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Cryptolepine-induced vasodilation in the isolated perfused kidney of the rat: role of G-proteins, K^+ and Ca^{2+} channels

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Abstract

The isolated perfused kidney of the rat was used to examine the contribution by guanosine triphosphate (GTP)-binding (G-) proteins, K^+ and Ca^{2+} channels to the vasodilator actions of cryptolepine (5-methylquindoline). In normal Krebs-Henseleit buffer (4.7 mM KCl), cryptolepine elicited dose-dependent reductions in perfusion pressure of phenylephrine-preconstricted kidneys. The reductions in perfusion pressure by cryptolepine at bolus doses of 2.5, 5, and 10 μg were -18.0 ± 3.4, -30.6 ± 5.3, and -38.3 ± 6.8 mm Hg, respectively ($n = 19$). In K^+-free (0 mM KCl) Krebs-Henseleit solution, the vasodilator response to cryptolepine was reduced by $44.7 \pm 5.7\%$ ($n = 5$; $P < 0.01$). The addition of ouabain (10^{-4} M) further reduced cryptolepine-induced vasodilation to $63.0 \pm 7.2\%$ ($n = 11$; $P < 0.01$) of the control. A combination of both conditions did not abolish the vasodilator responses to cryptolepine, suggesting the involvement of additional mechanisms. In 80, as opposed to 20 mM KCl, the reductions in perfusion pressure by cryptolepine, 2.5, 5, and 10 μg were markedly reduced to -0.8 ± 0.8, -2.3 ± 1.4, and -4.0 ± 2.1 mm Hg, respectively ($P < 0.01$; $n = 6$). Responses to acetylcholine and diazoxide, an adenosine triphosphate (ATP)-dependent K^+ channel activator, were also markedly reduced, suggesting the involvement of K^+ channels for these agents. Furthermore, tetraethylammonium (5 and 10 mM), a non-specific K^+ channel blocker, inhibited the vasodilator responses to cryptolepine ($n = 5$; $P < 0.01$) and to diazoxide and acetylcholine in a dose-related manner. However, glibenclamide (5 and 10 μM), an ATP-sensitive K^+ channel blocker, inhibited the vasodilator responses to diazoxide and acetylcholine but was without effect on cryptolepine-induced vasodilation. This suggests that cryptolepine activates K^+ channels which are tetraethyl ammonium- but not glibenclamide-sensitive. In pertussis toxin-treated rats, the vasodilator response to cryptolepine was not affected while that to acetylcholine and especially diazoxide was markedly inhibited. This suggests that, unlike diazoxide and acetycholine, the K^+ channels activated by cryptolepine are not coupled to pertussis toxin-sensitive G-proteins. In the presence of verapamil (5 μM) and cobalt chloride (1 mM), Ca^{2+} channel blockers, the vasodilator response to cryptolepine was inhibited ($n = 5$; $P < 0.01$), suggesting that Ca^{2+} flux across membranes is also involved in cryptolepine-induced vasodilation in the rat kidney.

Keywords: Cryptolepine (5-methylquindoline); Vasodilation; Kidney, rat; G-protein; K^+ channel; Ca^{2+} channel; Pertussis toxin

1. Introduction

Cryptolepine, a methylquindolanol alkaloid is a 3:4-benz-δ-carboline derivative of *Cryptolepsis sanguinolenta*, a plant native to West Africa (Ablordeppey et al., 1990). The plant is used to treat hypertension and the alkaloid extracted from it was shown to possess hypotensive activity and to cause a prolonged fall in blood pressure of dogs (Bamgbose and Noamesi, 1978). Other studies showed the antiplatelet, antithrombotic, and fibrinolytic activities of cryptolepine (Oyekan et al., 1986; Oyekan and Okafor, 1989; Oyekan and Ablordeppey, 1993a, b). An increase in platelet cyclic adenosine 3',5'-monophosphate (cAMP) (Oyekan and Ablordeppey, 1993a) and an antiphospholipase A_2 activity (Oyekan and Ablordeppey, 1993b) were suggested for the antiaggregatory action of cryptolepine.

Recently, we reported that the renal vasodilator effect of cryptolepine in the rat is endothelium-dependent, involving the release of a non-cyclooxygenase endothelium-dependent relaxing factor (EDRF), possibly nitric oxide (NO). The evidence for this was derived

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from the observation that N^{ω}-nitro-L-arginine, a specific inhibitor of the synthesis/release of EDRF/NO but not indomethacin, affected the vasodilator response to cryptolepine. The effector pathway for cryptolepine-induced vasodilation is cyclic guanosine 3',5'-monophosphate (cGMP) of vascular smooth muscle since methylene blue, an activator of soluble guanylate cyclase, inhibited cryptolepine vasodilation (Oyekan, 1994).

In the above study, the possibility was raised that the vasodilator action of cryptolepine may also be mediated via mechanisms involving an increase in potassium permeability, as the vasodilator response to cryptolepine was reduced in potassium-depolarized (50 mM K^+) tissues. EDRF was shown to increase K^+ permeability in several blood vessels (Bolton et al., 1983) and agents like acetylcholine, histamine, A23187, including cryptolepine and others, that release EDRF will therefore increase potassium permeability. An increase in potassium permeability may proceed through the direct activation of K^+ channels (Gordon and Martin, 1983; Gebremedhin et al., 1987) or activation of the sodium pump (Webb and Bohr, 1978; Rubanyi and Vanhoutte, 1985). For a number of vasodilators, cromakalin, diazoxide, acetylcholine, bradykinin, etc., activation of K^+ channels/hyperpolarization and stimulation of Na^+/K^+-ATPase are the mechanisms involved (Bolton et al., 1984; Komori and Suzuki, 1987; Chen and Suzuki, 1989; Komori and Vanhoutte, 1990).

