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EFFECT OF KAVA EXTRACT AND INDIVIDUAL KAVAPYRONES ON NEUROTRANSMITTER LEVELS IN THE NUCLEUS ACCUMBENS OF RATS

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(Final form, August 1998)

Abstract

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1. Kavapyrones have well-known psychotropic properties. The most common actions of the extract are relaxation and euphoria, depending on the circumstances of ingestion, whereas higher doses cause sleepiness and skeletal muscle relaxation. Several other actions have been reported such as anticonvulsant properties, neuroprotection and analgesia. No interactions with neuroreceptors have yet been found that would explain the multiple actions.
2. To reveal neuronal functions affected by the kavapyrones the authors studied their actions on the mesolimbic reward system using in vivo microdialysis.
3. A small dose of kava extract (20 mg/kg body weight i.p.) caused changes in rat behaviour and concentrations of dopamine in the nucleus accumbens. Higher doses (120 mg/kg i.p.) increased the levels of dopamine. With respect to the individual compounds, D,L-kawain induced in low doses a decrease in dopamine levels and in higher amounts either an increase or no change in dopamine concentrations. Yangonin resulted in a decrease of dopamine levels to below the detection limit and desmethoxyyangonin in an increase of dopamine levels. Dihydrokawain, methysticin and dihydromethysticin did not produce any significant changes of dopamine levels. D,L-kawain caused a decrease in 5-HT concentrations. Some of the other kavapyrones affected 5-HT levels as well.
4. The results suggest that the relaxing and slightly euphoric actions may be caused by the activation of the mesolimbic dopaminergic neurones. Changes of the activity of 5-HT neurones could explain the sleep-inducing action.

Keywords: alpha pyrones, dopamine, 5-HT, kava extract, microdialysis, nucleus accumbens, phytopharmaca.

Abbreviations: 3,4-dihydroxy-phenylacetic acid (DOPAC), gamma-aminobutyric acid (GABA), 5-hydroxy-3-indoleacetic acid (5-HIAA), 5-hydroxytryptamine (5-HT), homovanillic acid (HVA), nucleus accumbens (n. acc.).

Introduction

In the islands of the South Pacific, consumption of a beverage prepared from the rhizome, root tuber or stem of the pepper plant (Piper methysticum Forster) is widespread. In Europe the extract is used as a phyto-anxiolytic, which is prepared from the kava rhizome. The extract contains the dihydroalphapyrones D,L-kawain, dihydro-kawain, methysticin, dihydromethysticin as well as the alpha pyrones yangonin and desmethoxyyangonin. These and other alkaloids (11-methoxynoryangonin, 11-methoxymethysticin, tetrahydroyangonin, 5-hydroxy-dihydrokawain) seem to be the active compounds of the kava beverage (Shulgin 1973).

According to the review of Hänsel and Woelk (1994) the kava beverage produces a friendly and relaxed feeling and promotes social and business interactions, though somnolence can occur, as well vomiting after higher doses. Later on a pleasant sensation of relaxation comes without aggression, followed by motor ataxia, while the subjects remain fully conscious. Sleepiness dominates following ingestion of relatively large quantities. Some local anaesthetic effects on the tongue and palate have also been described. This alteration probably results in a loss of appetite and, later, a loss of body weight.

Kava extract shows a similar activity profile as the benzodiazepines. The benzodiazepine receptors or GABAergic neurones may therefore be the site of action. Davies et al (1992) demonstrated that the kava extract does not affect those mechanisms and they also failed to find any activation of 5-HT_{1A} receptors. In contrast to these observations, Jussofie et al (1994) found that the sedative effects of the kavapyrones may be mediated by an increase of $GABA_A$ receptor binding sites in vitro in the hippocampus and other brain regions.

Examination of the individual compounds revealed that dihydromethysticin produced strong and prolonged somnolence and slight ataxia. This observation is supported by the results of animal studies that have shown a considerable extension of the hexobarbital-sodium induced sleeping time in mice. This effect was less marked after methysticin. Dihydrokawain, kawain and yangonin had hardly any effect on sleep (Meyer 1979).

