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A Chloroform Extract of the Herb Feverfew Blocks Voltage-dependent Potassium Currents Recorded from Single Smooth Muscle Cells

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Abstract—We have studied the effects of a chloroform extract of fresh leaves from the herb feverfew (*Tanacetum parthenium*) on potassium currents in smooth muscle. The currents were recorded from single cells dissociated from the rat anococcygeus and the rabbit ear artery using the whole-cell patch-clamp technique. When applied to cells isolated from the rat anococcygeus, the extract reduced the inactivating voltage-dependent potassium current in a concentration-related manner, with an IC50 value (the concentration that reduced the current by 50%) of 56 $\mu g\ mL^{-1}$. Complete block of the current occurred at 1 $mg\ mL^{-1}$. In addition to reducing the peak current, feverfew decreased the time to peak of the current and increased the rate of decay of the current. These effects can be explained by the feverfew extract blocking open potassium channels. In single cells isolated from rabbit ear artery the feverfew extract again reduced the voltage-dependent potassium current, whilst at the same time having no effect on the spontaneous transient outward currents which arise as a consequence of activation of calcium-dependent potassium channels. These results suggest that chloroform extracts of feverfew leaf contain an as yet unidentified substance capable of producing a selective, open-channel block of voltage-dependent potassium channels.

The herb feverfew has a long history of use in traditional folk medicine where it is purported to alleviate headache, vertigo and other 'pains coming from a cold cause' (for review see Berry 1984). Recently, feverfew has been used specifically as a prophylactic for migraine, and extracts are now commercially available in health food shops. Two clinical trials indicate a beneficial role of feverfew in migraine prevention (Johnson et al 1985) and treatment (Murphy et al 1988).

The pathophysiology of migraine remains a controversial issue, particularly as regards the importance of vasodilatation of the meningeal blood vessels in producing headache (Saxena & Ferrari 1989; Moskowitz 1992). Given this, it is not surprising that the mechanisms by which feverfew produces its antimigraine effects are unclear. Recently, chloroform extracts of the herb have been shown to have complex effects on the contractility of vascular smooth muscle (Barsby et al 1992, 1993). Extracts of fresh leaves produce an irreversible and non-specific inhibition of contractility (Barsby et al 1992a). This effect appears to be due to the presence of sesquiterpene butyrolactones, such as parthenolide, contained in feverfew leaves (Bohlmann & Zdero 1982), and known to react covalently with sulphydryl or amino groups in proteins by a Michael-type addition reaction (Kupchan et al 1970). In marked contrast, chloroform extracts of powdered, dried leaves, such as those which can be purchased from health food stores and which do not contain parthenolide, produce reversible contractions of smooth muscle (Barsby et al 1993). It is possible that this latter effect might be important for feverfew's anti-migraine activity since the ability to produce vasoconstriction is a property shared by a number of anti-migraine drugs, including the ergot alkaloids and the 5-HT_{1D} receptor agonist sumatriptan (Saxena & Ferrari 1989).

In an attempt to elucidate the cellular mechanism by which extracts of feverfew produce smooth muscle contraction, we have studied the effects of chloroform extracts of the herb on the electrophysiological properties of single smooth muscle cells dissociated from two preparations, the rat anococcygeus and the rabbit ear artery. We now report that feverfew extracts produce a selective inhibition of voltage-dependent potassium currents by a mechanism reminiscent of an open channel blocking action.

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Materials and Methods

Preparation of cells

Rat anococcygeus. Male Wistar rats, > 250 g, were killed by cervical dislocation followed by exsanguination, and the anococcygeus muscles were removed as described by Gillespie (1972). Cells were dissociated using a protocol described previously (McFadzean & England 1992). Briefly, the tissue was chopped into 2 mm pieces and incubated for 10 min in 2 mL of a Ca^{2+}-free physiological salt solution (PSS) at 37°C and comprising (mM): NaCl 126, KCl 6, $MgCl_2$ 1·2, NaH_2PO_4 1·2, HEPES 10, glucose 11, adjusted to pH 7·2 with 1 M NaOH. The tissue was then incubated for 45 min in Ca^{2+}-free PSS containing bovine serum albumin (BSA, 5 mg, mL^{-1}), papain (1 $mg\ mL^{-1}$), collagenase (1·5 $mg\ mL^{-1}$) and dithioerythritol (DTT, 2·5 mM). The tissue was then washed with enzyme-free PSS and the single cells dissociated by passing the tissue pieces several times through a wide bore Pasteur pipette. The resulting cell-rich solution was centrifuged at 180 *g* for 1 min and the pellet resuspended in PSS containing 0·75 mM $CaCl_2$. The cells were plated onto cover slips and refrigerated at 4°C before electrophysiological experiments.

