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EFFECTS OF KAVA ON NEUROMUSCULAR TRANSMISSION AND MUSCLE CONTRACTILITY

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Summary

The effects of kava, a native drink from Oceania, on neuromuscular transmission and muscle contractility have been examined in mouse phrenic nerve-hemidiaphragm and frog sartorius muscle preparations using twitch tension and intracellular recording techniques. The extent of muscle paralysis induced by kava was similar in both directly and indirectly stimulated mouse hemidiaphragms. The neuromuscular blockade produced was poorly reversed by calcium and by neostigmine. Intracellular recordings from frog sartorius muscles showed that kava depressed the amplitude of both miniature end-plate potentials (mepps) and end-plate potentials (epps) but had no effect on the frequency of mepps. Kava greatly prolonged the duration of mepps and epps and also slowed and depressed directly elicited muscle action potentials. It is concluded that kava causes paralysis by mechanisms similar to local anaesthetics.

Introduction

The custom of drinking kava, an infusion prepared from the rhizome or stem of the plant *Piper methysticum* Forst. (Piperaceae), has been practised in many islands of Oceania since before the first contact with Europeans. However, kava is consumed not merely as a social beverage, but it has also acquired a central role in the lives of the people. It is used in ceremonies to welcome distinguished visitors (Ford, 1967), at formal gatherings, initiation or completion of work (Turner, 1861), validation of titles, celebration of marriages, births or deaths (Mead, 1930), as a libation to the gods (Firth, 1970), to cure illnesses and to remove curses (Mariner, 1827; Turner, 1861), in fact in almost all phases of life in the islands.

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The early missionaries and explorers maintained that the beverage partially paralysed the lower extremities making it difficult to walk (for references, see Holmes, 1967). Although kava is not alcoholic much has been made of its narcotic properties. It is now generally agreed that it is a refreshing, astringent drink which produces a numbing of the tongue and sometimes of the inner lining of the mouth. In the past, one of the methods of preparing the infusion has been by chewing pieces of the root or stem, mixing the cud with water and removing the fibrous materials. The chewer invariably experienced anaesthesia of the tongue and of the inner lining of the mouth, a loss of taste for an extended period of time and stiffness of various mouth muscles.

The active ingredients in kava are a series of approx. 12 α-pyrones (Hansel, 1968; Shulgin, 1973). Although Klohs et al. (1959) have claimed that purified kava pyrones show little of their biological activity when tested individually, other workers have reported otherwise. For instance, Meyer and Kretzschmar (1965) have found that administration of these compounds leads to ataxia and muscle paralysis but without loss of consciousness in a manner more reminiscent of mephenesin than of tubocurarine. The pyrones also allay anxiety and reduce fatigue although quite different physiological effects, some involving intoxication, have also been reported (Hansel, 1968). On the other hand, Meyer and May (1964) have shown that most of these compounds inhibit frog heart contraction, but with different potencies, in an action which has been compared with the local anaesthetic effectiveness of cocaine. At present it is not clear whether these local anaesthetic effects relate to the muscle paralysis which is noted when a large amount of kava is consumed. In the present study an investigation has been made on the effect of whole kava extract on muscle contractility and on neuromuscular transmission using twitch tension and electrophysiological techniques.

Materials and methods

Preparation of kava extract

Pieces of dried *Piper methysticum* stem from the island of Taveuni (Fiji) were ground to a fine powder. Five grams of this powder was suspended in 100 ml of Krebs-Henseleit (1932) or frog Ringer solution at 20—25°C and the suspension vigorously agitated for 10 min. The resulting mixture was centrifuged at 500 × *g* for 10 min. The supernatant was filtered through Whatman No. 4 filter paper and the filtrate was used in all subsequent experiments. Concentrations of kava are expressed as mass of kava powder per unit volume of solution.

