Gender-dependent ATPA-induced changes in long-term potentiation in the rat lateral amygdala

Manja Schubert,* Christian Drephal,†‡ and Doris Albrecht†,1
*Department of Physiology, Faculty of Medical and Health Science, University of Auckland, Auckland, New Zealand; †Institute of Neurophysiology, Charité—Universitätsmedizin Berlin, Berlin, Germany, and ‡Ev. Krankenhaus Bielefeld, Medizinische Klinik, Bielefeld, Germany

ABSTRACT There is increasing evidence that kainate receptors contribute to both postsynaptic and presynaptic signaling not only in the hippocampus but also in the amygdala. The present study demonstrates that low concentrations of the specific kainate GLU\textsubscript{K5} receptor agonist, ATPA, depressed baseline activity in the lateral nucleus of the rat amygdala (LA), induced by stimulation of external capsule fibers or by intranuclear stimulation in horizontal brain slices. ATPA reduced high-frequency-induced long-term potentiation (LTP) in males while it enhanced LTP in females during certain phases of the estrus cycle. In untreated slices from females, LA-LTP differed depending on the phase of the estrus cycle. In addition, we show for the first time that the p38 mitogen-activated protein (MAP) kinase inhibitor, SKF 86002, reduced LA-LTP. In males, the effects of ATPA and SKF 86002 were not additive. To the contrary, in females, the exposure to ATPA in control plus SKF 86002 increases LTP relative to control plus SKF 86002 alone. Thus, we demonstrate that the effectiveness of GLU\textsubscript{K5} stimulation on plasticity changes in the amygdala is gender-dependent and that the MAP kinase pathway might be involved in males.—Schubert, M., Drephal, C., Albrecht, D. Gender-dependent ATPA-induced changes in long-term potentiation in the rat lateral amygdala. \textit{FASEB J.} 22, 000–000 (2008)

Key Words: Lateral nucleus of the amygdala • high-frequency stimulation • UBP296 • SKF 86002 • horizontal brain slice

KAINATE RECEPTORS SHARE MANY STRUCTURAL, PHARMACOLOGICAL, AND BIOPHYSICAL PROPERTIES WITH AMPA RECEPTORS, WHICH ARE THE RECEPTORS MEDIATING THE MAJORITY OF FAST EXCITATORY SYNAPTIC TRANSMISSION. HOWEVER, KAINATE RECEPTORS APPEAR TO PLAY A DISTINCT ROLE IN SYNAPTIC TRANSMISSION (1, 2). THERE ARE SEVERAL DIFFERENT KAINATE RECEPTOR SUBUNITS THAT CAN COASSEMBLE TO FORM HETEROmeric KAINATE RECEPTORS (3, 4). RECENT DATA SUGGEST THAT KAINATE RECEPTORS PLAY AN IMPORTANT ROLE IN SEIZURE GENERATION (5). GLU\textsubscript{K5} KAINATE RECEPTORS CAN TRIGGER EPILEPTIFORM ACTIVITY IN THE AMYGDALA AND ARE THOUGHT TO PARTICIPATE IN LONG-TERM PLASTICITY MECHANISMS THAT UNDERLIE SOME FORMS OF EPILEPTOGENESIS (6). THESE DATA SUPPORT THE SUGGESTION THAT GLU\textsubscript{K5} KAINATE RECEPTORS REPRESENT A NOVEL TARGET FOR ANTI-EPILEPTIC DRUG DEVELOPMENT (7). THE EXPRESSION OF THE GLU\textsubscript{K5} SUBUNIT IS HIGHER IN THE AMYGDALA THAN IN THE HIPPOCAMPUS (8, 9). STUDIES ON THE FUNCTION OF GLU\textsubscript{K5} IN THE AMYGDALA HAVE FOCUSED ON THE BASOLATERAL NUCLEUS OF THE AMYGDALA (BLA). IN THE BLA, GLU\textsubscript{K5} KAINATE RECEPTORS ARE PRESENT ON SOMATODENDRITIC REGIONS OF PYRAMIDAL CELLS AND INTERNEURONS (6, 9). GLU\textsubscript{K5} KAINATE RECEPTORS ALSO MEDIATE A COMPONENT OF THE EVOKED EXCITATORY POSTSYNAPTIC CURRENTS (10).