Recent evidence also suggests that G-proteins couple the receptors of ligands that activate K^+ channels to the channels (Yamashita et al., 1987; Nakajima et al., 1988); G-proteins have therefore been implicated as mediators of the signal transduction of some K^+ channel-linked responses (Sasaki and Sato, 1987; Gilman, 1987). The aim of this study was therefore to study the contribution of K^+ channels and the Na^+/K^+-ATPase to cryptolepine-induced renal vasodilation.

The influence of pertussis toxin treatment on the vasodilator effect of cryptolepine was also investigated in order to characterize further the contribution of G-protein-coupled K^+ channels to the vasodilation. Moreover, in view of the fact that cryptolepine releases EDRF and the release of EDRF is calcium-dependent (Spedding et al., 1986), we investigated the contribution of calcium channels to the vasodilator response to cryptolepine in the presence of antagonists of calcium channels.

2. Materials and methods

These studies were performed using adult male Sprague-Dawley rats (weight, 328 ± 6 g), bred at the Laboratory Animal facility of the Faculty of Medical Sciences, the University of the West Indies, St Augustine, Trinidad.

2.1. Rat isolated perfused kidney

After rats were anaesthetized with pentobarbital sodium (60 mg/kg i.p.), the right kidney was exposed by midline laparotomy and the mesenteric and right renal arteries were cleared of surrounding tissue. Ties were loosely placed around these vessels and the vena cava just above and below the junction with the right renal vein. The right renal artery was then cannulated with a 19-gauge needle via the mesenteric artery to avoid interruption of blood flow and the vena cava was occluded and cut to provide an exit for the perfusate. The right ureter was also cut, and the animal was killed by an intracardiac injection of 10 mg pentobarbital. The kidney was then removed and suspended in a water-jacketed bath at 37°C.

Kidneys were perfused at constant flow by means of a Gilson peristaltic pump (Model MP2 Minipuls) with Krebs solution at 37°C and gassed with 95% O_2, 5% CO_2. The Krebs solution had the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, NaH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25 and glucose 11.5. K^+-depolarized (80 mM) and 20 mM K^+ Krebs solutions were prepared by substituting an equimolar amount of K^+ for Na^+, while K^+-free Krebs-Henseleit was made without any added K^+. Flow was adjusted to obtain a basal perfusion pressure of 75–90 mm Hg. The mean perfusate flow rate at this pressure was 8.6 ± 0.3 ml/min. To amplify vasodilator responses, vascular tone was elevated with phenylephrine (5×10^{-7} M), which increased perfusion pressure by 89.4 ± 3.9 mm Hg. Vascular perfusion pressure was measured with a Statham transducer and recorded on a Gilson Duograph recorder (Model ICT-2H).

Flow was maintained at a constant rate, and therefore changes in perfusion pressure were used as an index of changes in the resistance of the artery: a fall in perfusion pressure indicated vasodilatation, a rise, vasoconstriction. All responses were measured from the minimum of the pulse pressure and are the peak responses.

2.2. Experimental protocol

In some experiments, the contributions of the Na^+/K^+-ATPase and K^+ channels to the vasodilator actions of cryptolepine were evaluated. To this end, we used conditions that interfere with Na^+/K^+ transport, namely K^+-free Krebs-Henseleit solution and ouabain treatment, to examine the vasodilator response to cryptolepine. Thus, cryptolepine (2.5–10 μg) was tested during perfusion with K^+-free (0 mM K^+) Krebs-Henseleit solution or with normal (4.7 mM K^+) Krebs-Henseleit solution in the absence or presence of

ouabain (10^{-4} M). K^+-induced vasodilation in 0 mM K^+ was used as an indicator of Na^+/K^+-ATPase activity (Webb and Bohr, 1978) and the selectivity of ouabain on Na^+/K^+ pump was assessed against K^+-induced vasodilation by testing the vasodilator effect of 2 and 5 mg KCl in 0 mM K^+ in the presence of ouabain. In experiments where normal or 0 mM K^+ Krebs-Henseleit solution was used, arterial tone was elevated with phenylephrine (5×10^{-7} M).

The ability of K^+ channel openers to relax contractions produced by 20 mM KCl but their ineffectiveness against 80 mM KCl has become one of the standard tests for this group of drugs (Hamilton and Weston, 1989). Therefore, in other experiments, the kidneys were perfused with Krebs-Henseleit solution containing 20 mM or 80 mM K^+ (final concentration). Due to the elevation of tone by K^+, the concentration of phenylephrine used in kidneys perfused with 20 mM K^+ was halved, and with 80 mM K^+, no phenylephrine was added to elevate tone. The vasodilator response to cryptolepine (2.5–10 μg) as well as to acetylcholine (0.25 and 0.5 μg), nitroprusside (2.5 and 5 μg) and diazoxide (20 and 40 μg) was tested in both cases. In these experiments, phentolamine (10 μM) was added to block the effect of possibly indirectly released catecholamines from adrenergic fibres.

