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Prog. Neuro-Psychopharmacol. & Biol. Psychiat. 1997, Vol. 21, pp. 697–706

0278-5846/97 $32.00 + .00

PII S0278-5846(97)00042-0

EFFECTS OF KAWAIN AND DIHYDROMETHYSTICIN ON FIELD POTENTIAL CHANGES IN THE HIPPOCAMPUS

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(Final form, January 1997)

Abstract

Walden, Jörg, Jörg von Wegerer, Ute Winter, Mathias Berger and Heinz Grunze: Effects of kawain and dihydromethysticin on field potential changes in the hippocampus. Prog. Neuro-Psychopharmacol. & Biol. Psychiat. 1997, 21, pp. 697–706. © 1997 Elsevier Science Inc.

1. The kava-pyrones kawain and dihydromethysticin are constituents of Piper methysticum which exert anticonvulsant, analgesic and anxiolytic properties.
2. In the present study the effect of these kava-pyrones were tested on field potential changes (fp) induced by omission of the extracellular Mg^{2+}, recorded from the area CA1 and CA3 of the hippocampal slice preparation of guinea pigs. These fp are generated by an activation of NMDA receptors and voltage dependent calcium channels.
3. Kawain and dihydromethysticin reduced reversibly the frequency of occurrence of fp in a concentration range from 5 to 40 µmol/l and 10 to 40 µmol/l, respectively.
4. Reduction of the fp frequency after addition of subthreshold concentrations of 5 µmol/l kawain and 10 µmol/l dihydromethysticin indicated additive actions of both drugs.
5. Since the serotonin-1A agonist ipsapirone also exerts anxiolytic effects, subthreshold concentrations of kawain or dihydromethysticin were combined with a subthreshold concentration of ipsapirone in another set of experiments. Combining kawain and ipsapirone or dihydromethysticin and ipsapirone caused a reduction of the rate of fp to 0.76 and 0.81 of the baseline value, respectively.
6. The findings suggest that (i) single constituents of Piper methysticum may have additive actions, (ii) that the two components kawain and dihydromethysticin may enhance the effects of the anxiolytic serotonin-1A agonist ipsapirone and (iii) that activation of NMDA receptors and/or voltage dependent calcium channels may be involved in the elementary mechanism of action of some kava-pyrones.

Keywords: anxiolytic actions, dihydromethysticin, kava-pyrones, kawain, serotonin-1A agonists

Abbreviations: aminophosphonovalerat (APV), dihydromethysticin (DHM), dimethylsulphoxide (DMSO), fieldpotential (fp), ipsapirone (Ipsa), kawain (kawa), N-methyl-D-aspartate (NMDA)

Introduction

Several structurally related 4-methoxy-alpha-pyrone derivatives (kava-pyrones) are active constituents of rhizome extracts from Piper methysticum (Efron et al., 1979). Experimental and clinical studies have demonstrated analgesic, antiepileptic and anxiolytic properties of these drugs (Kretzschmar et al., 1970).

Despite several preclinical and clinical investigations the elementary mechanism of action of these drugs remains unknown (Hänsel and Woelk, 1995). Recent investigations analyzed the effects of some kava-pyrones on several neurotransmitter systems including their actions on the glutamatergic (Gleitz et al., 1996), the GABAergic (Jussufie et al., 1994) and the dopaminergic system (Schelosky et al , 1995).

Until now there have been no studies analyzing the interaction with the serotoninergic system in detail. This is, however, of major importance since a disturbance of the serotoninergic system has been described as respondsible for the pathophysiology of anxiety and depression Increased serotonergic neurotransmission is discussed as a long-term effect of antidepressant and anxiolytic drugs, and agonists of serotonin-1A receptors show anxiolytic and antidepressive actions (Blier and de Montigny, 1994). In addition, these serotonin-1A agonists activate voltage dependent calcium channels (Walden et al., 1993b; Anwyl, 1991). This is important since (i) a disturbed intracellular calcium ion homeostasis is described in the pathophysiology of affective disorders (Dubovsky, 1993, van Calker et al., 1993) and (ii) the antidepressant and anxiolytic drug imipramine has also calcium antagonistic effects besides other actions (Choi et al., 1992).