Recently, Li et al. (8) demonstrated the involvement of GLU\textsubscript{K5} kainate receptors in homosynaptic and heterosynaptic potentiation in the BLA of coronal brain slices. They showed a progressive enhancement of transmission during low-frequency stimulation (LFS) of external capsule (EC) fibers, which could be blocked by the specific GLU\textsubscript{K5} subunit receptor antagonist LY382884. In contrast to these data, in the lateral nucleus of the amygdala (LA), LFS of EC fibers did not induce significant long-term changes in neuronal activity in either coronal (11) or horizontal brain slices (12). However, by stimulating afferents within the LA, we observed persistent long-term depression (LTD) in both intracellular and extracellular recordings (12) using the same paradigm as Li et al. (8). In horizontal slices, stimulation of EC fibers activates excitatory afferences from cortical structures and includes afferences from the lateral entorhinal and perirhinal cortex that course through the EC and synapse in the LA and the BLA (13). Stimulation within the LA (intranuclear [IN] stimulation site) also activates local connections within the LA and afferences from other amygdaloid nuclei (13, 14). Theta-pulse stimulation of thalamic fibers in coronal slices also caused LTD in the LA (11).

We did not see a significant long-term increase in field potential (FP) amplitude 60 min after LFS of EC fibers in either drug-free controls or slices treated with a specific agonist of GLU\textsubscript{K5} receptor subunits, ATPA (1 μM) (unpublished extra- and intracellular recordings). Therefore, we wondered whether a stable LTP induced in horizontal slices by high-frequency stimulation (HFS) of EC fibers (15) can be influenced by stimulation of kainate GLU\textsubscript{K5} receptors. Moreover, consider-
ing the known gender differences in epilepsy (16), our study aimed to identify possible differences in LTP between males and females after GLUK5 stimulation. We report here for the first time that low concentrations of ATPA reduced high-frequency-induced LA-LTP in males while it enhanced LA-LTP in females. This sex-specific effect on LTP did not show input-specificity. We show that the effectiveness of ATPA is gender-dependent. Bi et al. (17) showed that cyclic changes in estrogen levels during the estrus cycle of female rats are associated with corresponding changes in the levels of activation of extracellular signal-regulated kinase 2 (ERK2) and the magnitude of LTP in the hippocampus. Endogenous estrogen produced a tonic phosphorylation/activation of ERK2/mitogen-activated protein (MAP) kinase throughout the female brain and an increase in tyrosine phosphorylation of NR2 subunits of N-methyl-D-aspartate (NMDA) receptors. Therefore, we were interested to test the influence of MAP kinase on gender-dependent ATPA-induced plasticity changes in the LA. The MAP kinase pathway is identified to be involved in plasticity changes in the amygdala (18–20). However, although the p38 MAP kinase plays a role in hippocampal plasticity changes, (21, 22) and MAP kinase pathways may differentially regulate postsynaptic glutamate receptor trafficking in the hippocampus, no data are available, to our knowledge, for a functional role of p38 MAP kinase in the amygdala. We show in the present study that SKF 86002, a p38 MAP kinase inhibitor, reduced LA-LTP in both males and females and that the MAP kinase pathway might be involved in the gender-specific, qualitative effects of ATPA on LA-LTP.

MATERIALS AND METHODS

Animals

For electrophysiological experiments, 8- to 10-wk-old male and female Wistar rats were used. Animals were housed in standardized conditions with an artificial 12-h dark-light cycle and a room temperature of 22°C. The estrus cycle was monitored by vaginal smears taken each morning (8:00–9:00 AM). Rats had free access to food and water. All experimental protocols were approved by government authorities and conformed to the European Council Directive.