Twitch tension experiments

Phrenic nerve-hemidiaphragm preparations from Porton mice (20—35 g)

were mounted in Krebs-Henseleit (1932) solution to which 2 g/ℓ dextrose was added, maintained at 32°C and gassed with oxygen containing carbon dioxide (5%). Resting tension was approximately 0.5 g. The phrenic nerve was stimulated at a frequency of 0.1 Hz with supramaximal rectangular pulses of 0.2 ms duration. For direct muscle stimulation, neuromuscular transmission was abolished by (+)-tubocurarine (3 μM). The hemidiaphragm was stimulated through a hook electrode inserted into the rib tissue with supramaximal rectangular pulses of 1 ms duration at a frequency of 0.1 Hz.

To assess reversibility of neuromuscular paralysis an 80—90% block of twitch height was established and calcium chloride (to a final calcium concentration of 5 or 10 mM) or neostigmine (3 μM) was added to the tissue bath. The extent of reversal was measured 5 min after addition of the reversal agent and the recovery values expressed as percentages of the control twitch height.

Intracellular recording

The isolated sciatic nerve-sartorius muscle preparation from *Rana pipiens* was used. The preparations were pinned to a resin-coated 10 ml perspex tissue bath and bathed in a normal frog Ringer solution (111 mM NaCl; 2 mM KCl; 2 mM $NaHCO_3$; 2 mM $CaCl_2$; 1 mM Tris) or in high magnesium (8 mM)—low calcium (1 mM) Ringer solution.

Standard microelectrode techniques were used. The preparations were observed at a magnification of 300 times with a binocular microscope fitted with a Leitz UM 20/0.33 long working distance objective. End-plate regions were localised by following nerve-twigs and penetrating muscle fibres until mepps with rise times of less than 1.5 ms could be recorded. Cells with resting potentials greater than −75 mV were rejected. To study evoked release of acetylcholine the sciatic nerve was stimulated at a frequency of 0.5 Hz with supramaximal pulses of 0.2 ms duration. After each addition of drug or change of solution, at least 20—30 min equilibration was allowed before recordings were made.

All values of mepp and epp amplitudes were corrected to a standard membrane potential of −80 mV (Katz and Thesleff, 1957) and epps were also corrected for non-linear summation (Martin, 1955), Epp quantal content was determined by the ratio of the mean amplitudes of epps and mepps (del Castillo and Katz, 1954).

Muscle action potentials were generated in frog muscles bathed in normal frog Ringer solution. A second microelectrode was inserted into a fibre about 600 μm from the recording electrode and stimulated with a rectangular 0.2 ms pulse of sufficient strength to trigger an action potential.

The results are presented in the text and tables as means ± S.E. of experiments on at least four separate preparations. Statistical comparisons were made by the use of the Mann-Whitney U-test, values of $P < 0.05$ being regarded as significant.

Results

Twitch-tension recording

At concentrations above 2 mg/ml kava produced a concentration-dependent decrease in tension of both indirectly and directly stimulated preparations. Such decrease in twitch tension was fully reversed on washing the preparation with Krebs solution. At the same concentration of the drug the blockade of indirectly elicited twitches was only slightly larger than that of directly elicited twitches. The concentration-inhibition relationships are shown in Fig. 1.

Increasing the calcium concentration to 5 mM produced a 5—10% reversal of kava-induced (20 mg/ml) blockade of indirectly elicited twitches. A 10—20% recovery was found in experiments in which calcium concentration was increased to 10 mM. The neuromuscular block produced by kava (20 mg/ml) was not reversed by neostigmine (3 μM). In some preparations addition of neostigmine quickened the development of the failure of contractions.