The aim of the present investigation was to test the effect of the kava-pyrones kawain and dihydromethysticin on calcium dependent potentials in the hippocampus. As a model of calcium dependent neuronal activity fp changes induced by omission of the extracellular Mg^{2+} were used (Mody et al., 1987; Pohl et al., 1992; Walden et al., 1992).

Methods

Experimental procedure

The experiments were carried out in hippocampal slices of guinea pigs (300 - 400 μm thick). The brain was rapidly removed from the guinea pig under ether anesthesia. The slices were preincubated for 2 h in a 28° C standard artificial cerebrospinal fluid containing (in mmol/l) NaCl 124, KCl 4, $CaCl_2$ 0.75, KH_2PO_4

1.24, $MgCl_2$ 1.3, $NaHCO_3$ 26 and glucose 10, equilibrated with 5% CO_2 in O_2 (carbogen). After preincubation the slices were transferred to a recording chamber which was continuously (2 ml/min) perfused by 32° C warm solution. The ionic composition was identical to preincubation exept for the Ca^{2+}-concentration which was raised from 0.75 to 1.75 mmol/l and the K^+-concentration which was augmented from 4 to 8 mmol/l (Bingmann and Speckmann, 1988; Walden et al., 1992). Slices were perfused in the recording chamber for a minimum of 1 hour with this standard solution before switching to a zero Mg^{2+}-solution. The pH of the carbogen equilibrated solution was 7.4 and did not change when $MgCl_2$ was omitted or when drugs were added. Kawain and dihydromethysticin (Krewel Meuselbach GmbH, Eitorf, Germany) were dissolved in DMSO in a final concentration of 0,1 % which has no effects on fp.

Assessment

Extracellular recordings were performed from stratum pyramidale of CA1 and CA3 areas of the hippocampal slice using glass microelectrodes (resistance 1 to 3 MΩ) filled with 2 mol/l NaCl. The tips of the microelectrodes were positioned under visual control and placed into the tissue with a step-motorized micromanipulator.

Data analysis

Signals were recorded by means of a conventional microelectrode amplifier (NPI electronics, Tamm, Germany), stored on an oscilloscope and plotted on a pen recorder. All experimental data are given as mean ± SEM.

Results

During perfusion of the slice with a zero Mg^{2+} solution typical fp developed within a few minutes (Fig. 1, CTRL-1). Several investigations have shown that calcium currents essentially contribute to the generation of these extracellular fp (Bingmann and Speckmann, 1988; Pohl et al., 1992; Walden et al., 1992) which are the correlate of intracellularly recorded paroxysmal depolarization shifts (Schmitz et al., 1995; Walden et al., 1992). The discharge rate of fP with 8 mmol/l K^+ in the control solution was 21-78/min (n=23, 36 ± 15). There was no difference in the frequency of occurrence of fp between CA3 and CA1 region of the hippocampal slice (Pohl et al., 1992).

When kawa or DHM (50 - 100 µmol/l)were added to the zero-Mg^{2+} solution the fp rate and amplitude were reduced with fp being eventually totally blocked after 20-40 min. After washout of the kava-pyrones

the fp rate fully recovered with a latency of reappearence of fp of 37-103 min. Typical experiments with kawa and DHM are shown in Fig. 1 and 2. These effects of kawa and DHM on low-Mg^{2+} induced fp were dose-dependent (Fig. 3).

20 µmol/l kawa or 20 µmol/l DHM reduced the rate of fp to 0.29 ± 0.12 (mean ± S.E.M.; n= 12) or to 0.43 ± 0.25 (mean ± S.E.M.; n=11) , respectively, after 30 min of superfusion. Concentrations of 5 µmol/l Kawa and 10 µmol/l DHM did not alter the frequency of occurrence of the EFP. These concentrations were considered as subthreshold concentration (Fig. 1C and 2C).

To investigate additive effects, in a first set of experiments the subthreshold concentrations of both kava-pyrones were combined and in a second experiment each subthreshold concentration was added to the serotonin-1A agonist ipsapirone.

