Restoration of vasodilation and CBF autoregulation by genistein in rat pial artery after brain injury

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Hong, Ki Whan, Hwa Kyoung Shin, Chi Dae Kim, Won Suk Lee, and Byung Yong Rhim. Restoration of vasodilation and CBF autoregulation by genistein in rat pial artery after brain injury. Am J Physiol Heart Circ Physiol 281: H308–H315, 2001.—This study determined whether, after fluid percussion injury (FPI), tyrosine kinase activation is coupled to inhibition of K+ channels and alteration in cerebral blood flow (CBF) autoregulation in the rat pial artery. Injury of moderate severity (2–2.5 atm) was produced by FPI in anesthetized rats equipped with a closed cranial window. The suppressed vasodilation of the pial arterioles to calcitonin gene-related peptide (CGRP) and levorakalim (LMK) and altered lower limit of CBF autoregulation after FPI were restored by genistein but not by daidzein, an inactive analog. Vasodilation to S-nitrosop-NAcetyl penicillamine (0.1–10 μmol/l) was, however, little influenced after FPI. The restored vasodilation was decreased by sodium orthovanadate, suggesting the reciprocal action of tyrosine phosphorylation and dephosphorylation. After FPI, CGRP-induced vasodilation restored by genistein (10 μmol/l) was strongly antagonized by iberiotoxin but not by glibenclamide, whereas LMK-induced vasodilation was, in contrast, inhibited by glibenclamide but not by iberiotoxin. Taken together, we suggest that, after FPI, activation of tyrosine kinase links the inhibition of K+ channels to impaired autoregulatory vasodilation in response to acute hypotension and alteration in CBF autoregulation in the rat pial artery.

Fluid percussion injury; tyrosine phosphorylation; cerebral blood flow; CGRP

Traumatic brain injury still remains a leading cause of morbidity and mortality despite recent improvements in the efficiency of traumatic care (11). Fluid percussion brain injury, as a model of human concussive trauma, causes considerable alterations in neurohumoral control of the cerebral circulation, including arterial dilation with a transient hypertensive response and transient decrease of cerebral blood flow (CBF) (6) and impairment of K+ channel function with reduced vasorelaxation (2, 3). A growing number of reports have documented that activation of tyrosine kinase with tyrosine phosphorylation plays a pivotal role in the signal transduction processes, including cell growth and differentiation, and in the receptor-mediated current inhibition (14). Tyrosine kinase phosphorylation was demonstrated to be implicated in the regulation of ATP-sensitive K+ (KATP), Ca2+-activated K+ (KCa), and inwardly rectifying K+ channel activities (20, 32). Although several recent studies have characterized the mechanism by which vasodilation is reduced after traumatic brain injury, less is known concerning the mechanism of reduced vasorelaxation in response to vasodilators. On the other hand, calcitonin gene-related peptide (CGRP) has been demonstrated to act in part through activation of KATP channels (25, 29) and to elevate intracellular levels of cAMP through activation of adenyl cyclase (5).

Thus the present study was designed to test the hypothesis that, after fluid percussion injury (FPI), tyrosine kinase activation is coupled to inhibition of K+ channels, which, in turn, may link to impaired autoregulatory vasodilation in response to acute hypotension and alterations in CBF autoregulation in the rat pial artery. To address this hypothesis, we employed CGRP, an endogenous K+ channel opener, and levorakalim (LMK), a KATP channel opener, and identified the effects of genistein and daidzein to restore 1) the suppressed ability of CGRP and LMK to dilate the pial arterioles, 2) the reduced autoregulatory vasodilation to transient hypotension, and 3) the alteration in the lower limit of CBF autoregulation. In this study, we confirmed the restoration of pial arteries to vasodilate in response to CGRP and LMK under treatment with genistein but not daidzein, an inactive structural analog of genistein. In line with these studies, we pharmacologically characterized the type of K+ channels involved in the vasodilation induced after genistein treatment with the use of glibenclamide, a KATP channel blocker, and iberiotoxin, a KCa channel blocker.