The alpha pyrones have anticonvulsant properties.The action may be explained by the observation of Gleitz et al (1996)who found that kawain interacts with voltage-dependant Na^+ and Ca^{2+} channels on 4-aminopyridine stimulated cerebrocortical synaptosomes thereby suppressing the 4-aminopyridine-induced increase in $[Na^+]_i$, $[Ca^{2+}]_i$ and the release of endogenous glutamate. Methysticin and dihydro-methysticin have a neuroprotective potency

comparable to that of memantine. Kawain, dihydrokawain and yangonin did not exert any neuroprotection (Backhauss and Krieglstein 1992).

In vitro studies with field potential changes demonstrated that kawain and dihydromethysticin may have additive actions and enhance the effects of the anxiolytic serotonin-1A agonist ipsapirone in the hippocampus of guinea pigs (Walden et al 1997).

The inconsistent findings of the in vitro studies prompted the authors to investigate the effects of the kavapyrones not at the receptor level but on an intact neuronal system within the central nervous system. We used the reward system as model system specifically for changes in the n. acc. which represents an important projection area. Following i.p. administration of the kavapyrones to rats the levels of dopamine, 5-hydroxy-tryptamine and some of their metabolites were determined.

Materials and Methods

Animals and Surgical Procedures

Male Wistar rats weighing 220-300 g (WIST: Charles River, Germany) were maintained on a 12-h light/dark cycle in a temperature and humidity-controlled environment for at least 7 days until surgery. Food and water were available ad libitum. The microdialysis experiments were performed as described previously (Sällström Baum et al 1996). Briefly described, under anaesthesia a guide cannula (all used equipment are from Carnegie Medicine/Sweden) was implanted into the brain of rats mounted into a stereotaxic device (David Kopf Instruments, USA). The tip of the guide cannula was lowered into the n. acc. (coordinates: anterior 1.2 mm, lateral 1.4 mm and ventral -6.5 mm (relative to bregma)). After 72 h recovery, individually held, the microdialysis probe was inserted through the guide cannula under light diethylether anaesthesia and connected to a microinjection pump. This probe was continuously perfused with artificial cerebrospinal fluid (aCSF; 137 mM Na^+, 1.2 mM Ca^{2+}, 2.4 mM K^+, 144.2 mM Cl^-, 1.2 mM Mg^{2+}, 0.9 mM $NaH_2PO_4.H_2O$ and 1.4 mM $Na_2HPO_4.2H_2O$;pH 7.0) for 20 h before the experiment commenced. The flow rate was 2.0 µl/min and twenty minute fractions were collected.

Cannula placements were confirmed at the end of the experiment by standard histological procedures.

Drug Administration

After five collected samples (basal level) the following drugs were injected i.p.: kava extract (batch No.420015/18.5. 1994), D,L-kawain (batch No.K20332),dihydrokawain, methysticin, dihydromethysticin, yangonin and desmethoxyyangonin respectively. The kava extract and the individual kavapyrones were a gift of the Krewel Meuselbach, Eitorf /Germany. All kavapyrones were assimilated in one or two drops of Cremophor (Mainland Pharmazeut. Fabrik, Frankfurt a.M.,Germany) and diluted as an emulsion with 0.9% sodium chloride. Control animals were administered the solvent without active substance. Three different doses of the kava extract were administered (20 mg/kg, 120 mg/kg and 220 mg/kg body weight). The D,L-kawain was applicated in doses of 30 mg/kg, 60 mg/kg and 120 mg/kg body weight. The other kavapyrones were injected in a dose of 120 mg/kg body weight. The proportions of pure kavapyrone in the used kava extract batch were not determined specifically. The individual kavapyrones were synthesized: The chemical analysis yielded a purity of 100 ± 0.3% determined by 1_H- and ^{13}C-NMR- spectra, HPLC and elemental analysis. Kretschmar and Teschendorf (1974) have reported that Piper methysticum extracts fluctuate within the following maximum and minimum levels D,L-kawain 19.5 to 23.8%, dihydrokawain 9.6 to 33.4%, methysticin 19.5 to 22.3%, dihydromethysticin 5.5 to 11.7%, yangonin 16.6 to 32.7% and desmethoxyyangonin 5.5 to 5.7%. This commercial extract contains in traces also flavokawin A and B, some pyrrolidine alkaloids, ketane, sterine, aliphatic alcohols and some organic acids. The relatively small proportion of these compounds could contribute to the kavapyrones effects.