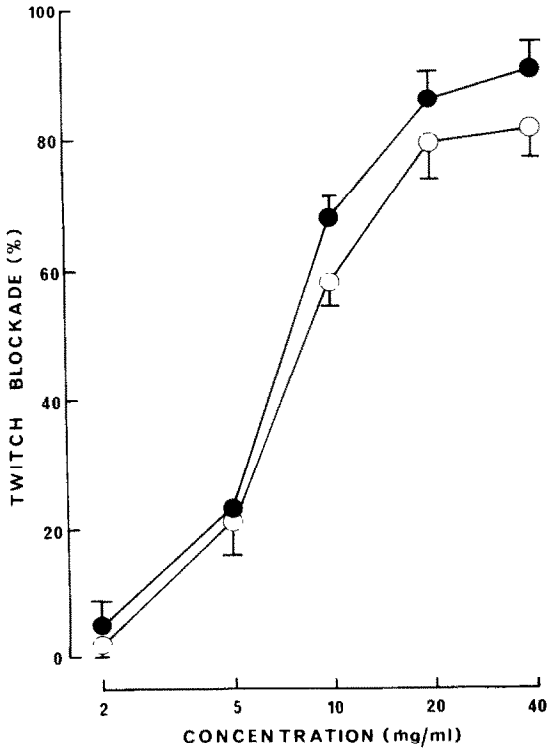

Fig. 1. Concentration—inhibition lines for effects of kava on indirectly (•) and directly (○) stimulated mouse hemidiaphragm preparations. Each point represents the mean obtained from four to six determinations. Standard error bars are shown unless smaller than symbols.

Effects on mepps and epps

The effects of kava on mepp amplitude and frequency were assessed in frog sartorius muscle preparations bathed in normal frog Ringer solution. There was a gradual decline in mepp amplitude starting from the time of application reaching a constant amplitude after 15—20 min. In the presence of kava (5 mg/ml) a mean mepp amplitude of 0.38 ± 0.03 mV was obtained which differed significantly ($P < 0.05$) from the control value of 0.68 ± 0.7 mV (Fig. 2). However, the reduction in mepp frequency from a control value of 0.89 ± 0.13 s^{-1} to 0.68 ± 0.12 s^{-1} was not significant ($P < 0.05$).

Amplitudes of control epps recorded from frog sartorius muscle preparations bathed in high magnesium—low calcium Ringer solution fluctuated randomly. In the presence of kava epp amplitude and fluctuation of epps were considerably reduced (Fig. 3): but kava did not affect the quantal content of epps. The quantal content in 5 mg/ml kava was 12 ± 3 which was not significantly different ($P > 0.05$) from the control value of 15 ± 3.

The effect of kava (5 mg/ml) on the time course of epps was qualitatively the same as its effect on the time course of mepps. The times to peak amplitude of both mepps and epps were significantly decreased ($P < 0.05$) (Table 1). The decay of mepps and epps was separated into two distinct phases: an initial phase which was shortened and hence a significant reduction ($P < 0.05$) in time to 50% decay was obtained (Table 1), and a

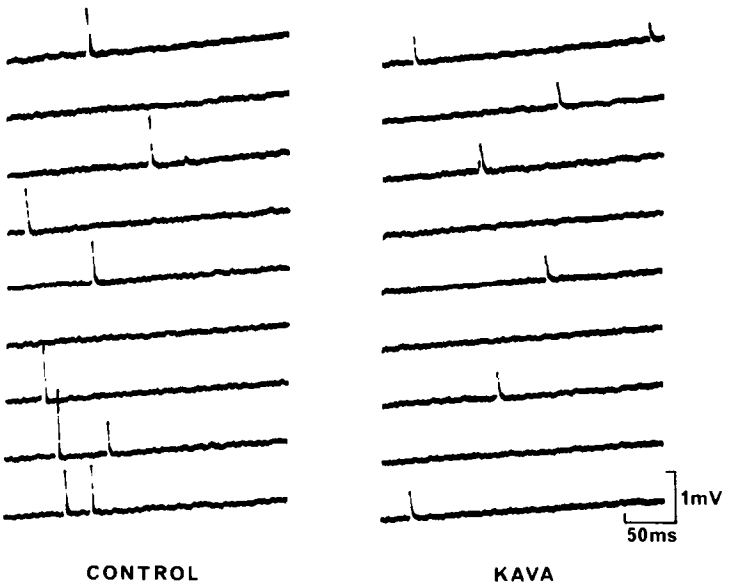

Fig. 2. Mepps recorded intracellularly from frog sciatic nerve-sartorius muscle preparation before (control) and after treatment with kava (5 mg/ml) in normal frog Ringer solution.

