Dependency of Pial Arterial and Arteriolar Diameter on Perivascular Osmolarity in the Cat

A MICROAPPLICATION STUDY

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ABSTRACT

The effect of perivascular osmolarity on the diameter of pial arteries was studied in cats by the microapplication technique. Between 251 and 360 mosmoles/liter, concentration-response curves were obtained for single vessels. Constriction occurred when perivascular osmolarity was decreased below 317 mosmoles/liter, and dilation occurred at osmolarities above this value. The effect was the same whether the osmolarity was changed by addition of mannitol or NaCl. Reduction of sodium concentration from 156 to 133 mEq/liter at constant osmolarity did not affect arteriolar diameter, but greater reductions in sodium concentration induced vasoconstriction. The results indicate that the resistance of pial arteries can be influenced by local changes in perivascular osmolarity.

KEY WORDS

perivascular ions, vascular smooth muscle, cerebral blood vessels, local arteriolar reactions, cerebral arteriolar resistance

Changes in osmolarity have been shown to be one determinant of arteriolar resistance in many vascular beds (1-12). For the cerebral vessels, however, there is no direct evidence that changes in osmolarity in a physiological range are effective in altering arteriolar resistance. Several authors (13-15) have found an increase in cerebral blood flow following the intravenous injection of hypertonic solutions. This increase, however, might not reflect quantitatively the direct dilatory effect of the hyperosmolarity on the smooth muscles. It is well known that the infusion of hypertonic solutions leads to a shift of cerebrospinal fluid (CSF) into the blood, thus lowering the pressure in the CSF (15, 16). This event may induce autoregulatory reactions because of the increase in the transmural pressure difference. Therefore, the changes in cerebral vascular resistance observed following the infusion of hypertonic solutions can be considered only as a net effect of different mechanisms opposing each other. In addition, other factors like changes in hematocrit might contribute to the observed changes in resistance.

Hypertonic solutions have been locally applied by Forbes and Nason using the window technique (17). They rinsed the surface of the brain with solutions containing 30% NaCl or 50% urea and found marked vasodilation. However, such an increase in osmolarity is far greater than any physiological change, and the effect of osmolarity needs to be studied under more physiological conditions.

For this reason the microapplication technique was used; this technique allows local changes in the composition of the perivascular fluid of a single pial arteriole and measurement of the effect on segmental vascular diameter. The technique has been used in the past to evaluate the role of other local factors such as pH and K⁺ in the regulation of pial arteriolar resistance (18).

Methods

Experiments were performed on ten cats of either sex anesthetized with glucocorticosterone (40-50 mg/kg, iv). The cats were ventilated artificially. Pco₂, pH, and Po₂ of the arterial blood were measured with Astrup equipment at 38°C; pH was 7.32 ± 0.04 (SD), Pco₂ was 30.1 ± 1.8 (SD) mm Hg, and Po₂ was 125 ± 18 (SD) mm Hg. The value of arterial Pco₂ is close to that obtained in conscious cats (19, 20). The pH, when compared with that in conscious cats (7.45 and 7.38), is somewhat low; this finding might be explained by a slight metabolic acidosis resulting from the anesthesia. End-tidal CO₂ was monitored with an infrared gas analyzer and was 4.3 ± 0.2 (SD) ml CO₂/100 ml expired air. The arterial blood pressure was recorded continuously using a strain gauge and a Hellige recorder. Cats with a mean arterial blood pressure less than 100 mm Hg were not used. Body temperature was
maintained between 37°C and 38°C. To counteract fluid losses, an infusion of Tyrode's solution (2.5 ml/kg hour⁻¹) was given intravenously. Parts of the parietal and the temporal lobes of the brain were exposed by craniotomy (opening in the skull was 1.5 x 3 cm), and the dura was removed. Great care was taken to carry out this operation as gently as possible without touching the brain. The brain surface was bathed by mineral oil heated to between 37°C and 38°C.

