１．研究課題名：

レーザードップラ血流計を用いたラット体性感覚野賦活に伴う二次信号の計測とその発生機序に関する研究

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５．研究期間：1996 年―2000 年

６．要約

ラット体性感覚野賦活時における神経活動の電気的計測とレーザードップラ血流計による微小血流動態の測定を行った。ラット後肢の電気刺激や皮質直接刺激に対する体性感覚野の神経応答と血流量の関係を調査した結果、脳活動の大きさと血流増加量には相関関係があること、局所血流調節が細動脈の弛緩・拡張のみならず毛細血管レベルでも行われている可能性があることを明らかにした。また、賦活時の血流増加量が脳組織の酸素消費量や物質の代謝量とは独立に決定されること、高酸素分圧下での賦活血流過剰供給の一端に一酸化窒素合成系の変化が関与している可能性が明らかとなった。

７．研究目的

神経細胞が蓄えることのできるエネルギー量は極めて僅かである。そのため、一定の血流が常時脳に供給されることは、脳機能の恒常性を維持する上で非常に重要である。脳の自動調節能や頭蓋内圧緩衝能は、そのような観点から生理学的に有効な機能であるといえる。一方、機能的に高度に分化した脳では、局所的な神経細胞群が興奮と抑制を繰り返しながら密接に連絡している。したがって、脳全体の恒常性とは別に、局所的な機能変化に追従できる調節機構、つまり微小循環調節が必要となる。

脳の神経活動と活動領域における血流増加機構の存在が報告されて以来、その調節メカニズム解明のために様々な研究が行われてきた[1, 2]。しかし、その大部分はマクロ的な脳血流変化の調節機序の解明であり、脳の微小循環調節については不明

な点が多く残されている。解明されるべき問題として、賦活時の微小循環調節に関与している血管部位はどこなのか、その血管部位はどのような機序で調節され、どのような目的で血流は変化するのか、などを挙げることができる。本研究ではレーザードップラ血流計（LDF）を用い、ラット大脳皮質体性感覚野賦活時の微小循環調節機構について様々な角度から前述の問題にアプローチした。具体的には、（1）脳賦活の大きさと局所血流動態の相関について、（2）賦活血流の調節血管の検索、（3）賦活時における血流増加の目的、（4）賦活血流量調節機序の解明である。

8．材料と方法

αクロラロース及びミオブロックで持続麻酔した Sprague-Dawley ラット（300g 450g）を用いた。正常時の血中ガス分圧は人工呼吸器による呼吸管理によって一定（$PaCO_2$=32　40mmHg, PaO_2=90　120mmHg）に調節した。体性感覚野の興奮は後肢皮下への電極挿入による 0.1 ミリ秒パルスの電気刺激（1.5mA、5 秒）、あるいは体性感覚野への刺激電極挿入による直接電気刺激（1m 秒パルス、10μA、5 秒）により引き起こした。局所血流量は LDF を用いて測定し、対応部位の頭骨を薄くした状態で行った（図 1）。LDF の時間分解能は 0.03m 秒、計測範囲は 1mm^3 である。本 LDF は血流量、赤血球濃度、赤血球速度（血流量／赤血球濃度）をそれぞれ検出できる。刺激に対する体性感覚野の神経応答は、タングステン電極を対応部位に挿入することで記録した。神経活動の電気的記録および LDF シグナルは MacLab による加算平均を行った。有意差の検定には t 検定を用いた。

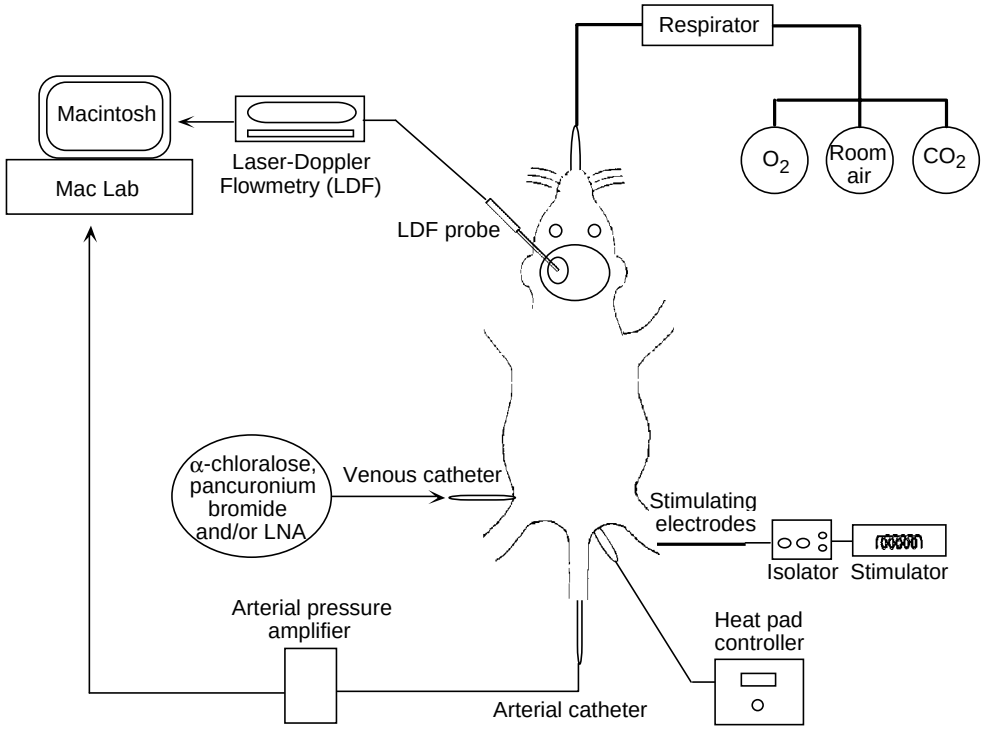

図 1．実験の模式図

αクロラロース及びミオブロックで持続麻酔したラットをステレオに固定し、人工呼吸器で呼吸管理した。血流変化はレーザー・ドップラ血流計(LDF)を用いて計測し、血圧モニターからの出力と共に Mac Lab で加算平均した。

１）刺激頻度と局所血流量の計測

脳神経活動と賦活血流量の関係を調査するため、後肢への 0.2、0.5、5、10、50 Hz の電気刺激、あるいは体性感覚野への 5、10、50 Hz の直接電気刺激により誘発される局所血流変化を計測した。それぞれの刺激頻度における血流量は、20 回の連続刺激（80 秒間隔）に対する加算平均により求めた。

２）血中ガス分圧の揺らぎに対する血流動態の計測

正常ガス分圧下における賦活血流量の計測後、高酸素分圧、低二酸化炭素分圧あるいは高二酸化炭素分圧下での賦活血流量を計測した。体性感覚野の興奮は後肢への 5Hz の電気刺激により行い、それによって誘発される血流量変化は、それぞれの状態で 50 回の連続刺激に対する LDF シグナルの加算平均により求めた。

高酸素分圧化は呼吸器へのガス供給を 100%酸素に切り替えることで、低二酸化炭素分圧化は呼吸器のストローク量を大きくすることで、高二酸化炭素分圧化は正常ガスに二酸化炭素を約 2.5%の割合で混合することによりもたらした。

３）一酸化炭素合成阻害と局所血流量の変化

正常時での賦活血流量計測後、正常酸素分圧下あるいは高酸素分圧下で一酸化炭素（NO）合成阻害時の賦活血流量を計測した。体性感覚野の興奮誘発には後肢への 5Hz 電気刺激を用い、それぞれの状態で 30 回の連続刺激に対する LDF シグナルの加算平均を行った。

高酸素分圧化は呼吸器へのガス供給を 100%酸素に切り替えることで、また NO の合成阻害は NO 合成酵素阻害剤（N^{ω}-nitro-L-arginin: LNA）を静脈より注入することにより行った。

９．結果

１）刺激頻度と局所血流量の計測

血流の立ち上がりのタイミングは刺激頻度に関係なく刺激開始後約 0.5 秒であり、赤血球速度及び濃度の立ち上がりに有意な時間差は認められなかった。また、後肢刺激の場合、体性感覚野賦活時の局所血流量（賦活血流量）は刺激頻度が 5Hz で最大となり、これは誘発電位の発現頻度の最大値と一致した（図 2a）。直接刺激によって誘発される賦活血流量は刺激頻度の増大とともに大きくなった（図 2b）。それぞ

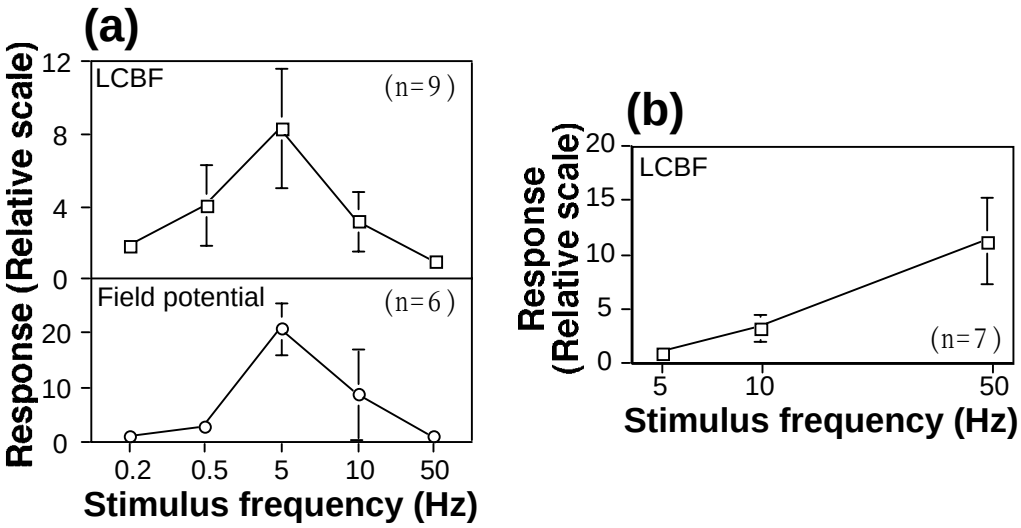

図 2. 脳賦活と局所血流量の関係

（a）末梢刺激（後肢電気刺激）に対する局所血流増加量と誘発電位の発現頻度。（b）皮質直接電気刺激に対する刺激頻度と局所血流増加量の関係。

れの刺激に対する赤血球速度及び濃度の反応量に顕著な差は認められなかった（図3）。

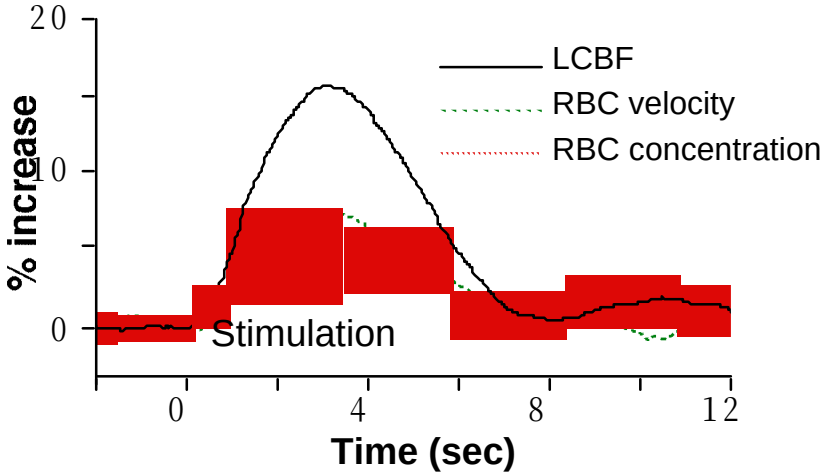

図 3. 刺激中の血流動態

ハッチラインは刺激時間を示す。縦軸は血流増加量をパーセントで表示した。

2）血中ガス分圧の揺らぎに対する血流動態の計測

高酸素分圧下（PaO_2=479.4±77.2mmHg）のベースライン血流量は正常ガス分圧時（PaO_2=105.5±7.8mmHg）と比較し約 5.0%減少した（$p<0.01$）。この時、赤血球濃度は有意に低下し、赤血球速度はわずかに上昇した（図 4）。高酸素分圧下の基

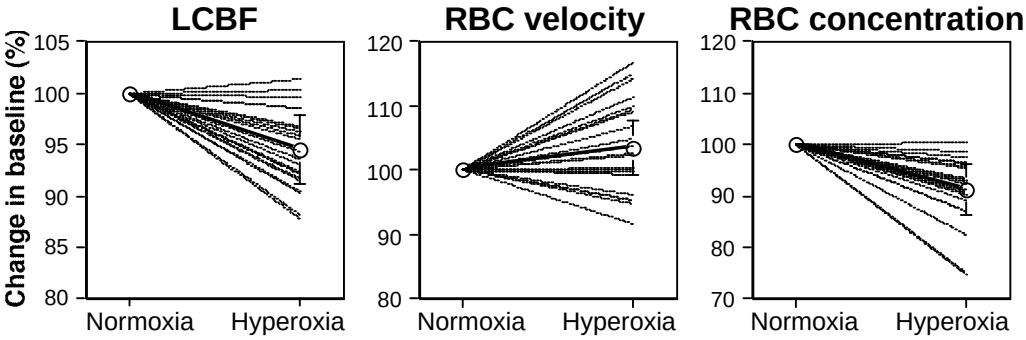

図 4．高酸素分圧時のベースラインレベルの変化

正常ガス分圧時の値を 100%とし、高酸素分圧時の血流量、赤血球速度、赤血球濃度のベースラインレベルの変化をパーセントで示した。一本の破線は試行したラット 1 個体に対応し、実線及び縦棒はそれぞれ平均値及び SD を示す。

準化賦活血流量（ベースライン血流量で基準化した賦活血流量）は正常ガス分圧時と比べ約 64.4%増加し（$p<0.01$）、その立ち上がりは約 150 ミリ秒早くなった（図 5a）（$p<0.01$）。高酸素分圧下の赤血球速度と濃度の反応量は正常ガス分圧時のそれらと比較し有意に増加した（図 5b）（$p<0.05$）。

一方、高二酸化炭素分圧（$PaCO_2$=73.4±13.3mmHg）及び低二酸化炭素分圧（$PaCO_2$=26.4±1.1mmHg)下の安静時血流量（ベースライン血流量）を正常二酸化炭素分圧（$PaCO_2$=34.7±2.5mmHg）下のそれと比較した結果、それぞれ 47%増加、11%減少した（図 6a）。赤血球速度及び濃度がベースライン血流量変化に及ぼす影

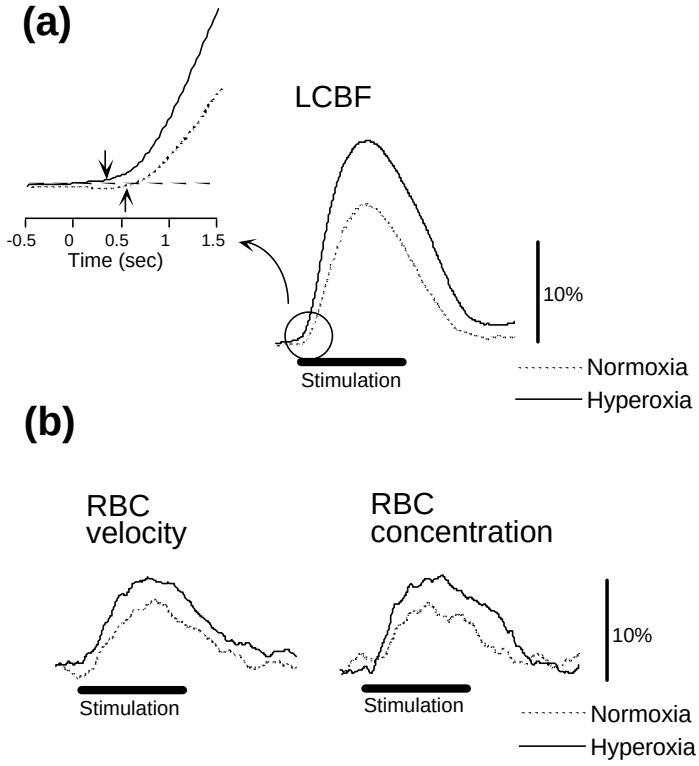

図 5．高酸素分圧時の血流動態

（a）血流量変化。挿入図の矢印はそれぞれのガス分圧下における血流の平均立ち上がり時間を、横軸の Time=0 は刺激の開始示している。（b）赤血球速度および濃度の変化。反応曲線はそれぞれのベースラインレベルで基準化したものを示す。縦棒は 10%の増加を、横棒は刺激時間（5Hz、5sec）を示している。

響は、赤血球速度の変化がより大きく関与していた（図 6b）。また、各々の二酸化炭素分圧下における賦活血流量は、二酸化炭素分圧の増加に伴って増加した（図 6a）。

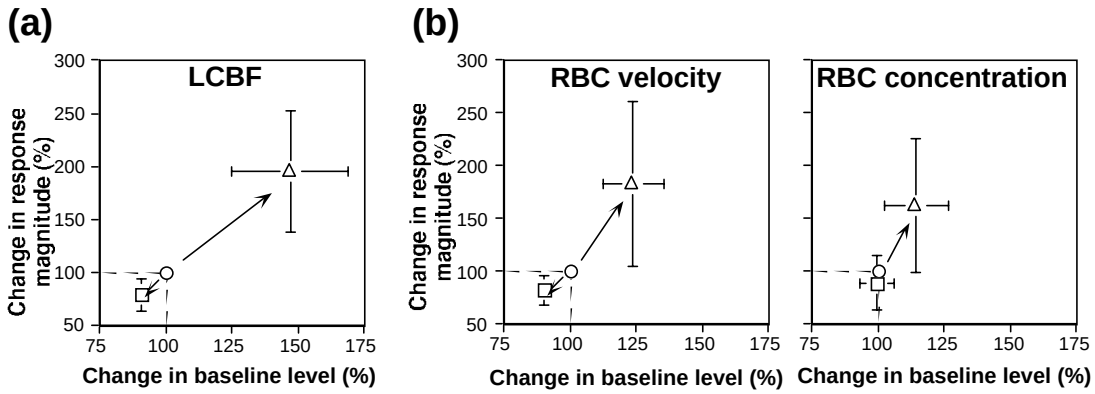

図 6. ベースラインレベルと反応量の関係

正常二酸化炭素分圧時（○）の値を 100%とし、低二酸化炭素分圧時（□）、高二酸化炭素分圧時（△）の血流量、赤血球速度、赤血球濃度の変化をパーセントで示した。横軸はベースラインレベル、縦軸は反応量を示す。

基準化賦活血流量、赤血球速度と濃度の反応量は、それぞれの二酸化炭素分圧間で有意差は認められなかった（図 7）。また、どの二酸化炭素分圧状態においても、赤血球速度と濃度の反応量の間に顕著な差は認められなかった。

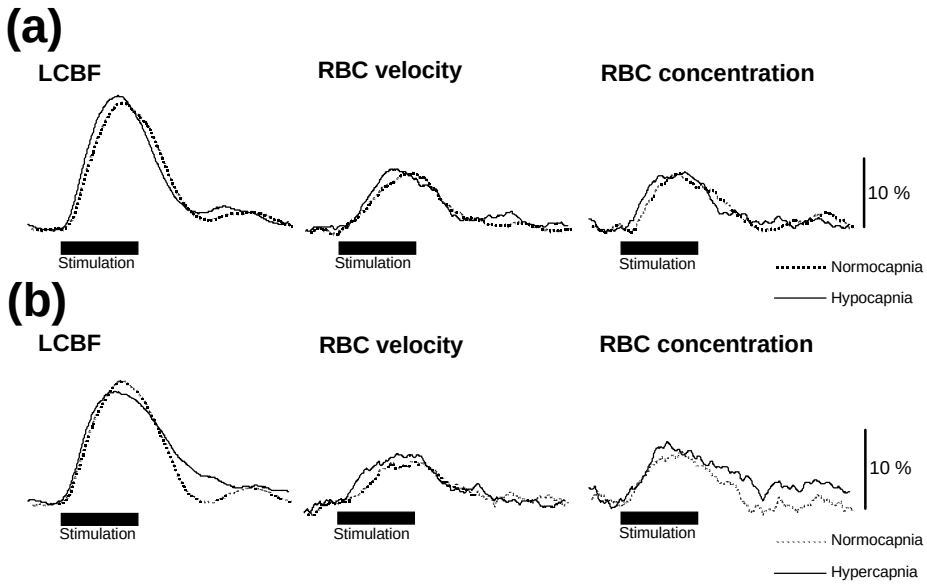

図 7．低二酸化炭素分圧時（a）及び高二酸化炭素分圧時（b）時の血流動態

反応曲線はそれぞれの二酸化炭素分圧時のベースラインレベルで基準化して示した。縦棒は 10%の増加を、横棒は刺激時間（5Hz、5sec）を示している。

3）一酸化炭素合成阻害と局所血流量の変化

高酸素分圧下で LNA 投与後は正常酸素分圧下で LNA 投与前と比較し、ベースライン血流量が約 18%低下し、基準化賦活血流量も約 37%減少した（図 8）（$p<0.01$）。賦活血流量の立ち上がりに有意差は認められなかった。一方、正常酸素分圧で LNA

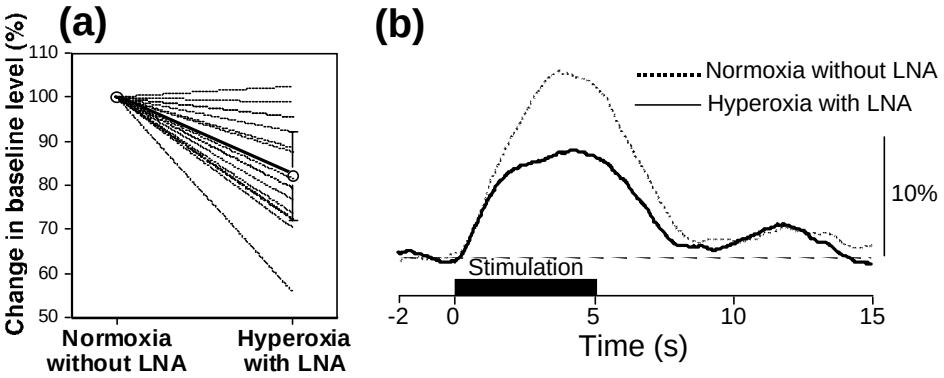

図 8．高酸素分圧時 NO 合成抑制下における血流変化

（a）ベースラインレベルの変化　正常時の値を 100%とし、高酸素分圧時 LNA 投与下のベースライン血流量の変化をパーセントで示した。（b）血流量変化。反応曲線はそれぞれのベースライン血流量で基準化したものを示す。縦棒は 10%の増加を、横棒は刺激時間（5Hz、5sec）を示している。

投与後は投与前と比べ、ベースライン血流量は約 14%低下したが（$p<0.01$）、基準化賦活血流量やその立ち上がりに有意差は認められなかった（図 9）。

10．考察

1）脳賦活血流量は神経活動の大きさに比例する

脳血流量と脳代謝の相関関係が計測法の発達により明らかにされ、賦活時の神経活動の大きさと血流増加量はほぼ比例関係にあるという概念は多くの研究者が認めるところである。しかし、*in vivo* 下の動物で末梢刺激によって引き起こされた脳賦活の大きさと局所血流増加量を調査した文献は少ない[3-6]。本研究では、ラット後肢を様々な頻度で電気刺激し、脳賦活部位における局所血流変化を LDF を用いて計測した。同時に、皮質に金属電極を挿入し LDF 計測部分の局所神経応答を記録した。その結果、誘発電位の発現頻度と局所血流増加量はほぼ相関することを明らかにした（図 2a）。また、脳を直接電気刺激し強制的に高頻度で脳神経を興奮さた場合、刺激頻度に応じた血流増加を確認した（図 2b）。しかしながら、脳活動を賦活部位における神経活動の総和と定義した場合、興奮する神経細胞の数が血流増加量を決定するのか、神経の発火頻度なのか、あるいはその両者なのか、現在のところ決着

