Cellular mechanisms underlying cutaneous pressure-induced vasodilation: in vivo involvement of potassium channels

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Submitted 6 October 2004; accepted in final form 18 February 2005

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The local control of the cutaneous circulation includes a vasodilator response to local, nonpainful pressure application (12). This neurally mediated pressure-induced vasodilation (PIV) is observed in humans (12), rats (14), and mice (31). This increase in cutaneous blood flow delays the occurrence of ischemia due to applied pressure, thus protecting the skin against pressure. In diabetic patients, a rapid decrease of cutaneous blood flow in response to locally applied pressure was observed (11), as this protective mechanism is absent in diabetes (21). Because the absence of this protective mechanism of the skin against pressure could be involved in the development of pressure sores, e.g., diabetic foot, the study of the underlying cellular mechanisms of PIV deserves further investigations.

The development of PIV depends on the activation of capsaicin-sensitive nerve fibers leading to the release of calcitonin gene-related peptide (CGRP), vasoactive intestinal peptide (VIP), and/or pituitary adenylate cyclase-activating polypeptide (PACAP) (9, 12, 14). These neuropeptides then act at the endothelial level to synthesize and release endothelial factors. We have demonstrated the need of an intact endothelium for the development of PIV. Indeed, endothelial nitric oxide (NO) and prostaglandins play major roles in PIV, because PIV is absent after acute inhibition of NO synthase or cyclooxygenase (14). Additionally, in physiological conditions, PIV was unchanged after EDHF inhibition (18). It has been extensively reviewed that a number of endogenous vasodilators (e.g., CGRP, VIP, and PACAP) and both NO and prostaglandins may act in part by activation of K⁺ channels (24, 26). Indeed, the opening of K⁺ channels increases K⁺ efflux, causing membrane hyperpolarization. This hyperpolarization is associated with the synthesis and the release of endothelial-derived relaxing factors in endothelial cells (25), whereas it leads to the relaxation in smooth muscle cells (32).

Vascular smooth muscle large-conductance Ca²⁺-activated K⁺ (BKCa) channels have been reported to be important in NO-dependent vasodilation (35), whereas the activation of endothelial small-conductance Ca²⁺-activated K⁺ (SKCa) channels is involved in EDHF-related relaxation (37). Although it was reported that NO promotes the opening of voltage-sensitive K⁺ (KV) channels in pulmonary arterial smooth muscle cells (39), the opening of these channels was not involved in the relaxation in response to endothelium-derived NO in the rat basilar artery (1). The neuropeptides CGRP, VIP, and PACAP, involved in the development of PIV (9, 14), may activate ATP-sensitive K⁺ (KATP) channels (26) to relax the blood vessels. Moreover, it has been frequently reported that vascular smooth muscle KATP channels are activated mainly by the prostaglandin/cAMP pathway and also by the NO/cGMP pathway (24, 26, 32), leading to blood vessels relaxation. With this information as a background, our goals were to examine the in vivo involvement of BKCa, SKCa, KV, and KATP channels in the cellular mechanisms leading to PIV using a pharmacological approach in anesthetized rats. To assess whether the K⁺ channels involved in PIV were endothelial or vascular smooth muscle in origin, the vasodilator...
responses to both exogenous nitrovasodilator [sodium nitroprusside (SNP)] and endogenous receptor-mediated endothelial factor release [acetylcholine (ACh)] were also performed with or without blockade of these K⁺ channels.

METHODS

Animal instrumentation. The studies were performed in Wistar rats (200–300 g). Before the experiments, animals had free access to standard laboratory food and water. Animals were housed in a regulated environment with a constant ambient temperature of 24°C. Procedures for the maintenance and use of the experimental animals were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23, Revised 1996). Experimental procedures were approved by the local ethics committee for animal experimentation.

To present a hairless area for cutaneous blood flow measurements, local pressure application, and iontophoretic stimulation, hair was removed from the skin from the skull to the back of the animals with a depilatory lotion. This was performed 2 days before the experiment to prevent cutaneous irritation from confounding the results.

