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KAVA PYRONES EXERT EFFECTS ON NEURONAL TRANSMISSION AND TRANSMEMBRANEOUS CATION CURRENTS SIMILAR TO ESTABLISHED MOOD STABILIZERS - A REVIEW

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Abstract

Grunze, Heinz, Jens Langosch, Karin Schirrmacher, Dieter Bingmann, Jörg von Wegerer and Jörg Walden: Kava pyrones exert effects on neuronal transmission and transmembraneous cation currents similar to established mood stabilizers. A review. Prog. Neuro-Psychopharmacol. & Biol. Psychiat., 2001, 25, pp. 1555–1570. ©2001 Elsevier Science Inc.

1. Antiepileptic drugs that are successful as mood stabilizers, e.g. carbamazepine, valproate and lamotrigine, exhibit a characteristic pattern of action on ion fluxes. As a common target, they all affect Na^+- and Ca^{2+} inward and K^+ outward currents.
2. Furthermore, they have a variety of interactions with the metabolism and receptor occupation of biogenic amines and excitatory and inhibitory amino acids, and, by this, also influence long- term potentiation (LTP) to different degrees.
3. The kava pyrones (±)-kavain and dihydromethysticin are constituents of Piper methysticum. Anticonvulsant, analgesic and anxiolytic properties have been described in small open trials.
4. In the studies summarized in this article the effects mainly of (±)-kavain were tested on neurotransmission and especially on voltage gated ion channels. It is assumed that effects on ion channels may significantly contribute to clinical efficacy.
5. Experimental paradigms included current and voltage clamp recordings from rat hippocampal CA 1 pyramidal cells and dorsal root ganglia as well as field potential recordings in guinea pig hippocampal slices.
6. The findings suggest that (i) kava pyrones have a weak Na^+ antagonistic effect that may contribute to their antiepileptic properties (ii) that they have pronounced L- type Ca^{2+} channel antagonistic properties and act as an positive modulator of the early K^+ outward current. These two actions may be of importance for mood stabilization. (iii) Furthermore, kava pyrones have additive effects with the serotonin-1A agonist ipsapirone probably contributing to their anxiolytic and sleep- inducing effects. (iv) Finally, they show a distinct pattern of action on glutamatergic and GABAergic transmission without affecting LTP. The latter, however, seems not to be true for the spissum extract of Kava where suppression of LTP was observed.
7. In summary, kava pyrones exhibit a profile of cellular actions that shows a large overlap with several mood stabilizers, especially lamotrigine.

Keywords: anxiety, bipolar disorder, calcium, dihydromethysticin, epilepsy, γ- aminobutyric acid (GABA), glutamate, (±)kavain, kava pyrones, Kava spissum, long- term potentiation, mood stabilizer, Piper methysticum, potassium, serotonin, sodium, voltage gated ion channels.

Abbreviations: action potential (AP), artificial cerebrospinal fluid (ACSF), dihydromethysticin (DHM), dimethylsulfoxide (DMSO), dorsal root ganglion (DRG), excitatory postsynaptic potential (EPSP), extracellular fieldpotential (EFP), field excitatory postsynaptic potential (fEPSP), γ- aminobutyric acid (GABA), inhibitory postsynaptic potential (IPSP), long- term potentiation (LTP), N-methyl-D-aspartate (NMDA), paroxysmal depolarization shift (PDS), population spike (PS), stratum radiale (SR), tetraethylammonium chloride (TEA-Cl), tetrodotoxin (TTX).

Introduction

Extracts of Piper methysticum contain various concentrations of the seven pyrones: dihydrokavain, kavain, dihydromethysticin, methysticin, yangonin, desmethoxyyangonin and tetrahydroyangonin as major active compounds. These extracts have shown evidence for positive psychotrophic effects in the treatment of anxiety and mood disorders in open and controlled trials (Kretschmer, 1970;Pittler and Ernst, 2000;Volz and Kieser, 1997). Interestingly, kava pyrones and another successful herbal remedy, aswal (a dried root extract from Withania somnifera), are also effective anticonvulsants. However, their mechanisms of action on the cellular level are still somewhat speculative. For kava pyrones, effects on the GABAergic (Jussofie et al., 1994), glutamatergic (Gleitz et al., 1996), and dopaminergic (Baum et al., 1998) transmission have been described, but little is known about effects on the serotoninergic system. The latter may, however, be critically involved in anxiety and affective disorders as deducted from the clinical efficacy of the 5HT 1A agonist buspirone (Blier et al., 1997) and of selective serotonin re-uptake inhibitors.