Glass micropipettes with sharpened tips (8-10μ, o.d.) were filled with test solutions and sealed between oil to reduce CO₂ diffusion from the test solution. In separate experiments we found that CO₂ loss from the test solution stored in the micropipette was negligible. When mock spinal fluid was stored in a micropipette for 2 hours, which is the maximal storage time in a pipette during an experiment, no measurable loss in the total CO₂ content was observed, indicating a constant bicarbonate (HCO₃⁻) concentration in the test solution. The filled micropipette was mounted on a micromanipulator, and the tip was positioned in the immediate vicinity of a superficial artery or arteriole. In general, we examined vessels surrounded only by a fine layer of arachnoidal tissue and not those embedded in the cerebral surface, thus excluding the possibility of neuronal damage. In the few cases in which vessels embedded in the cerebral surface were examined, the results were the same. Hence, no distinction has been made between the two groups. By applying pressure to a syringe attached to the micropipette, 1-3 μl of fluid was injected into the perivascular space.

A Bausch and Lomb Stereo-Zoom microscope at a magnification of 50x was used. The setting of the magnification was unchanged throughout the experiment. To ensure constant magnification, a micrometer was measured before and after each experiment. A possible error in calculating the vascular diameter could have occurred when photographs were taken at varying objective-object distances. A small change in objective-object distance could result from volume changes of the brain caused by respiratory movements or blood pressure waves. Since the micropipette tip was kept in a fixed position outside a vessel, such movements of the brain surface would have led to a displacement of the pipette in the tissue. This phenomenon, however, did not occur in these studies, indicating only minimal changes in objective-object distance. In a test experiment, using a glass capillary as a model of a vessel, we quantified the error that occurs when the same diameter is measured at varying objective-object distances (defocusing). When the distance was reduced 200μ, at a total distance of 7.5 cm, a degree of defocusing occurred which under experimental conditions would not have been tolerated. Under these conditions the measured diameter was 63.7 ± 1.3μ (SD) (n = 50) compared with 61.7 ± 0.5μ (n = 19). Similar differences and standard deviations were obtained for larger diameters. Since the degree of defocusing under experimental conditions was much less than 200μ, the possible error induced by defocusing falls within the standard deviation of the method (see below).

In some experiments the pial vessels were photographed with a Nikkor camera, using Ilford FP4 black-and-white film. Vascular diameter was measured from magnified photographic prints using a caliper. The term "vascular diameter" as used in this paper has been defined in a previous paper (21). In the other experiments the vascular diameter was measured with the image-splitting method of Baez (22), using a 625-line Siemens television camera and a Servogor recorder. The vascular diameter was measured before and 20 and 40 seconds after the beginning of the injection of test solution. The reproducibility of the image-splitting method was assessed from repeated measurements of the diameter of the same vessel. For vessels with diameters ranging from 30 to 200μ, the standard deviation was 1.2μ (n = 74). This value is in the same range as that for the photographic method described elsewhere (21).

Before applying test solutions of different osmolarity or sodium (Na⁺) concentration, the reactivity of the vessels was tested by changing the HCO₃⁻ concentration in the perivascular space. All vessels exhibited the typical response, i.e., constriction at 22 mEq HCO₃⁻/liter and dilation at 5 mEq HCO₃⁻/liter (18). All solutions used in the following experiments had a pH of 7.15, a potassium (K⁺) concentration of 3 mEq/liter, a calcium (Ca²⁺) concentration of 5 mEq/liter, and a HCO₃⁻ concentration of 11 mEq/liter. The remaining anions were chloride (Cl⁻). All solutions were equilibrated with 5% CO₂. Three types of experiments were performed: type 1, application of mock spinal fluid containing 133 mEq Na⁺/liter in which the osmolarity was altered by the addition of varying amounts of mannitol, resulting in osmolarities of 251, 271, 292, 312, 331, and 360 mosmole/liter; type 2, change in osmolarity by the addition of NaCl instead of mannitol, resulting in the same osmolarities as in type 1; type 3, reduction of NaCl concentration at constant osmolarity. The Na⁺ concentrations were 150, 143, 133, 95, and 57 mEq/liter in which the osmolarity was adjusted to 301 mosmole/liter by the addition of mannitol.