The role of K^+ channels in the vasodilator action of cryptolepine was further assessed with tetraethylammonium (5 and 10 mM), a non-specific K^+ channel blocker, and glibenclamide (5 and 10 μM), an ATP-dependent K^+ channel blocker. In these studies, each inhibitor was tested against the vasodilator response to cryptolepine (2.5, 5, and 10 μg), acetylcholine (0.25 and 0.5 μg), and to diazoxide (20 and 40 μg), an ATP-dependent K^+ channel activator. In order to ascertain the selectivity of these interventions, the vasodilator response to sodium nitroprusside (2.5 and 5 μg) was also tested as a positive control. The inhibitors were included in the perfusate from the beginning of the experiment so that the inhibitor-tissue contact time was at least 30 min before testing of the agonists.

In other experiments, the contribution by Ca^{2+} channels to the vasodilator action of cryptolepine was examined. Dose-response curves were obtained to cryptolepine (2.5–10 μg) in kidneys perfused with normal Krebs-Henseleit solution containing Ca^{2+} channel antagonists. We studied the effect of verapamil (5 μM), an organic Ca^{2+} channel blocker, and cobalt chloride (1 mM), an inorganic Ca^{2+} channel blocker, on the vasodilator responses to the usual doses of cryptolepine, acetylcholine and nitroprusside. The protocol followed was similar to that described above for K^+ channel blockers except that the dose of phenylephrine was increased 10-fold in verapamil-treated kidneys in order to obtain an elevation of tone similar to that in other experiments.

2.3. Treatment of rats with pertussis toxin

Rats were injected with a single dose of pertussis toxin 50 μg/kg body weight i.p. Control rats were injected with normal saline. 72 h later, kidneys were isolated from pertussis toxin-treated and control rats and set up as described. Vasodilator responses to cryptolepine, acetylcholine, nitroprusside, and diazoxide were tested as usual in kidneys preconstricted with phenylephrine. The concentration of phenylephrine needed to elevate arterial tone in pertussis toxin-treated kidneys to levels similar to those of vehicle-injected controls was 2.5×10^{-6} M.

2.3.1. Drugs

Phenylephrine, acetylcholine, sodium nitroprusside, pentobarbitone, KCl, tetraethylammonium, ouabain, cobalt chloride (all from Sigma, St. Louis, MO, USA) were dissolved in Krebs-Henseleit solution. Fresh solutions of ouabain were prepared and protected from light. Verapamil (Sigma) stock (100 mg/ml) was prepared in dimethyl sulfoxide (DMSO) and made up in Krebs-Henseleit solution. It was protected from light during the experiment. Pertussis toxin (Sigma) supplied in lysine/glycerol buffer pH 7.2, was diluted with normal saline (0.9% NaCl). Cryptolepine, synthesized (Ablordeppey et al., 1990) by Dr S.Y. Ablordeppey, Department of Chemistry, University of Virginia, Virginia, USA was dissolved in Krebs-Henseleit solution.

2.4. Statistical analysis

All data were expressed as means ± S.E. In some cases, the data were expressed as percent change from pretreatment or control values. Analysis of variance (ANOVA) was used to compare dose-response curves for cryptolepine between control and treated groups, Duncan's test (Antonipillai et al., 1989) being used to determine the significance of differences in mean values within each group. For the other agonists, Student's t-test for unpaired data was employed. In experiments with K^+-free Krebs-Henseleit solution, the effect of ouabain treatment was analyzed with Student's t-test for paired data. In all cases, $P \leq 0.05$ was regarded as significant. All statistical analyses were done using the 'Statworks' application on the Macintosh Plus computer.

3. Results

3.1. Cryptolepine-induced vasodilation during alteration of K^+ concentration or ouabain infusion

The basal perfusion pressure in kidney vessels perfused with Krebs solution was 89.2 ± 3.6 mm Hg (n =

29). No significant differences existed in this value with the different experimental manipulations. In kidneys perfused with normal (4.7 mM K^+) or K^+-free (0 mM K^+) Krebs-Henseleit solution, infusion of phenylephrine (5×10^{-7} M) caused a sustained elevation of arterial perfusion pressure (89.4 ± 6.9 mm Hg). In normal Krebs-Henseleit solution, cryptolepine 2.5, 5, and 10 μg reduced the perfusion pressure by −18.0 ± 3.4, −30.6 ± 5.3, and −38.3 ± 6.8 mm Hg, respectively (n = 18; pooled data). In kidneys perfused with K^+-free Krebs-Henseleit solution (n = 5), the basal perfusion pressure was 93.2 ± 4.2 mm Hg; and following the administration of phenylephrine, the pressure increased by 85.3 ± 4.0 mm Hg. Under these conditions, KCl 2 and 5 mg elicited reductions in perfusion pressure of −16.8 ± 2.0 and −31.3 ± 4.7 mm Hg, respectively (data not shown; see Fig. 1a). However, the change in perfusion pressure induced by cryptolepine was reduced ($P < 0.01$), the reduction amounting to −44.7 ± 5.7% (Fig. 1a). Following the infusion of ouabain (10^{-4} M), perfusion pressure was elevated by 33.7 ± 5.9 mm Hg (n = 5) from a preinfusion value of 163.5 ± 7.1 mm Hg. Despite this increase in arterial tone, the reductions in perfusion pressure by KCl (Fig. 1a) and cryptolepine (Fig. 1a) were lessened ($P < 0.01$). Ouabain inhibition of cryptolepine-induced vasodilation amounted to −63.0 ± 7.2% (n = 11; $P < 0.01$).