Preparation and recording

Detailed methods for slice preparation and electrode positioning have been previously described (15). The electrode positioning is shown in Fig. 1A. The rats were anesthetized with isoflurane and decapitated. For extracellular recordings, the brains were removed quickly and placed in ice-cold artificial cerebrospinal fluid (ACSF) (in mM: 124 NaCl, 3 KCl, 1.6 CaCl₂, 1.8 MgSO₄, 1.25 NaH₂PO₄, 10 glucose, and 26 NaHCO₃). Hemisected horizontal slices (400 μm) were prepared with a vibroslicer (Campden Instruments, Silbey, UK). Slices were placed in an interface chamber and allowed to equilibrate for 120 min at 35°C. They were superfused continuously with ACSF (1.5 ml/min). The pH was maintained at 7.4 (95% O₂ and 5% CO₂). Glass microelectrodes (Science Products, Hofheim, Germany) were filled with ACSF (tip resistance 1 MΩ) and placed in the LA to record FPs. Bipolar stimulation electrodes were used to stimulate either EC fibers or fibers running through the LA (IN stimulation site).

Stimulation parameters

An input/output (I/O) response curve was constructed by varying the intensity of single-pulse stimulation and, for each
intensity, averaging 6 responses. The stimulus intensity that evoked a mean FP equal to 50% of the maximum response then was used for all subsequent stimulations, *(i.e.,* tetanus and subsequent single-pulse stimulations). Single stimuli (duration 100 μs) were presented every 10 s. Once a stable baseline of responses was obtained for at least 20 min, HFS was delivered as 2 trains at 100 Hz (*duration: 1 s, 30 s apart*). Drug-induced changes in baseline activity were considered. Subsequent responses to single stimuli were recorded for at least 60 min, and their amplitude was quantified as percent change with respect to baseline.

**Drug application**

In drug-treated slices, HFS was induced 30 min after drug perfusion. We alternated between control and treatment experiments to account for potential day-to-day, as well as time-of-day, differences. ATPA [(RS)-2-amino-5-(3-hydroxy-5-tertbutyliso-xazol-4-yl) propionic acid, kainate GLU₉₅ agonist], UBP296 [(RS)-3-(2-carboxybenzyl) willardine, GLU₉₅ antagonist], and SKF 86002 (p38 MAP kinase inhibitor) were obtained from Tocris Bioscience (Bristol, UK). ATPA is a substituted analog of AMPA that binds to GLU₉₅, with high affinity. ATPA also can activate GLU₉₅/K₂ (23). UBP296 is highly selective, as it selectively depresses glutamate-induced calcium influx in cells containing GLU₉₅ in either homomeric or heteromeric forms (24).

**Data analysis**

Data were collected and averaged with the custom-made software Signal 2 (Cambridge Electronic Design, Cambridge, UK). We defined the FP amplitude as the absolute DC voltage of a vertical line running from the minimal point of the FP to its intersection with a line running tangent to the points of FP onset and offset. It is assumed that the recorded negative wave reflects a summation of both excitatory post-synaptic potentials and synchronized action potentials (population spike component) (25, 26). Watanabe et al. (26) have carried out intracellular recordings of evoked potentials and confirmed that the latency of peak negative FPs (5–6 ms) corresponds well with that of intracellularly recorded action potentials, indicating that the extracellularly recorded sharp negativity is a population spike. Therefore, we analyzed the amplitude of FPs in the present study. In addition, the slope measure in the lateral amygdala is more sensitive to variability and signal noise, making it more difficult to analyze (25).

To calculate the significance of differences between groups, the Mann-Whitney test was used (Prism 4 software; GraphPad, San Diego, CA, USA). *P < 0.05* was considered significant. To express and compare changes of FP amplitudes between the animal groups, we averaged responses from the 57- to 60-min period after HFS.

**RESULTS**

**ATPA-induced changes in long-term potentiation**

In ATPA-perfused slices from male rats, we observed a reduction in HFS-induced LA-LTP in comparison with untreated controls (IN-control: 151.3±10.8%, n=10, vs. ATPA: 135.8±6.1%, n=9; Fig. 1B). ATPA was used at a concentration of 1 μM in all recordings. At this concentration, the agonist acts only on GLU₉₅-containing kainate receptors (23). In females, ATPA caused an enhancement of LA-LTP compared to controls (IN-control: 152.5±5.4%, n=11, vs. ATPA: 187.1±11.8%, n=8; Fig. 1C). As shown in Fig. 1D, there were no significant differences in control recordings between males and females, in agreement with our previous results in untreated rats (15).