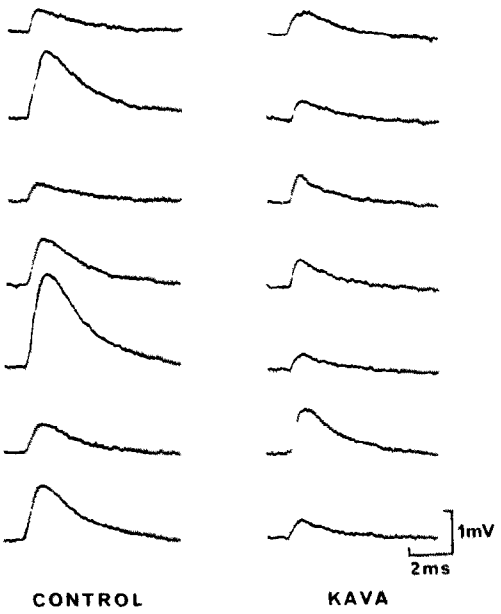

Fig. 3. Epps recorded intracellularly from frog sciatic nerve-sartorius preparation before (control) and after treatment with kava (5 mg/ml) in modified frog Ringer solution.

greatly prolonged second phase (Fig. 4). The overall effect was a marked prolongation of both mepps and epps.

Effects on muscle action potentials

In concentration ranges similar to those inducing muscle paralysis kava produced marked changes in muscle action potential parameters (Table 2). For example, during the first 20—30 min of exposure to kava (20 mg/ml) maximum rates of rise and fall were reduced and the overshoot became

TABLE 1

EFFECT OF KAVA (5 mg/ml) ON TIME COURSE OF MEPPS AND EPPS RECORDED FROM FROG SCIATIC NERVE-SARTORIUS MUSCLE PREPARATION

Mepps were recorded in normal Ringer and epps in modified Ringer solutions.

	Rise time (ms)	50% decay (ms)
Mepps		
Control	0.93 ± 0.05	2.85 ± 0.19
Drug	0.68 ± 0.05[a]	1.78 ± 0.09[a]
Epps		
Control	1.35 ± 0.15	2.76 ± 0.33
Drug	0.93 ± 0.13[a]	1.48 ± 0.16[a]

[a]Significantly different ($P < 0.05$) from control.

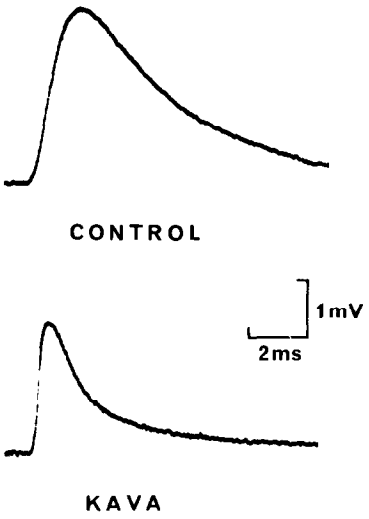

Fig. 4. Single representative end-plate potentials recorded from frog sciatic nerve-sartorius preparation bathed in modified frog Ringer solution (control) and after treatment with kava (5 mg/ml).

progressively smaller until the action potential failed to reach the zero potential at around 60 min. After 60—90 min it became impossible to generate action potentials (Fig. 5). No changes in resting membrane potentials were noted, even at the highest concentration tested.

Discussion

Kava causes muscle relaxation by a direct action on muscle contractility rather than by an inhibition of neuromuscular transmission. Thus, in the twitch tension experiments there was little difference between concentrations required to block directly and indirectly stimulated preparations. Further, blockade of twitches evoked by nerve stimulation was poorly

TABLE 2

EFFECT OF KAVA (20 mg/ml) ON OVERSHOOT AND MAXIMUM RATES OF RISE AND FALL OF MUSCLE ACTION POTENTIALS 60 min AFTER EXPOSURE

	Control	Drug
Maximum rate of rise ($V\ s^{-1}$)	408 ± 25	253 ± 32[a]
Maximum rate of fall ($V\ s^{-1}$)	121 ± 5	56 ± 7[a]
Overshoot (mV)	36 ± 4	5 ± 7[a]

[a]Significantly different ($P < 0.05$) from control.