In the first subset of experiments in which subthreshold concentrations of kawa and DHM were combined the fp rate was reduced to 0.67 ± 0.14 (mean ± S.E.M.; n=12; Fig. 4 and 5). In the second series of

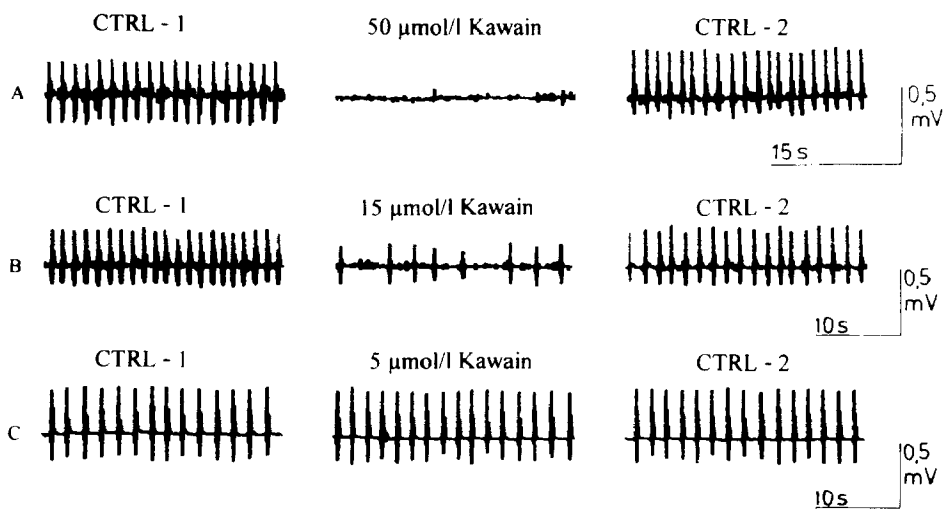

Fig. 1: Effect of kawain on extracellular field potentials induced by omission of Mg^{2+} from the extracellular bath solution. Recordings before (CTRL-1), 30 min during and 60 min after (CTRL-2) the superfusion with 50 µmol/l (A), 15 µmol/l (B) and 5 µmol/l kawain.

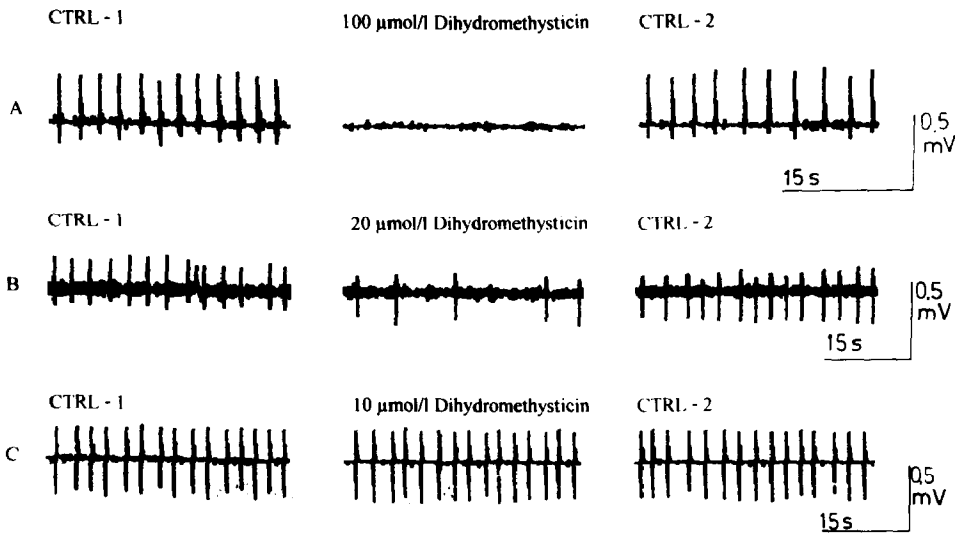

Fig. 2: Effect of dihydromethysticin on extracellular field potentials induced by omission of Mg^{2+} from the extracellular bath solution. Recordings before (CTRL-1), 30 min during and 60 min after (CTRL-2) the superfusion with 100 µmol/l (A), 20 µmol/l (B) and 10 µmol/l dihydromethysticin..