Rabbit ear artery. New Zealand White rabbits, 1·5–2·5 kg, of either sex were killed by overdose of pentobarbitone (Sagatal) and the main artery removed from each ear. The tissue was incubated for 10 min in 2 mL of the Ca^{2+}-free PSS followed by a dispersion protocol detailed by Benham & Bolton (1986). Briefly, three 30-min incubations were performed in PSS containing, in all incubations, DTT (2·5 mM) and BSA (20 mg mL^{-1}) and in successive incubations, 0·6 mg mL^{-1} and 1·7 units mL^{-1}, 0·5 mg mL^{-1} and 2·1 units mL^{-1}, 0·4 mg mL^{-1} and 2·5 units mL^{-1} of collagenase and elastase, respectively. On completion of the enzyme treatment, the tissues were washed and prepared for electrophysiological recordings in exactly the same way as outlined for preparation of the anococcygeus muscle cells.

Electrophysiological recordings

Membrane currents were recorded using the whole-cell variant of the patch-clamp technique (Hamill et al 1981). Cells were perfused with an extracellular solution comprising (mM): NaCl 120, KCl 6, $MgCl_2$ 1·2, $CaCl_2$ 1·5, Na_2HPO_4 1·2, HEPES 10, glucose 11, adjusted to pH 7·2 with NaOH and saturated with O_2. Recording micropipettes had DC resistances in the range 5–10 Mohms and were filled with an isotonic solution comprising (mM): KCl 126, $MgCl_2$ 1·2, NaCl 5, HEPES 10, glucose 11, EGTA 1 adjusted to pH 7·2 with KOH. In experiments on rabbit ear artery cells the perforated-patch technique was employed (Horn & Marty 1988). The polyene antibiotic nystatin was added to the micropipette-filling solution at a final concentration of 125 $\mu g\ mL^{-1}$. Perforation of the patch occurred within 5 to 15 min of sealing onto the cell and resulted in series resistances of typically 10–15 Mohms. No series resistance compensation was employed. All experiments were performed at room temperature (21–24°C).

Acquisition and analysis of membrane currents were performed using pClamp software (Axon Instruments Inc). Currents were digitized using a digital-to-analogue converter (TL-1 Axon Instruments) and stored on the hard disk of a personal computer (Vig 2; Viglen Ltd).

All grouped data are expressed as the mean ± s.e.m.

Preparation of feverfew extracts

Extracts were prepared according to Begley et al (1989). Fresh leaves from *Tanacetum parthenium* (L.) Sch. Bip., Compositae, grown at Castle Donington, Leicestershire, were ground with 20:1 v/w chloroform in a pestle and mortar. The chloroform was evaporated by drying the preparation under a stream of N_2 and the resulting green oil resuspended in methanol at a concentration of 100 mg mL^{-1}.

Drugs and solutions

All enzymes, salts and nystatin were obtained from the Sigma Chemical Co. (Poole, UK).

Results

The effects of feverfew on the voltage-dependent potassium current in cells dissociated from the rat anococcygeus

Step depolarization of rat anococcygeus cells from holding potentials more negative than −30 mV to command potentials more positive than −40 mV evoked a slowly inactivating outward current, the properties of which have been described recently (McFadzean & England 1992). Typical currents are shown in Fig. 1, where a cell, held at −70 mV was depolarized to three different command potentials, −20, 0 and +20 mV. The cell was then exposed to the feverfew extract at a concentration of 56 $\mu g\ mL^{-1}$ and the voltage steps repeated. In the presence of the extract, the amplitude of the current evoked at all the test potentials was markedly reduced. Furthermore, the time course of current decay was altered, the initial rate of decay of the current appearing to speed up in the presence of the extract. These effects were seen in all cells tested (n = 41). The methanol vehicle had no effect on the current at concentrations up to 0·01% (data not shown). In Fig. 1C, the currents evoked by stepping to +20 mV in the absence and presence of feverfew extract are superimposed along with a current evoked following a 5-min washout of the extract. It can be seen that the current

