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Effect of α_2-adrenergic drugs dexmedetomidine and atipamezole on extracellular amino acid levels in vivo

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Abstract

α_2-Adrenoceptors are known to be involved in a variety of physiological functions and pathological conditions, including epilepsy and the extent of excitotoxin-induced cell death. In this study we evaluated whether selective α_2-adrenergic drugs can modulate the release of neurotransmitter amino acids. The effect of the α_2-adrenoceptor agonist dexmedetomidine (5 μg/kg, s.c.) and the α_2-adrenoceptor antagonist atipamezole (0.1 mg/kg and 1 mg/kg, s.c.) on the release of extracellular glutamate, aspartate and γ-aminobutyric acid (GABA) was studied with microdialysis in the hippocampus of freely moving rats under basal and K^+-evoked conditions. Atipamezole (1 mg/kg) decreased K^+-evoked glutamate efflux by 30% compared to the control group ($P < 0.05$) but did not affect significantly the effluxes of aspartate and GABA. Dexmedetomidine and the lower dose of atipamezole (0.1 mg/kg) did not significantly alter the evoked overflow of amino acids. The results suggest that α_2-adrenergic drugs have only modest effects on the K^+-stimulated overflow of extracellular neurotransmitter amino acids in rat hippocampus.

Keywords: Aspartate; Atipamezole; Dexmedetomidine; Glutamate; GABA (γ-aminobutyric acid); Microdialysis; Hippocampus

1. Introduction

Glutamate, released into the extracellular fluid as a neurotransmitter, plays an important role in central nervous system (CNS) functions such as learning and memory (McEntee and Crook, 1993). Some glutamate release is thought to be regulated by the noradrenergic system through α_2-adrenoceptors. Kamisaki and colleagues (Kamisaki et al., 1993) have found that noradrenaline and the α_2-adrenoceptor agonist, clonidine, decreases K^+-evoked overflow of glutamate from rat spinal cord synaptosomes. This is interesting, because glutamate plays a crucial role in epilepsy and neuronal damage (Dingledine et al., 1990; Lasley 1991; Meldrum 1991) and it is considered that γ-aminobutyric acid (GABA)-mediated inhibition is diminished in epileptic tissue (Lasley, 1991). Additional evidence of excitatory amino acid involvement in epilepsy has come from the microdialysis study of animal models of focal epilepsy, including intracerebral cobalt (Dodd et al., 1980) and kainate application (Wade et al., 1987) as well as in the electrically kindled amygdala (Minamoto et al. 1992), in which seizure activity is associated with an increased release of glutamate. Elevated extracellular concentrations of aspartate, glutamate and taurine have also been found in human epilepsy (During, 1991).

α_2-Adrenoceptors mediate regulatory control over a wide range of physiological, behavioral and endocrine functions, and are thought to play a role in conditions such as hypertension, anxiety, endogenous depression and cognitive functions (Berlan et al., 1992; Ruffolo et al., 1993). α_2-Adrenoceptors on noradrenergic nerve endings and cell bodies participate in autoinhibition of the noradrenergic system (Cedarbaum and Aghajanian, 1977). In addition, α_2-adrenoceptors have been demonstrated to be located also in several neurons other than noradrenergic neurons. Therefore, noradrenaline and α_2-adrenergic drugs influence the release of many other neurotransmitters such as 5-hy-

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droxytryptamine (5-HT), dopamine and acetylcholine (Frankhuyzen and Mulder, 1982; Xu et al., 1993; Beani et al., 1986).

Manipulation of central α_2-adrenoceptors can influence ischemic neuronal damage as well as the onset and severity of experimental seizures in many animal models of epilepsy (Löscher and Czuczwar, 1987; Gustafson et al., 1990; Shibata et al., 1992). Agonists of α_2-adrenoceptors, like clonidine, can alleviate and an α_2-adrenoceptor antagonist, like yohimbine, can potentiate experimental convulsions in rats (Baran et al., 1985; Tsuda et al., 1990).

