Cerebral Blood Flow Regulation

II. Vasodilator Mechanisms

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SUMMARY Rapid vascular resistance adjustments in the brain and in the circle of Willis have been continuously measured and enhanced by signal averaging methods. Nafldofuril, a drug chemically similar to local anesthetics and to beta adrenergic blocking agents, increases local CBF by reducing resistance in brain and in extracerebral supply arteries. It has also been reported to affect brain metabolism. Other similar drugs merit study for potential effects on CBF and brain metabolism which may be useful in treatment.

CALCULATIONS OF CEREBRAL vascular resistance have been used as an index of cerebral blood flow (CBF) regulation. However, many of the methods for measuring CBF cannot be used to detect rapid flow changes, and so cannot be used to recognize vascular responses occurring within a short time interval. Continuous measures of CBF, with simultaneous determinations of perfusion pressure, are required to monitor resistance changes which occur within 2-5 seconds or serially over an interval of 1-2 minutes. A continuous verifiable measure of local CBF is possible by a thermal diffusion method. From these measures, calculations of resistances depict an uninterrupted sequence of change in the extracerebral and cerebral arteries. The responses to CO₂, epinephrine, norepinephrine, and bilateral common carotid artery occlusion in this two-compartment system have been previously described. Studies of this type may be helpful in identifying the interaction of control mechanisms as they affect supply arteries, collateral arteries and the cerebral vessels.

Nafldofuril, a drug structurally similar to local anesthetics and beta adrenergic blocking agents, has been reported to increase CBF and cerebral metabolism. In addition to its local anesthetic properties, it causes bradycardia and peripheral vasodilation. The present studies were undertaken to identify its site of action on cerebral circulation. The results are pertinent to further understanding of the relationship between the neural, myogenic and metabolic components of CBF regulation.

Methods

All experiments utilized adult mongrel cats and were carried out conforming to the "Guiding Principles in the Care and Use of Animals," approved by the Council of the American Physiological Society.

Local CBF is recorded by a modification of Gibbs' method using a heated thermocouple probe which allows direct recording of thermal conductivity on a linear scale. The system is based on continuously recording the electrical power required to maintain a chosen temperature difference between a heated needle probe and an unheated reference probe. The chosen temperature difference is automatically and continuously maintained by a servo controller which adjusts the power input into a small heater winding on the probe. The difference between the thermal conductivity value of dead brain as compared to living brain represents the heat removed by blood flow. There is a direct relationship between flow and the square of the heating current. Since the flow measurements are affected by heat exchange between brain and environment as well as between brain and blood, an error in flow calculations may be introduced if temperature gradients are allowed to develop independent of blood flow. This potential error is caused by a difference of temperature in the environment of the reference junction as compared to the environment of the heated junction. The magnitude of this error can be measured as discussed in our previous publication. During each experiment, precautions are maintained to detect this source of error and to avoid it. Under these conditions the square of the heating current is a linear measure of blood flow. At the termination of the experimental procedure, after sacrifice, the current required to maintain the selected temperature gradient establishes an absolute zero flow reference for all other flow measures in the experiment. Results from one test animal to another are totally reproducible.