To investigate a possible input-specificity of gender-specific changes in LTP after GLU₉₅ stimulation, we also tested the input from the EC to LA neurons. In horizontal slices, the stimulation of this input mainly activates fibers from the perirhinal and entorhinal cortex (13). HFS caused stable EC-induced LTP in the LA of both males and females (152.9±4.6%, n=8, vs. 155.7±5.4%, n=9; *P>0.05*; Fig. 2A, C). As described previously (15), EC-induced LA-LTP does not exhibit gender differences. Similar to the results obtained by stimulation of afferents within the LA, ATPA depressed EC-induced LA-LTP in males (134.0±7.5%; n=7). As described previously in the hippocampus (24), the GLU₉₅ selective kainate receptor antagonist UBP296 (1 μM) blocked the effects of ATPA (162.5±10.0%, n=6; *P<0.001*; Fig. 2A). In contrast, ATPA enhanced EC-induced LA-LTP in females (188.3±16.2%, n=8). Figure 2D shows that 1 μM UBP296 partially depressed the effect of 1 μM ATPA in females (166.5±7.6%, n=7), whereas a higher concentration (5 μM UBP296) was needed to block the effect of ATPA completely (159.7±8.5%, n=8).

**ATPA-induced changes in baseline activity**

Considering the gender-dependent effects of ATPA on LA-LTP, we also investigated the ability of ATPA to depress baseline FP amplitude in the LA, as described for the hippocampus (27). In males, ATPA reduced baseline activity induced by EC stimulation by 7.2% ± 4.1% (n=12; *P<0.05*). In females, baseline activity was significantly reduced by 10.9 ± 6.2% (n=9; *P<0.05*). When afferents within the amygdala were stimulated, ATPA also significantly depressed baseline activity in both males and females. In addition, ATPA significantly decreased I/O curves in both males and females in comparison to drug-free controls (*P<0.05*; data not shown).

**Estrus cycle-dependent changes in plasticity**

We next evaluated the magnitude of EC-induced LA-LTP in slices from females in either proestrus or diestrus conditions. An overview of these experiments is shown in Fig. 3E. We did not find significant differences in I/O curves (Fig. 3A). We found that ATPA enhanced EC-induced LA-LTP in slices from both adult female rats with high (proestrus) endogenous estrogen levels (control: 141.8±6.9%, n=7, vs. ATPA: 156.0±4.0%, n=7; *P<0.05*; Fig. 3B) and with low estrogen levels (diestrus) (control: 132.5±8.5%, n=9, vs. ATPA: 146.6±9.9%, n=10; *P<0.05*; Fig. 3D). ATPA also enhanced LTP in slices from females in estrus (control: 148.4±8.9%, n=8, vs. ATPA: 172.5±15.4%, n=10; *P<0.05*; Fig. 3C). Kainate GLU₉₅
receptor stimulation had the strongest effect in slices of females at estrus. As shown previously, in the CA1 region of the hippocampus (17), in drug-free controls, there was a significant difference in the magnitude of LA-LTP between females in different estrus states ($P<0.05$; Fig. 3E). Slices prepared from females in proestrus or estrus

**Figure 2.** Stimulation of EC: gender-dependent, ATPA-induced changes in plasticity in the LA. A) ATPA-induced suppression of HFS-induced LA-LTP in males ($n=7$ slices) in comparison to drug-free controls ($n=8$ slices; $P<0.001$), which was blocked by the specific GLU_K antagonist UBP296 ($n=6$ slices). ATPA and UBP296 were washed in a concentration of 1 μM. C) In females, 1 μM ATPA induced a strong enhancement of LTP ($n=8$ slices) in comparison to controls ($n=9$ slices; $P<0.001$). This enhancement was completely blocked by 5 μM UBP296 ($n=8$ slices). Data points in A and C represent averaged amplitudes (mean±se) of FPs normalized with respect to baseline values. B, D) Bar histograms of data points from A and C, as averaged at 57–60 min after HFS and normalized with respect to baseline (mean±se; $*P<0.05$). Application of HFS (2×100 Hz, duration 1 s, interval 30 s) at time 0.