Medetomidine is a potent, highly specific and selective α_2-adrenoceptor agonist. It is a racemic mixture of two enantiomers and it has clearly been shown that the pharmacological effects of medetomidine are caused by its dextro enantiomer, dexmedetomidine (MacDonald et al., 1991; Savola and Virtanen, 1991). In receptor binding and isolated organ studies medetomidine has a higher intrinsic activity, a higher affinity for α_2-adrenoceptors and a higher relative α_1/α_2-selective ratio than other tested α_2-adrenoceptor agonists, detomidine, clonidine, UK 14,304 or xylazine. Medetomidine inhibits dose dependently the release of noradrenaline, serotonin and dopamine in rat brain (MacDonald et al., 1988). Moreover, it does not have affinity or effects on any tested receptors other than α_2-adrenoceptors (Virtanen et al., 1988). Medetomidine has no selectivity for α_{2A}- or α_{2B}-adrenoceptor subtypes (Uhlén and Wikberg, 1991).

Atipamezole is relatively novel, highly specific and selective α_2-adrenoceptor antagonist (Scheinin et al., 1988; Virtanen et al., 1989). In receptor binding studies it is reported to have about a 100 times higher affinity for α_2-adrenoceptors and over 100 times higher α_1/α_2-selective ratio than idazoxan or yohimbine. Furthermore, in studies with isolated organs atipamezole is a more potent α_2-adrenoceptor antagonist and has about 200 times higher relative α_1/α_2-blocking ratio than idazoxan (Virtanen et al., 1989). It has almost equal affinity for different α_2-adrenoceptor subtypes (Renouard et al., 1994). Atipamezole also penetrates rapidly into brain (Biegon et al., 1992) and causes a dose-dependent increase in central noradrenaline and serotonin release (Scheinin et al., 1988).

Recently dexmedetomidine has been found to suppress kainic acid-induced convulsions and hippocampal neuronal damage in rats. In contrast, atipamezole slightly potentiated kainic acid-induced seizures (Halonen et al., Brain Res., in press). We hypothesized that α_2-adrenergic drugs might affect the seizure threshold by modulating the release of neurotransmitter amino acids. In the present study we evaluated the effect of dexmedetomidine and atipamezole on the levels of glutamate, aspartate and γ-aminobutyric acid (GABA) in the extracellular fluid under basal and K^+-stimulated conditions. K^+ stimulation was chosen, because it is known to produce an increased release of glutamate (Paulsen and Fonnum, 1989; Anderson and DiMicco, 1992) and epileptiform activity in brain slices (Traynelis and Dingledine, 1988). Hippocampal microdialysis in freely moving rats was used and the amino acids were measured by reverse-phase high performance liquid chromatography (HPLC) with fluorescence detection.

2. Materials and methods

2.1. Animals

Male Han-Wistar rats (weighting 280–375 g) were used. The animals were kept under regular lighting conditions (12-h light/dark cycle) and given food and water ad libitum. All experiments were approved by the local ethics committee.

2.2. Drugs

Atipamezole (code MPV-1248, 4-[2-ethyl-2,3-dihydro-1*H*-inden-2-yl]-1*H*-imidazole) hydrochloride and dexmedetomidine (*d*-4-[1-(2,3-dimethylphenyl)ethyl]-1*H*-imidazole) hydrochloride were synthetized by Orion-Farmos, Finland. All other reagents and standards of the highest quality were obtained from standard commercial sources. Atipamezole (0.1 mg/kg and 1 mg/kg) and dexmedetomidine (5 μg/kg) were dissolved in 0.9% saline and injected s.c. in a volume of 0.5 ml/kg. Control rats received 0.9% saline in a volume of 0.5 ml/kg.

2.3. Experimental procedures

Rats were anesthetized with i.p. injection of equithesin (60 mg/kg pentobarbital, 100 mg/kg chloral hydrate) and positioned in a stereotaxic frame (Narischige) with bregma and lambda at the same height. A microdialysis guide cannula (CMA/10, Carnegie Medicin Ab, Sweden) was implanted into the left hippocampus (5.8 mm posterior, 5.5 mm lateral, 4.5 mm ventral to the bregma) according to the atlas of Paxinos and Watson (1986). The guide cannula was fixed by using screws and dental acrylic cement. A microdialysis probe (CMA/10, Carnegie Medicin Ab, Sweden, membrane 3 mm, outer diameter 0.5 mm, molecular cut-off 20000 daltons) was inserted slowly into the guide cannula after the surgery. The animal was allowed to recover overnight.