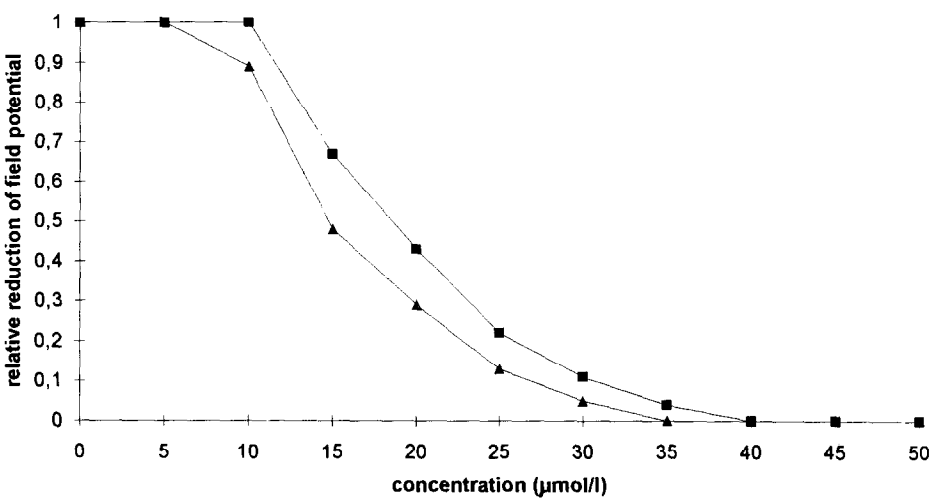

Fig. 3: Dose-response relation of the action of kawain (triangles)and dihydromethysticin (squares) on the rate of extracellular field potentials (fp). Ordinate: relative reduction of fp. Mean values out of 12 (kawain) and 11 (dihydromethysticin) experiments for each concentration.

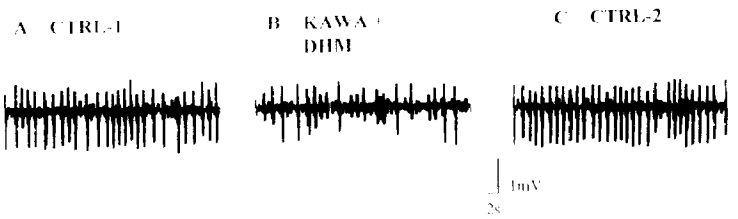

Fig. 4: Additive effects of kawain and dihydromethysticin on extracellular field potentials in the CA1 area of the hippocampal slice induced by omission of Mg^{2+} from the bath solution. Systemic administration of subthreshold concentrations of 5 µmol/l kawain and 10 µmol/l dihydromethysticin decreased the rate of fp.

investigations 5 µmol/l ipsapirone was found to be a subthreshold concentration for the serotonin-1A agonist (Walden et al., 1993b). A combination of ipsapirone with 5 µmol/l kawa or 10 µmol/l DHM decreased the rate of fp to 0.76 ± 0.13 (mean ± S.E.M.; n=6) and to 0.81 ± 0.09 (mean ± S.E.M.; n=7), respectively (Fig. 5).

Since an activation of NMDA receptors is involved in the generation of low-Mg^{2+} induced fp changes (Walther et al., 1986) the effect of the NMDA antagonist 5-aminophosphonovalerat (APV) on the fp repetition rate was tested. APV reduced the frequency of occurrence of EFP in a dose-dependent manner. The subthreshold concentration of APV which does not affect fp was 0.5 µmol/l. A combination of the subthreshold concentration of kawa or DHM with the subthreshold concentration of 0.5 µmol/l APV was, however, without effect.

Discussion

This study demonstrated that the kava-pyrones kawain and dihydromethysticin reduce the frequency of occurrence of fp in the hippocampus in a dose dependent manner. Moreover, both drugs show additive effects with each other and with the serotonin-1A agonist ipsapirone.

