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The influence of (±)-kavain on population spikes and long-term potentiation in guinea pig hippocampal slices

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Abstract

Little is known about the mechanisms of action of kava pyrones which are the pharmacological active compounds of the plant *Piper methysticum* Forst. We investigated the effects of the synthetic kava pyrone (±)-kavain on long-term potentiation (LTP) in the CA1-region of guinea pig hippocampal slices. (±)-Kavain reduced the amplitudes of extracellular field potential changes evoked by electrical stimulation in a concentration dependent manner. These effects were reversible. In experiments with LTP no changes were found in the presence of (±)-kavain. In conclusion, our findings suggest (±)-kavain to be an effective drug in modulating excitatory signals in the hippocampus of guinea pigs. Additionally, no alterations on synaptic plasticity in hippocampal neurons for this kava pyrone can be presumed. © 1998 Elsevier Science Inc. All rights reserved.

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1. Introduction

The psychoactive beverage kava prepared from rhizomes, roots and stalks of the intoxicating pepper, *Piper methysticum* Forst., plays an important role in the traditional medicine and socio-cultural life of the inhabitants of the South Pacific islands [14,22]. The beverage is consumed to counteract fatigue, to reduce anxiety and to generate a state of well being. However, after consuming higher dosages individuals fall into a deep sleep [3,10,14]. Extracts of *Piper methysticum* contain various concentrations of the seven pyrones: dihydrokavain, kavain, dihydromethysticin, methysticin, yangonin, desmethoxyyangonin and tetrahydroyangonin as major active compounds [10,16]. For these pyrones a number of pharmacological effects have been described: sedation, potentiation of barbiturate [16] and of ethanol sleeping time [11]; reduction of central muscle tone and inhibition of polysynaptic reflexes for kavain and dihydromethysticin [16]. Furthermore, they show anticonvulsive, local anaesthetic and antiarrhythmic properties [16]. Activation of opioid and dopamin receptors by kava pyrones was excluded [16,22]. (±)-Kavain, a synthetic kava pyrone, inhibits the veratridine-induced increase in intracellular Ca^{2+} and glutamate release possibly by specific inhibition of voltage dependent Na^{+}-channels [8,9].

Pharmacological properties of kava pyrones are comparable to those of benzodiazepines, but no significant binding to GABA and benzodiazepine receptors was detected [6]. Pharmaco-EEG brain mapping analyses of the effects of kava pyrones and the benzodiazepine diazepam show substantial differences between each other and versus placebo. Moreover, less elevated psychophysiological capability in kava treated subjects was found [7]. Long-term potentiation (LTP) is a widely accepted model for learning and synaptic plasticity [2].

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The benzodiazepines diazepam, triazolam, and the non-competitive AMPA receptor antagonist GYKI 52466 have the capability of blocking LTP [4,18,19].

In this study, we investigated the effect of (±)-kavain on the amplitudes of field potential changes (population spikes) and on the induction of LTP in the CA1-region of hippocampal slices from guinea pigs.

2. Materials and methods

Hippocampal slices were prepared as described previously [1]. In brief, white female guinea pigs (Charles River, Sulzfeld, Germany; 280–350 g) were anaesthetized with ether, brains were removed and transverse hippocampal slices (ca. 400 μm thick) were prepared under ice cold medium. After preparation, all slices were incubated in a holding chamber in 25°C medium, equilibrated with 95% O_2 plus 5% CO_2, for at least 90 min. The medium was of the following composition (in mM): NaCl 124, KCl 3, KH_2PO_4 1.24, $MgSO_4$ 1.3, $CaCl_2$ 2, $NaHCO_3$ 26, and glucose 10. After transferring slices in a recording chamber, they were superfused at a rate of 2 ml min^{-1} with equilibrated 25°C medium. (±)-Kavain was applied by changing the superfusion medium by means of three-way taps. At the constant flow rate of 2 ml min^{-1}, about 45 s were required until the drug reached the bath. Concentration-response curves of (±)-kavain were obtained by applying increasing concentrations of this compound for 10 min. There were 15 min intervals between subsequent applications. For comparison of LTP, the drug was present in the bath 15 min before, during, and 20 min after high frequency stimulation (HFS).