All microdialysis experiments were performed in awake, freely moving animals. The animal was placed in a plexiglas cage and the probe was connected to the microdialysis system (CMA/100 Microinfusion Pump and CMA/110 Liquid Switch, Carnegie Medicin, Swe-

den). Perfusion (2 μl/min) was started with a Ringer solution containing 147 mM NaCl, 4 mM KCl and 2.3 mM $CaCl_2$ (pH 6). For K^+ stimulation (20 min) the medium was changed to a second solution containing 51 mM NaCl, 100 mM KCl and 2.3 mM $CaCl_2$. Samples were collected at 20-min intervals, starting 60 min after the beginning of the microdialysis. After three basal samples, the experimental drug or saline was administered s.c. to the rat. K^+ stimulation was started 20 min later. In a separate experiment, the Ca^{2+} sensitivity of K^+-induced amino acid release was tested by replacing $CaCl_2$ with 20 mM $MgCl_2$.

Motor seizure activity of the rats was rated during the stimulation according to the 5-stage behavioural scale of Racine (1972). (1) Mouth and facial clonic movements. (2) Head nodding. (3) Forelimb clonus. (4) Rearing. (5) Rearing and falling.

2.4. Histological analysis of the location of the probe

After each experiment, the probe was removed and the anesthetized animal was killed by decapitation. The brain was removed from the skull and fixed in 4% formalin in 0.1 M phosphate buffer, pH 7.4. The brain was sectioned on a vibratome at 50 μm and stained with thionin. The location of the probe was verified microscopically.

2.5. Preparation of microdialysate samples

The derivatization stock reagent was prepared by dissolving 10 μg *o*-phthalaldehyde in 800 μl methanol, followed by 10 μl 3-mercaptopropionic acid and 100 μl 1 M potassium borate buffer (pH 10.4). The working solution was prepared by dissolving 150 μl stock solution with the double volume of 1 M potassium borate buffer (pH 10.4). Precolumn derivatization was accomplished by mixing 15 μl dialysate sample and 8 μl working solution 4 min prior to its injection to the column.

2.6. GABA, glutamate and aspartate assay

GABA, glutamate and aspartate were measured by reverse-phase HPLC using a precolumn *o*-phthalaldehyde derivatization method. Briefly, separation was achieved on a Nova-Pak C18 (3.9 × 150 mm, 4 μm, 60 Å, part No. 37520) column with detection by a Merck Hitachi F1000 Fluorescence Spectrophotometer (ex. 330 nm/em. 450 nm). The mobile phase A was 0.10 M Na_2HPO_4 (pH 6.4) and 10% methanol (v/v), and B was 62.5% methanol at a rate 1.2 ml/min. Linear gradient conditions were: 100% A/0% B for 3 min, 0% B → 48% B in 17 min, 48% B for 2 min, 48% B → 100% B in 3 min, 100% B for 3 min, 100% B → 100% A in 3 min, 100% A for 5 min. Analysis of one microdialysis sample lasted 40 min. The limit of detection for amino acids was 30 fmol. In vitro recovery of the probes for GABA was 16%, for glutamate 17% and for aspartate 14%.

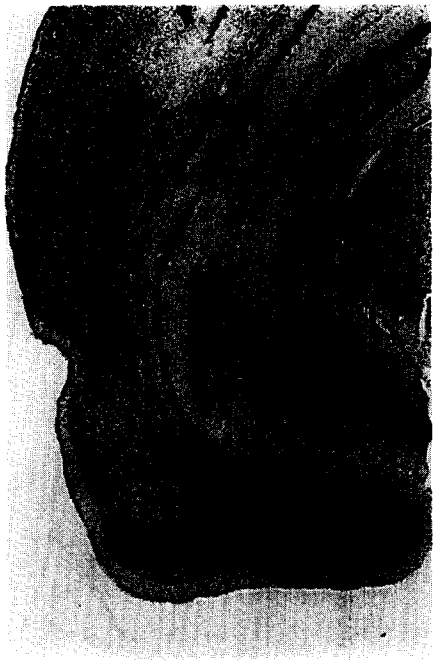

Fig. 1. Representative probe placement in the ventral hippocampus; 50 μm coronal sections stained with thionin.