Analysis of the Dialysate

The analysis of the dialysate were performed as described earlier (Sällström Baum et al 1996). The catecholamines were separated by the HPLC-ECD system using a Nova-Pak C18 column (4µm,150 x 3.9 mm ID; Waters/USA). The isocratic mobile phase (pH 3.2) consisted of 25 mM NaH_2PO_4, 0.81 mM octanesulfonic acid, 0.19 mM EDTA, and 12.3% methanol (vol/vol). The electrode was set at 350 mV. The detection limit for authentic dopamine and 5-HT was approximately 5 fmol injected directly onto the column.

Statistical Analysis

The data are expressed as percentages of the basal values (mean ± S.E.). The mean of the five samples collected prior to treatment was defined as 100 %. The course after treatment was analyzed using Friedman ANOVA with repeated measures. Pearson's correlation was used when appropriate.

Results

Control Conditions

The following mean basal levels were determined: dopamine 19.82 ± 0.56 fmol/50 µl, DOPAC 893.57 ± 4.84 fmol/50 µl and HVA 29.56 ± 0.47 pmol/50 µl (20 min dialysate (40µl) and addition 10 µl perchloric acid; n=16, mean ± S.E.). I.p. injection of Cremophor/0.9% NaCl (vehicle) did not change the mean levels of either neurotransmitter or the metabolites. The mean basal levels of extracellular 5-HT were 8.62 ± 0.262 fmol/50 µl perfusate (n=12; the levels were below the detection limit in four rats) and those of 5-HIAA 4.31 ± 0.035 mol/50 µl perfusate (mean ± S.E.).

Effect of the Kava Extract on Extraneuronal Levels of Dopamine, 5-HT and Their Metabolites

All administered doses of the kava extract (i.p.) changed the extracellular dopamine content in the n.acc. (20 mg/kg χ^2=40.8, p=0.024; 120 mg/kg χ^2=59.9, p=0.0001; 220 mg/kg χ^2=58.6, p=0.0002). Application of 20 or 120 mg/kg i.p. caused an increase of dopamine during the observation period. The highest dose given (220 mg/kg) resulted in a short decrease followed by an increase above baseline. Application of vehicle resulted in no significant change in dopamine levels (χ^2=9.9,p=0.9) (Fig1).

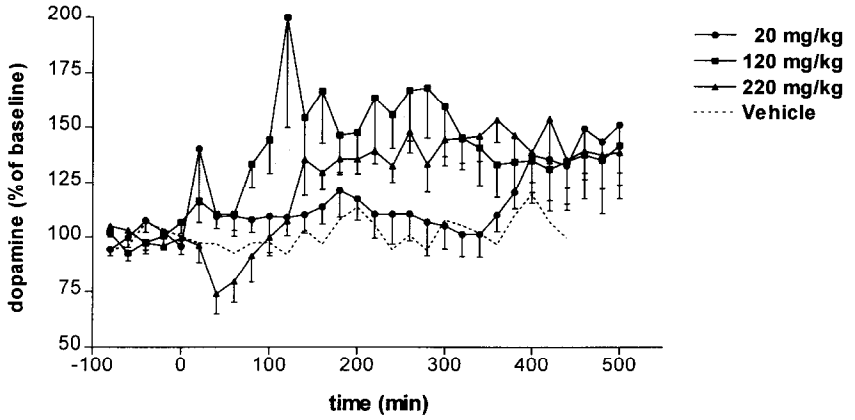

Fig 1. The effect of kava extract (20 mg/kg (n=9), 120 mg/kg (n=12) and 220 mg/kg (n=8)) administered i.p. on the extraneuronal concentration of dopamine in the n.acc. Mean of controls (n=8) is depicted as a dotted line. Results are presented as mean (%) of basal values -S.E.

The concentration of the dopamine metabolite DOPAC was not affected by doses below 220 mg/kg. When 220 mg/kg kava extract was administered an increase of up to several thousand % above baseline was found in some rats. Others showed no change (Fig 2).