Materials and Methods

Preparation of animals. Male Sprague-Dawley rats (250–300 g) were anesthetized with urethane (1 g/kg ip) and placed...
on a heating pad (Homeothermic Blanket System, Harvard Apparatus; South Natick, MA) to maintain a constant rectal temperature (37 ± 0.5°C). After a tracheostomy, the animal was mechanically ventilated with room air with a respirator (model 683, Harvard Apparatus) after immobilization with 5 mg/kg gallamine triethiodide. The mean PCO2 was monitored with an end-tidal CO2 analyzer (CapStar-100, IITC Life Science; Woodland Hills, CA). Catheters were placed in a carotid artery for measurement of systemic arterial blood pressure (Statham P23D pressure transducer, Gould; Cleveland, OH), a femoral cutdown was performed, and a femoral arterial catheter was inserted for withdrawing and sampling of the arterial blood. The blood was collected before and after installation of a cranial window for blood gas and pH determination (STAT Profile 3, Nova Biomedicals; Boston, MA).

**Measurement of vessel diameter.** With the animal in a stereotaxic frame, pial microvessels were visualized through an implanted closed cranial window. The head was fixed in a prone position with a stereotactic apparatus (Stoelting: Waukegan, IL) and a square-shaped (5 × 5 mm) burr hole was made over the right parietal cortex. The dura was resected with caution. Pial precapillary microvessels were visualized through a stereomicroscope (model SMZ-2T, Nikon). It was fed to a television monitor for direct observation, and the caliber was measured using a Width analyzer (C3161, Hamamatsu Photonics, Hamamatsu). The composition (in mmol/l) of the artificial CSF was as follows: 132 NaCl, 2.9 KCl, 1.4 MgCl2, 24.6 NaHCO3, 1.2 CaCl2, 6.7 urea, and 3.7 d-glucose (pH 7.4). Three hours after FPI, the closed window was removed, and the intracranial pressure was maintained constant at 5–6 mmHg throughout the experiment by adjusting the height of the free end of the plastic tubing, which was connected to the outlet of the window. Only one arteriole was observed under the window in each rat.

**Protocol for in vivo experiments.** To determine the vasodilatory responses of the resting pial arteries to CGRP (0.001–0.1 μmol/l), LMK (0.1–10 μmol/l), and S-nitroso-N-acetyl penicillamine (SNAP; 0.1–10 μmol/l), the cortical surface was suffused with artificial CSF containing increasing concentrations of each agent. When measuring the autoregulatory vasodilation to hypotension in the rat 3 h after FPI, we first determined the vasodilatory function of the pial artery to stepwise hypotension and vasoconstrictor action to its reverse of blood pressure by infusion of blood from the reservoir under suffusion of the cortical surface with artificial CSF containing vehicle. Thereafter, we reexamined the autoregulatory responses under treatment with genistein. The decrease in blood pressure at each step was maintained for 2 min, and changes in vessel diameter during the last minute were measured. The inhibitory effect of either genistein (10 μmol/l) or daidzein (10 μmol/l) was determined on the vasodilation induced by CGRP or LMK. The genistein, daidzein, glibenclamide, and iberiotoxin were applied at 30 min before and during application of CGRP or LMK and hypotensive procedure, respectively. Alterations in pial arterial diameter and blood pressure were expressed as percent changes in the baseline diameter and mean arterial blood pressure.

**Measurement of lower limit of CBF autoregulation.** The animal’s head was fixed in a stereotaxic instrument. Animals were mechanically ventilated with room air with a respirator (model 683, Harvard Apparatus). After a craniotomy, CBF to the pial artery over the parietal cortical surface was measured by using laser-Doppler flowmetry (Laserflo BPM, Vasamedics; St. Paul, MN) equipped with a 1-mm-diameter needle probe (model F-433-5 needle probe, Vasamedics). After the dura mater was carefully sectioned, the probe was placed lateral to, but near to, the pial artery in the open window and advanced into the CSF ~0.2 mm above the surface of the cortex. After the two baselines at intervals of 10 min were measured, the prewarmed artificial CSF saturated with 9% O2-5% CO2 (37°C) was applied locally to the open window with a bolus volume of 100 μl for three times every 10 min. The baseline CBF level was not altered due to bleeding in this experiment. The laser-Doppler flowmetry outputs were measured as arbitrary units, and the changes in CBF were expressed as a percentage of the baseline CBF.