The calculations of resistances are based on measures of local CBF, systemic blood pressure (BP) and cerebral perfusion pressure (CPP), measured as retrograde lingual artery wedge pressure. During occlusion of both common carotid arteries, proximal to branching, all cephalic flow is directed through the vertebral system. The occipital artery does communicate with cephalic branches of the carotid artery. However, our measurements of lingual artery pressure during bilateral common carotid artery occlusion, are not altered by clamping or releasing the occipital branch of the vertebral artery. The primary functional communication between the vertebral arteries, the carotid arteries, and therefore, the lingual artery is the circle of Willis. Since the resistance values are based on calculations derived from pressure and flow measurements in linear, arbitrary units, they have no absolute numerical value, but reflect proportional changes in brain vascular resistance (Ric) and collateral vascular resistance (Rec). Ric is derived from local caudate flow and the cerebral perfusion pressure. It parallels brain vascular resistance only as well as the flow in the caudate parallels total hemispheric flow. Rec 1, in the preclosure interval, is derived from a measure of pressure drop between aorta and lingual artery. It therefore represents resistance in the cervical segment of the carotid artery. Rec 2, derived from the flow and pressure measurements during BCO, when all flow is by way of the vertebral system, represents vascular resistance in the circle of Willis and the vertebral arteries.
Animals were initially anesthetized with 30 mg/kg of pentobarbital given intra-peritoneally, with additional 10 mg/kg intravenous (IV) doses given as needed to maintain light anesthesia. Statham resistance bridge pressure transducers were used to monitor systemic blood pressure (BP) by a catheter threaded into the descending aorta via the femoral artery, and cerebral perfusion pressure (CPP), by a catheter threaded retrograde through the lingual artery adjacent to the distal common carotid artery. Both common carotid arteries were exposed and dissected free by blunt dissection. A hydraulic blood vessel occluder was then placed around each artery proximal to branching. A tracheostomy was performed to allow measurement of expired CO2 with a Beckman infra-red gas analyzer. Thermal diffusion blood flow probes were stereotactically placed into left and right caudate nuclei with their respective reference probes placed into the ipsilateral thalamus. A thermocouple with ice water reference was attached to each thalamus reference probe to monitor absolute brain temperature. Heart rate was measured from the BP pulses. All parameters were recorded on a Grass Model 7B oscillograph with simultaneous recording on an FM tape recorder.

Each animal was sacrificed at the end of the experiment to obtain zero flow and calibration measures for the CBF probes and the pressure transducers. The brain was fixed in situ with intra-arterial formalin for histological verification of probe locations.

The effects of naftidrofuryl were determined by comparing the responses to six bilateral carotid occlusion (BCO) challenges with and without drug administration in each animal. During drug treatment, an initial loading dose of 2.0 mg/kg IV naftidrofuryl was given followed by continuous IV infusion of 0.5 mg/kg/min. Total infusion of fluid was less than 10 cc in each animal over the entire experiment. For each animal to serve as its own control, two conditions were chosen, drug treatment (Condition A) preceded the control period (Condition B). In the other five animals (BA), the sequence was reversed. Fifteen minutes were allowed after the initial loading dose and the beginning of infusion for the drug to reach maximum effect before the BCOs were begun. In those sessions in which the drug administration occurred first, 30 minutes elapsed after cessation of infusion before repeating the procedure, to allow time for the drug effects to fade.

For each animal to serve as its own control, two constraints must be met. First, the pharmacologic agent must have a rapid onset of effect to prevent delay between B and A conditions in the BA animals. Second, the agent must have a rapid decay time to prevent drug effects from carrying over in the AB animals. Pilot studies indicated that naftidrofuryl has a rapid onset of action on CBF and its effect approaches a maximum in approximately 15 minutes. However, the drug action was found to decay slowly so that even 30 minutes after cessation of infusion, there was some effect remaining. The persistence of the drug effect in the AB animals tends to obscure differences between A and B conditions. Therefore, the comparison of both A groups with both B groups yields a cautious estimate of the pharmacologic properties of the drug.

Data were collected using the following format:

A. One minute pre-occlusion baseline.
B. One minute BCO challenge.
C. Three minutes post-occlusion recovery time.

Each of these five minute periods is referred to as an epoch.

Off-line analysis was carried out as follows: Each data channel was digitized at the rate of 51 points per minute, for a total of 255 points per channel per epoch. The resistances, Ric, Rec and Rtot, total cerebrovascular resistance, were calculated point by point, across time, as follows: Ric = CPP/CBF; Rec = (BP - CPP)/(CBF + (Ext. carotid flow)); Rtot = Ric + Rec.

