Perivascular Potassium and pH as Determinants of Local Pial Arterial Diameter in Cats

A MICROAPPLICATION STUDY

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ABSTRACT

The effects of and the interaction between local perivascular variation in potassium and bicarbonate concentration on the diameter of pial arteries were studied in cats by the microapplication technique. At 11 mEq/liter of bicarbonate, a direct correlation existed between vascular diameter and K+ concentrations between 0 and 10 mEq/liter. At K+ concentrations between 10 and 20 mEq/liter, no further increase in vascular diameter was observed. At a K+ concentration of 5 mEq/liter, an inverse relationship existed between pial arterial diameter and perivascular bicarbonate concentrations between 0 and 22 mEq/liter. At K+ concentrations of 0 and 10 mEq/liter, the pial arterial diameter was determined strongly by the K+ concentration and was only slightly, if at all, influenced by bicarbonate concentrations between 5 and 22 mEq/liter. At lower bicarbonate concentrations the local acidity induced a marked vasodilation. The results indicate that the diameter of pial arterioles in cats is affected by periarteriolar concentrations of K+ and H+; the degree of the vasoreaction induced by H+ is modulated by K+.

KEY WORDS vascular smooth muscle cerebral arteriolar resistance local control of cerebral blood flow cerebral blood vessels local arteriolar reactions perivascular ions

Extravascular potassium is considered to be an important factor for the regulation of arteriolar resistance in many vascular beds. Although changes in the K+ concentration of cerebrospinal fluid (CSF) have been measured during respiratory acidosis and alkalosis (1–3) and also during hypoxia (4), the sensitivity of cerebral vessels to changes in extracellular K+ concentration has not yet been tested. Furthermore, an increased K+ concentration has been measured in the CSF during seizure activity (5). A more pronounced increase in the K+ concentration during neuronal activity has been observed in the perineuronal space of the central nervous system of the leech (6). Since changes in the cerebral blood flow have been measured during respiratory acidosis and alkalosis (7–9) and during enhanced activity of the cerebral cortex (10–12), it would be of interest to investigate whether changes in blood flow are at least partially mediated by alterations in the extravascular K+ concentration. Therefore, the influence of the perivascular K+ concentration on the resistance of pial arteries was studied. Using the microapplication technique, the K+ concentration in the perivascular space of single pial arteries was changed and the alterations in vessel diameter measured.

In a previous study (13) the effect of extreme changes in perivascular pH on pial arteriolar diameter was reported. These observations suggested that the perivascular pH might be, in addition to other factors, a determinant of cerebral vascular resistance to blood flow. To incorporate such a pH-dependent vascular reaction into the regulation of cerebral blood flow in the present...
study, the perivascular pH was changed in a physiological range, and the induced changes in vascular diameter were observed. Concentration-response curves for bicarbonate were constructed and their dependency on perivascular K⁺ concentration investigated.

Methods

Experiments were performed on 21 cats of either sex anesthetized with glucochloralose (40-50 mg/kg, iv). Some cats were allowed to breathe spontaneously, the others were artificially ventilated. The Pco₂, Po₂, and pH of the arterial blood were measured with Astrup equipment. Pco₂ was 28.6 ± 4.4 (SD) mm Hg, Po₂ was 95.2 ± 9.4 (SD) mm Hg, and pH was 7.32 ± 0.03 (SD). The value of arterial Pco₂ is similar to values obtained in conscious cats (14, 15). The procedures for this study, with the exception of the preparation of mock spinal fluid containing various concentrations of K⁺ instead of l-norepinephrine, are identical to those cited in the preceding paper (16).

The mock spinal fluid used in these experiments was prepared fresh daily from two stock solutions containing Na⁺, K⁺, Ca²⁺, and Cl⁻ in various concentrations. Both were equilibrated with 5% CO₂, sodium bicarbonate (420 mg/100 ml) was added to one of them, and the solutions were then covered with mineral oil. Different amounts of both solutions were mixed to produce solutions with different bicarbonate and chloride concentrations and constant cation concentration. The final solution had an osmolarity of 280 mosmoles/liter. The K⁺ concentrations (0, 3, 5, 10, or 20 mEq/liter) determined the Na⁺ concentrations (149, 146, 144, 139, or 129 mEq/liter). The Ca²⁺ concentration was 5 mEq/liter. The anion composition was variable, since the bicarbonate concentration (0, 5, 11, or 22 mEq/liter) determined the Cl⁻ concentration (155, 150, 144, or 133 mEq/liter). The pH (38°C) at 0 mEq/liter of bicarbonate was 4.8, at 5 mEq/liter it was 6.8, at 11 mEq/liter 7.15, and at 22 mEq/liter 7.45.