Biogenic amines are of interest in the pathophysiology of affective disorders. Increased intracellular Ca^{2+} concentrations measured in peripheral blood cells gave clear evidence for a disturbed intracellular ion concentration as an underlying pathology, especially in bipolar disorder (Dubovsky and Franks, 1983). However, K^+ currents may play a crucial role not only in neuroprotection (Rundfeldt, 1999), but also in mood stabilization (Grunze et al., 2000). Compared to Ca^{2+}, the role of transmembraneous Na^+ fluxes was of less interest as this appeared to be a rather unspecific mechanism of action of many antiepileptic drugs independent from their usefulness in bipolar disorder. However, a recent controlled trial of the Na^+ channel blocker phenytoin showed evidence for antimanic efficacy (Mishory et al., 2000).

A striking fact is that -besides actions on different neurotransmitter receptors and intracellular modulation of second messenger pathways- all mood stabilizer successful in clinical practice interfere

either directly with voltage gated ion channels (carbamazepine, valproate, lamotrigine, (Grunze et al., 1999) or have an indirect impact on ion concentrations, mainly by modulating the release from intracellular Ca^{2+} stores (lithium).

Thus, the authors were interested whether kava pyrones may at least partially overlap in their mode of cellular action with established mood stabilizers. This article summarizes our previously published main findings on the action of kava pyrones on voltage gated Ca^{2+}, K^+ and Na^+ channels, postsynaptic potentials as well as on serotonergic transmission. Previously unpublished, we investigated whether kava pyrones interfere with long term potentiation (LTP) as a cellular model of learning and memory. Memory impairment, e.g. with lithium treatment, is an issue that has raised increasing attention during recent years as it may be one of the key reasons for discontinuation of a drug (Calabrese and Woyshville, 1995;Karniol et al., 1978). It is likely that a substance which does not show marked interference with LTP, e.g. lamotrigine (Langosch et al., 2000;Otsuki et al., 1998), may have a more favorable cognitive side effect profile.

Methods

Experiments described herein were carried out at the Institute of Physiology, GH Essen, Germany, and the Departments of Psychiatry in Freiburg and Munich, Germany. Thus, modified experimental procedures and different equipment were used. However, each set of experiments was conducted only in one place to ensure that controls are valid.

Drugs:

(±)Kavain, Kava spissum and dihydromethysticin (DHM) used in all experiments were from one batch supplied by Krewel Meuselbach GmbH, Eitorf, Germany, guarantying identical preparations. Shortly before the individual experiment, they were dissolved in saline or dimethylsulfoxide (DMSO, Sigma Chemicals, 0.01- 0.1 % final concentration). The effect of DMSO by itself at the different concentrations was always tested in conjunction with the experiments and taken into account when calculating (±)kavain, Kava spissum or DHM responses.

Experiment 1: Extracellular Recordings

The experiments were carried out in hippocampal slices of prepubescent guinea pigs of both gender. Preparation procedures and composition of the artificial cerebrospinal fluid (ACSF) were employed as previously described (Walden et al., 1997).(±)-Kavain, Kava spissum and DHM were dissolved in

DMSO at a final concentration of 0,1 % and administered systemically with the bath solution. Extracellular spontaneous synchronized field potentials (EFP) were recorded from the stratum pyramidale of CA1 and CA3 areas using glass microelectrodes (resistance was in the range of 1 to 3 M Ω) filled with 2 mol/l NaCl.

In physiological ACSF, field excitatory postsynaptic potentials (fEPSP) and population spikes (PS) were evoked by constant low frequency stimulation with two insulated bipolar tungsten electrodes, placed in the stratum radiatum of CA1. PS and fEPSP were evoked by constant stimulation with 0,066 Hz and recorded from the stratum pyramidale. For induction of LTP, 2 bursts of 10 pulses were delivered at a rate of 100 Hz, separated by 10 seconds, through the same electrode. LTP was determined by both an increase of the PS amplitude and fEPSP slope. In this paradigm, one electrode was used for the determination of baseline and post-tetanic potentials under control (= drug-free) conditions, the other input for repeating the experiment in the presence of the drug. Both parts of the experiment were carried out one hour apart.

