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K^+-induced vasodilation in the rat kidney is dependent on the endothelium and activation of K^+ channels

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Abstract

Increased extracellular K^+ is reported to cause endothelium-independent vasodilation and K^+ has been proposed as an endothelium-derived hyperpolarizing factor. However, the endothelium is endowed with K^+ channels that may also be responsive to increased K^+. We examined the vasodilator effect of bolus administration of 20, 40 and 60 μmol KCl in the rat isolated kidney in which perfusion pressure was elevated with phenylephrine. KCl produced dose-dependent vasodilator responses that were virtually abolished by removal of the endothelium which also abolished the vasodilator effect of bradykinin without affecting that to nitroprusside. The vasodilator effect of KCl was unaffected by inhibition of cyclooxygenase, nitric oxide synthase or cytochrome P450 but reduced by inhibition of K^+ channels with tetraethylammonium (TEA). Barium chloride reduced the vasodilator effects of KCl but charybdotoxin/apamin was without effect. These results indicate that KCl results in endothelium-dependent vasodilation that is independent of nitric oxide (NO), prostaglandins and cytochrome P450 but dependent on activation of endothelial K^+ channels.

Keywords: K^+; Renal vasodilation; Endothelium-dependent; K^+ channel

1. Introduction

Increases in extracellular K^+, to levels that may be achieved in vivo, have long been recognised to relax vascular smooth muscle (Bunger et al., 1976) as a result of hyperpolarization (Edwards et al., 1988) which, in turn, is thought to inhibit voltage-dependent Ca^{2+} channels and reduce intracellular Ca^{2+} (Nelson and Quayle, 1995). As a result of its vasorelaxant activity, K^+ has been invoked as a mediator of autoregulatory responses in the coronary and cerebral vascular beds (Kuschinsky et al., 1972; Edwards et al., 1988; McCarron and Halpern, 1990; Knot et al., 1996). The hyperpolarizing effect of K^+ has been reported to result from activation of Ba^{2+}-sensitive K^+ channels, i.e., the inwardly rectifying channel (McCarron and Halpern, 1990; Knot et al., 1996; Edwards et al., 1998), and/or stimulation of Na^+ K^+ ATPase (McCarron and Halpern, 1990; Prior et al., 1998; Edwards et al., 1998). Most studies have revealed that the dilator effect of K^+ which is variously reported to be transient or sustained, is independent of the endothelium (McCarron and Halpern, 1990; Knot et al., 1996; Prior et al., 1998; Edwards et al., 1998). However, endothelial cells also possess K^+ channels and Na^+ K^+ ATPase which should also be responsive to increases in extracellular K^+. Hyperpolarization of endothelial cells, in contrast to vascular smooth muscle cells, results in increased intracellular Ca^{2+} and activates processes involved in the release of endothelial factors such as nitric oxide (NO), prostaglandins and, presumably, endothelium-derived hyperpolarizing factor (EDHF) (Luckhoff and Busse, 1990). Consequently, the effects of increases in extracellular K^+ may not simply reflect a direct action on vascular smooth muscle.

We used the isolated kidney of the rat to examine the vascular effects of K^+ in terms of dependency on the endothelium, release of endothelial-derived factors such as NO, epoxyeicosatrienoic acids (EETs) and prostanoids and activation of K^+ channels.