The Na⁺, K⁺, and Ca²⁺ concentrations were determined using a Zeiss flame photometer. The total CO₂ content was measured by a Natelson-Mikro-van-Slyke apparatus, and the HCO₃⁻ concentration was then calculated. The osmolarity of all solutions was measured using a Precision Systems osmometer.

The vascular diameter before a concentration-response curve was determined was taken as the control diameter in all three types of experiments. In all three groups, concentration-response curves were obtained for single vessels. The curves shown in the figures are mean values (±SE) of the single curves. In the experiments of types 1 and 2 the osmolarity was increased in steps, and in those of type 3 the NaCl concentration was reduced in steps.

Results

Osmolarity Changed by Mannitol

The effect of changes in perivascular osmolarity was tested by application of mock spinal fluid containing identical electrolyte concentrations at
increasing concentrations of mannitol. Figure 1 shows photographs taken from pial arteries before and after the perivascular injection of a hypotonic mock spinal fluid. It is apparent that the vessel diameter following hypotonic injection into the perivascular space was considerably reduced. Figure 2 shows the concentration-response curve obtained from the mean values of the concentration-response curves for individual vessels. The arteries tested had a diameter ranging from 30 to 230μ, with a mean value of 102μ. The changes in vascular diameter are expressed as a percent of the control diameter to compare the data obtained from vessels of different size. This procedure is valid for all the figures presented in this paper, since the percent reaction was independent of vascular diameter over the range from 80 to 230μ. A correlation between control diameter and percent reaction also could not be determined in vessels 30-80μ in diameter, but the mean percent reaction was more
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pronounced than it was in the larger vessels. At perivascular osmolarities below 317 mosmoles/liter the arterioles constricted, and the degree of constriction showed a concentration dependency (Fig. 2). Similarly, an increase in osmolarity above 317 mosmoles/liter led to dilation. The statistical analysis revealed that a nonlinearity of the relationship between osmolarity and change in vascular diameter could not be verified ($P < 0.01$). Therefore, a linear regression function was calculated. The correlation coefficient of 0.735 demonstrates that the slope of this correlation is significantly ($P < 0.01$) different from zero.

CHANGES IN NaCl CONCENTRATION AT CONSTANT OSMOLARITY

The range of the concentration-response curve for osmolarity (Fig. 2) made it necessary to reduce the Na$^+$ concentration to 133 mEq/liter in all solutions tested. This value is lower than that reported for the cortical subarachnoidal (159 mEq/liter) and cisterna magna CSF (158 mEq/liter) (23). It was therefore necessary to test the effect of reduced NaCl concentration on the diameter of pial arterioles. The results are depicted in Figure 3. The Na$^+$ concentration was gradually reduced from 156 to 57 mEq/liter at a constant osmolarity of 301 mosmoles/liter (by addition of mannitol). As can be seen, a reduction in Na$^+$ concentration to 133 mEq/liter caused no alterations in vascular diameter when compared with the reaction at a Na$^+$ concentration of 156 mEq/liter. A further decrease in Na$^+$ concentration to 95 and 57 mEq/liter resulted in a reduction of vascular diameter of 8% and 19%, respectively. The statistical analysis was performed by the multiple comparison of dependent observations (24), since inspection of Figure 3 indicated a nonlinear relationship between Na$^+$ concentration and change in vascular diameter. The constriction was statistically significant at 95 mEq/liter ($P < 0.05$) and at 57 mEq/liter ($P < 0.01$) when compared with the reactions at 133, 143, and 156 mEq/liter, which were not significantly different from each other.