Experiment 2: Whole Cell Recordings. Rat Hippocampus, Current Clamp Mode

Whole cell patch clamp recordings were performed from CA 1 pyramidal neurons of Long-Evans rats (age 20- 40 d, both gender), using a submerged hippocampal slice preparation. Standard methods for preparation and composition of the ACSF were employed as described elsewhere (Grunze et al., 1996). Only cells with an resting membrane potential between -58 and -65 mV and an input resistance of 50-100 MΩ were used. Cells were classified as pyramidal type cells by their electrophysiological properties. (±)-Kavain (100 µmol/l, dissolved in 0,5% DMSO, final DMSO concentration 0.05 %) was administered with the ACSF for 20 minutes, and its effects on membrane parameters (resting membrane potential, input resistance), action potentials and postsynaptic potentials were recorded. Reversibility of changes was checked after a wash-out period of 15-20 minutes.

Experiment 3: Whole Cell Recordings Rat Dorsal Root Ganglion Cells, Voltage Clamp Mode

Detailed description of methods is given in the (Schirrmacher et al., 1999) paper. Cultured dorsal root ganglion (DRG) cells were selected although these cells are probably not involved in epileptogenesis or mood control. Nevertheless, these cells facilitate the analysis of postsynaptic drug effects, because they allow for a better control of local drug concentrations than e.g. neurones in slice preparations. In brief, DRG from 14 neonatal rats (2- to 4-day-old) were excised after decapitation and enzymatically prepared. The neurones were used for electrophysiological recordings 12h after plating for another 2 or

3 days in culture. Coverslips were transferred to a recording chamber superfused with Hepes buffered saline and mounted on an inverted microscope. For whole-cell recordings glass pipettes with a resistances of 2-4MΩ were used in conjunction with an EPC9 (HEKA, Germany) patch clamp probe. Cells were clamped at -80mV. Series resistance compensation was automatically performed by the patch clamp software EPC9Screen.. Raw data were filtered at 2.3kHz. All data were corrected for leak currents by a p/4 protocol (Chad and Eckert, 1986). Ca^{2+} and Na^{+} currents were evoked at a step rate of 1/ 5s. Ca^{2+} currents were elicited by stepping from the holding potential of -80mV to 0mV for 70ms and then back to the holding potential. Na^{+} currents were evoked by step depolarizations from the holding potential of -80mV to 0mV for 25ms. To identify single ionic currents the ACSF was modified by ion substitution and/or by using specific channel blocking agents. To reduce K^{+} conductance, tetraethylammonium chloride (TEA-Cl) was added to the saline in exchange for Na^{+}. To isolate Ca^{2+} currents, $CaCl_2$ was replaced by $BaCl_2$. Na^{+} currents were suppressed by the addition of tetrodotoxin (TTX, 0.4 µmol/l) where appropriate. Furthermore, the pipette solution used for measuring Ca^{2+} and Na^{+} currents also contained CsCl (140 mmol/l), which completely blocked K^{+} channels from the inside. (±)-Kavain was dissolved in DMSO. The final DMSO concentration was 0.01%. Alternatively, (±)-kavain was dissolved directly in the hydrous salt solution and stirred for several hours. In contrast to the other set of experiments, patch clamp experiments were performed at room temperature (21±1°C). Experiments were excluded in which the currents showed a run-down of more than 10% of the total current within 3-5 min after whole-cell mode was attained.

Results

Experiments 1:Extracellular Recordings

After prolonged exposure to low magnesium media, an increase of spontaneous EFP in the CA 1 and 3 area of the hippocampus with superimposed population spikes can be observed (Mody et al., 1987).The intracellular correlate of these field potentials are paroxysmal depolarization shift (PDS) with bursts of action potentials, which are caused by an increased and prolonged Ca^{2+} influx into the cell and resemble giant excitatory postsynaptic potentials (Grunze and Walden, 1997).

In our experiments, these typical extracellular field potentials (EFP) developed within a few minutes during perfusion of the slice with a zero Mg^{2+} solution. The discharge rate of EFP with 8 mmol/l K^{+} in the ACSF was in the range of 21-78/min (n=23) with no difference in the rate of EFP between CA3 and CA1.