For the experiments, animals were anesthetized by an intraperitoneal injection of thiopental at a dose of 50 mg/kg body wt. The level of anesthesia was determined by testing eye reflexes and tail pinch. Rats were placed in an incubator (MMS; Chelles, France) warmed to 30°C to maintain stable body temperature throughout the experiment (37.5 ± 0.5°C), which was monitored with a rectal thermometer. The left femoral vein was cannulated for drug or vehicle administration. A catheter was also placed in the right femoral vein for K⁺ channel opener administration when necessary. Mean arterial blood pressure (MABP) was measured throughout the recordings by pressure transducer (Mallinckrodt, Medica; Dublin, Ireland) connected to the left femoral arterial catheter. The animals were placed in the prone position, and the head was fixed on a frame.

Cutaneous blood flow, MABP, and rectal temperature were continuously recorded by a data-acquisition system (Biopac; Santa Barbara, CA) at a sample frequency of 20 Hz and analyzed off-line by commercially available software (Acqknowledge Biopac). All animals were killed at the end of the experiment by an overdose of thiopental. Cutaneous blood flow and MABP were recorded for 1 min after the death of the rat to assess the "physiological zero" of the measurements, which was subsequently subtracted from the corresponding measured values.

Assessment of PIV. A weighbridge was adapted to hold a laser-Doppler probe (PF408, Perimed). The probe was connected to a laser-Doppler flowmeter (PF4001 Master, Perimed) and had a 12.6-mm² circular contact surface area. This probe allowed for simultaneous local pressure application and cutaneous blood flow measurements at the local pressure application site. The weighbridge was carefully equilibrated, and the probe was then positioned perpendicularly to the skin in the center of the hairless area of skull. The technical details of this method have been described (13). Data collection began with a 1-min basal period before the onset of increasing local pressure. Externally applied pressure was then increased progressively at 11.1 Pa/s (5.0 mmHg/min) for 15 min. Each group consisted of 10 rats. For each protocol, control rats received a volume of vehicle corresponding to the drug administration in treated rats.

To examine the involvement of K⁺ channels, we treated the rats with tetraethylammonium (TEA), a nonspecific K⁺ channel blocker, which was injected intravenously (40 mg/kg) 10 min before the onset of increasing local applied pressure.

BKCa and SKCa channel involvement in PIV mechanotransduction (protocols 2 and 3). To study the role of BKCa channels in PIV, ibotrextoxin, a specific blocker, was infused intravenously (6.0 µg·kg⁻¹·min⁻¹) in treated rats (protocol 2). The infusion was begun 10 min before the local pressure application and was maintained throughout the experiment. The vasodilator hydralazine produces direct relaxation of smooth muscle arteries and arterioles involving the opening of Ca²⁺-activated K⁺ channels leading to a systemic hypotension, which is reduced by iberiotoxin treatment, as previously reported (3). Because hydralazine has a small effect on skin, we examined the systemic blood pressure-lowering effect of a bolus of hydralazine (0.4 mg/kg) injected at the end of the experiment via the other femoral vein to verify the efficacy of iberiotoxin. To specify the role of SKCa channels in PIV, apamin, probably the most specific blocker of these channels (38), was injected intravenously (0.5 mg/kg) 10 min before the local pressure application in treated rats (protocol 3).

Kv channel involvement in PIV mechanotransduction (protocol 4). To specify the role of Kv channels in PIV, we used 4-aminopyridine (4-AP), a specific blocker of these channels in vascular smooth muscle (24). Treated rats received a bolus of 4-AP (1.3 mg/kg) injected intravenously 15 min before the local pressure application. Because 4-AP is highly alkaline in solution, the pH of the saline was adjusted to that of the 4-AP solution (pH 8.5). Bradykinin causes an endothelium-dependent hypotensive response that is dependent on the opening of Kv channels (4). Therefore, we examined this hypotensive response induced by an intravenous bolus of bradykinin (30 µg/kg) injected at the end of the experiment via the other femoral vein to test the efficacy of 4-AP.

