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Short communication

In vivo microdialysis study of (±)-kavain on veratridine-induced glutamate release

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Abstract

This is the first microdialysis study to address the effects of (±)-kavain on veratridine-induced glutamate release in freely moving rats. (±)-Kavain (100 mg/kg, p.o.) significantly reduced veratridine-induced glutamate release compared with that of vehicle-treated controls. Maximum extracellular glutamate levels were obtained 20–40 min after veratridine stimulation (500 μM, added to the perfusate). In the control group the increase was 301% and in the (±)-kavain group the increase was significantly reduced to 219% (the basal value was 100%). These results demonstrate that (±)-kavain reduces veratridine-induced glutamate release in vivo, which confirms previous in vitro data. © 1998 Elsevier Science B.V.

Keywords: Kavain; Na^+ channel blocker; Veratridine; Glutamate release; Microdialysis; Anticonvulsive drug

1. Introduction

Glutamate is one of the most important excitatory neurotransmitters in the brain (Choi, 1988), and has a central role in the corticocortical and corticostriatal pathways in the brain (Fonnum, 1984; Tsumoto, 1990). The mechanisms of glutamate release and uptake are rather complex. They include the release of glutamate from neurons by vesicular exocytosis (Lu et al., 1991) and from cytoplasmic pools the release by non-exocytotic ion-coupled transporter systems (Szatkowski et al., 1990). Effective uptake systems for glutamate are located on astrocytes and neurons (Paulsen and Fonnum, 1989).

The development of the in vivo microdialysis technique has provided a more extensive insight into the process of neurotransmitter release into the extracellular space (Ungerstedt et al., 1982; Tossman et al., 1983). Although the origin of glutamate is rather difficult to estimate (Ungerstedt, 1984; Westerink et al., 1987), the microdialysis technique makes it possible to measure the balance between neuronal release and uptake into the surrounding nerve terminals and glial elements (Tossman and Ungerstedt, 1986; Herrera-Marschitz et al., 1996). Alterations in extracellular glutamate concentrations are of particular interest for pharmacological studies of drugs which influence neurodegenerative processes or epileptic seizure activity.

Gleitz et al. (1996a) reported the Na^+ channel blocking properties of (±)-kavain in rat cortical synaptosome preparations. They demonstrated that (±)-kavain interacts with voltage-dependent Na^+ and Ca^{2+} channels and subsequently suppresses the veratridine-induced release of endogenous glutamate in vitro. We used the microdialysis technique in order to obtain information about the inhibition of veratridine-induced glutamate release by (±)-kavain in vivo.

2. Materials and methods

2.1. Animals

Adult male albino Wistar rats (Hsd/Win:WU, Fa. Harlan-Winkelmann Borchen, Germany), weighing about 300 g at the time of implantation, were used in this study. The ambient room temperature was maintained at 23 ± 2°C and the relative humidity at 55 ± 5%. The animals were maintained under a 12-h light–dark cycle (lights on from 0700

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to 1900) and were housed individually in Plexiglas cages ($40 \times 20 \times 24$ cm^3) and fed on standard food (Altromin®) and tap water.

2.2. Microdialysis experiments

One day before the experiment, a microdialysis probe was implanted under chloral hydrate anesthesia (400 mg/kg i.p.). The probe tip was aimed at the head of the left caudate nucleus (coordinates from bregma: A: 1.25, L: 2.6, V: 5.0 according to the atlas of Paxinos and Watson, 1986). The probe was fixed to the skull with stainless steel screws and methylacrylic cement. The microdialysis probe was constructed as described earlier (Lienau and Kuschinsky, 1997) (membrane diameter 0.38 mm, membrane length 3 mm) and perfused with a modified Ringer's solution (Na^+ 147 mM, K^+ 4 mM, Ca^{2+} 1.3 mM, Mg^{2+} 1 mM, Cl^- 155.6 mM dissolved in bidistilled water) at a flow rate of 2 μl/min. In order to perform postoperative washout, the probe was perfused with modified Ringer's solution during implantation and for 60 min afterwards. On the following day, the rats were placed in a system for freely moving animals (CMA 120 Carnegie Medicin, Stockholm, Sweden). Microdialysis experiments started 18 to 24 h after probe implantation with a 120- to 180-min equilibration period. Afterwards, three 20-min fractions were collected for calculation of the basal glutamate level, which was set at 100%. Then, (±)-kavain 100 mg/kg or vehicle (neutral oil) was administered via an intragastric tube and after 60 min the perfusion fluid was switched to a solution which additionally contained 500 μM veratridine.

