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Extract of kava (*Piper methysticum*) and its methysticin constituents protect brain tissue against ischemic damage in rodents

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The purpose of the present study was to test whether kava extract and its constituents kawain, dihydrokawain, methysticin, dihydromethysticin and yangonin provide protection against ischemic brain damage. To this end, we used a model of focal cerebral ischemia in mice and rats. Ischemia was induced by microbipolar coagulation of the left middle cerebral artery (MCA). To quantify the size of the lesion in mice, the area of the infarct on the brain surface was assessed planimetrically 48 h after MCA occlusion by transcardial perfusion of carbon black. In the rat model infarct volume was determined 48 h after MCA occlusion by planimetric analysis and subsequent integration of the infarct areas on serial coronal slices. Compounds were administered i.p., except the kava extract, which was administered orally. The effects of the kava extract and its constituents were compared with those produced by the typical anticonvulsant, memantine. The kava extract, methysticin and dihydromethysticin produced effects similar to those of the reference substance memantine. The kava extract (150 mg/kg, 1 h before ischemia) diminished the infarct area ($P < 0.05$) in mouse brains and the infarct volume ($P < 0.05$) in rat brains. Methysticin, dihydromethysticin (both 10 and 30 mg/kg, 15 min before ischemia) and memantine (20 mg/kg, 30 min before ischemia) significantly reduced the infarct area in mouse brains. All other compounds failed to produce a beneficial effect on the infarct area in mouse brains. In conclusion, the kava extract exhibited neuroprotective activity, which was probably mediated by its constituents methysticin and dihydromethysticin.

Kava extract; Methysticin; Memantine; Middle cerebral artery occlusion; (Rat); (Mouse)

1. Introduction

Neuronal hyperexcitation in the course of cerebral ischemia (Suzuki et al., 1983) caused by excessive release of the excitatory amino acids L-glutamate and L-aspartate (Benveniste et al., 1984) may lead to neuronal necrosis (Nadler et al., 1981). Various studies indicate that compounds capable of blocking neuronal stimulation protect brain tissue against damage (Meldrum et al., 1985; Choi et al., 1987; Goldberg et al., 1988; Bode-Greuel et al., 1990). Thus, anticonvulsants such as the non-competitive NMDA antagonist dizocilpine (Meldrum, 1986; Wong et al., 1986), memantine (Kornhuber et al., 1989) and phencyclidine (Hayes and Balster, 1985) reduce brain injury caused by cerebral ischemia (Sauer et al., 1988; Seif el Nasr et al., 1990; Backhauß et al., 1992). Kava extract obtained from the roots of *Piper methysticum* Forst. contains α-pyrone constituents such as methysticin, dihydromethysticin, kawain, dihydrokawain and yangonin which have been reported to possess anticonvulsive properties (Meyer, 1964; Meyer and Meyer-Burg, 1964; Kretzschmar and Meyer, 1965, 1969). We therefore attempted to find out whether kava extract and its α-pyrone constituents were able to reduce infarct size in mouse and rat models of focal cerebral ischemia. In addition, we compared the effects of these drugs with those produced by the anticonvulsant memantine in the mouse middle cerebral artery (MCA) occlusion model.

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2. Materials and methods

2.1. Animals

Male NMRI mice (Savo, Kissleg, F.R.G.) weighing 30–40 g and male Fischer 344 rats (Charles River Wiga, Sulzfeld, F.R.G.) weighing 250–300 g were used in the experiments. The animals had free access to food (Altromin, Lage, F.R.G.) and water and were kept under standardized environmental conditions (12

h light/dark cycle, 23 ± 1°C and 55 ± 5% relative humidity). There were no significant differences between the mean body weight of treated and untreated animals.

2.2. Drugs and drug treatment

The following compounds obtained from Dr. Willmar Schwabe (Karlsruhe, F.R.G.) were tested: kava extract (WS 1490, containing 70% kava lactones), kawain, dihydrokawain, methysticin, dihydromethysticin and yangonin. Memantine was obtained from Merz (Frankfurt, F.R.G.). Kava extract was titrated with a mixture of polyethyleneglycol 400 and water (20:80) to give a milky emulsion which was administered orally (p.o.). The other compounds were dissolved in neutral oil or saline (memantine) and injected i.p. Controls received vehicle alone.

2.3. Physiological variables

Arterial $PaCO_2$, PaO_2, pH (Corning 178, Corning Medical, Giessen, F.R.G.), and plasma glucose (Beckman Glucose Analyser II, Munich, F.R.G.) were measured in blood samples of 40 μl. Mean arterial blood pressure (MABP) was measured with a Statham P23DB transducer (Heto Rey, Puerto Rico, Recomed, Hellige, Freiburg, F.R.G.).