Stock solutions of (±)-kavain were dissolved in dimethyl sulfoxide (DMSO).The resulting DMSO concentration in the medium was 0.5%. The highest drug concentration achievable using this procedure was 300 μM. Baseline and wash-out values were measured from medium containing 0.5% DMSO. Furthermore, DMSO alone, diluted at 0.5% in the medium had no effects on synaptic transmission [18].

The CA1 region of hippocampus was identified under transmission microscopy using a binocular microscope (×40). Glass microelectrodes filled with 3M NaCl, having a tip resistance of 1–2 MΩ were used to detect extracellular field potentials in the stratum pyramidale of the CA1 region. Signals were passed through a switched amplifier (SEC 1L, NPI electronic, Tamm, FRG) and filtered at 3 kHz. The data was digitized at 15 kHz using a laboratory interface, and then stored and analyzed with the laboratory computer (DABAS-system, Science Products, Hofheim, FRG [23]). Test stimuli were generated by isolated pulse stimulators (A-MSystems, Everett, WA). Field potential-changes (population spikes) were evoked by constant stimulation of the schaffer collateral pathway ($f = 0.066$ Hz, duration 50 μs, $I = 0.8$–4 mA) with an insulated bipolar tungsten electrode (tip diameter 0.05 mm), placed in the stratum radiatum of CA1. The intensity of test stimulation was adjusted to evoke half-maximal population spikes. A second stimulation electrode with the same parameters was positioned also in the stratum radiatum and was used to obtain baseline values in LTP-experiments. Both inputs were stimulated alternately. LTP was induced by high frequency stimulation (HFS). A total of two bursts with 100 Hz, a duration of 1 s, and a 10 s interval were applied with the same intensity used for test stimuli. The lack of either short or long term potentiation in the pathway not receiving HFS, reflected the independence of both inputs. Although it was recognized, that the field EPSP slope, recorded from the stratum radiatum may reflect a more direct measurement of the synaptic efficacy than the population spike amplitude, the latter measurements were important to understand the effects of (±)-kavain at the integration of the synaptic signals into a neuronal output.

The population spike amplitudes were measured from the negative to positive maximum values within a 3–18 ms interval after the stimulus. Data were normalized and means ± S.E.M. of n trials are shown. One way ANOVA followed by Dunnet's test was performed for multiple comparisons. The Student's paired t-test was used to compare means as appropriate. A probability level of 0.05 or less was considered to be statistically significant.

3. Results

We found (±)-kavain to reduce the amplitude of population spikes in a concentration-dependent manner. Concentrations of 1, 10, 100, and 300 μM decreased the amplitude by 12.5 ± 4.7, 21.7 ± 6.9, 33.7 ± 3.7, and 52.5 ± 8.9%, respectively ($n = 6$; $P <$ 0.05, significant differences against baseline). Superfusion with bath medium for 20 min after applying the highest drug concentration increased the amplitude to 69.0 ± 11.2% ($n = 6$; $P < 0.05$, significant difference; Fig. 1).

In other experiments, LTP was evoked by tetanic stimulation with one of two stimulus electrodes. The recorded data were considered as baseline values. Afterwards, superfusion with (±)-kavain (100 μM) was performed. In order to compensate the inhibitory action of (±)-kavain on the population spikes, the test pulse intensity was readjusted before applying HFS. After a drug contact time of 15 min, stable responses were reached, and a second LTP in the same slice was induced with the other stimulation electrode. Neither initial potentiation (control = 221 ± 22%; (±)-kavain