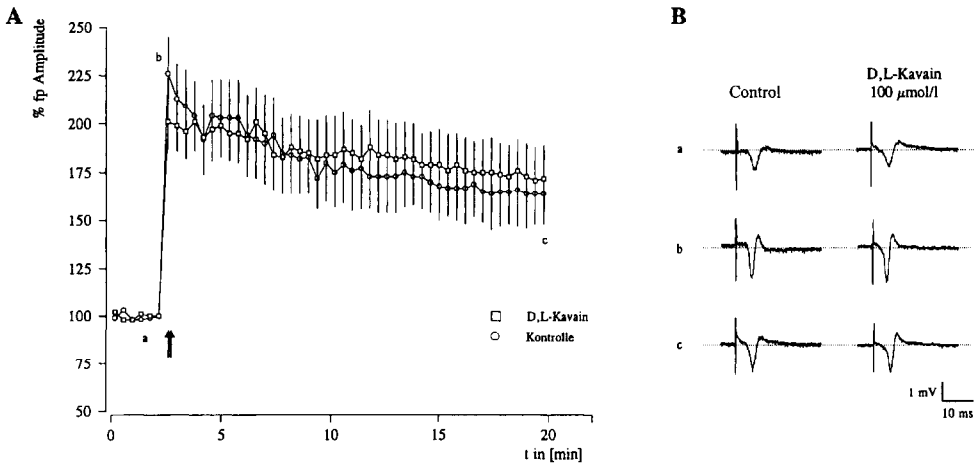

Fig. 1.: ± kavain does not impair LTP. Left: PS amplitudes show a lasting increase compared to baseline (100%) after tetanic stimulation both under control conditions (O) and with 100 µM (±)-kavain (□), means ± S.E.M. of 7 experiments. $p<0.05$, no significant difference between control and PS amplitudes with (±)- kavain. Right: Examples for PS recordings: (a) before tetanus, (b) posttetanic potentiation, (c) lasting PS potentiation after 20 min (LTP). (previously unpublished results)

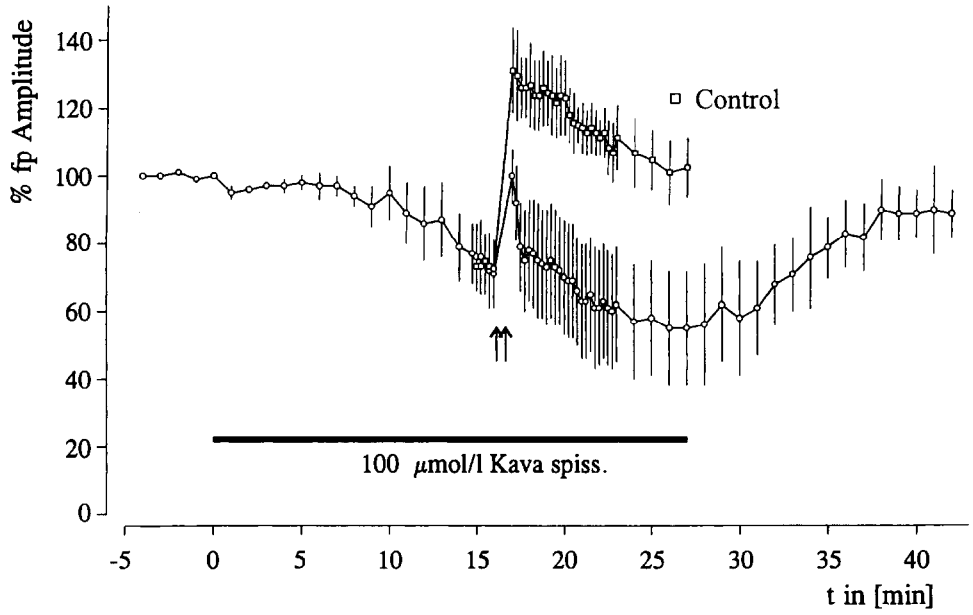

Fig.2.: Kava spissum impairs LTP. Superfusion with 100µM kava spissum (horizontal bar, 30 min duration) leads already to a decrease of PS amplitudes. Control conditions (□) were normalized for this effect. After tetanic stimulation (↑↑) only posttetanic potentiation can be seen in the presence of kava spiss, but no LTP. Mean ± S.E.M. of three experiments. Between t = -5 to 15 and from 23 min onwards 4 consecutive stimulations are expressed as one data point (previously unpublished results).

