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Article

Anxiolytic-like effects of Kava-Kava in the elevated plus maze test—a comparison with diazepam

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Abstract

Kava-Kava, a drug derived from a traditional psychoactive beverage used in the South Pacific, is known for tranquilizing and anxiolytic effects. Extracts made from the roots of the Kava plant (*Piper methysticum* G. Forster) have anxiolytic and mild sedative effects in man. To our knowledge, there are only few data concerning the efficacy of Kava-Kava in animal tests of anxiety. This study was carried out to compare the anxiolytic potential of Kava-Kava extract LI 150 with diazepam. Acute effects of diazepam and a Kava-Kava preparation, compared to their respective controls, were examined in Wistar rats using the elevated plus maze (X-maze). The time spent on open arms, the percentage of open-arm visits and parameters describing the risk assessment were evaluated. LI 150 (120–240 mg/kg po) affected the behaviour measured in the X-maze test, inducing an anxiolytic like behaviour similar to diazepam (15 mg/kg po). These data support the use of Kava-Kava in the treatment of anxiety.

Keywords: Anxiety; Anxiolytic; Diazepam; Kava-Kava extract LI 150; Rat; X-maze

1. Introduction

Pathological anxiety is one of the most common emotional disorders and treatment of phobias or panic attacks is still not trivial. Pharmacological treatment plays an important role in the therapeutic concept.

Benzodiazepines have been the most widely used anxiolytics in general practice for many years (Holm, 1988; Rang et al., 1995) and are relatively safe drugs for a short-term treatment of anxiety despite their drug dependence potential and side effects (Ballinger, 1990; Lader, 1999). The benzodiazepine diazepam is a standard anxiolytic and is also employed in behavioural pharmacology as the reference compound for an anxiolytic-like effect even if the screened drug is not acting via benzodiazepine receptors.

Nevertheless, there is considerable interest in the development of new anxiolytics. New synthesized compounds as well as drugs derived from traditional herbs may have a possible therapeutic relevance in the treatment of anxiety (Beaubrun and Gray, 2000). Especially, the use of "mild," "natural" and tolerable phytopharmaceuticals are in public favour for this purpose (Lake, 2000).

Kava-Kava, a beverage made from the roots of a bush in the tropical Pacific island region, is known for its tranquilizing and anxiolytic effects in man. Phytopharmacological preparations are made from the roots of the Kava plant *Piper methysticum* G. Forster. These extracts containing kavapyrones are supposed to have mild anxiolytic effects. In comparison to other anxiolytics, e.g. benzodiazepines, less of the adverse effects generally associated with these drugs were detected with Kava preparations (Pittler and Ernst, 2000). Although Kava-Kava has been characterised for its anxiolytic potential in clinical studies (Kinzler et al., 1991; Volz and Kieser, 1997; Boerner et al., 2000), there are, to our knowledge, only few data on the activity of Kava-Kava in animal tests of anxiety available (Smith et al., 2001).

It is well known that placebo effects can have an substantial effect on the outcome in clinical studies. However, testing the effects of Kava-Kava on anxiety-related behaviour in an animal test eliminates the risk of placebo-induced effects. Hence, the animal test seems to be a more

Abbreviations: CE, closed-arm entries; c-returns, returning to the protecting closed arms; SAP, stretch attended posture; TE, total arm entries; X-maze, elevated plus maze

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objective tool to assess the anxiolytic potential of a drug. Additionally, it is not known exactly which compounds of the Kava-Kava extract are pharmacologically active. An evaluation of the behavioural effects of multiple compounds can be performed more easily in animal tests than in multiple clinical studies. To our knowledge, the mechanisms underlying the actions of Kava-Kava seem to affect serotonergic, dopaminergic and glutaminergic neurotransmission (Malsch and Kieser, 2001). To determine the exact neurobiological mechanism behind Kava-Kava anxiolytic effects in more detail, it is also convenient to use animals. For this reason, the Kava-Kava extract LI 150 was compared with the standard anxiolytic diazepam in an animal test for anxiety not requiring punishment (Green, 1991; Lader, 1991; Stephens and Andrews, 1991).

