Influence of Serotonin and Norepinephrine on Flow Capacity/Pressure Characteristics of Feline Isolated Cerebral Arteries

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SUMMARY Isolated feline pial vessel segments (250–400 μm in diameter, 4 mm long) were mounted in Krebs solution in organ baths on L-shaped metal holders connected to pressure transducers. From the distance between the outer limits of the wires (f) and the force recorded by the transducers (F), the transmural pressure and the flow capacity were calculated. Changes in these parameters were produced by increasing the distance between the holders. Norepinephrine (10⁻⁵M) and serotonin (10⁻⁶M) were added to the tissue bath at different resting wall tensions. Both norepinephrine and serotonin resulted in contractile responses that were maximal at a resting wall tension of 2 nM/mm. Norepinephrine and serotonin displaced the flow capacity/pressure curve to the right up till at least 112 mm Hg, indicating a displacement of the autoregulatory range to the right.

AS AN INCREASING NUMBER of biological substances such as acetylcholine, serotonin (5-HT), norepinephrine (NE) are known to influence vascular tone, the question arises as to what extent vasoactive agents may influence the normal autoregulatory responses. In the cerebrovascular system it is at present only the perivascular sympathetic nerves that have been found to modulate the upper and lower limits of autoregulation.1 2

In order to address this fundamental issue and to establish a convenient model we have studied the mechanical reactions of isolated feline cerebral arteries to 5-HT and NE at conditions of changes in intraluminal pressure.

Methods

Preparation and Mounting

Cats of either sex were anaesthetised by intraperitoneal administration of sodium pentobarbital (30 mg/kg), and sacrificed by exsanguination and decapitation. The skull was opened, the brain removed, placed in a Petri dish and soaked in a cold Krebs buffer solution. The middle cerebral arteries and pial arteries distributing from these vessels were subsequently removed and placed in a glass beaker containing the cold buffer solution. With the aid of an operating microscope, arterial segments were dissected free and placed in a glass beaker containing the cold buffer solution. Part of the material was used in the experiments immediately, whereas the rest was stored in a refrigerator (+4°C) for up to 24h.

Vessel segments of approximately 4 mm in length and 400 μm in diameter were mounted in 2.5 or 5 ml temperature-controlled organ baths containing a system of L-shaped metal holders for recording of isometric circular contractions (wire diameters 100 or 200 μm). Contractions were measured by means of Grass FT-03 transducers, amplified and recorded on a Grass polygraph.

The tissue baths contained a Krebs buffer solution of the following composition (mM): NaCl, 119; KCl, 4.6; CaCl₂, 1.5; MgCl₂, 1.2; NaHCO₃, 15; NaH₂PO₄, 1.2 and glucose 11.0. In experiments involving a calcium free solution, CaCl₂ was omitted from the standard Krebs solution and EGTA 10 μM added. The bath and stock solutions were kept at 37°C and aerated continuously with a mixture of 95% O₂ and 5% CO₂ to maintain a pH of 7.4. Chemicals used were of analytical grade.

Experimental Procedure

The arterial preparation were not subjected to any load during the equilibration period (1–1 ½ hrs). After this period the parameters mentioned below were measured in conjunction with stretching the vessels (giving more tone) and administration of drugs. During the experiments two parameters were measured repeatedly: The distance between the outer limits of the wires (f), and the force recorded by the transducer (F). The internal circumference (L) of the vessel was calculated as follows:

\[ L = 2f + d (\pi - 2) \]

\[ (d = \text{diameter of the wires}) \]

The internal radius \( r = \frac{2f + d (\pi - 2)}{2\pi} \)

The circumferential wall tension (WT) was calculated from the formula

\[ \text{WT} = \frac{F}{2e} \]

\( (e = \text{length of vessel segment}) \)

The transmural pressure (p) was calculated from Laplace’s equation:

\[ p = \frac{\text{WT}}{r} \]

In addition to the transmural pressure, the recorded variables (f and F) also allow an estimation of the flow characteristics of the vessel segment.
Poiseuille's formula for laminar flows in tubes is used:

\[
\frac{dV}{dt} = \frac{\pi \times (P_2 - P_1)}{8 \varepsilon L} \times r^4.
\]

\(V = \text{volume}, \ t = \text{time}, \ \varepsilon = \text{viscosity}, \ P_1 = \text{pressure at the beginning of the tube}, \ P_2 = \text{pressure at the end of the tube}, \ L = \text{length of the tube}\).

By calculating \(\frac{dV}{dt}\) we can describe the effects of lummen size and intraluminal pressure on the flow characteristics of the vessel segment seen in isolation, disregarding all the other influences on cerebral perfusion which operate in vivo.

For this purpose we can, for instance assume that the pressure at the end of the tube \((P_2)\) is near zero, provided the imaginary tube is long enough, and the length of the tube \(L\), as well as the viscosity of the fluid, are constants.

Poiseuille's formula can then be simplified to

\[
\frac{dV}{dt} = K \times P_1 \times r^4 \quad (K \text{ is a constant}).
\]

or, by substitution of \(P_1\) by \(\frac{WT}{r}\) (Laplace's equation:

\[
\frac{dV}{dt} = K \times \frac{WT}{r} \times r^4,
\]

where \(K\) is an unknown constant.