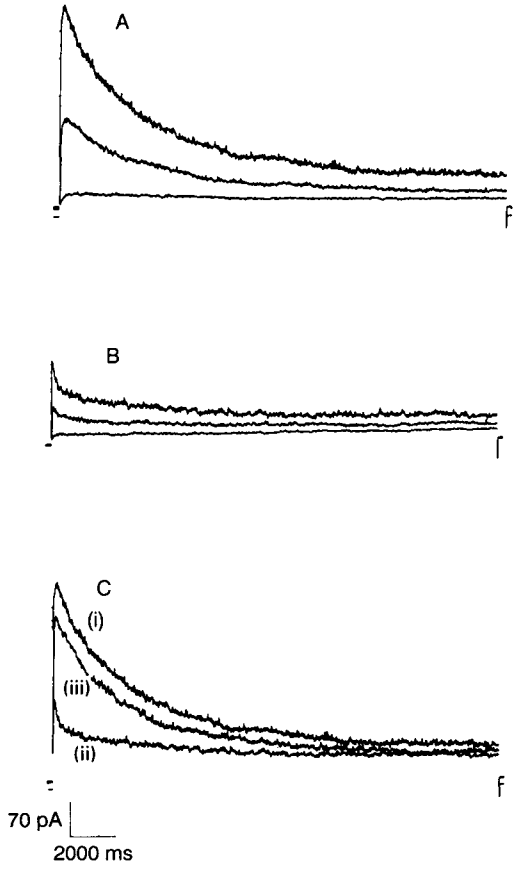

FIG. 1. The effect of feverfew on the inactivating outward current in single cells of the rat anococcygeus muscle. A and B both show a family of potassium currents evoked by 10 s step depolarizations of a cell from a holding potential of −70 mV to command potentials of −20, 0 and +20 mV (lower to upper traces, respectively). Currents evoked from an untreated cell are illustrated in A whereas currents in B were obtained when the same cell was bathed in 56 $\mu g\ mL^{-1}$ of a chloroform extract of feverfew leaves. C shows the currents evoked by the step to +20 mV before (i) and during (ii) the application of extract, superimposed with a current recorded after washing the cell with extract-free PSS for 5 min (iii).

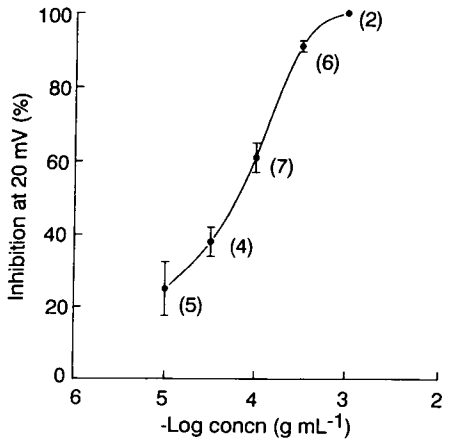

FIG. 2. The response to feverfew extract, measured as the percentage inhibition of the peak current evoked at a command potential of +20 mV following a step from a holding potential of −90 mV, plotted against the concentration of the extract. Each point shows the mean ± s.e.m. for the number of observations in parentheses.

returned to around 80% of its control amplitude. No further recovery was obtained in cells washed for up to 15 min.

The effects of feverfew extract were concentration-dependent as shown in Fig. 2. Currents were evoked by stepping to +20 mV from a holding potential of −90 mV. The response to the extract was quantified as the percentage inhibition of the peak current recorded before addition of the extract. From these data, the IC50 (the concentration of the extract producing 50% inhibition of the control current) was calculated to be 56 μg mL^{-1}.

The effects of feverfew on the time-course of the current

In addition to reducing the peak amplitude, feverfew extract also altered the kinetics of the inactivation of the outward current. This effect is illustrated in Fig. 3. Currents were evoked by step depolarizations from a holding potential of −70 mV to a command potential of +30 mV. Fig. 3A shows currents recorded in the absence and presence of the extract (56 μg mL^{-1}) and again shows the apparent increase in the rate of inactivation of the current produced by the extract. In Fig. 3B, the same currents are shown on an expanded time scale, and with the current recorded in the presence of extract scaled so that the peak current is the same as that in control cells. This shows that as well as increasing the rate of decay, the extract reduced the time to peak of the current.