を得ていない。

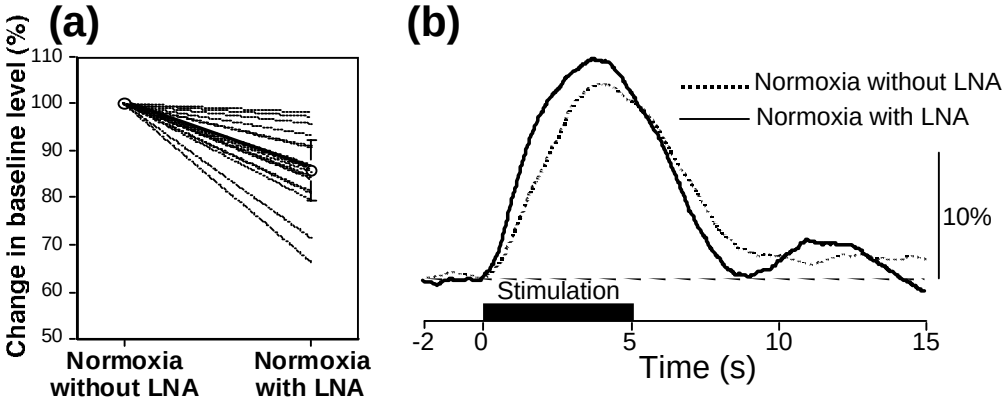

図 9．正常酸素分圧時 NO 合成抑制下における血流変化

（a）ベースラインレベルの変化：正常時の値を 100%とし、正常酸素分圧時 LNA 投与下のベースライン血流量の変化をパーセントで示した。（b）血流量変化：反応曲線はそれぞれのベースライン血流量で基準化したものを示す。縦棒は 10%の増加を、横棒は刺激時間（5Hz、5sec）を示している。

２）脳賦活血流増加量は代謝要求量に対して独立である

脳賦活時の血流増加の目的は、代謝によって消費された酸素や栄養物質の供給であると考えられる。脳賦活時には血管から賦活部位への局所的な酸素やグルコースの供給が報告されている[7-9]。高酸素分圧時（PaO_2=500mmHg）の脳組織中酸素分圧は正常酸素分圧時と比較し約 20%増加する[10]。したがって、賦活血流量が代謝によって消費された酸素量に依存して決定されると仮定すると、高酸素分圧時の賦活血流量は正常酸素分圧時と比べ低下するものと考えられる。しかしながら、高酸素分圧状態での脳賦活は正常酸素分圧時に比べより多くの血流増加を誘発することが PET や fMRI を用いた人での賦活実験で報告されている[11, 12]。LDF を用いた本研究でも同様の現象を確認している（図 5a）。これらのことは、賦活血流量が代謝による酸素要求量とは独立に決定されることを示唆している[13]。また、組織への過剰な酸素供給、あるいは脳組織の高酸素分圧は神経活動に伴う血流調節機構に何らかの影響を与えると考えられる。

低二酸化炭素分圧時には正常二酸化炭素分圧時と比べベースライン血流量が約 11 %減少し、高二酸化炭素分圧時には約 47%増加している（図 6a）。したがって、賦活時の血流増加が酸素以外の物質（例えばグルコースなど）の供給にある場合、低二酸化炭素分圧時にはより多くの血流の供給が必要であるし、逆に高二酸化炭素分圧時には少ない供給で十分であると考えられる。しかし、本実験から賦活血流量はベースライン血流量に比例することが明らかとなった（図 6a）。このことは、賦活血流量が代謝によって消費された酸素以外の物質の要求量に対しても独立であることを示唆している[14]。

前述のように、賦活血流量は神経活動の大きさに比例する（図 2）。賦活血流の増加が代謝によって消費された物質の要求量に対して独立であることから、神経活動の大きさに対応した賦活血流量の増加は、単に神経活動に比例した血管反応を反映しているものと考えられる。神経活動に伴う血管拡張には二酸化炭素、一酸化炭素、NO、カリウム、アデノシンなどの化学的調節に加え、神経性調節も報告されている

[2]。賦活時の血管反応にはこれらの協調・拮抗の複雑な系の関与が考えられる。

３）賦活血流の調節血管

抵抗血管（細動脈）が局所脳血流の調節に大きく関与していることは明らかであるが、毛細血管の積極的な関与を示唆する報告も存在する[5, 6, 8, 15]。「全ての毛細血管は様々な血流速度で導通があり、賦活によって流速の遅い血管内で血液の流れが早くなる」という概念は多くの研究者が認めるところである[1]。しかし、微小循環レベルの血流調節が微小循環血管系のどの部分（毛細血管網直前の細動脈、毛細血管あるいは細静脈）で行われているか、その詳細は良く分かっていない。LDF は単位体積当たりの赤血球の濃度と赤血球の平均速度を独立に検出することができる。脳賦活時の毛細血管網の血流動態を LDF を用いて計測した場合、抵抗血管によってのみ微小循環が調節されているのであれば赤血球の速度増加は赤血球の濃度増加よりも先行するであろうし、また、毛細血管が先行して微小循環を調節しているのであれば赤血球の濃度は赤血球の速度よりも早く増加するはずである。しかし我々の観察では、赤血球の濃度と速度の増加はいずれも血流増加と同時に立ち上がり、血流増加に関与する両者の変化の割合はほぼ同等であった（図 3）。

血中二酸化炭素分圧の変化において、ベースライン血流量に寄与する割合は赤血球濃度の変化よりも速度の変化が大きい（図 6b）。これは、二酸化炭素分圧の変化に伴う血流変化には抵抗血管の変化がより大きく関与していることを意味している[14]。一方、賦活時における赤血球速度と濃度の反応量はほぼ同等である（図 7）。これは、賦活時における血管反応が抵抗血管のみならず毛細血管レベルでも起こっていることを意味する[14]。抵抗血管と毛細血管には電気的なやり取りがあることも報告されている[16]。以上のことは、抵抗血管と毛細血管による血流の二重調節機構の存在を示唆する。

４）脳賦活血流量に及ぼす一酸化窒素の影響

正常酸素分圧下では LNA 投与による安静時血流量は低下したが、基準化賦活血流量は投与前のそれと比較し有意な変化は認められなかった（図 9）。これは、低二酸化炭素分圧時の血流変化と同様である（図 6a, 7a）。正常酸素分圧下での LNA 投与は抵抗血管以上の比較的太い血管の NO 合成を抑制するが、賦活血流量を調節する局所機構に与える影響は少ないと考えられる。一方、高酸素分圧下では LNA 投与後の基準化賦活血流量は有意に減少している（図 8）。高酸素分圧時、NO 活性の上昇がラット胎児で報告されている[17]。したがって、高酸素分圧下における LNA 投与後の基準化賦活血流量の減少は、過剰に上昇した局所レベルでの NO 活性が LNA によって抑制を受けた結果であると推察される。高酸素分圧下における血流の過剰供給が、NO 活性の上昇によってもたらされる可能性が示唆された。

11．今後の展開

本研究は脳活動に伴う二次信号発生機序の一部分を解明したに過ぎない。しかしながら、ここで得られた結果は循環生理学的知識の集積にとどまらず、「動物脳の高

次機能の解明」といった現代生物学・医学の重要なテーマを理解する上で有用なモデルに成りうると考えられる。例えば、本研究では脳賦活血流増加量は代謝要求量に対して独立であることを明らかにした。しかしこれは、生物体における物質の消費と供給の概念からは不可思議な現象である[18]。動物は生存に必要ないくつかの補償機能を生物進化の過程で獲得した。脳における血流調節機構においても同様の補償機能を得たものと考えられる。生物進化のどの段階でこのような特殊性を獲得したかを調査することで、その機能の必要性が解明されるであろう。

動物行動をつかさどる脳の特徴はその複雑さにあり、脳科学の最終目標はその様な複雑な脳機能の基盤を生理学的、行動学的に明らかにすることにあると考えられる。これまでの研究では、個々の研究分野における「一定の脳生理機構」については一応の知見を引き出すことに成功してきた。脳の世紀と言われ、脳研究に関わる様々な光学機器や生物学的手法が生み出され、今までに不明とされてきた脳機能が次々と明らかにされている。しかしながら、それらが組み合わさった、より複雑な系についてはほとんど手がつけられていないのが現状である。今後は、これまでに得られた知見を土台とした上で、さらに複雑な脳機能の解析へと進展するのがこの研究分野の進むべき方向であろうと確信する。

12. 参考文献

[1] Kuschinsky W, Paulson OB. Capillary circulation in the brain. Cerebrovasc. Brain Metab. Rev., 4, 261-286 (1992)

[2] Villringer A, Dirnagl U. Coupling of brain activity and cerebral blood flow: basis of functional neuroimaging. Cerebrovasc. Brain Metab. Rev., 7, 240-276 (1995)

[3] Ibáñez V, Deiber MP, Sadato N, Toro C, Grissom J, Woods RP, Mazziotta JC, Hallett M. Effects of stimulus rate on regional cerebral blood flow after median nerve stimulation. Brain, 118, 1339-1351 (1995)

[4] Leniger-Follert E, Hossmann KA. Simultaneous measurements of microflow and evoked potentials in the somatosensory cortex of the cat brain during specific sensory activation. Pfluegers Arch 380, 85-89 (1979)

[5] Matsuura T, Fujita H, Seki C, Kashikura K, Kanno I. Hemodynamics evoked by microelectrical direct stimulation in rat somatosensory cortex. Comp. Biochem. Physiol. A, 124, 47-52 (1999)

[6] Matsuura T, Fujita H, Seki C, Kashikura K, Yamada K, Kanno I. CBF change evoked by somatosensory activation measured by laser-Doppler flowmetry: independently evaluation of RBC velocity and RBC concentration. Jpn. J. Physiol., 49, 289-296 (1999)

[7] Fox PT, Raichle ME, Mintun MA Dence C. Nonoxidative glucose consumption

during focal physiologic neural activity. Science, 241, 462-464 (1988)
[8] Malonek D, Dirnagl U, Lindauer U, Yamada K, Kanno I and Grinvald A. Vascular imprints of neuronal activity: Relationships between the dynamics of cortical blood flow, oxygenation, and volume changes following sensory stimulation. Proc. Natl. Acad. Sci. USA, 94, 14826-14831 (1997)
[9] Menon RS, Ogawa S, Strupp JP, Anderson P, Ugurbil K. BOLD based functional MRI at 4 tesla includes a capillary bed contribution: echo-planar imaging time course mirrors previous optical imaging using intrinsic signals. Magn. Reson. Med., 33, 453-459 (1995)
[10] Shinozuka T, Nempto EM and Winter PM. Mechanisms of cerebral O_2 sensitivity from hyperoxia to moderate hypoxia in the rat. J. Cereb. Blood Flow Metab., 9, 187-195 (1989)
[11] Kanno I, Fujita H, Hatazawa J. Enhancement of CBF response for V1 stimuli during hyperoxia: behavior of oxygen in neuronal activation revisited. J. Cereb. Blood Flow Metab., 17, S646 (1996)
[12] Kashikura K, Kershaw J, Kashikura A, Matsuura T, Kanno I. Hyperoxia enhanced activation-induced hemodynamic response in human V1:an fMRI study. Neuroreport, 11, 903-906 (2000)
[13] Matsuura T, Fujita H, Kashikura K, Kanno I. Modulation of evoked cerebral blood flow under excessive blood supply and hyperoxic conditions. Jpn. J. Physiol., 50, 115-123 (2000)
[14] Matsuura T, Fujita H, Kashikura K, Kanno I. Evoked local cerebral blood flow induced by somatosensory stimulation is proportional to the baseline flow. Neurosci. Res., in press.
[15] Duelli R, Kuschinsky W. Changes in brain capillary diameter during hypocapnia and hypercapnia. J. Cereb. Blood Flow Metab., 13, 1025-1028 (1993)
[16] Beach JM, Mcgahren ED, Duling BR. Capillaries and arterioles are electrically coupled in hamster cheek pouch. Am. J. Physiol., 275, H1489-H1496 (1998)
[17] Potter CF, Kuo N, Farver CF Mcmahon JT, Chang C, Agani FH, Haxhiu MA, Martin RJ. Effect of hyperoxia on nitric oxide synthase expression, nitric oxide activity, and ling injury in rat pips. Pediatr Res., 45, 8-13 (1999)
[18] Schmidt-Nielsen K, Scaling: Why is animal size so important? Cambridge Univ. Press, Cambridge (1984)

13. 研究業績

13-1. 原著論文

[1] Matsuura T, Fujita H, Seki C, Kashikura K, Yamada K, Kanno I: CBF change evoked by somatosensory activation measured by laser-Doppler flowmetry: independently evaluation of RBC velocity and RBC concentration. Jpn. J. Physiol., 49, 289-296 (1999)

[2] Matsuura T, Fujita H, Seki C, Kashikura K, Kanno I: Hemodynamics evoked by microelectrical direct stimulation in rat somatosensory cortex. Comp. Biochem. Physiol. A, 124, 47-52 (1999)

[3] Matsuura T, Fujita H, Kashikura K, Kanno I: Modulation of evoked cerebral blood flow under excessive blood supply and hyperoxic conditions. Jpn. J. Physiol., 50, 115-123 (2000)

[4] Matsuura T, Fujita H, Kashikura K, Kanno I: Evoked local cerebral blood flow induced by somatosensory stimulation is proportional to the baseline flow. Neurosci. Res., in press.

[5] Matsuura T, Kashikura K, Kanno I: Role of nitric oxide in the evoked cerebral blood flow under hyperoxia.（提出中）

[6] Matsuura T, Kashikura K, Kanno I: Hemodynamic response under the hyperoxic condition.（提出中）

[7] Bakalova R, Matsuura T, Kanno I: Frequency dependence of evoked local CBF induced by somatosensory stimulation in rat under normo- and hypercapnia.（提出中）

[8] Matsuura T, Kashikura K, Kanno I: Change in RBC behavior induced by somatosensory activation with various frequencies.（準備中）

13-2. 総説など

[1] 松浦哲也、藤田英明、柏倉健一、菅野巖：脳賦活時の微小循環調節. 比較生理生化学、16、180-190（1999）

13-3. 国際学会発表

[1] Matsuura T, Fujita H, Seki C, Kashikura K, Kanno I: Hemodynamics of RBC velocity and volume evoked by micro electrical stimulation of rat brain. 27th annual meeting of Society for neuroscience. Oct. 25-30, 1997, New Orleans, USA.

[2] Matsuura T, Fujita H, Seki C, Kashikura K, Yamada K, Kanno I: Local CBF change evoked by hind paw stimulation measured using LDF in rat brain. 28th annual meeting of Society for neuroscience. Nov. 7-12, 1998, Los Angeles, USA.

[3] Matsuura T, Fujita H, Kashikura K, Kanno I: Hyperoxia enhanced and quickened the evoked cerebral blood flow in rat. 5th International Congress

of Comparative Physiology and Biochemistry. Aug. 23-28, 1999, Calgary, Canada.

[4] Matsuura T, Fujita H, Kashikura K, Kanno I: Modulation of evoked local cerebral blood flow under the hypercapnic and the hyperoxic conditions. 30th annual meeting of Society for neuroscience. Nov. 4-9, 2000, New Orleans, USA.

13-4. 国内学会発表

[1] 松浦哲也、藤田英明、関千江、柏倉健一、菅野巖：ラット大脳の電気刺激によって誘発される局所脳血流変化の計測．第 8 回日本比較生理生化学会大会、平成 9 年 7 月 18 日　20 日、札幌

[2] 松浦哲也、藤田英明、関千江、柏倉健一、菅野巖：ラット体性感覚野の局所脳血流量調節に及ぼす酸素の影響．第 21 回日本神経科学・第 41 回日本神経化学合同大会、平成 10 年 9 月 21 日　23 日、東京

[3] 松浦哲也、藤田英明、関千江、柏倉健一、山田勝也、菅野巖：ラット後肢電気刺激に対する大脳皮質体性感覚野の興奮と局所脳血流量の変化．日本動物学会・第 69 回大会、平成 10 年 9 月 26 日　28 日、東広島

[4] 松浦哲也、菅野巖：ラット体性感覚野賦活時の局所脳血流動態の計測．第 31 回東北生理談話会、平成 10 年 10 月 24 日　25 日、山形

[5] 松浦哲也、藤田英明、柏倉健一、菅野巖：高酸素及び高二酸化炭素分圧下で賦活されたラット体性感覚野の局所血流動態．第 22 回日本神経科学大会、平成 11 年 9 月 6 日　8 日、大阪

[6] 松浦哲也、藤田英明、柏倉健一、菅野巖：ラット大脳皮質体性感覚野の局所血流動態に及ぼす二酸化炭素の影響．日本動物学会・第 70 回大会、平成 11 年 9 月 27 日　29 日、山形

[7] 松浦哲也、藤田英明、柏倉健一、菅野巖：高酸素分圧下のラット脳賦活血流量に及ぼす一酸化窒素の影響．第 11 回日本比較生理生化学会大会、平成 12 年 8 月 3 日　5 日、山口

[8] 松浦哲也、藤田英明、柏倉健一、菅野巖：ラット脳賦活時の局所血流増加量はベースライン血流量に比例する．第 23 回日本神経科学・第 10 回日本神経回路学会合同大会、平成 12 年 9 月 4 日　6 日、横浜

[9] 松浦哲也、藤田英明、柏倉健一、菅野巖：ラット体性感覚野賦活時の局所血流増加量は酸素要求量に対して独立である．日本動物学会・第 71 回大会、平成 12 年 9 月 21 日　23 日、東京

14. Measurement and mechanism of secondary signal evoked by somatosensory activation using laser-Doppler flowmetry in rats.

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19. Abstract

We measured the field potential and local cerebral blood flow (LCBF) using laser-Doppler flowmetry in α-chloralose anesthetized rats during activation of the somatosensory cortex. In the present study, we obtained the following results: 1) LCBF increased nearly proportionally to the intensity of neuronal activity, 2) both arteriole diameter change and capillary volume change contributed to initial LCBF responses, 3) the amount of evoked LCBF is not determined by the demand for the delivery of metabolic substrates, 4) nitric oxide is involved in LCBF response under the hyperoxic condition.

CBF change evoked by somatosensory activation measured by laser-Doppler flowmetry: independent evaluation of RBC velocity and RBC concentration.

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要旨　ラット後肢を様々な頻度（0.2、0.5、5、10，50 Hz）で電気刺激(1.5 mA、2、5、20秒)し、大脳皮質体性感覚野における局所血流動態(血流量、赤血球の速度及び容積の変化）をレーザードップラ血流計を用いて測定するとともに、計測部位における誘発電位を記録した。その結果、血流量は刺激頻度が５Hzで最大となり、これは誘発電位の発現頻度の最大値と一致した。血流量の立ち上がりのタイミングは刺激頻度に関係なく刺激開始後約0.5秒であり、速度及び容積の立ち上がりに時間的な差は認められなかった。以上のことから、局所脳血流量増加の割合は脳における神経活動の大きさと比例関係にあり、賦活時の局所脳血流量は複数の調節メカニズムよって支配されている可能性が示唆された。

ABSTRACT

The purpose of this study was to examine the timing and magnitude of cerebral blood flow (CBF) responses to neuronal activation. We measured the changes in local CBF (LCBF), RBC velocity and RBC concentration by laser-Doppler flowmetry (LDF), and field potential recordings during activation of the somatosensory cortex of the rat in response to electrical stimulation of the hind paw. Electrical stimuli, 0.1 ms pulses of 1 - 1.5 mA for 5 s, were applied at 0.2, 0.5, 5, 10 and 50 Hz under α-chloralose anesthesia. LCBF showed the maximum increase at 5 Hz, and rose approximately 0.5 s after the onset of stimulation regardless of the frequency. The maximum frequency of the field potentials also obtained at 5 Hz. During activation of the somatosensory cortex, the onset of rise in red blood cell (RBC) concentration did not precede that of RBC velocity, and the peak RBC concentration was noted earlier than that of both

LCBF and RBC velocity, suggesting that both arteriolar diameter and active changes in the capillary contributed to the LCBF response.

Keywords
field potential, functional activation, hind paw stimulation, laser-Doppler flowmetry, local cerebral blood flow.

INTRODUCTION

The mechanism of coupling between neuronal activation and blood flow still remains to be elucidated, despite the numerous investigations were undertaken over the past few decades. We focus in this study on the mechanism of regulation of the early changes in cerebral blood flow (CBF) following neuronal activation. We examine which vessel, i.e., the arteriole, capillary or venule, primarily regulates the changes in local CBF (LCBF). Previous studies suggest that the diameter of the pial arteriole determines the cortical blood flow during neuronal activation of the cortex (1). Recently, Malonek and Grinvald (2) observed in the cat visual cortex, that changes in the total hemoglobin content (corresponding to cerebral blood volume) preceded those in the oxyhemoglobin concentration (with resultant increase in CBF). Their observation suggests that the capillary changes occur prior to arteriolar dilatation.

The purpose of our current study was to investigate the regulatory mechanisms of the change in LCBF evoked by neuronal activation using laser-Doppler flowmetry (LDF). Cerebral microcirculation is often monitored by LDF, and a number of laboratories have used LDF to demonstrate the changes in CBF related to cerebral activation in response to various stimuli (1, 3, 4, 5, 6). On the other hand, there have been only few attempts to observe the hemodynamics of red blood cell (RBC) velocity and RBC concentration independently, although validation of these parameters was achieved by an *in vitro* experiment (4). Because these parameters are affected by the high level of noise in *in vivo* studies, we accumulated multiple data by repeated triggering in order to reduce the noise level. We then examined the relationship between the changes in these

hemodynamic parameters during neuronal activation in response to hind paw electrical stimulation.

Another objective of this study was to estimate the relationship between neuronal activity and the changes in LCBF. Although a relationship between the stimulus frequency and CBF response has been reported (7, 8), these experiments evaluated the amplitude of neuronal activity using surface electrodes on the skull. Such evaluation inevitably has limitations for the accurate estimation of neuronal activity. In contrast, in this study, we recorded the field potential of a local area using an electrode inserted into the cortex, and evaluated the relationship between this recording and LDF parameters.

This is a report of the independent evaluation of the *in vivo* dynamics of RBC velocity and RBC concentration during sensory stimulation using LDF. Such data, namely of change in the LCBF during neuronal activation, are of potential value for a better understanding of brain imaging techniques.

MATERIALS AND METHODS

Animal preparation

Male Sprague-Dawley rats (300 - 380 g) were anesthetized with halothane (4% induction and 1.5% during surgery) in 30% O_2 and 70% N_2O. The tail artery was cannulated for monitoring blood pressure and blood gas sampling, and the left femoral vein was cannulated for intravenous drug administration. Following tracheotomy, α-chloralose (100 mg kg^{-1}, i.v.) was administered, and halothane and nitrous oxide were discontinued. After tracheotomy, the rats were immobilized with pancuronium bromide (0.7 mg kg^{-1}, i.v.), and mechanically ventilated with a respirator (SN-480-7, Shinano, Japan) throughout the experimental period. Anesthesia was maintained with α-chloralose (35 mg kg^{-1} hr^{-1}, i.v.) and muscle relaxation with pancuronium bromide (0.8 mg kg^{-1} hr^{-1}, i.v.). Body temperature was maintained at 37.5 ± 0.2 °C with the aid of a heating pad (ATC-101, Unique medical, Japan). Arterial blood was sampled from the tail artery for blood gas analysis three times throughout the experiments; before, during and just after the measurements. Arterial blood pressure was monitored during the experiments and the mean value (MABP) was calculated from the

average of those measured at the three aforementioned time points. The rats were fixed in a stereotactic frame, and the parietal bone was thinned to translucency at the left somatosensory cortex, over an area of 3 x 3 mm^2, centered 2.5 mm caudal and 2.5 mm lateral to the bregma.

LDF measurement

LCBF was measured with an LDF (Ne-He, wavelength of 780 nm, maximal intensity of 0.8 mW; Periflux 4001 Master, Perimed, Sweden) and an LDF probe with a tip diameter of 0.46 mm (Probe 411, Perimed, Sweden). LDF measures blood flow based on the Doppler effect with laser light. The frequency shift of the scattered radiation is caused by moving RBCs in the blood vessels. The amount by which the frequency is shifted by the Doppler effect corresponds to the RBC concentration, and the average shift in frequency is related to RBC velocity. Our LDF system simultaneously provided three parameters; flux (= LCBF), RBC velocity and RBC concentration, where RBC velocity = LCBF / RBC concentration (9). The area of LDF measurement has been reported to be about 1 mm^3 (10, 11). It has been confirmed that RBC behavior in the capillary is the main contributor to the LDF signals (4). A time constant of 0.03 s was used for all LDF signals (LCBF, RBC velocity and RBC concentration). The LDF probe was located on the somatosensory area of the hind paw, perpendicular to the brain surface. It was attached to the thinned skull and then fine-positioned for maximum signal change during stimulation, avoiding areas with large blood vessels.