3.2. Cryptolepine-induced vasodilation in the presence of 20 and 80 mM KCl and K^+ channel blockers

Depletion of K^+ and treatment with ouabain did not abolish the vasodilator effect of cryptolepine, and the role of K^+ channels was therefore investigated. In kidneys perfused with Krebs-Henseleit solution containing 20 mM KCl, the elevation of perfusion pressure was 47.2 ± 2.6 mm Hg (n = 6; KCl alone) or 85.9 ± 4.4 mm Hg (n = 6; KCl [20 mM] and phenylephrine [2.5 ×

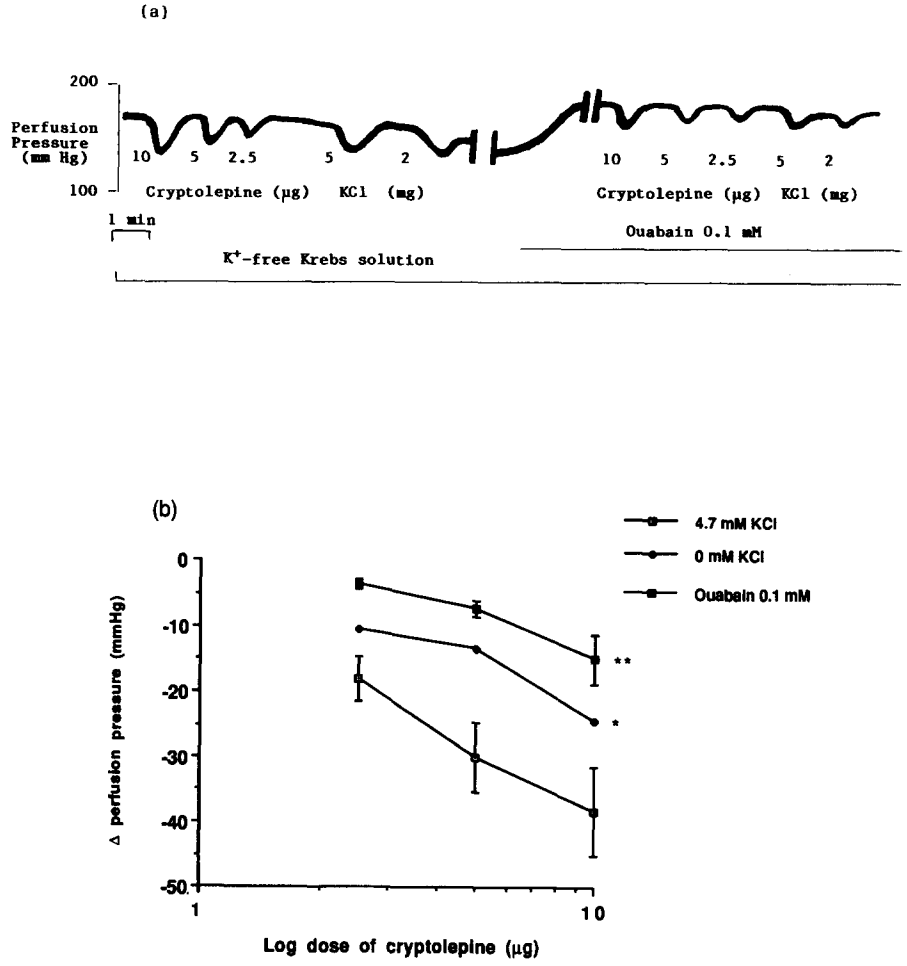
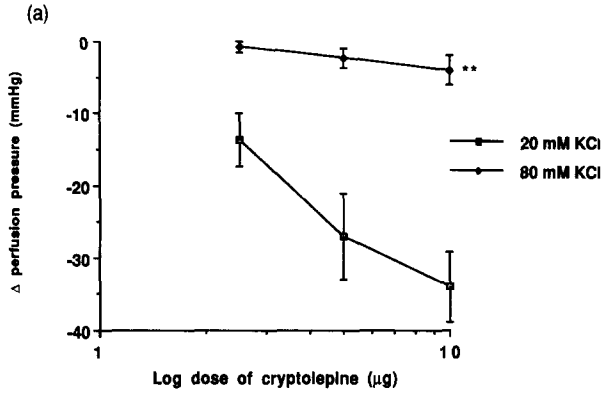


Fig. 1. (a) Representative tracings of the vasodilator response to cryptolepine 2.5, 5 and 10 μg and KCl 2 and 5 mg in K^+-free Krebs-Henseleit solution with or without ouabain (0.1 mM). Arterial tone was elevated with phenylephrine (5×10^{-7} M). The vasodilator responses to cryptolepine and KCl were inhibited when ouabain was infused. The tracing is representative of five experiments. (b) The vasodilator response to cryptolepine 2.5, 5 and 10 μg in kidneys perfused with Krebs-Henseleit solution containing 4.7 or 0 mM KCl and preconstricted with phenylephrine (5×10^{-7} M). $^*P < 0.05$, $^{**}P < 0.01$. Data were compared between control (4.7 mM KCl) and kidneys perfused with K^+-free Krebs-Henseleit solution with or without ouabain (0.1 mM).

