Humoral Responses of Smooth Muscle from Rabbit Subarachnoid Artery Compared to Kidney, Mesentery, Lung, Heart, and Skin Vascular Smooth Muscle

BY THORALF M. SUNDT, JR., M.D., AND R. K. WINKELMANN, M.D.

Abstract:

Smooth muscle strips from rabbit subarachnoid arteries did not respond to catecholamines in concentrations which caused strips from skin, mesentery, and kidney vessels to contract. The strips from subarachnoid arteries responded to serotonin, histamine, and angiotensin II but not to bradykinin, acetylcholine, methacholine, and adenosine phosphate compounds. Heart and lung vascular strips did not respond to catecholamines but responded to acetylcholine; cardiac strips responded to dilute methacholine. Lung strips often failed to respond to histamine in usual concentrations and did respond to bradykinin. The subarachnoid strips had a distinctive pattern of response as compared to vascular tissue from other organs. Results of other isolated vessel studies are reviewed along with pertinent investigations of cerebral autoregulation, vasospasm, and autonomic nerves. The possible significance of these findings is discussed.

Additional Key Words: cerebral autoregulation, autonomic nervous system, catecholamines, vasospasm, serotonin, subarachnoid hemorrhage.

Subarachnoid arteries might be expected to have a unique responsiveness because of their distinctive structure. They have a thinner intima and media than do vessels of comparable size elsewhere in the body. Although they have a thick internal elastic membrane of considerable importance, they lack an external elastic membrane and are largely surrounded by cerebrospinal fluid. In common with vessels elsewhere in the body, they have a rich autonomic nerve supply; however, the function of this nerve supply in the control of cerebral circulation has yet to be identified.

Bohr et al. found a variety of responses of vascular smooth muscle under different conditions of investigation. In some species there was an absence of response to catecholamines in cerebral vessels and a response to...
serotonin but, in cerebral vessels with myogenic tone, catecholamines could exert a modulating effect. 5

The present work was designed to study, in a species with minimal myogenic tone, the pattern of responsiveness of subarachnoid artery vascular smooth muscle to a variety of vasoconstrictive agents as contrasted with arteries from other relatively large beds. The species differences in response from region to region are discussed, and the data are analyzed and interpreted with existing studies of vascular smooth muscle, spasm, and autoregulation. We observed a specific pattern of response for rabbit subarachnoid vessel strips as contrasted to the distinct response patterns noted in vascular strips from skin, mesentery, kidney, heart, and lung.

Methods
We utilized the helical strip technique as modified by Sams and Winkelmann. 8 All specimens were from rabbits. We chose the basilar subarachnoid artery because this artery traverses a relatively long distance along the brain stem without substantial decrease in size. In the rabbit, this artery ranges from 200 μ to 400 μ in diameter. Arterial segments utilized from other tissues had similar diameters and were from the skin, heart, mesentery of the small intestine, kidney, and lung. Cutaneous vessels were from the branches of the median artery of the ear.

The dissected vessel was cut into a helical strip and suspended in a vertical perfusion bath containing physiological saline. The circular muscle of the arterial wall is so oriented in such strips that contraction of the muscle produces a decrease in the length of the strip. The strip contractions were detected by a Grass force-displacement transducer, and the responses were displayed, after amplification, through a Grass 5-D polygraph. An initial tension of 100 mg was used on the strips during an equilibration period of two hours. This equilibration was necessary to maximize the phasic changes with specific agents. The physiological salt solution bathing the vessels was at pH 7.35 and had the following millimolar composition: NaCl 118.9; KCl 4.7; KH₂PO₄ 1.2; MgSO₄ 1.2; NaHCO₃ 14.9; glucose 5.6; CaCl₂ 2.5; sucrose 49.9; and ethylenediaminetetraacetic acid (EDTA) 0.026. The vessels were maintained at 37°C by circulation of warm water around the perfusion vessel. The physiological salt solution was oxygenated in a warm reservoir before introduction into the perfusion bath.

The vascular strips were tested against epinephrine, norepinephrine, histamine, serotonin (5-hydroxytryptamine), acetylcholine, methacholine, angiotensin, bradykinin, adenosine-3',5' cyclic monophosphate (cyclic AMP), adenosine diphosphate (ADP), and adenosine triphosphate (ATP). Thresholds were determined and the strength of contraction, compared to contractions in response to standard potassium chloride solution, was measured. Frequently, one subarachnoid artery strip was compared directly to a strip from one of the other vascular beds in the same chamber. The vessels tested were obtained from a series of ten rabbits. Forty strips from subarachnoid arteries were tested and compared with 15 vascular strips from each of the other areas. Concentrations of the stimulating agents were very dilute at first and were increased stepwise to the final highest concentration indicated.

