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Focal elevations in neocortical interstitial K^+ produced by stimulation of the fastigial nucleus in rat

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Abstract

We studied whether K^+ , a potent cerebrovasodilator released by active neurons, participates in the increase in cortical cerebral blood flow (CBF) elicited by stimulation of the cerebellar fastigial nucleus (FN). Rats were anesthetized by continuous administration of halothane (1–3%), paralyzed and artificially ventilated. FN was stimulated electrically (8 s trains, 50 Hz, 5–10 V) through microelectrodes positioned stereotactically. K^+_o (mM) was measured in sensory cortex by K^+ -sensitive micropipettes. In some experiments neocortical CBF was monitored continuously by laser-doppler flowmetry. Stimulation of the FN produced significant increases in K^+_o that averaged 0.91 ± 0.16 mM (range 0.5–2.9 mM; $n = 19$) and were confined to sites corresponding to the intermediate cortical laminae ($P < 0.05$, ANOVA). To determine whether such K^+_o elevations were able to produce increases in CBF comparable to those elicited by FN stimulation, cortical K^+_o was increased by superfusing the sensory cortex with 20–30 mM K^+ in Ringer. K^+_o elevations of 2.8 ± 0.6 mM increased CBF by $17 \pm 2\%$ ($n = 5$), an increase considerably smaller than that elicited by FN stimulation in cerebral cortex. We conclude that K^+ is unlikely to mediate the cortical cerebrovasodilation. Furthermore, the restricted spatial distribution of the K^+_o increase indicates that the cortical neural activity evoked by FN stimulation is highly focal. Thus the findings support the hypothesis that, in cortex, the vasodilation is mediated by activation of a restricted group of neural elements, perhaps neurons in laminae III–IV.

Keywords

Cerebral blood flow; Cerebral cortex; Cerebellum; Ion-sensitive microelectrode; Laser-doppler flowmetry

Electrical stimulation of the rostral pole of the cerebellar fastigial nucleus (FN) increases cerebral blood flow (CBF) globally, an effect mediated through neural pathways entirely contained within the brain and via cholinergic mechanisms^{8,10–12,18}. The increases in CBF are greatest in the cerebral cortex (up to 215% of control) wherein they are not associated with corresponding changes in local cerebral glucose utilization¹⁷. This finding indicates that in cortex the vasodilation is not secondary to increased local metabolic demands, thereby representing an example of ‘primary neurogenic vasodilation’.

In cerebral cortex, selective destruction of local neurons by the excitotoxin ibotenic acid abolishes the increases in CBF within the area of the lesion, indicating that the cortical vasodilation is mediated by local neurons¹⁰. The mechanisms by which these neurons interact

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with local blood vessels to increase CBF have not been elucidated. A recent study in which the temporal profile of the increases in cortical CBF was determined using laser doppler flowmetry (LDF) has shown that the flow increase develops slowly (50–60 s) and persists for several minutes after termination of the stimulation⁸. The slow time course of the vasodilation is consistent with the hypothesis that local neurons mediate the changes in CBF by releasing vasoactive agents into the interstitium. These agents would then reach local microvessels by diffusion and determine the increases in CBF.

Several vasoactive agents can be released by active neurons and astroglia⁷. One such agent which has long been considered to be involved in cerebrovascular regulation during neuronal activity is K^+ (ref. ¹⁶). Indeed, this ion is a powerful cerebrovasodilator¹⁵ which accumulates into the extracellular space as result of neuronal activity (see ref. ²⁴ for review). K^+ may reach the perivascular space either by simple diffusion through the interstitium or via ionic currents involving astrocytes and their perivascular endfeet²⁰. In this study we sought to determine whether FN stimulation increases interstitial neocortical K^+ concentration (K^+_o) and, if so, whether the magnitude of the K^+_o elevations is sufficient to produce increases in local CBF. A preliminary report of part of this study has been made⁹.