OSMOLARITY CHANGED BY NaCl

It has been demonstrated that moderate reduction of NaCl concentration at constant osmolarity had no effect on the vascular diameter. Despite this observation the possibility exists that the slope of the concentration-response curve for osmolarity (Fig. 2) is influenced by changes in NaCl concentration. Therefore in a separate series of experiments the osmolarity of the test solutions was increased by the addition of NaCl instead of mannitol. The results are shown in Figure 4, which also contains the concentration-response curve for mannitol from Figure 2. The diameters of the arteries tested ranged from 30 to 210μ (mean 101μ) and were practically identical to those tested in the mannitol group (Fig. 2). The statistical analysis revealed no nonlinearity of the relationship between osmolarity changed by addition of NaCl and change in vascular diameter ($P < 0.01$). Although the slopes of the concentration-response curves in Figure 4 are not significantly different, the regression lines are not coincident ($P < 0.01$).

Discussion

The data presented in this paper demonstrate the sensitivity of the pial arteries to changes in

![Figure 3](http://circres.ahajournals.org/)

Concentration-response curve for Na$^+$ at a constant osmolarity of 301 mosmoles/liter. The Cl$^-$ concentration was lowered in parallel with the Na$^+$ concentration. The curve shows means ± SE; n = number of vessels tested.
perivascular osmolarity. A reduction in osmolarity from isotonicity constricted the vessels, whereas an increase in osmolarity dilated them. These results are qualitatively consistent with the results of several experiments that increased osmolarity of either the blood (13-15) or the surface fluid of the cortex by use of the window technique (17). The microapplication method used in the present study, however, excludes the possibility that secondary effects, such as changes in hematocrit or in local metabolic rate, were responsible for the vascular reaction. It is also unlikely that changes in the transmural pressure difference, induced by the perivascular injection method, accounted for the observed local vascular reactions. The injection method used was the same in all experiments; therefore, a change in the transmural pressure difference would be equally distributed throughout all the experiments. Hence, the concentration-dependent vascular reactions cannot be related to changes in the transmural pressure differences. Furthermore, the osmotically induced concentration-response curves showed constrictory as well as dilatory reactions, a result difficult to reconcile with a transmural pressure mechanism.

The results reported in this paper are consistent with findings obtained using isolated vascular preparations and in vivo perfusion techniques. The isolated portal vein reduces its mechanical activity when the bathing medium is hypertonic and increases its activity when the medium is hypotonic (1, 25-27). A dependency of vascular resistance to blood flow on hyperosmolarity of the blood has been described for various vascular beds (1-12). In those experiments, the hyperosmolarity was achieved either by infusing hypertonic solutions into the blood or by increasing the metabolic rate of an organ. A quantitative comparison of these results with our data, however, is difficult for several reasons. The effective osmolarity and the ionic concentrations acting at the smooth muscle cells may be different when the same solution is applied at the intravascular site or at the perivascular site, as done in the present study. In addition, the time course of vascular reactions necessarily influences the figures obtained at different time intervals after the onset of the change in osmolarity. Finally, the changes in vascular resistance as calculated from blood pressure and blood flow rate through an entire organ are not necessarily comparable with the changes in vascular diameter of a single vessel, which was measured in this paper.

For the cerebral circulation only a few reports are available indicating that changes in cerebral blood flow, which are associated with changes in cerebral function, may have been caused in part by changes in the osmolarity of the CSF. During asphyxia, when cerebral blood flow is known to be increased (28), Bito and Myers (29) found that the osmolarity of cortical subarachnoidal CSF was increased by 12-17 mosmoles/liter. During changes in neuronal activity and in acid-base status, measurements of CSF osmolarity have not yet been carried out. However, changes in CSF electrolyte...
concentrations have been measured under these conditions (28, 30-36), and they suggest that the osmolarity of CSF has changed simultaneously.

If osmolarity is one determinant of arteriolar resistance in the brain, the cerebral vasodilatation observed after the infusion of hypertonic solutions into the blood may be explained on the basis of the observed findings: an increase in blood osmolarity induces osmotic water flux from the brain to the blood across the blood-brain barrier (37). This water flux will result in an increase in extravascular osmolarity in the brain resulting in vasodilatation.