When (±)-kavain or DHM were added to the zero-Mg^{2+} solution at concentrations of 20, 50 or 100 µ mol/l, respectively, EFP were reduced in amplitude and rate until they eventually vanished totally after 20-40 min. The effects of (±)-kavain and DHM on low-Mg^{2+} induced EFP were dose-dependent. Following withdrawal of the kava pyrones from the solution EFP were fully reestablished after 37-103 min (Walden et al., 1997).

Reduction of Ca^{2+} influx into the cell can either be caused by a direct blockade of voltage dependent channels, or can also be gated through 5 HT 1A receptor activation in this paradigm (Koike et al., 1994). To investigate additive effects, a sub-threshold concentration of (±)-kavain (5 µmol/l) or DHM (10µmol/l) was added to a sub- threshold concentration of the serotonin-1A agonist ipsapirone (5 µmol/l). Each individual concentration of a drug showed no effect on the EFP rate by itself. However, when combining ipsapirone with (±)-kavain or DHM the repetition rate of EFP decreased to 0.76 ± 0.13 (mean ± S.E.M.; n=6) and to 0.81 ± 0.09 (mean ± S.E.M.; n=7), respectively (Walden et al., 1995).

In addition, we tested the effect of (±)-kavain on synaptic plasticity by inducing LTP. These experiments have not been published previously. They were carried out in physiological ACSF. At concentrations sufficient to modulate excitatory postsynaptic potentials (EPSP) and their synchronized correlate in the low- magnesium model, the EFP, no effect on the induction and maintenance of LTP could be observed, suggesting that NMDA receptor blockade is not a main action of (±)-kavain and the reduction of Ca^{2+} influx by (±)-kavain does not reach an extent sufficient to interfere with normal learning and memory (Fig. 1). However, using 100µM Kava spissum, LTP was clearly suppressed and only a small posttetanic potentiation was noticed (Fig.2).

Experiment 2: Whole Cell Recordings. Rat Hippocampus, Current Clamp Mode

All experiments were carried our with 100 µmol/l (±)-kavain. The resting membrane potential, action potential (AP) threshold, AP duration and input resistance remained unchanged with (±)kavain in all 8 neurons investigated. Repetitive AP firing was elicited by injecting currents from 150- 450 pA. (±)Kavain reduced the AP frequency in 5/8 neurons between 20-66 % with a mean of 47±10.4 % (Fig. 3, A, B). Furthermore, in 6/9 cells a 15 ±5 % decrease of the AP amplitude was observed. High frequency repetitive firing was elicited by injecting 950 pA for 3 seconds in 4 cells. All of them showed an voltage dependent attenuation of the firing. These effects, observed with different antiepileptic drugs, have previously been attributed to a blockade of voltage dependent Na^{+} channels

(Macdonald and Kelly, 1995). In 5/9 cells we observed after addition of (±)-kavain a marked (>60 %) prolongation of the time period from the onset of the depolarizing stimulus until the first AP, and also an obvious increase of the interval between AP's.

Recording postsynaptic potentials elicited by stratum radiatum stimulation with a bipolar tungstem electrode, we saw with (±)-kavain in all cells a >50% reduction of the excitatory postsynaptic potential (EPSP, Fig 3C), accompanied only in one cell by a small increase of the inhibitory postsynaptic potential (IPSP).