10^{-7} M]). Cryptolepine-induced vasodilation was unaffected (Fig. 2a) while acetylcholine-induced vasodilation was moderately reduced ($n = 6$; $P < 0.05$; Fig. 2b) when compared to responses in normal (4.7 mM KCl) Krebs-Henseleit solution. However, in kidneys depolarized with 80 mM KCl (elevation of perfusion pressure was 95.1 ± 6.4 mm Hg from a basal value of 93.8 ± 2.9 mm Hg; $n = 6$), cryptolepine-induced vasodilation was markedly inhibited ($n = 6$; $P < 0.01$; Fig. 2a). The vasodilator response to diazoxide (20 and 40 μg) and that to acetylcholine (0.25 and 0.5 μg) were markedly inhibited ($n = 6$; $P < 0.01$). On the other hand, there was no difference in the response to nitroprusside in 20 and 80 mM KCl (Fig. 2b).

As shown in Fig. 3a and b, tetraethyl ammonium, 5 and 10 mM, inhibited the vasodilator responses to cryptolepine, acetylcholine and diazoxide in a dose-related manner. The inhibition by tetraethyl ammonium was only significant at the higher concentration em-



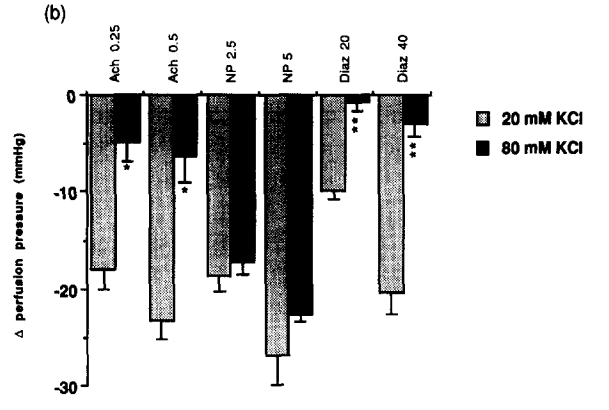


Fig. 2. The reduction in perfusion pressure by cryptolepine (a), acetylcholine (Ach, 0.25 and 0.5 μg), diazoxide (Diaz, 20 and 40 μg) and nitroprusside (NP, 2.5 and 5 μg) in Krebs-Henseleit solution containing 20 and 80 mM KCl. $^{*}P < 0.05$, $^{**}P < 0.01$. Data were compared between kidneys that were perfused with 20 mM KCl and those perfused with 80 mM KCl.

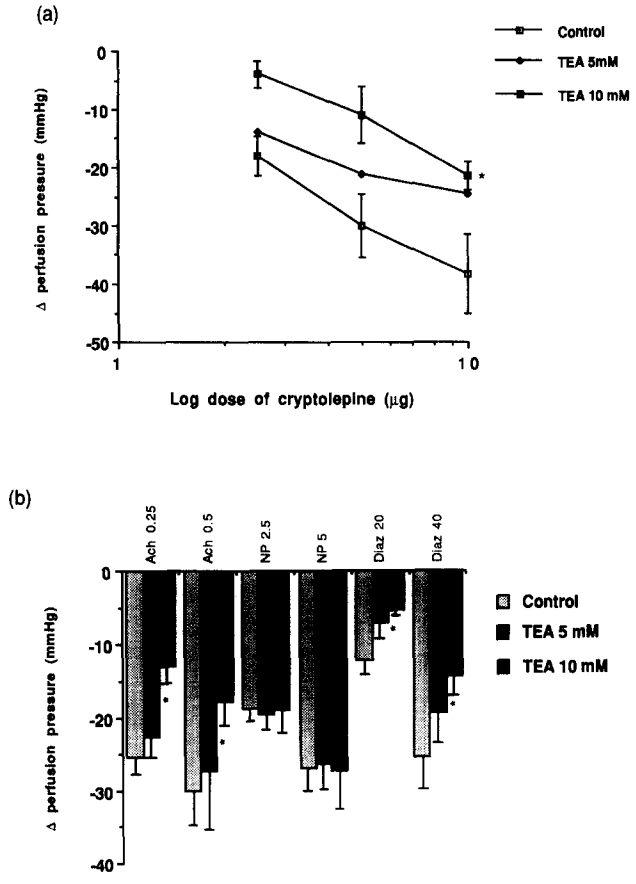


Fig. 3. The vasodilator responses to cryptolepine (a) and acetylcholine (Ach, 0.25 and 0.5 μg) and diazoxide (Diaz, 20 and 40 μg (b) in the presence of tetraethyl ammonium (TEA, 5 and 10 mM) in kidneys preconstricted with phenylephrine. $^{*}P < 0.05$. Data comparisons were done between the control (0 mM TEA) and kidneys treated with TEA (5 and 10 mM).

ployed. Thus, at 10 mM tetraethyl ammonium, the responses to cryptolepine 2.5, 5, and 10 μg were reduced ($n = 5$; $P < 0.01$) from their respective control values (Fig. 1b) to −4 ± 2.4, −11.0 ± 4.9 and −21.5 ± 2.5 mm Hg, respectively. The vasodilator response to acetylcholine 0.25 and 0.5 μg was reduced ($n = 5$; $P < 0.05$) as was that to diazoxide 20 and 40 μg ($n = 5$; $P < 0.05$–0.01). The vasodilator response to nitroprusside was unaffected at any concentration of tetraethyl ammonium used (Fig. 3b). The basal perfusion pressure in kidneys treated with tetraethyl ammonium was 87.4 ± 4.2 mm Hg.