We will then define \(K \times \frac{WT}{r} \times r^4\) as the flow capacity of the vessel segment. The flow capacity (choosing an arbitrary constant) can be presented in a diagram as a function of the transmural pressure \(P = \frac{WT}{r}\).

This will allow us to compare the effect of for instance different vasoactive agents on flow capacity on a given vessel segment at different transmural pressures from the recorded values of \(f\) and \(F\) during the experiment.

**Drugs**

One aim of the investigation has been to evaluate the effect of potent vasoactive agents such as 5-HT and NE on the flow capacity pressure relationship. These agents were added to the Krebs solution in the tissue bath at different resting tensions \((P): (0.5, 1, 2, 4, 8 \text{ mN}),\) and in a few instances 16 mN achieved by varying the distance between the wires \((1 \text{ mN} \text{ is the force giving 1 g an acceleration of 1 m per sec}^2)\).

The following drugs were used: serotonin (5-HT) and norepinephrine (NE) (both Sigma) and nimodipine (Bayer). These substances were dissolved in 0.9% saline. The final concentrations of the drugs in the tissue baths were \(10^{-6}\text{M}, 10^{-5}\text{M}, \) and \(10^{-4}\text{M}\) respectively. Previous experience with this in vitro model has shown that the concentrations of 5-HT and NE eliciting half maximum contraction at a resting tension of 4 mN are \(3.2 \pm 1.1 \times 10^{-6}\text{M}\) and \(7.5 \pm 2.1 \times 10^{-7}\text{M}\) respectively.

**Results**

By gradually increasing the distance between the wires \((and \ r)\), the passive tone \((WT)\) in Krebs solution is increased. At low values of \(r\) (around 0.3 mm) this increment is small, at higher values of \(r\) the increment is higher. For comparison, the wall tension is considerably lower in Ca++ free medium (fig. 1). This finding is in accordance with the results of Högestätt et al. The active tone achieved by addition of 5-HT and NE is influenced by the radius of the vessel and the resulting passive tone. The maximal difference between active and passive tone is achieved at a passive WT of 2 mN/mm when 5-HT and NE are added to the bath. This difference is essentially unchanged by increasing the WT to 4 mN/mm (figs. 1 and 2).

The calculated flow capacity transmural pressure curves were similar for the two vasoactive agents tested. An example is shown in figure 3:

The curve is displaced to the right up till at least about 15 mN/mm² (corresponding to 112.5 mm Hg) after activation. On the other hand, Ca++ deprivation displaces the curve to the left.

**Discussion**

The present study has confirmed earlier work showing that the vasoconstrictor effect of NE increases with increasing resting tension of the vessel wall up to a wall tension of about 2 mN/mm in feline pial arteries. It is apparent from the present study that the vasoconstrictor effect of 5-HT similarly reaches a maximum at about 2 mN/mm and then is nearly constant up till at least about 4 mN/mm.

![Figure 1](http://stroke.ahajournals.org/)

**Figure 1.** Passive and serotonin (5HT) induced active wall tension (WT) as a function of vessel radius \((r)\).
The present technique is not a direct test of autoregulation of pial vessels since the narrowing of the vessel lumen in response to increased pressure is prevented. It does, however, allow an estimation of relative flow capacity of the vessel at different diameters and intraluminal pressures by a simple mathematical calculation. It therefore becomes possible to compare the flow capacity of the vessel seen in isolation at different intra-arteries do possess autoregulation. Stromberg and Fox and MacKenzie et al observed that feline pial arteries

Before discussing the models' relevance to the analysis of the regulation of cerebral perfusion and its autoregulation it is important to consider whether these arteries do possess autoregulation. Stromberg and Fox and MacKenzie et al observed that feline pial arteries

with diameters between 50 and 450 μm dilated with decreases and constricted with increases in systemic blood pressure. They also noted that the difference between aortic blood pressure and pressure in the largest pial surface arteries became greater at higher systemic blood pressure. This finding has been confirmed by Shima et al. Similarly, it has been found that calf middle cerebral arteries do exhibit an autoregulatory response in an in vitro model. Thus, even if the smaller arteries and arterioles account for a greater proportion of the vascular resistance and involvement in its regulation, the participation of the larger pial arteries in the autoregulatory process seems well documented. In contrast, the role of large inflow vessels proximal to the circle of Willis is debatable.

The finding that feline middle cerebral arteries do constrict at rather high transmural pressures as a response to 5-HT as well as NE does, of course, not imply that these transmitters are involved in the autoregulatory response itself. But the observation is in agreement with the finding that sympathetic stimulation tends to displace the normal autoregulation range to the right, as the autoregulation curve becomes less steep. The norepinephrine induced vasoconstriction would therefore tend to displace the breakthrough point of the autoregulation curve to the right. The effect of 5-HT is similar to that of NE suggesting that the newly discovered serotonergic innervation of pial arteries and arterioles with fibres emanating from the dorsal raphe nuclei may serve as an intrinsic regulatory system capable of adjusting the autoregulatory curve.

**References**

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**Figure 2.** Passive and norepinephrine (NE) induced active wall tension as a function of vessel radius.

**Figure 3.** Flow capacity/pressure characteristics of isolated pial pressure. Effect of serotonin (SHT) and Ca^2+ deprivation.

**Diagram:**

- **Flow capacity/pressure characteristics of isolated pial pressure. Effect of serotonin (SHT) and Ca^2+ deprivation.**

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