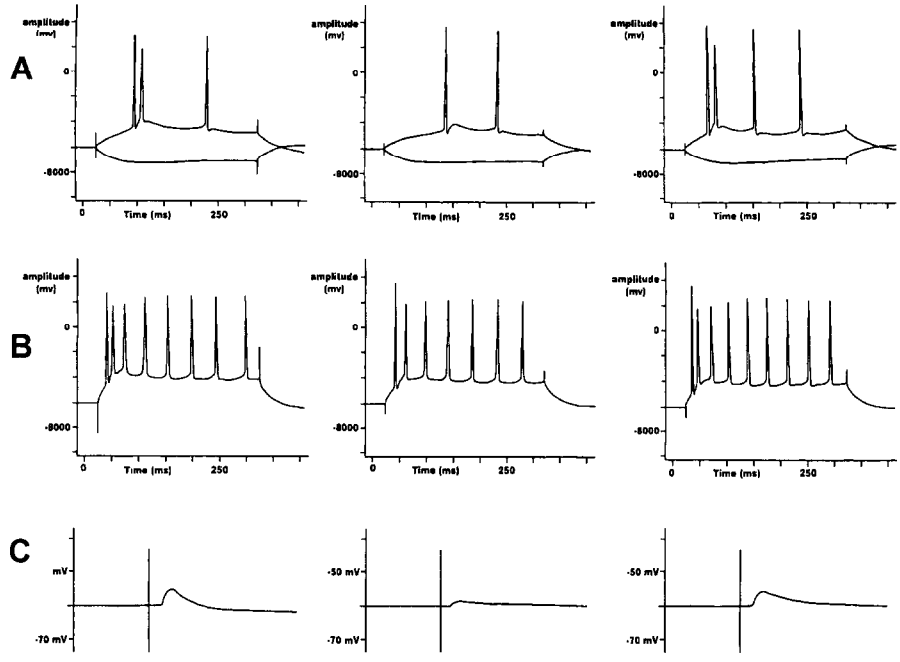

Fig 3: Whole cell patch clamp recording from CA 1 hippocampal neuron, baseline (left column), 10 min after addition (middle column) and 15 min after washout of kavain (right column). Together with AP frequency reduction (holding potential -60 mV; A: response to 150 pA and B: response to 450 pA), kavain (100TM) also diminishes synaptic transmission (C: evoked potentials, bipolar stimulus electrode in the stratum radiale (SR) (adapted from Grunze and Walden, 1998)

Experiment 3: Whole Cell Recordings Rat Dorsal Root Ganglion Cells, Voltage Clamp Mode

Effects of (±)-kavain on voltage-gated Ca^{2+} and Na^{+} channel currents were examined in 25 DRG derived from different neonatal rats. We analyzed the influence of (±)-kavain on the amplitude of whole cell currents measured under voltage clamp conditions.

Effects of (±)-Kavain Dissolved in Hydrous Salt Solution: In Ba^{2+}/TTX-containing ACSF, depolarisation of the membrane potential from -80mV to 0mV evoked inward currents which reached a peak within 5-10ms and decayed slowly and, therefore, are Ca^{2+} channel currents. Switching to (±)-kavain containing ACSF a decrease of the peak and steady-state channel currents shortly after application was observed. The decrease of the peak amplitude of the Ca^{2+} channel currents during (±)-kavain exposure was not reversible after wash.

Effects of (±)-kavain on voltage-activated Na^+ currents were studied in normal HEPES-buffered solution using a similar pulse protocol as for the Ca^{2+} currents. Exposure to (±)-kavain led to a decrease of the voltage-dependent Na^+ current amplitude to 81 ±6 % (n=5) of the baseline value. This effect was only partly reversible in 1 out of 5 cells. Non- reversibility was considered to be due to the bad solubility of (±)-kavain in hydrous solutions which may cause an incomplete wash-out of precipitated kavain crystals.

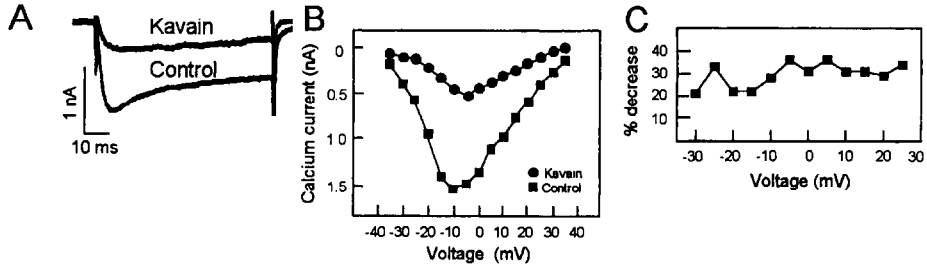

Fig.4: A: Reduction of the voltage -activated Ca^{2+}channel currents elicited by a depolarization from -80 mV to 0 mV for 70 ms by 100 µM (±)- kavain. B: Current- voltage relation of the voltage -activated Ca^{2+}channel currents (peak values) before and after application of 100µM (±)- kavain. C: Percent decrease of the peak amplitude of the Ca^{2+} current in the voltage range between -30 and 30 mV.(Adapted from (Schirrmacher et al., 1999)