Effects of Kava-Pyrones on Calcium Channels

The zero-Mg^{2+} induced fp are thought to be generated by removal of Mg^{2+} from the NMDA receptor and a consecutive activation of voltage dependent calcium channels (Bingmann and Speckmann, 1988; Mody et al., 1987; Pohl et al., 1992; Walther et al., 1986) These fp can be blocked by organic calcium channel blockers (Pohl et al., 1992; Walden et al., 1992, 1993a). Thus, it may be assumed that kawain and

dihydromethysticin may exert besides other actions also effects on neuronal calcium channels. This is of special interest because calcium antagonism is discussed as a treatment strategy in epilepsies (Speckmann et al., 1986) as well as in affective disorders (Brunet et al., 1990; Dubovsky, 1993; Schirrmacher et al., 1993; Walden et

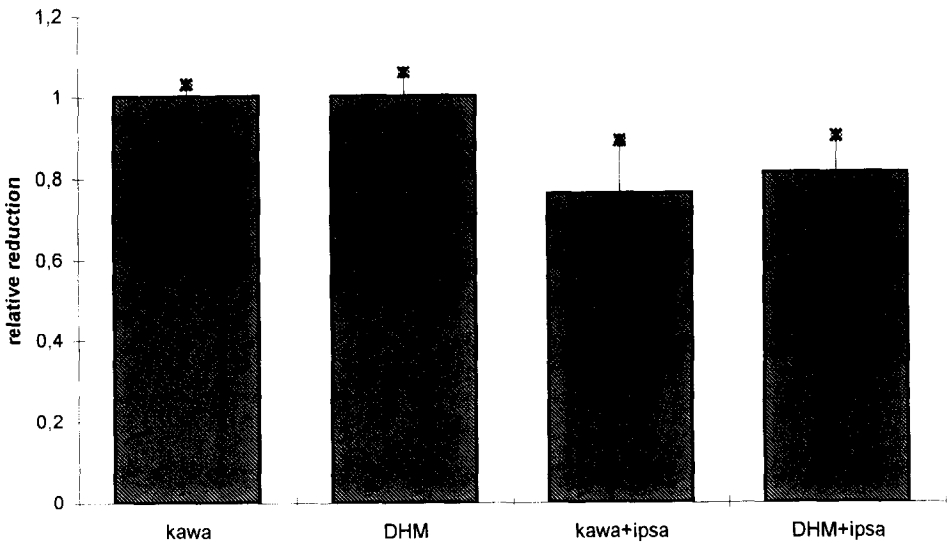

Fig. 5: Effects of combinations of subthreshold concentrations of kawain (5 µmol/l) and dihydromethysticin (10 µmol/l) with a subthreshold concentration (5 µmol/l) of the serotonin-1A agonist ipsapirone.

al., 1995). This interpretation is supported by the fact that (i) different kava-pyrones exert antiepileptic effects , e.g. methysticin (Schmitz et al., 1995) and (ii) other antidepressant drugs have been demonstrated to exert calcium channel blocking effects, e.g. imipramine which is also clinically used in anxiety disorders (Choi et al., 1992).

Physiological Significance

The concentrations of kawain and dihydromethysticin in our experiments were in the range from 10 to 100 µmol/l. Significant pharmacological effects of kava-pyrones are expected with a steady state plasma concentrations of about 50 to 150 µmol/l (Kretzschmar and Meyer, 1968), which corresponds to the

These experiments do not differentiate between the types of calcium channels which may be affected by kawain and dihydromethysticin, and the possibility can not be ruled out that modulations of second messenger systems impinging on calcium channel phosphorylation may be involved. For example, diverse neurotransmitters can profoundly alter the response of calcium channels to depolarizing voltage pulses (Dolphin, 1995). This type of modulation has a powerful impact on neuronal activity and is a fundamental cellular mechanism to regulate calcium entry.

Concerning the generation of low-Mg^{2+} induced fp the commonly accepted hypothesis is the activation of NMDA receptor coupled channels (Mody et al., 1987). This is, however, not an exclusive explanation. Organic calcium antagonists have also been reported to block low-Mg^{2+} elicited field potentials (Pohl et al., 1992; Straub et al., 1992),although they do not act on NMDA receptors (Jones and Heinemann, 1987, Speckmann et al., 1986). Nevertheless the origin of the described additive effect still has to be analyzed in detail, e.g.using whole cell voltage clamp technique.

Conclusion

The kava-pyrones kawain and dihydromethysticin exert additive effects with each other and with the serotonin-1A agonist ipsapirone. It is concluded that this pharmacological behavior - which may be due to actions on voltage dependent calcium channels - may participate to the antidepressive and anxiolytic effects of these compounds in men.

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