Methods for surgical preparation of rats, electrical stimulation of the FN, measurement of K^+_o using ion-sensitive micropipettes (ISMs) and measurement of CBF by LDF have been described in detail in earlier publications^{5,8,14,18} and will be summarized below. Studies were performed on 11 male Wistar rats (300–400 g) anesthetized with halothane (5% induction, 2–3% during surgery and 1% during recordings), in a 30% oxygen-nitrogen mixture. Body temperature was maintained at 37 ± 0.5 °C by a water heated body holder. A catheter was inserted in the femoral artery and the trachea was cannulated. Animals were then placed on a stereotaxic frame (mod. 1730, Kopf, Tujunga, CA), paralyzed with tubocurarine (2 mg/kg, i.m.) and artificially ventilated by a mechanical ventilator (mod. 683, Harvard Apparatus, South Natick, MA). The arterial catheter was used for continuous recording of arterial pressure (AP) and for blood sampling. Arterial pCO_2 , pO_2 and pH were measured at multiple times on 0.2 ml of blood by using a blood gas analyzer (mod. 168, CIBA-Corning Diagnostic, Medfield, MA) (Table I). For stimulation of the FN, the midline portion of the caudal occipital bone was removed to expose the cerebellar vermis and the dorsal medulla. For recording of cortical K^+_o and CBF, a 3×3 mm craniotomy hole was drilled at a site 2–3 mm lateral and 1–2 mm caudal to bregma. The underlying cortex corresponds to the leg area of the primary sensory cortex as from this region elevations in K^+_o and CBF can be obtained from stimulation of the sciatic nerve (Iadecola and Kraig, unpublished observations). The dura was usually removed. The craniotomy site was continuously superfused with Ringer warmed at 37 °C and aerated with 95% O_2 and 5% CO_2 (pH 7.3–7.4). The composition of the Ringer in mM was: Na^+ 143.5; K^+ 3.0; Ca^{2+} 1.5; Mg^{2+} 1.4; Cl^- 115; HCO_3^- 26.4; gluconate 9.6; and glucose 5.0 (modified from ref. ³). The FN was stimulated electrically through monopolar platinum-iridium microelectrodes (diameter 150 μm)¹⁸. The ground was a metal clip attached to the animal's scalp. At the end of the experiment animals were sacrificed by increasing the inspired concentration of halothane to 5%. After AP had dropped to 0 mm Hg for several minutes, rats were decapitated and their brains removed and frozen in Freon 12 for subsequent histological verification of stimulated sites.

K^+ electrodes were fabricated from concentric borosilicate glass capillaries pulled to a 1–2 μm tip. As described in detail elsewhere^{5,14}, one barrel was backfilled with 100% *N,N*-dimethyltrimethyl-sylilamine (Fluka, Basel, Switzerland), heated to 200–300 °C and then filled with a valinomycin based cocktail (Fluka) that was modified to lower its electrical resistance¹. The reference barrel was backfilled with 150 mM NaCl. For brain recordings, ISMs were connected to an Axoprobe-A1 differential amplifier (Axon Instruments, Burlingame, CA) and inserted into the brain using a micromanipulator (see below). A 1 M KCl 3% agar bridge

placed on the adjacent temporalis muscle served as an indifferent electrode. Signals (mV) were filtered at 2 Hz, displayed on the polygraph, digitized (mod. DR-484, Neurodata, New York, NY) and stored on videotape. Before and after recording from the brain, ISMs were calibrated with test solutions containing 3, 6, 12, 24, 48, 96 mM KCl and 150 mM NaCl. The relationship between mV and K^+ concentration (mM) so obtained was then fitted to the Nikolsky equation² using least-square linear regression analysis¹⁹. The Na interference value yielding the best fit was determined by an iterative procedure and this value was used in the final calculations. Slope and Na interference of the micropipettes were 56.8 ± 0.6 and 0.03 ± 0.02 ($n = 11$) per decade change in K^+ , respectively.