**Fluid percussion injury.** The fluid percussion device used to produce experimental brain injury was as follows: the device consisted of a Plexiglas cylindrical reservoir (60 cm long and 4.5 cm in diameter) bounded at one end by a Plexiglas cork-covered piston mounted on O-rings, which was filled with isotonic saline. The opposite end of the reservoir was fitted with a 2-cm-long metal housing on which a transducer was mounted and connected to a 5-mm tube that terminated with a male Luer-Lok fitting. The exposed end of the tube was covered with a rubber pad. When trauma was applied, the tube was connected to a female Luer-Lok fitting that was implanted over the exposed dura of the rat. FPI was induced by striking the piston with a 4.5-kg pendulum, which struck the piston of the device from a predetermined height for one time (~25 ms in duration). The intensity of the blow was adjusted between 2.0 and 2.5 atm.

**Drugs.** CGRP, genistein, daidzein, sodium orthovanadate, glibenclamide, and iberiotoxin were purchased from Sigma (St. Louis, MO). CGRP and iberiotoxin were dissolved in 0.1% bovine serum albumin to make a stock solution of 0.01 mM. Genistein and daidzein were dissolved in DMSO to make a stock solution of 1 mM and diluted when used. Sodium orthovanadate was prepared in distilled water as a stock solution of 0.5 mM (pH 10) to prevent the formation of decavanadate. Glibenclamide was sonicated in 1 ml of NaOH (0.1 N) and diluted with 5% glucose to make a stock solution of 10 mM. LMK was obtained from the Korea Research Institute of Chemical Technology (Daejon, Korea) and dissolved in ethanol-polyethylene glycol (50:50% vol/vol) to make a stock solution of 1 mM. SNAP was from RBI (Natick, MA).

**Statistical analysis.** Results are expressed as means ± SE. Student’s t-test was used for analyzing values between the data of vehicle and inhibitor-treated groups [concentration giving 25% maximal response (EC25) and lower limit of CBF autoregulation]. Two-way repeated measures ANOVA was used for the comparison of time-dependent arterial diameter changes in response to agonists between inhibitor-treated and untreated groups. ANOVA for repeated measurement followed by Dunnett’s method was used for statistical analysis of CBF between baseline value and the value at each blood pressure level. P < 0.05 was accepted as statistically significant. The n values reflect the results for one vessel in each animal. Data presented as percent change were compared by nonparametric means with the Wilcoxon signed-rank test.
RESULTS

Mean arterial blood pressure in the control group (113.4 ± 7.8 mmHg) was not significantly different from that in rats subjected to FPI (106.5 ± 1.3 mmHg). Baseline pial arterial diameter of the FPI group 3 h after injury was 42.5 ± 5.6 μm (n = 20), which differed little from the control value (39.4 ± 2.2 μm, n = 43) (Table 1).

CGRP-, LMK-, and SNAP-induced vasodilation. Suffusion with artificial CSF containing CGRP (0.001–0.1 μmol/l) and LMK (0.1–10 μmol/l) exerted concentration-dependent vasodilations of the pial arteries, with EC25 values of 0.002 ± 0.001 μmol/l and 0.91 ± 0.36 μmol/l, respectively. CGRP and LMK, at the concentration ranges used in this study, caused little change in systemic arterial blood pressure. The vasodilations induced by CGRP and LMK were markedly blunted after FPI, with increased EC25 values of 0.113 ± 0.024 μmol/l (P < 0.001) and 41.35 ± 13.23 μmol/l (P < 0.001) with significantly decreased dilation magnitudes (at 1 μmol/l CGRP: from 94.9 ± 15.3% in control to 26.7 ± 5.2%, P < 0.001; and 10 μmol/l LMK: from 87.8 ± 18.6% in control to 23.8 ± 14.4%, P < 0.001), respectively (Fig. 1). However, 0.1–10 μmol/l SNAP-induced vasodilations were little influenced after FPI in both larger (77.6 ± 3.1 μm) and smaller arteries (43.3 ± 2.0 μm) (Fig. 2).

Restoration of vasodilation by inhibitors of tyrosine kinase and phosphatase. Vasodilations to CGRP and LMK, which were significantly blunted after FPI, markedly recovered under suffusion of cranial surface with artificial CSF containing 10 μmol/l genistein (tyrosine kinase inhibitor), respectively, but not by 10 μmol/l daidzein, an inactive analog of genistein (data not shown). The higher concentration of genistein (>10 μmol/l) alone caused vasodilation of the pial artery. The vasodilation induced by either CGRP or LMK was largely restored after genistein treatment, with significantly decreased EC25 values of 0.006 ± 0.002 μmol/l (P < 0.001) and 1.00 ± 0.33 μmol/l (P < 0.001), respectively. Interestingly, the restored CGRP-induced vasodilation was again sensitively inhibited by application of 0.1–1 mmol/l sodium orthovanadate, whereas LMK-induced vasodilation was decreased by much higher doses, 1–10 mmol/l (Fig. 3). These findings suggest a reciprocal action of tyrosine phosphorylation and dephosphorylation on the pial arterial beds. Suffusion with artificial CSF containing genistein (<10 μmol/l) showed little effect on pial arterial diameter.