Hind paw stimulation

Activation of the cortex was carried out by electrical stimulation of the hind paw with rectangular pulses (0.1 ms) through a pair of small needle electrodes inserted under the skin of the right hind paw. Stimulation was carried out for about one hour after preparation. In all rats, a current stimulus of 1 to 1.5 mA, which elicited a change in LCBF, was applied at a frequency of 5 Hz for a duration of 5 s. In the experiments for the analysis of frequency dependency (study 1), we used 9 rats, and varied the frequency (0.2, 0.5, 5, 10 and 50 Hz) of the electrical stimuli for 5 s duration at a selected intensity. The order of the stimulus

frequencies was selected randomly; at each stimulus frequency, 20 successive stimuli were applied at 80-s intervals. In the experiments performed for determination of the time-course of change in RBC velocity and RBC concentration (study 2), we used 14 rats and applied electrical stimuli of a selected intensity at 5 Hz for a duration of 5 s; 50 successive stimuli were applied at 80-s intervals. Blank sampling of LDF signals, without electrical stimulation, was performed after application of each set of stimuli. Data acquisition of LDF was synchronized to the respirator cycle.

Electrophysiology

After LDF measurements, we recorded the field potentials to confirm that the LDF probe was indeed located at the activation area. A tungsten microelectrode (12 MΩ), for recording the field potentials, was inserted into the cortex through the thinned portion of the skull at an angle of 45 degrees relative to the LDF probe. The tip of the electrode was set at a depth of about 0.5 mm from the surface of the cortex, just beneath the LDF probe. An Ag-AgCl indifferent electrode was placed between the skull bone and the scalp. In 6 of the 9 rats in study 1, the field potentials in response to various stimulus frequencies were also recorded to estimate the correlation between change in LCBF and neuronal activation.

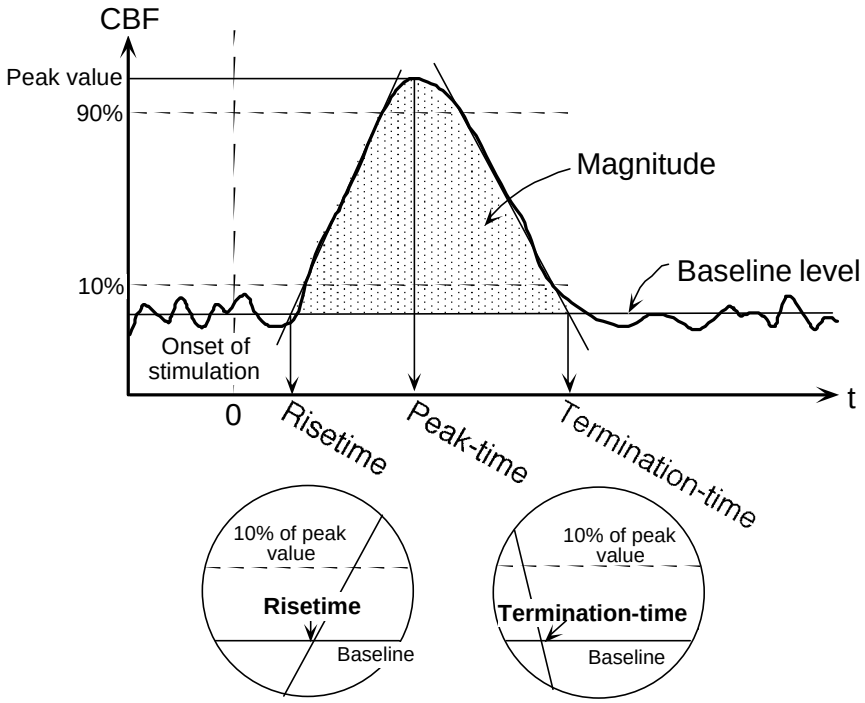

Fig. 1. Schematic diagram illustrating the calculation method of the time parameters and response magnitude. The risetime was determined as the time at the intersection of the baseline (pre-stimulus level) by the extrapolated line drawn on the normalized response curve from 90 % to 10 % of the peak, the termination-time was also determined as the time at the intersection of the baseline by a similar extrapolated line. The peak-time was the time at which the response curve reached the maximum height. The response magnitude was calculated as an integral of the response curve from the risetime to the termination-time.

Data analysis

Data from the successive measurements of the LDF signals, field potentials and arterial blood pressure values were accumulated by using MacLab data acquisition software (AD Instruments, Japan), digitized at 40 Hz, and saved on a disk for off-line analysis. The LDF data were normalized to the blank. The risetime of LDF signal was determined as the time at the intersection of the baseline (pre-stimulus level) by the extrapolated line which was drawn on the normalized response curve from 90 % to 10 % of the peak, the termination-time was also determined as the time of the intersection of the baseline by a similarly extrapolated line (Fig. 1). The peak-time was the time at which the response curve reached the maximum height. The response magnitude was calculated as an integral of the response curve from the risetime to the termination-time, and was considered to reflect the total amount of increase in blood flow. LDF data which

included spontaneous oscillations in the pre-stimulus period larger than the evoked response were excluded from the analysis. In the field potential analysis, the number of spike-shaped potentials which exceeded the noise level during the stimulus period was counted.

In study 1, parameters (i.e. risetime, peak-time, termination-time and magnitude) among the each stimulus frequency were statistically analyzed by ANOVA (repeated measurements) and multiple comparisons (Bonferroni). In study 2, significant differences between the risetime, peak-time and termination-time of RBC velocity and RBC concentration were statistically examined using the Wilcoxon test.

RESULTS

The mean arterial blood pressure (MABP) was 109 ± 9 mm Hg (mean ± SD, n = 23). MABP was stable throughout the experiment and did not change significantly during stimulation (Fig. 2). The blood gas values were as follows; PO_2 = 108 ± 13 mm Hg, PCO_2 = 34.7 ± 2.5 mm Hg and pH = 7.43 ± 0.04 (n = 23). These values were maintained within a stable range by regulating the stroke volume and oxygen concentration of the inspired gas.

Characteristics of changes in field potentials in response to stimulus frequency

At frequencies ≤ 5 Hz, each electrical stimulus evoked a corresponding field potential. At frequencies ≥ 10 Hz, the field potentials did not correspond to the electrical stimulation pulses except for the first one (Fig. 2 and Table 1), implying that the field potentials recorded in response to current pulses subsequent to the first pulse were either markedly decreased in amplitude or entirely absent at stimulus frequencies ≥10 Hz. On the other hand, the latency of onset of the first potential after stimulation was consistent for all stimulus frequencies, and was 10.1 ± 0.7 ms.

LCBF with change in stimulus frequency

The risetime, peak-time and termination-time of the LCBF response curve was measured as a function of the stimulus frequency (study 1). The

risetime was nearly constant, at approximately 0.5 s, regardless of the stimulus frequency, between 0.2 and 50 Hz (NS, Fig. 3a). The response curve started to decline even when the field potentials continued to be recorded (Figs. 2 and 3a; 0.5, 5 and 10 Hz), that is, the peak-time was noted before the cessation of neuronal activity. The peak-time, termination-time and magnitude increased with the stimulus frequency in the range of 0.2 - 5 Hz, although the values of all the three parameters decreased at frequencies ≥ 10 Hz (Figs. 2 and 3).

In most rats, spontaneous oscillations were observed, but could be averaged out by accumulation at 20 data points at stimulus frequencies of 0.5, 5 and 10 Hz. However, the majority of these oscillations still remained after the stimulation period at stimulus frequencies of 0.2 and 50 Hz (Fig. 2), i.e. the shortest neuronal activity.

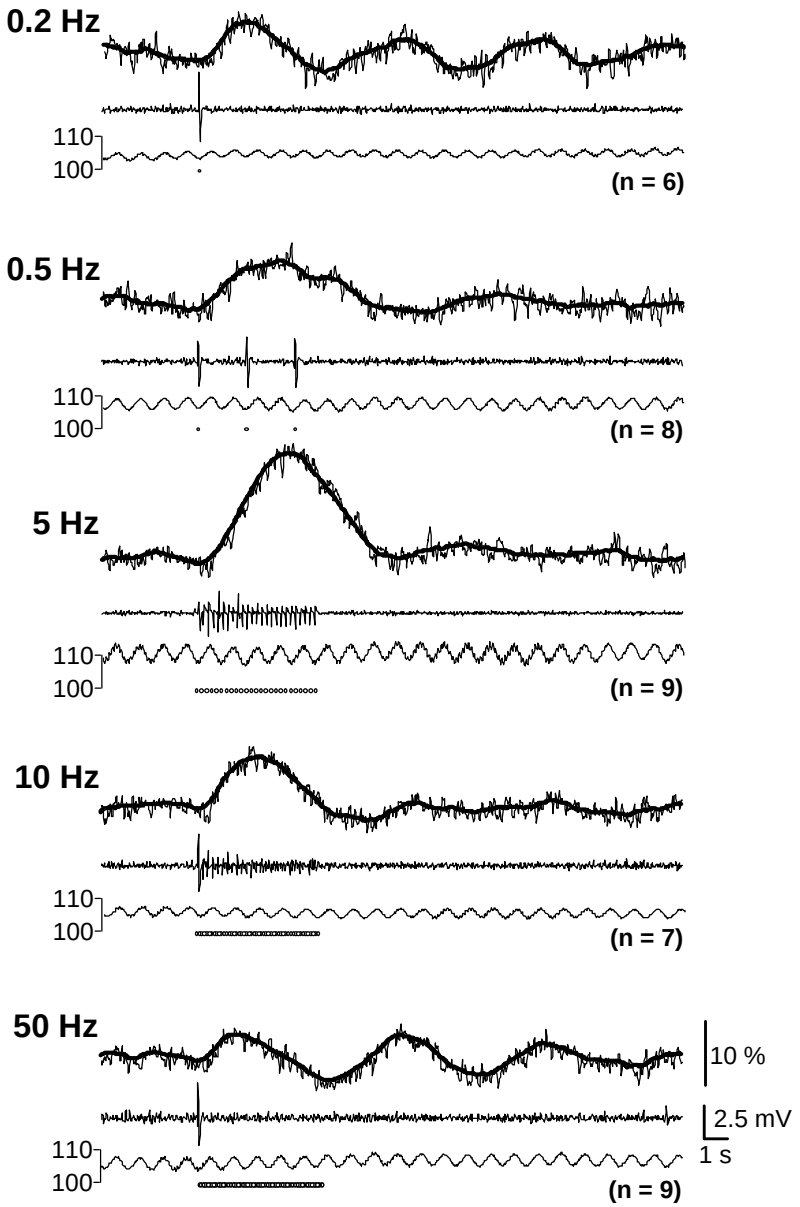

Fig. 2. Time-course of changes in LCBF, cortical activation and arterial blood pressure responses for various frequencies of 5 s stimulation of the hind paw. Each data set consists of the changes in LCBF (top), field potentials (2nd), arterial blood pressure values (mm Hg, 3rd) and the mark of electrical pulse (bottom). The LCBF change was normalized to blank stimulation, and averaged by the number of animals used. Representative field potential and blood pressure recordings show a sample of the recording of one animal. Note that the frequency of the field potential and of the individual electrical stimulation coincided at stimulus frequencies ≤ 5 Hz, but did not fully coincide at those of ≥ 10 Hz except for the first potential, and that there was no change in the mean arterial blood pressure during stimulation.

Time-course of change in RBC velocity and RBC concentration

An important objective of this study was to investigate the time-course of changes in RBC velocity and RBC concentration independently (study 2). We measured the time-courses of changes in these parameters during application of electrical pulses of 5 s duration at 5 Hz, because the response curves of RBC

velocity and RBC concentration showed poor signal-to-noise ratio at all except this frequency.

Table 1. *Frequency of field potentials for various stimulus frequencies*

Stimulus frequency (Hz)	Mean number of field potentials detected
0.2	1.0 ± 0.0
0.5	3.0 ± 0.0
5	20.0 ± 4.8
10	8.8 ± 10.4
50	1.0 ± 0.0

Mean ± SD (n = 6)

There were no significant differences in risetime and termination-time among LCBF, RBC velocity and RBC concentration (Fig. 4). On the other hand, the peak-time of RBC concentration occurred earlier than those of RBC velocity and LCBF ($P<0.05$, Fig. 4). The peak-times of LCBF, RBC velocity and RBC concentration were 3.2± 0.3 s, 3.5± 0.5 s and 3.0 ± 0.3 s respectively (n = 14).

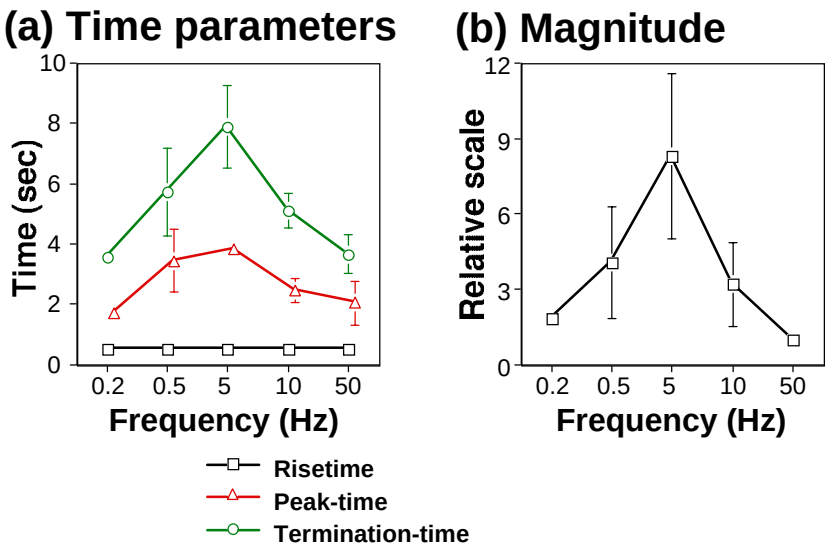

Fig. 3. Effect of various frequencies of 5 s stimulation on the time parameters and the relative response magnitude of LCBF. (a) Changes in risetime, peak-time and termination-time with the onset of stimulation (see Fig. 1). (b) Relative magnitude (area under the LCBF response curve) of the increase in LCBF, with the response magnitude obtained at a stimulus frequency of 50 Hz being considered as 1.0. Note that the LCBF rose about 0.5 s after the onset of stimulation regardless of the stimulus frequency (NS), and that the LCBF response showed maximal increase at the stimulus frequency of 5 Hz ($P<0.01$). Error bars are ± 1 standard deviation.

DISCUSSION

We have studied the relationship between the field potential recorded and changes in LCBF in response to electrical stimulation of the rat hind paw.

Changes in LCBF were detected by LDF, and the time-courses of RBC velocity and RBC concentration were evaluated independently. We showed that even a single pulse of electrical stimulation of the hind paw evoked an increase in LCBF.

Attributes of LDF

Before discussing the physiological interpretation of our data, we must know the

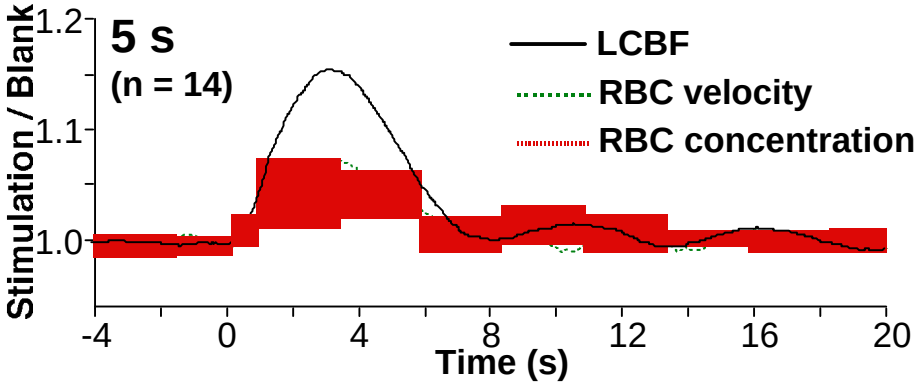

Fig. 4. Time-course of change in LCBF (solid line), RBC velocity (rough dotted line) and RBC concentration (close dotted line) in response to 5 s stimulations at a frequency of 5 Hz of the hind paw. Data of the change in LCBF, RBC velocity and RBC concentration were averaged by the number of animals used. Hatched lines indicate stimulus period. Note that the RBC concentration peaked before the RBC velocity and LCBF ($P<0.05$), and that there was no significant time lag between the risetimes of RBC velocity and RBC concentration.

attributes of LDF. LDF reflects only RBC behavior, and LCBF is calculated as a product of RBC concentration and RBC velocity. True LCBF is a function of both RBC flow and plasma flow. It is known that 10 –20 % of capillaries are perfused only by plasma (12), which does not contribute to LDF signals. Therefore, our data may not represent true LCBF if the hematocrit were to vary. In the microvasculature, RBCs flow at the center of the vessel at a speed greater than the plasma velocity (Fahraeus effect; 13), which results in a reduced microvascular hematocrit. In the capillary bed, on the contrary, hematocrit is known to increase slightly by the pathway effect (14). It is also known that, at microvascular bifurcations, RBC streams into a vessel of which the flow velocity is faster (network Fahraeus effect; 13, 14). These phenomena are probably influenced by the arteriole and/or capillary changes during cortical activation. However, it was found that the local hematocrit did not change (15), or decreased only slightly with increase in LCBF (16). Moreover, Dirnagl *et al.* demonstrated that relative CBF

measured by LDF correlated well with that measured by [^{14}C] iodoantipyrine autoradiography (17), suggesting that relative LCBF as detected by LDF reflects the true LCBF.

Relationship between neuronal activation and increase in LCBF

The intensity of cortical activation is evaluated by the amplitude of the evoked potential using a surface electrode. In the cat somatosensory cortex, the maximum hyperemia and maximum amplitude of the evoked potential were both obtained for stimulation at 2 - 3 Hz (8). On the other hand, Ibáñez *et al.* (7) reported that regional CBF showed a maximum increase following median nerve stimulation at 4 Hz, despite a relative reduction in the amplitude of the somatosensory evoked potential. Such surface-evoked potentials are difficult to evaluate from the electrophysiological point of view because of summation of both temporal and spatial electrical activities in a large area. The changes in amplitude reflect a sum of the excitatory and inhibitory activities. Therefore, the amplitudes of surface-evoked potentials do not directly reflect the intensity of cortical activation.

In this study, the field potential was measured through an electrode inserted into the cortex. The frequencies of the field potentials and the individual electrical pulses coincided for stimulation at frequencies ≤ 5 Hz, but not for that at frequencies ≥ 10 Hz in most rats (Fig. 2). This implies that the number of field potentials recorded in response to stimulation at frequencies ≥ 10 Hz was much smaller than that recorded at a stimulus frequency of 5 Hz, or that the field potentials recorded after the second pulses were markedly decreased in amplitude at stimulus frequencies ≥ 10 Hz (Fig. 2, Table 1). It is unlikely that cortical neurons have a reaction limit for stimulation at ≥ 10 Hz, because individual somatosensory neurons in the rat and cat cortex react to stimuli ≥ 10 Hz (18, 19). While it is possible that such reductions in somatosensory neuronal activation are due to either inhibitory mechanisms, habituation of the peripheral nerve of the hind paw, or limitations of transmission of the sensory input, we considered that it was most likely to be due to inhibitory mechanisms (7). Indeed, intracortical inhibition was confirmed as a frequency-dependent response above 20 Hz (18),

suggesting that inhibitory neurons in the cortex are activated at higher frequencies. These observations suggest that maximum cortical activation by stimulation of the somatosensory area of the hind paw occurred at a stimulus frequency of 5 Hz in our study.

LCBF also showed the maximum increase at a stimulus frequency of 5 Hz ($P<0.01$). At stimulus frequencies $\geq$ 10 Hz, the increase in LCBF was attenuated with increasing stimulus frequency (Figs. 2 and 3b). The relationship between stimulus frequency and increase in LCBF was previously investigated in the rat somatosensory cortex. Ngai and colleagues demonstrated that the change in diameter of the pial arteriole was maximal in response to sciatic nerve stimulation at 5 Hz (20), and the LCBF showed a similar response profile with pial arteriole change (1). In the rat whisker barrel cortex, the peak amplitude of blood flow evoked with stimulation at a frequency of 3 Hz was significantly greater than that at other frequencies (21). These results suggest that the LCBF in the somatosensory area is maximal when the peripheral nerves are stimulated at a frequency of approximately 5 Hz.

The oxygen supply to the tissue may be increased during cortical activation. This phenomenon is evident from the results of optical imaging, that is, an increase in deoxyhemoglobin concentration occurs at the early period of cortical activation (2). In the present study, we showed that LCBF increased in the similar manner to the intensity of cortical activation, indicating the coupling between cortical activation and increase in LCBF. On the other hand, Fox and Raichle found a quantitative uncoupling of LCBF and oxidative metabolism (22). These suggest that the increase in LCBF depends on cortical activation, but oxygen consumption is independent from cortical activation. However, the elucidation of this odd phenomena, i.e., the uncoupling between an increase in LCBF and oxygen consumption, needs further investigation, such as simultaneous measurement of LCBF and direct tissue PO_2 with high temporal resolution during cortical activation.

Although spontaneous oscillations could be averaged out by accumulation of data at stimulus frequencies of 0.5, 5 and 10 Hz, a considerable proportion still remained at those of 0.2 and 50 Hz (Fig. 2). Morita *et al.* (23) reported that pronounced vasodilatation led to total abolition of vasomotion. This

suggests the possibility that spontaneous oscillations are decreased by large increases in LCBF. On the other hand, Golanov *et al.* (24) proposed a neurogenic origin for at least one kind of low-frequency oscillation in the cortex. Our results suggested that spontaneous oscillations were synchronous with stimulation when the strength of cortical activation was small. This was supported by the relatively lower amplitude during the pre-stimulus period than following stimulation, of the accumulation curve. These findings confirm that neural activity is probably related to the oscillations, but the source of these oscillations still remains controversial (25).

Risetime of change in LCBF

In order to discuss the time-course of change in LCBF, it is necessary to confirm that the LDF probe was located at the cortical activation area of the somatosensory cortex for the hind paw. The risetime of LCBF may be influenced by the location of the LDF probe. In all the rats in our experiments, the microelectrode was inserted into the cortex at the same location as the LDF probe, and the field potentials were recorded. The mean latency of the field potentials was 10.1 ± 0.7 ms. This agreed with the latency reported in the literature (26). This indicates that the LDF probe was located at the activation area of the somatosensory cortex of the hind paw.

The LCBF response, for all the stimulation frequencies examined, rose rapidly about 0.5 s after the onset of stimulation (Fig. 3a). Several investigators have reported a much later risetime of the LCBF response to somatosensory stimulation. Schmitz *et al.* described that the CBF typically increased within 2 - 3 s after onset of forepaw stimulation (6). A similar time-course for the increase in CBF following cortical stimulation has been observed using modified hydrogen clearance (8). However, the accuracy of these results was limited either by low signal-to-noise (S/N) ratio or poor time resolution. We improved the S/N ratios by accumulating 20 to 50 events of repeated trials and time resolution by using a small time constant (0.03 s) in LDF. Therefore, the risetime of LCBF of 0.5 s was considered more reliable compared to the previous report (6, 8).

The physiological interpretation of this 0.5 s response, with respect to capillary and arteriole functions, is still open to debate. The mechanisms of coupling between neuronal activation and LCBF are still under investigation. Several possible mechanisms have been proposed (27, 28), e.g. reaction caused by a metabolic by-products, regulation by cations or chemical substrates (3, 6, 29, 30, 31), and neurogenic regulation (32). In addition, a direct reaction on the capillary has been proposed (12, 27, 33, 34). Since our method only provides information based on RBC motion within the capillary, further investigations are required for elucidation of the regulatory mechanisms.

Possible microcirculatory regulation

In this study, we demonstrated the independent dynamics of RBC velocity and RBC concentration using LDF. The increase in RBC velocity at the capillary level is supposed as being induced by an increase in transluminal pressure due to a decrease in resistance of the upstream arteriole. On the other hand, the increase in RBC concentration within the capillary suggests a direct change in the capillary; i.e. physiological recruitment (35) or capillary dilatation (12, 33).

Some possible mechanisms of LCBF regulation are proposed. The first is that active capillary changes occur earlier than those in the arteriole (2, 36). If this were the case, the RBC concentration would increase earlier than the RBC velocity. Actually, there is a report that the capillaries contract under hyperoxia and dilate under hypoxia (34). In addition, the caliber of the capillary, previously considered to be unchangeable (37), was reported to be altered by carbon dioxide (12, 33). The second possibility is that the changes in LCBF are primarily controlled only by resistance vessels, i.e. arteriole (38, 39), and passive capillary changes may be involved in addition. If this were the case, the RBC velocity in the capillary bed would change earlier than RBC concentration. However, we found no significant time differences between the risetimes of RBC velocity and RBC concentration during somatosensory stimulation, and also found that both RBC velocity and RBC concentration contributed equally to the observed changes in LCBF (Fig. 4). Therefore, we propose a third possibility that both capillary and

arteriole changes occur simultaneously and contribute equally to the initial increase in LCBF.

