did not change the effect of MK-801, either. There were no gender differences.

The resting behaviour was not profoundly influenced by the shock (Tables 1, 2). MK-801 caused a significant reduction both in the duration and frequency of resting. GyKI 52466 was ineffective per se and in combination. Females were more active also in this respect (spent less time in rest than males) and the reducing effect of MK-801 was also more expressed in this gender.

Hormone levels (ACTH, PRL) measured before the footshock session were not significantly modified by any drug treatment. However, there was a tendency for lower ACTH levels after the fifth MK-801 administration alone or in combination in both the sexes (Table 3.). The ACTH levels were increased by the shame procedure (transfer to a new environment) in males but not in females. The 5 min footshock was able to induce a further ACTH-rise in both the genders. The pretreatment with the glutamate receptor antagonists was ineffective on the ACTH elevations in both genders. PRL levels were not changed by the new environment. However the footshock induced a remarkable (approx. 10-fold) rise in both genders. In males, MK-801 in combination with GYKI 52466 diminished the footshock-induced PRL elevation, while in females MK-801 alone was also effective on the same parameter. The levels of plasma PRL at 5 min positively correlated to the freezing time (r=+0.36).

Discussion

This work has demonstrated a profound gender difference in the behaviour of animals during the footshock-induced fear. Female rats tried to avoid the electrical stimulus more actively than males, as they spent more time with exploration and jumping and less time with freezing or rest. Ionotropic glutamate receptor antagonists had anxiolytic action. The NMDA antagonist, MK-801 was more effective in females, as it prevented the footshock-induced freezing per se, while in males it was effective only in combination with the AMPA/kainate antagonist GYKI 52466. The locomotor side effect of MK-801 was not visible after repeated administration as there was no difference between the different treatment groups in exploratory behaviour. The freezing behaviour was positively correlated with the changes in PRL but not with ACTH levels.

Table 3

Hormone levels before and at the end of footshock

			Control	GYKI 52466	MK-801	GYKI+MK
Male	Min	n (C/F)	7/13	8/10	8/12	8/14
ACTH (fmol/ml)	0		115.2±21.14	152.7±37.5	93.9±31.8	90.8±23.6
	5-0	C	141.8±41.1$	112.9±34.1$	126.5±40.6$	186.8±48.0$
		F	208.0±51.9(*)	422.1±74.4(**)	343.3±69.8(*)	284.8±37.8(*)
PRL (ng/ml)	0		2.44±0.76	5.14±1.8	2.66±0.53	2.75±0.71
	5-0	C	4.11±2.17	0.6±1.4	3.03±1.5	-0.8±0.98
		F	20.08±5.17(*)	23.6±2.5(**)	20.85±3.4(*)	5.33±1.98*
Female		n (C/F)	9/9	9/13	9/12	8/13
ACTH (fmol/ml)	0		121.5±36.8	211.7±46.7	53.8±13.1	90.03±28.0
	5-0	C	13.48±15.4	-35.2±29.1	-14.1±17.1	17.2±38.0
		F	187.9±28.3(*)	84.7±52.9(*)	116.2±23.1(*)	23.68±25.72
PRL (ng/ml)	0		2.21±0.48	3.57±1.26	1.99±0.37	1.56±0.12
	5-0	C	0.13±0.33	-1.35±1.8	0.1±0.33	-0.22±0.08
		F	28.9±13.2(*)	30.57±16.46(*)	6.23±6.8*#	-0.18±0.13**#

C-control, unshocked, underwent shame procedure; F- footshock for 5 min
*p<0.05, **p<0.01 vs. saline treated group; (*)p<0.05, (**)p<0.01 vs. unshocked rats; $p<0.05 vs. 0 min; #p<0.05 vs. male rats

The ACTH levels were increased by the transfer to a new environment in males but not in females. The 5 min footshock was able to induce a further ACTH-rise in both the genders. The ACTH levels were not significantly modified by any drug treatment. The footshock induced a remarkable rise in PRL levels. In males MK-801 in combination with GyKI 52466 diminished the footshock-induced PRL elevation. In females MK-801 alone was also effective on the same parameter.

Our current psychopharmacological treatments for anxiety disorders evidence a number of shortcomings, including side effects and lack of primary effects. Although several new drugs have been developed, most of them are based on outmoded theories on the pathogenesis of these disorders (i.e. monoamine hypotheses), thus frustrating our ability to create more specific and effective interventions. Several lines of evidence, in humans and in animal models, support the contention that neurotransmission via the NMDA receptor is dysregulated in depression (Pittenger et al. 2007). Glutamatergic drugs were already demonstrated to have anxiolytic action for many different paradigms including fear-potentiated startle, punished responding, and the elevated plus maze (Amiel and Mathew 2007; Simon and Gorman 2006). Human clinical drug trials have demonstrated the efficacy of glutamate antagonists for the treatment of obsessive-compulsive disorder, posttraumatic stress disorder, generalized anxiety disorder and social phobia. Recent data from magnetic resonance imaging studies provide an additional link between the glutamate system and anxiety (Cortese and Phan 2005).