Effects of K+ channel antagonists. In the pial arteries subjected to FPI, CGRP-induced vasodilation, which was restored in the presence of genistein (10 μmol/l), was strongly antagonized by iberiotoxin (0.1 μmol/l)

Table 1. Physiological variables: MABP and blood gas analysis

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control</th>
<th>FPI</th>
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<tbody>
<tr>
<td>n</td>
<td>20</td>
<td>43</td>
</tr>
<tr>
<td>MABP, mmHg</td>
<td>113.4 ± 7.8</td>
<td>106.5 ± 1.3</td>
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<tr>
<td>PCO2, mmHg</td>
<td>32.5 ± 5.7</td>
<td>31.4 ± 6.2</td>
</tr>
<tr>
<td>PO2, mmHg</td>
<td>103.9 ± 10.4</td>
<td>98.3 ± 4.3</td>
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<tr>
<td>pH</td>
<td>7.39 ± 0.05</td>
<td>7.41 ± 0.04</td>
</tr>
<tr>
<td>Baseline diameter, μm</td>
<td>39.4 ± 2.2</td>
<td>42.5 ± 5.6</td>
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Values are means ± SE; n = no. of rats. MABP, mean arterial blood pressure; FPI, fluid percussion injury.
but not by glibenclamide (10 μmol/l), whereas LMK-induced vasodilation was, in contrast, inhibited by glibenclamide but not by iberiotoxin (Fig. 4).

**Autoregulatory vasodilation.** We examined whether the autoregulatory vasodilator responses of the pial microvessels were altered after FPI. As shown in Fig. 5, changes in pial arterial diameter in response to a stepwise hypotension were plotted as a function of changes in systemic mean arterial blood pressure, and mean slope was calculated from each linear regression line. Three hours after FPI, the pial artery showed little vasodilatory response to lowering of the blood pressure, as evidenced by reduced slopes of regression lines (slope for vasodilation: from $-1.99 \pm 0.27$ in control to $-0.43 \pm 0.14$, $P < 0.001$; and slope for vasoconstriction: from $-1.60 \pm 0.17$ to $-0.58 \pm 0.14$, $P < 0.001$). Autoregulatory vasodilation to hypotension, which was markedly blunted after FPI, was largely recovered under pretreatment with 10 μmol/l genistein (slope for vasodilation: $-1.36 \pm 0.13$, $P < 0.001$; and slope for vasoconstriction: $-1.14 \pm 0.13$, $P < 0.001$) but not by daidzein.

**Lower limit of CBF autoregulation.** CBF was well preserved despite a decrease in mean arterial blood pressure to $55–60$ mmHg. When the blood pressure level further decreased, CBF fell steeply and depended on the magnitude of the fall in blood pressure thereafter. The lower limit of autoregulation was defined as the blood pressure at which CBF decreased by 10% of its value at resting mean arterial blood pressure. After FPI, the lower limit of CBF autoregulation significantly shifted to a higher blood pressure level (from $56.3 \pm 3.3$ mmHg in control to $82.4 \pm 2.4$ mmHg, $P < 0.001$, $n = 7$) (Fig. 6). Under local treatment with 10 μmol/l genistein, the lower limit of CBF autoregulation, which was largely shifted to higher blood pres-

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**Fig. 2.** Percent changes in pial arterial diameter in response to S-nitroso-N-acetyl penicillamine (SNAP; 0.1–10 μmol/l) in the control group and rats subjected to FPI. Values are means ± SE of 4 experiments. The vasodilation induced by SNAP was little influenced after FPI in both larger (77.6 ± 3.1 μm in diameter; top) and smaller arteries (43.3 ± 2.0 μm in diameter; bottom). EC_{25}, concentration giving 25% maximal response.