On the other hand, glibenclamide (5 and 10 μM) did not affect the vasodilator response to cryptolepine (Fig. 4a) and nitroprusside (Fig. 4b) at any of the concentrations used. However, the vasodilator responses to acetylcholine and diazoxide were inhibited ($n = 5$; $P < 0.05$–0.01; Fig. 4b). Glibenclamide exerted no effect on perfusion pressure in the basal state and following the addition of phenylephrine.

3.3. The effect of pertussis toxin treatment on cryptolepine-induced vasodilation

The basal perfusion pressure in kidneys isolated from rats after 72 h of treatment with pertussis toxin was 86.3 ± 3.5 mm Hg ($n = 5$). However, these kidneys exhibited lower responsiveness to the usual concentration of phenylephrine (5×10^{-7} M). A 5-fold increase in the concentration of phenylephrine (2.5×10^{-6} M) was needed to elevate the arterial tone to levels similar (85.3 ± 4.8 mm Hg; $n = 5$) to those in vehicle-injected (control) kidneys (91.6 ± 3.3 mm Hg; $n = 5$). Responses to cryptolepine in control kidneys were not different from those of the kidneys not injected, so data from the kidneys from vehicle-treated rats were pooled with those from control uninjected rats. Fig. 5a and b shows that, in kidneys from pertussis toxin-treated rats, while the vasodilator responses to cryptolepine and nitroprusside were unaffected, acetylcholine- and diazoxide-induced vasodilation was reduced ($P < 0.05$). The vasodilator response to acetylcholine was reduced from −25.3 ± 2.5 mm Hg (control; $n = 18$) to −15.0 ± 2.7 mm Hg (0.25 μg) and from −30.7 ± 4.7 mm Hg (control; $n = 18$) to −21.3 ± 2.3 mm Hg (0.5 μg; $P < 0.05$) while diazoxide-induced vasodilation was reduced from

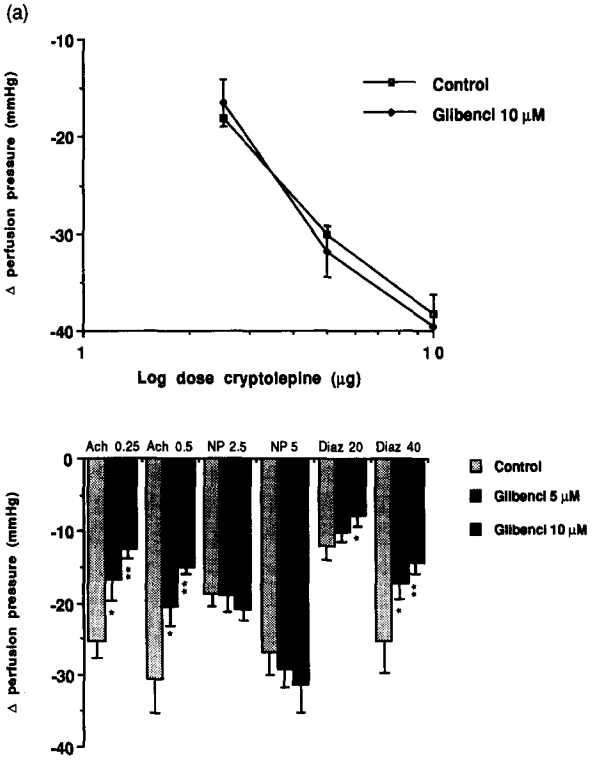


Fig. 4. The effects of glibenclamide (Glibencl, 5 and 10 μM) on the vasodilator responses to cryptolepine, acetylcholine (Ach), nitroprusside (NP) and diazoxide (Diaz) in kidneys preconstricted with phenylephrine. (Data for 5 μM not shown for clarity.) $^{*}P < 0.05$, $^{**}P < 0.01$. Data were compared between control and glibenclamide-treated kidneys.