On the other hand, glutamate, NMDA or AMPA/kainate agonists produce an increase in motor behaviour (Donzanti et al. 1984; Klockgether et al. 1986; Kalivas et al. 1989; Layer et al. 1991). Moreover, the antagonists of the NMDA receptor-channel complex induce phencyclidine-like side effects which include head weaving, body rolling, sniffing and disturbances of motor coordination. MK-801 produces locomotor activation by itself in a dose-dependent way (Welsch-Kunze and Kuschinski 1990). The HPA axis is also activated after a single MK-801 administration, but this effect disappears after repeated treatment (Pechnick et al. 1987, 1989). To reduce the possible side effects we used repeated administrations and combined the NMDA antagonist with the non-NMDA antagonist GyKI 52466. We have chosen footshock-induced freezing behavior in rats as it has already been proved that this is a good animal model for assessing anxiolytics (Conti et al. 1990). In males the combination was more effective than the MK-801 alone, thus we could prove our theory that inhibition of more than one glutamate receptor is more effective, in this way the side effects can be reduced.

The literature is inconsistent about the involvement of the NMDA or AMPA/kainate receptors in stress-induced fear. MK-801 was already reported to be ineffective on footshock-induced immediate freezing (Zuena et al. 2008), however it diminished the conditioned response (Saulskaya and Marsden 1995). In contrast, the competitive NMDA antagonists NPC 12626 and CPP effectively reduced the immediate freezing time (Conti et al. 1990). MK-801 reduced the chronic stress-induced behavioural deficits (fighting attacks), too (Ossowska et al. 1997). It has also been reported that the activation of non-NMDA receptors in amygdala is necessary for the expression of conditioned fear (Campeau et al. 1992; Kim et al. 1993). Bilateral intra-amygdala infusions of the AMPA receptor antagonist CNQX decreased reactivity to footshock, blocked shock-induced decreases in locomotor activity, and had an anxiolytic effect in the elevated plus maze (Mesches et al. 1996). One possible explanation of the discrepancies is the dose and the different treatment protocol used (single contra repeated administration). We have chosen a relatively low dose to minimize the side effects and proved the efficacy of a combined treatment in male rats.

According to our knowledge, this study is the first attempt revealing gender specific sensitivity of the glutamatergic neurotransmission in footshock-induced fear. In females the MK-801 administration alone was already maximally effective (completely abolished freezing behavior). This fact also might have some therapeutic consequences with the usage of different doses in the two genders. Our results are supported by Zuena et al. (2008) who demonstrated gender specific changes in metabotrop glutamate receptor level after prenatal stress.

Our hypothesis was that the stress-induced behavioural changes were the consequence of changes in serum levels of the HPA axis hormones. We could not prove our hypothesis as there was no significant correlation between the freezing behaviour and the plasma ACTH and corticosterone (data not shown) level in either gender. This is in accordance with the observations by Goldstein et al. (1994), who found that the NMDA glycine site antagonist (+)-HA-966 did not affect stress-induced serum corticosterone elevation but did attenuate the freezing response. On the other hand plasma PRL levels revealed a positive correlation with the freezing time during footshock. Several studies supported the role of excitatory amino acids in the control of PRL secretion (Zelena et al. 1999; Nagy et al. 2005). On the other hand, the role of PRL level elevation during stress is questionable. A recent study presented that social interaction reduces the PRL elevation in the conditioned fear paradigm, therefore its level may serve as a signal for environmental changes (Insana and Wilson 2008). Besides that PRL may regulate the immune response, too (Lahat et al. 1993). Prolactin is hardly involved in sleep regulation, therefore the changes in its

level may contribute to sleep-disturbances in stressed patients (Stenberg 2007). It is more probable that PRL is not a link between glutamate and the behavior but the glutamatergic drugs affects the two systems in parallel. The hypothalamus is very important in neuroendocrine regulation (Lightman 2008). The hypothalamus appears to be densely packed with behaviourally relevant, adjacent, discrete areas from which the aggression, grooming, escape, teeth chattering etc, can be elicited (Brandao et al. 2003). This leads to a complex regulation, where not only hormone changes may affect the behaviour of the animal, but the drugs can affect the two systems simultaneously.

Out of the study we proved the involvement of endogenous glutamate neurotransmission in stress-induced fear. Therapeutic usage may involve a combination of different receptor antagonists. Special attention should be given to the gender, as females seem to be more sensitive, therefore lower doses would be necessary. During the treatment the PRL levels should also be monitored.

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