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2. Methods

2.1. Animals and experimental procedure

Male Wistar rats weighing 300–450 g were used in these studies. The European Community guidelines for the use of experimental animals were adhered to. The isolated perfused kidney was prepared as previously described (Rapacon et al., 1996). Briefly, rats were anesthetized with pentobarbital (65 mg/kg i.p.) and the right kidney exposed by midline laparotomy. The renal artery was cannulated via the mesenteric artery to prevent interruption of blood flow and the kidney perfused in situ with oxygenated Krebs' buffer at 37 °C. The vena cava was cut to allow exit of the perfusate and the ureter transected. Perfusate flow (8–10 ml/min) was adjusted to obtain a basal perfusion pressure of 60–100 mm Hg which was increased to approximately 200–250 mm Hg to elevate vascular tone and amplify vasodilator responses by adding phenylephrine to the perfusing solution. Once a stable elevated perfusion pressure was obtained, vasodilator responses to bradykinin (30 ng) and nitroprusside (1 μg) were first determined in all groups to assess the effectiveness and specificity of any interventions. Responses to bradykinin are dependent on the endothelium and, in the rat kidney, on cytochrome P450 and K^+ channel activation, whereas responses to nitroprusside are mediated by a direct effect on the vascular smooth muscle. Subsequently, vascular responses to KCl (20, 40 and 60 μmol) were compared in untreated kidneys, those in which the endothelium was removed, and those in which prostaglandin synthesis, NO synthesis, cytochrome P450, K^+ channels or gap junctions were inhibited. The endothelium was removed by perfusing the kidney with distilled water (4 ml) and air (2–3 ml) whereas prostaglandin synthesis, NO synthesis and cytochrome P450 were inhibited with indomethacin (2.8 μM), nitroarginine (50 μM) and clotrimazole (1 μM), respectively. Tetraethylammonium (TEA; 10 mM) was used as a general inhibitor of K^+ channels whereas barium chloride (30 μM) was used to inhibit inwardly rectifying K^+ channels which have been implicated in the vasorelaxant effect of K^+ (McCarron and Halpern, 1990; Knot et al., 1996; Edwards et al., 1998). We also used a combination of charybdotoxin (50 nM) and apamin (100 nM) to inhibit responses attributed to EDHF (Busse et al., 2002). Experiments were also conducted in the presence of glibenclamide (1 and 10 μM) to address the contribution of hyperosmolarity to the vasodilator effect of KCl as ATP-sensitive K^+ channels are reported to mediate the vasodilator effect of hyperosmolar solutions (Ishizaka and Kuo, 1997). Carbenoxolone (200 μM) was used as an inhibitor of gap junctions, based on the report by Chaytor et al. (2000). In all experiments, the various pharmacological inhibitors were added to the perfusate before elevating perfusion pressure with phenylephrine.

In untreated kidneys and those exposed to indomethacin and TEA, 7.5×10^{-7} M phenylephrine was used to elevate renal perfusion pressure. In the presence of nitroarginine, the requirement for phenylephrine was less ($2–4\times10^{-7}$ M) whereas in kidneys subjected to endothelial denudation, the requirement for phenylephrine to raise perfusion pressure was increased to 10^{-6} M.

2.2. Materials

All drugs were purchased from Sigma Chemical, St. Louis, MO. Indomethacin was dissolved in 4.2% $NaHCO_3$, glibenclamide and clotrimazole in ethanol and the other agents in distilled water.

2.3. Statistical analysis

Results were compared by analysis of variance and when significance was shown, individual points were compared by a Student's *t*-test; $p<0.05$ was considered statistically significant. Data are expressed as means±S.E.M.

3. Results

3.1. Basal and elevated renal perfusion pressures in the various treatment groups

Basal perfusion pressures in the various groups were 83±4 mm Hg for the control group (n=7), 94±2 mm Hg for endothelium denuded group (n=5), 82±6 mm Hg for the indomethacin-treated group (n=3), 78±3 mm Hg for the nitroarginine- and indomethacin-treated group (n=7), 80±2 mm Hg for the clotrimazole-treated group (n=6), 77±5 mm Hg for the glibenclamide-treated group (n=4) and 91±2 mm Hg for the TEA-treated group (n=5). Removal of the endothelium resulted in a rapid elevation in perfusion pressure which then returned to a value below the basal perfusion pressure to 66±4 mm Hg. Elevated perfusion pressures were 233±5 mm Hg for the control group, 208±12 mm Hg for the endothelium denuded group, 244±1 mm Hg for the indomethacin-treated group, 242±7 mm Hg for the indomethacin plus nitroarginine-treated group, 240±10 mm Hg for the clotrimazole-treated group, 242±11 mm Hg for the glibenclamide-treated group and 215±19 mm Hg for the TEA-treated group.

The studies to address the effects of barium chloride, the combination of apamin/charybdotoxin and carbenoxolone were conducted independently of those described above and, therefore, had their own control groups. The basal perfusion pressure for the control group for barium chloride was 76±4 mm Hg (n=5) and 82±6 mm Hg for the treated group (n=4); the elevated perfusion pressures were 218±8 mm Hg and 216±12 mm Hg, respectively. The basal perfusion pressures for the charybdotoxin/apamin-treated group (n=3) and its respective control group (n=3) were 75±6 mm Hg and 73±4 mm Hg and the corresponding elevated perfusion pressures were 235±4 mm Hg and