2.7. Statistical analysis

Data are reported as fmol/min ± S.E.M. and as percentages of basal values (% ± S.E.M.). The statistical significance of the results was tested by Mann-Whitney *U*-test. A level of $P < 0.05$ was considered critical for assigning statistically significant differences.

3. Results

3.1. Basal and depolarization-evoked releases of amino acids

The proper placement of the probes in the ventral hippocampus between CA1 pyramidal cells and stratum lacunosum moleculare was verified in every animal by histological assessment (Fig. 1). The baseline concentrations of glutamate in the dialysate varied between animals and in some animals the baseline decreased without reaching a steady state level. Animals displaying unusually high basal levels or inconsistent baseline levels were excluded from this study.

60 min after the start of microdialysis with standard Ringer, the basal release of extracellular amino acids was constant. The basal values (mean fmol/min ±

S.E.M.; $n = 24$) were as follows: glutamate, 1246 ± 116; aspartate, 218 ± 30; and GABA, 62 ± 6. Stimulation with 100 mM KCl increased glutamate release by 191%, aspartate by 241% and GABA by 2349% above their control values. Fig. 2 shows the effect of the high K^+ stimulation on the dialysate levels of aspartate, glutamate and GABA in the control and drug treated groups.

3.2. Effects of adrenergic agents

The α_2-adrenoceptor agonist, dexmedetomidine, and the α_2-adrenoceptor antagonist, atipamezole, did not affect the basal levels of amino acids in the dialysate (Fig. 2A,C,E). Dexmedetomidine (5 μg/kg), atipamezole (0.1 mg/kg or 1 mg/kg) or saline was injected subcutanously 20 min before the K^+ stimulation, and