**Figure 3.** Excitability in different phases of the estrus cycle (I/O curves, A), and ATPA-induced enhancement in LA-LTP in slices from female rats at different phases of the estrus cycle (proestrus, B; estrus, C; diestrus, D). A) I/O curves did not differ significantly. B) ATPA ($n=7$ slices) caused a weak but significant facilitation of LTP in slices derived from rats in the proestrus phase in comparison with controls ($n=8$ slices; $P<0.05$). C) ATPA ($n=10$ slices) also significantly enhanced LTP during the estrus phase in comparison with controls ($n=7$ slices; $P<0.05$). The magnitude of LA-LTP also significantly differed in the different estrus phases in drug-free conditions ($P<0.05$). LTP was induced by HFS of EC fibers. Application of HFS (2×100 Hz, duration 1 s, interval 30 s) at time 0. Data points in B, C, and D represent averaged amplitudes (mean±se) of FPs normalized with respect to baseline values. E) Bar histogram of data points B–D, as averaged at 57–60 min after HFS and normalized with respect to baseline (mean±se; $*P<0.05$).
exhibited greater LA-LTP than slices from females in diestrus.

**Crosstalk with p38 MAP kinase**

To gain insight as to whether the gender-dependent effects of GLU_K5 stimulation are mediated by the MAP kinase pathway, we tested the effect of SKF 86002, a p38 MAP kinase inhibitor, alone and coadministered with ATPA. As shown in Fig. 4, SKF 86002 (10 μM) significantly reduced the magnitude of LA-LTP in both males (EC-control: 150.3±5.7%, n=9, vs. SKF 86002: 138.9±5.4%, n=7) and females (EC-control: 148.6±5.5%, n=7, vs. SKF 86002: 128.5±5.0%, n=7). In males, the effect of coadministration of SKF 86002 and ATPA (142.5±6.7%, n=8) did not differ significantly from the effect of ATPA when applied alone (134.0±7.5%, n=7). Therefore, the effects of ATPA and SKF 86002 in males were not additive. In contrast, the suppressive effect of SKF 86002 on LA-LTP in females was diminished by the coadministration of SKF 86002 and ATPA (153.4±5.5%, n=8). Thus, we did not obtain significant differences in LTP recordings in females between drug-free controls and coapplication of SKF 86002 and ATPA. These results suggest an involvement of p38 MAP kinase in the effect of ATPA on LA-LTP in males.

**DISCUSSION**

Our results show that 1 μM ATPA depresses baseline activity in the amygdala induced by single pulses, similar to data described for the CA1 region of the hippocampus (23). The effect of ATPA on baseline activity is not gender dependent. In contrast, tetanic stimulation produces gender-dependent changes in LA-LTP in the presence of ATPA. Moreover, p38 MAP kinase seems to be involved in the mediation of these kainate GLU_K5-dependent and gender-dependent effects on plasticity changes in the amygdala.

Our present results, obtained in untreated, drug-free slices, are in accordance with previous data showing that the magnitude of HFS-induced LA-LTP and LFS-induced LA-LTD did not differ between genders (12, 15). In previous studies, we demonstrated that EC-induced LA-LTP in horizontal slices is dependent on both NMDA receptors and L-type calcium channels, whereas LA-LTP induced by high frequency stimulation of afferents within the LA was dependent on NMDA receptors alone (15). In these pharmacological investigations, there were no differences observed between genders. However, the present study reveals that kainate GLU_K5 receptor stimulation depresses LA-LTP in males and enhances it in females. This effect was not input-specific, (i.e., EC or IN stimulation caused similar effects).

In addition, we show that the magnitude of the effect of ATPA is dependent on estrus state. It is known that gonadal steroids have a profound impact on the morphology of dendrites and patterns of synaptic connectivity (28–30). In agreement with previous reports (31, 32), we did not find significant group differences in the pre-LTP I/O curves. However, the magnitude of LA-LTP was significantly enhanced during estrus in com-