The X-maze, based on the Y-maze (Montgomery, 1955), is a well-established test for anxiety-like behaviours that has been used in rats (Handley and Mithani, 1984; Pellow et al., 1985), mice (Lister, 1987) and in guinea pigs (Rex et al., 1993). Additionally, the X-maze test is now the most popular and widely used animal test for anxiety and results obtained can be compared with the literature or rated more easily (e.g. Hogg, 1996).

Aim of the study was the determination of acute Kava-Kava-induced effects on anxiety-related behaviour in rats.

2. Material and methods

2.1. Animals

Male Wistar rats (Shoe: Wist, Tierzucht Schönwalde, Germany) of 170–230-g body weight were used. They were group housed, five per cage ($45 \times 60 \times 25$ cm), at room temperature (22 ± 2 °C) and with a 12-h light/dark cycle (light on at 06:00 h) illuminated with 170 lx. Standard pellet food (Altromin 1326) and water were freely available at all times. To ensure adaptation to the new environment, the rats were housed in the departmental holding room for 2 weeks before testing. The rats were assigned randomly to the procedure. The tests were performed in a sound-proof, brightly illuminated room between 08:30 and 11:00 h.

2.2. Drugs

The following drugs were used: diazepam (AWD, Dresden, Germany) and Kava-Kava preparation LI 150, an 96% ethanol extract of *P. methysticum* roots, resulting in a dry powder containing 30% kavapyrones (Lichtwer Pharma, Germany) or specific vehicle [diazepam: 0.9% NaCl containing ethanol (186 mg/10 ml) and Macrogol 400; Kava-Kava: 0.9% NaCl containing sodium carboxymethyl-amylopektin (UAP, Laborchemie Apolda, Germany]. The animals received either diazepam (15 mg/kg), LI 150 (adjusted to 120, 180 and 240 mg/kg kavapyrone) or their respective vehicle 60 min prior testing. All drugs were suspended ultrasonically in vehicle immediately prior to use. The drugs were given orally in a volume of 10 ml/kg.

2.3. Apparatus

The behavioural experiments were performed using an elevated plus maze (X-maze) illuminated with 210 lx on the open arms, 190 lx in the centre and 160 lx in the closed arms. The X-maze was 64 cm high with four arms (44×17 cm), with a wall on two opposite arms (height 25 cm).

2.4. Experimental procedure

The animals were placed in the middle of the X-maze facing a corner of the centre platform (equal choice of entering an open or closed arm) 60 min after receiving vehicle, LI 150 or diazepam. The experiments were performed for 5 min (Pellow et al., 1985; Rodgers and Cole, 1994). The animals were observed using a real-time video system and the behaviour was assessed using a computer-aided system (VideoTrack V.2.16, CPL Systems, UK). A computerized measurement of the distance travelled was included. In every group, 10 animals were examined.

The behavioural parameters measured were entries into the open arms in percent of the total entries into all arms, time spent on the open arms, number of stretch attended postures (SAP), the number of head dips and the number of returning to the protecting closed arms (c-returns) as measures of anxiety-related activity and the total number of entries into all arms, the number of entries into the closed arms and the distance travelled as measures of locomotor activity. All performed procedures and experiments were approved by the Berlin Senate's Animal Protection Board "Landesamt für Arbeitsschutz, Gesundheitsschutz und technische Sicherheit Berlin, Fachgruppe Veterinärwesen, Lebensmittelwesen und Gentechnik."

2.5. Statistical analysis

The diazepam data were analysed using Student's t test (one treatment versus control) and the Kava-Kava data were analysed using one-way ANOVA followed by Dunnett's test (three treatments versus control). The data are expressed as mean ± S.E.M. A difference of the means of $P < .05$ was considered as statistically significant.