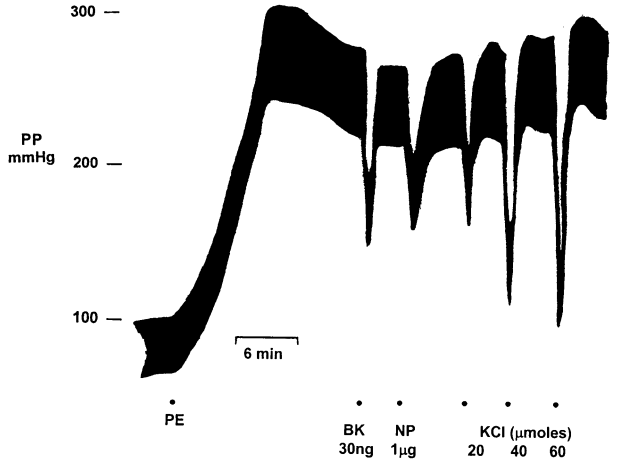

Fig. 1. Recording of perfusion pressure (PP) in the isolated kidney constricted with phenylephrine showing vasodilator responses to bradykinin (BK), nitroprusside (NP) and KCl.

247±4 mm Hg. Basal perfusion pressures for the carbenoxolone-treated group and the respective control group were 89±4 and 66±6 mm Hg whereas the elevated perfusion pressures were 220±6 and 230±21 mm Hg, respectively.

3.2. Effect of removal of the endothelium on vasodilator responses

In phenylephrine preconstricted kidneys (n=7), 20, 40 and 60 μmol KCl caused rapid and reproducible dose-dependent falls in perfusion pressure of 31±4, 69±7 and 98±8 mm Hg, respectively (Figs. 1 and 2). Bradykinin (30 ng) and nitroprusside (1 μg) decreased perfusion pressure by 54±7 mm Hg and 46±5 mm Hg, respectively. In kidneys perfused briefly with air and water to remove the endothelium (n=5), the vasodilator response to bradykinin was almost abolished, 3±1 mm Hg (p<0.01), confirming the effectiveness of the procedure (Fig. 2). However, the vasodilator effect of nitroprusside was unaffected, 57±10 mm Hg, indicating that the capacity of vascular smooth muscle to relax was unimpaired. In the absence of endothelium the vasodilator effect of KCl was greatly impaired (p<0.01), 20,40 and 60 μmol eliciting falls in perfusion pressure of only 6±2, 14±4 and 20±4 mm Hg, respectively (Fig. 2).

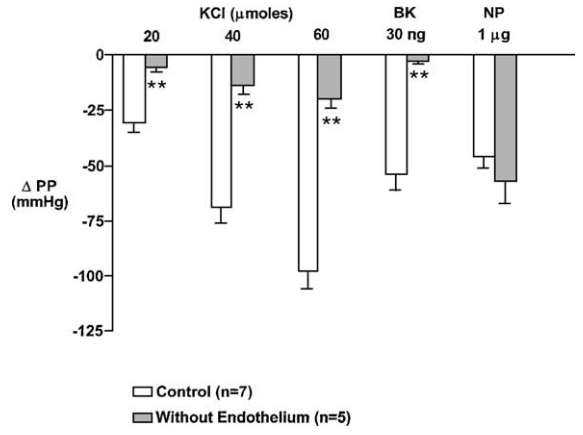

Fig. 2. Vasodilator responses, expressed as decreases in perfusion pressure (PP), to bradykinin (BK), nitroprusside (NP) and KCl in phenylephrine-constricted kidneys under control conditions (n=7; open bars) and following removal of the endothelium (n=5; stippled bars). **p<0.01.

3.3. No role for prostaglandins, cytochrome P450 and NO in vasodilator responses to KCl

To examine a role for prostaglandins and/or NO in the vasodilator effect of KCl, indomethacin and a combination of indomethacin and nitroarginine were used. In kidneys treated with indomethacin alone (n=3) to inhibit prostaglandin synthesis, vasodilator responses to KCl, bradykinin and nitroprusside were not different from those obtained in untreated kidneys (n=7). Thus, 20, 40 and 60 μmol KCl decreased perfusion pressure by 34±9, 70±17 and 104±12 mm Hg, respectively, whereas bradykinin and nitroprusside reduced perfusion pressure by 58±5 and 53±7 mm Hg, respectively (Fig. 3). Similarly, in kidneys treated with both indomethacin and nitroarginine (n=7), the vasodilator effect of KCl was almost identical to that obtained in the control group (Fig. 3); the response to bradykinin was also not different whereas the vasodilator effect of nitroprusside was greatly enhanced, 99±5 mm Hg (p<0.01). Inhibition of cytochrome P450 with clotrimazole (n=6) was without effect on vasodilator responses to KCl or nitroprusside but reduced that to bradykinin, 18±6 mm Hg (Fig. 3).