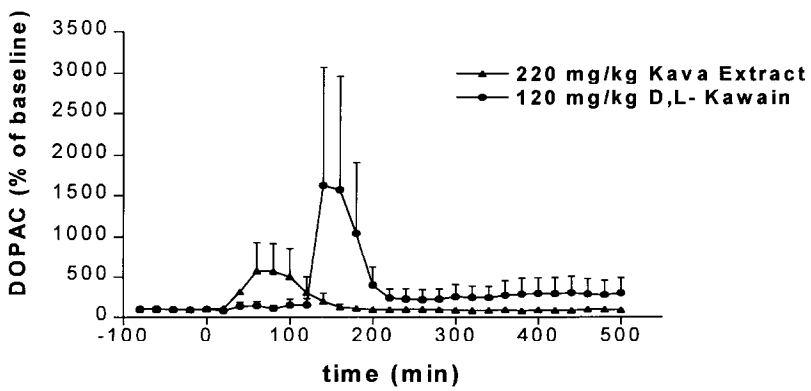

Fig 2. The effect of kava extract (220 mg/kg, n=8) and D,L-kawain (120 g/kg,n=6) on the extraneuronal concentration of DOPAC. Results are expressed as mean(%) of basal values +S.E.

Due to the large interindividual differences, no statistical significance could be determined (Friedman ANOVA). The correlation coefficient between dopamine and DOPAC was

r=-0.9631 ($p<0.05$) at 80 min after application. Application of vehicle resulted in no significant change in DOPAC levels (χ^2=10.3, p=0.9).

The concentration of HVA changed after as little as 20 mg/kg showing an increase of 20% (χ^2=37.7, p=0.04). Application of 120 mg/kg caused the HVA levels (χ^2=49.6, p=0.002) to fall by 20% with a subsequent 15% increase over baseline. The highest dose given did not significantly change the HVA levels. Application of vehicle resulted in no significant change in HVA levels (χ^2=17.1, p=0.7) (Fig 3).

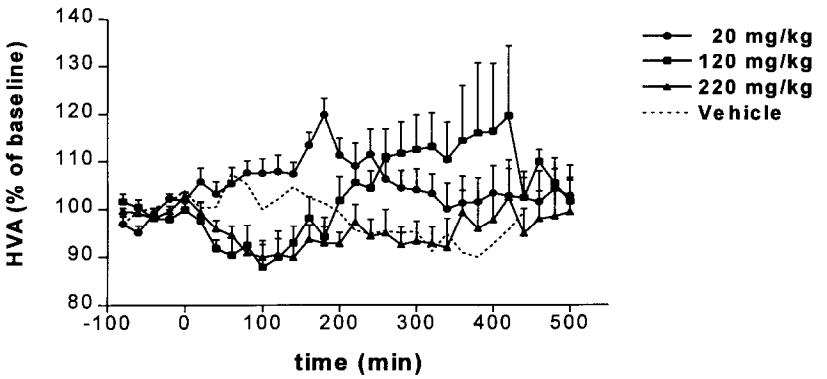

Fig 3. The effect of kava extract (20 mg/kg (n=9), 120 mg/kg (n=12) and 220 mg/kg (n=8)) on the extraneuronal concentration of HVA in the n.acc. Results are presented as mean (%) of basal values +S.E.

None of the applied doses of kava extract elicited a significant change in the extracellular concentrations of 5-HT or 5-HIAA. The reaction of the individual rats differed. About half of the rats showed an increase (p=0.035) and the other half no change of 5-HT. The 5-HT levels correlated with those of 5-HIAA between 200-240 minutes after application (200 min: r=0.8449, p=0.008; 220 min: r=0.8341, p=0.010; 240 min: r=0.7869, p=0.020). The two compounds did not change in control rats during the observation period (5-HT: χ^2=22.3, p=0.2; 5-HIAA:χ^2=17.5, p=0.7). A summary of the effects is compiled in Table 1.

Effects of the Individual Kavapyrones on the Extraneuronal Levels of Dopamine, DOPAC and HVA

D,L-kawain (30 mg/kg i.p.) induced a decrease in dopamine levels which lasted for more than 8 hours (χ^2=49.6, p=0.002), a change in DOPAC (χ^2=44.9, p=0.008), and no change in

HVA levels (Fig 4). Neither dopamine nor DOPAC concentrations were affected by a medium dose of 60 mg/kg, but HVA concentrations were reduced (χ^2=50.1, p=0.002). After a high dose of 120 mg/kg some rats showed a large increase of DOPAC above baseline (Fig 2). Neither dopamine, nor HVA were changed to a comparable high extent, although dopamine levels correlated with those of DOPAC at 100-120 min after application (r=-0.9603, P<0.05).