KATP channel involvement in PIV mechanotransduction (protocol 5). To examine the role of KATP Channels in PIV, the specific blocker glibenclamide was injected intravenously (20 mg/kg) 10 min before the local pressure application in treated rats. Reductions in peripheral vascular resistance and blood pressure caused by low concentrations of pinacidil are antagonized by glibenclamide (26). To test the efficacy of glibenclamide to block KATP channels, a bolus of pinacidil (0.3 mg/kg), an opener of KATP channels, was injected at the end of the experiment via the other femoral vein. At the end of the experiment, the efficacy of glibenclamide was also tested by measuring the expected hypoglycemia via blood glucose measurement using an Accu-Check Active glucometer (Roche; Lyon, France).

To determine the effect of glibenclamide-induced hypoglycemia in PIV, we tested the development of PIV in a separate group of rats after the administration of gliclazide, another high-potency sulfonylurea. Gliclazide was injected intravenously (15 mg/kg) 10 min before the onset of local pressure application. Because gliclazide shows a specificity for pancreatic β-cell KATP channels over those of the heart and smooth muscle (22), it would not have any effect on the injection of a bolus of pinacidil (as for glibenclamide efficacy). To test the efficacy of gliclazide, we also measured the gliclazide-induced hypoglycemia.

Assessment of endothelium-independent and -dependent vasodilation. Endothelium-independent and -dependent vasodilator responses were assessed with and without blockade of the K⁺ channels that had previously caused a reduction of PIV. Endothelium-independent vasodilation was induced by iontophoretic delivery of SNP (67 mM) using cathodal current (100 µA) for 30 s. Endothelium-dependent vasodilation in the cutaneous microcirculation was induced by iontophoretic delivery of ACh (5.5 mM) using anodal current (100 µA) for 20 s. The iontophoretic delivery was performed over an area of 1.2 cm² using a laser-Doppler probe (481-1, Perimed) positioned on the hairless back skin of the rat. This probe also allowed for cutaneous blood flow recording. Data collection began with a 1-min basal period before the onset of current application and was continuously recorded for 15 min. Preliminary studies were performed to verify that there was no effect of iontophoretic delivery of deionized water with the use of anodal and cathodal currents of 100 µA for 20 s and 30 s, respectively. We studied the involvement of NO and prostaglandins in ACh-induced vasodilation by inhibiting NO synthase and cyclooxygenase with N³ nitro-L-arginine (20 mg/kg iv) and indomethacin (5 mg/kg ip), respectively. The ACh-induced vasodilation was almost abolished after NO synthase inhibition (6.2 ± 1.3% vs. 56.3 ± 6.5%, P < 0.0001) and reduced after cyclooxygenase inhibition (22.0 ± 3.0% vs. 58.9 ± 4.8, P < 0.0001), demonstrating the major role of
NO in the in vivo endothelium-dependent vasodilation induced by iontophoretic delivery of ACh, with a significant participation of prostaglandins.

**BKCa channel involvement in SNP- and ACh-induced vasodilation (protocol 6).** To investigate the role of BKCa channels in endothelial-independent and -dependent vasodilation, rats were treated with iberiotoxin (as described in protocol 2) for SNP- and ACh-induced vasodilation, respectively.

**KATP channel involvement in SNP- and ACh-induced vasodilation (protocol 7).** To examine the role of KATP channels in endothelial-independent and -dependent vasodilation, rats were treated with glibenclamide (as described in protocol 5) for SNP- and ACh-induced vasodilation, respectively.

**Drugs.** All substances (TEA, iberiotoxin, hydralazine, apamin, 4-AP, bradykinin, glibenclamide, pinacidil, and gliclazide) were purchased from Sigma (St. Louis, MO). All drugs were dissolved in saline except for glibenclamide, which was dissolved in a mixture of 5% Tween 80–95% saline. SNP and ACh were obtained from Nitrate (SERB; Paris, France) and Sigma, respectively, and they were dissolved in deionized water. All substances were prepared on the day of the study.