3.4. Contribution of K^+ channels to the vasodilator effect of KCl

We next investigated the role of K^+ channels in the vasodilator effect of KCl using TEA as an inhibitor of K^+

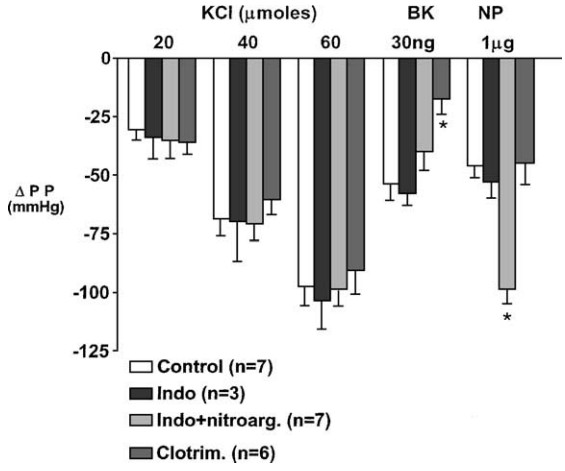

Fig. 3. Changes in perfusion pressure (PP) in response to bradykinin (BK), nitroprusside (NP) and KCl in phenylephrine-constricted kidneys under control conditions (n=7; open bars), following treatment with 2.8 μM indomethacin alone (n=3; dense stippled bars), a combination of 50 μM nitroarginine and 2.8 μM indomethacin (n=7;stippled bars) or 1 μM clotrimazole (n=6; shaded bars). *p<0.05.

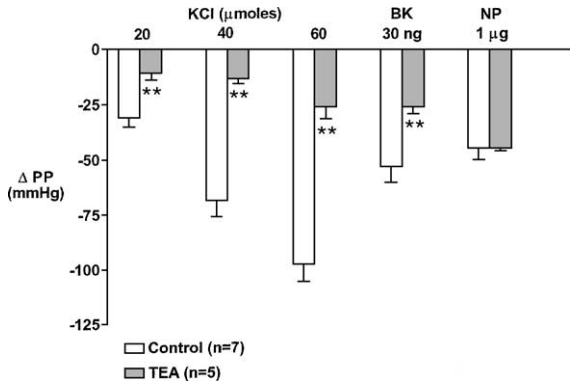

Fig. 4. Effect of inhibition of K^+ channels with 10 mM TEA (n=5; open bars) on changes in perfusion pressure in response to KCl, bradykinin (BK) and nitroprusside (NP). Control, n=7, open bars; TEA, n=5, stippled bars. **p<0.01.

channels. As we reported previously (Rapacon et al., 1996), TEA reduced the vasodilator effect of bradykinin, 27±3 mm Hg (n=5) compared to 54±7 mm Hg for the control group (n=7), but was without effect on the vasodilator activity of nitroprusside, 46±1 mm Hg (Fig. 4). In the presence of TEA, 20, 40 and 60 μmol KCl reduced perfusion pressure by only 11±3, 14±2 and 27±5 mm Hg, respectively (Fig. 4). Moreover, 120 μmol KCl, which produced maximal vasodilation in untreated kidneys, elicited a vasoconstrictor response in the presence of TEA, increasing perfusion pressure by 125±27 mm Hg (data not shown).

We considered that the vasodilator effect of bolus administration of KCl may result from a hyperosmolar effect that has been reported to be endothelium-dependent and mediated via activation of ATP-sensitive K^+ channels that are blocked by glibenclamide (Ishizaka and Kuo, 1997). However, in the presence of glibenclamide (n=4) which abolished the vasodilator response to cromakalim (Rapacon et al., 1996), vasodilator responses to KCl were unaffected,

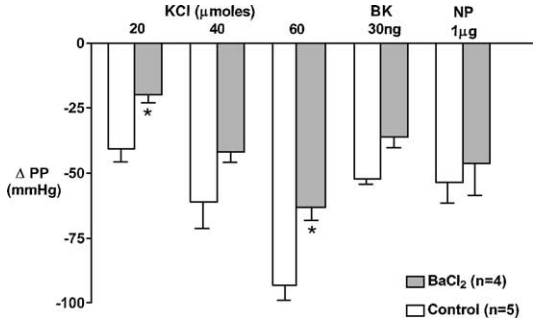

Fig. 5. Changes in perfusion pressure (PP) in response to bradykinin (BK), nitroprusside (NP) and KCl in phenylephrine-constricted kidneys under control conditions (n=5; open bars) and following treatment with 30 μM $BaCl_2$ (n=4; stippled bars). *p<0.05.