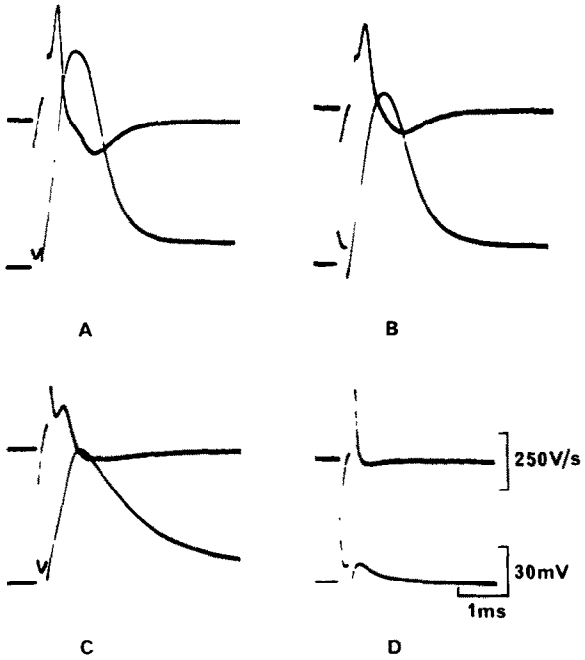

Fig. 5. Effect of kava (20 mg/ml) on directly elicited muscle action potentials in the frog sartorius muscle before (A), and 20 min (B), 40 min (C) and 60 min (D) after incubation with the drug. The lower record is the change in transmembrane potential of the muscle membrane and the upper record is the first derivative of the muscle membrane potential.

reversed by calcium or neostigmine. At the neuromuscular junction the main action of calcium is antagonism of block produced by prejunctionally active compounds like magnesium (del Castillo and Engbaek, 1954) whereas that for neostigmine is reversal of postjunctional block produced by tubocurarine and similarly acting compounds (Goodman and Gilman, 1965), Therefore, kava appears to have little specific action at the neuromuscular junction. This conclusion was confirmed by the intracellular recording studies which showed that the epp quantal content in the presence of kava was not significantly lower than control values, indicating that it had little or no effect on transmitter release. Additionally, mepp frequency was not affected. A reduction in mepp frequency is characteristic of compounds which act prejunctionally to reduce transmitter release (Hubbard, 1961).

Since the amplitudes of both mepps and epps were reduced by kava it can be concluded that kava depresses postjunctional sensitivity. The marked prolongation of mepps and epps in kava is similar to that previously reported for lignocaine and other local anaesthetics (Furukawa, 1957; Maeno, 1966; Steinbach, 1968) and suggests that kava may affect receptor ion channels. Such prolongation of end-plate potential has been attributed to an anticholinesterase action (Hunt and Kuffler, 1950), to a prolonged release of

transmitter probably arising from a prolongation of the nerve action potential (Takeuchi and Takeuchi, 1959), to a change in the time constant of the muscle (Takeuchi and Takeuchi, 1959) or to changes in the postjunctional time course of the transmitter and its associated changes in ionic permeability (Furukawa, 1957; Maeno, 1966; Steinbach, 1968). The blocking actions of kava on responses to direct stimulation in the twitch tension experiments are unlikely to be due to an effect on muscle membrane potential as this was virtually unchanged during the investigations. On the other hand, blockade of receptor ion channels in the muscle membrane could result in a decrease in action potential conduction and hence in muscle contractility. In the frog muscle, kava was found first to slow the rate of rise and to prolong the falling phase of the action potential and then to block the electrical excitability of the membrane. Such effects are consistent with an action on ion channels. A local anaesthetic action has previously been suggested for kava pyrones (Meyer and May, 1964). These authors showed that the kava pyrones inhibited frog heart contraction. This action was compared with those of cocaine which showed a similar protection against ventricular fibrillation.

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