3. Results

3.1. Diazepam

The vehicle-treated control animals spent 25.3 ± 5.1 s on the open arms. Approximately 16.1 ± 3.2% of all entries into the arms of the X-maze took place into the aversive open arms. The animals of the control group looked 7.3 ± 1.1

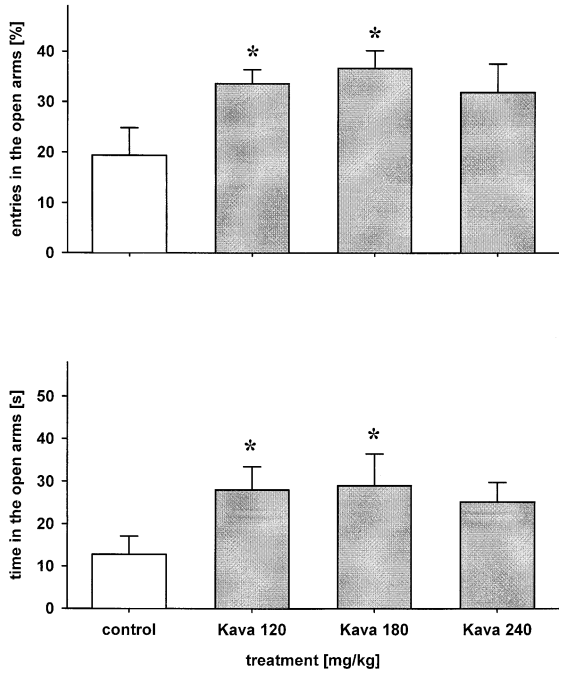

Fig. 1. LI 150 (120, 180 and 240 mg/kg po; $n=10$) induced an "anxiolytic" behaviour on exposure to the X-maze with increased entries into the open arms and more time spent on the open arms compared to the controls ($n=10$). * $P<.05$, one-way ANOVA followed by Dunnett's test. Data presented as means ± S.E.M.

times over the edge of the open arms downward (head dips) and they stretched themselves for 2.8 ± 0.5 times from the protected and closed arms into the open, aversive arms (SAP). The control animals returned 5.0 ± 0.7 times to the protecting closed arms without entering the aversive open arms (c-returns).

After the application of 15-mg/kg po diazepam, the animals spent more time (55.7 ± 15.5 s) on the open arms. The percentage of entries into the open arms increased (44.0 ± 6.7%). The number of head dips rose to 11.9 ± 2.7. The animals seemed prepared to take more risks as measured by a decreased number of the c-returns (2.8 ± 0.8) and the SAP (1.8 ± 0.3).

In a preliminary dose-finding study, lower diazepam doses, which were effective in previous experiments after intraperitoneal administration (e.g. Rex et al., 1996), did not induce an anxiolytic-like behaviour on the X-maze following oral administration. Higher doses (20 mg/kg) had only a sedating effect.

The locomotor activity was not significantly changed by diazepam [12.8 ± 1.8 m, 7.37 ± 1.12 closed-arm entries (CE), 13.17 ± 2.01 total arm entries (TE)] in comparison to the control group (9.4 ± 1.9 m, 8.52 ± 1.01 CE, 10.74 ± 1.24 TE).

3.2. Kava-Kava

The Kava-Kava controls treated with UAP spent 12.8 ± 4.3 s on the aversive open arms and 19.4 ± 5.4% of all entries were directed into the aversive open arms (Fig. 1). The animals of the UAP control group looked 5.6 ± 1.2 times downward over the edge of the open arms (head dips). These animals stretched themselves for 2.7 ± 0.4 times from the closed arms into the open arms (SAP) and they returned 7.0 ± 1.1 times to the protecting closed arms without entering the aversive open arms (c-returns) (Fig. 2).

After the application of the Kava-Kava preparation LI 150, the proportion of the entrances into the open arms rose following 120 mg/kg and after 180 mg/kg [$F(3,36)=2.887$, $P<.05$]. With the highest dose of 240 mg/kg, the proportion of the entrances into the open arms was increased but not significantly (Fig. 1).

The time, which the animals spent on the open arms, doubled after treatment with Kava-Kava in all three doses [$F(3,36)=4.057$, $P<.05$] compared to the controls (Fig. 1). The animals treated with LI 150 seemed to take more risks on exposure to the X-maze, detectable by the increased number of head dips from the open arms after 120 and 180 mg/kg [$F(3,36)=3.974$, $P<.05$], compared with the controls (Fig. 2). The number of c-returns was decreased following treatment with the Kava-Kava extract of 180 and 240 mg/kg [$F(3,36)=4.564$, $P<.01$] compared to the controls. The frequency of the SAP in the Kava-Kava-treated animals did not differ from the SAP frequency in the control group (Fig. 2).