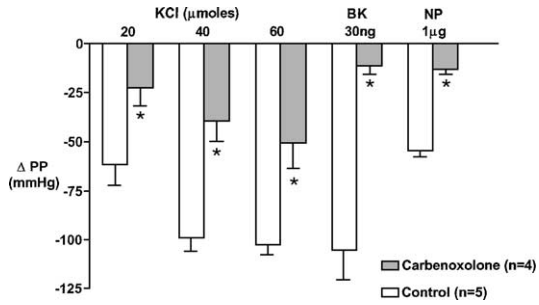

Fig. 6. Changes in perfusion pressure (PP) in response to bradykinin (BK), nitroprusside (NP) and KCl in phenylephrine-constricted kidneys under control conditions (n=5; open bars) and following treatment with 200 μM carbenoxolone (n=4; stippled bars). *p<0.05.

20, 40 and 60 μmol reducing perfusion pressure by 35±7, 76±10 and 108±8 mm Hg, respectively, compared to 34±9, 70±17 and 104±12 mm Hg, respectively, for the control group (n=7). Moreover, we found no effect of dextrose administered in a bolus dose to achieve the same osmolarity as that for KCl.

As TEA reduced the vasodilator responses to KCl, indicating a role for K^+ channels, we next investigated the contribution of EDHF using the combination of charybdotoxin/apamin; susceptibility to inhibition by this combination is considered a criterion for EDHF-mediated responses (Busse et al., 2002). Charybdotoxin/apamin did not affect the response to nitroprusside, 40±7 mm Hg (n=3) compared to 42±4 mm Hg for the control group (n=3) but reduced that to bradykinin, 20±10 mm Hg versus 45±5 mm Hg for the control group. However, responses to KCl were unaffected; in the presence of charybdotoxin/apamin, 20, 40 and 60 μmol KCl reduced perfusion pressure by 40±5, 79±9 and 107±6 mm Hg, respectively, compared to 40±2, 75±9 and 99±9 mm Hg, respectively, in the control group.

We also tested the effects of barium chloride on the vasodilator responses to KCl as K^+-induced vasodilation has been attributed to activation of inwardly rectifying K^+ channels (McCarron and Halpern, 1990; Knot et al., 1996; Edwards et al., 1998). Barium chloride reduced the responses to KCl (Fig. 5) with the greatest effect on the lowest dose of KCl used (n=5). In contrast, the vasodilator responses to bradykinin and nitroprusside were not affected by barium chloride treatment.

3.5. Effect of the gap junction inhibitor, carbenoxolone on vasodilator responses to KCl

In the presence of carbenoxolone (200 μM) to inhibit gap junctions (n=4), responses to 20, 40 and 60 μmol KCl were reduced to 23±9, 40±10 and 51±13 mm Hg, respectively, from control (n=5) values of 62±10, 99±7 and 103±5 mm Hg, respectively (Fig. 6). In the presence of carbenoxolone, responses to bradykinin and nitroprusside were also reduced.