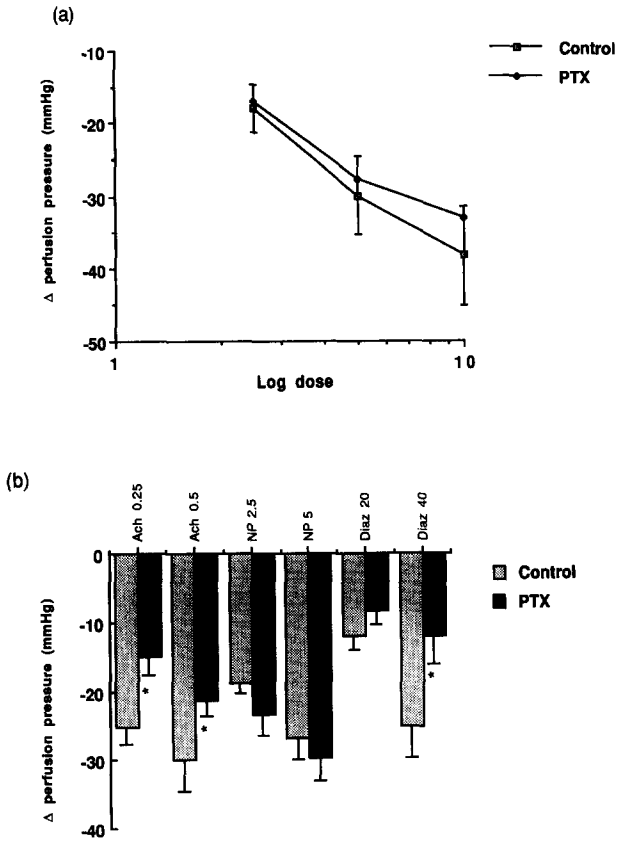


Fig. 5. Effects of pertussis toxin (PTX) treatment on the vasodilator response to cryptolepine (a), acetylcholine (Ach), diazoxide (Diaz) and nitroprusside (NP) in kidneys perfused with phenylephrine (2.5×10^{-6} M) to elevate arterial tone. $^{*}P < 0.05$. Data were compared between control kidneys perfused with phenylephrine (5×10^{-7} M; Control) and PTX-treated kidneys.

−12.0 ± 2.0 mm Hg (control) to −8.3 ± 2.3 mm Hg (20 μg) and from −25.3 ± 4.5 mm Hg (control) to −12.0 ± 4.2 mm Hg (40 μg) ($P < 0.05$).

3.4. Effect of Ca^{2+} channel antagonists on cryptolepine-induced vasodilation of the kidney

Kidneys treated with verapamil (5 μM), the organic Ca^{2+} channel blocker, exhibited markedly reduced responses to phenylephrine (5×10^{-7} M). Such tissues required 2.5×10^{-6} M of phenylephrine to achieve an arterial tone similar (86.7 ± 4.2 mm Hg; $n = 5$) to that of vehicle-treated kidneys. The final concentration of DMSO (vehicle) in Krebs-Henseleit solution was not greater than 0.005% and, at this concentration, there was no effect on the vasoconstrictor response to phenylephrine (5×10^{-7} M), neither was there any effect on the responses to the vasodilators used. Verapamil (5 μM) markedly reduced the vasodilation induced by cryptolepine ($n = 5$; $P < 0.01$; Fig. 6a), while the inhibition of acetylcholine-induced vasodilation was moderate ($n = 5$; $P < 0.05$) and that by nitroprusside was not affected (Fig. 6b).

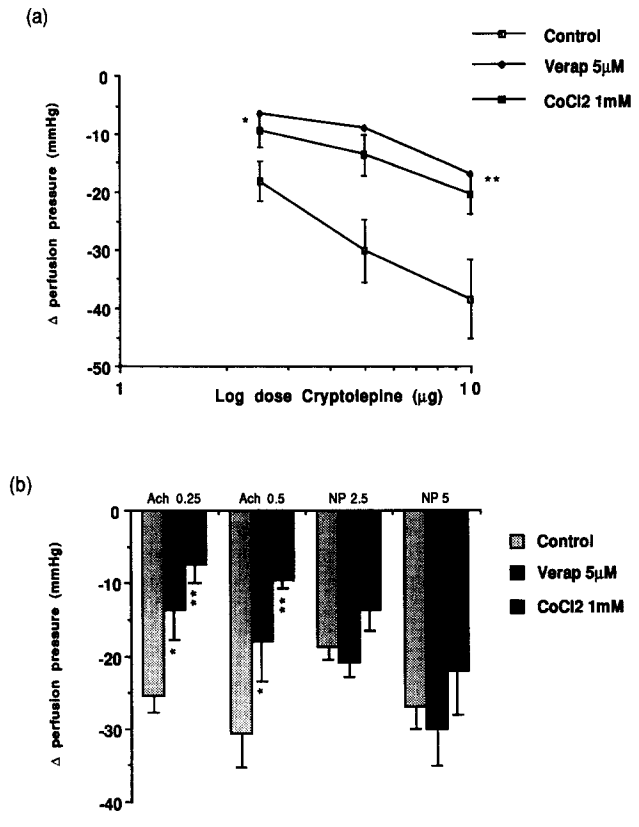


Fig. 6. The reductions in perfusion pressure by cryptolepine (a) and acetylcholine (Ach, 0.25 and 0.5 μg) and nitroprusside (NP, 2.5 and 5 μg) (b) in kidneys perfused with Krebs buffer containing verapamil (Verap, 5 μM) and cobalt chloride ($CoCl_2$, 1 mM). Verapamil-treated kidneys were perfused with phenylephrine (2.5×10^{-6} M). Control and $CoCl_2$-treated kidneys were preconstricted with phenylephrine (5×10^{-7} M). $^{*}P < 0.05$, $^{**}P < 0.01$. Data were compared between control kidneys and those treated with verapamil and $CoCl_2$.

$CoCl_2$ (1 mM), the inorganic Ca^{2+} channel blocker, also inhibited the vasodilator responses to cryptolepine and acetylcholine without affecting the vasodilator response to nitroprusside (Fig. 6a and b). There was no effect on basal perfusion pressure but phenylephrine-induced tone was reduced. A 5-fold increase in the usual concentration of phenylephrine was therefore required to increase tone to levels (85.8 ± 4.8 mm Hg) comparable to those in control kidneys.

4. Discussion

The results of the present study demonstrated that the vasodilator activity of cryptolepine in the rat kidney is sensitive to Ca^{2+} flux across membranes and mediated through changes in K^+ conductance which appear to involve activation of both Na^+/K^+-ATPase and pertussis toxin-insensitive K^+ channels.