We showed that the RBC concentration peaked before the LCBF and RBC velocity ($P<0.05$) (Fig. 4). This earlier peaking of RBC concentration suggests the occurrence of active capillary changes, because, if only arteriole changes were involved in LCBF regulation, the changes in RBC concentration would follow those of RBC velocity and LCBF. In summary, it is possible that the capillary and arteriole are controlled by independent mechanisms, and that the LCBF is regulated by both capillary and arteriole changes, although arteriolar dilatation, physiological recruitment and capillary dilatation would have to interact in a highly complex manner to regulate the LCBF.

ACKNOWLEDGMENTS

We are grateful to Drs. Masahito Nemoto and Yuko Miura for their helpful discussions, Drs. Yasuji Yoshida and Shingo Kawamura for technical advice, and Mr. Yozo Ito for technical assistance. We also thank Dr. Shohab Youssefian, Akita Prefectural College of Agriculture, for critical reading of the manuscript.

REFERENCES

1. Ngai AC, Meno JR and Winn HR: Simultaneous measurements of pial arteriolar diameter and laser-Doppler flow during somatosensory stimulation. J Cereb Blood Flow Metab 15: 124-127, 1995
2. Malonek D and Grinvald A: Interactions between electrical activity and cortical microcirculation revealed by imaging spectroscopy: Implications for functional brain mapping. Science 272: 551-554, 1996
3. Akgören N, Dalgaard P and Lauritzen M: Cerebral blood flow increases evoked by electrical stimulation of rat cerebellar cortex: relation to excitatory synaptic activity and nitric oxide synthesis. Brain Res 710: 204-214, 1996
4. Barfod C, Akgören N, Fabriciu, M, Dirnagl U and Lauritzen M: Laser-Doppler measurements of concentration and velocity of moving blood cells in rat cerebral circulation. Acta Physiol Scand 160: 123-132, 1997

5. Lindauer U, Villringer A and Dirnagl U: Characterization of CBF response to somatosensory stimulation: model and influence of anesthetics. Am J Physiol 264: H1223-H1228, 1993
6. Schmitz B, Böttiger BW and Hossmann KA: Brief hypercapnia enhances somatosensory activation of blood flow in rat. J Cereb Blood Flow Metab 16: 1307-1311, 1996
7. Ibáñez V, Deiber MP, Sadato N, Toro C, Grissom J, Woods RP, Mazziotta JC and Hallett M: Effects of stimulus rate on regional cerebral blood flow after median nerve stimulation. Brain 118, 1339-1351, 1995
8. Leniger-Follert E and Hossmann KA: Simultaneous measurements of microflow and evoked potentials in the somatosensory cortex of the cat brain during specific sensory activation. Pfluegers Arch 380: 85-89, 1979
9. Nilsson GE: Signal processor for laser Doppler tissue flowmeters. Med and Biol Eng and Comput 22, 343-348: 1984
10. Fukuda O, Endo S, Kuwayama N, Harada J and Takaku A: The characteristics of laser-Doppler flowmetry for the measurement of regional cerebral blood flow. Neurosurgery 36: 358-364, 1995
11. Nilsson GE, Tenland T and Öberg PÅ: Evaluation of a laser Doppler flowmeter for measurement of tissue blood flow. IEEE Trans Biomed Eng BME-27: 597-604, 1980
12. Villringer A, Them A, Lindauer U, Einhäupl K and Dirnagl U: Capillary perfusion of the rat brain cortex. An in vivo confocal microscopy study. Circ Res 75: 55-62, 1994
13. Pries AR, Ley K and Gaehtgens P: Generalization of the Fahraeus principle for microvessel networks. Am J Physiol 251: H1324-H1332, 1986
14. Pries AR, Secomb TW and Gaehtgens P: Relationship between structural and hemodynamic heterogeneity in microvascular networks. Am J Physiol 270: H545-H553, 1996
15. Chen JL, Wei L, Acuff A, Bereczki D, Hans FJ, Otsuka T, Finnegan W, Patlak C and Fenstermacher L: Slightly altered permeability-surface area products imply some cerebral capillary recruitment during hypercapnia. Microvasc Res 48: 190-211, 1994

16. Todd MM, Weeks JB and Warner DS: Cerebral blood flow, blood volume, and brain tissue hematocrit during isovolemic hemodilution with hetastarch in rats. Am J Physiol 263: H75-H82, 1992
17. Dirnagl U, Kaplan B, Jacewicz M and Pulsinelli W: Continuous measurement of cerebral cortical blood flow by laser-Doppler flowmetry in a rat stroke model. J Cereb Blood Flow Metab 9: 589-596, 1989
18. Hellweg FC, Schultz W and Creutzfeldt OD: Extracellular and intracellular recording from cat's cortical whisker projection area: thalamocortical response transformation. J Neurophysio 40: 463-479, 1977
19. Simons DJ: Response properties of vibrissa units in rat SI somatosensory Neocortex. J Neurophysiol 41: 798-820, 1978
20. Ngai AC, Ko KR, Morii S and Winn HR: Effect of sciatic nerve stimulation on pial arterioles in rats. Am J Physiol 254: H133-H139, 1988
21. Woolsey TA, Rovainen CM, Cox SB, Henegar MH, Liang GE, Liu D, Moskalenko YE, Sui J and Wei L: Neuronal units linked to microvascular modules in cerebral cortex: response elements for imaging the brain. Cerebral Cortex 6: 647-660, 1996
22. Fox PT and Raichle ME: Focal physiological uncoupling of cerebral blood flow and oxidative metabolism during somatosensory stimulation in human subjects. Proc Natl Acad Sci USA 83:1140-1144, 1986
23. Morita Y, Hardebo JE and Bouskela E: Influence of cerebrovascular sympathetic, parasympathetic, and sensory nerves on autoregulation and spontaneous vasomotion. Acta Physiol Scand 154: 121-130, 1995
24. Golanov EV, Yamamoto S and Reis DJ: Spontaneous waves of cerebral blood flow associated with a pattern of electrocortical activity. Am J Physiol 266: R240-R214, 1994
25. Mayhew JEW, Askew S, Zheng Y, Porrill J, Westby GWM, Redgrave P, Rector DM and Harper RM: Cerebral vasomotion: a 0.1-Hz oscillation in reflected light imaging of neuronal activity. Neuroimage 4: 183-193, 1996
26. Claus D and Neundörfer B: Remarks peripheral and central neural conduction in the rats. Z EEG-EMG 14: 160-163, 1983

27. Villringer A and Dirnagl U: Coupling of brain activity and cerebral blood flow: basis of functional neuroimaging. Cerebrovasc Brain Metab Rev 7: 240-276, 1995
28. Lou HC, Edvinsson L and MacKenzie ET: The concept of coupling blood flow to brain function: revision required? Ann Neurol 22: 289-297, 1987
29. Dirnagl U, Lindauer U and Villringer A: Role of nitric oxide in the coupling of cerebral blood flow to neuronal activation in rats. Neurosci Lett 149: 43-46, 1993
30. Ko KR, Ngai AC and Winn HR: Role of adenosine in regulation of regional cerebral blood flow in sensory cortex. Am J Physiol 259: H1703-H1708, 1990
31. Urbanics R, Leniger-Follert E and Lübbers DW: Time course of changes of extracellular H^+ and K^+ activities during and after direct electrical stimulation of the brain cortex. Pflugers Arch 378: 47-53, 1978
32. Sato A and Sato Y: Regulation of regional cerebral blood flow by cholinergic fibers originating in the basal forebrain. Neurosci Res 14: 242-274, 1992
33. Duelli R and Kuschinsky W: Changes in brain capillary diameter during hypocapnia and hypercapnia. J Cereb Blood Flow Metab 13: 1025-1028, 1993
34. Tomita M, Fukuuchi Y, Tanahashi N, Kobari M, Takeda H, Yokoyama M, Ito D and Terakawa S: Contraction / dilatation of cultured vascular endothelial cells inducted by hyperoxia / hypoxia. J Cereb Blood Flow Metab 15: Suppl S271, 1995
35. Kuschinsky W and Paulson OB: Capillary circulation in the brain. Cerebrovasc Brain Metab Rev 4: 261-286, 1992
36. Malonek D, Dirnagl U, Lindauer U, Yamada K, Kanno I and Grinvald A: Vascular imprints of neuronal activity: Relationships between the dynamics of cortical blood flow, oxygenation, and volume changes following sensory stimulation. Proc Natl Acad Sci USA 94: 14826-14831, 1997
37. Mchedlishvili G and Varazashvili M: Hematocrit in cerebral capillaries and veins under control and ischemic condition. J Cereb Blood Flow Metab 7: 739-744, 1987

38. Paulson OB and Newman EA: Does the release of potassium from astrocyte endfeet regulate cerebral blood flow? Science 237: 896-898, 1987
39. Segal SS and Duling BR: Flow control among microvessels coordinated by intercellular conduction. Science 234: 868-870, 1986

Hemodynamics evoked by microelectrical direct stimulation in rat somatosensory cortex.

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ラット大脳皮質体性感覚野を様々な頻度（2, 5, 10, 50Hz）で電気刺激（10μA, 5秒）し、刺激部位における局所血流量（赤血球の速度×濃度）の変化をレーザードプラー血流計を用いて測定した。その結果、血流量の立ち上がりのタイミングは刺激頻度に関係なく刺激開始後約0.5秒であり、速度及び濃度の立ち上がりに時間的な差は認められなかった。また刺激頻度の増加に伴い、刺激部位における局所血流量増加の割合は大きくなり、刺激終了後ベースレベルに回復するまでの時間は遅延した。以上のことから、電気刺激による局所脳血流の増加は、抵抗血管の弛緩および毛細血管の変化によって引き起こされる可能性が考えられる。

Abstract

The aim of this study was to estimate the timing (latency) of the increase in red blood cell (RBC) velocity and RBC concentration, and the magnitude of response in local cerebral blood flow (LCBF) for neuronal activation. We measured LCBF change during activation of the somatosensory cortex by direct microelectrical stimulation. Electrical stimuli of 5, 10 and 50 Hz of 1 ms pulse with 10 - 15 μA, were given for 5 s. LCBF, RBC velocity and RBC concentration were monitored by laser-Doppler flowmetry (LDF) in α-chloralose anesthetized rats (n=7). LCBF, RBC velocity and RBC concentration increased nearly proportionally to stimulus frequency, i.e., neuronal activity. LCBF rose approximately 0.5 s after the onset of stimulation, and there was no significant time lag of the latencies among LCBF, RBC velocity and RBC concentration at the same stimulus frequency. We interpret these results to mean that the onset of LCBF increase on cortical activation is reflected by a rapid change in arteriole (resistance vessel) dilation and capillary volume. The data also elucidate the linear relationship between LCBF increase and cortical activity.

Key words: Arteriole, Somatosensory cortex, Capillary, Cerebral blood flow, Direct electrical stimulation, Functional activation, Hemodynamics, Laser-Doppler flowmetry, Microcirculation.

Introduction

It is well known that local cerebral blood flow (LCBF) mirrors the activity of the cortex. However, this coupling mechanism is poorly understood. There are two possible locations of the vasculature where microcirculation is initially regulated; the arteriolar site (resistance vessel) and the capillary site. In the case of the arteriolar site (21, 24), blood velocity in the capillary should increase during cortical activation (capillary perfusion heterogeneity; 12). Some investigators observed that the diameter of the pial arteriole reflects the amount of blood flow during neuronal activation of the cortex (19). In the case of the capillary site, capillary cerebral blood volume (CBV) should increase via a direct reaction of the capillary (5, 25, 29). Malonek *et al.* noted in cat visual cortex that the CBV increase preceded the CBF increase, and supposed that the capillary change occurs before arteriolar dilation (16). However, at present, there is no direct method to observe *in vivo* the reaction of the capillary or arteriole in the deep layer of the cortex at the site of activation.

Laser-Doppler flowmetry (LDF) has been used to monitor cerebral microcirculation in the capillary bed. A number of laboratories have used LDF to demonstrate CBF changes related to cerebral activation by various stimuli (1, 2, 19, 23). Recently, LDF has been improved to monitor red blood cell (RBC) velocity and RBC concentration independently (2). The validations of these parameters were achieved by *in vitro* experiments (2). Moreover, it was confirmed that RBC behavior in the capillary bed is the main contributor to the LDF signal (2), suggesting that the vascular behavior of the arteriole and the capillary during cortical activation is indirectly obtained from the change in RBC velocity or RBC concentration. However, there have been only a few attempts to measure the hemodynamics of RBC velocity and RBC concentration, and no estimation of the timing (latency) of the increase in these LDF parameters on cortical activation.

This is probably because the response curves of these parameters suffer from a poor signal-to-noise ratio in *in vivo* studies, e.g., only 20 % LCBF change on peripheral stimulation. Therefore, in the present study, we evoked a large LCBF change by direct electrical stimulation of the rat somatosensory cortex, and reduced the noise level by accumulation of multiple repeated triggering data. The aim of this study was to measure the hemodynamics of RBC velocity and RBC concentration on somatosensory activation, and to elucidate the mechanisms regulating LCBF change.

Materials and Methods

Animal preparation

Seven male Sprague-Dawley rats (300-380 g) were anesthetized with halothane (4% induction and 1.5% during surgery) in 30 % O_2 and 70 % N_2O. We cannulated the tail artery for monitoring blood pressure and blood gas sampling, and the left femoral vein for drug administration (Fig. 1). Following tracheotomy, α-chloralose (100 mg/kg, i.v.) was administered, and then halothane and nitrous oxide were discontinued. After tracheotomy, the animals were immobilized with pancuronium bromide (0.7 mg/kg, i.v.), and ventilated with a respirator (SN-480-7, Shinano, Japan) throughout the experiments. Anesthesia was maintained with α-chloralose (35 mg/kg/hr, i.v.) and pancuronium bromide (0.8 mg/kg/hr, i.v.). Body temperature was maintained at 37.5 ± 0.2 °C with a heating pad (ATC-101, Unique Medical, Japan). Arterial blood was sampled for blood gas analysis three times during the experimental period; before, during and immediately after the measurement. Arterial blood pressure was monitored during the experiments and the mean value (MABP) was calculated by averaging these three time points.

The rats were fixed in a stereotactic frame, and the parietal bone was thinned until translucent at the left somatosensory cortex (mainly barrel area), over an area of 3 x 4 mm^2 centered at 3 mm caudal and 6 mm lateral to the bregma.

Recording and stimulation procedures

Activation of the cortex was carried out by direct electrical stimulation of

the cortex with rectangular pulses (1 ms). For electrical stimulation, a pair of tungsten microelectrodes (tip diameter of about 10 µm, tip distance of about 200 µm; 12 MΩ) was perpendicularly inserted into the cortex of the brain (Fig. 1) (14). The electrode tip was 0.5 mm from the cortical surface. Stimulation was carried out about one hour after preparation. Electrical stimulation was varied in frequencies of 5, 10 and 50 Hz with 5 s duration. In each rat, a current stimulus of 10 to 15 µA, which elicited a change in LCBF, was applied at 5 Hz for 5 s.

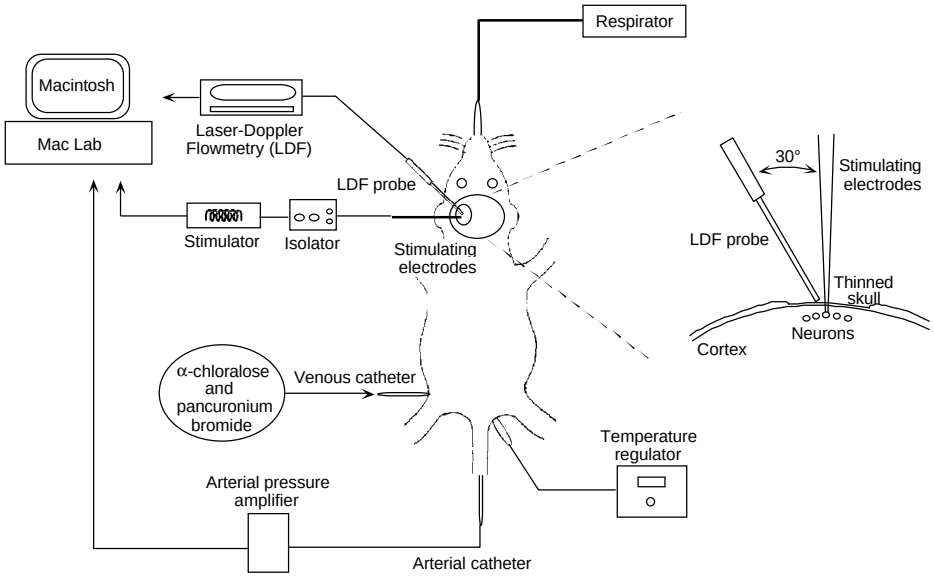


Fig. 1. Scheme of experimental arrangement. The tail artery and the left femoral vein were cannulated for monitoring blood pressure, blood gas sampling and intravenous drug administration. LCBF, RBC velocity and RBC concentration for cortical activation were monitored by laser-Doppler flowmetry (LDF) in α-chloralose anesthetized rats. Cortical activation was elicited by direct microelectrical stimulation.

LCBF was measured by LDF (TDF-LN1, Unique Medical, Japan). The tip diameter of the LDF probe was 0.55 mm (Probe LP-N, Unique Medical, Japan). LDF measures RBC behavior in the capillary based on the Doppler effect with laser light (wavelength of 780 nm). The frequency shift of the scattered radiation is caused by moving RBCs in the blood vessels. The amount of light whose frequency is shifted by the Doppler effect corresponds to the RBC concentration, and the average shift in frequency is related to RBC velocity. The LDF system used here can simultaneously measure three parameters, LCBF, RBC velocity and RBC concentration, where RBC velocity = LCBF / RBC concentration. The measurement area of LDF was approximately 1 mm^3 (6, 20). We set a time constant of 0.1 s for all LDF signals (LCBF, RBC velocity and RBC concentration).

The LDF probe was placed at the same location as the tip of the stimulus electrode, making an angle of 60° with respect to the brain surface. The LDF probe was attached to the thinned skull, avoiding areas with large blood vessels.

At each stimulus frequency, 20 successive sets of stimuli were applied at 80 s intervals. To avoid habituation, a weak stimulus was applied first, then the frequency was increased stepwise. Blank sampling of LDF signals, without electrical stimulation, was carried out before application of each set of stimuli. Data acquisition of LDF was synchronized to the respirator cycle.

Data collection

The LDF signals and arterial blood pressure value were recorded continuously on MacLab data acquisition software (AD Instruments, Japan) and the outputs were accumulated 20 times. These data were saved on a disk for off-line analysis. The LDF data were normalized by blank sampling (26). The latency was determined as the intersection with the baseline by the line extrapolated from the normalized response curve from 90 % to 10 % of the peak. The response area was calculated by integrating the response curve from the rise point to the termination point where the curve reaches the baseline, and was considered to reflect the total amount of increase in the supply of blood. Each latency and response area among the stimulus frequencies was statistically analyzed by ANOVA (repeated measurements) and multiple comparisons (Bonferroni).

Results

The mean arterial blood pressure (MABP) was 115 ± 9 mm Hg (mean ± SD, n = 7). MABP was stable throughout the experiment and did not change significantly during stimulation. The blood gas values were PO_2 = 112 ± 20 mmHg, PCO_2 = 32.7 ± 4.0 mm Hg and pH = 7.46 ± 0.04 (n = 7). These values were maintained within a stable range by regulating the stroke volume of the ventilation pump and the oxygen concentration of the ventilation gas.

Electrical stimulation of the cortex for 5 s evoked a frequency-dependent increase in LCBF ($p<0.05$, Figs. 2 and 3). The increases in RBC velocity and RBC

concentration were also nearly proportional to the stimulus frequency ($p<0.05$, Figs. 2 and 3). When the stimulus frequency was 5 - 10 Hz, there was no obvious difference between the response area of RBC velocity and that of RBC concentration. However, the response area of RBC velocity was larger than that of RBC concentration at 50 Hz stimulation (Fig. 3).

The latency of the LCBF at 5 and 10 Hz stimulation was approximately 0.5 s, and that at 50 Hz was approximately 0.3 s (Figs. 2 and 4). With LCBF, RBC velocity and RBC concentration, there was no significant difference in the latency at the same stimulus frequency (Fig. 4). The increase of these parameters at 50 Hz occurred earlier than those at 5 Hz and 10 Hz stimulation (LCBF at 50 Hz – LCBF at 5 Hz; $P=0.07$, others; $p<0.05$), although there was no significant difference between 5 Hz and 10 Hz (Fig. 4).

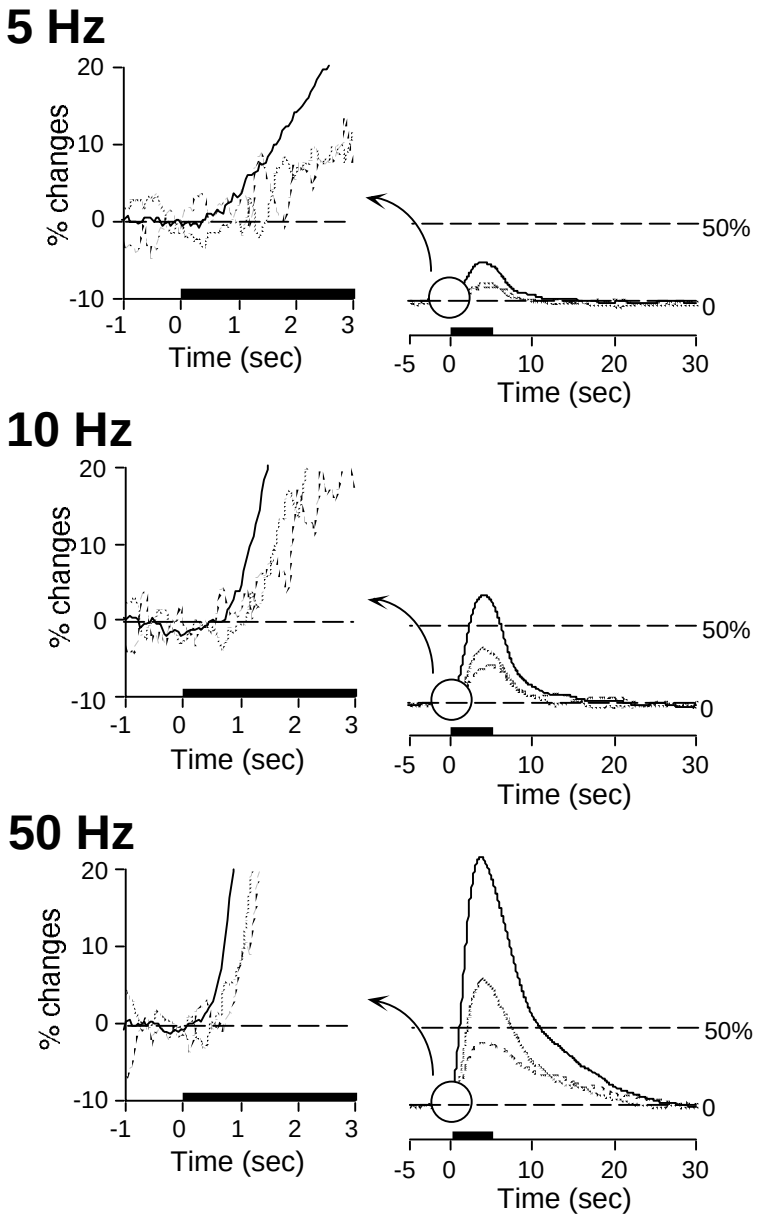


Fig. 2. Time course of LCBF (solid line), RBC velocity (dotted line) and RBC concentration (dashed line) in response to various frequencies for 5 s. Data of change in LCBF, RBC velocity and RBC concentration were normalized by blank sampling, and averaged by the number of animals used (n = 7). Time zero (t=0) and horizontal bars on the x-axis indicate the stimulus onset and stimulus period, respectively. Note that LCBF, RBC velocity and RBC concentration showed frequency-dependent increase, and that they rose at approximately 0.5 s after the onset of stimulation.

It is worth noting that LCBF, RBC velocity and RBC concentration peaked within 3 - 4 seconds after the onset of stimulation and began to decrease before the termination of stimulation regardless of the stimulus frequency (Fig. 2).