Results

SUBARACHNOID VESSELS
These vascular smooth muscle strips responded poorly or not at all under the conditions of this study to catecholamine in concentrations which caused contraction of other organ vascular smooth muscle (table 1). Thirty-three of 40 strips did not respond at all to epinephrine up to 3 × 10⁻⁷ gm/ml. The seven responsive strips

<table>
<thead>
<tr>
<th>Vessel source</th>
<th>Epinephrine (10⁻⁸ gm/ml)</th>
<th>Norepinephrine (10⁻⁸ gm/ml)</th>
<th>Isoproterenol (10⁻⁷ gm/ml)</th>
<th>Serotonin (10⁻⁶ gm/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.6</td>
</tr>
<tr>
<td>Lung</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3.0</td>
</tr>
<tr>
<td>Heart</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3.0</td>
</tr>
<tr>
<td>Kidney</td>
<td>2.0</td>
<td>1.8</td>
<td>2.0</td>
<td>1.2</td>
</tr>
<tr>
<td>Mesentery</td>
<td>3.0</td>
<td>4.8</td>
<td>2.0</td>
<td>0.9</td>
</tr>
<tr>
<td>Skin</td>
<td>1.0</td>
<td>1.0</td>
<td>2.0</td>
<td>1.4</td>
</tr>
</tbody>
</table>

*0 means no response.
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TABLE 2

Thresholds for Response of Strips of Rabbit Vessels to Various Agents

<table>
<thead>
<tr>
<th>Vessel source</th>
<th>Histamine (10^{-5} gm/ml)</th>
<th>Bradykinin* (10^{-5} gm/ml)</th>
<th>Angiotensin II* (10^{-5} gm/ml)</th>
<th>Acetylcholine (10^{-5} gm/ml)</th>
<th>Methacholine (10^{-5} gm/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>2</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lung</td>
<td>0 (9/16)†</td>
<td>+ (10/18)</td>
<td>+</td>
<td>2.0</td>
<td>0</td>
</tr>
<tr>
<td>Heart</td>
<td>3.2</td>
<td>0</td>
<td>+</td>
<td>2.0</td>
<td>0.048</td>
</tr>
<tr>
<td>Kidney</td>
<td>1.9</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mesentery</td>
<td>2.0</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Skin</td>
<td>0.7</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Shown only as presence or absence of response.
†In parentheses, number responding/number tested.

gave a minimal response to an average dose of 5 \times 10^{-6} \text{ gm/ml}. This is 1,000 times higher than the threshold previously determined for rabbit kidney and skin vascular strips. Twenty-nine of 40 strips did not respond to norepinephrine at concentrations up to 3 \times 10^{-5} \text{ gm/ml}. The 11 which responded did so at an average of 9 \times 10^{-6} \text{ gm/ml}. Isoproterenol failed to give a response in six of nine strips tested; three minimal responses occurred at 2 \times 10^{-5} \text{ gm/ml}.

Serotonin (5-hydroxytryptamine) produced a contraction in almost all vessels studied; only three strips failed to give a response. The threshold response varied widely, from 10^{-5} to 10^{-4} \text{ gm/ml}. The average was 1.6 \times 10^{-5} \text{ gm/ml} and was similar to the response of other organ vascular tissue.

The preparations responded to histamine at 10^{-5} \text{ gm/ml} and to angiotensin II at 10^{-4} \text{ gm/ml} (table 2). No response to acetylcholine at 10^{-5} \text{ gm/ml}, methacholine at 10^{-6} \text{ gm/ml}, or bradykinin at 10^{-6} \text{ gm/ml} was observed. Of the adenosine phosphate compounds, AMP and

FIGURE 1

Response of rabbit brain and skin arterial strips to adenine nucleotides.
ADP caused no response but ATP caused a contractile response in seven of 30 strips at $2 \times 10^{-3}$ gm/ml (fig. 1). Cyclic AMP produced no response in brain vessel strips and only a rare response in skin strips (table 3).