4. Discussion

The results of this study confirm those of others showing that increases in extracellular K^+ elicit vasodilation. It has been reported that K^+ dilates the coronary and cerebral vascular beds where it has been suggested to play a role in autoregulation of blood flow (Bunger et al., 1976; Kuschinsky et al., 1972; Edwards et al., 1988; McCarron and Halpern, 1990). More recently, it was proposed that K^+, released from the endothelium in response to acetylcholine, may fulfil the role of a hyperpolarizing factor in rat hepatic artery (Edwards et al., 1998). Thus, increases in K^+ equivalent to those produced by acetylcholine were reported to produce sustained, endothelium-independent relaxation of vascular smooth muscle associated with hyperpolarization (Edwards et al., 1998). Our results indicate that this is not the case for the renal vascular bed of the rat, supporting the results of Jiang and Dusting (2001) who reported an endothelium-dependent relaxant effect to K^+ in isolated renal arteries of the rat. Similar findings were reported for the rat perfused mesenteric vasculature (Harris et al., 2000), porcine coronary arteries (Beny and Schaad, 2000) and human subcutaneous resistance arteries (McIntyre et al., 2001). Under our experimental conditions, increases in K^+ by bolus administration of KCl resulted in dose-dependent vasodilator effects that were clearly dependent on the presence of an intact endothelium. Thus, removal of the endothelium almost abolished the vasodilator activity of K^+ without affecting the ability of nitroprusside to reduce perfusion pressure, indicating that the reduced response to K^+ was not the result of diminished capacity for vasodilation. Similarly, the slightly lower elevated perfusion pressure in this group could not account for the diminished response to KCl as responses to nitroprusside remained intact. These observations are in contrast to those reported by others demonstrating a direct vasorelaxant effect of K^+ on vascular smooth muscle that results from hyperpolarization (McCarron and Halpern, 1990; Knot et al., 1996; Prior et al., 1998; Edwards et al., 1988). We found that the vasodilation to K^+ was endothelium-dependent but we cannot exclude a contribution of hyperpolarization of vascular smooth muscle. The explanation for the differences is not readily apparent but may relate to different vascular beds under study, bolus administration of KCl in our experiments compared to increases in perfusate levels in the other studies and the possibility that endothelial K^+ channels may also be activated and, ultimately, lead to release of a vasorelaxant factor. Alternatively, removal of the endothelium in some way impairs the activity of vascular smooth muscle K^+ channels and masks any direct effect of K^+, a possibility we cannot exclude as sensitivity to the vasoconstrictor effect of phenylephrine decreased, suggesting that the method employed to remove the endothelium may have damaged the underlying vascular smooth muscle. With respect to the first possibility, vascular tissue from different sites may not respond the same to K^+ depending on the size of the vessel and the presence of different types of K^+ channels. For example, rings of rat aorta with or without endothelium and under basal tension of 2 g or precontracted with U46619, an endoperoxide analogue, contract rather than relax in response to incremental increases in K^+ in the bathing fluid (unpublished observations; data not shown) indicating depolarization rather than hyperpolarization. In three experiments we also examined the effect of incremental increases in perfusate K^+. Increasing perfusate K^+ from a control level of 5 mM to 10, 15 and 20 mM resulted in vasodilation of phenylephrine-constricted kidneys that was not sustained and perfusion pressure returned to the pretreatment level (data not shown). This may be due to activation of the electrogenic Na^+ pump whereby the resultant hyperpolarization in response to elevation of K^+ concentration, for example, higher than 10 mM, is known to be transient.

Although the possibility of a hyperosmolar effect on the endothelium and activation of ATP-sensitive K^+ channels has been presented (Ishizaka and Kuo, 1997), our results exclude this possibility, assuming a similar mechanism obtained in the rat kidney, as glibenclamide did not affect the vasodilator activity of KCl. Moreover, we found no effect of dextrose administered in a bolus dose to achieve the same osmolarity as that for KCl. We also excluded the possibility that pH may contribute to the vasodilator effect of KCl in the perfused kidney as neutralising the solution did not reduce the vasodilator activity.

Based on our observations, there is the distinct possibility that the vasodilator activity of K^+ in the perfused kidney results from an effect on endothelial K^+ channels which, in turn, leads to the release of a vasorelaxant factor or, alternatively, results in the transmission of the hyperpolarization from endothelium to the underlying smooth muscle via electrical conduction (Von de Weid and Beny, 1993). That K^+ channels and, thereby, hyperpolarization, are involved is inferred from the results with TEA which reduced the vasodilator effect of KCl by 65% without affecting dilator responses to nitroprusside. The concentration of TEA used is reported to block most types of K^+ channels (Kuriyama et al., 1995) and, therefore, provides no insight to the specific type of channel involved in the response to K^+. Although these results show that K^+ acts on channels in the endothelium, we cannot say that the effect of TEA is confined to the endothelium. However, the results are consistent with K^+ acting on endothelial K^+ channels to initiate events resulting in release of a vasorelaxant mediator that may or may not activate K^+ channels in vascular smooth muscle. A contribution for EDHF was excluded as the combination of apamin/charybdotoxin was without effect on KCl-induced vasodilation whereas responses to bradykinin, which exhibit an EDHF component, were reduced. Thus, this combination of K^+ channel inhibitors is considered a criterion for EDHF involvement in vasodilator responses in rat arteries (Edwards et al., 1998). In contrast, in the presence of barium to inhibit inwardly