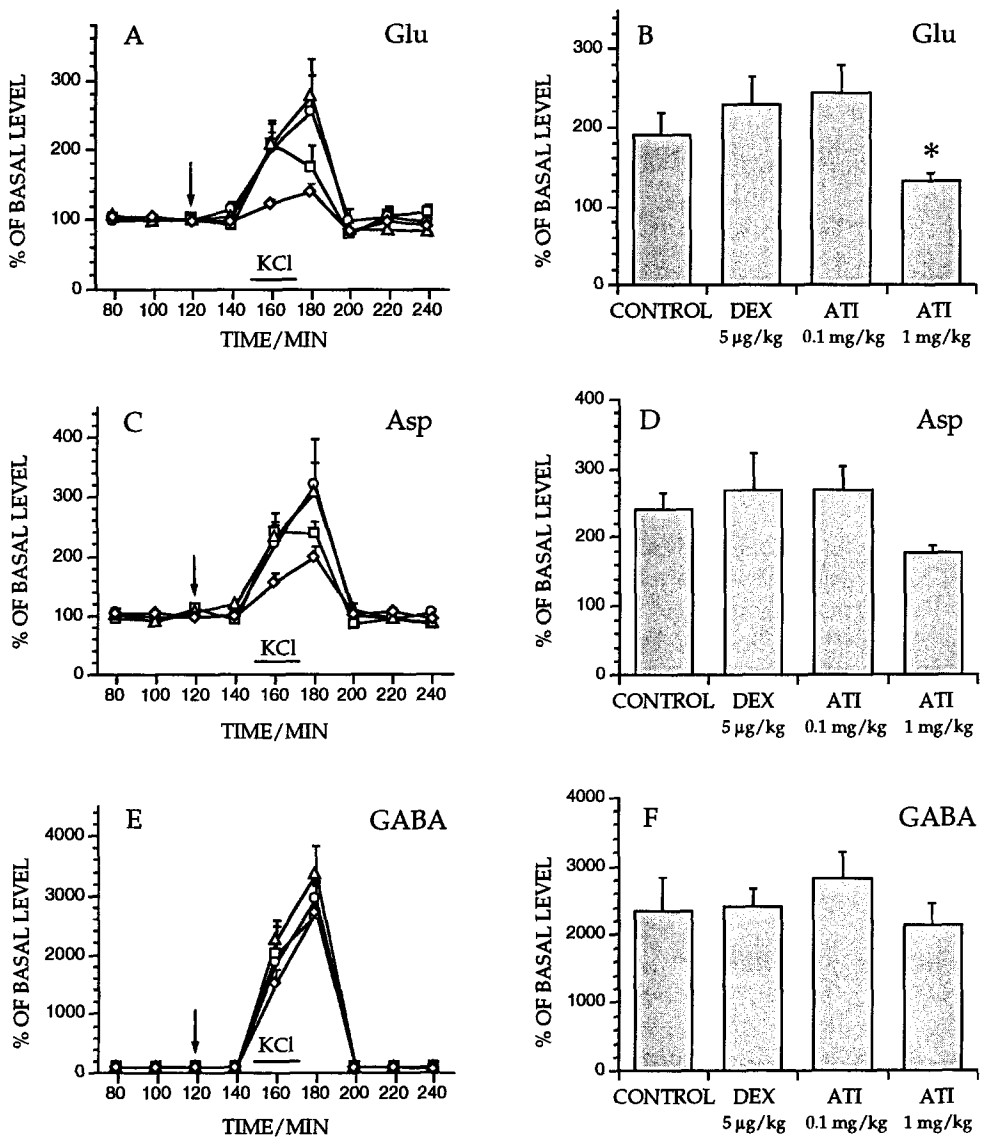

Fig. 2. Effect of saline (□), dexmedetomidine (5 μg/kg) (○), atipamezole (0.1 mg/kg) (△) and atipamezole (1 mg/kg) (◇) on the basal and K^+-evoked extracellular concentrations of glutamate, aspartate and GABA in dialysate collected by microdialysis from the rat ventral hippocampus. Time course of changes in extracellular levels of glutamate (A), aspartate (C) and GABA (E). High K^+ (100 mM) was perfused between 150 min and 170 min (horizontal bar). The arrow indicates when the drug was injected into the rat. All values are expressed as percentages of the basal levels (mean ± S.E.M., $n = 5$–8). The concentrations of extracellular glutamate (B), aspartate (D) and GABA (F) in the rat hippocampus during KCl (100 mM) stimulation in controls, dexmedetomidine (DEX)- and atipamezole (ATI)-treated animals. The KCl-induced increase of amino acid overflow is expressed as a percentage of the respective basal values (means ± S.E.M., $n = 5$–8). * $P < 0.05$ (Mann-Whitney U-test).

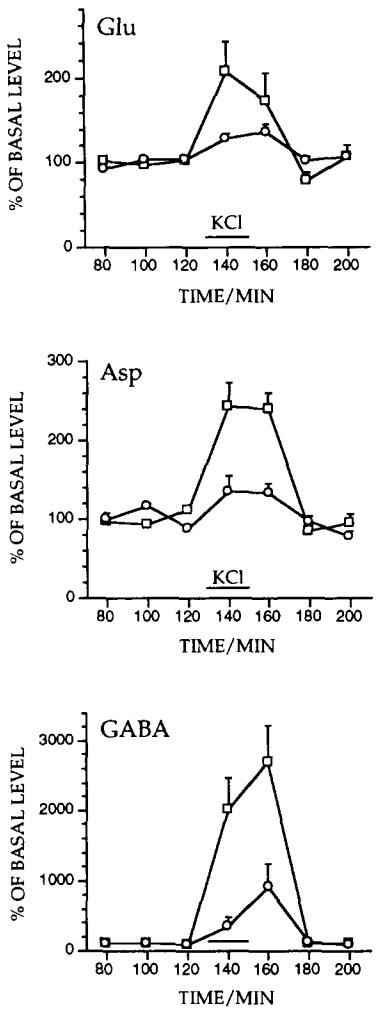

Fig. 3. Microdialysis of rat hippocampus showing the Ca^{2+} dependency of K^+-evoked release of transmitter amino acids. Perfusion was done with (□) and without Ca^{2+} (○). High K^+ (100 mM) was perfused between 130 min and 150 min (horizontal bar). All values are expressed as percentages of the basal levels (means ± S.E.M., $n = 5$). Omitting Ca^{2+} and adding 20 mM $MgCl_2$ to the ringer solution significantly ($P < 0.05$, Mann-Whitney U-test) reduced the extracellular levels of neurotransmitter amino acids.