**Data analysis.** The signals were averaged every 30 s to reduce the effects of instantaneous variability due to vasomotion. Basal values were calculated as means over the 1-min basilar period before the onset of increasing local pressure (protocols 1–5) or current application (protocols 6 and 7). Cutaneous blood flow was expressed in arbitrary units (AU). Cutaneous vascular conductance (CVC) was calculated as the ratio of cutaneous blood flow to MABP (AU/mmHg). PIV was reported as the peak change (percent increase) in CVC from baseline after the onset of local pressure application. The pressure for PIV occurrence was defined as the local applied pressure corresponding to the peak change in CVC from baseline in response to the iontophoretic delivery of SNP and ACh, respectively. All data are expressed as means ± SE. For statistical analysis, unpaired t-tests or one-way ANOVA followed by the Bonferroni post test was used when more than two groups were compared. To test for significant differences among groups, we performed one-way ANOVA with Dunnett’s multiple-comparison test (baseline as control). A two-tailed P value of <0.05 was regarded as statistically significant. Statistical evaluations were performed using Prism version 2.0 (Graphpad Software’ San Diego, CA).

**RESULTS**

For all protocols, within each group, no significant variation was found in MABP throughout the experiment. However, basal MABP was increased in rats that received 4-AP (protocol 4: 110.9 ± 1.6 vs. 102.9 ± 2.3 mmHg, P < 0.05). Table 1 summarizes the average PIV responses in controls and rats treated with each of the K+ channel blockers for protocols 1–5.

**K+ channel involvement in PIV mechanotransduction (protocol 1).** TEA reduced, but did not abolish, PIV in treated rats compared with control rats (Fig. 1 and Table 1). PIV was observed at locally applied pressures that were not different between treated rats and control rats (Table 1).

**BKCa and SKCa channel involvement in PIV mechanotransduction (protocols 2 and 3).** PIV was reduced in rats treated with iberiotoxin compared with their respective control rats, whereas PIV was not different between apamin-treated rats and control rats (Table 1). In each protocol, PIV was observed at locally applied pressures that were not different between treated rats and their respective control rats (Table 1). Compared with controls, hydralazine-induced hypotension was reduced in rats treated with iberiotoxin (14.3 ± 3.0% vs. 33.8 ± 2.1%, P < 0.001), showing the efficacy of the treatment.

**KV channel involvement in PIV mechanotransduction (protocol 4).** PIV was not different between rats treated with 4-AP and control rats (Table 1). The bradykinin-induced hypotension was reduced in rats treated with 4-AP (20.8 ± 3.0%) compared with controls (34.9 ± 2.2%, P < 0.001), showing the efficacy of the 4-AP administration.

**KATP channel involvement in PIV mechanotransduction (protocol 5).** PIV was reduced in glibenclamide-treated rats compared with control rats, whereas PIV was unchanged in gliclazide-treated rats (Table 1). As expected, the hypoglycemic effect of sulphonylurea was observed via blood glucose measurements in glibenclamide-treated rats (44.0 ± 1.7 mg/dl, P < 0.001) and gliclazide-treated rats (48.4 ± 2.4 mg/dl, P < 0.001) compared with controls (87.5 ± 4.0 mg/dl), showing the efficacy of the treatments. Moreover the pinacidil-induced decrease in MABP was reduced in glibenclamide-treated rats (12.5 ± 2.1%, P < 0.05) and unchanged in gliclazide-treated rats (31.9 ± 4.7%) compared with controls (32.7 ± 3.7%), as expected.

**BKCa channel involvement in vasodilation to iontophoresis of SNP or ACh (protocol 6).** Basal CVC was not different between iberiotoxin-treated rats (0.2 ± 0.0 AU/mmHg) and control rats (0.2 ± 0.0 AU/mmHg) before SNP delivery.