Results

POTASSIUM

The effect of perivascular K⁺ concentration on the diameter of pial arteries was studied on 12 arteries from four cats. The results depicted in Figure 1 summarize the concentration-response curves. K⁺ was applied perivascularly in increasing concentrations from 0 to 20 mEq/liter at a constant bicarbonate concentration of 11 mEq/liter. The vascular diameter...
was diminished at 0 and 3 mEq/liter when compared to control. At 10 mEq/liter of K\(^+\), the vessels were all dilated. An increase in perivascular K\(^+\) concentration to 20 mEq/liter was not followed by a further increase in vascular diameter. Assuming a linear correlation at K\(^+\) concentrations between 0 and 10 mEq/liter, the correlation coefficients were calculated for each curve and ranged between 0.87 and 0.99, with a mean of 0.96. Therefore, a highly significant relationship existed between vascular diameter and extravascular K\(^+\) concentrations from 0 to 10 mEq/liter.

**BICARBONATE**

The effect of perivascular bicarbonate concentration on the diameter of pial arteries was studied on ten arteries from two cats, and the results are depicted in Figure 2. The lines connect values obtained from individual vessels when the bicarbonate concentration was decreased in steps from 22 mEq/liter to 0 mEq/liter at a constant K\(^+\) concentration of 5 mEq/liter. As can be seen, the vascular diameter increased over the entire range of decreasing bicarbonate concentration from 22 to 0 mEq/liter.

In Figure 3 the relationship between changes in vascular diameter and perivascular bicarbonate concentration is again shown. However, in contrast to Figure 2, which contains concentration-response curves obtained on individual vessels, Figure 3 summarizes the mean values obtained by random injection of test solutions of different bicarbonate concentrations at a K\(^+\) concentration of 5 mEq/liter. The vessels constricted at bicarbonate concentrations above 11 mEq/liter and dilated at lower concentrations. Each value depicted in Figure 2 was significantly (P < 0.01) different from its neighboring values. It is apparent that the courses of the concentration-response curves of Figures 2 and 3 were similar. This indicates that the systematic decrease in bicarbonate concentration from 22 mEq/liter to 0 mEq/liter, as performed in all experiments of Figure 2, did not change the sensitivity of the vessels to bicarbonate compared to the sensitivity seen with random injections.
BICARBONATE VERSUS POTASSIUM

Because of the influence of both K+ concentration and bicarbonate concentration on pial arterial diameter, a shift of the concentration-response curve for bicarbonate, shown in Figure 3, was expected, depending on the K+ concentration in the injection fluid. This is shown in Figure 4, which summarizes concentration-response curves for bicarbonate at 0 and 10 mEq/liter of K+. The mean values shown were derived from concentration-response curves on individual vessels and from changes in diameter observed following random injections. Both techniques yielded the same slope of the concentration-response curve.

Between 5 and 22 mEq/liter of bicarbonate the vessels remained dilated when the K+ concentration was 10 mEq/liter, whereas at a K+ concentration of 0 mEq/liter the vessels remained constricted. When the bicarbonate concentration was below 5 mEq/liter, the vascular diameter increased at both K+ concentrations.

To determine whether a correlation existed between bicarbonate concentrations of 5 and 22 mEq/liter and vascular diameter at 0 and 10 mEq/liter of K+, regression lines were calculated from the values obtained at 5 and 22 mEq/liter of bicarbonate in 0 and 10 mEq/liter of K+, respectively, and compared to the regression line obtained in 5 mEq/liter of K+. This kind of analysis was necessary because the functions at 0 and 10 mEq/liter of K+ (5-22 mEq/liter of bicarbonate) were linear but those of 5 mEq/liter of K+ were not.

The correlation coefficient calculated for the values obtained at 10 mEq/liter of K+ was -0.153 and not significantly different from zero. This indicates that no dependency existed between the bicarbonate concentration (5-22 mEq/liter) and the vascular diameter at 10 mEq/liter of K+. At 0 mEq/liter of K+, the correlation coefficient was -0.448, indicating a significant dependency between the bicarbonate concentration (5-22 mEq/liter) and the vascular diameter (P<0.01). However, this value was significantly lower than the correlation coefficient obtained at 5 mEq/liter of K+ (P<0.01). These data indicate that the correlation between vascular diameter and bicarbonate concentrations of 5-22 mEq/liter was dependent on the K+ concentration. Compared to 5 mEq/liter of K+, the correlation was weakened at 0 mEq/liter of K+ and abolished at 10 mEq/liter of K+.