The perivascular osmolarity at which no vascular response was observed in this study was 317 mosmoles/liter (at a K⁺ concentration of 3 mEq/liter). This osmolarity does not necessarily reflect the naturally occurring local osmolarity. The injection fluid contained ions (K⁺, HCO₃⁻) which themselves are potent vasoactive constituents (18). Their concentrations in the injection fluid might have been slightly different from those in the original perivascular fluid. Hence, the level of osmolarity at which no vascular reaction occurred may have been influenced by the deviation in the concentrations of vasoactive ions from those existing in the original perivascular fluid. For example, in earlier experiments (18) we found no vascular reaction when the K⁺ concentration in the injection fluid was 5 mEq/liter at a total osmolarity of 280 mosmoles/liter. Here, the constrictory effect of lowered osmolarity was compensated by the increase in K⁺ concentration.

In contrast to changes in K⁺ concentration and H⁺ concentration within the physiological range, changes in perivascular Na⁺ concentration between 156 and 133 mEq/liter had no effect on pial arteriolar diameter when the osmolarity was kept constant. Johansson (38), using the portal vein preparation, also found no change in mechanical activity when the Na⁺ concentration was reduced by 20 mEq/liter at unchanged osmolarity. Vasocostriction of pial arterioles occurred only when Na⁺ concentration was reduced below 95 mEq/liter. Since in these experiments Cl⁻ concentration was lowered to the same levels, the ionic specificity of this reaction remains to be established. Sitrin and Bohr (39) have suggested that Na⁺ and Ca²⁺ compete for the smooth muscle cell in such a way that a reduction in extracellular Na⁺ concentration leads to an increase in Ca²⁺ influx or a decrease in Ca²⁺ efflux. The resulting increase in intracellular Ca²⁺ concentration is thought to be responsible for the contraction of the smooth muscle cell under the conditions of lowered extracellular Na⁺ concentration.

The response of the pial arteries is practically the same whether osmolarity is changed between 251 and 360 mosmoles/liter by adding mannitol or by adding NaCl. The difference between the two curves with identical slopes in Figure 4 might be due to the fact that two groups of cats were used. At the lowest osmolarity of 251 mosmoles/liter the solutions tested in both groups were completely identical in their composition. Yet, the decrease in vascular diameter in these two groups differed by 5% (P < 0.05), and this difference was maintained over the entire range of osmolalities tested. Therefore, the difference between the two curves is not explained by the different substances used to increase osmolarity (mannitol vs. NaCl). Similar conclusions were drawn by other authors (5, 6) who studied the effect of increased blood osmolarity on vascular resistance in the gracilis muscle and the forelimb of the dog and in the forearm of man. In these studies dextrose was used instead of mannitol.

As to the mechanisms underlying the effect of osmolarity on pial arteriolar resistance, the results obtained on taenia coli and portal vein might be relevant. For hypoosmolarity two mechanisms have been discussed: (1) changes in intracellular electrolyte concentration and (2) changes in permeability. When the portal vein is bathed with hypotonic solutions, the smooth muscle cells swell (40) and thereby reduce their intracellular K⁺ concentration. However, the calculated decrease in intracellular K⁺ concentration is too small to quantitatively account for the increase in mechanical activity (26). Efflux studies have disclosed an increase in the permeability ratio for sodium and potassium under these conditions (41). The resulting depolarization would facilitate mechanical activity. In contrast to the change in the permeability ratio for sodium and potassium at hypoosmolarity, no change in the ratio was found at hyperosmolarity (41). However, the intracellular K⁺ concentration was increased at hyperosmolarity (42), a result consistent with the simultaneous decrease in cellular volume. Tomita (43) and Kuriyama and Mekata (44), using the taenia coli preparation, found hyperpolarization under these conditions. Kuriyama et al. (45), on the other hand, found depolarization of smooth muscle cells in the portal vein exposed to hyperosmolarity. Whether the relaxation of the
smooth muscle cells during depolarization is mediated by a negative inotropic effect of hypertonic solutions, as suggested by Mellander et al. (1), remains to be established.

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References
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