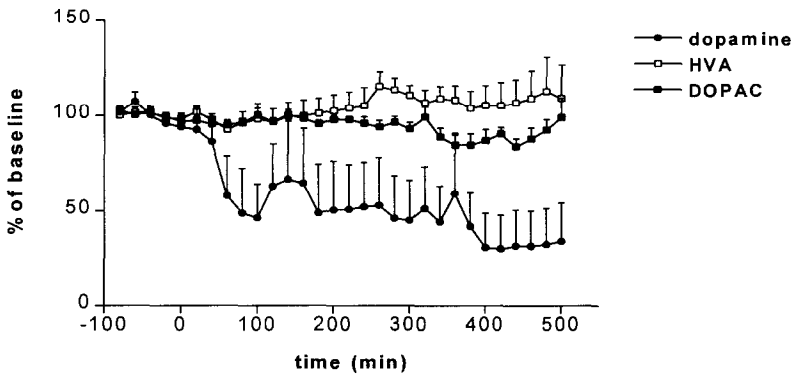

Fig 4. The effect of D,L-kawain (30 mg/kg,n=6) on the extraneuronal concentration of dopamine, DOPAC and HVA. Results are expressed as mean (%) of basal values +S.E.

Dihydrokawain (120 mg/kg i.p.) caused no significant changes in dopamine, DOPAC or HVA levels. However, interestingly the level of dopamine increased steadily. Methysticin (120 mg/kg i.p.) influenced neither the dopamine, DOPAC nor the HVA concentrations. Dihydromethysticin (120 mg/kg) failed to affect the dopamine, DOPAC or HVA levels.

Yangonin (120 mg/kg i.p.) reduced the dopamine levels (χ^2=43.2, p=0.013). After a blockade of the dopamine release between 100 and 120 min neuronal function showed a slight recovery, though after 420 min, dopamine levels were again reduced (Fig 5). A similar time course was observed following the administration of 220 mg/kg kava extract (Fig 1). Yangonin caused a nonsignificant decrease in DOPAC levels with a minimum between 300 and 400 min and the HVA levels showed a slight, but not significant fall (Fig 5).

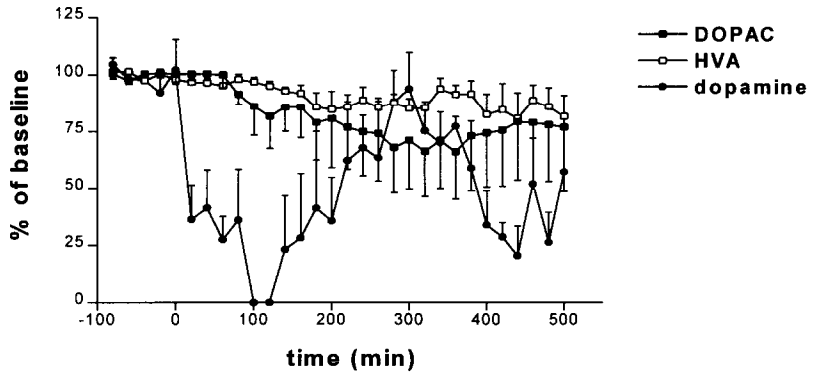

Fig 5. The effect of yangonin (120 mg/kg,n=5) on the extraneuronal concentration of dopamine, DOPAC and HVA. Results are expressed as mean (%) of basal values ±S.E.

Desmethoxyyangonin showed a totally different activity profile from yangonin. The dopamine concentrations rose steadily (χ^2=39.3,p=0.034). In contrast, DOPAC fell with a nonsignificant minimum between 300 and 360 min The HVA concentrations also fell (χ^2=114.6, p=0.000) (Fig 6). A summary of the effects is compiled in Table 1.

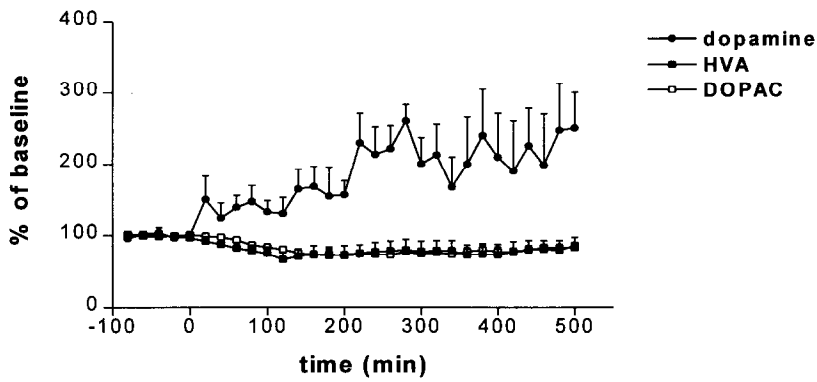

Fig 6. The effect of desmethoxyyangonin (120 mg/kg,n=8) on the extraneuronal concentrations of dopamine, DOPAC and HVA. Results are expressed as mean (%) of basal values +S.E.