their effects on basal and evoked overflow of amino acids were compared with controls. Atipamezole (1 mg/kg) decreased significantly K^+-evoked glutamate release by 30%, but it had no significant effect on the release of aspartate and GABA (Fig. 2B,D,F). Dexmedetomidine and the lower dose of atipamezole (0.1 mg/kg) did not alter significantly the evoked overflow of amino acids.

3.3. Ca^{2+}-dependent overflow of amino acids (Fig. 3)

We could not see any significant suppression of amino acid release by simply omitting Ca^{2+} from the perfusion solution during the basal or K^+-evoked conditions. Therefore we replaced Ca^{2+} with 20 mM $MgCl_2$, which is often used to block Ca^{2+} channels. Perfusion with Ca^{2+}-free Ringer with 20 mM $MgCl_2$ had no significant effect on the basal outflow of amino acids but inhibited the K^+-evoked release. The K^+-evoked release of glutamate, aspartate and GABA was suppressed by 31% ($P < 0.05$), 44% ($P < 0.05$) and 73% ($P < 0.05$) during the stimulation, respectively.

3.4. Motor seizure activity during microdialysis

Control rats and dexmedetomidine-treated rats did not exhibit motor seizure activity. In contrasts one out of eight rats treated with the lower dose of atipamezole (0.1 mg/kg) had facial clonic movements and head nodding during the stimulation and two out of seven with the higher dose of atipamezole (1 mg/kg) had head nodding and forelimb clonus (mean seizure score ± S.E.M.: 0.25 ± 0.25 and 0.71 ± 0.47, respectively). However, there was no significant difference in the concentrations of amino acids between controls and animals with seizures.

4. Discussion

In the present study we demonstrated that the α_2-adrenoceptor agonist, dexmedetomidine (5 μg/kg), and the lower dose of the α_2-adrenoceptor antagonist, atipamezole (0.1 mg/kg), do not have any marked effect on the extracellular levels of glutamate, aspartate and GABA, under basal and K^+-stimulated conditions in the rat hippocampus. Interestingly, the higher dose of atipamezole (1 mg/kg) decreased the K^+-evoked outflow of glutamate.

The basal concentrations of glutamate, aspartate and GABA measured in this study were within the range reported in previous studies (Andiné et al., 1991; Minamoto et al., 1992; Lehmann, 1989), although methodological factors, such as probe construction, perfusion rates and amino acid determination produce some differences in the levels of amino acids analyzed by different groups. Similar to Morari et al. (1993), we found marked differences in the basal concentrations of glutamate between animals, whereas basal GABA levels were quite similar within the animals. The region of probe placement was chosen because the hippocampus and the CA1 region have a low threshold for seizure generation and neuronal damage. In addition the hippocampus receives noradrenergic innervation from the locus coeruleus (Swanson et al., 1987). It has also been demonstrated that atipamezole rapidly penetrates into the brain and ventral hippocampus (Biegon et al., 1992).

We found in our previous study (Halonen et al., in press) that dexmedetomidine suppressed kainic acid-