The locomotor activity was not significantly changed by LI 150 (120 mg/kg: 10.3 ± 0.9 m, 7.76 ± 0.65 CE, 11.78 ± 0.90

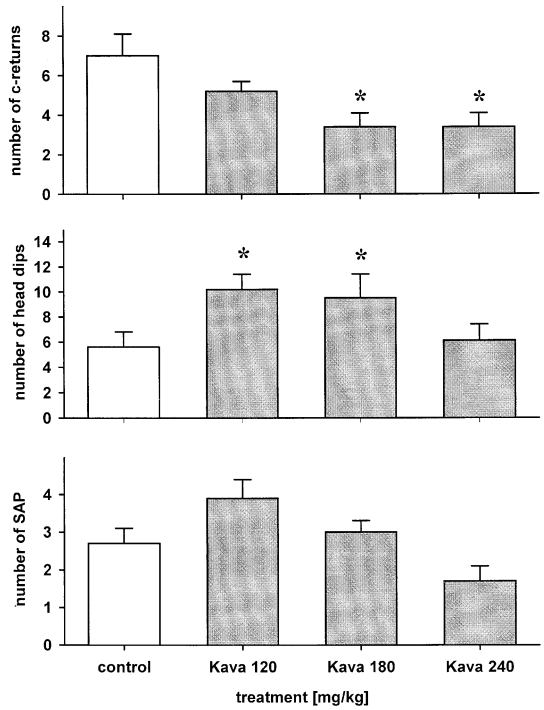

Fig. 2. Effects of LI 150 (120, 180 and 240 mg/kg po; $n=10$) on the number of c-returns, head dips and the SAP during exposure to the X-maze compared to vehicle-treated controls ($n=10$). * $P<.05$, one-way ANOVA followed by Dunnett's test. Data are presented as means ± S.E.M.

TE; 180 mg/kg: 9.6 ± 1.1 m, 6.38 ± 0.79 CE, 10.11 ± 1.26 TE; 240 mg/kg: 8.5 ± 1.1 m, 6.72 ± 0.91 CE, 9.78 ± 1.32 TE) in comparison to their control group (7.8 ± 0.7 m, 7.35 ± 0.80 CE, 9.19 ± 0.99 TE).

4. Discussion

In the therapy of pathological anxiety, a combination of therapeutic interventions is mostly indicated. Beside a psychotherapeutic approach, anxiolytics are a mandatory part of the treatment of anxiety.

There is a variety of animal tests for the investigation of ''anxiolytic'' or ''anxiogenic'' effects of substances (Stephens and Andrews, 1991). The X-maze is a well-established animal test causing a fear status by comprehensible stimuli and the use of innate behaviours of the animals, is one of the most widely used models to assess anxiety in small rodents (Hogg, 1996) and is a validated and reliable test for detecting both anxiolytic- and anxiogenic-like effects of agents (Pellow and File, 1986; Rodgers and Cole, 1994).

In this animal model, an anxiolytic- or anxiogenic-like effect is evaluated by the relation of entries into the open arms to the total entries and the time spent on the open arms of the X-maze in comparison to the same parameters of the control group (Pellow et al., 1985). An increase of the time and the proportion of the entrances into the open arms without a changed locomotor activity is regarded as a powerful marker for an ''anxiolytic'' substance effect (Pellow et al., 1985).

Additional measurements for anxiety-related behaviour are parameters describing the risk assessment of the animals as exploring only the nonaversive closed arms, the stretching of the animals in the aversive open arms without leaving the closed arms completely and the vertical exploration on the open arms by head dips (Rodgers and Cole, 1994). These risk-assessing parameters are supposed to provide more information about the drug effects. An increased downward exploration indicated by increasing number of head dips and a reduction of the parameters for a risk assessment like stretching into the open arms or the returns into the protecting closed arms are further and supporting indicators for a fear-reducing substance effect.