As described in detail elsewhere⁸, LDF was performed using an instrument (BPM 403, Vasamedic, St. Paul, MN) equipped with a 2 mW helium-neon laser (wavelength of 632.8 nm). The probe (tip diameter 0.8 mm), mounted on a micromanipulator, was positioned near the K^+ electrode and approximately 0.5 mm above the pial surface. Probe positioning near large pial vessels was avoided. The analog output of the instrument was fed into a DC amplifier and displayed on the polygraph. Once a suitable placement was obtained, the probe was left at that site for the duration of the experiment. After sacrificing the animal the zero level of CBF was recorded on the polygraph. CBF elevations were expressed as percent increase relative to the resting value.

After establishment of the anesthesia insertion of catheters, artificial ventilation and exposure of the sensory cortex and caudal brainstem, a stimulating electrode mounted on a micromanipulator with a 10° posterior inclination was positioned on the calamus scriptorius and the stereotaxic coordinates recorded as stereotaxic zero¹⁸. The electrode was then moved 5 mm rostral and 0.8 mm lateral to stereotaxic zero, lowered into the cerebellum until the vertical stereotaxic zero was reached. As described elsewhere¹⁸, the electrode was withdrawn in 0.5 mm steps and at each step exploratory stimuli, consisting of 8 s trains of 0.5 ms pulses at 50 Hz and with a stimulus intensity of 1–2 V, were delivered. An active site in FN was defined as one in which stimulation resulted in a stimulus-locked 10–20 mm Hg AP elevation.

Once the most active site in FN was localized the electrode was left there. With the aid of a surgical microscope the ISM was then positioned over the target cortical area and the K^+ concentration of the Ringer superfusate recorded. The pipette was inserted into the cortex and lowered 300 μ m below the pial surface. The FN was then stimulated (8 s trains, 50 Hz, 5–10 V) and the K^+_o changes in cerebral cortex recorded. To determine the cortical depth profile of the K^+_o changes, the micropipette was withdrawn at 250 μ m steps and, after stabilization of the K^+ signal, the FN was stimulated at each step. In experiments in which the relationship between K^+_o and CBF was studied, rats were prepared as described above with the exception that the cerebellum was not exposed and the FN was not stimulated. The ISM was inserted 400–600 μ m below the pial surface of the target cortical area and the LDF probe was positioned near to it (Fig. 3). The K^+ concentration of the superfusate was then gradually increased (20–30 mM) until recurrent waves of cortical spreading depression occurred.

Data are expressed as mean $\pm$ S.E.M. Multiple comparisons were evaluated by the analysis of variance with repeated measures and the Scheffé test. Two-group comparisons were analyzed by Student's *t*-test. Values were considered statistically significant for $P < 0.05$.

Resting K^+_o in sensory cortex was 3.2 ± 0.1 mM (mean $\pm$ S.E.M., $n = 13$) (range: 2.4–3.9 mM). Electrical stimulation of the FN (8 s trains, 50 Hz, 5–10 V) produced sustained and stimulus-locked elevations in cortical K^+_o ranging from 0.2 to 2.9 mM ($n = 163$ in 9 rats) (Fig. 1). The increases in K^+_o were related to the intensity of the stimulus and could not be elicited by stimulation of sites near but not within the FN (Fig. 1). To examine the possibility that the elevations in K^+_o were related to the increase in AP elicited by FN stimulation, the relationship

between magnitude of the responses evoked from all cortical depths and AP elevations was examined by linear regression analysis. This analysis showed that the elevations in K^+_o were independent of the increases in AP ($r = 0.013$; $P > 0.05$; $n = 163$).

As illustrated in Fig. 2 the magnitude of the K^+ responses was dependent on the cortical depth from which they were recorded. Maximal K^+ elevations occurred at $750\ \mu\text{m}$ below the pial surface wherein K^+_o increased by $0.91 \pm 0.16\ \text{mM}$ ($P < 0.05$; analysis of variance and Scheffé test) (Fig. 2). The K^+_o elevations recorded at depths larger and smaller than $750\ \mu\text{m}$ did not reach statistical significance ($P > 0.05$). These smaller increases are probably due to diffusion of the ion along the concentration gradient generated by the restricted K^+ source or, less likely, to lower-level release distributed over a wider volume.