![Figure 4](image-url) **Figure 4.** Gender-dependent changes in plasticity in the LA induced by either SKF 86002 alone or by the coadministration SKF 86002 with ATPA. Data were obtained using stimulation of EC fibers. A) SKF 86002-induced suppression of HFS-induced LA-LTP in males (n=7 slices) compared to controls (n=9; P<0.05). The coadministration of SKF 86002 and ATPA (n=8 slices; P<0.05) also reduced LA-LTP in comparison with controls. ATPA and SKF 86002 were washed in a concentration of 1 μM and 10 μM, respectively. C) In females, SKF 86002 reduced LA-LTP (n=8 slices) in comparison to controls (n=7 slices; P<0.05). The coadministration of SKF 86002 and ATPA (n=8 slices) showed additive effects. Thus, SKF86002 does not occlude the action of ATPA in females, and the pathway blocked by SKF86002 apparently is not required for ATPA to increase LTP. Data points in A and C represent averaged amplitudes (mean±SE) of FPs normalized with respect to baseline values. Application of HFS (2×100 Hz, duration 1 s, interval 30 s) at time 0. B, D) Bar histograms of data points from A and C, as averaged at 57–60 min after HFS and normalized with respect to baseline (mean±SE; *P<0.05).
comparison with the diestrus or the proestrous state. A reason for the stronger LTP in the estrus states might be that the estrogen receptor β mRNA and protein are expressed at high levels in the estrus state and at low levels in the proestrus phase, as shown for the hippocampus (33).

Estrogens are known to potentiate kainate currents (34) and the excitatory effects of kainic acid (35, 36). In contrast to the depressive effects of ATPA on baseline activity in the hippocampus (27), the activation of postsynaptic kainate receptors have been shown to occur only at HFS of mossy fiber afferents (37). Therefore, it seems to be of essential relevance that one of the striking characteristics of GLU_{K5} kainate receptors mediated synaptic response is its remarkable enhancement by train stimulation (10). Estrogen may play a role in the modulation of high-frequency-induced transmission and represent a possible explanation for the LTP-enhancing effect of ATPA on LA-LTP in female rats only. GLU_{K5}-containing kainate receptors could act as facilitatory autoreceptors, amplifying glutamate release from the presynaptic terminal in response to extracellular glutamate accumulation. In this way, kainate receptor-mediated depolarization may help to trigger LTP induction. Because SKF 86002 does not occlude the action of ATPA in females, it can be concluded that the pathway blocked by SKF 86002 apparently is not required for ATPA to increase LA-LTP.

The terminals of GABAergic neurons in the amygdala express 2 subtypes of GLU_{K5}-containing kainate receptors, with different agonist affinities and opposing mechanisms of action. Activation of somatodendritic GLU_{K5} kainate receptors in pyramidal cells enhances amygdaloid excitability resulting from pyramidal cell depolarization and enhanced glutamate release (38). Activation of somatodendritic GLU_{K5} kainate receptors in interneurons suppresses amygdalar excitability because of interneuronal depolarization and enhanced GABA release (38). In males, ATPA reduced the magnitude of LA-LTP. A similar ATPA-induced depression of LA-LTP was found in intracellular recordings in 4-month-old male rats (39). Male rats contain significantly more kainate receptors in the hippocampus than female rats (40). Although it has been demonstrated that, in males, the density of GLU_{K5} receptors in the amygdala is higher than in the hippocampus (9), no data are known for females. Braga et al. (9) also demonstrated that low concentrations of ATPA enhances GABAergic transmission, which would increase the threshold for LTP induction. Enhancement of GABAergic transmission by 1 μM ATPA also could explain the suppressive influence of ATPA on baseline activity in both males and females. It is known that MAP kinase is phosphorylated after kainate stimulation in neurons (41). In males, the effects of ATPA and SKF 86002 seem to occlude each other and therefore might be on the same pathway.

In summary, the gender-specific effects of ATPA on LA-LTP could result from the influences of sex hormones, which may alter GLU_{K5} receptor expression or GLU_{K5}-induced currents. Thus, our results could be interpreted as the molecular and neurophysiological correlates of gender- and hormone-specific alterations in behavior and functional memory and may represent one explanation for gender differences in epilepsy (16).

This work was supported by the Deutsche Forschungsgemeinschaft (German Research Council; SFB-03/TP D3). We thank Dr. H. Siegmund for expert help in data analysis and K. Berlin for technical assistance. We also thank San Francisco Edit for improving the English usage.

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Received for publication July 20, 2007. Accepted for publication October 18, 2007.