Signal averaging techniques were used to enhance the resolution of the responses to the BCOs. For each of 10 animals, the data from each channel were separately averaged across the six treated and six untreated epochs. The averages of six trials for each parameter in each of the ten animals were averaged across all animals. In this way, a single average representing 60 trials was obtained for each parameter during condition A and B. Analysis revealed that CBF and temperature measures from the left and right hemispheres did not differ significantly; therefore, these bilateral measures and the derived resistance measures were combined for all subsequent analyses. The CBF tracings and derived resistances therefore represent the average of 120 trials each of A and B epochs.

Differences between untreated and treated intervals were evaluated for each parameter using point-by-point matched pair t-tests, using p ≤ .001 as the two-tail criterion level of significance; therefore 255 t-tests were calculated for each parameter. Histograms were constructed to indicate points in the epochs yielding significant differences, with the direction of histogram deflection corresponding to the direction of mean difference.

In applying the criterion cut-off to each of the 255 matched-pair t-tests between treated and untreated epochs, no correction was introduced to compensate for the fact that a large number of comparisons were being made and that successive points within each epoch were highly correlated. These tests were not applied with the idea that each of the 255 sample points in each epoch represented independent events, but rather, in an attempt to define broad regions of time where differences in response occurred between treated and untreated conditions. If the same statistical procedures compared only untreated epochs, very few, if any, significant differences would be obtained using our chosen criterion level. If the 255 samples in each epoch were, in fact, independent, probability theory would yield an expectation that only one in 1000 comparisons would attain significance under these conditions due to chance factors. This statistical technique has previously been applied to the analysis of cerebral evoked responses. Comparison of treated and untreated epochs, on the other hand, resulted in a large number of significant differences, but these tended to cluster into three major time regions: the periods of time before and after occlusion, the early portion of the occlusion period, and the late portion of the occlusion period. Thus, rather than being concerned with 255 separate tests of statistical significance, the t-tests empirically factored out three major time regions

*The resistance calculations for Rec do not include external carotid flow since it represents a small proportion of total common carotid flow.
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FIGURE 1. Cerebral blood flow and vascular resistance values (averages of 120 measures) are depicted in a resting state, during bilateral carotid artery occlusion, and for a three minute recovery period. In the resting state, total resistance, Rtot, is primarily brain resistance, Ric. Rec 1 represents carotid artery resistance. During bilateral occlusion brain flow is by way of vertebral arteries and collateral channels. Rec 2 represents resistance in the collateral system which includes the circle of Willis. Collateral resistance is initially increased by BCO; this is followed by a reactive adjustment which decreases Rec 2. Brain resistance, Ric, initially decreases as CBF falls. It rises as CBF is restored. Therefore, the resistance changes in the brain are in opposite direction to the changes in the extracerebral supply system during bilateral carotid artery occlusion.

of communality within which any of the several r-values obtained are equally appropriate to describe the magnitude of difference.

Results

The series of vascular responses which restore and sustain CBF during BCO are shown in figure 1. Prior to BCO when all supply channels are open, the brain vascular resistance, Ric, accounts for over 90% of the total cerebrovascular resistance, Rtot. The remainder is in the supply vessels, including carotid artery, and circle of Willis.

BCO decreases CBF and increases Rtot. The increment in total resistance is the result of redirection of blood flow from the carotid arteries directly into each ipsilateral hemisphere, to an indirect course by way of vertebral arteries and circle of Willis. Rec 1 is changed to Rec 2 by BCO. The fall in CBF reduces Ric (fig. 1). Within 10–15 seconds CBF begins to be restored because of dilation of the collateral system, i.e., circle of Willis; the reduction of Rec 2 decreases Rtot. Brain resistance arteries remain open but do not dilate further; in fact, Ric increases as CBF increases. However, CBF is not reduced by the increase in brain resistance because Rec 2 continues to decline. This sequence of vasomotor adjustments during BCO takes place while systemic BP remains stable, although increased above baseline levels. Upon release of BCO, there is an overshoot of CBF for 15–20 seconds. This happens as normal flow channels are resumed while Ric is still below baseline and the abrupt shift from Rec 2 back to Rec 1 markedly reduces Rtot. Then, in response to the CBF overshoot, Ric increases above baseline. This additional increase in brain resistance restores CBF to the pre-occlusion level within 30 seconds of release of BCO.