**MSENTERY, SKIN, AND KIDNEY VESSELS**

These vessels responded similarly and vigorously to concentrations of catecholamines which did not cause cerebral vessel response (fig. 2). Serotonin gave comparable positive responses in vascular tissues from all sources (table 1). Results with histamine, bradykinin, angiotensin II, acetylcholine, and methacholine were similar in mesentery, skin, and kidney vascular strips (table 2). The mesentery and skin strips were very responsive to ADP; the kidney, mesentery, and skin strips were very responsive to ATP (table 3). Cyclic AMP caused no response in mesentery and kidney strips.

**HEART AND LUNG**

These vascular strips were unique in that epinephrine and norepinephrine did not cause contraction at $10^{-4}$ gm/ml (fig. 2). Isoproterenol caused no response at $10^{-5}$ gm/ml. Serotonin caused contraction at $10^{-5}$ gm/ml, as did angiotensin II. The response to histamine in the cardiac vascular strips was comparable to that in skin, brain, kidney, and mesentery strips (table 2). However, the lung strips did not respond to histamine in comparable concentration (fig. 3); at a ten-fold increase in concentration, only 7 of 16 strips gave a positive contraction with histamine. Bradykinin produced a response at $10^{-6}$ gm/ml only in lung vascular tissue, but only half (10 of 18) of the strips responded. All the other vascular strips failed to respond at $10^{-5}$ gm/ml.

**Discussion**

To interpret the data from this report, it is necessary to review briefly investigations pertinent to the control of cerebral circulation and its alterations in cerebral infarction and subarachnoid hemorrhage.

**CEREBRAL AUTOREGULATION**

That total cerebral blood flow remains relatively constant throughout a wide range of body activities and peripheral blood pressures in health is seemingly well established. Regional flow probably depends on local metabolic demands. This ability of the brain to control its own blood supply is referred to as "cerebral autoregulation" and, when functional, it has been shown to be acutely sensitive to alterations in $P_{\text{CO}_2}$. When cerebral autoregulation is impaired, as in subarachnoid hemorrhage or cerebral infarction, cerebral blood flow no longer is acutely reactive to alterations in $P_{\text{CO}_2}$ and becomes acutely sensitive to alterations in peripheral blood pressure, blood volume, and cardiac output.

The exact manner in which $P_{\text{CO}_2}$ alters brain blood flow is yet to be clarified. There is

<table>
<thead>
<tr>
<th>Vessel source</th>
<th>Adenosine-3',5' cyclic monophosphate</th>
<th>Adenosine-3' diphosphate</th>
<th>Adenosine-3' triphosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>0</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>Lung</td>
<td>0</td>
<td>+</td>
<td>+ +</td>
</tr>
<tr>
<td>Heart</td>
<td>+</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>Kidney</td>
<td>0</td>
<td>+</td>
<td>+ +</td>
</tr>
<tr>
<td>Mesentery</td>
<td>0</td>
<td>+ +</td>
<td>+ + +</td>
</tr>
<tr>
<td>Skin</td>
<td>+</td>
<td>+ + +</td>
<td>+ + + +</td>
</tr>
</tbody>
</table>

*+ = < 25% responding; ++ = 25 to 50% responding; +++ = 51 to 75% responding; ++++ = 76 to 100% responding.*
some evidence that there is a central regulator in the brain stem which is sensitive to alterations in $P_{CO_2}$. However, most investigators think that $P_{CO_2}$ acts by changing local extracellular tissue pH. Luxury perfusion or reactive hyperemia after restitution of flow to areas of ischemia has been shown to correlate with a tissue lactic acidosis.

There is some speculation that $P_{CO_2}$ alters blood flow through reflex activity with cerebral autonomic nerves, and Fraser et al. have shown that $\alpha$ blocking agents do in fact alter the responsiveness of cerebral vessels to alterations in $P_{CO_2}$, suggesting a reflex through the sympathetic nervous system. An alternate hypothesis would be the release of a local humoral agent, in response to metabolic alterations, which in turn would act through a receptor mechanism on the small vessels. Our present state of knowledge has not identified such a humoral agent, but obviously it should be one that produces a strong reaction on cerebral vessels, such as serotonin. The lack of response to acetylcholine makes this less attractive as a mediating factor.

**CEREBRAL AUTONOMIC NERVES**

The work of Forbes and Cobb and Penfield over three decades ago demonstrated the...
presence of cerebral autonomic nerves. However, it was not until the recent availability of fluorescent techniques and electron microscopy that this vast network was fully appreciated. Nelson and Rennels have shown, with electron microscopy, the presence of granulated vesicles throughout the entire pial microcirculation. Fraser et al. and Peerless and Yasargil have demonstrated, in separate species, the loss of catecholamines from these granulated vesicles after subarachnoid hemorrhage. The loss of this fluorescence also has been demonstrated after cervical sympathectomy. It appears then that a subarachnoid hemorrhage in effect produces an end-organ sympathectomy.