**Fig. 3.** Inhibiting effect of sodium orthovanadate on the restored vasodilations under pretreatment with genistein (10 μmol/l) by CGRP (0.1 μmol/l) and LMK (10 μmol/l). Values are means ± SE of 3–4 experiments. The restored vasodilation by either CGRP or LMK was not altered throughout experiment in the absence of sodium orthovanadate.
DISCUSSION

The major findings of this study in the rat pial artery were that 1) vasodilation induced by either CGRP and LMK, but not SNAP, was significantly blunted after FPI; 2) these reduced vasodilations were significantly restored to the control level under treatment with genistein, and the restoration was again reversed to reduction in diameter by sodium orthovanadate; 3) pretreatment with genistein well preserved the reduced autoregulatory vasodilation to hypotension and restored the lower limit of CBF autoregulation, which was shifted to a higher blood pressure after injury.

FPI produces some aspects of the biochemical, physiological, neurological, and morphological responses observed in human blunt head trauma (12). It has been documented that FPI causes a transient increase in mean arterial blood pressure and elevation of intracranial pressure within 5–10 min (31) and pial arterial vasoconstriction with decreased CBF within 10 min of injury (3). Because these sequences have been shown during an early time period, in our preliminary study, the experiment was performed 3 h after injury. At this time, we observed some of postinjury hemorrhagic points on the contratralateral surface of cortex. Pial arterioles have been reported as the primary sites of autoregulatory function by a change of their calibers in response to blood pressure changes (19).

Changes in CBF, especially during severe hypotension, are largely dependent on the capacity of autoregulatory vasodilation in response to hypotension and reactivity of arterioles. Maintenance of CBF is very important from a therapeutic point of view, as claimed in a report (13) demonstrating that the severity of neurotic symptoms in the chronic stage of cerebral infarction was correlated with CBF levels. Because the magnitude of vasodilatory reserves available for the autoregulation is significantly decreased after FPI, it can be predicted that the ability to compensate to acute hypotension would be limited in the patient subjected to brain trauma. The lower limit of CBF autoregulation was reported to shift to a higher blood pressure in patients with chronic hypertension, thereby being highly vulnerable to brain ischemia in response to antihypertensive therapy or when subjected to acute hypotension (22, 30). In line with these facts, the morbidity and mortality of severe head trauma increase with poorer outcome when hypotension accompanies the brain injury (27). On the basis of these reports, the therapeutic approach to preserve the autoregulatory vasodilation in response to hypotension (CBF autoregulation) appears to be very important to reduce the morbidity and mortality of the patients. However, the signal transduction pathways involved in the genesis of the physiological and morphological sequelae after FPI remain unclear.

In our study, the vasodilation to either CGRP or LMK in the rat pial artery was significantly reduced, as was the autoregulatory vasodilation to acute hypotension after FPI. Interestingly, pretreatment with genistein well preserved not only the vasodilation to these agonists but also the autoregulatory vasodilation to hypotension, which was blunted after FPI. However, the improvement after genistein was not complete. It remains for further study what mechanism mediates the remaining impairment to CGRP and LMK after FPI. Evidence has accumulated that tyrosine phosphorylation plays a modulatory role in the ion channel regulation, including inwardly rectifying K+ channels and delayed rectifier K+ channels (17, 28). Xiong et al. (32) confirmed that genistein increased whole cell KCa channel currents in vascular smooth muscle cells from the rat tail artery, suggesting that tyrosine kinase modulates KCa channel activity.

Genistein is a naturally occurring flavonoid derived from the fermentation broth of Pseudomonas sp., which inhibits protein tyrosine kinase activity (1). This compound was shown to have no discernible effect on protein kinase C and protein kinase A (7). Contained herein is the first report illustrating that pretreatment with genistein, an inhibitor of tyrosine kinase, results in restoration of vasodilation in the rat pial artery after brain injury. These findings permit us to infer that the...
restoration of reduced vasodilation by genistein, but not by daidzein, was due to inhibition of tyrosine kinase phosphorylation. The evidence cited above implicating activation of tyrosine kinase in the modulation of K⁺ channels strengthens our results that the phosphorylation of tyrosine kinase in the cerebral vessels is implicated in the impairment of K⁺ channels after traumatic brain injury. Supporting this view is the

Fig. 5. Graph showing alterations in the autoregulatory vasodilator and vasoconstrictor responses to changes in arterial blood pressure after FPI without and with pretreatment with genistein (10 μmol/l). The cortical surface was suffused with artificial cerebrospinal fluid without and with genistein after 30 min and during stepwise hypotension and reverse of blood pressure. Values are means ± SE of 6 experiments. Significant differences (P < 0.001) were shown between the blood pressure-diameter relation of the control and FPI groups and between that of FPI and FPI + genistein group by two-way repeated measures analysis of variance.