induced convulsions and neurotoxicity. In the dose-response studies of dexmedetomidine (1, 2, 3, 5, 7.5, 10 μg/kg, s.c.), the most effective dose was 5 μg/kg. With this dose about 60% of rats were protected from any behavioural convulsions, whereas only 20% of the rats were protected when the dose was 1 or 10 μg/kg. Anticonvulsant activity has also been reported with another α_2-adrenoceptor agonist, clonidine (Baran et al., 1985). Recently, dexmedetomidine has also been reported to improve the neurologic and histologic outcome in transient cerebral ischemia in the rat (Hoffman et al., 1991). Moreover, another α_2-adrenoceptor agonist, clonidine, was found to reduce the K^+-evoked overflow of glutamate in synaptosomes from the rat spinal cord (Kamisaki et al., 1993). This suggests that activation of α_2-adrenoceptors by α_2-adrenoceptor agonists might mediate their anticonvulsant activity partly by decreasing glutamate overflow. Nevertheless, dexmedetomidine had no effect on the basal or K^+-stimulated release of glutamate and aspartate, nor could we observe any drug-related changes in the outflow of inhibitory transmitter, GABA. Comparison of the results between in vivo and in vitro studies is difficult due to interrupted neuronal connections in cell or slice preparations. However, there are some factors which might have influenced our results. First, glutamate, aspartate and GABA in the perfusate are derived from both the metabolic pool and synaptic processes. The perfusion with Ca^{2+}-free solution indicated that K^+-evoked release only partially reflects ongoing neuronal activity. Second, perfusion with a solution of high K^+ concentration influences all cells in the vicinity of the probe. Therefore, the levels of neurotransmitter amino acids in the microdialysate reflect the changes in the efflux from both neurons and glia cells.

Earlier findings in our laboratory have shown that the α_2-adrenoceptor antagonist, atipamezole (1 mg/kg, s.c.), has a proconvulsive effect in a kainic acid model of status epilepticus (Halonen et al., in press). The dose of 0.1 mg/kg also produced a slightly proconvulsant effect in later studies (Valtonen et al., personal findings). Therefore it was thought that atipamezole might increase the release of glutamate during stimulation. However, the lower dose of atipamezole (0.1 mg/kg) did not influence the release of glutamate. In contrast, the higher dose of atipamezole (1 mg/kg) suppressed glutamate outflow in the hippocampus but did not affect significantly the levels of extracellular aspartate and GABA. The exact mechanism by which atipamezole decreases glutamate release is not known. GABAergic inhibition does not seem to be involved, because the extracellular concentration of GABA was not elevated compared to controls.

Noradrenaline exerts a complex action in the rat hippocampus, acting at α- and β-receptors (Segal et al., 1991). It has been demonstrated that α_2-adrenoceptor antagonists increase the turnover of noradrenaline and produce a dose-dependent increase of noradrenaline release (MacDonald et al., 1988; Scheinin et al., 1988). Noradrenaline release is further enhanced by stimulation with high levels of K^+ (Kalén et al. 1988). It has been found that noradrenaline has a biphasic modulatory action in vitro in the hippocampus (Mueller et al., 1981, 1982; Mynlieff and Dunwiddie, 1988). Low doses of noradrenaline have stimulatory and high doses of noradrenaline have inhibitory effects on neuronal excitation. Therefore, the greatly increased release of noradrenaline with the higher dose of atipamezole might be able to initiate the expression of some inhibitory mechanisms at the target neurons. In addition to direct effects of noradrenaline and noradrenergic drugs on glutamatergic neurons, α_2-adrenergic drugs modulate the release of other neurotransmitters, such as serotonin, dopamine and acetylcholine (Göthert et al., 1981; Frankhuyzen and Mulder, 1982; Beani et al., 1986; Mongeau et al. 1993; Xu et al., 1993), which might have indirect effects on the release of glutamate in the hippocampus. For these reasons the modulation of noradrenaline release by atipamezole might result in complex effects on noradrenergic and subsequently on glutamatergic neurotransmission.

Increased glutamatergic excitation is thought to be involved in the initiation or propagation of epileptic seizures. However, experimental convulsions do not always produce noticeable changes in the extracellular levels of glutamate (Lehmann et al., 1985; Millan et al., 1991) even in the presence glutamate uptake blockade (Meldrum, 1992). Similarly, we did not detect any changes in extracellular glutamate or aspartate concentrations among the atipamezole-treated rats which had mild convulsions during K^+ stimulation compared to seizure-free animals or the control group. It is known that glutamate and aspartate have highly effective uptake mechanisms which maintain the extracellular levels of neurotransmitter amino acids below toxic concentrations (Attwell et al., 1991). Thus, it seems that the microdialysis procedure might not be sensitive enough to detect rapid changes in synaptosomal neurotransmitter release.

In conclusion, this study showed that α_2-adrenergic drugs have only modest effects on the levels of extracellular neurotransmitter amino acids under basal and K^+-stimulated conditions in rat hippocampus.

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