Discussion

No effect of direct electrical stimulation on vasculature

We used direct electrical stimulation of the somatosensory cortex to

evoke a large LCBF change. Direct electrical stimulation of the cortex is sometimes used for microcirculation research (1, 2). However, this stimulation was expected to stimulate a larger area of the cortex including various segments of the microcirculation, and not only somatosensory neurons, but also vascular smooth muscle and endothelium. This possibility can be excluded as follows: First, it was reported that LCBF change was abolished by tetrodotoxin (TTX) even when the cortical neuron was stimulated directly (1). This means that vascular smooth muscle is not activated by weak direct electrical stimulation, because it is known that TTX does not change the tension of the vascular smooth muscle (9, 10). Second, the neurogenic control of vascular smooth muscle is a very slow process, that is, LCBF should be sustained at an increased level during stimulation (3, 22). Our experiment did not support this postulation. Finally, if the electrical stimulation affected the capillary directly, the RBC concentration should change prior to RBC velocity. However, our present results rule this out. Therefore, we conclude that there is no effect of direct electrical stimulation on the vascular smooth muscle or the endothelium of the capillary.

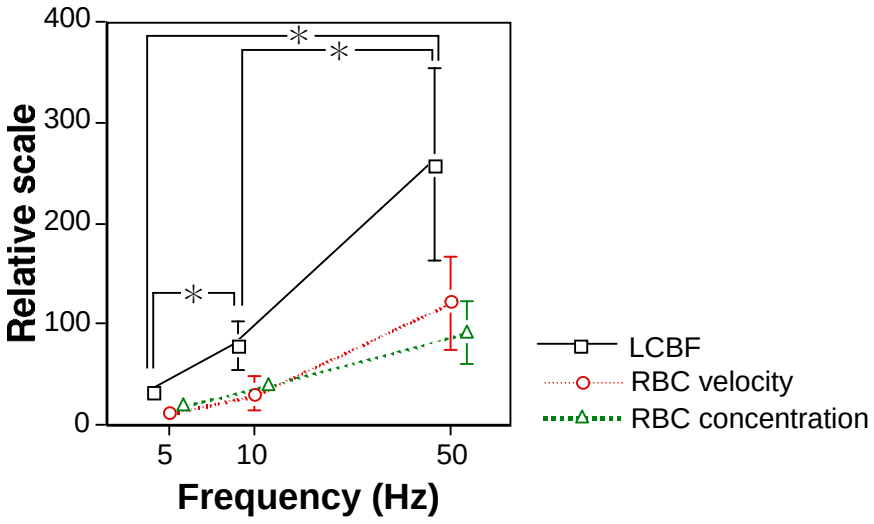


Fig. 3. Effect of 5 s stimulation at various frequencies on the response area. The response area was calculated by integrating the response curve from the rise point to the termination point where the curve reaches the baseline. Note that LCBF, RBC velocity and RBC concentration increased nearly proportionally to stimulus frequency (*, $p<0.05$ for LCBF, RBC velocity and RBC concentration; ANOVA with Bonferroni post hoc test). Error bars indicate ± 1 standard deviation (n = 7).

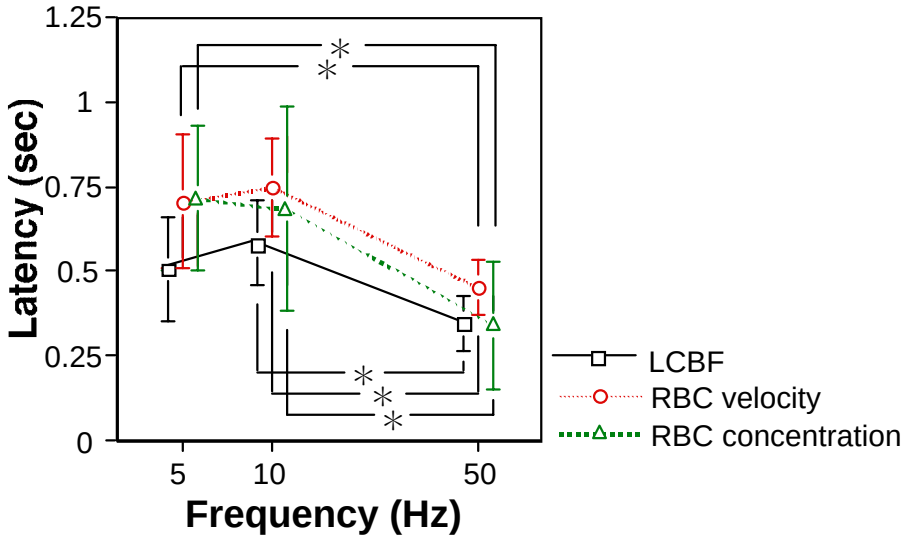
LCBF regulation

Microcirculatory regulation at the vessel level on cortical activation has

been described. Some investigators support the hypothesis that the diameter of the feeding artery determines LCBF during neuronal activation (e.g. 19, 21, 24). Others suggest that active capillary volume change is the main contributor to LCBF change (e.g. 16, 25). The increase in RBC velocity at the capillary level indicates an increase in perfusion pressure due to dilation of the resistance vessels, but the increase in RBC concentration indicates a capillary change; e.g., physiological recruitment (12) and capillary dilation (5, 29). Therefore, in the case of regulation by only arteriole dilation, the change in RBC velocity in the capillary bed should be larger and occur earlier than that of RBC concentration. In the case of capillary regulation, RBC concentration should increase earlier, and should contribute more than RBC velocity. In the present study, we have shown that there was no significant time lag in the latency between RBC velocity and RBC concentration on neuronal activation (Fig. 4), and that both RBC velocity and RBC concentration contributed to the observed changes in LCBF (Figs. 2 and 3). These results suggest that both arteriole dilation and capillary volume change contribute simultaneously to the initial regulation of LCBF.

Several factors might be responsible for the vessel diameter change in neurovascular coupling on cortical activation, such as metabolic by-products and neurogenic control. Some vasodilator products, e.g., CO_2, proton (H^+), potassium (K^+), adenosine, and nitric oxide (NO), have been reported as mediators of increase in LCBF during neuronal activity (15, 28). The levels of K^+ and NO should rise immediately after the onset of neuronal activation (4, 27), although those of H^+ and adenosine should increase later than the onset of LCBF increase (11, 28). It is known that neurogenic control is a slow process (3, 22). In this study, the LCBF showed a very rapid response, approximately 0.5 s, and both RBC velocity and RBC concentration rose at the same time as LCBF. These results suggest that NO and K^+ mediators should have an effect on both the capillary and arteriole in initial LCBF regulation, and evoke the rapid LCBF increase on cortical activation through the combined action of mediators responsible for the coupling.

Fig. 4. Latency with the onset of stimulation. The latency was determined by the intersection with the baseline of the extrapolated line which was drawn from the normalized response curve (Fig. 2) from 90 % to 10 % of the peak. Note that there is no significant difference (ANOVA) in the latencies among LCBF, RBC velocity and RBC concentration at the same stimulus frequency, and that the



latencies of these parameters at 50 Hz were earlier than those at 5 Hz and 10 Hz stimulation (*, $p<0.05$; ANOVA with Bonferroni post hoc test), although there was no significant difference (ANOVA) between 5 Hz and 10 Hz. Error bars indicate ± 1 standard deviation (n = 7).

LCBF, RBC velocity and RBC concentration peaked before the termination of stimulation (Fig. 2). Ngai *et al.* observed that there was an initial peak followed by a plateau during the course of stimulation (19). This biphasic change may correspond to an early transient reaction followed by a steady-state plateau. The results of the present study suggest that the 5 s stimulus disrupts the biphasic response, leaving only the early transient reaction.

Relationship between neuronal activity and LCBF response

The relationship between neuronal activity and LCBF response has been well described. The greatest LCBF increase was obtained at around 5 Hz in various experiments employing peripheral nerve stimulation (8, 13, 18, 30), and corresponded to the maximum amplitude of the evoked potential (13) and the maximum appearance of the field potential in the cortex (17). With higher frequency, the neuronal activity of the somatosensory area was reduced due to inhibitory mechanisms when stimuli were applied via peripheral nerves (7). However, in the present study, as we employed a direct cortical stimulation, LCBF, RBC velocity and RBC concentration increased nearly proportionally to stimulus frequency until 50 Hz (Fig. 3). Direct electrical stimuli could cause the excitement of neurons around the electrode until 50 Hz without initiating an inhibitory response, because of the interval between stimuli (20 ms at 50 Hz). Consequently, LCBF increases nearly proportionally to the level of cortical activity.

Barfod *et al.* demonstrated that RBC velocity continued to increase while RBC concentration reached a steady state during hypercapnia (2). In this experiment, the response area of RBC velocity was larger than that of RBC concentration at 50 Hz stimulation, although there was no obvious difference at 5-10 Hz (Fig. 3). These results suggest that RBC velocity is more dominant than RBC concentration due to a physical limit on the change in the capillary volume during a huge LCBF increase, i.e., extreme neuronal activity.

On peripheral stimulation, the latency of LCBF was approximately 0.5 s, regardless of the extent of LCBF increase (17), suggesting that the latency is independent of LCBF level. However, in this experiment, the LCBF response at 50 Hz stimulation took about 0.3 s, although the LCBF for 5 and 10 Hz stimulation rose approximately at 0.5 s after the onset of stimulation (Fig. 4). This suggests that extreme neuronal activity at 50 Hz stimulation advanced the latency of LCBF, though its mechanism remains unknown.

Acknowledgments

We gratefully acknowledge the technical assistance of Mr. Yozo Ito and Mr. Ryoetsu Sato of the Akita Research Institute of Brain and Blood Vessels. We are also grateful to Dr. Katsuya Yamada, Department of Physiology, School of Medicine, Akita University, for helpful discussion.

References

1. Akgören, N.; Dalgaard, P., Lauritzen, M. Cerebral blood flow increases evoked by electrical stimulation of rat cerebellar cortex: relation to excitatory synaptic activity and nitric oxide synthesis. Brain Res. **710**: 204-214; 1996.
2. Barfod, C.; Akgören, N.; Fabricius, M.; Dirnagl, U.; Lauritzen, M. Laser-Doppler measurements of concentration and velocity of moving blood cells in rat cerebral circulation. Acta. Physiol. Scand. **160**: 123-132; 1997.
3. Biesold, D.; Inanami, O.; Sato, A.; Sato, Y. Stimulation of the nucleus basalis of Meynert increases cerebral cortical flow in rats. Neurosci. Lett. **98**: 39-44; 1989.
4. Dirnagl, U.; Lindauer, U.; Villringer, A. Role of nitric oxide in the coupling of

cerebral blood flow to neuronal activation in rats. Neurosci. Lett. **149**: 43-46; 1993.

5. Duelli, R.; Kuschinsky, W. Changes in brain capillary diameter during hypocapnia and hypercapnia. J. Cereb. Blood Flow Metab. **13**: 1025-1028; 1993.
6. Fukuda, O.; Endo, S.; Kuwayama, N.; Harada, J.; Takaku, A. The characteristics of laser-Doppler flowmetry for the measurement of regional cerebral blood flow. Neurosurgery **36**: 358-364; 1995.
7. Hellweg, F. C.; Schultz, W.; Creutzfeldt, O. D. Extracellular and intracellular recording from cat's cortical whisker projection area: thalamocortical response transformation. J. Neurophysio. **40**: 463-479; 1977.
8. Ibáñez, V.; Deiber, M. P.; Sadato, N.; Toro, C.; Grissom, J.; Woods, R. P.; Mazziotta, J. C.; Hallett, M. Effects of stimulus rate on regional cerebral blood flow after median nerve stimulation. Brain **118**, 1339-1351; 1995.
9. Ito, T.; Kuriyama, H. Membrane properties of the smooth muscle fiber of the guinea-pig portal vein. J. Physiol. **214**: 427-441; 1971.
10. Itoh, T.; Kuriyama, H.; Suzuki, H. Excitation-contraction coupling in smooth muscle cell of the guinea-pig mesenteric artery. J. Physiol. **321**: 531-535; 1981.
11. Ko, K. R.; Ngai, A. C.; Winn, H. R. Role of adenosine in regulation of regional cerebral blood flow in sensory cortex. Am. J. Physiol. **259**: H1703-H1708; 1990
12. Kuschinsky, W.; Paulson, O. B. Capillary circulation in the brain. Cerebrovasc. Brain Metab. Rev. **4**: 261-286; 1992.
13. Leniger-Follert, E.; Hossmann, K. A. Simultaneous measurements of microflow and evoked potentials in the somatosensory cortex of the cat brain during specific sensory activation. Pflüeger's Arch. **380**: 85-89; 1979.
14. Leniger-Follert, E.; Lübbers, D. W. Behavior of microflow and local PO2 of the brain cortex during and after direct electrical stimulation. Pflüger's Arch. **366**: 39-44; 1976.
15. Lou, H. C.; Edvinsson, L.; MacKenzie, E. T. The concept of coupling blood flow to brain function: revision required? Ann. Neurol. **22**: 289-297; 1987.
16. Malonek, D.; Dirnagl, U.; Lindauer, U.; Yamada, K.; Kanno, I.; Grinvald, A.

Vascular imprints of neuronal activity: Relationships between the dynamics of cortical blood flow, oxygenation, and volume changes fllowing sensory stimulation. Proc. Natl. Acad. Sci. USA **94**: 14826-14831; 1997.

17. Matsuura, T.; Fujita, H.; Seki, C.; Kashikura, K.; Yamada, K.; Kanno, I. Hemodynamics evoked by somatosensory activation measured using LDF in rat brain. Zool. Sci. **15** (suppl.); 121; 1998.
18. Ngai, A. C.; Ko, K. R.; Morii, S; Winn, H. R. Effect of sciatic nerve stimulation on pial arterioles in rats. Am. J. Physiol. **254**; H133-H139; 1988
19. Ngai, A. C.; Meno, J. R.; Winn, H. R. Simultaneous measurements of pial arteriolar diameter and laser-Doppler flow during somatosensory stimulation. J. Cereb. Blood Flow Metab. **15**: 124-127; 1995.
20. Nilsson, G. E.; Tenland, T.; Öberg, P. Å. Evaluation of a laser-Doppler flowmeter for measurement of tissue blood flow. IEEE Trans. Biomed. Eng. BME-**27**: 597-604; 1980.
21. Paulson, O. B.; Newman, E. A. Does the release of potassium from astrocyte endfeet regulate cerebral blood flow? Science **237**: 896-898; 1987.
22. Sato, A.; Sato, Y. Regulation of regional cerebral blood flow by cholinergic fibers originating in the basal forebrain. Neurosci. Res. **14**: 242-274; 1992.
23. Schmitz, B.; Böttiger, B. W.; Hossmann, K. A. Brief hypercapnia enhances somatosensory activation of blood flow in rat. J. Cereb. Blood Flow Metab. **16**: 1307-1311; 1996.
24. Segal, S. S.; Duling, B. R. Flow control among microvessels coordinated by intercellular conduction. Science **234**: 868-870; 1986.
25. Tomita, M.; Fukuuchi, Y.; Tanahashi, N.; Kobari, M.; Takeda, H.; Yokoyama, M.; Ito, D.; Terakawa, S. Contraction/dilatation of cultured vascular endothelial cells inducted by hyperoxia/hypoxia. J. Cereb. Blood Flow Metab. **15**: Suppl S271; 1995.
26. To's, D. Y.; Frostig, R. D.; Lieke, E. E.; Grinvald, A. Functional organization of primate visual cortex revealed by high resolution optical imaging. Science **249**: 417-420; 1990.
27. Urbanics, R.; Leniger-Follert, E.; Lübbers, D. W. Time course of changes of

extracellular H^+ and K^+ activities during and after direct electrical stimulation of the brain cortex. Pfluger's Arch. **378**: 47-53; 1978.

28. Villringer, A.; Dirnagl, U. Coupling of brain activity and cerebral blood flow: basis of functional neuroimaging. Cerebrovasc. Brain Metab. Rev. **7**: 240-276; 1995.
29. Villringer, A.; Them, A.; Lindauer, U.; Einhäupl, K.; Dirnagl, U. Capillary perfusion of the rat brain cortex. An in vivo confocal microscopy study. Circ. Res. **75**: 55-62; 1994.
30. Woolsey, T. A.; Rovainen, C. M.; Cox, S. B.; Henegar, M. H.; Liang, G. E.; Liu, D.; Moskalenko, Y. E.; Sui, J.; Wei, L. Neuronal units linked to microvascular modules in cerebral cortex: response elements for imaging the brain. Cerebral Cortex **6**: 647-660; 1996.

Modulation of evoked cerebral blood flow under the excessive blood supply and the hyperoxic condition

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要旨　高二酸化炭素分圧下で血流が脳に過剰に供給されている状態、あるいは高酸素分圧下で脳組織中に過剰に酸素が存在する状態で、ラット体性感覚野賦活時の局所血流変化をレーザードップラ血流計を用いて計測し、正常ガス分圧時のそれらと比較した。高二酸化炭素分圧下のベースライン血流量(LCBF $_{base}$)は正常ガス分圧時のそれと比較し約 46.5%増加したが($p<0.01$)、LCBF $_{base}$ で基準化した賦活時の脳局所血流量(evoked LCBF)に有意な変化は認められなかった。一方、高酸素分圧下の LCBF $_{base}$ は正常ガス分圧時と比較し約 5.0%減少し ($p<0.01$)、evoked LCBF の反応量は正常ガス分圧時と比べ約 64.4%増大した($p<0.01$)。それぞれの状態における神経活動に顕著な変化は認められなかった。これらは、神経活動に伴う血流増加が代謝による酸素要求量とは独立に決定されていることを示唆している。

Abstract: We measured the field potential and local cerebral blood flow (LCBF) using laser-Doppler flowmetry in α-chloralose anesthetized rats during activation of the somatosensory cortex by electrical stimulation of the hind paw under independent administration of additional carbon dioxide and oxygen. The aim of this study was to test the hypothesis that the increase in LCBF during activation of the cortex (evoked LCBF) is not directed toward supplying oxygen for oxidative metabolism. Under the hypercapnic condition ($PaCO_2$=74.9±14.3 mm Hg), the baseline LCBF was about 46.5 % higher than that under the normocapnic condition ($PaCO_2$=35.7±2.1 mm Hg) ($p<0.001$), but after normalization for each baseline (divided by the prestimulus level), there was no significant difference in the peak value and the rise-time of normalized evoked LCBF. On the other hand, the baseline level of LCBF under the hyperoxic condition (PaO_2=479.4±77.2 mm Hg) was about 5.0 % lower than that under the normoxic condition

(PaO_2=105.5±7.8 mm Hg) ($p<0.01$), suggesting mild vasoconstriction under the condition of hyperoxia at rest. The peak value of normalized evoked LCBF under the hyperoxic condition was about 6.5 % higher than that under the normoxic condition ($p<0.05$). In addition, the rise-time of evoked LCBF was earlier under the hyperoxic condition (0.37±0.16 s) than that under the normoxic condition (0.52±0.12 s) ($p<0.01$). The field potential measured during stimulation under the hypercapnic and hyperoxic conditions was not significantly different in comparison with that under normal gas conditions. These results support our hypothesis and suggest that the excess oxygen is involved in the mechanism underlying the regulation of LCBF.

Key words: Cerebral blood flow; Laser-Doppler flowmetry; Hind paw stimulation; Hypercapnia; Hyperoxia; Neuronal activation.

INTRODUCTION

It is well known that cerebral blood flow (CBF) reflects the neuronal activity of the cortex. However, physiological interpretation of the mechanisms regulating local CBF (LCBF) is still awaited, because the association between neuronal activation, metabolism and change in LCBF is highly complex. Several possible mechanisms have been proposed [1], including reaction with metabolic by-products, regulation by cation or chemical substrates and neurogenic regulation. The likely mediators of LCBF regulation are potassium [2, 3], hydrogen ions [3], adenosine [4] and nitric oxide [5, 6]. Oxygen and carbon dioxide may also be involved in LCBF regulation during cortical activation [7-9].

One important role of the increase in LCBF during cortical activation is the supply of oxygen to the brain tissue. In fact, it has been reported that deoxyhemoglobin levels rise immediately after cortical activation [10], and an “initial dip”, that is, a negative signal during the first few seconds before the appearance of a positive signal, was observed in the blood oxygenation level dependent contrast signal of high-tesla functional MRI [11, 12]. On the other hand, Fox and Raichle [13] demonstrated a lack of coupling between LCBF and oxygen metabolism. In our previous study, we reported that LCBF increased nearly proportionally to the intensity of cortical activation [14, 15]. These suggest that

the degree of increase in LCBF is dependent on neuronal activity, but not on oxygen consumption. Thus, it is hypothesized that the increase in LCBF is not directed toward supplying oxygen for oxidative metabolism, but is caused by the mechanisms operative on the blood vessel which is proportional to neuronal activity. If this hypothesis were correct, the same level of cortical activity should yield the same degree of absolute LCBF increase during cortical activation (evoked LCBF) even if the oxygen concentration in the tissue or the blood supply is increased to superabundant levels.

Studies of evoked LCBF at various levels of global CBF have been described by some investigators [16-18]. However, there have been only a few attempts to measure the evoked LCBF under conditions of hyperoxia [19], and no estimation so far of the time course of changes in the evoked LCBF under conditions of excessive blood supply or hyperoxia. In general, hypercapnia leads to an increase in the blood supply due to the dilation of resistance vessels, and hyperoxia causes excess oxygen tension in the brain tissue. In the present study, we tested the above hypothesis by measuring the neuronal activity using an electrode inserted into the cortex and the evoked LCBF using a laser-Doppler flowmetry (LDF), during activation of the somatosensory cortex under hypercapnic, hyperoxic and normal gas conditions (normocapnia and normoxia). Such data are of potential value for understanding the mechanism underlying the LCBF regulation during cortical activation.

MATERIALS AND METHODS

A Sprague-Dawley rat was anesthetized with halothane (4 % for induction and 1.5 % during surgery) in 30 % oxygen and 70 % nitrous oxide. The tail artery and the left femoral vein were cannulated for monitoring the blood pressure, blood gas sampling, and intravenous drug administration. Following tracheotomy, α-chloralose (75 mg kg^{-1}, i.v.) was administered, and halothane and nitrous oxide were discontinued. The rat was immobilized with pancuronium bromide (0.7 mg/kg, i.v.), and ventilated with a respirator (SN-480-7, Shinano, Japan) throughout the experimental period. Anesthesia was maintained with α-chloralose (45 mg kg^{-1} hr^{-1}, i.v.), and muscle relaxation with pancuronium

bromide (0.8 mg kg^{-1} hr^{-1}, i.v.). The body temperature was maintained at about 37.0 °C with the aid of a heating pad (ATC-101, Unique Medical, Japan). Arterial blood was sampled for blood gas analysis before and immediately after each examination (Fig. 1). Arterial blood pressure was monitored during the experiments and the mean arterial blood pressure (MABP) was calculated as the average at three time points (i.e., before, during and immediately after each examination). The rat was fixed in a stereotactic frame, and the parietal bone was thinned to translucency at the left somatosensory cortex, over an area of 3 x 3 mm, centered 2.5 mm caudal and 2.5 mm lateral to the bregma.

To ensure a stable condition of the animal, the measurements were performed 2~3 hours after the preparation. Activation of the cortex was carried out by electrical pulse stimulation of the hind paw (pulse width 0.1 ms), using a pair of small needle electrodes inserted under the skin of the right hind paw. Electrical stimuli of 5 Hz and 5 s duration with the intensity of 1.5 mA were administered. This stimulus intensity did not cause any change in the systemic MABP during the stimulation [14]. Fifty successive stimuli were applied at 60 s intervals. Under the normal gas condition (normoxia-normocapnia), the $PaCO_2$ levels were maintained within a range of 32-40 mm Hg and the PaO_2 levels in the range of 90-120 mm Hg by regulating the stroke volume of ventilation and the fractional concentration of oxygen in the inspired gas, respectively. For the experiments under the hypercapnic condition, we used 13 rats, and first examined the LCBF responses to normocapnia, followed by those to hypercapnia after 20 min of hypercapnic ventilation. For hypercapnic ventilation, approximately 2.5 % carbon dioxide was mixed with the gas administered under the normal gas condition. For the experiments under the hyperoxic condition also, we used 13 rats, and first examined the LCBF responses to normoxia, followed by those to hyperoxia after 20 min of oxygen ventilation. In 12 other rats used for the control experiments, the LCBF responses under the normal gas condition were examined twice under the same time schedule as that for the hypercapnia and hyperoxia experiments (Fig. 1).