rectifying K^+ channels, which have been reported to contribute to K^+-induced vasorelaxation (Chrissobolis et al., 2000), we found that the vasodilator effects of KCl were impaired suggesting a role for endothelial inwardly rectifying K^+ channels in the response. The effect of barium could not be attributed to impairment of vasodilation as responses to both bradykinin and nitroprusside were unaffected. These results indicate that the EDHF-mediated response to bradykinin does not involve barium-sensitive K^+ channels and are consistent with those reported by others (Beny and Schaad, 2000). It is of interest to note that inwardly rectifying, barium-sensitive K^+ channels were restricted to the endothelium of rat mesenteric artery (Crane et al., 2003). In addition to inwardly rectifying K^+ channels, the endothelium possesses Na^+K^+ATPase that is activated by elevated K^+ and would also contribute to hyperpolarization due to the exchange of three Na^+ leaving the cell for two K^+ entering the cell. The resulting hyperpolarization could then be electrotonically transmitted to the underlying vascular smooth muscle. Alternatively, hyperpolarization of the endothelium would increase the electrochemical gradient for Ca^{2+} entry that leads to the release of a diffusible factor that relaxes vascular smooth muscle.

We excluded the possibility that the endothelium-dependent effect of K^+ was mediated by a prostaglandin, NO or a product of cytochrome P450 as responses to KCl were not affected by indomethacin, nitroarginine or clotrimazole, respectively. The concentration of indomethacin we used has been shown to reduce stimulated prostaglandin synthesis by over 85% (Sarubbi et al., 1989) while the concentration of nitroarginine is sufficient to inhibit NO synthesis (Cachofeiro and Nasjletti, 1991). This conclusion is supported by the observations that the requirement for phenylephrine to elevate renal perfusion pressure was reduced by at least a factor of two and that in the presence of nitroarginine, vasodilator responses to nitroprusside were markedly increased, an expected consequence of removing background levels of NO. We have previously shown that clotrimazole reduces the NO- and prostaglandin-independent coronary and renal vasodilator effects of bradykinin in the rat (Fulton et al., 1992, 1995) and this was confirmed in the present study where the vasodilator effect of bradykinin was reduced. However, it should be pointed out that if K^+-induced vasodilation is mediated by multiple endothelial factors as is the case with bradykinin, inhibition of one component may be insufficient to produce impairment of the response. As a counterpoint to this caveat, the effect of bradykinin was reduced by both TEA and clotrimazole in the absence of inhibition of NO and prostaglandin synthesis. The exclusion of the established endothelium-derived mediators in the renal vasodilator effect of K^+ necessarily invokes an alternate mechanism of transmission of the signal from the endothelium to the vascular smooth muscle. We considered the most likely mechanism to be electrotonic transmission via gap junctions as was reported by Harris et al. (2000). Consequently, we used carbenoxolone to inhibit gap junctions. Although vasodilator responses to KCl were reduced in the presence of carbenoxolone, suggesting a role of gap junctions, these results must be treated with caution as the renal vasodilator effects of bradykinin and nitroprusside were impaired to an even greater degree, possibly indicating effects of carbenoxolone unrelated to inhibition of gap junctions, for example, carbenoxolone is a derivative of glycyrrhetinic acid which has been shown to inhibit Na^+K^+ATPase. Earlier studies with carbenoxolone (unpublished) revealed that 100 μM concentrations were without effect on renal vasodilator responses to bradykinin.

In summary, K^+ administered intraarterially to the kidney results in endothelium-dependent vasodilation that is independent of prostaglandins, NO and cytochrome P450 but requires activation of K^+ channels in the endothelium.

Acknowledgements

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References

Beny, J.L., Schaad, O., 2000. An evaluation of potassium ions as endothelium-derived hyperpolarizing factor in porcine coronary arteries. Br. J. Pharmacol. 131, 965–973.

Bunger, R., Haddy, F.J., Querengasser, A., Gerlach, E., 1976. Studies on potassium-induced coronary dilation in the isolated guinea pig heart. Pflugers Arch. 363, 27–31.

Busse, R., Edwards, G., Feletou, M., Fleming, I., Vanhoutte, P.M., Weston, A.H., 2002. EDHF: bringing the concepts together. Trends Pharmacol. Sci. 23, 374–380.

Cachofeiro, V., Nasjletti, A., 1991. Increased vascular responsiveness to bradykinin in kidneys of spontaneously hypertensive rats: effects of nitro-L-arginine. Hypertension 18, 683–688.

Chaytor, A.T., Marsh, W.L., Hutcheson, I.R., Griffith, T.M., 2000. Comparison of glycyrrhetinic acid isoforms and carbenoxolone as inhibitors of EDHF-type relaxations mediated via gap junctions. Endothelium 76, 265–278.

Chrissobolis, S., Ziogas, J., Chu, Y., Faraci, F.M., Sobey, C.G., 2000. Role of inwardly rectifying K^+ channels in K^+-induced cerebral vasodilatation in vivo. Am. J. Physiol. 279, H2704–H2712.