Samples were collected every 20 min and analyzed by high-performance liquid chromatography (HPLC) with fluorescence detection after automatic precolumn derivatization with *o*-phthaldialdehyde, according to the method of Lindroth and Mopper (1979), by using an autosampler with cooling module set at 4°C (Waters 717 plus, Millipore, Milford, USA). Aliquots (5 μl) were mixed with the same volume of *o*-phthaldialdehyde solution (Sigma, Munich, Germany) and the reaction product was injected after 1 min onto a vertex column (Nucleosil 120-3 C18, 125 × 3.0 mm, Knauer, Berlin, Germany) maintained at 20°C (column thermostat 5–85°C, Knauer, Berlin, Germany). The mobile phase, containing 0.1 M sodium acetate/0.05 N boric acid buffer (pH 7.0) and methanol (77:23), was delivered by a high-pressure pump (Waters 616, Millipore, Milford, USA) at a flow rate of 0.4 ml/min, and degassed by an on-line degasser (Knauer, Berlin, Germany). A linear gradient up to 100% methanol was used to wash the column after each analysis. The fluorescence monitor (RF 535, Shimadzu, Kyoto, Japan) was set to 330 nm excitation wavelength and 450 nm emission wavelength. Data were recorded and analyzed by the HPLC computer system Gynkosoft (Gynkotek, Germering, Germany). The areas of the glutamate peaks were integrated and calculated by means of external standard calibration. The detection limit for glutamate was approximately 0.1 μmol/l and its in vitro recovery was about 12%. The values of extracellular glutamate are expressed as a percentage of the basal glutamate level and are not corrected for recovery.

2.3. Drugs

(±)-Kavain was purchased from Fa. Roth, Karlsruhe, Germany. The HPLC reagents were obtained from Sigma, Munich, Germany, in the highest purity available. (±)-Kavain was suspended in neutral oil (miglyol®). The volume was 10 ml/kg for intragastric application. The doses are expressed in terms of the base.

2.4. Statistics

In order to compare the effects of the different treatments, the area under the glutamate release curve was calculated by using of a data calculation system (Origin®). Statistical analysis was performed on the median values by using the Kruskal–Wallis *H*-test and the non-parametric Mann–Whitney *U*-test.

3. Results

Fig. 1 shows the influence of (±)-kavain and tetrodotoxin on the veratridine-induced increase in extracellular glutamate in striata from freely moving rats. The basal glutamate levels of each group were not significantly different and were set at 100% (neutral oil + veratridine = 2.5 ± 0.4 μM, (±)-kavain + veratridine = 1.5 ± 0.3 μM, tetrodotoxin + veratridine = 2.1 ± 0.3 μM, mean values ± S.E.M.).

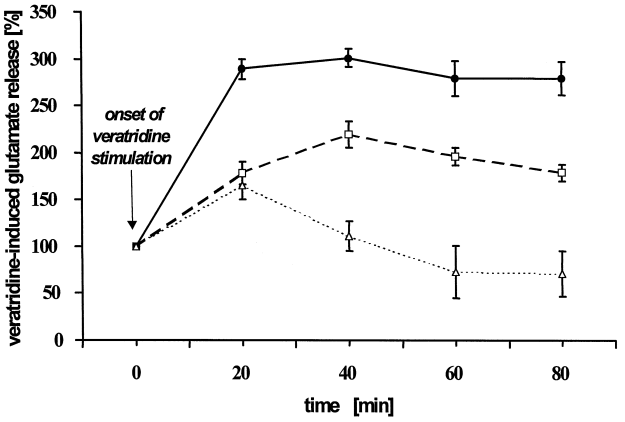

Fig. 1. Influence of (±)-kavain (□) and tetrodotoxin (△) on veratridine-induced glutamate increase compared with that of vehicle-treated controls (●). (±)-Kavain (100 mg/kg p.o.) was administered 60 min before local stimulation with veratridine. Tetrodotoxin (5 μM) and veratridine (500 μM) were administered via the probe. Abscissa: time after the start of veratridine stimulation, ordinate: extracellular glutamate in percent of basal levels. The mean of three 20-min pre-drug dialysates were taken as 100% basal values. Mean values ± S.E.M. for $n = 6$ rats are given.