Naftidrofuryl increases CBF prior to BCO (fig. 2). It reduces resistance primarily by dilation of cerebral arteries (Ric is reduced, fig. 3). But Rec 1 is also reduced (fig. 4). At the onset of BCO, the rate of CBF decline is steeper in the treated animals than in the untreated, but both groups reach the same low point in 10–15 seconds. CBF then increases in

FIGURE 2. Comparison of averages of 120 measures each of CBF in untreated (solid line) and treated (dashed line) conditions in response to one minute periods of bilateral carotid occlusion. The ordinate is in arbitrary linear units of flow, with the origin representing zero flow obtained at sacrifice. The solid line represents averaged data in untreated animals. The dashed line represents averaged data during drug administration. The histograms at the bottom represent regions where the difference between data points obtained in treated and untreated animals was significant at the p ≤ .001 confidence level. The direction the histogram projects from the dashed reference-line corresponds to the direction of difference between treated and untreated values.

Naftidrofuryl increases CBF prior to BCO (fig. 2). It reduces resistance primarily by dilation of cerebral arteries (Ric is reduced, fig. 3). But Rec 1 is also reduced (fig. 4). At the onset of BCO, the rate of CBF decline is steeper in the treated animals than in the untreated, but both groups reach the same low point in 10–15 seconds. CBF then increases in

FIGURE 3. Comparison of averages of 120 measures each of intracerebral artery resistance (Ric) in untreated (solid line) and treated (dashed line) conditions. Prior to bilateral occlusion resistance is lowered by drug administration. The initial change at the time of BCO equalizes resistance in the two groups. During the last 30 seconds of BCO, the drug maintains resistance lower than in the untreated condition. At release of BCO resistance returns to baseline.
Comparison of averages of 120 measures each of carotid artery resistance $R_{ec\_1}$, and collateral system resistance, including the circle of Willis, $R_{ec\_2}$ (untreated condition, solid line; treated condition, dashed line). The drug reduces resistance in the carotid artery prior to occlusion. During the final 30 seconds of bilateral carotid artery occlusion, resistance in the circle of Willis and vertebral arteries is reduced by the drug. After release of BCO, carotid resistance is at baseline.

Both groups but is maintained at a higher level in the treated animals (fig. 2). This increase in CBF in the treated group during BCO is due to reduced $Ric$ and $R_{ec\_2}$ during the last 30 seconds of BCO. On release of BCO, there is no overshoot of CBF in the treated, as occurs in the untreated group. However, the overshoot in the untreated remains below CBF in the treated group.

BP is lower in the treated group prior to BCO. BP responses to BCO in the treated and untreated groups are parallel (fig. 5).

Drug administration dilates the carotid artery reducing $R_{ec\_1}$ in the pre-occlusion interval (fig. 4); CPP is thereby maintained (fig. 6) in spite of the hypotension (fig. 5). There is a smaller drop in CPP in the treated group, during BCO, but both groups stabilize at the same level until release of BCO (fig. 6). CBF is greater during BCO in treated as compared to untreated conditions because of dilatation of the circle of Willis (fig. 4) and the cerebral vessels (fig. 3). The pattern of vasomotor responses at onset of BCO is similar in both untreated and treated conditions. The drug vasodilates supply vessels, circle of Willis and cerebral arteries. However, the reduction of $R_{ec\_1}$, $R_{ec\_2}$, and $Ric$ which is responsible for an increase in CBF during BCO, changes the limits of autoregulation. In the treated animals, CBF does not reach pre-occlusion levels as it does in the untreated. At release of BCO, there is no overshoot but the level of CBF remains increased by the drug (fig. 2).

Bradycardia is a persistent drug effect (fig. 7). End tidal $CO_2$ was not altered by drug treatment and remained constant during BCO (fig. 8).