2.4. Surgical procedures and induction of ischemia

MCA occlusion in mice was performed as described by Welsh et al. (1987). Animals were anesthetized with tribromoethanol (600 mg/kg i.p.) and after a skin incision had been made between the left ear and the orbit the parotid gland and the temporalis muscle were removed by electrical coagulation (Erbotom, Erbe, Tübingen, F.R.G.). A small bore hole was drilled on the temporolateral surface of the skull (Proxxon 28500/N, Proxxon, Niersbach, F.R.G.) and the stem of the left MCA was occluded by microbipolar coagulation. Body temperature was maintained at 37 ± 1°C with a heating lamp during preparation of the animals. After the surgical procedure the animals were kept at an environmental temperature of 30°C for 120 min. After 48 h mice were perfused transcardially with carbon black (0.5 ml) and the brains were removed and stored for at least 24 h in phosphate buffer (pH 7.4) with 4% formalin. The unstained infarct area on the brain surface was determined planimetrically by means of an image analyzing system (IBAS 2, Kontron, Eching, F.R.G.) according to Backhauß et al. (1992).

Occlusion of the left MCA in rats was performed under light halothane/nitrous oxide anesthesia according to Tamura et al. (1981). Briefly, after a skin incision between orbit and ear, the temporalis muscle was divided and partly removed. After subtemporal craniectomy beside the foramen ovale, the MCA and its lenticulostriate branches were occluded by microbipolar coagulation. Body temperature was maintained at 37 ± 1°C, as in the mouse procedure. After 48 h rats were perfusion-fixed with phosphate buffer (pH 7.4) containing 4% formalin. Brains were removed, embedded in Paraplast (Monoject Scientific, Kildare, Ireland) and sliced in coronal sections every 0.5 mm. The slices were stained with cresyl violet, and infarct volume was determined by planimetric analysis and subsequent integration of the infarct areas on serial coronal slices. The cortical, striatal and total infarct volumes were determined separately.

2.5. Statistics

All values are presented as means ± S.D. Multiple comparisons were made with the Kruskal–Wallis H-test combined with the Duncan's test. Single comparisons were made with the Mann–Whitney U-test.

3. Results

After MCA occlusion neither controls nor drug-treated animals exhibited convulsions. Kava extract

TABLE 1

Physiological variables of controls and kava extract-treated rats. Values were determined 1 h after drug administration (p.o.). The values are given as means ± S.D. of n experiments.

	Control n = 5	Kava extract (150 mg/kg) n = 5
PaO_2 (mm Hg)	123.4 ± 19.4	118.8 ± 17.5
$PaCO_2$ (mm Hg)	49.2 ± 3.2	49.8 ± 2.8
Arterial pH	7.43 ± 0.008	7.44 ± 0.02
Plasma glucose (mg/100 ml)	180.6 ± 8.8	171 ± 19.4
MABP (mm Hg)	109 ± 10.3	111 ± 10.8
Temperature (°C)	37.9 ± 0.46	37.3 ± 0.98
Body weight (g)	289 ± 11.4	295 ± 7.1

TABLE 2

Physiological variables of controls and methysticin-treated rats. Values were determined 15 min after drug administration (i.p.). The values are given as means ± S.D. of n experiments.

	Control n = 5	Methysticin (10 mg/kg) n = 5	Methysticin (30 mg/kg) n = 5
PaO_2 (mm Hg)	119.4 ± 12.5	111.6 ± 12.4	116.3 ± 9.9
$PaCO_2$ (mm Hg)	44.6 ± 2.6	45.9 ± 2.7	46.1 ± 1.4
Arterial pH	7.41 ± 0.044	7.40 ± 0.038	7.41 ± 0.022
Plasma glucose (mg/100 ml)	172 ± 9.3	171.2 ± 6.1	175.4 ± 13.7
MABP (mm Hg)	124.4 ± 13	121.4 ± 9.7	121 ± 6.5
Temperature (°C)	36.7 ± 0.3	36.5 ± 0.3	36.9 ± 0.2
Body weight (g)	291 ± 8.9	295 ± 7.1	293 ± 9.7