LCBF was measured with an LDF (Ne-He, wavelength of 780 nm, maximal intensity of 0.8 mW; Periflux 4001 Master, Perimed, Sweden) equipped with an LDF probe with a tip diameter of 0.46 mm (Probe 411, Perimed, Sweden).

The area of LDF measurement was about 1 mm^3 [20].

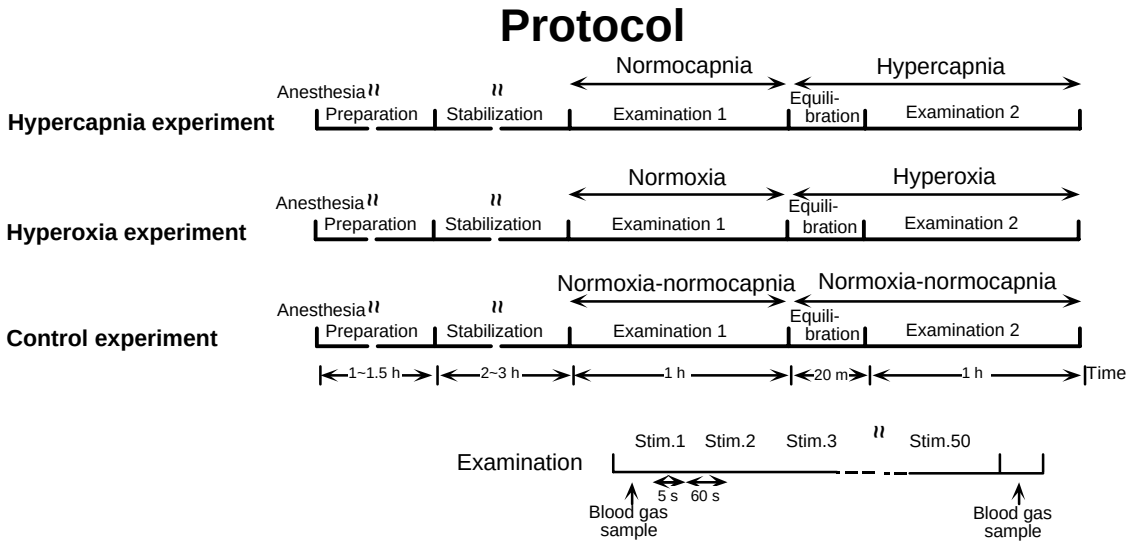

Fig. 1. Protocol of hypercapnia, hyperoxia and control experiments. Examination was carried out about 2 ~3 hours after the preparation of the animals. In the hypercapnia (n=13) and hyperoxia experiments (n=13), the LCBF responses under the normal gas condition were initially examined, and then those under hypercapnic or hyperoxic conditions, 20 min after changing the inspired gas. In the control experiment (n=12), the LCBF responses under the normal gas condition were examined twice under the same time schedule as that for the hypercapnia and hyperoxia experiments. At each examination, 50 successive stimuli of 5 Hz frequency and 5 s duration were applied at 60 s intervals, and outputs from 50 successive measurements were accumulated using data acquisition software.

The time constant was set to 0.03 s. The LDF probe was placed in the somatosensory area of the hind paw, perpendicular to the brain surface. It was attached to the thinned parietal bone and then fine-positioned for detecting maximum signal changes during stimulation, avoiding areas with large blood vessels.

The LDF signals and arterial blood pressure were recorded continuously using the MacLab data acquisition software (AD Instruments, Australia), and outputs from 50 successive measurements were accumulated. Data were digitized at 40 Hz, and saved on a disk for off-line analyses. The LDF data were normalized to the prestimulus value (baseline level), which we define in this paper as normalized LCBF. The rise-time of the LDF signal was determined as the intersection of the baseline by the extrapolated line which was drawn on the normalized response curve from 90 to 10 % of the peak. The termination-time was also determined as the time at the intersection of the baseline by a similar extrapolated line (detail in ref. 14). The response magnitude was calculated as the

integral of the normalized response curve from the rise-time to the termination-time, and was considered to reflect the total amount of increase in blood flow. The peak response value (percent of baseline) was calculated from the normalized response curve. These LDF data (i.e. rise-time, response magnitude and peak response value) obtained at the first (normal gas condition) and second examinations (hypercapnic, hyperoxic, and normal gas conditions) were statistically analyzed by the t-test. For statistical analysis of the baseline levels, we also applied the paired t-test on the raw LDF data. We then normalized the baseline levels and peak response values to those of the normal gas condition.

To confirm the neuronal activity during the somatosensory stimulations, the field potentials under hypercapnic, hyperoxic and normal gas conditions were recorded in another 14 rats (7 rats for the hypercapnia and 7 other rats for the hyperoxia experiments). A tungsten microelectrode (12 MΩ) was inserted into the somatosensory area of the hind paw through the thinned portion of the skull, and was fixed using dental cement. The tip of the electrode was set at a depth of about 0.5 mm from the surface of the cortex. An Ag-AgCl indifferent electrode was placed between the skull bone and the scalp. We first recorded the field potentials under the normal gas condition, followed by those under the hypercapnic or hyperoxic conditions in the same time schedule as that for the LCBF measurement. Fifty successive signals of the field potential recordings were also accumulated using the MacLab data acquisition software. In the field potential analysis, the number of spike-shaped potentials which exceeded the noise level during the stimulation period was counted. The mean amplitude of the field potentials was calculated as the average of the negative components of each potential. The electrophysiological data (latency, mean amplitude and number of field potentials) were statistically analyzed by the t-test. Values are given as means ± SD.

RESULTS

Animal condition during the experiments

As shown in Table 1, the physiological variables were within normal range in all the experimental animals throughout the experimental period, except for $PaCO_2$ under the condition of hypercapnia and PaO_2 under the condition of

hyperoxia. The PaO_2 under the hypercapnic condition

Table 1. *Physiological data during examination for each blood gas condition*

Blood gas condition	MABP (mm Hg)	$PaCO_2$ (mm Hg)	PaO_2 (mm Hg)
Hypercapnia experiment (n=20)			
Normocapnia	100.3±11.3	35.7±2.1	110.4±8.1
Hypercapnia	91.1±13.1*	74.9±14.3*	116.6±11.3
Hyperoxia experiment (n=20)			
Normoxia	109.3±7.0	34.7±1.4	105.5±7.8
Hyperoxia	109.7±8.4	35.5±3.0	479.4±77.2*
Control experiment (n=12)			
Normal gas condition (1st)	107.0±8.9	36.1±1.5	119.8±9.2
Normal gas condition (2nd)	103.2±9.6	36.7±1.1	115.1±10.8

There were significant differences between the hypercapnic and normocapnic, and hyperoxic and normoxic conditions (*; $p<0.001$). Data of hypercapnia and hyperoxia experiments include both groups of LCBF and field potential measurements. Mean ± SD.

and $PaCO_2$ under the hyperoxic condition were within normal range relative to those under the normal gas condition (NS). The MABP under the hypercapnic condition was lower than that under the normocapnic condition ($p<0.001$), while the MABP during the hyperoxia experiment remained stable throughout the experimental period. The systemic arterial blood pressure showed no changes during the stimulations.

Neuronal activity of the somatosensory cortex during hind paw stimulation under the hypercapnic and hyperoxic conditions

The latency, mean amplitude and number of field potentials were measured as a function of the neuronal activity during somatosensory stimulation. The mean latency of onset of the first potential was about 10.2 ms under the normal gas condition, and 10.0±0.7 ms and 10.1±0.7 ms, respectively, under the hypercapnic and hyperoxic conditions (Table 2). These values were not

significantly different from each other.

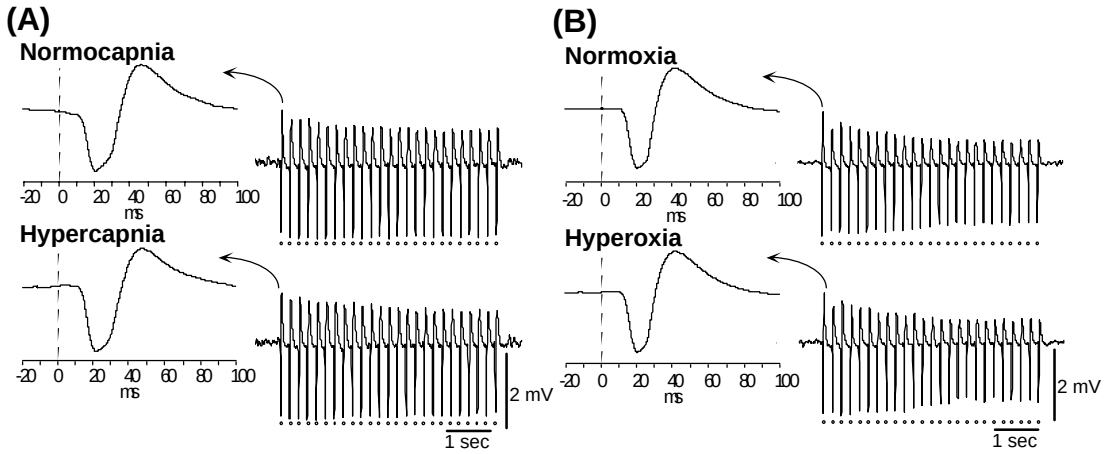

Fig. 2. Field potential recordings from the somatosensory cortex for 5 Hz stimulation of the hind paw under the normocapnic and hypercapnic conditions (A), and under the normoxic and hyperoxic conditions (B). Left sides show temporally extended first field potential, and time zero in horizontal axes indicate stimulus onset. Dots on the right side indicate the marks of electrical stimulation. Note that there was no significant difference in latency, amplitude and number of field potentials between either the hypercapnic and normocapnic conditions or the hyperoxic and normoxic conditions.

In most rats under the normal gas condition, each electrical stimulus evoked a corresponding field potential (Fig. 2) [14]. Under the hypercapnic and hyperoxic conditions, the field potentials showed the same frequencies as those under the normal gas condition (Fig. 2 and Table 2). Moreover, there was no significant difference in the mean amplitude of field potentials either between the hypercapnic and normocapnic conditions or between the hyperoxic and normoxic conditions (Fig. 2 and Table 2).

Effect on LCBF change

The baseline LCBF under the hypercapnic condition was about 46.5 % higher than that under the normocapnic condition ($p<0.001$) (Fig. 3A). On the other hand, the baseline LCBF under the hyperoxic condition was about 5.0 % lower than that under the normoxic condition ($p<0.01$) (Fig. 3B). In the control experiments, there was no significant difference in the baseline LCBF between the first and second examinations (data not shown).

Table 2. *Latency, amplitude and number of field potentials under the hypercapnic, hyperoxic and normal gas conditions*

Blood gas condition	Latency of first field potential (ms)	Mean amplitude of field potentials (mV)	Number of field potentials detected
Hypercapnia experiment (n=7)			
Normocapnia	10.2±0.3	1.27±0.67	22.8±3.6
Hypercapnia	10.0±0.7	1.25±0.62	22.8±3.6
Hyperoxia experiment (n=7)			
Normoxia	10.2±0.5	1.04±0.51	23.3±2.9
Hyperoxia	10.1±0.7	1.00±0.53	23.3±2.9

There were no significant differences in latency, amplitude and number of field potentials between the hypercapnic and normocapnic, and the hyperoxic and normoxic conditions. Mean ± SD.

The peak normalized evoked LCBF under the hypercapnic condition was decreased slightly compared to that under the normocapnic condition but the difference was not significant (Fig. 3A). On the other hand, the peak normalized evoked LCBF under the hyperoxic condition was about 6.5 % greater than that under the normoxic condition ($p<0.05$) (Fig. 3B). During the control

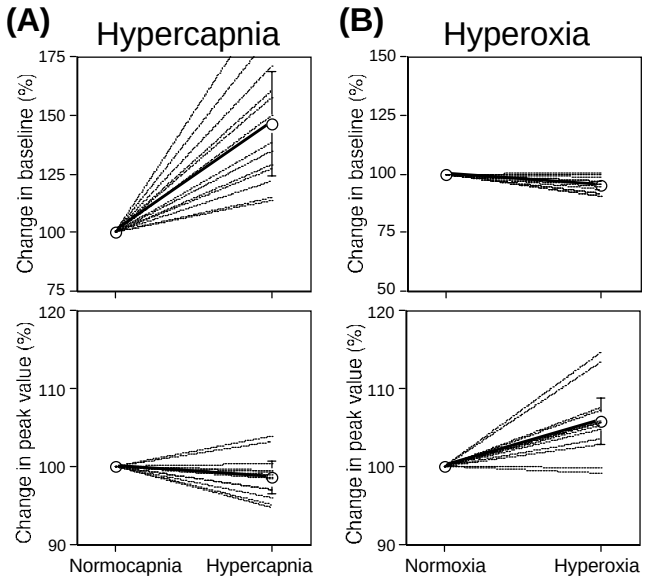

Fig. 3. Changes in the baseline LCBF (top) and peak normalized evoked LCBF (bottom), with LCBF obtained under the normal gas condition being considered as 100 %. (A) Percent change in LCBF under the hypercapnic condition. (B) Percent change in LCBF under the hyperoxic condition.

Each line corresponds to one rat. Bold lines and error bars indicate the mean value and SD, respectively. These data were normalized by those of the normal gas condition after statistical analysis. Note that the baseline level under the hypercapnic condition and that under the hyperoxic condition were, respectively, about 46.5 % higher and 5.0 % lower than that under the normal gas condition ($p<0.01$), and that the peak normalized evoked LCBF under the hyperoxic condition was greater than that the under normoxic condition ($p<0.05$), although there was no significant difference in the peak normalized evoked LCBF between the hypercapnic and normocapnic conditions.

experiments, there was no significant difference in the peak normalized evoked LCBF between the first and second examinations.

The response magnitude of the evoked LCBF under the hyperoxic condition was about 64.4 % greater than that under the normoxic condition ($p<0.001$), although there was no significant difference between those under the hypercapnic and normocapnic conditions, and also no significant difference from that in the control experiment (Fig. 4).

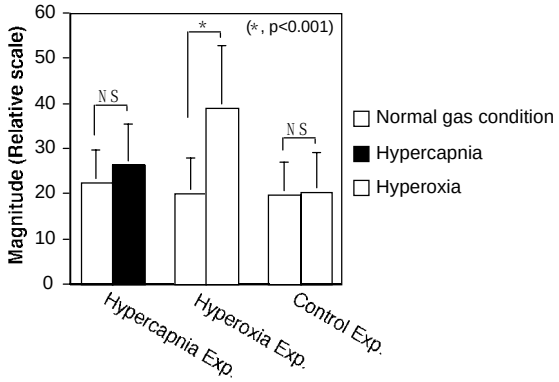

Fig. 4. Comparison of response magnitudes (area under the LCBF response curve). Open bar, black bar and gray bar indicate the response magnitude under the normal gas, hypercapnic and hyperoxic conditions, respectively. Note that the response magnitude under the hyperoxic condition was significantly greater than that under the normoxic condition as indicated by an asterisk ($p<0.001$). Error bars indicate SD.

Time course of changes in the evoked LCBF

The rise-time of the evoked LCBF was nearly constant, at approximately 0.5 s, under the normal gas condition (Table 3). Under the hypercapnic and normocapnic conditions, the rise-times were 0.54 ± 0.23 s and 0.54 ± 0.20 s, respectively (Table 3). There was no significant difference in the rise-times between the hypercapnic and normocapnic conditions (Fig. 5A). However, the risetime under the hyperoxic condition was earlier than that under the normoxic condition ($p<0.01$) (Fig. 5B). The mean rise-time of the evoked LCBF under the hyperoxic condition was 0.37±0.16 s, whereas it was about 0.52±0.12 s under the

normoxic condition (Table 3).

Within 3 - 4 s after the onset of stimulation, the evoked LCBF reached its peak value regardless of the blood gas condition (Fig. 5). The time at which the evoked LCBF reached its peak value was not significantly different between either the hypercapnic and normocapnic conditions or the hyperoxic and normoxic conditions. However, under the hypercapnic and hyperoxic conditions, the evoked LCBF returned to the baseline level more slowly in comparison with that under the normal gas condition (Fig. 5).

DISCUSSION

We measured the LCBF during activation of the rat somatosensory cortex by electrical stimulation of the hind paw under independent administration of additional carbon dioxide and oxygen. The changes in LCBF were detected using an LDF, and the time courses of the changes in LCBF were evaluated by the accumulation of multiple data in order to reduce the noise level. The described shorter rise-time and enhancement of the evoked LCBF response under the hyperoxic condition is the first report of LCBF measured using an LDF.

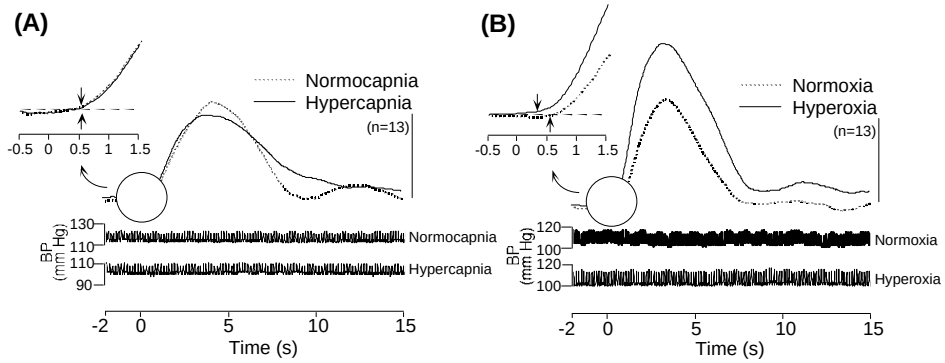

Fig. 5. Normalized LCBF responses evoked by stimulation. (A) Time courses of changes in evoked LCBF under the normocapnic (dotted line) and hypercapnic (solid line) conditions. (B) Time courses of changes in evoked LCBF under the normoxic (dotted line) and hyperoxic (solid line) conditions. The response curves were normalized to the baseline level, and were averaged by the number of animals used. Arterial blood pressure (BP) recordings are those of a representative animal. Vertical bars, 10 % increase in LCBF; time zero (t=0 s), stimulus onset; hatched lines, stimulation period; horizontal arrows, mean rise-times. Note that the LCBF under the hyperoxic condition increased about 0.15 s earlier than that under the normoxic condition ($p<0.01$), although the difference in rise-time between the hypercapnic and normocapnic conditions was not significant. Electrical stimulation of the hind paw did not affect the BP.

The evoked LCBF is independent of the metabolic oxygen demand

In this study, we confirmed that the field potentials detected during

stimulation under the hypercapnic and hyperoxic conditions were not significantly different from those under the normal gas condition (Fig. 2 and Table 2), indicating that neuronal activity was not affected by hypercapnia and hyperoxia. This suggests that energy metabolism was not altered by changes in the blood gas conditions in our experiments.

Table 3. *Rise-time of evoked LCBF*

Blood gas condition	Rise-time (s)
Hypercapnia experiment (n=13)	
Normocapnia	0.54±0.23
Hypercapnia	0.54±0.20
Hyperoxia experiment (n=13)	
Normoxia	0.52±0.12
Hyperoxia	0.37±0.16*
Control experiment (n=12)	
Normal gas condition (1st)	0.51±0.08
Normal gas condition (2nd)	0.55±0.45

There was significant difference in the rise-time between the hyperoxic and normoxic conditions (*; $p<0.01$). Mean ± SD.

The baseline LCBF under the hypercapnic condition was about 46.5 % higher than that under the normocapnic condition (Fig, 3A). The PO_2 in the cerebral cortex has been reported to be 20 % higher under the hyperoxic condition (500 mm Hg) than that under the normoxic condition (100 mm Hg) [21]. If the evoked LCBF response was to increase the supply of oxygen for oxidative metabolism, the response of evoked LCBF under the hypercapnic or hyperoxic condition would be lower than that under the normal gas condition. Nevertheless, the present study showed that there was no significant difference in the magnitude of the evoked LCBF response between the hypercapnic and normocapnic conditions after normalization for each baseline (Figs. 4 and 5A), and that the normalized evoked LCBF under the hyperoxic condition was greater than

that under the normoxic condition (Figs. 4 and 5B). These results support our hypothesis that the increase in LCBF is not directed toward supplying oxygen for oxidative metabolism.

The brain has a high rate of aerobic metabolism. During cortical activation, the brain tissue exhibits an increased demand not only for oxygen supply but also the supply of other substrates (e.g. glucose) as an energy source of neuronal spiking. There is the possibility that the evoked LCBF plays the role of supplying other substrates for metabolism. However, this possibility was not found to be true based on the results of the hypercapnia experiment in which the substrates were supplied in abundance. In addition, we and others confirmed that the evoked LCBF increased nearly proportionally to the intensity of neuronal activity [14, 15, 22]. These suggest that the evoked LCBF is a basic response of the blood vessels to neuronal activity.

Effect of hypercapnia on evoked LCBF

Despite the increase in the baseline LCBF with an increase in the $PaCO_2$ level (Fig. 3A), the peak normalized evoked LCBF and magnitude of the evoked LCBF response were not affected by the $PaCO_2$ level (Figs. 3A and 4). The relationship between the baseline LCBF and the evoked LCBF under various $PaCO_2$ levels has been reported in several previous studies performed using the ^{133}Xe-inhalation technique [17] and positron emission tomography (PET) [16, 18]. They described that the absolute LCBF during neuronal activation changed proportionally with the change in the baseline LCBF [16-18]. This means that the normalized evoked LCBF was the same regardless of the $PaCO_2$ level, which is consistent with the results of our study (Fig. 4). These indicate that the normalized evoked LCBF is not affected by changes in the baseline LCBF.

It is possible that the physiological interpretation of these phenomena consists of either changes in the activity level of neurogenic regulation, differences in metabolic rate, or differences in the level of vasodilation induced in resistance vessels by $PaCO_2$. The firing rate of the facial nerve, which provides vasodilator fibers, does not change under the hypercapnic condition [23]. These suggest that the activity level of neurogenic regulation is independent of the $PaCO_2$ level. From

a brief hypercapnia experiment, Schmitz *et al.* [24] reported the possibility that the metabolic rate is influenced by the $PaCO_2$ level. However, their experiment was performed following a brief period of hypercapnia and not during hypercapnia. A most likely interpretation is based on "the law of initial value" which suggests that the LCBF response is evoked in addition to the hyperperfusion induced by $PaCO_2$ [17, 18].

In the present study, we showed that hypercapnia had no effect on the rise-time of the evoked LCBF (Fig. 5A and Table 3), indicating that the onset of the LCBF response is independent of the baseline LCBF. However, the return of the evoked LCBF to the prestimulus (baseline) level under the hypercapnic condition was delayed in comparison to that under the normal gas condition (Fig. 5A). This suggests that carbon dioxide affects the behavior of the blood vessels during the phase of return of the LCBF to the baseline level.

Effect of hyperoxia on evoked LCBF

The baseline LCBF under the hyperoxic condition was decreased in comparison with that under the normoxic condition (Fig 3B). There are many reports on the changes in CBF during hyperoxia at rest [25-28]. All of these showed that the CBF decreased under the hyperoxic condition in comparison with that under the normoxic condition, suggesting vasoconstriction. However, the mechanisms underlying the vasoconstriction during hyperoxia are not yet clear. Several possible mechanisms have been proposed; hypocapnia with hyperoxia, probably due to the Bohr effect [28], direct action of oxygen on the vessels [29, 30], and neurogenic regulation [27]. In the present paper, it is difficult to explain hyperoxia-induced hypocapnia, because the $PaCO_2$ level did not change during the hyperoxia experiment (Table 1) [25, 26]. On the other hand, there are some *in vitro* experiments which have shown that the resistance of the vessels can be changed by varying the partial pressure of oxygen [29-31], suggesting that the direct action of oxygen on the capillary and/or other vessels is one of the factors underlying the vasoconstriction. Neurogenic regulation could also be another factor underlying the vasoconstriction [27].

The present study showed that the peak normalized evoked LCBF under

the hyperoxic condition was approximately 6.5 % higher than that under the normoxic condition (Fig. 3B), and the response magnitude of LCBF under the hyperoxic condition was approximately 64.4 % higher than that under the normoxic condition (Fig. 4). There are two possible mechanisms underlying the enhancement of the normalized evoked LCBF under the hyperoxic condition. One is that the normalized evoked LCBF under the hyperoxic condition shows only an apparent increase, because of the approximately 6.5 % higher peak value and the approximately 5.0 % decrease in the baseline LCBF under the hyperoxic condition in comparison with that under the normoxic condition. However, the decrease in absolute evoked CBF under the hypocapnic condition correlated positively with the baseline CBF [16, 18], suggesting that a decrease in the baseline level of LCBF does not affect the normalized evoked LCBF (see above discussion).