Table 1. Basal CVC, PIV, and local pressure for PIV responses in controls and rats treated with each of the K+ channel blockers

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Blocker</th>
<th>Signaling Pathway</th>
<th>Basal CVC, AU/mmHg</th>
<th>PIV, %</th>
<th>Pressure for PIV Occurrence, kPa</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TEA</td>
<td>K+ channels</td>
<td>1.4±0.1</td>
<td>16.2±3.0*</td>
<td>1.1±0.4</td>
</tr>
<tr>
<td>Controls</td>
<td>1.4±0.1</td>
<td>72.4±3.4</td>
<td>1.2±0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Iberiotoxin</td>
<td>Large-conductance Ca2+-activated K+ channels</td>
<td>1.6±0.2</td>
<td>38.6±3.7*</td>
<td>0.9±0.2</td>
</tr>
<tr>
<td>Controls</td>
<td>1.5±0.1</td>
<td>104.0±4.2</td>
<td>1.2±0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Apamin</td>
<td>Small-conductance Ca2+-activated K+ channels</td>
<td>1.2±0.1</td>
<td>80.8±8.3</td>
<td>1.6±0.4</td>
</tr>
<tr>
<td>Controls</td>
<td>1.2±0.1</td>
<td>94.4±6.5</td>
<td>1.4±0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>4-AP</td>
<td>Voltage-sensitive K+ channels</td>
<td>1.1±0.1</td>
<td>91.3±12.6</td>
<td>1.0±0.2</td>
</tr>
<tr>
<td>Controls</td>
<td>1.2±0.2</td>
<td>105.2±13.9</td>
<td>1.4±0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Glibenclamide</td>
<td>ATP-sensitive K+ channels</td>
<td>1.5±0.1</td>
<td>44.7±6.6*</td>
<td>1.8±0.3</td>
</tr>
<tr>
<td>Gliclazide</td>
<td>1.4±0.1</td>
<td>97.4±13.1</td>
<td>1.5±0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>1.7±0.1</td>
<td>95.8±14.2</td>
<td>1.2±0.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 10 rats/group. Basal cutaneous vascular conductance (CVC), peak change in CVC from baseline in response to local pressure application pressure-induced vasodilation (PIV) and the local applied pressure corresponding to the peak change in CVC (pressure for PIV occurrence) in controls and rats treated with each of the K+ channel blockers were compared by unpaired t-test (protocols 1–4) or one-way ANOVA followed by Bonferroni post test (protocol 5). Differences other than those indicated were not detected. TEA, tetraethylammonium; 4-AP, 4-aminopyridine. *P < 0.01 and †P < 0.001 compared with controls.

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SNP-induced vasodilation was reduced in iberiotoxin-treated rats (15.6 ± 3.5%) compared with control rats (48.1 ± 6.9%, \(P < 0.001\); Fig. 2A). Basal CVC was not different between iberiotoxin-treated rats (0.3 ± 0.0 AU/mmHg) and control rats (0.2 ± 0.0 AU/mmHg) before ACh delivery. ACh-induced vasodilation was not different between glibenclamide-treated rats (48.0 ± 7.0%) and control rats (59.6 ± 4.7%; Fig. 2B).

**DISCUSSION**

Local pressure applied to the skin stimulates capsaicin-sensitive nerve fibers to release CGRP, VIP, and PACAP (9, 14). These neuropeptides stimulate endothelial cells to release NO and prostaglandins. These factors elicited a cutaneous vasodilation against the increase of the local applied pressure, delaying the occurrence of tissue ischemia (14). The different \(K^+\) channels are known to play a role in endothelial and smooth muscle cell hyperpolarization and, hence, in vascular dilation. In the present study, the reduction of PIV after TEA administration indicates an important role for \(K^+\) channels in vivo in the mechanotransduction of PIV. More specifically, the present findings showed that iberiotoxin and glibenclamide administrations limited PIV, demonstrating that \(BK_{Ca}\) and \(K_{ATP}\) channels play major roles in the underlying cellular mechanisms leading to cutaneous PIV.