We next sought to determine whether the magnitude of the increase in K^+_o elicited by FN stimulation was sufficient to produce relaxation of the smooth muscles of local blood vessels and could mediate the vasodilation through such mechanism. To achieve this goal, the target cortical region was superfused with Ringer containing an elevated concentration of K^+ so as to gradually increase K^+_o locally. At the same time CBF and K^+_o were monitored continuously by LDF and K^+ -selective micropipettes, respectively. A representative experiment is shown in Fig. 3. Elevations in K^+_o averaging $2.8 \pm 0.6\ \text{mM}$ increased CBF by $17 \pm 2\%$ ($n = 5$) while larger changes ($11.4 \pm 1.6\ \text{mM}$) increased CBF by $83 \pm 15\%$ ($n = 4$), prior to inducing cortical spreading depression. Therefore increases in K^+_o similar to those elicited by FN stimulation produce increases in CBF which are considerably smaller than those elicited by stimulation of the FN.

We have investigated whether K^+ , an ion which has long been considered to be involved in cerebrovascular regulation during neuronal activation^{4,5,16}, could participate in the cerebrovasodilation elicited by FN stimulation. We have found that indeed electrical stimulation of the FN elicits increases in cortical K^+_o that are greatest at sites corresponding to the intermediate cortical laminae. The focality of the K^+ increases and the sharp decline of the changes at depths greater and smaller than $750\ \mu\text{m}$ is consistent with K^+ being generated from a restricted planar source¹⁹. Indeed, the K^+ increases elicited by FN stimulation in sensory cortex are smaller and more focal than those evoked by stimulation of the ventrobasal complex of the thalamus, which activates neocortical neural elements largely confined to lamina IV⁶.

For several reasons, it is unlikely that K^+_o mediates the cerebrovasodilation elicited by FN stimulation. First, the increases in K^+_o occur only in selected cortical laminae while the increases in CBF, as determined by the autoradiographic iodoantipyrine technique, involve all cortical laminae equally^{10,17}. Second, when cortical K^+_o was elevated by K^+ superfusion to the same level reached during FN stimulation, the resulting increases in CBF (+10–20%) were negligible in comparison with those elicited by FN stimulation (+100–120%)^{10–12,17,18}. Third, the time-course of the elevations in K^+_o and CBF differ substantially. While the K^+_o elevations are rapid and temporally 'locked' to the stimulus, the elevations in CBF are slow and persist well beyond termination of the stimulation⁸. Therefore, other vasoactive agents are likely to participate in the cerebrovasodilation.

It is well established that neural activity results in an increased K^+ concentration in the extracellular space^{6,13,21–23}. Whether the K^+_o increases result from activity in neuronal cell bodies or terminals has not been definitely established²⁴. Several lines of evidence suggest that a substantial portion of K^+ release originates from synaptic transmission^{21–23}. Therefore, it is likely that the increases in neocortical K^+ elicited by FN stimulation result from enhanced synaptic activity, presumably, from terminals of local neurons. Irrespective of the cellular origin of K^+ , the findings of this study are consistent with K^+ being generated from a neural source confined to the middle cortical laminae.

The identity of the local neurons mediating the cortical cerebrovasodilation has not been determined. Indirect evidence suggest that these neurons may represent a restricted group. First, stimulation of the FN does not produce electroencephalographic evidence of widespread cortical activation^{8,11}. Second, in most cortical regions FN stimulation does not increase local glucose utilization, as established by the 2-deoxyglucose method¹⁷. Thus the neurons mediating the vasodilation may be so few that their activation does not increase the global glucose utilization of the region in which they are situated. The present study provides further support to this hypothesis as it demonstrates that the cortical neural activity evoked by FN stimulation, reflected by increases in K^+_o is highly restricted. Therefore, only selected cortical neural elements might be activated by FN stimulation. The findings also raise the possibility that the active neural elements responsible for the vasodilation are located in the middle cortical laminae.