Table 1

Effects of Kavapyrone Administration (120 mg/kg i.p.) on the Levels of Dopamine, DOPAC, HVA, 5-HT and 5-HIAA in the N. Acc. of Rats. ↑: Increased Levels [a], ↓: Decreased Levels [a], ns=no significant changes on the $p<0.05$ level

	Dopamine	DOPAC	HVA	5-HT	5-HIAA
Vehicle	ns	ns	ns	ns	ns
Kava extract	↑ (p = 0.00)	ns	↑ (p = 0.00)	ns	ns
D, L-Kawain	ns	ns	ns	↓ (p = 0.02)	ns
Dihydrokawain	ns	ns	ns	↑ (p = 0.04)	ns
Methysticin	ns	ns	ns	ns	ns
Dihydromethysticin	ns	ns	ns	ns	↓ (p = 0.00)
Yangonin	↓ (p = 0.01)	ns	ns	ns	↑ (p = 0.02)
Desmethoxyyangonin	↑ (p = 0.03)	ns	↓ (p = 0.00)	ns	ns

[a]Changes were calculated by using Friedman ANOVA (presented in parenthesis)

Effects of the Individual Kavapyrones on the Extraneuronal Levels of 5-HT and 5-HIAA Levels

A medium dose of D,L-kawain (60 mg/kg) induced a decrease in the 5-HT concentrations (χ^2=44.1,p=0.01) and in 5-HIAA concentrations (χ^2=39.5, p=0.03). A high dose of 120 mg/kg induced a decrease in the 5-HT concentrations (χ^2=40.4, p=0.02) with no significant effect on 5-HIAA levels (Fig 7).

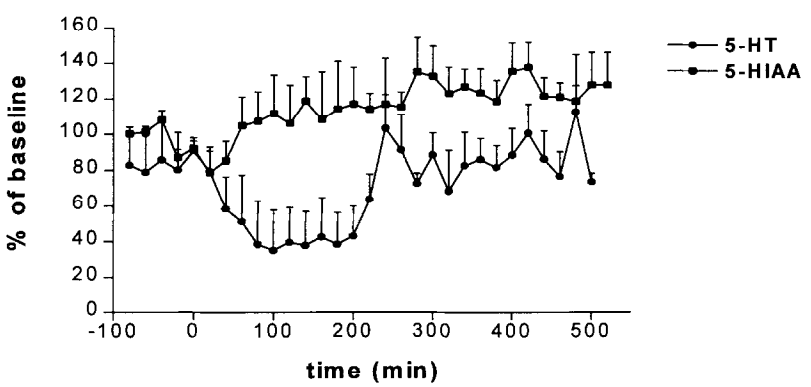

Fig 7. The effect of D,L - kawain (120 mg/kg,n=9) on the extra-neuronal concentrations of 5-HT and 5-HIAA. Results are expressed as mean (%) of basal values +S.E.

Dihydromethysticin led to a steady increase in 5-HT followed by reduced 5-HIAA levels (χ^2=56.7, p=0.000). In contrast, desmethoxyyangonin (120 mg/kg i.p.) showed a different

action profile. About half of the rats showed a prolonged reduction lasting for more than eight hours and the other half a slight rise in 5-HT concentrations. We then compared those rats with a reduction and those with an increase separately. Between 140 and 220 min, the two groups were significantly different ($P<0.05$). Pearson's correlation showed that when 5-HT increased then 5-HIAA decreased (80 min: $r=-0.9672$, $p=0.033$). However, when 5-HT decreased then 5-HIAA decreased as well (100 min:$r=0.9848$, $p=0.015$). Dihydrokawain caused a moderate increase in release of 5-HT ($\chi^2=37.7$, $p=0.04$) with a nonsignificant change of 5-HIAA. Yangonin reduced the levels of 5-HIAA ($\chi^2=41.2$, $p=0.02$) with no significant change of 5-HT. Methysticin altered neither the levels of 5-HT nor those of 5-HIAA. A summary of the effects is compiled in Table 1 for a dose of 120 mg/kg body weight as an example.