Our results demonstrated that iberiotoxin reduced SNP-induced vasodilation, showing the important role of \(BK_{Ca}\) channels in vascular smooth muscle relaxation induced by exogenous nitrovasodilator, as previously reported (35). ACh-
induced vasodilation was equally reduced with iberiotoxin compared with SNP-induced vasodilation, showing that the reduction in the ACh response was fully explained by vascular dysfunction at the smooth muscle level. We then proposed that endothelial BKCa channels do not participate in the vasodilator response to iontophoretic delivery of ACh. Accordingly, the inhibition of endothelial BKCa channels via intraluminal administration of iberiotoxin was previously shown not to interfere with receptor-mediated endothelial synthesis or release of NO (34). In contrast, an in vitro study (7) showed the participation of BKCa channels in maintaining increased intracellular Ca2+ in endothelial cells after stimulation with agonists such as ACh. However, we stated that the in vivo reduction of ACh-induced vasodilation with iberiotoxin cannot be explained by an alteration of the synthesis or release of NO and prostaglandins. It is therefore more likely that in our experimental conditions, vascular smooth muscle, but not endothelial, BKCa channels are those involved in ACh-induced vasodilation.

It is known that endothelial NO lowers intracellular Ca2+ in smooth muscle cells mainly by activating BKCa channels via the cGMP/PKG pathway. Because NO plays a major role in PIV, we suggest that the activation of vascular smooth muscle BKCa channels causes membrane hyperpolarization mainly by the NO/cGMP pathway, leading to the development of PIV. cAMP-dependent phosphorylation after prostaglandin release also stimulates BKCa channels in several types of smooth muscle probably via cAMP/PKA (30). Because PIV is also prostaglandin dependent (14), we cannot exclude the possibility that vascular smooth muscle BKCa channels were partially activated by the prostaglandin/cAMP pathway in the PIV observed in the present study.

The present findings also showed that KATP channels play a major role in the cellular mechanisms of PIV. The release of NO may activate KATP channels via cGMP in smooth muscle cells (24, 26, 32). However, previous reports (7, 19) agree that endothelial KATP channels do not mediate agonist-induced release of endothelial factors. In the present study, we found that the ACh- and SNP-induced vasodilator responses were not altered after KATP channel blockade. Similarly, in a previous report (36), SNP did not activate a glibenclamide-sensitive current in cells. This strongly suggests that the alteration of PIV after KATP channel blockade cannot be explained by a reduction of NO synthesis or release, suggesting that KATP channels activated in PIV do not involve the NO/cGMP pathway.

In contrast, KATP channels do appear to be involved in cAMP-dependent vasodilator responses to prostaglandins (6). Because prostaglandins are also involved in PIV (14), we suggest that the opening of vascular smooth muscle KATP channels contributed to the mechanism of PIV via a prostaglandin-activated cAMP/PKA pathway. However, additional studies are needed to further clarify the exact cellular mechanisms.

Another role for glibenclamide may be that it abolishes the synergistic interaction between endothelial NO and prostaglandins (17). In our present results, this negative effect in the interaction between NO and prostaglandins could also explain the reduction of PIV with glibenclamide. For example, isoflurane selectively attenuated endothelium-dependent dilation of canine pulmonary arteries by interfering with the synergistic interaction between NO and prostaglandins, possibly via an effect on KATP channels (16). Similarly, we recently reported that PIV was altered with high doses of isoflurane by an alteration in endothelial function (8). We thus hypothesize that the opening of KATP channels could also participate to the development of PIV by impairing the synergic interaction between NO and prostaglandins. However, further studies are needed to verify this hypothesis.

KATP channels are heterooctameric proteins comprising two subunits: the pore-forming subunit Kir6.x (Kir6.1 or Kir6.2) and the regulatory subunit sulphonylurea receptor SURx (SUR1, SUR2A, or SUR2B), which belongs to the ATP-binding cassette protein superfamily (15). Generally, vascular smooth muscle-type KATP channels contain Kir6.1/SUR2B (23). Because sulphonylurea agents exert their physiological effects in many cell types via binding to specific SUR, we suggest that glibenclamide agents reduce PIV because the SUR2B subunit of KATP channels are involved in PIV. Diabetic rats exhibit a downregulation of SUR2B in vascular smooth muscle, although the expression of Kir6.1 and Kir6.2 was unchanged (27). This suggests that the expression of specific KATP channel subunits is altered in diabetes. This alteration could at least in part contribute to the absence of PIV observed in diabetes (21, 31).