In summary, we have demonstrated that stimulation of the FN produces increases in cortical K^+_o which are restricted to the intermediate cortical laminae. While these changes in K^+_o are unlikely to be the sole mediator of the increases in cortical CBF elicited by FN stimulation, their localized spatial distribution supports the hypothesis that the cortical vasodilation is mediated by activation of a restricted group of neural elements, perhaps neurons in laminae III–IV.

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References

1. Amman D, Chao P, Simon W. Valinomycin-based K^+ selective microelectrodes with low electrical membrane resistance. *Neurosci Lett* 1987;74:221–226. [PubMed: 3574760]
2. Amman, D. Ion-Selective Microelectrodes. Springer; Berlin: 1986.
3. Bretag AH. Synthetic interstitial fluid for isolated mammalian brain tissue. *Life Sci* 1969;8:319–329. [PubMed: 5781321]
4. Cameron IR, Caronna J. The effect of local changes in potassium and bicarbonate concentration on hypothalamic blood flow in the rabbit. *J Physiol* 1976;262:415–430. [PubMed: 11341]
5. Chesler M, Kraig R. Intracellular pH of astrocytes increases rapidly with cortical stimulation. *Am J Physiol* 1987;253:R666–R670. [PubMed: 3116863]
6. Cordingley GE, Somjen GG. The clearing of excess potassium from extracellular space in spinal cord and cerebral cortex. *Brain Research* 1978;151:291–306. [PubMed: 209864]
7. Iadecola C, Raichle ME. Local regulation of the cerebral microcirculation: toward a neurobiological understanding. *Trends Neurosci.* in press.
8. Iadecola C, Reis DJ. Continuous monitoring of cerebrocortical blood flow during stimulation of the cerebellar fastigial nucleus: a study by laser-doppler flowmetry. *J Cereb Blood Flow Metab* 1990;10:608–617. [PubMed: 2117016]
9. Iadecola C, Kraig RP. Stimulation of the fastigial nucleus produces a focal elevation in cerebrocortical interstitial K^+ *Soc Neurosci Abstr* 1990;16:291.
10. Iadecola C, Arneric S, Baker H, Tucker L, Reis D. Role of local neurons in the cerebrocortical vasodilation elicited from cerebellum. *Am J Physiol* 1987;252:R1082–R1091. [PubMed: 3591980]
11. Iadecola C, Underwood M, Reis D. Muscarinic cholinergic receptors mediate the cerebrovasodilation elicited by stimulation of the cerebellar fastigial nucleus in rat. *Brain Research* 1986;368:375–379. [PubMed: 3516306]
12. Iadecola C, Mraovitch S, Meeley M, Reis D. Lesions of the basal forebrain in rat selectively impair the cortical vasodilation elicited from cerebellar fastigial nucleus. *Brain Research* 1983;279:41–52. [PubMed: 6416613]