Behavioural Changes

During the experimental period general behaviour of the rats was observed. As little as 20 mg kava extract/kg body weight induced sedation. After 400 min, no behavioural differences from controls were detected. D,L-kawain caused muscle relaxation for 120 min and permanent sedation during the observation period. The fur was rough. Dihydrokawain raised the muscle tone (duration:100 min approximately). Methysticin caused sedation. Dihydromethysticin led to muscle relaxation and diarrhoea. No obvious change was found after yangonin while after desmethoxyyangonin muscle relaxation was seen for about 60 minutes.

Discussion

Methysticin, dihydromethysticin, kawain and dihydrokawain constitute the major compounds of kawa resin and are generally considered to be responsible for the pharmacological activity of kava in man and experimental animals (Shulgin 1973, Meyer 1979). In this study it could be shown that also minor components of the kava extract cause pharmacological effects.

Pharmacological Studies

The mechanism of action of the kavapyrones has not yet been established. The reduction of anxiety (Kinzler et al 1991, Volz et al 1997), the promotion of sleep (Kretschmar and Teschendorf 1974), the anticonvulsant effect (Meyer 1979, Gleitz et al 1996), and the muscle relaxation are reminiscent of the activity profile of the benzodiazepines. The anticonvulsant properties of kavapyrones have been compared also with those of procaine (Gleitz et al 1996). Recent experimental studies have shown that the kavapyrones do not interact with the $GABA_A$ receptor, with the binding site of the benzodiazepines, nor with the $GABA_B$ receptor (Davies et al 1992). Neurophysiological investigations have failed to reveal any benzodiazepine like effects. For example, in studies with event-related potentials in which kava extract led to an increase in word recall oxazepam and kava extract caused opposite effects (Munte et al 1993). Herberg (1993) found that the effects of ethanol (moderate doses) were not modified by concomitant kava ingestion. In a study by Jamieson and Duffield (1990) naloxon failed to block the analgetic effects of kava. The authors concluded that kava does not interact with opioid receptors. Interestingly, the neuroprotective effects of methysticin and dihydromethysticin have been found to be comparable in potency to memantine (Backhauss and Krieglstein 1992). Kawain, dihydrokawain and yangonin were ineffective.

Regarding other psychotropic effects, Holm et al (1991) demonstrated that kava extract does not affect cortical and cognitive mechanisms (consciousness). With both D,L-kawain (28 mg/kg p.o.) and kava extract (10-50 mg/kg i.p.) muscle tone was seen to be diminished in about 50% of the cats (Holm et al 1991). One problem with pharmacological studies on kavapyrones is their poor solubility in water. Hänsel et al (1994) describes that the lowest effective doses in a mobility test were 60 mg/kg body weight kawain and 90 mg/kg body weight dihydrokawain intraperitoneally administered. In contrast, the authors were able to demonstrate that as little as 20 mg/kg kava extract caused a significant change in extraneuronal concentrations of dopamine in the n. acc., possibly due to a different agent used for solubilization. The effects remained detectable for several hours, which could be due to the lipophilic properties of the active compound of the extract.

Effects of Kava Extract on Dopaminergic Mechanisms

The sensation of relaxation produced by kava extract could be due to the activation of the mesolimbic dopaminergic reward system. Stimulation of dopaminergic mechanisms in the

area postrema of the brain stem could cause the emetic effects described after higher doses. Interestingly, some rats reacted to the highest dose of the extract (220 mg/kg) with a decrease in dopamine levels which correlated with an increase in DOPAC concentrations, whereas the other rats reacted with an increase in dopamine and no change of DOPAC. No explanation for the interindividual differences in the reaction to the extract could be determined. Thus, the high dose represents a threshold dose to which the rats reacted with different sensitivity. The former effect suggests a reserpine-like action of the extract. Reserpine blocks ATP-Mg^{2+}-dependent reuptake of dopamine into the neuronal storage vesicles. Following the transport through the plasma membrane, some dopamine accumulates in the cytoplasm, where it is oxidized by mitochondrial monoamineoxidase yielding the aldehyde. The remaining portion is generally taken up by the storage vesicles. Blockade of the latter mechanism would explain why large amounts of DOPAC, which is formed by oxidation of the aldehyde, were excreted. In contrast to the effect of reserpine, the action of kava extract is reversible. The study with the individual compounds revealed that this effect could be caused by D,L-kawain. The fact that D,L-kawain acted with a delay compared to the extract could be explained by the observation that kawain as well as yangonin have a facilitated entry into the brain, when administered as constituent of an extract compared with the pure compound (Keledjian et al 1988). These authors found that dihydrokawain, methysticin and dihydromethysticin showed hardly any effect in the test system employed. Yangonin given as part of the extract reached 20 times higher levels in the brain than when given as individual compound. Yangonin is also slower eliminated than other pyrones(Keledjian et al 1988).