The other possible mechanism is the existence of an interaction mechanism with oxygen during the LCBF change, and our results suggest this possibility. A likely mechanism for the enhancement of the normalized evoked LCBF is interference by hyperoxia of the functions of other mediators of cortical activation, such as potassium [2, 3], nitric oxide [5, 6], hydrogen ions [3] and adenosine [4]. It is known that the concentrations of potassium and nitric oxide increase promptly after the onset of neuronal activation [3, 5, 6], whereas those of hydrogen ions and adenosine increase after the onset of LCBF response [3, 4]. In the present study, we found that the evoked LCBF under the hyperoxic condition increased earlier by approximately 0.15 s than that under the normoxic condition (Fig. 5B and Table 3). This phenomenon was not caused by the excessive increase in the normalized evoked LCBF, because the rise-time of 0.5 s is consistent regardless of the degree of LCBF increase within the normal range of neuronal activity [14, 15]. Therefore, the role of oxygen may be to prompt the action of potassium and/or nitric oxide involved in the initial LCBF regulation. Moreover, we showed that the return of the evoked LCBF to the prestimulus (baseline) level under the hyperoxic condition was delayed in comparison to that under the normoxic condition (Fig. 5B). This suggests a possibility that oxygen also affects the production of mediators (e.g. hydrogen ions and adenosine) involved in the regulation in the later part of the evoked LCBF response. Enhancement of the neurogenic regulation by oxygen is a possible mechanism. However, there is no

report of any increase in the level of neuronal activity or neurotransmission to suggest stimulated neurogenic regulation during oxygen uptake. Therefore, it is unclear whether the neurogenic regulation is relevant to LCBF enhancement under the hyperoxic condition. The direct action of oxygen is also another possibility. Thus, the oxygen tension or oxygen content may be relevant to LCBF regulation [7, 8].

ACKNOWLEDGMENTS

The technical assistance of Mr. Yozo Ito and Ms. Mikiko Ogiwara, Akita Research Institute of Brain and Blood Vessels, is gratefully acknowledged. We are also grateful to Dr. Katsuya Yamada, Department of Physiology, School of Medicine, Akita University, for the helpful discussion.

REFERENCES

1. Villringer A and Dirnagl U: Coupling of brain activity and cerebral blood flow: basis of functional neuroimaging. Cerebrovasc. Brain Metab Rev 7: 240-276, 1995
2. Kuschinsky W, Wahl M, Bosse O and Thurau K: Perivascular potassium and pH as determinants of local pial arterial diameter in cats. Circ Res 31: 240-247, 1972
3. Urbanics R, Leniger-Follert E and Lübbers DW: Time course of changes of extracellular H^+ and K^+ activities during and after direct electrical stimulation of the brain cortex. Pflügers Arch 378: 47-53, 1978
4. Ko KR, Ngai AC and Winn HR: Role of adenosine in regulation of regional cerebral blood flow in sensory cortex. Am J Physiol 259: H1703-H1708, 1990
5. Dirnagl U, Lindauer U and Villringer A: Role of nitric oxide in the coupling of cerebral blood flow to neuronal activation in rats. Neurosci Lett 149: 43-46, 1993
6. Dirnagl U, Niwa K, Lindauer U and Villringer A: Coupling of cerebral blood flow to neuronal activation: role of adenosine and nitric oxide. Am J Physiol 267: H296-H301, 1994
7. Duling BR and Berne RM: Longitudinal gradients in periarteriolar oxygen

tension. Circ Res 27: 669-678, 1970

8. Federspiel WJ and Sarelus IH: An examination of the contribution of red cell spacing to the uniformity of oxygen flux at the capillary wall. Microvasc Res 27: 273-285, 1984
9. Lassen NA: Intrinsic regulation of blood flow in the brain. In: Peripheral circulation, Ed. Johnson PC, John Wiley and Sons Inc, New York, pp. 337-358, 1978
10. Malonek D, Dirnagl U, Lindauer U, Yamada K, Kanno I and Grinvald A: Vascular imprints of neuronal activity: Relationships between the dynamics of cortical blood flow, oxygenation, and volume changes following sensory stimulation. Proc Natl Acad Sci USA 94: 14826-14831, 1997
11. Ernst T and Hennig J: Observation of a fast response in functional MR. Magn Reson Med 32: 146-149, 1994
12. Menon RS, Ogawa S, Strupp JP, Anderson P and Ugurbil K: BOLD based functional MRI at 4 tesla includes a capillary bed contribution: echo-planar imaging time course mirrors previous optical imaging using intrinsic signals. Magn Reson Med 33: 453-459, 1995
13. Fox PT and Raichle ME: Focal physiological uncoupling of cerebral blood flow and oxidative metabolism during somatosensory stimulation in human subjects. Proc Natl Acad Sci USA 83: 1140-1144, 1986
14. Matsuura T, Fujita H, Seki C, Kashikura K, Yamada K and Kanno I: CBF change evoked by somatosensory activation measured by laser-Doppler flowmetry: independent evaluation of RBC velocity and RBC concentration. Jpn J Physiol 46: 289-296, 1999
15. Matsuura T, Fujita H, Seki C, Kashikura K and Kanno I: Hemodynamics evoked by microelectrical direct stimulation in rat somatosensory cortex. Comp Biochem Physiol A 124: 47-52, 1999
16. Kanno I, Shimosegawa E, Fujita H and Hatazawa J: Uncoupling of absolute CBF to neuronal activity. Adv Exp Med Biol 413: 209-214, 1997
17. Maximilian VA, Prohovnik I and Risberg J: Cerebral hemodynamic response to mental activation in normo- and hypercapnia. Stroke 11: 342-347, 1980
18. Shimosegawa E, Kanno I, Hatazawa J, Fujita H, Iida H, Miura S, Murakami

M, Inugami A, Ogawa T, Itoh H, Okudera T and Uemura K: Photic stimulation study of changing the arterial partial pressure level of carbon dioxide. J Cereb Blood Flow Metab 15: 111-114, 1995

19. Kanno I, Fujita H, Hatazawa J: Enhancement of CBF response for V1 stimuli during hyperoxia: behavior of oxygen in neuronal activation revisited. J Cereb Blood Flow Metab 17: S646, 1996
20. Nilsson GE: Signal processor for laser Doppler tissue flowmeters. Med Biol Eng Comput 22: 343-348, 1984
21. Shinozuka T, Nempto EM and Winter PM: Mechanisms of cerebral O_2 sensitivity from hyperoxia to moderate hypoxia in the rat. J Cereb Blood Flow Metab 9: 187-195, 1989
22. Leniger-Follert E and Hossmann KA: Simultaneous measurements of microflow and evoked potentials in the somatosensory cortex of the cat brain during specific sensory activation. Pflüegers Arch 380: 85-89, 1979
23. Goadsby PJ and Hoskin KL: Cerebral blood flow is not coupled to neuronal activity during stimulation of the facial nerve vasodilator system. Brain Res 647: 192-198, 1994
24. Schmitz B, Böttiger BW and Hossmann KA: Brief hypercapnia enhances somatosensory activation of blood flow in rat. J Cereb Blood Flow Metab 16: 1307-1311, 1996
25. Bergofsky EH and Bertun P: Response of regional circulation to hyperoxia. J Appl Physiol 21: 567-572, 1996
26. Jacobson I, Harper AM and McDowall DG: The effects of oxygen at 1 and 2 atmospheres on the blood flow and oxygen uptake of the cortex. Surg Gynecol Obstet 119: 737-742, 1964
27. Nakajima S, Meyer JS, Amano T, Shaw T, Okabe T and Mortel KF: Cerebral vasomotor responsiveness during 100% oxygen inhalation in cerebral ischemia. Arch Neurol 40: 271-276, 1983
28. Tindall GT, Wilkins RH and Odom GL: Effect of hyperbaric oxygenation on cerebral blood flow. Surg Forum 16: 414-416, 1965
29. Carrier OJ, Walker JR and Guyton AC: Role of oxygen in autoregulation of blood flow in isolated vessels. Am J Physiol 206: 951-954, 1964
30. Detar R and Bohr DF: Oxygen and vascular smooth muscle contraction. Am J

Physiol 214: 241-244, 1968

31. Tomita M, Fukuuchi Y, Tanahashi N, Kobari M, Takeda H, Yokoyama M, Ito D and Terakawa S: Contraction / dilatation of cultured vascular endothelial cells inducted by hyperoxia / hypoxia. J Cereb Blood Flow Metab 15: S271, 1995

Evoked local cerebral blood flow induced by somatosensory stimulation is proportional to the baseline flow

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要旨　様々な二酸化炭素分圧下に置かれたラット大脳皮質体性感覚野の局所血流量をレーザードップラ血流計を用いて測定した。高二酸化炭素分圧（73.4±13.3mmHg）及び低二酸化炭素分圧 （26.4±1.1mmHg)下の安静時血流量（ベースライン血流量）を正常二酸化炭素分圧（34.7±2.5mmHg）下のそれと比較した結果、それぞれ47%増加、11%減少した。また、各々の二酸化炭素分圧下で後肢を電気刺激 （1.5mA、5Hz、5 秒）し体性感覚野賦活時の局所血流量（賦活血流量）を測定した結果、二酸化炭素分圧の増加に伴った賦活血流量の増加が認められた。以上の結果は、賦活血流量がベースライン血流量に比例することを示しており、賦活血流量が代謝による酸素消費量や物質の代謝量と独立に決定されることを示唆する。

Abstract

The purpose of this study was to determine the relationship between the increase in local cerebral blood flow during neuronal activation (evoked LCBF) and the baseline flow level. We measured the hemodynamics in α-chloralose-anesthetized rats using laser-Doppler flowmetry during somatosensory stimulation under the hypocapnic, normocapnic and hypercapnic conditions. The baseline levels of LCBF and red blood cell (RBC) velocity under hypocapnia ($PaCO_2$=26.4±1.1 mmHg) were, respectively, 10 and 11% lower than those under normocapnia ($PaCO_2$=34.2±1.4 mmHg) ($p<0.01$). The evoked response magnitude of LCBF and RBC velocity under hypocapnia were, respectively, 22 and 18% lower than those under normocapnia. There was no significant difference in the baseline level and evoked response magnitude of RBC concentration. On the other hand, the baseline levels of LCBF, RBC velocity and

RBC concentration under hypercapnia ($PaCO_2$=73.4±13.3 mmHg) were, respectively, 47, 24 and 14% higher than those under normocapnia ($PaCO_2$=34.7±2.5 mmHg) ($p<0.01$). The evoked response magnitude of LCBF, RBC velocity and RBC concentration under hypercapnia were, respectively, 96, 82 and 62% greater than those under normocapnia. After normalization with respect to each baseline level, there was no significant difference in normalized evoked response magnitude of LCBF, RBC velocity and RBC concentration, either between hypocapnic and normocapnic conditions or between hypercapnic and normocapnic conditions, indicating that evoked LCBF is proportional to the baseline flow. These results suggest that the amount of evoked LCBF is not determined by the demand for metabolic substrates.

Keywords: Cerebral blood flow; Hemodynamics; Hind paw stimulation; Hypercapnia; Hypocapnia; Laser-Doppler flowmetry; Somatosensory activation.

1. Introduction

In studies of local cerebral blood flow (LCBF) with respect to neuronal activity, it is considered that the same levels of neuronal activity should yield the same degrees of evoked LCBF, because of the concept of coupling between evoked LCBF and neuronal activity (Lou et al., 1987; Villringer and Dirnagl, 1995). The regulatory mechanisms of evoked LCBF may be responsible for neurogenic control and reactions of metabolic by-products, such as potassium (Kuschinsky et al., 1972; Urbanics et al., 1978), hydrogen ions (Urbanics et al., 1978), adenosine (Ko et al., 1990) and nitric oxide (Dirnagl et al., 1993; Dirnagl et al., 1994). However, the exact mechanism underlying the coupling between neuronal activity and evoked LCBF is not fully understood, because of the highly complex mechanisms of neuronal activity and metabolism, and evoked LCBF.

A number of researches have used brain imaging techniques to demonstrate changes in LCBF related to neuronal activity. Laser-Doppler flowmetry (LDF) allows continuous and noninvasive measurements of microcirculatory blood flow in the brain, and have been used to investigate the coupling mechanisms of

neuronal activity and evoked LCBF (Ances et al., 1999; Detre et al., 1998; Lindauer et al., 1993; Matsuura et al., 1999ab; Schmitz et al., 1996). When evoked LCBF is used to estimate brain activity, we need to accumulate LDF signals in order to reduce the noise level (Ances et al., 1999; Detre et al., 1998; Matsuura et al., 1999ab). However, over a long period of LCBF measurement, the baseline level of LCBF will sometimes change with changes in physiological parameters. The most unstable parameter is $PaCO_2$ level. Carbon dioxide (CO_2) is a strong vasodilator and is involved in LCBF regulation (Lassen, 1978). It is known that the baseline flow shows 2-5% increase per mmHg $PaCO_2$ (Barfod et al., 1997; Detre et al., 1998; Kanno et al., 1997). This suggests that evoked LCBF may be affected by changes in the baseline flow which is induced by a change in the $PaCO_2$ level, in spite of a constant level of neuronal activity. Shimosegawa et al. (1995) observed by human positron emission tomography (PET) that the magnitude of evoked LCBF is proportionally correlated with the baseline flow induced by a change in the $PaCO_2$ level. In general, LDF signals, which are detected during neuronal activation, are normalized with respect to the baseline level. Thus, it is hypothesized that normalized evoked LCBF detected by LDF remains constant even with changes in the baseline level, when the level of neuronal activity is constant. However, the effects of the baseline flow on evoked LCBF using LDF have not yet been investigated. In the present study, we measured evoked LCBF induced by somatosensory stimulation and the level of baseline flow using LDF under hypocapnic, normocapnic and hypercapnic conditions, to investigate the relationship between evoked LCBF and baseline flow.

Another objective of this study was to investigate the effect of CO_2 on blood vessels and the time course of LCBF in response to somatosensory stimulation. LDF has a shorter time constant (0.03 s) and has been improved to independently monitor red blood cell (RBC) velocity and RBC concentration in the capillary bed (Barfod et al., 1997). Therefore, we can obtain the time course of evoked LCBF response by accumulating data of repeated measurements, as well as the vascular behavior of capillaries and resistance vessels (arterioles) during neuronal activation (Detre et al., 1998; Matsuura et al. 1999ab). Such data may possibly lead to a better

understanding of the principles of brain imaging measurements during neuronal activation.

2. Materials and methods

2.1. Animal preparation

All experiments were in accordance with the guidelines of the Physiological Society of Japan and were approved by the Animal Care and Use Committee of the Akita Research Institute of Brain and Blood Vessels.

Sprague-Dawley rats (350-460 g) were anesthetized with halothane (4% for induction and 1.5% during surgery) in 30% oxygen and 70% nitrous oxide. The tail artery and the left femoral vein were cannulated for blood pressure monitoring, blood gas sampling, and intravenous drug administration. Following tracheotomy, α-chloralose (75 mg/kg, i.v.) was administered, and halothane and nitrous oxide administrations were discontinued. The rat was immobilized with pancuronium bromide (0.7 mg/kg, i.v.), and ventilated with a respirator (SN-480-7, Shinano, Japan) throughout the experimental period. Anesthesia was maintained with α-chloralose (45 mg/kg/hr, i.v.), and muscle relaxation, with pancuronium bromide (0.8 mg/kg/hr/, i.v.). The body temperature was maintained at about 37.0 °C using a heating pad (ATC-101, Unique Medical, Japan). The rat was fixed in a stereotactic frame, and the parietal bone was thinned to translucency at the left somatosensory cortex, over an area of 3×3 mm, centered at 2.5 mm caudal and 2.5 mm lateral to the bregma. Under the normocapnic condition, $PaCO_2$ levels were maintained in the range of 32-40 mmHg, and PaO_2 levels in the range of 90-120 mmHg by regulating the stroke volume of ventilation and the fractional concentration of oxygen in the gas inspired, respectively.

2.2. LDF measurement and stimulation procedures

LCBF was measured with an LDF (Periflux 4001 Master, Perimed, Sweden) equipped with an LDF probe with a tip diameter of 0.46 mm (Probe 411, Perimed, Sweden). Our LDF system can simultaneously measure three parameters; flux (= LCBF), RBC velocity and RBC concentration, where RBC velocity = LCBF /

RBC concentration (Nilsson, 1984). The area of LDF measurement was about 1 mm^3 (Nilsson et al., 1980). A time constant of 0.03 s was used for measuring all LDF signals (LCBF, RBC velocity and RBC concentration). The LDF probe was placed in the somatosensory area of the hind paw, perpendicular to the brain surface. It was attached to the thinned parietal bone, avoiding areas with large blood vessels. There was no effect of skull and dura thickness on LDF measurement, because no significant difference in LCBF response obtained through a thinned skull and that with the dura removed has been confirmed (Lindauer et al., 1993). To ensure a stable condition of the animal, measurements were performed 2-3 hr after the preparation of the parietal bone.

In the present study, two different paradigms were used. In *study 1*, LCBF, $PaCO_2$, heart rate and arterial blood pressure of rats at rest were successively measured after 2.5% CO_2 inhalation (n=10). This paradigm was to investigate the effect of hypercapnia on the baseline LCBF and other physiological parameters. In *study 2*, LCBF, RBC velocity and RBC concentration during somatosensory stimulation of rats were measured by LDF under the three different conditions of hypocapnia, normocapnia and hypercapnia.

In *study 2*, the activation of the cortex was carried out by electrical pulse stimulation of the hind paw (pulse width 0.1 ms), using a pair of small needle electrodes inserted under the skin of the right hind paw. Electrical stimuli of 5 Hz and 5 s duration with 1.5 mA intensity were administered. The stimulus intensity did not cause any change in the systemic arterial blood pressure during stimulation (Matsuura et al., 1999a, 2000). Fifty successive stimuli were applied at 60-s intervals. This interval is sufficient to avoid habituation of neuronal activity and vessel behavior (Ances et al., 2000). For experiments under the hypocapnic condition, ten rats were used. We first examined LCBF under normocapnia, followed by that under hypocapnia after 20 min of hyperventilation (Fig. 1). For

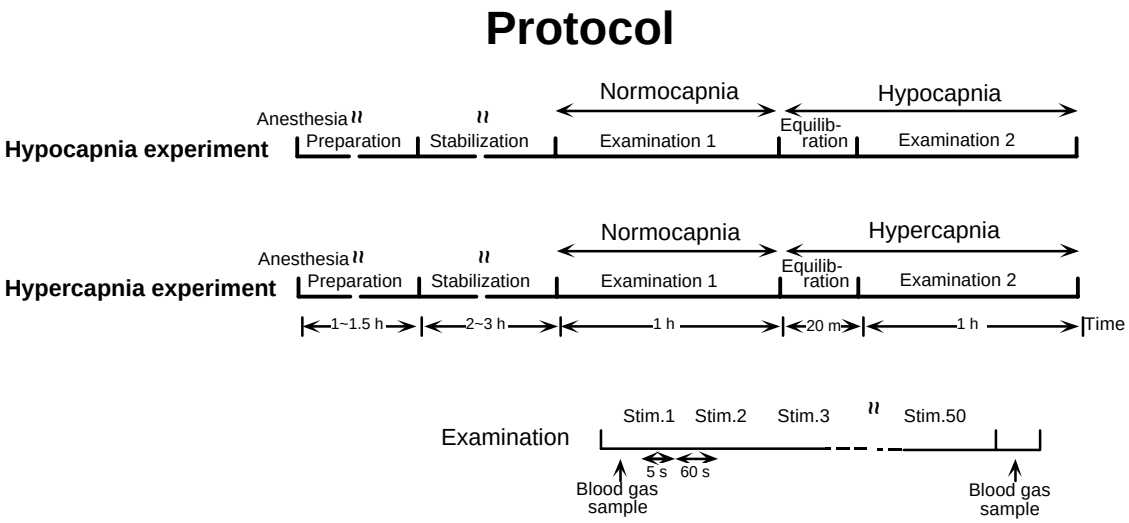

Fig. 1. Protocol of hypocapnia and hypercapnia experiments (*Study 2*). Examination was carried out about 2 ~3 hr after preparation of parietal bones. In both experiments, LCBF responses under the normocapnic condition were initially examined, and then those under the hypocapnic or hypercapnic condition, after a 20-min change in the respiratory condition. During each examination, 50 successive stimuli of 5 Hz and 5 s duration were applied at 60-s intervals, and outputs of 50 successive measurements were accumulated using the MacLab data acquisition software.

experiments under the hypercapnic condition, another set of ten rats was used. We also first examined LCBF under normocapnia, followed by that under hypercapnia after 20 min of hypercapnic ventilation (Fig. 1). For hypercapnic ventilation, approximately 2.5% CO_2 was mixed with the gas administered under the normal gas condition. An equilibration time of 20 min was observed to be the time at which the baseline LCBF reached a peak value after 2.5% CO_2 inhalation (see results of *study 1*). Arterial blood pressure was monitored during the experiments and the mean arterial blood pressure (MABP) was calculated as the average at three time points (i.e., before, during and immediately after each examination). Arterial blood was sampled for blood gas analysis before and immediately after each examination (Fig. 1).

2.3. Data analyses

The LDF signals and arterial blood pressure in *study 2* were recorded continuously using the MacLab data acquisition software (AD Instruments, Australia), and outputs of 50 successive measurements were accumulated in order to reduce the noise level of LDF signals. Data were digitized at 40 Hz and saved on a disk for off-line analyses. The rise time of the evoked LCBF was determined as the

time at the intersection of the baseline and the extrapolated line, which was drawn on the response curve from 90 to 10% of the peak. The termination time was also determined as the time at the intersection of the baseline and a similar extrapolated line (detail in Matsuura et al., 1999a). The peak time was the time at which the response curve of LCBF reached the maximum height. The response magnitude was calculated as the integral above the baseline level of the response curve from the rise time to the termination time. The physiological data and time parameters of evoked LCBF obtained at the first (normocapnic condition) and second examinations (hypercapnic and hypocapnic conditions) were statistically analyzed using the *t*-test. For statistical analysis of the baseline levels and response magnitude, we also applied the paired *t*-test on the raw LDF data. We then normalized the values of the baseline levels and response magnitude with respect to those of the normocapnic condition. In this paper, we showed the normalized response curve and normalized response magnitude. The normalized response curves of LDF signals, which indicate time courses of percent change from baseline level, were calculated by dividing raw data by each baseline values (normalization with respect to the baseline level). The normalized response magnitude is the integral of the normalized response curve from the rise time to the termination time. Values are presented as means±SD.

3. Results

3.1. Effects of CO_2 on LCBF and physiological parameters

Hypercapnia, which was induced by adding 2.5% CO_2, increased the $PaCO_2$ level and LCBF with a slight decrease in MABP and heart rate (Fig. 2). LCBF reached a peak value after about 20 min of hypercapnic ventilation and then continued to gradually decline during hypercapnia, while the $PaCO_2$ level continued to increase during hypercapnic ventilation (Fig. 2). After 20 min, the $PaCO_2$ level had increased to 63.0±4.2 from 35.4±2.0 mmHg and LCBF had increased to 85.3±27.4%, corresponding to an increase of 3.1±1.7 %/mmHg (Table 1). However, after 70 min, the $PaCO_2$ level had increased to 75.1±5.4 mmHg, and LCBF increased to 53.1±18.4% relative to that before CO_2 inhalation, corresponding to an increase of 1.3±0.5 %/mmHg (Table 1).

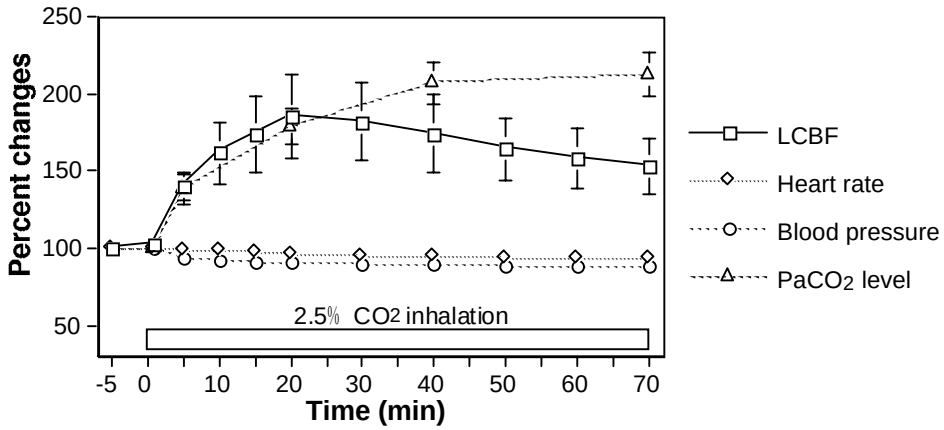

Fig. 2. Time course of changes in LCBF, $PaCO_2$ level, heart rate and MABP in response to 2.5% CO_2 inhalation; values of the parameters obtained before CO_2 inhalation (normocapnia) were considered as 100%. Note that LCBF reached a peak value after about 20 min of CO_2 inhalation and then declined during hypercapnia, while the $PaCO_2$ level continued to increase with a slight decrease in heart rate and MABP. Error bars are ±SD (n=10).