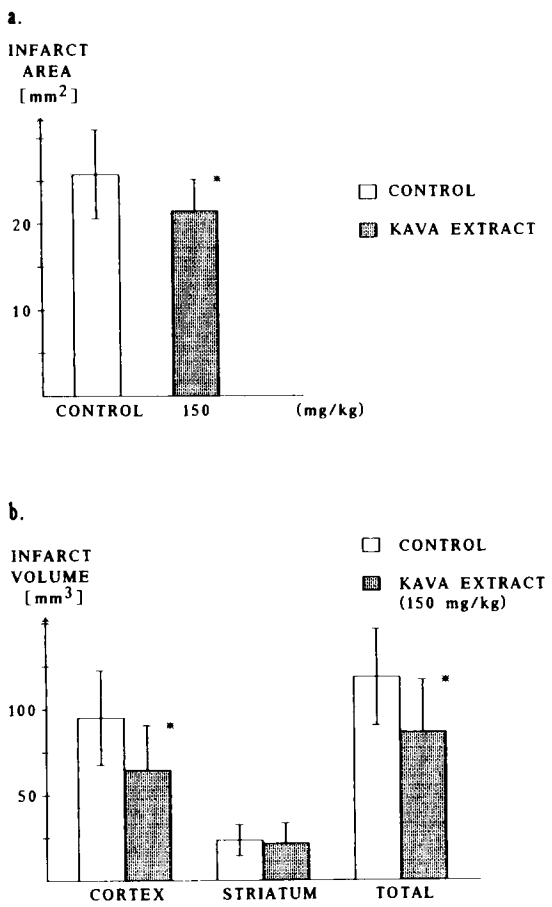

Fig. 1. Reduction of infarct size by kava extract. Ischemia was induced by MCA occlusion. After 48 h the infarct area was determined planimetrically on the mouse brain surface and the infarct volume was assessed in rat brains by planimetric analysis and subsequent integration of the infarct areas on serial coronal slices. Kava extract was administered p.o. 1 h before ischemia. The values are given as means ± S.D. of (a) 10 (controls and kava extract), (b) 9 (controls) and 10 (kava extract) experiments. Mann–Whitney U-test: * $P < 0.05$.

TABLE 3

Kawain, dihydrokawain and yangonin failed to diminish infarct area on the mouse brain surface. Ischemia was induced by microbipolar occlusion of the left MCA. After 48 h, infarct area was determined planimetrically. Kawain, dihydrokawain (both 15 min before ischemia) and yangonin (30 min before ischemia) were administered i.p. The values are given as means ± S.D. of n experiments.

Drug	Dose (mg/kg)	Infarct area on the brain surface (mm^2)	n
Control	0	23.8 ± 3.2	9
Kawain	30	20.9 ± 3.9	10
Kawain	60	24.8 ± 3.0	10
Control	0	26.1 ± 3.1	10
Dihydrokawain	30	22.2 ± 3.6	9
Dihydrokawain	60	25.9 ± 4.9	10
Control	0	21.7 ± 2.9	10
Yangonin	70	21.5 ± 3.9	10
Yangonin	140	22.5 ± 4.9	10

and methysticin did not alter the physiological parameters of the rats (tables 1 and 2). In the rat model of focal ischemia kava extract (150 mg/kg p.o., 1 h before ischemia) significantly diminished cortical and total infarct volume without affecting striatal infarct volume (fig. 1b). The body weight of the rats (means ± S.D.) was 288.8 ± 9.9 g (controls) and 290.2 ± 10.2 g (kava extract-treated). Similar results were obtained from experiments with mice with MCA occlusions. Kava extract (150 mg/kg p.o., 1 h before ischemia) significantly reduced the infarct area on the brain surface (fig. 1a), as did methysticin and dihydromethysticin (both 10 and 30 mg/kg i.p., 15 min before ischemia) (fig. 2a, b). Kawain, dihydrokawain (both 30 and 70 mg/kg i.p., 15 min before ischemia) and yangonin failed to show a beneficial effect on the mouse model (table 3). Yangonin permeates slowly and in small amounts into brain tissue (Keledjian et al., 1988). It was therefore administered 30 min before ischemia (i.p.) in dosages of 70 and 140 mg/kg. Memantine (20