In control conditions, inactivation of the current could adequately be described by a single exponential function with a time constant of 3056 ± 52 ms (n = 4). In the presence of the extract however, a double exponential was required to

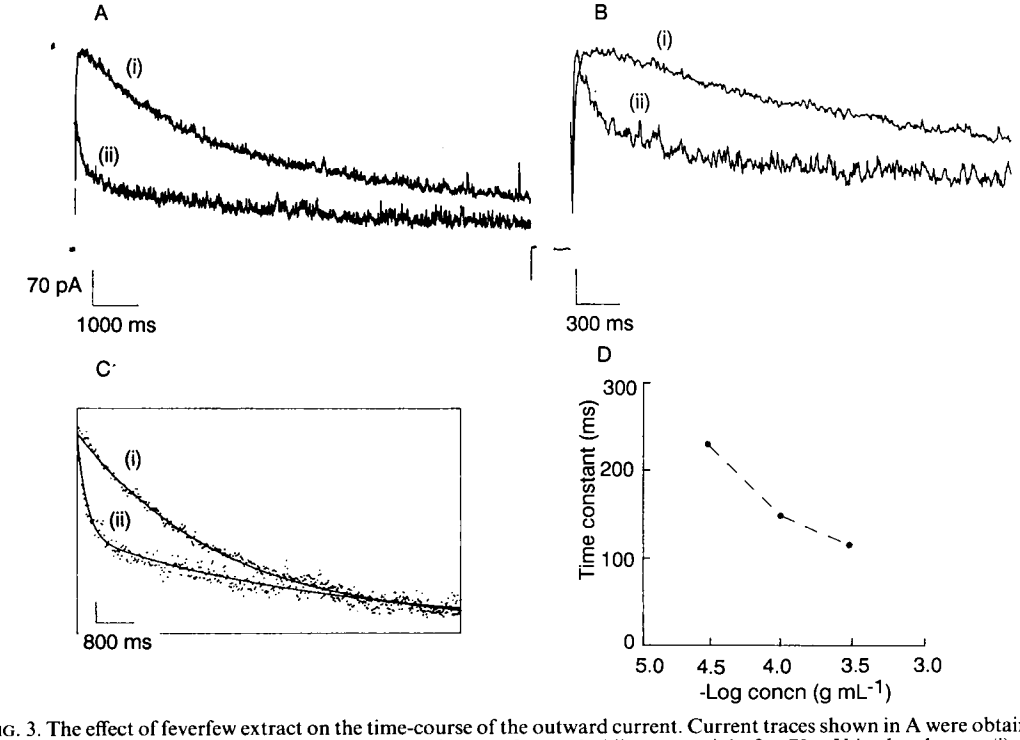

FIG. 3. The effect of feverfew extract on the time-course of the outward current. Current traces shown in A were obtained from an anococcygeus cell depolarized to 20 mV for 10 s from a holding potential of −70 mV in the absence (i) and presence (ii) of 56 μg mL^{-1} extract. B shows the same data but on an expanded time scale and with the current recorded in the presence of extract, scaled so that the peak current corresponds to that recorded in the absence of the extract. Feverfew reduced the time to peak of the outward current and increased the rate of decay of the current. C shows exponential fits (solid lines) to the current data points obtained in the absence (i) and presence of extract (ii). In the absence of the extract the current decay could be fitted by a single exponential function of the form $Y = A_0 + A_1 e^{-t/\tau}$, where A_0 is the initial amplitude of the current and A_1 is the amplitude at time t. The time constant of the exponential (τ) was 3164 ms. In the presence of the extract the decay of the current could be described as the sum of two exponential functions ($Y = A_0 + A_1 e^{-t/\tau 1} + A_2 e^{-t/\tau 2}$) with time constants $\tau 1$ and $\tau 2$ of 185 and 5081 ms, respectively. D shows a plot of the values obtained for the fast time constant ($\tau 1$) against the concentration of extract. Data points were obtained from a single cell exposed to three concentrations of the herb extract.