Discussion

METHODS

The microapplication technique used in this study made it possible to locally alter the composition of the perivascular fluid surrounding pial arteries and arterioles and to observe the local response of this vascular segment in vivo. The fact that only minute amounts of test substances were injected excluded the influence of these substances on the general circulation and particularly on acid-base status. The perivascular site of application also was identical with the site from which naturally produced vasomotor substances under many physiological circumstances act locally on the smooth muscle cells. This technique is particularly well suited to studying the effect of vasoactive substances on cerebral vessels. When the test substances are included...
in the blood, the existence of a blood-brain barrier in the vascular endothelium (17, 18) might modify, in an unpredictable way, the concentration of the test substance at the smooth muscles.

To compare the data obtained on vessels of different diameters, all induced changes in vascular diameter were expressed as percent of control diameter. Such a presentation of the data appears to be valid since the observed percent changes in diameter were not dependent on the control diameter. The only exception noted was during the application of 22 mEq/liter of bicarbonate, when it was our impression that smaller vessels (25-110μ) reduced their diameter relatively more than did larger vessels (110-260μ). However, no attempt was made to quantify such a possible dependency.

**POTASSIUM**

During the last years several authors have stressed the importance of K+ for the local regulation of blood flow through various organs (19-22). Such a potassium-dependent mechanism has not yet been evaluated for the brain, although analysis of the CSF has shown that its K+ concentration may vary (1-5) under certain circumstances which are associated with changes in cerebral blood flow.

The present results clearly showed that the diameter of the pial arteries and arterioles depended on the perivascular K+ concentration when all other factors were kept constant. The fact that a concentration-response relationship existed between K+ concentrations of 0-10 mEq/liter and vascular diameter might be taken as strong support for the assumption that in the brain the perivascular K+ concentration could play a role in the adjustment of arteriolar resistance. At K+ concentrations which exceeded the normally occurring variations in extracellular K+ concentrations, the responsiveness of the vessels to changes in K+ concentration was much reduced, as seen by the flattened course of the concentration-response curve at K+ concentrations between 10 and 20 mEq/liter. We did not examine the vascular response to K+ concentrations above 20 mEq/liter, but such concentrations are known to induce vasoconstriction (23). Consistent with our data are recent observations of Knabe et al. (24). Using a similar microapplication method, they found vasoconstriction following perivascular injection of potassium-free mock spinal fluid and vasodilation at K+ concentrations between 6.5 and 26.2 mEq/liter. A quantitative comparison with our data, however, is not possible since these authors reported their observations on a qualitative basis only. Contrary to our findings and those of Knabe et al., Cameron and Segal (25) found pial arteriolar constriction following periarteriolar injection of 6 and 10 mEq/liter of K+ in the rabbit. The reason for this discrepancy is not immediately apparent unless one attributes these differences to species differences.

Little is known about the magnitude of changes in K+ concentration in cerebral extracellular fluid under physiological conditions. During respiratory acidosis, which is associated with cerebral vasodilation, Schindler et al. (1) and Cameron (2) found a concomitant increase in K+ concentration of CSF on the order of 0.3-1.2 mEq/liter. There is evidence (2) that this increase is caused by an efflux of K+ from the cells into the extracellular fluid. Hence, the local increase in K+ concentration in the extracellular fluid immediately adjacent to the cells and the arterioles might be more pronounced than that observed in the fluid of the cisterna magna. There is also experimental evidence that the extracellular K+ concentration increases during neuronal activity which is known to be associated with cerebral vasodilation (10-12). From their measurements of membrane potentials in the central nervous system of the leech, Baylor and Nicholls (6) calculated an increase in K+ concentration of up to twofold.

The mechanism by which the perivascular K+ concentration affects the vascular contractile tone is still unknown. Several possibilities might be considered. (1) A decrease in K+ concentration might facilitate a release of norepinephrine from nerve endings surrounding pial arterioles, as has been observed in
nerve endings isolated from rat or rabbit brain (26). Norepinephrine has been found to exert a vasoconstrictor action on pial arterioles when applied from the perivascular site (27).

(2) It has been shown for smooth muscle that a reduction of the extracellular K+ concentration induces a transient increase in mechanical activity associated with a depolarization of the membrane (28). For the taenia coli it has been proposed that this depolarization is caused by an inhibition of an electrogenic sodium pump (29). (3) In the portal vein preparation a reduction of the extracellular K+ concentration from 6 to 0 mEq/liter leads to a decrease in the K+ conductance of the cell membrane (30). This in turn might decrease the membrane potential and thus increase the spike frequency and the mechanical activity of the smooth muscle cells.