In our previous study (Oyekan, 1994), cryptolepine was reported to be an endothelium-dependent vasodilator that releases endothelium-derived relaxing factor (EDRF), possibly nitric oxide.

EDRF is capable of increasing potassium permeability, hence, agonists that release EDRF stimulated the efflux of K^+ from vascular tissues through Na^+/K^+-ATPase and/or K^+ channels (Webb and Bohr, 1978; Bolton et al., 1983; Peterson and Maruyama, 1984). Since cryptolepine releases EDRF, it was necessary to assess whether it also elicits an increase in K^+ permeability. Procedures known to modify the activity of the Na^+/K^+ pump, namely, infusion of ouabain or K^+-free Krebs-Henseleit solution (Webb and Bohr, 1978; Feletou and Vanhoutte, 1988) were therefore employed to examine the vasodilator effect of cryptolepine. In K^+-free solution, KCl elicited vasodilation and cryptolepine-induced vasodilation was inhibited. Following the infusion of ouabain, the vasodilator responses to cryptolepine and KCl were inhibited. These results suggest that the vasodilation elicited by cryptolepine is dependent on activation of the Na^+/K^+ ATPase. However, neither K^+-free solution, ouabain, nor their combination completely inhibited cryptolepine-induced vasodilation, suggesting the involvement of additional mechanism(s). Since an increase in K^+ conductance can also be brought about through activation of K^+ channels, we examined the vasodilator response to cryptolepine in the presence of K^+ channel blockers. The data from this study suggest that cryptolepine activates K^+ channels which are tetraethyl ammonium-sensitive but glibenclamide-insensitive. This is evidenced by the observation that tetraethyl ammonium, especially at 10 mM, blocked the vasodilator response while glibenclamide, the ATP-dependent K^+ channel blocker, was without effect. However, the vasodilator responses to acetylcholine, and diazoxide, an ATP-dependent K^+ channel activator, were also inhibited while the response to nitroprusside was unaffected.

G-proteins couple a large number of membrane-bound receptors to a variety of subcellular effector systems and thereby transduce receptor-generated signals to alterations in cellular activity. Several distinct G-proteins have been identified and characterized: G_s which stimulates adenylyl cyclase and calcium channels, G_i which inhibits adenylyl cyclase and activates K^+ channels, G_o which may act to inhibit calcium channels and G_z (G_p) which activates phospholipase C (see Flavahan and Vanhoutte, 1990). Pertusssis toxin, which is generally used as a probe to examine the role of various G-proteins in signal transduction mechanisms, catalyzes nicotinamide adenosine diphosphate (NAD)-dependent adenosine diphosphate (ADP)-ribosyl-transferase of the α-subunits of G_i- and G_o- and possibly G_p-proteins at a cysteine residue, resulting in inactivation of the G-proteins. GTP-binding proteins such as G_i (inhibitory G-protein) have generally been implicated in the regulation of the activation of

K^+ channels coupled to receptors for acetylcholine, dopamine and histamine (Sasaki and Sato, 1987). The results of this study with acetylcholine are in agreement with this report but contrast with those of Flavahan and Vanhoutte (1989) and Adeagbo and Malik (1990) for porcine coronary artery and rat mesentery. This is a reflection that agonists have differential requirements for signal transduction mechanisms in different tissues. This is not unusual as another important vasodilator mechanism, namely, activation of guanylate cyclase, was shown not to be important in the rat mesentery (Adeagbo and Malik, 1990). In this study, acetylcholine- and diazoxide-induced vasodilation was inhibited by pertussis toxin treatment. However, the vasodilator response to cryptolepine was not affected. This suggests that K^+ channels associated with vasodilator responses to diazoxide and acetylcholine, but not cryptolepine, are coupled to a pertussis toxin-sensitive GTP binding protein.

The inhibition of vasodilator responses to cryptolepine and acetylcholine by Ca^{2+} channel antagonists is an interesting finding. This finding suggests that Ca^{2+} flux across membranes plays a crucial role in the vasodilator responses to these agonists. Since the synthesis and/or release of EDRF is a Ca^{2+}-dependent process (Furchgott, 1983; Long and Stone, 1985), it is not surprising that the vasodilator effects of these EDRF-releasing agonists, cryptolepine and acetylcholine, were inhibited by Ca^{2+} channel blockers. The lack of effect of the Ca^{2+} channel antagonists on vasodilation induced by nitroprusside demonstrates the specificity of the effect to both acetylcholine and cryptolepine.

This study showed that many mechanisms are involved in the vasodilator effect of cryptolepine. The mechanisms, though many are interwoven, are directly or indirectly related to the central mechanism involving the release of EDRF. Thus, the release of EDRF, a Ca^{2+}-dependent process, is followed by an increase in K^+ permeability which elicits K^+ efflux through Na^+/K^+-ATPase and/or K^+ channels. Therefore, it is difficult to determine individually the relative contributions of the different mechanisms to the response observed. However, the marked inhibition of vasodilation following inhibition of Ca^{2+} and K^+ channels suggests that these mechanisms are the more important ones.

In conclusion, data from this study demonstrate that the vasodilator effect of cryptolepine is regulated by Ca^{2+}. In addition, the effect is mediated via K^+ efflux, notably through activation of tetraethyl ammonium-sensitive but glibenclamide-insensitive K^+ channels and Na^+/K^+-ATPase. The K^+ channels activated by cryptolepine are not coupled to a pertussis toxin-sensitive GTP-binding protein.

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