Fig. 6. Relationships of changes in cerebral blood flow (CBF) to changes in mean arterial blood pressure in the cortical pial arteries in the control group and rats subjected to FPI without and with pretreatment with genistein (1 and 10 μmol/l) and daidzein (100 μmol/l). Arrows, lower limits of CBF autoregulation, defined as the mean arterial blood pressure at which CBF decreased by 10% of the baseline value. Values are means ± SE of 5 experiments. ***P < 0.0001 vs. control; **P < 0.01 vs. FPI.
demonstration of inhibition of restored vasodilation by sodium orthovanadate, a potent inhibitor of protein tyrosine phosphatase (10), indicating that tyrosine kinase activation is involved in the reduction of vasodilation to K⁺ channel openers and acute hypotension after FPI. As suggested by Di Salvo et al. (7), it appears likely that sodium orthovanadate-induced contraction of the pial artery is linked to enhanced protein tyrosine phosphorylation. These findings further suggest the reciprocal action of tyrosine phosphorylation and dephosphorylation on the pial arterial beds, consistent with the results demonstrated by Kwak et al. (20). However, it remains unexplained why the concentration of sodium orthovanadate needed to inhibit the vasodilation restored in the presence of genistein in the case of CGRP is different from that of LMK.

On the other hand, membrane potential of the vascular smooth muscle cells is a major determinant of vascular tone, and K⁺ channel opening plays a pivotal role in regulation of the tone of cerebral blood vessels by increasing K⁺ efflux and producing hyperpolarization (4, 24). Several types of K⁺ channels, including KATP, KCa, and KCa, have been suggested to regulate the tone of cerebral vessels by pharmacological study (24). CGRP, an endogenous activator of K⁺ channels, produces hyperpolarization of cerebral vessels in vitro (26) and causes vasodilation, which is mediated by activation of the opening of KATP channels (18, 21, 25) and KCa channels (23) through cAMP-linked protein kinase phosphorylation (5). Moreover, Hong et al. (15, 16) provided evidence that CGRP is implicated in the autoregulatory vasodilation of the pial artery in response to hypotension via mediation of KATP channel activation (21).

Interestingly, we found that the genistein-induced restoration of CGRP vasodilation was strongly antagonized by iberiotoxin but not by glibenclamide, whereas the LMK-induced vasodilation was inhibited by glibenclamide but not by iberiotoxin. These results indicate that vasodilation to CGRP is predominantly mediated via activation of KCa channels, whereas that of LMK is via KATP channels. At present time, it goes beyond the scope of our in vivo study to illustrate the mechanism(s) by which CGRP and LMK differently control the two K⁺ channels under treatment with genistein, an inhibitor of tyrosine kinase. In contrast, implication of increased prostaglandin synthesis (9) and endothelin-1 (2) was demonstrated in the genesis of the physiological and morphological sequelae after FPI. It may be possible that vasoconstrictor responses mediated by activation of tyrosine kinase with these agents (8, 14) indirectly cause attenuation of vasodilator response to agonists. If it is the case, inhibition of kinase with genistein may have attenuated the constrictor responses. Nevertheless, it is uncertain which purported physiological ligands activate this kinase. Furthermore, the exact mechanism by which tyrosine kinase modulates these channels remains to be determined.

In the present study, FPI failed to suppress the vasodilation to SNAP, a releaser of nitric oxide, suggesting that K⁺ channels may not normally contribute to dilator responses of the pial artery to nitric oxide. Further study is required to elucidate whether FPI suppresses cGMP-analog-induced vasodilation, and genistein may preserve its vasodilation in the rat pial artery.

In conclusion, our results highlight an implication of tyrosine kinase phosphorylation as a major signaling mediator in the alterations of autoregulatory cerebral circulation after FPI. After FPI, the activation of tyrosine kinase may account for the reduction of vasodilator responsiveness to CGRP and LMK, which is mediated by inhibition of either KATP channels or KCa channels. Interestingly, tyrosine kinase inhibitors, including genistein, well preserved the altered autoregulatory vasodilation to hypotension and restored the lower limit of CBF, which were shifted to a higher blood pressure. However, the exact mechanism(s) by which tyrosine kinase is activated and by which activated tyrosine kinase, in turn, modulates the activity of K⁺ channels remains to be identified.

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