Effects of (±)-Kavain Dissolved in DMSO : As (±)-kavain showed effects on both, Ca^{2+} and Na^+ channel currents when directly dissolved in the bath solutions with unknown drug concentrations, we prepared stem solutions containing (±)-kavain dissolved in DMSO. Figure 4 illustrates reduction of the voltage-dependent Ca^{2+} currents by 100 µmol/l (±)-kavain administered with the ACSF. The peak amplitude of the Ca^{2+} currents was reduced to 35% of the control value after a step-depolarisation from -80mV to 0mV (Fig.4A). The voltage-activated Ca^{2+} channel currents were reduced over the entire voltage range (Fig.4B). In this experiment a shift in the peak of the current-voltage relationship of 5mV was observed. Between -30 and 30mV the decrease of the peak amplitude by 100µmol/l (±)-kavain was

similar indicating that high- and low-voltage activated Ca^{2+} channel currents are affected in a similar way. (Fig.4C).

Voltage-dependent Na^+ currents were elicited by depolarising pulses from a holding potential of -80mV to 0mV for 25ms. The Na^+ current increased within 1ms to a peak of 5.3nA and then decayed almost to zero. The half time of this decay was 3ms. A 22 min lasting exposure to (±)-kavain dissolved in normal saline induced a transient decrease of the amplitude of the Na^+ current of 500pA, which was 10% of the initial value. Subsequent addition of 100µmol/l (±)-kavain (in DMSO) hardly affected this peak current (trace3). However, addition of 400µmol/l (±)-kavain transiently decreased the Na^+ current amplitude from 5 nA to 1.7nA which was 34% of the initial value.

Superfusion of the DRG cells with 100µmol/l or 400µmol/l (±)-kavain reduced the Na^+ currents to 65% (n=2) or 41±4% (n=5), respectively, which in most cases were transient or reversible after washing. However, 50µmol/l (±)-kavain did not affect Na^+ currents (96±9%, n=6). In one of 6 cells, the Na^+ current was reversibly reduced to 77% of the initial value by 50µmol/l(±)-kavain. Thus, low concentrations of (±)-kavain up to 50µmo/l hardly affected voltage-dependent Na^+ currents in DRG cells, whereas higher concentrations of up to 400µmol/l induced prominent reductions of this current.

Discussion

Effects of (±)-Kavain on Excitatory Amino Acids and Biogenic Amines Involved in Neurotransmission

The stability of the resting membrane potential during the addition of (±)-kavain (Experiment 2) makes at least a strong GABAergic component of its action unlikely, as direct application of GABA or its agonists drag the resting membrane potential towards more positive potentials. The lack of acute, strong GABA agonistic properties is also supported by the lack of changes of the IPSP following stratum radiatum stimulation. This is in contrast with the results of Jussofie (Jussofie et al., 1994) who found an up- regulation of [3H] muscimol binding in the hippocampus in the presence of (±)-kavain. However, concentrations used in those experiments were 5-fold higher than in our experiments which may explain the diverging findings. Glutamatergic synaptic transmission appears to be diminished in the presence of (±)-kavain, as the reduction of the EPSP in our intracellular (Experiment 2) and the decrease of the population spike amplitude in our extracellular experiments (Experiment 1) suggest. These findings are in line with previous reports from Gleitz (Gleitz et al., 1996) on the influence of (±)-kavain on presynaptic transmission. Diminished EPSP's resemble more likely an effect on

AMPA/Kainate receptors than NMDA receptors as LTP was not affected in our extracellular experiments. This was true for (±)- kavain, but may be different for kava spissum. Kava spissum may contain additional compounds which are only soluble in ethanol and inhibit the expression of LTP.