**Discussion**

We have previously demonstrated that it is possible to obtain reproducible numerical values for local CBF by establishing appropriate temperature controls. The method has now been extended by applying signal averaging techniques similar to those used to detect sensory evoked cerebral responses. In this way random CBF fluctuations are reduced and an enhanced picture of event-related CBF changes is obtained. This combined flow measurement-data averaging system provides a powerful tool for the examination of rapid and transient CBF regulating actions. The separation of the cerebrovascular system into two compartments makes it possible to examine the two areas of the cerebral vascular supply that may act independently to regulate CBF. It is also possible to detect local CBF changes with minimum disruption of vascular and cerebral physiology. The application of these methods demonstrates that naftidrofuryl dilates the carotid artery, circle of Willis and cerebral arteries, increasing local CBF in the caudate nuclei of both hemispheres equally.

The naftidrofuryl molecule is similar to other local anesthetics. It contains a hydrophylic amino group with an ester linkage to a lipophylic aromatic residue. Procaine is typical of drugs with this configuration; others, like lidocaine, have an amide bond between the intermediate group and the aromatic residue. Propanolol, although not commonly considered along with these agents, is also a potent local anesthetic. It has a terminal amino group and an aromatic residue but with an ether linkage. Pronethanol and...
some other beta adrenergic blocking drugs have an aliphatic linkage. In spite of differences in linkage and in the amino and aromatic groupings, naftidrofuryl, local anesthetics, and the beta adrenergic blocking agents have similar pharmacologic properties. There is a quinidine-like effect on myocardium and there are vasomotor effects in addition to variable anesthetic potency. The prominent vascular action of local anesthetics is vasodilation by direct spasmyotic effect on the smooth muscle cell of the vascular wall. While propanolol, by its blocking action on beta adrenergic receptors, causes vasoconstriction, other drugs in this category are vasodilators. Propanolol, too, may also have a secondary dilator action. These drugs, like other local anesthetics, may directly affect the vascular wall in addition to their action on the beta adrenergic receptors.

The metabolic effects of various beta adrenergic blocking agents are disproportionate to their cardiovascular effects, and the relationship between central nervous system effects, i.e. depression, hallucinations and nightmares, and their metabolic action is not known. Except on cocaine, there is relatively limited documentation on the clinical central nervous system effects of local anesthetics. There are comprehensive studies of anticonvulsant properties and use in the treatment of epilepsy. These observations are not correlated with metabolic actions in the brain. Naftidrofuryl has been found to increase brain utilization of glucose, other local anesthetics have been reported to suppress glucose oxidation in brain homogenates, and propanolol has been reported to reduce CBF and brain metabolism.

Comprehensive measurements of the CBF effects of other local anesthetics and beta adrenergic blocking agents are not available. Naftidrofuryl has been shown to increase CBF by dilating cerebral as well as extra-cerebral supply arteries. Several mechanisms of action are suggested by the pharmacologic similarities discussed and by the drug's effect on brain metabolism. Vasodilation in the brain (reduced Ric) may be secondary to pH change associated with the increased brain metabolism. On the other hand, a direct spasmyotic effect on the vascular wall or action on the beta adrenergic receptors is also possible. It is unlikely that any change in cerebral glucose utilization would have a vasodilator effect on the circle of Willis or other supply arteries. Vasodilation in these areas (reduced Rec 1 and Rec 2) would have to be the result of a direct myogenic or neurogenic action of the drug. If the muscle cell receptor site and the neural receptor site react with the drug, then initiation or blocking of the conversion of ATP to cyclic AMP or other intracellular messengers would be a common mechanism for vasomotor control whether by action on the muscle cell or by autonomic neuron influence. This would also be a direct link between brain metabolism and blood flow regulation. Additional studies to determine the CBF and metabolic effects as well as the mode of action of these drugs might provide a means for pharmacologic control of CBF and brain metabolism and for effective treatment of cerebrovascular disorders.

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Cerebral blood flow regulation. II. Vasodilator mechanisms.
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Stroke. 1977;8:189-193
doi: 10.1161/01.STR.8.2.189
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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