**BICARBONATE**

The demonstration of a concentration-response relationship between pial arteriolar diameter and perivascular bicarbonate concentration (Figs. 2, 3) lends further support to the concept that vascular resistance in the brain is locally modulated by extracellular pH (7, 9, 13, 31–35) in a physiological range and that this mechanism contributes to a homeostasis of intracerebral pH. The concentration-response curve crossed the abscissa at a bicarbonate concentration of 11.5 mEq/liter when the K+ concentration of the injected fluid was 5 mEq/liter, as shown in Figure 3. These particular concentrations, at which no vascular reaction occurred, differed from those measured in CSF of cats with a comparable acid-base status when the fluid was obtained from the cisterna magna (K+ = 2.88 mEq/liter [36], HCO₃⁻ = 20 mEq/liter [37]) or from the cortical subarachnoid space (K+ = 2.52 mEq/liter [36]).

Since both bicarbonate and K+ affect the vascular diameter, it is impossible to calculate the true local ionic composition of the perivascular fluid from the concentration-response curves. However, it appears unlikely that the ionic composition as found in the fluid of the cisterna magna or the cortical subarachnoid space resembles that of the perivascular fluid in the brain. This follows from the observation that injection of a fluid identical with that obtained from the cisterna magna would produce a marked vasoconstriction. Therefore, it is suggested that the ionic composition of the extracellular fluid in the brain is not homogeneous but that it differs between perivascular space and large CSF-containing cavities. Preliminary results which argue in favor of this hypothesis have revealed a difference in total CO₂ content between cisternal CSF and cortical subarachnoid CSF. After opening the skull, the dura was covered with warmed mineral oil immediately. Then the subarachnoid space was punctured and the fluid was sampled anaerobically as was the fluid of the cisterna magna. At an arterial PO₂ of 28 mm Hg (pH = 7.33, PO₂ = 140 mm Hg) the total CO₂ content of the CSF in the cisterna magna in four cats was 19.8 ± 0.9 (SD) mmol/liter and of the cortical subarachnoid space 15.9 ± 0.6 mmol/liter. The difference is statistically significant (P < 0.01). Moreover, measurements with pH microelectrodes in the immediate vicinity of the pial arteries have revealed a pH of about 7.2 (Knabe, Heuser, Gebert, and Betz, personal communication and own preliminary results). These data indicate the existence of a pH gradient between cisternal CSF, cortical subarachnoid CSF, and local perivascular CSF. Consistent with a lower bicarbonate concentration in the perivascular space is the observation that mock spinal fluid containing 11 mEq/liter of bicarbonate (pH = 7.15), as used in this study, had no effect on vascular diameter per se (Fig. 3).

It appears unlikely that the lower bicarbonate concentration in the perivascular space, when compared to that of the cisterna magna fluid, was caused by CO₂ diffusion from the tissue into the oil covering the cerebral surface. The oil was almost stagnant and allowed to equilibrate for at least \( \frac{3}{4} \) hour before starting the experiments. Furthermore the delivery of CO₂ to the surface of the brain (metabolism, blood flow) exceeded by several orders of magnitude the amount of CO₂ which might have diffused from the oil into the air.
Several observations support the assumption that the data obtained from the pial superficial cerebral arterioles are representative for the majority of the cerebral arterioles. Similar to the correlation between periarteriolar pH and vessel diameter at a K⁺ concentration of 5 mEq/liter, as demonstrated in Figures 2 and 3, a dependency exists between total cerebral blood flow and either pH in the CSF (32-35) or pH measured on the cortical surface (7, 31). Autoregulation of cerebral blood flow is reflected in concomitant changes in diameter of pial arteries (38).

**INTERACTION BETWEEN BICARBONATE AND POTASSIUM**

There is some evidence that the potassium-dependent vascular reaction at K⁺ concentrations of 0 and 10 mEq/liter dominates the bicarbonate-dependent one in the range of bicarbonate concentrations from 5 to 22 mEq/liter. As can be deduced from Figure 4, the degree of vasodilation or vasoconstriction was primarily determined by the K⁺ concentration, as expected from the slope of the curves shown in Figure 1. However, at a bicarbonate concentration below 5 mEq/liter an additive effect of bicarbonate on vascular diameter became apparent. Whether this demonstrates the existence of two specific mechanisms, one sensitive to H⁺, the other sensitive to K⁺, remains to be established.

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