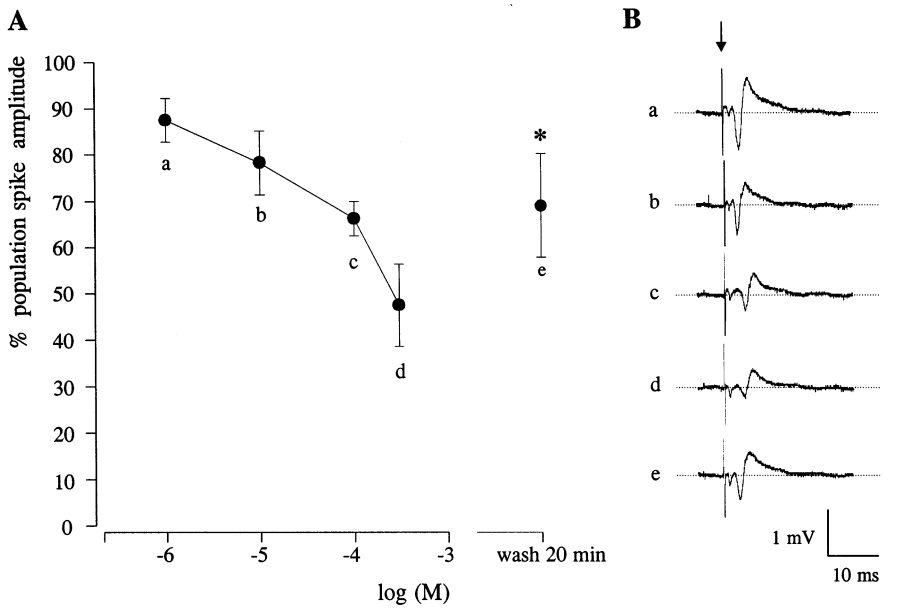

Fig. 1. Decrease of field potential changes (population spikes) by (±)-kavain. Population spikes were evoked by constant current stimulation of the schaffer collateral pathway and detected in the stratum pyramidale of the CA1 region of hippocampus. (A) The Concentration-response curve was received by applying increasing concentrations of the drug for 10 min (1–300 μM). The interval between subsequent applications was 15 min. Wash-out values were measured 20 min after finishing (±)-kavain (300 μM) applications. Means ± S.E.M. from six experiments. $^*P < 0.05$, significant difference between d and e. Representative tracings from one experiment are shown in (B). Arrow indicates stimuli.

100 μM = 201 ± 14%; $n = 6$) nor amplitudes after 20 min (control = 164 ± 16%; (±)-kavain = 172 ± 17%; $n = 6$) showed significant differences ($P < 0.05$; Fig. 2). Furthermore, concentrations of 1 and 10 μM (±)-kavain had no effects on LTP ($n = 3$, not shown).

4. Discussion

Our findings suggest that (±)-kavain is an effective drug in modulating the integration of synaptic signals into a neuronal output in guinea pig hippocampal slices. Responses to test stimuli were decreased in a concentration dependent manner and a recovery was seen after washing out the substances, suggesting reversible effects on receptors or channels. A full recovery was not found within a 20 min washout interval, because of a high tissue affinity implied by the lipophility of (±)-kavain. Further investigations to characterize the receptors or channels involved were not the aim of this study, but it is well known, that kainate, AMPA, NMDA and metabotropic glutamate receptors exist in the CA1 region of hippocampus [21,24]. Pre- and/or postsynaptic mechanisms could be possible targets of (±)-kavain. Recently, (±)-kavain was reported to inhibit veratradin-induced glutamate release and intracellular Ca^{2+}-increase in isolated synaptosomes [9]. Activation of inhibitory GABA receptors by kava pyrones is conceivable [13], but other authors did not find any significant binding of these compounds to these sites [6]. Another possible mechanism of action of kava pyrones is inhibiton of Na^+-channels, according to the local anaesthetic properties of kava pyrones [8,16]. Recent findings support this hypothesis. (±)-Kavain inhibits voltage-dependent Na^+-channels in isolated rat hippocampal neurones [15]. Methysticin, another kava pyrone also reportedly inhibited voltage-dependent Na^+-channels and displayed anticonvulsant properties [15,20].