The function of the cerebral autonomic nervous system is yet to be clarified. However, such a rich adrenergic nerve supply implies a functional role. It has been shown that cerebral vascular resistance can be altered by both \( \alpha \) and \( \beta \) adrenergic agents, implying a modulation of cerebral vascular tone by these drugs. It is not known whether such an alteration is a physiological response or a response derived from the conditions of the experiment. However, it must be considered that there is a modulating effect on cerebral vessels with an intact autonomic nerve supply.

An important role of the sympathetic nervous system in the body as a whole is its buffering action on circulating catecholamines. Most circulating catecholamines are taken up by the intact granulated vesicles. When such granulated vesicles are impaired, as with sympathectomy and, in the case of the cerebral circulation, a subarachnoid hemorrhage, the Cannon effect ensues with a sensitization of these vessels to circulating catecholamines. It is entirely possible, as previously suggested, that a primary function of the cerebral autonomic nervous system is to buffer against the circulating catecholamines and protect the cerebral circulation from their vasoconstrictive action. The sensitization of vessels to catecholamines after sympathectomy persists for 10 to 14 days in the extremities and it is probable that a similar period obtains in the brain. During this interval, a sympathectomized end-organ will be hyperactive to circulating catecholamines.

That the sympathetic nervous system has little major control on cerebral blood flow after stimulation in no way contradicts the above thesis. This system could exert some modulating effect on myogenic tone (to be discussed below) and serve primarily as a buffer when functional. When damaged or destroyed, as in head trauma or subarachnoid hemorrhage, the entire system might then become vulnerable. In this setting the system could become hypersensitive to a variety of humoral substances in the subarachnoid space which could act as direct vasoconstrictors and also potentiate catecholamines. Our results indicate that serotonin might be one of these agents.

**CEREBRAL VASOSPASM**

An immense amount of research on the nature of cerebral vasospasm has provided a firm foundation for future studies. However, the exact nature of cerebral vasospasm has yet to be elucidated. It seems clear at this point that there is: (1) a loss of autoregulation with subarachnoid hemorrhage and vasospasm; (2) a loss of fluorescence of adrenergic nerve endings after subarachnoid hemorrhage; (3) some evidence that \( \alpha \) adrenergic blocking agents are useful in the treatment of vasospasm; (4) evidence that \( \beta \) adrenergic drugs are helpful in cerebral vasospasm in combination with lidocaine hydrochloride; (5) a difference between mechanical vasospasm and humoral vasospasm; and (6) a humoral agent present in blood that is liberated after a subarachnoid hemorrhage and produces profound and generalized vasospasm.

**IN VITRO STUDIES**

Bohr and his group have completed a variety of delicate experiments on isolated resistance vessels, utilizing primarily two models. One model involves direct recording of changes in tension or length of an isolated strip or ring of vascular smooth muscle, similar to the method of the present investigation. In the other model they investigated the reactivity of single isolated resistance vessels 50 \( \mu \) to 250 \( \mu \) in outside diameter. The helical strips were from arteries 250 \( \mu \) to 500 \( \mu \) in outside diameter.

**Isolated Resistance Vessel Perfusion Studies**

With this model they found myogenic tone in certain vascular beds but not in others. There was some species variability. It is important to note that they found no myogenic tone in the brain vessels of the rabbit, in contrast to the presence of myogenic tone in the brain vessels.
HUMORAL RESPONSES OF SMOOTH MUSCLE

of the rat, dog, and, most importantly, monkey. Spontaneous myogenic tone was more prevalent in smaller vessels (50 μ to 100 μ in outside diameter) than in larger ones. It was postulated that vessels smaller than 50 μ in outside diameter might have more myogenic tone than the ones investigated but arterioles of that caliber could not be studied. Of the species investigated, myogenic tone appeared to be most prominent in the rat and least prominent in the rabbit.

The property of intrinsic myogenic tone in the vascular smooth muscle was an important component of total peripheral resistance. Cerebral vessels in the species investigated dilated with smooth muscle relaxants, such as isoproterenol or papaverine, and constricted in response to injection of epinephrine, norepinephrine, or potassium chloride. When plasma was added to the muscle bath, vasoconstriction occurred in cerebral vessels. This vasoconstrictive action was not blocked by phenoxybenzamine. The plasma appeared to potentiate the constrictor action of catecholamines, a likely phenomenon reproduced in subarachnoid hemorrhage.