13. Kortytová H. Arousal-induced increase of cortical $[K^+]$ in unrestrained rats. *Experientia* 1977;33:242–244. [PubMed: 844574]
14. Kraig R, Chesler M. Astrocytic acidosis in hyperglycemic and complete ischemia. *J Cereb Blood Flow Metab* 1990;10:104–114. [PubMed: 2298827]
15. Kuschinsky W, Wahl M, Bosse O, Thürau K. Perivascular K^+ and pH as determinants of local pial arterial diameter in cats: a microapplication study. *Circ Res* 1972;31:240–247. [PubMed: 5049739]
16. Lou HC, Evinsson L, MacKenzie ET. The concept of coupling blood flow to brain function: revision required? *Ann Neurol* 1987;22:289–297. [PubMed: 2890324]
17. Nakai M, Iadecola C, Ruggiero D, Tucker L, Reis D. Electrical stimulation of cerebellar fastigial nucleus increases cerebrocortical blood flow without change in local metabolism: evidence for an intrinsic system in brain for primary vasodilation. *Brain Research* 1983;260:35–49. [PubMed: 6824954]
18. Nakai M, Iadecola C, Reis D. Global cerebral vasodilation by stimulation of rat fastigial cerebellar nucleus. *Am J Physiol* 1982;243 :H226–H235. [PubMed: 7114233]
19. Nicholson C, Phillips JM. Ion diffusion modified by tortuosity and volume fraction in the extracellular microenvironment of the rat cerebellum. *J Physiol* 1981;321:225–257. [PubMed: 7338810]
20. Paulson OB, Newman EA. Does the release of potassium from astrocyte endfeet regulate cerebral blood flow? *Science* 1987;237:896–898. [PubMed: 3616619]
21. Richter DW, Camerer H, Sonnhof U. Changes in extracellular potassium during spontaneous activity of medullary respiratory ions. *Pflügers Arch* 1978;376:139–149.
22. Singer W, Lux HD. Extracellular potassium gradients and visual receptive fields in the cat striate cortex. *Brain Research* 1975;96:378–383. [PubMed: 1175021]
23. Ten Bruggencate G, Nicholson C, Stöckle H. Climbing fiber evoked potassium release in cat cerebellum. *Pflügers Arch* 1976;367:107–109.
24. Walz A, Hertz L. Functional interactions between neurons and astrocytes. II Potassium homeostasis at the cellular level. *Progr Neurobiol* 1983;20:133–183.

Fig. 1.

Upper panel: elevations in extracellular K^+ and arterial pressure (AP) elicited by electrical stimulation of the fastigial nucleus (FN) in anesthetized rat. Notice that the K^+ elevations are stimulus-locked and related to the intensity of the stimulation (5 V 5 Volts; 10 V 10 Volts; 50 Hz; 8 s duration). Lower panel: locations of sites in the FN from which increases in K_o^+ and AP could be elicited by electrical stimulation. Notice that maximal increases in K_o^+ and AP could be elicited only from sites within the FN.

Fig. 2.

Depth profile of the changes in neocortical K⁺_o elicited by electrical stimulation of the fastigial nucleus in anesthetized rat. Upper panel: sites in sensory cortex where the elevations in K⁺_o occurred in a representative experiment. Largest K⁺_o changes are seen in laminae III-IV. Lower panel: distribution of the K⁺_o elevations ([ΔK⁺]_o) according to cortical depth (mm below pial surface). Significant K⁺_o elevations occur only at a depth of 0.75 mm ($P < 0.05$; ANOVA and Scheffé test). The depth profile suggests that the increases in K⁺ result from a single laminar source in laminae III-IV.

Fig. 3.

Upper panel: apparatus used to study the relationship between cortical cerebral blood flow and extracellular K⁺. A laser-doppler probe (ICBF) was placed near a K⁺-sensitive micropipette inserted 400–600 μm below pia. The exposed cortex was superfused with Ringer containing increasing concentrations of K⁺ and the resulting changes in CBF and K⁺_o monitored. Lower panel: relationship between changes in cerebral blood flow (ΔCBF) and changes in K⁺_o (ΔK⁺_o) in the sensory cortex of a representative rat. Notice that an increase in K⁺_o similar to that produced by stimulation of the fastigial nucleus (2–3 mM; arrow) elevated CBF by less than 20%.

TABLE I

Arterial pressure, blood gases and hematocrit of the rats used in this study

AP (mm Hg)	107 ± 4
pCO ₂ (mm Hg)	33.9 ± 1.2
pO ₂ (mm Hg)	124 ± 13
pH	7.43 ± 0.02
Hematocrit (%)	44 ± 1

Mean ± S.E.M., $n = 11$; AP, arterial pressure.