The slight, but not significant, diversity in the behaviour of the two control groups were probably caused by the different treatment-specific vehicles.

Diazepam is used as a standard anxiolytic and has been frequently employed in behavioural pharmacology as a reference compound to potentially anxiolytic-acting substances (Taukulis and Goggin, 1990; Guimaraes et al., 1990; Wright et al., 1992).

In the present study, a single acute administration of diazepam led to an ''anxiolytic'' behaviour. Compared to the controls, diazepam increased the numbers of entrances into the open arms and prolonged the stay on these arms in addition to a reduction of the parameters for a risk assessment. The present results confirm previous findings in our laboratory (Rex et al., 1996) and a number of reports of others (e.g. Pellow et al., 1985; Dalvi and Rodgers, 1999; Fernandes et al., 1999).

Diazepam is effective in a multiplicity of fear tests, apart from the X-maze test, as in the black and white box test (Merlo-Pich and Samanin, 1989), the social interaction test (Costall et al., 1988) and defensive burying attempt (Rohmer et al., 1990).

In contrast to previous studies, the effective diazepam dose used in this study seems very high. However, in a preliminary study, we found that lower doses of diazepam did not cause significant changes in behaviour on exposure to the X-maze when given per os.

The relatively high concentration of diazepam necessary to induce an ''anxiolytic'' behaviour can be caused by the oral application. Diazepam has an extensive first-pass effect in the rat, which leads to less active metabolites (Löscher and Frey, 1981) accented by the high liver capacity and the relatively slow absorption from the intestine.

Additionally, the oral administration seems to cause a kind of arousal, which would interfere with a possible aversion-reducing activity of diazepam. The control animals receiving their drug per os showed in general a higher locomotor activity and seemed to react more ''anxious'' and the diazepam-induced anxiolytic effect was lower compared to animals receiving the treatment intraperitoneally (Rex et al., 1996, 1998).

The Kava-Kava extract LI 150 given acutely led in the examined doses to a clear ''anxiolytic'' effect during the stay on the X-maze. The middle dose of 180 mg/kg induced the most marked effects and led to a rise in the classic anxiety-related behavioural parameters. Following administration of LI 150, the animals seemed to be prepared to take more risks. An increased readiness to take risks is regarded as a characterization of reduced fear (Rodgers and Johnson, 1995). Kava-Kava induced an inverted U-shape dose–response curve, which is not unusual for anxiolytics (Rex et al., 1996, 1998). The descending limb of the inverted ''U'' may be attributable to other effects of the anxiolytics interfering with the expression of the behaviour used to measure anxiolytic effects (Jones et al., 1994).

In clinical studies, Kava-Kava extract was found to be superior to placebo and effectively relieving anxiety (Kinzler et al., 1991; Volz and Kieser, 1997; Woelk et al., 1993; Boerner et al., 2000). However, to our knowledge, there is no published material available on the ''anxiolytic'' effects of the substance in experimental animal studies.

In our study, Kava-Kava showed an acute ''anxiolytic'' effect, although Kava preparations showed their anxiolytic properties in therapeutical studies in humans after 2–4 weeks of therapy duration (Volz and Kieser, 1997). Acute clinical effects of Kava-Kava were also shown already 120 min after drug administration through qualitative EEG analysis (Schulz et al., 1998).

Often, anxiolytics like the serotonin 1A agonists show anxiolytic effects in man only after chronic treatment. However, an anxiolytic-like effect in rats is also visible following a single administration of the drugs (Chopin and Briley, 1987; Griebel, 1995; Rex et al., 1998).

5. Conclusions

This study support the usefulness of animal models of anxiety to predict the therapeutic value in the treatment of anxiety in humans.

Our study shows that the Kava-Kava extract LI 150 had marked effects on the anxiety-related behavioural parameters on exposure to the X-maze in rats. LI 150 causes an ''anxiolytic'' behaviour comparable with the effects of diazepam. Future work will be focused on the neurobiological mechanisms of action and possible interactions of Kava-Kava with classical neurotransmitters.

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