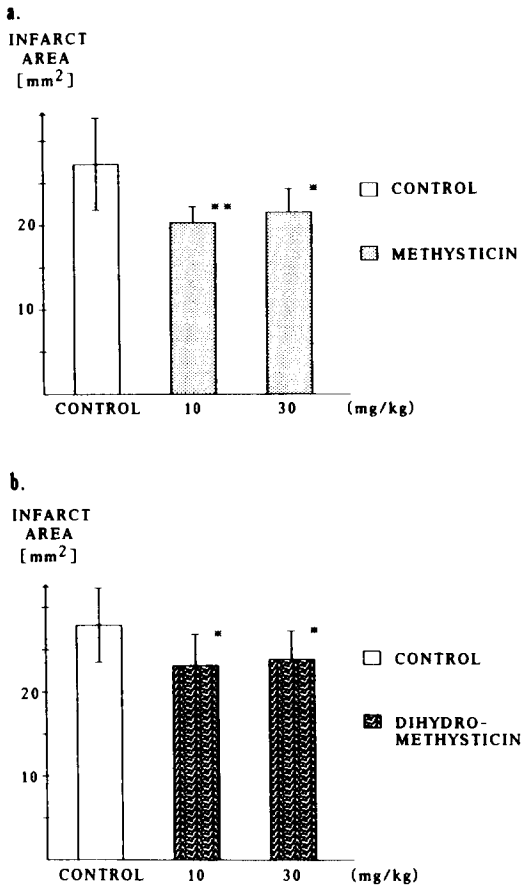

Fig. 2. Reduction of infarct area by methysticin (a) and dihydromethysticin (b). The infarct area on the mouse brain surface was determined planimetrically 48 h after MCA occlusion. The compounds were administered i.p. 15 min before MCA occlusion. Values are shown as means ± S.D. from (a) 9 (controls) and 10 (methysticin), (b) 10 (controls and dihydromethysticin) experiments. Kruskal–Wallis H-test: * $P < 0.05$, ** $P < 0.01$.

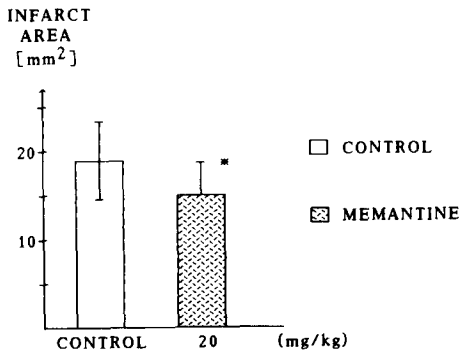

Fig. 3. Reduction of infarct area by memantine. Ischemia was induced by MCA occlusion. After 48 h the infarct area on the mouse brain surface was assessed planimetrically. The drug was administered i.p. 30 min before ischemia. The values are presented as means ± S.D. of 12 (controls) and 13 (memantine 20 mg/kg) experiments. Mann-Whitney U-test: * $P < 0.05$.

mg/kg i.p., 30 min before ischemia) significantly reduced the infarct area in mouse brains (fig. 3).

4. Discussion

Various central effects of kava extract and its constituents have already been demonstrated (Furgiuele et al., 1965; Meyer and Kretzschmar, 1966; Kretschmer, 1970). Our interest was especially attracted to the anticonvulsive effects (Meyer, 1964; Meyer and Meyer-Burg, 1964; Kretzschmar and Meyer, 1965; 1969) because anticonvulsant drugs could also act as neuroprotective agents. Various anticonvulsant drugs such as dizocilpine (Meldrum, 1986; Wong et al., 1986), memantine (Kornhuber et al., 1989) and phencyclidine (Hayes and Balster, 1985) have already been shown to protect brain tissue against ischemic damage (Sauer et al., 1988; Seif el Nasr et al., 1990). Meldrum and Swan (1989) pointed out that there may be a relationship between anticonvulsive and neuroprotective activity.

It thus seemed reasonable to test the kava extract and some of its constituents for their putative neuroprotective potency. Using two models of focal ischemia, we found out that the kava extract and at least two of its α-pyrone constituents, methysticin and dihydromethysticin, were indeed capable of reducing the infarct size in mouse and rat brain after MCA occlusion. In comparison to these effects, the anticonvulsant memantine, which was found by Seif el Nasr et al. (1990) to be neuroprotective, was also protective and reduced the size of the brain lesion after MCA occlusion in mice. The reduction of infarct area caused by kawain and dihydrokawain was not statistically significant, and yangonin did not seem to be active at all in the mouse model used. The pharmacokinetic properties of kawain and dihydrokawain could be responsible for their ineffectiveness. These agents have only a short-lasting anticonvulsive effect (Kretzschmar and Meyer, 1969), and it may be argued that the short half-life of these compounds in rodents hampers a possible neuroprotective effect. Methysticin and dihydromethysticin have been described as the most active anticonvulsive constituents of the kava extract (Kretzschmar and Meyer, 1969). In addition, the anticonvulsive effects of the agents were observed in the same dosage range (up to 30 mg/kg) as the protective effects against ischemic brain damage, thereby showing a relationship between the anticonvulsive and neuroprotective activity of these agents.

In summary, the neuroprotective effect of kava extract was observed in two models of focal ischemia. Similar effects were found for methysticin and dihydromethysticin. These results suggest that the neuroprotective effect of the kava extract is mainly mediated by its methysticin constituents. Further experiments are necessary to clarify the mechanism of the neuroprotective action of these compounds.

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