Effects of Yangonin on Dopaminergic Mechanisms

That the effect of the high dose of the kava extract on dopamine concentrations was weaker than that of 120 mg/kg body weight may have been due to yangonin, which alone caused a fall in dopamine levels (Fig 1 and 5). The threshold dose of the action of yangonin should probably be between 120 and 220 mg/kg of the kava extract which may explain that lower doses of the extract cause a relatively strong increase in the dopamine levels. Since the extraneuronal concentration of dopamine correlates with the activity of the neurones (Di Chiara 1990) the findings suggest that yangonin causes a decrease in the firing rate of the mesolimbic dopamine containing neurons. The fall in the dopamine concentrations following

administration of yangonin subsequently led to a delayed decrease in DOPAC and HVA levels (Fig 5).

A possible explanation for the reduction in dopamine by yangonin is that it stimulates glycinergic neurones, which in turn inhibit the firing rate of dopaminergic neurones. This effect is caused by specific glycine receptors. However, the strychnine antagonistic like action of yangonin (which is probably effective via glycinergic mechanisms) is weak (Kretschmar and Teschendorf 1974). One should also consider that methysticin has a much stronger antistrychnine effect than yangonin. Methysticin increases dopamine release and thus glycinergic mechanisms can not be responsible. The most important neurons stimulating dopaminergic actions in the ventral tegmentum are probably glutamatergic neurons. Inhibition of those neurons could explain the neuroprotective effect of methysticin. But in that test system yangonin had no significant effect (Backhauss and Krieglstein 1992). Thus, despite the strong action of yangonin on dopamine levels, previous reports provide no explanation about the underlying mechanism.

Effects of Kavapyrones on Serotonergic Mechanisms

Other findings of the present study were the changes of the 5-HT concentrations following administration of the kava extract or the individual pyrones. D,L-kawain led to a decrease, in some rats even to below the detection limit. Desmethoxyyangonin caused an increase in some rats and a decrease in others. Yangonin, dihydrokawain and methysticin elicited hardly any change.

The action of kava extract showed a remarkable variability. About half of the rats had increased levels, some no change and a third group decreased levels. The levels of 5-HIAA correlated in those rats with increased 5-HT positively and remained unaffected in the other rats. If the serotonergic neurones are also affected in other brain regions, this might contribute to the reported relaxant action of the kava extract. There are reports of a dense serotonergic innervation in the shell of the n. acc. and it contains abundant GABA-immunoreactive neurons. Van Bockstaele et al (1996) showed with immunogold-silver labeling that 5-HT containing axon terminals may postsynaptically inhibit GABAergic neurons and their targets within the shell of the rat n.acc. 5-HT multiply the inhibitory effect of dopamine on the spontaneously activity dopaminergic neurons in ventral tegmentum (Brodie et al 1996). A single dose of fluoxetine (a specific inhibitor of 5-HT reuptake which induce not

only anxiolysis but also antidepressant effects) induced an increase in 5-HT synthesis in dorsal hippocampus, ventral thalamus, hypothalamus and substantia nigra. In contrast, after chronic treatment a decrease of 5-HT was observed in substantia nigra, caudate and n. acc., which suggests that 5-HT has a delayed influence on the brain 5-HT synthesis rate in structures with serotonergic terminals (Muck et al 1996). It is interesting to note that individuals having drank kava beverage report the need for sleep only after a delay, and that it was preceeded by the relaxing effects.

Conclusions

The kava extract activates dopaminergic neurons with a ceiling effect probably due to an increasing contribution of yangonin which is a potent dopamine antagonist.This compound is present in low concentrations in the extract and could prevent the abuse of high doses of the extract if the extract has a potential for abuse due, for example, to the activation of the mesolimbic dopaminergic system. Because this specific compound reduces the activity of dopamine-containing neurons the ingestion of high doses will not further promote the euphoric actions.

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