Table 1. *Changes in LCBF and $PaCO_2$ level after 2.5% CO_2 inhalation compared with those before CO_2 inhalation*

	Normocapnia	after 5 min	after 20 min	after 40 min	after 70 min
% increase in LCBF	-	40.0±9.3	85.3±27.4	74.1±25.6	53.1±18.4
$PaCO_2$ (mm Hg)	35.4±2.0	48.7±3.2	63.0±4.2	72.9±4.5	75.1±5.4
%/mm Hg	-	3.5±1.7	3.1±0.9	2.0±0.6	1.3±0.5

Mean±SD (n=10).

3.2. Physiological changes under hypocapnia and hypercapnia

In the hypocapnia and hypercapnia experiments, physiological variables were within the normal range throughout the experiments, except for the $PaCO_2$ level under the hypocapnic and hypercapnic conditions. As shown in Table 2, MABP under the hypercapnic condition was lower than that under the normocapnic condition, although it was stable under the hypocapnic condition. There was no systemic change in the arterial blood pressure caused by electrical stimulation of the hind paw (Matsuura et al. 1999a; Matsuura et al. 2000). On the other hand, the PaO_2 level under the hypocapnic condition was slightly higher than that under the normocapnic condition ($p<0.01$) due to hyperventilation under the hypocapnic

condition. There was no significant change in PaO_2 levels under normocapnic and hypercapnic conditions.

3.3. Changes in hemodynamics under hypocapnic and hypercapnic conditions

In the hypocapnia experiment, the baseline levels of LCBF and RBC velocity under the hypocapnic condition were, respectively, 10.0±0.04 and 10.8±0.03% lower than those under the normocapnic condition ($p<0.01$), while that of RBC concentration remained constant (Fig. 3). This indicates that the "on-off recruitment" of RBCs is not the underlying mechanism of decreased LCBF under hypocapnia. The response magnitude of LCBF and RBC velocity under the hypocapnic condition were, respectively, 21.8±14.9 and 17.9±13.7% lower than those under the normocapnic condition ($p<0.05$), while there was no significant difference in the response magnitude of RBC concentration between the hypocapnic and normocapnic conditions (Fig. 3). On the other hand, the

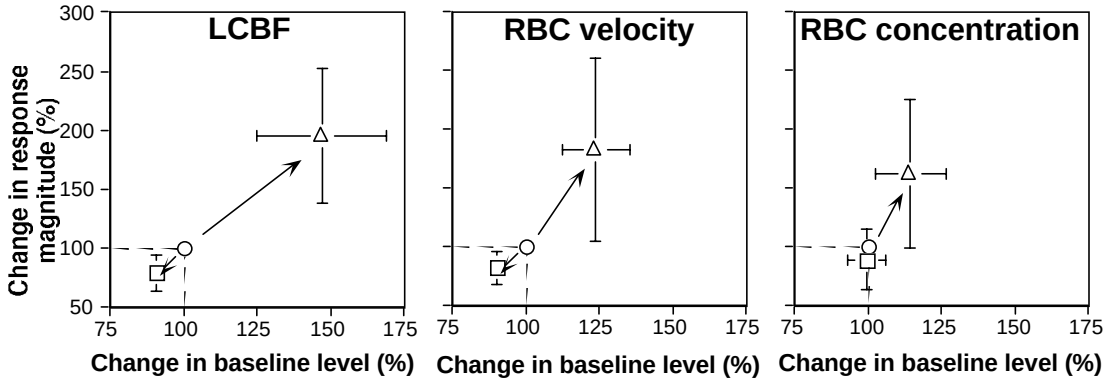

Fig. 3. Changes in baseline levels and response magnitudes of LCBF, RBC concentration and RBC velocity; values of the parameters obtained under the normocapnic condition were considered as 100%. Note that the response magnitude of LCBF, RBC velocity and RBC concentration changed proportionally with baseline levels. Data were normalized with respect to those of the normocapnic condition after statistical analysis. Error bars indicate ±SD. □, hypocapnia (n=10); ○, normocapnia; and △, hypercapnia (n=10).

concentration between the hypocapnic and normocapnic conditions (Fig. 3). On the other hand, the baseline levels of LCBF, RBC velocity and RBC concentration under the hypercapnic condition were, respectively, 47.0±22.0, 23.9±11.5 and 14.4±11.8% higher than those under the normocapnic condition ($p<0.05$, Fig. 3). The response magnitude of LCBF and RBC velocity under the hypercapnic condition were,

respectively, 95.5±57.4, 82.3±78.2% greater than those under the normocapnic condition ($p<0.05$, Fig. 3). The response magnitude of RBC concentration under the hypercapnic condition was 61.7±63.3% greater than that under the normocapnic condition (Fig. 3), although this difference did not reach statistical significance.

However, after normalization for each baseline level, there was no significant difference in normalized response magnitude of evoked LCBF, RBC velocity and RBC concentration either between the hypocapnic and normocapnic conditions or between the hypercapnic and normocapnic conditions (Figs. 4 and 5). These indicate that the evoked response magnitude of LCBF, RBC velocity and RBC concentration induced by neuronal activation proportionally changed with the baseline level of hemodynamics.

Table 2. *Physiological data during examination under each blood gas condition*

Blood gas condition	MABP (mmHg)	$PaCO_2$ (mmHg)	PaO_2 (mmHg)
Hypocapnia experiment (n=10)			
Normocapnia	107.7±12.2	34.2±1.4	106.5±6.3
Hypocapnia	110.4±8.9	26.4±1.1*	114.7±7.9*
Hypercapnia experiment (n=10)			
Normocapnia	100.3±12.5	34.7±2.5	109.8±11.2
Hypercapnia	89.3±13.1*	73.4±13.3*	114.8±14.4

There were significant differences between hypocapnic and normocapnic conditions, and between hypercapnic and normocapnic conditions (*$p<0.01$). Mean±SD.

3.4. Time course of evoked LCBF under hypocapnia and hypercapnia

Under the normocapnic condition, LCBF rose approximately 0.5 s after the onset of stimulation, peaked before termination of stimulation and returned to the baseline (prestimulation) level within 7-10 s after the onset of stimulation (Fig. 4 and Table 3). Under the hypocapnic condition, there was no significant difference in the rise time, peak time and termination time compared with those

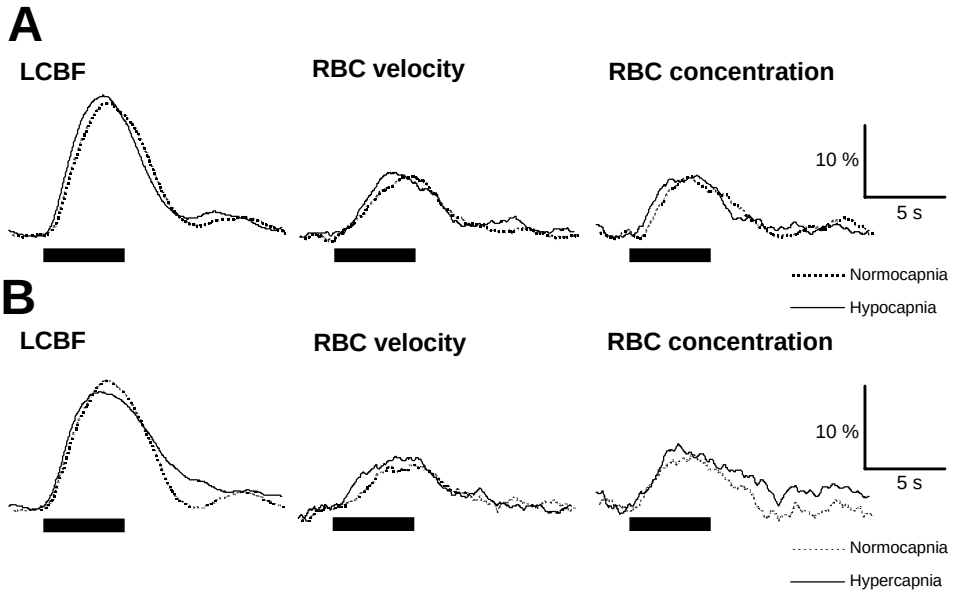

Fig. 4. Normalized response curve of LCBF, RBC velocity and RBC concentration evoked by stimulation. (**A**) Time course of hemodynamic response under the normocapnic (dotted line) and hypocapnic (solid line) conditions (n=10). (**B**) Time course of hemodynamic response under the normocapnic (dotted line) and hypocapnic (solid line) conditions (n=10). Data were normalized with respect to each baseline level and averaged by the number of animals used. Horizontal and vertical bars indicate stimulation period and 10% increase with respect to the baseline level, respectively.

under the normocapnic condition. Under the hypercapnic condition, there was no significant difference in rise time and peak time compared with those under the normocapnic condition. Termination time under the hypercapnic condition came later than that under the normocapnic condition ($p<0.01$), i.e., 14.06±2.97 s and 9.50±2.20 s, respectively (Table 3). These results indicate that the onset of the LCBF in response to stimulation is independent of the baseline flow level.

Table 3. *Time parameters in LCBF response curve*

Blood gas condition	Rise time (s)	Peak time (s)	Termination time (s)
Hypocapnia experiment (n=10)			
Normocapnia	0.52±0.06	3.67±0.35	9.07±0.99
Hypocapnia	0.52±0.08	3.65±0.57	8.52±1.37
Hypercapnia experiment (n=10)			

Normocapnia	0.53±0.22	3.94±0.63	9.50±2.20
Hypercapnia	0.54±0.18	3.88±0.62	14.06±2.97*

There was a significant difference between hypercapnic and normocapnic conditions (*p<0.01). Mean±SD.

4. Discussion

4.1. Changes in baseline level under hypocapnia and hypercapnia at rest

In *study 2*, percentage changes in the baseline LCBF levels relative to the change in $PaCO_2$ levels (1.4 %/mmHg during hypocapnia and 1.2 %/mmHg during hypercapnia) were lower than previously reported data (Barfod et al., 1997; Detre et al., 1998; Kanno et al., 1997). Most previous studies were performed within 20 min after the onset of hypercapnia; in this study, the baseline levels

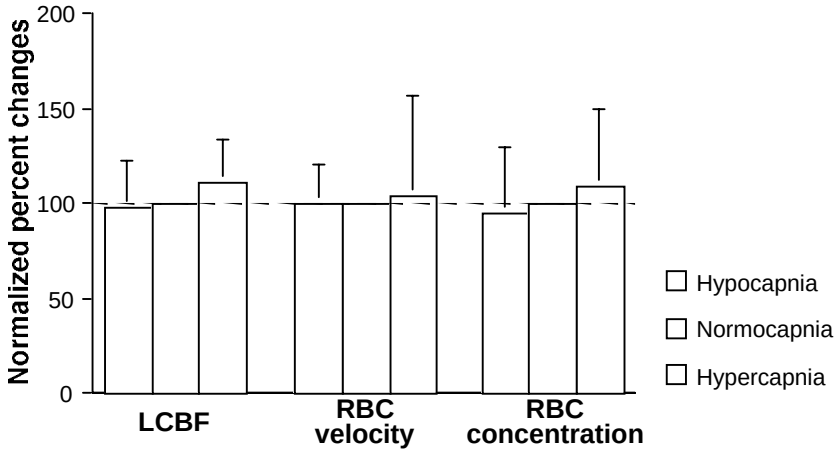

Fig. 5. Comparison of normalized response magnitudes of LCBF, RBC concentration and RBC velocity; values of the parameters obtained under the normocapnic condition were considered as 100%. Note that after normalization with respect to each baseline level, there was no significant difference in normalized response magnitude of LCBF, RBC velocity and RBC concentration either between the hypocapnic and normocapnic conditions or between the hypercapnic and normocapnic conditions, suggesting that LDF signals normalized by the baseline level reflect neuronal activity even with a change in the baseline flow. Data were normalized with respect to those of the normocapnic condition after statistical analysis. Error bars indicate SD.

of LCBF and $PaCO_2$ were averaged values obtained from 20 to 70 min after the onset of hypercapnia. In *study 1*, we confirmed the relationship between changes in LCBF and $PaCO_2$ levels after 2.5% CO_2 inhalation (Fig. 2). LCBF reached a peak value after about 20 min, while the $PaCO_2$ level continued to increase during hypercapnic ventilation (Fig. 2). Therefore, the percent change in the baseline level of LCBF relative to the change in $PaCO_2$ level became smaller with time after CO_2

inhalation (Table 1).

The patterns of changes in the baseline levels of RBC velocity and RBC concentration under resting condition were different (Fig. 3). The baseline level of RBC velocity increased with a rise in $PaCO_2$ level. The baseline level of RBC concentration under the hypercapnic condition was higher than that under the normocapnic condition, although there was no significant difference between hypocapnic and normocapnic conditions. There are many reports which investigate the relationship between RBC velocity and RBC concentration after CO_2 inhalation. Most investigators observed an increase in RBC concentration in addition to increase in RBC velocity during hypercapnia (e.g. Barfod et al., 1997; Villringer et al., 1994). However, Detre et al. found a decrease in RBC concentration under the hypercapnic condition (Detre et al., 1998). Interpretation of this discrepancy is made difficult by the use of anesthetic agents, the extent of anesthesia, and animal conditions.

An increase in RBC concentration under the hypercapnic condition indicates an increase in capillary volume under hypercapnia. Capillaries had been considered as rigid tubes. However, a moderate degree of distension of capillaries was observed by microscopy of histological sections of rats subjected to hypercapnia as compared to those subjected to hypocapnia (Duelli and Kuschinsky, 1993), and an increase in capillary diameter was detected by confocal laser scanning microscopy (Villringer et al., 1994). The observed changes in capillary volume under hypercapnia at rest are most likely due to passive distention due to a decrease in resistance upstream. The larger change in RBC velocity than that in RBC concentration also agrees with the change in perfusion level in the capillary bed. The CO_2 reactivity is mediated by pH variations around the resistance vessel, and the pH variations influence the tone of the vascular smooth muscle (Lassen, 1978). Nitric oxide and adenosine, vasodilators, also play a role in this reactivity (Estevez and Phillis, 1997; Wang et al., 1992). These suggest that a change in $PaCO_2$ level causes a change in the production of these mediators during resting condition, which are associated with the dilation of resistance vessels, causing a change in precapillary pressure.

4.2. Evoked LCBF is proportional to baseline flow

The relationship between evoked LCBF and baseline flow has been described previously. In human PET study, Kanno et al. (1997) and Shimosegawa et al. (1995) reported that the absolute evoked LCBF positively correlated with the baseline level of LCBF, which was consistent with our observation (Fig. 3). In the present study, we also showed that after normalization with respect to each baseline level, normalized response magnitudes of the evoked LCBF under the hypocapnic and hypercapnic conditions were not significantly different from those under the normocapnic condition (Figs. 4 and 5). It has been confirmed that the recordings of field potentials under the hypercapnic and normocapnic conditions were not significantly changed throughout the experimental period (Matsuura et al., 2000). These results suggest that evoked LCBF estimated by LDF, which was normalized with respect to the baseline level, reflects neuronal activity in the somatosensory cortex, even if the baseline level of LCBF is changed by the $PaCO_2$ level.

The change in perfusion level in the capillary bed induced by $PaCO_2$ during resting condition affects a change in evoked LCBF. Maximilian et al. (1980) investigated the evoked CBF during normocapnia and hypercapnia, and described that hypercapnic and metabolic flow increases do not interact but are additive. In the present study, we showed that the evoked response magnitude of RBC velocity is proportional to the baseline level (Fig. 3), indicating that the precapillary pressure during neuronal activation increased above the resting perfusion pressure of the capillary bed. However, during neuronal activation, the regulatory mechanism of evoked LCBF is a highly complex process. The normalized response magnitude of RBC concentration is the same as that of RBC velocity (Fig. 5), although the change at baseline level of RBC concentration is smaller than that of RBC velocity (Fig. 3). These results suggest that the process of evoked LCBF regulation is different from that of controlling the baseline LCBF as induced by CO_2. The evoked LCBF may be regulated not only by changes in resistance vessels, but also by a change in capillary volume (Matsuura et al., 1999ab), and changes in these vessels may affect the proportional change in evoked LCBF with respect to baseline flow.

The termination time of evoked LCBF under the hypercapnic condition was delayed as compared to that under the normocapnic condition (Table 3 and Fig 4). During neuronal activation, the concentrations of hydrogen ions and adenosine increase after the onset of LCBF response, and remain elevated throughout the activation (Ko et al., 1990; Urbanics et al., 1978). Contribution of nitric oxide and adenosine to vasodilation during hypercapnia was also reported (Estevez and Phillis, 1997). Our observations suggest that addition of CO_2 affects these mediators during neuronal activation, which are associated with regulation of vascular behavior at the return phase of LCBF to the baseline level.

4.3. Evoked LCBF is independent of demand for delivery of metabolic substrates

The brain has a high rate of aerobic metabolism. During neuronal activation, the brain tissue exhibits an increased demand for oxygen and other substrates (e.g., glucose), which serve as an energy source of neuronal spiking. Change in $PaCO_2$ levels should alter the oxygen affinity of hemoglobin (Bohr effect). Actually, the level of oxygen saturation in arterial blood increased under the hypocapnic condition and decreased under the hypercapnic condition compared to that under the normocapnic condition (data not shown). This supposes that proportional change in evoked LCBF is coursed by a change in oxygen delivery of hemoglobin at rest. However, in our previous study, we found that the evoked LCBF is independent of metabolic oxygen demand (Matsuura et al., 2000).

The evoked LCBF also plays a role in supplying other substrates for metabolism. If the response of evoked LCBF is determined by the supply of other substrates for energy metabolism, evoked LCBF do not have to increase proportionally to the baseline level of LCBF, because an increase in the baseline LCBF indicates an increase in the level of many substrates under resting condition. The results in the present study suggest that evoked LCBF is not determined by the demand for the delivery of metabolic substrates, and it is a basic response of the blood vessels to neuronal activation. It has been reported that the function of activation-dependent coupling serves to remove the lactate produced as a result of glycolysis (Villringer and Dirnagl, 1995). This is one possibility, the coupling

between evoked LCBF and neuronal activity. However, further investigations are required to elucidate this mechanism.

Acknowledgement

We gratefully acknowledge Dr. Yuko Miura for helpful discussions and Mr. Yozo Ito of the Akita Research Institute of Brain and Blood Vessels for technical assistance.

References

Ances, B.M., Greenberg, J.H., Detre, J.A., 1999. Activation-flow coupling with forepaw stimulation in female and male rats. Neurosci. Res. 35, 37-41.

Ances, B.M., Greenberg, J.H., Detre, J.A., 2000. Effects of variations in interstimulus interval on activation-flow coupling response and somatosensory evoked potentials with forepaw stimulation in the rat. J. Cereb. Blood Flow Metab. 20, 290-297.

Barfod, C., Akgöen, N., Fabricius, M., Dirnagl, U., Lauritzen, M., 1997. Laser-Doppler measurements of concentration and velocity of moving blood cells in rat cerebral circulation. Acta. Physiol. Scand. 160, 123-132.

Detre, J.A., Ances, B.M., Takahashi, K., Greenberg, J.H., 1998. Signal averaged laser Doppler measurements of activation-flow coupling in the rat forepaw somatosensory cortex. Brain Res. 796, 91-98.

Dirnagl, U., Lindauer, U., Villringer, A., 1993. Role of nitric oxide in the coupling of cerebral blood flow to neuronal activation in rats. Neurosci. Lett. 149, 43-46.

Dirnagl, U., Niwa, K., Lindauer, U., Villringer, A., 1994. Coupling of cerebral blood flow to neuronal activation: role of adenosine and nitric oxide. Am. J. Physiol. 267, H296-H301.

Duelli, R., Kuschinsky, W., 1993. Changes in brain capillary diameter during hypocapnia and hypercapnia. J. Cereb. Blood Flow Metab. 13, 1025-1028.

Estevez, A.Y., Phillis, J.W., 1997. Hypercapnia-induced increases in cerebral blood flow: roles of adenosine, nitric oxide and cortical arousal. Brain Res. 758, 1-8.

Kanno, I., Shimosegawa, E., Fujita, H., Hatazawa, J., 1997. Uncoupling of absolute

CBF to neuronal activity. Adv. Exp. Med. Biol. 413, 209-214.

Ko, K.R., Ngai, A.C., Winn, H.R., 1990. Role of adenosine in regulation of regional cerebral blood flow in sensory cortex. Am. J. Physiol. 259, H1703-H1708.

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.

Lassen, N.A., 1978. Intrinsic regulation of blood flow in the brain. In: Johnson PC (Ed) Peripheral circulation, John Wiley and Sons Inc, New York, pp. 337-358.

Lindauer, U., Villringer, A., Dirnagl, U., 1993. Characterization of CBF response to somatosensory stimulation: model and influence of anesthetics. Am. J. Physiol . 264, H1223-H1228.

Lou, H.C., Edvinsson, L., MacKenzie, E.T., 1987. The concept of coupling blood flow to brain function: revision required? Ann. Neurol. 22, 289-297.

Matsuura, T., Fujita, H., Seki, C., Kashikura, K., Yamada, K., Kanno, I., 1999a. CBF change evoked by somatosensory activation measured by Laser-Doppler flowmetry: independent evaluation of RBC velocity and RBC concentration. Jpn. J. Physiol. 49, 289-296.

Matsuura, T., Fujita, H., Seki, C., Kashikura, K., Kanno, I., 1999b. Hemodynamics evoked by microelectrical direct stimulation in rat somatosensory cortex. Comp. Biochem. Physiol. A. 124, 47-52.

Matsuura, T., Fujita, H., Kashikura, K., Kanno, I., 2000. Modulation of evoked cerebral blood flow under excessive blood supply and hyperoxic conditions. Jpn. J. Physiol. 50, 115-123.

Maximilian, V.A., Prohovnik, I., Risberg, J., 1980. Cerebral hemodynamic response to mental activation in normo- and hypercapnia. Stroke 11, 342-347.

Nilsson, G.E., 1984. Signal processor for laser Doppler tissue flowmeters. Med. and Biol. Eng. and Comput. 22, 343-348.

Nilsson, G.E., Tenland, T., Öberg, P.Å., 1980. Evaluation of a laser Doppler flowmeter for measurement of tissue blood flow. IEEE Trans. Biomed. Eng. BME-27, 597-604.

Schmitz, B., Böttiger, B.W., Hossmann, K.A., 1996. Brief hypercapnia enhances somatosensory activation of blood flow in rat. J. Cereb. Blood Flow Metab. 16,

1307-1311.

Shimosegawa, E., Kanno, I., Hatazawa, J., Fujita, H., Iida, H., Miura, S., Murakami, M., Inugami, A., Ogawa, T., Itoh, H., Okudera, T., Uemura, K., 1995. Photic stimulation study of changing the arterial partial pressure level of carbon dioxide. J. Cereb. Blood Flow Metab. 15, 111-114.

Urbanics, R., Leniger-Follert, E., Lübbers, D.W., 1978. Time course of changes of extracellular H^+ and K^+ activities during and after direct electrical stimulation of the brain cortex. Pflügers Arch. 378, 47-53.

Villringer, A., Dirnagl, U., 1995. Coupling of brain activation and cerebral blood flow: basis of functional neuroimaging. Cerebrovasc. Brain Metab. Rev. 7, 240-276.

Villringer, A., Them, A., Lindauer, U., Einhaupl, K., Dirnagl, U., 1994. Capillary perfusion of the rat brain cortex. An *in vivo* confocal microscopy study. Circ. Res. 75, 55-62.

Wang, Q., Paulson, O.B., Lassen, N.A., 1992. Effect of nitric oxide blockade by N^G-nitro-L-arginine on cerebral blood flow response to changes in carbon dioxide tension. J. Cereb. Blood Flow Metab. 12, 947-953.