Crane, G.J., Walker, S.D., Dora, K.A., Garland, C.J., 2003. Evidence for a differential cellular distribution of inward rectifier K channels in the rat isolated mesenteric artery. J. Vasc. Res. 40, 159–168.

Edwards, F.R., Hirst, G.D.S., Silverberg, G.D., 1988. Inward rectification in rat cerebral arterioles, involvement of potassium ions in autoregulation. J. Physiol. 404, 455–466.

Edwards, G., Dora, K.A., Gardener, M.J., Garland, C.J., Weston, A.H., 1998. K^+ is an endothelium-derived hyperpolarizing factor in rat arteries. Nature 396, 269–272.

Fulton, D., McGiff, J.C., Quilley, J., 1992. Contribution of NO and cytochrome P450 to the vasodilator effect of bradykinin in the rat kidney. Br. J. Pharmacol. 107, 722–725.

Fulton, D., Mahboubi, K., McGiff, J.C., Quilley, J., 1995. Cytochrome P450-dependent effects of bradykinin in the rat heart. Br. J. Pharmacol. 114, 99–102.

Harris, D., Martin, P.E.M., Evans, W.H., Kendall, D.A., Griffith, T.M., Randall, M.D., 2000. Role of gap junctions in endothelium-derived hyperpolarizing factor responses and mechanisms of K^+-induced relaxation. Eur. J. Pharmacol. 402, 119–128.

Ishizaka, H., Kuo, L., 1997. Endothelial ATP-sensitive potassium channels mediate coronary microvascular dilation to hyperosmolarity. Am. J. Physiol. 273, H104–H112.

Jiang, F., Dusting, G.J., 2001. Endothelium-dependent vasorelaxation independent of nitric oxide and K^+ release in isolated renal arteries of rats. Br. J. Pharmacol. 132, 1558–1564.

Knot, H.J., Zimmerman, P.A., Nelson, M.T., 1996. Extracellular K^+-induced hyperpolarizations and dilatations of rat coronary and cerebral arteries involve inward rectifier K^+ channels. J. Physiol. 492, 419–430.

Kuriyama, H., Kitamura, K., Nabata, H., 1995. Pharmacological and physiological significance of ion channels and factors that modulate them in vascular tissues. Pharmacol. Rev. 47, 391–573.

Kuschinsky, W., Wahl, M., Bosse, O., Thurau, K., 1972. Perivascular potassium and pH as determinants of local pial arterial diameter in cats. Circ. Res. 31, 240–247.

Luckhoff, A., Busse, R., 1990. Activators of potassium channels enhance calcium influx into endothelial cells as a consequence of potassium currents. Naunyn-Schmiedeberg's Arch. Pharmacol. 342, 94–99.

McCarron, J.G., Halpern, W., 1990. Potassium dilates rat cerebral arteries by two independent mechanisms. Am. J. Physiol. 259, H902–H908.

McIntyre, C.A., Buckley, C.H., Jones, G.C., Sandeep, T.C., Andrews, R.C., Elliot, A.I., Gray, G.A., Williams, B.C., McKnight, J.A., Walker, B.R., Hadoke, P.W., 2001. Endothelium-derived hyperpolarizing factor and potassium use different mechanisms to induce relaxation of human subcutaneous resistance arteries. Br. J. Pharmacol. 133, 902–908.

Nelson, M.T., Quayle, J.M., 1995. Physiological roles and properties of potassium channels in arterial smooth muscle. Am. J. Physiol. 268, C799–C822.

Prior, H.M., Webster, N., Quinn, K., Beech, D.J., Yates, M.S., 1998. K^+-induced dilation of a small renal artery: no role for inward rectifier K^+ channels. Cardiovasc. Res. 37, 780–790.

Rapacon, M., Mieyal, P., McGiff, J.C., Fulton, D., Quilley, J., 1996. Contribution of calcium-activated potassium channels to the vasodilator effect of bradykinin in the isolated, perfused kidney of the rat. Br. J. Pharmacol. 118, 1504–1508.

Sarubbi, D., McGiff, J.C., Quilley, J., 1989. Renal vascular responses and eicosanoid release in diabetic rats. Am. J. Physiol. 257, F762–F768.

Von de Weid, P.Y., Beny, J.-L., 1993. Simultaneous oscillations in the membrane potential of pig coronary artery endothelial cells and smooth muscle cells. J. Physiol. 471, 13–24.