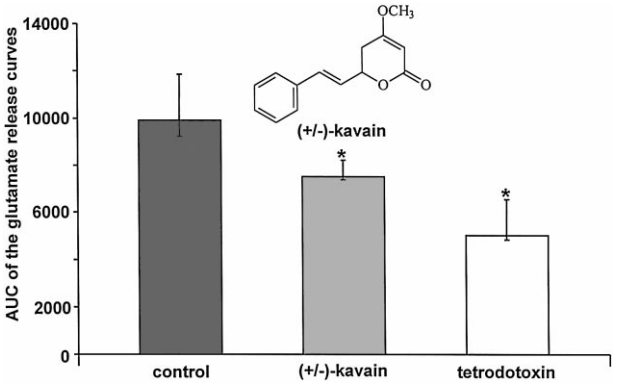

Fig. 2. Reduction of the veratridine-induced glutamate release by (±)-kavain and tetrodotoxin. Median values of the area under the curve (AUC) ± interquantile ranges for $n = 6$ rats are shown. For statistical comparison Kruskal–Wallis *H*-test followed by Mann–Whitney *U*-test were used ($P < 0.01$).

After a stable baseline was obtained, rats ($n = 6$) were treated with 100 mg/kg (±)-kavain or neutral oil (control group) via an intragastric tube. This route of administration was used to overcome the poor water solubility of kavain. Since we found high (±)-kavain brain tissue levels 60 min after intragastric application (Boonen and Ferger, unpublished results), the perfusion fluid was switched after 60 min to a modified Ringer's solution which contained 500 μM of the Na^+ channel activator veratridine in order to stimulate glutamate release. In both groups an increase in the extracellular glutamate concentration was measured after switching to the veratridine-containing perfusion fluid (Fig. 1).

In contrast to the control group, where an increase of the glutamate level to 289% and 301% after 20 and 40 min, respectively was found, the (±)-kavain treated rats showed a smaller veratridine-induced glutamate rise. Twenty minutes after veratridine treatment, the glutamate level increased to 178% of the basal level and after 40 min to 219% (Fig. 1).

Rats treated with the Na^+ channel blocker tetrodotoxin (5 μM) via the microdialysis probe simultaneously with veratridine showed a strong inhibition of veratridine-induced glutamate release. We found an increase to 165% of basal values after 20 min and to only 111% after 40 min (Fig. 1).

The reduction in the veratridine-induced glutamate release elicited by (±)-kavain or tetrodotoxin was statistically significant when calculated as the area under the curve (Fig. 2).

4. Discussion

Glutamate is one of the most widely distributed and most important neurotransmitters in the central nervous system, and excessive glutamate release is able to induce various neurological disorders, e.g., overstimulation of NMDA receptors after ischemia causes neuronal cell death (Benveniste et al., 1984) or excessive release of excitatory acid during periods of epilepsy may lead to neuronal cell death (Olney et al., 1986). Butcher and Hamberger (1987) found that veratrine induced a pronounced release of glutamate into the extracellular fluid of rat striatum and that decortication reduced this release. This suggests that extracellular glutamate found under the conditions used might be, at least for a large part, of neuronal origin.

In the present microdialysis study we demonstrated for the first time that (±)-kavain reduced veratridine-induced glutamate increase in vivo. Perfusion with veratridine led to a maximally stimulated release of extracellular glutamate (to about 300%) in the second intrastriatal dialysate after 20–40 min. In the (±)-kavain-treated group, the peak concentration of extracellular glutamate was obtained during the same collection period (20–40 min after onset of veratridine-stimulation) but was less pronounced, about 220% of basal extracellular glutamate levels. The co-perfusion of veratridine and tetrodotoxin, a selective inhibitor at site I of Na^+ channels (Catterall, 1992), led to an almost complete suppression of veratridine-induced glutamate release and served as a test for the involvement of sodium ions.

Kavapyrones are highly lipophilic substances which easily penetrate the blood–brain barrier. Keledjian et al. (1988) found maximal brain concentrations of 100 μM kavain after i.p. administration of 100 mg/kg of each compound to mice, which is consistent with our results (Boonen and Ferger, unpublished results). The reduction of extracellular glutamate concentrations under stimulated conditions could be an important mechanism of action of kavapyrones and other drugs with neuroprotective and anticonvulsive properties (Meldrum and Swan, 1989).

The inhibition of veratridine-stimulated glutamate release, demonstrated by acute administration of (±)-kavain in a high dosage (100 mg/kg) in vivo, seems to be an important extension of previous in vitro data (Gleitz et al., 1996a,b).

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