They found a definite dependence of the response on the perfusion pressure. At low perfusion pressure there was an increased response to vasoconstrictive agents, and at very high perfusion pressures there was a decreased response.

Several possible mechanisms were suggested by these authors to explain the presence or absence of myogenic tone. It was their judgment that the difference between tonically and nontonically contracted vascular smooth muscle was related to more dependency on extracellular calcium concentration in the smooth muscles that develop spontaneous tone. They thought that in this latter group there was probably greater cell membrane permeability to calcium, explaining the difference in excitation-contraction coupling, and that the vasodilators used might act either by stabilizing the membrane or by improving calcium extrusion. Vessels without myogenic tone would show no response to vasodilators, consistent with the results in our study, and such vessels also might be expected to have minimal response to catecholamines if these act primarily by modulating basic myogenic tone.

Spiral Strip Studies
Bohr and colleagues found, in the dog, only minimal response to catecholamines but consistent response to serotonin, in agreement with the results of the present investigation. In their work, a tension of 100 mg to 200 mg was applied to a resistance vessel strip and of approximately 500 mg to a preparation from a large vessel. These tensions produced optimal phasic responses, but it should be noted that the properties studied in this type of experiment are different from those studied in the myogenic tone preparation. Their results suggested to them that consistent differences existed between the responses of vascular smooth muscle in vivo and those obtained in vitro: "the preparation, as it is studied in the isolated bath, although under tension is relaxed, while vascular smooth muscle in vivo is known to be tonically contracted." It was thought that analysis of differences between in vivo and in vitro responses should lead to knowledge relevant to the evaluation of environmental influences in vivo, such as might reside in the plasma or in parenchymal metabolites.

The work of Uchida and Bohr indicates that it is possible for catecholamines to modulate vascular myogenic tone independently from the effect these vasoconstrictive agents have in a phasic response. Therefore, the rabbit vessels were selected for our study because of their lack of myogenic tone. These strips usually have negligible inherent active tone and do not show rhythmic contractions. This made them especially well suited for quantitative studies with stimulating drugs, since the variable influence of tone and rhythmic contractions on response did not have to be evaluated.

CORRELATION OF PRESENT STUDY WITH AVAILABLE DATA
The results of the present study indicate that, in vitro, cerebral vessels respond differently from vessels elsewhere in the body. Under the limits imposed by this investigation, active vascular smooth muscle contraction was evaluated rather than alterations in basic intrinsic myogenic tone. The various humoral agents tested were those that have been suggested as cerebral vasodilators or incriminated as vasoconstrictors in a pathological setting. Also investigated were humoral agents which might
serve as transmitter substances in the physiological state to produce changes in blood flow by small vessel constriction. These vessels were sensitive to serotonin and much less so to the other agents.

It appears that, although the sympathetic system might exert some modulating effect on myogenic tone in different physiological states, it does not produce a profound change in cerebral blood flow without having significant potentiation by local humoral or ionic changes. The sympathetic nervous system might serve in part as a homeostatic mechanism which guards against circulating catecholamines and holds the cerebral circulation in a state of mild neurogenic stimulation. In states of severe generalized vasospasm, this vasoconstrictive action cannot by itself be the sole explanation for the marked vessel reactivity.

Of all the agents studied, serotonin appeared to be the most active vasoconstrictor. The literature indicates the sensitization of ischemic vessels to serotonin, and it is possible that, in the state of ischemia, serotonin could potentiate the action of catecholamines or by itself be a profound vasoconstrictor. It is found not only in the brain but in circulating platelets; in areas of decreased flow with platelet aggregation, serotonin might easily be released from the brain or platelets (or both) and explain the areas of focal pallor which develop.

The rabbit brain vessel strips have an amine receptor (serotonin, histamine) and a peptide receptor (angiotensin II). Unlike other vascular smooth muscle beds, they lack, or possess in some inhibited form, a catecholamine receptor responsive to physiological or even low pharmacological amounts of catecholamine. Study of the special receptor responses of this vascular smooth muscle and its modulation by \( \alpha \) and \( \beta \) blocking agents, vasodilating agents, and other humoral mediators such as prostaglandin may provide a direct means for deciding which receptors are important in vivo in normal and pathological cerebral events.

References


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