(±)-kavain and DHM behaved additive with the serotonin-1A agonist ipsapirone in reducing the rate of EFP. Also mood stabilizers like lithium, valproate and lamotrigine are able to increase central serotonergic transmission (Hwang and Van, 1979;Odagaki et al., 1990;Southam et al., 1998). Activation of somatodendritic 5HT 1A autoreceptors leads to a decline of serotonine release, which may be related to sleep- inducing properties of kava pyrones. This is in line with recent in vivo microdialysis findings in rat nucleus accumbens (Baum et al., 1998). Not only a shift of the membrane potential, but also diverse neurotransmitters can profoundly alter the response of Ca^{2+} channels to depolarizing voltage pulses (Dolphin, 1995;Walden et al., 1995).This is also true for the delayed rectifier type of K^+ channels, where 5HT 1A receptor activation plays a strong modulatory role (Penington et al., 1993b;Penington et al., 1993a). Thus, additional action on serotonergic transmission may hasten direct effects on voltage gated ion channels as described below.

Effects of (±)-Kavain on Voltage- Gated Na^+, Ca^{2+} and K^+ Channels

Injecting depolarizing currents in Experiment 2, we saw in the majority of experiments, evidence for an inhibition of voltage dependent Na^+ channels, namely a reduction of the AP frequency in high frequency repetitive firing and a small reduction of the AP amplitude. These observations from hippocampal slices are backed up by our DRG voltage clamp experiments. The data on the inhibitory influence of (±)-kavain on Na^+ currents in DRG cells are consistent with those on veratridine-activated Na^+-channels in synaptosomes prepared from the rat cerebral cortex (IC_{50}=86 µM, (Gleitz et al., 1995). Threshold concentration for this effect seems to be approximately 50µmol/l.

The prolongation of the time interval from stimulus start to AP, as well as the prolongation of the interspike interval (Experiment 2), may be contributed to a positive modulatory effect of (±)-kavain on the fast transient K^+ outward current I_A or, in the hippocampus, also I_D (Storm, 1990). A similar effect has also been seen with the anticonvulsants carbamazepine (Zona et al., 1990), valproate (Walden et al., 1993)and lamotrigine (Grunze et al., 1998).

Voltage gated Ca^{2+} channels appear to be a main target of (±)-kavain. This is backed up both by our extracellular (Experiment 1) and our voltage clamp experiments (Experiment 3). Albeit high-threshold L-type Ca^{2+} channels appear the main site of drug action,(±)-kavain was at 100 µmol/l also a powerful inhibitor on low-voltage activated Ca^{2+} channel currents (Experiment 3). Effects of (±)-kavain on Ca^{2+} channel currents were thus similar to those of the antiepileptic drug carbamazepine in DRG cell cultures (Schirrmacher et al., 1995). Our extracellular experiments suggest that even smaller

concentrations of (±)-kavain or DHM are effective under pathological (epileptic) conditions. The observed partial recovery in Experiment 3within 2-5min, even in the presence of the drug, resembles desensitisation characteristics also known for other substances.

Kava pyrones exert a variety of effects predominantly anticonvulsive, analgesic and centrally muscle relaxant (Kretschmer, 1970;Meyer and Kretzschmar, 1966) which might be explained by inhibition of Na^+ currents. Anxiolytic effects may be attributable to its serotonergic properties. For mood stabilization, effects on Ca^{2+} and K^+ channels may be of interest. If the effects of (±)-kavain in the present experiments are summarized, we found for concentrations between 50- 150 µmol/l (corresponding to steady state plasma concentrations needed for pharmacological in vivo effects):

- serotonergic and antiglutamatergic (non- NMDA), but only weak, if at all, GABAergic action
- no inhibition of LTP
- pronounced antagonistic effects on voltage gated Ca^{2+} channels
- effects on the early K^+ outward current I_D
- moderate voltage gated Na^+ channel blockade.

This profile has an intriguing similarity to the one of lamotrigine (LTG). This antiepileptic drug has antidepressant effects in unipolar and bipolar depressed patients (Calabrese et al., 1999) and prophylactic mood stabilizing properties in bipolar rapid cycling patients (Walden et al., 2000), especially bipolar II patients (Calabrese, oral communication).

Conclusion

Kavain exerts multiple effects on ion channels, all pointing into the direction of limiting excitability. Furthermore, by not impairing LTP it may be relatively lacking of cognitive side effects. Summarizing all effects described in this paper, (±)-kavain seems to have at least on the cellular level a profile of action which can justify a further follow -up of its efficacy for mood disorder in controlled trials.

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