Inhibition of KATP channels in pancreatic β-cells by glibenclamide or gliclazide is thought to contribute significantly to the hypoglycemic effect of these drugs (2), which was also observed in the present study. Because PIV was not reduced in rats treated with gliclazide compared with controls, it is unlikely that the hypoglycemic effect per se was responsible for the reduction of PIV with glibenclamide in the present study.

Because in physiological conditions, PIV does not rely on EDHF release (18), we did not expect that SKCa channels were involved in PIV. As expected, apamin, a specific blocker of SKCa channels, did not modify PIV. This is also in accordance with the idea that SKCa channels are stimulated by substance P (10), a substance that is not involved in the development of PIV (14).

Additionally, in our experimental conditions, Kv channels were not involved in the development of PIV. These channels were reported to be involved in the relaxation in response to endothelium-derived NO in the rat basilar artery (1). External pressure could directly activate K+ channels, as reported in the intracellular transduction of shear stress. Indeed, direct activation of endothelial BKCa channels by a mechanical stimulus is an essential step in shear stress, which results in the synthesis of NO and prostaglandins, and elicits dilation (25, 34). Because the development of PIV requires local neural activity (14), we exclude a direct activation of K+ channels by the local pressure application.

CGRP, VIP, and PACAP may directly activate KATP channels to induce vasodilation (26, 33). However, a direct activation of the vascular smooth muscle BKCa and KATP channels by CGRP, VIP, or PACAP in the cellular mechanisms of PIV was also excluded, because we demonstrated the endothelial dependence in the development of PIV (14). Several studies have shown that the presynaptic blockers of K+ channels could stimulate neurotransmitter release (28) or have no effect in modulating release of vasodilator substances (29). For instance, no effect was observed on CGRP release with glibenclamide (20). We excluded that a negative effect on neurotransmitter release was responsible for the reduction of PIV observed in the present study.
Because some channel blockers tested (apamin, 4-AP, and gliclazide) were found to have no effect on the development of PIV, it could be argued that this was due to insufficient doses of blocker. Although an inhibitory effect was not verified for apamin, it was used at a dose 3–10 times higher than that shown to potentiate the pressor response to angiotensin II (5). This dose was also previously shown to antagonize the inhibitory effects of morphine on antidiomeric stimulation-evoked responses (38). An in vivo effect was demonstrated for gliclazide, which induced hypoglycemia similar to that seen with glibenclamide. Moreover, efficiency was directly verified for 4-AP, which inhibited bradykinin-induced hypotension. We are therefore confident that, for those channel blockers that did not alter PIV, this was most unlikely to be due to insufficient channel blockade.

In conclusion, the present findings demonstrate the involvement of BK<sub>Ca</sub> and K<sub>ATP</sub> channels in the underlying cellular mechanisms that lead to the development of local cutaneous PIV. In contrast, our data indicate little or no role for K<sub>V</sub> and SK<sub>Ca</sub> channels in the mechanotransduction of PIV. We used local administration of SNP and ACh to demonstrate in vivo that iberiotoxin, a specific blocker of BK<sub>Ca</sub> channels, blocked the downstream effect of NO on smooth muscle. In contrast, glibenclamide, a specific blocker of K<sub>ATP</sub> channels, could block the downstream effect of prostaglandins on the smooth muscle cells or act upstream on another target. We suggest that the opening of BK<sub>Ca</sub> and K<sub>ATP</sub> channels contribute to the hyperpolarization of vascular smooth muscle cells to produce PIV development mainly via the NO and PG pathways, respectively.

ACKNOWLEDGMENTS

We thank the local Animal Care Unit of the University of Angers for facilities. We also thank Maud Poirier for technical assistance.

REFERENCES


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