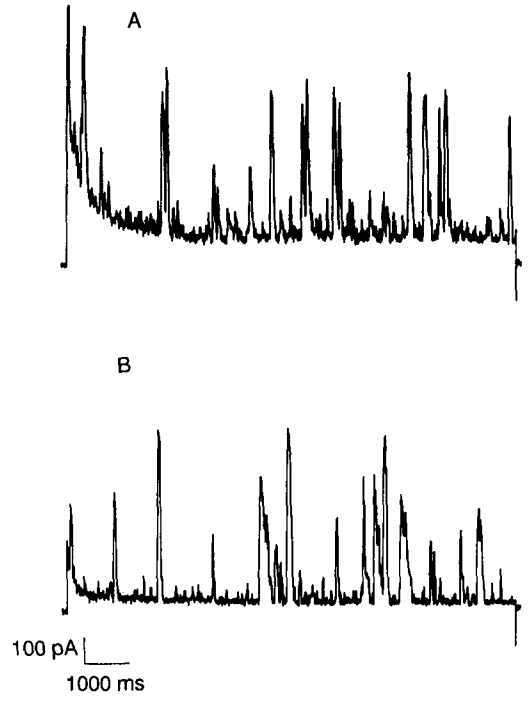

FIG. 4. The effect of feverfew extract on currents recorded from a cell dissociated from the rabbit ear artery. Currents were evoked by step depolarizations from a holding potential of −70 mV to a command potential of 0 mV in the absence (A) and presence (B) of 100 μg mL^{-1} extract.

achieve an acceptable fit of the experimental data. At a feverfew extract concentration of 56 μg mL^{-1} the time constants for the exponentials were 3904 ± 872 and 159 ± 30 ms (n = 4, same cells) with mean amplitudes of 41·5 ± 6 and 58·5 ± 6%, respectively. The fits obtained from a representative cell are shown (Fig. 3C).

The appearance of a fast component of current decay in the presence of feverfew extract is reminiscent of a mechanism of action known as open channel block (Hille 1991). Another characteristic of this mechanism of drug action is that the fast component of decay should speed up with increases in drug concentration. This was also seen to be the case with the extract as illustrated in Fig. 3D, which shows the time constant for the fast component of inactivation obtained from a single cell exposed to several concentrations of the extract, plotted against the concentration.

The effect of feverfew extract on outward currents in cells dissociated from the rabbit ear artery

To determine whether the blocking action of the extract was selective for voltage-dependent potassium currents, we looked at the effect of the extract on Ca^{2+}-dependent potassium currents in cells dissociated from the rabbit ear artery. The results of one such experiment are illustrated in Fig. 4, which shows currents evoked on depolarizing a rabbit ear artery cell from a holding potential of −70 mV to a command potential of 0 mV in the absence (Fig. 4A) and presence (Fig. 4B) of extract (100 μg mL^{-1}). Under control conditions the step depolarization evokes a time-dependent outward current similar to that seen in the rat anococcygeus. Superimposed on this, however, are spontaneous transient outward currents (STOCs) which have been shown to result from the spontaneous release of calcium from intracellular stores activating calcium-dependent potassium channels (Benham & Bolton 1986). Feverfew extract blocked the voltage-dependent potassium current whilst having no effect on the STOC activity.

Discussion

The main finding of the present study is that a chloroform extract of feverfew leaves reduces voltage-dependent potassium current in single smooth muscle cells without affecting current through calcium-dependent potassium channels. The effect of the extract on the voltage-dependent current appears to occur as a result of an as yet unidentified substance in the extract blocking open potassium channels.

The properties of the voltage-dependent potassium current found in rat anococcygeus cells and blocked by feverfew have been described recently (McFadzean & England 1992). The current is activated by depolarizations positive to −40 mV. With prolonged depolarizations however, the current inactivates with a time-course that can be described as a single exponential process with a time constant of 2–3 s. The extract reduced the amplitude of the current, and in addition changed the time course of the current such that the time to peak of the current was reduced in the presence of the herb extract and the current inactivated with a time course which was biexponential. The faster of the two components was concentration-dependent, the time constant decreasing with an increase in the concentration of the extract. These effects are consistent with a mechanism of action whereby the active principle blocks the potassium channels in their open state (Hille 1991). A similar mechanism of action has been described for a number of drugs acting on voltage-dependent potassium channels, including internally applied tetraethylammonium analogues (French & Shoukimas 1981), 4-aminopyridine (Thompson 1982) and verapamil (Terada et al 1987). Both 4-aminopyridine and verapamil produce similar effects to feverfew extract on the potassium current recorded in rat anococcygeus cells. In comparison, neither quinine nor tetraethylammonium, whilst reducing the amplitude of the current, has any effect on its time course of inactivation (McFadzean & England 1992).