The concentration-response curve of (±)-kavain suggests a half maximal inhibition at concentrations between 200–300 μM. Precise calculations of an EC_{50}-value are difficult to perform because the curve exhibits no clear maximum depression values. This might be attributed to the lipophility of the substance which causes problems in achieving higher concentrations of the drug in a medium which contains 0.5% DMSO only.

Kava pyrones and benzodiazepines share several pharmacologic properties. The benzodiazepines diazepam and triazolam do not decrease field potential changes evoked by continuous electrical stimulation [4]. 2,3-Benzodiazepines, however, like GYKI 52466, antagonists at AMPA receptors, do [18]. Nevertheless, all of these drugs are capable of blocking LTP [4,18]. Inhibition of LTP is believed to affect complex processes such as learning and memory [2,12,17]. We observed no alterations for the synthetic kava pyrone (±)-kavain on LTP at a concentration of 100 μM. These results are compatible with previous reports find-

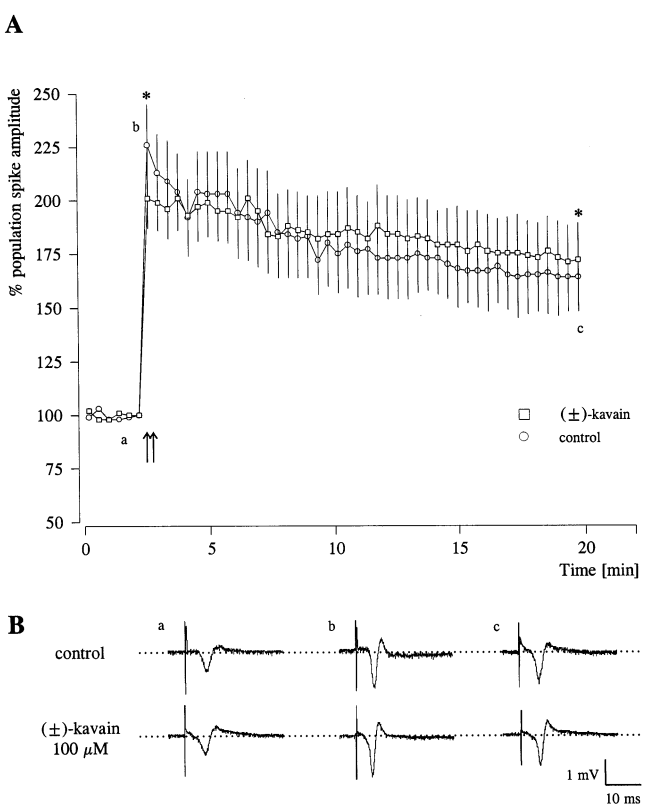

Fig. 2. (±)-Kavain showed no significant effects on high frequency induced long-term potentiation (two bursts 100 Hz, duration 1 s, interval 10 s). Field potential changes (population spikes) were elicited by two stimulation electrodes in the stratum radiatum of the CA1 region of the hippocampus. Both inputs were stimulated alternately. (A) After high frequency stimulation with one electrode to obtain control values, superfusion with (±)-kavain (100 μM) was performed until the end of the experiment. After 15 min superfusion and readjusting the stimulus intensity, a second LTP was induced in the same slice using the other one. Means ± S.E.M. of seven experiments. $^{*}P < 0.05$, no significant difference with respect to control. Arrows indicating high frequency stimulation. Representative tracings from one experiment are shown in (B).

ing psychophysiological capability in low dose kava-treated individuals unaffected or elevated in some cases [7]. There is a large body of evidence, that NMDA receptors are involved in mechanisms of induction and maintenance of LTP in the CA1 region of hippocampus [5] and therefore no action of (±)-kavain on these receptors can be postulated.

In conclusion, (±)-kavain seems to be an effective drug in modulating excitatory signals in guinea pig hippocampal neurones, but no alterations on synaptic plasticity can be presumed.

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