The channel-blocking activity of feverfew appears to show some selectivity for voltage-dependent potassium channels since it had no effect on the STOC activity in cells dissociated from the rabbit ear artery. STOCs have previously been shown to be caused by activation of calcium-dependent potassium channels by calcium released spontaneously from intracellular stores (Benham & Bolton 1986). As yet we have no information on whether the extract blocks other types of potassium channels or indeed channels other than potassium channels. Furthermore we do not know if the extract shows any selectivity within the family of voltage-dependent potassium currents. We are currently undertaking a series of experiments to look at the effect of the extract on a range of ion currents to determine just how selective the blocking action is.

Inhibition of voltage-dependent potassium channels would be expected to increase the excitability of smooth muscle and potentiate the effects of depolarizing stimuli

which act to open voltage-dependent calcium channels, thereby providing calcium for the contractile process (Bolton 1986). Chloroform extracts of dried and powdered feverfew leaves, such as those available in health food stores and used as a popular anti-migraine remedy, produce reversible contractions of vascular smooth muscle (Barsby et al 1993) which could conceivably be due to blockade of potassium channels. However, extracts of fresh leaves, including the extract used in the current series of experiments, rather than causing contractions of smooth muscle, produce an irreversible, non-specific inhibition of contractility (Barsby et al 1992). The irreversibility of this response, compared with the almost complete reversibility of the potassium channel blockade, suggests that these two activities are not related. In support of this, preliminary experiments have shown that the potassium current is not affected by parthenolide, a sesquiterpene butyrolactone present in our extract and thought to be responsible for the irreversible inhibition of smooth muscle contractility (Barsby et al 1993).

Might the cellular action described here be related to the anti-migraine activity of feverfew? Currently there are two opposing but overlapping theories as to the pathophysiology of migraine. The vascular theory proposes that vasodilatation of the meningeal blood vessels is responsible for the disorder, and that drugs such as the ergot alkaloids owe their anti-migraine activity to their ability to constrict extracerebral blood vessels. This theory has been accepted by many and has been used to explain the anti-migraine effects of 5-HT_{1D} receptor agonists such as sumatriptan (Saxena & Ferrari 1989). The vasoconstrictor, and by implication the potassium-channel-blocking effect, of feverfew fits in well with the vascular theory. More recently, however, this viewpoint has been challenged, and an alternative theory, the so-called neurogenic theory, has been put forward by Moskowitz (1992). This states that migraine occurs when as-yet unknown triggers activate perivascular sensory afferents of the trigeminal nerve innervating the meninges of the brain. The nerve impulses are transmitted both orthodromically, to higher centres, and antidromically to cause the release of vasoactive neuropeptides resulting in neurogenic inflammation. The inflammatory response involves vasodilatation of the meningeal blood vessels, particularly the arteries and arteriovenous anastomoses, with associated plasma extravasation and release of inflammatory mediators. In this scheme, drugs such as sumatriptan act pre-junctionally to block the release of neuropeptides involved in mediating the neurogenic inflammatory response. The vascular changes that occur are regarded as epiphenomena, and this makes it more difficult to explain feverfew's anti-migraine effect on the basis of its vasoconstrictor activity. However, it should not be overlooked that the voltage-dependent potassium channels present in smooth muscle cells are similar to those found in neurones and it is therefore possible that feverfew might interfere in some way with the neurogenic response.

In conclusion, the present study provides evidence that a chloroform extract of feverfew leaves contains an as-yet unidentified substance which selectively inhibits voltage-dependent K^+ currents by an open channel-blocking mechanism. This effect could account for the spasmogenic action of powdered leaf extracts observed previously and may also be relevant to the anti-migraine effects of this traditional remedy.

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