Effects of Forskolin on Cerebral Blood Flow: Implications for a Role of Adenylate Cyclase

DOUGLAS G. WYSHAM, M.D., ABIGAIL F. BROTHERTON, PH.D., AND DONALD D. HEISTAD, M.D.

SUMMARY. We have studied cerebral vascular effects of forskolin, a drug which stimulates adenylate cyclase and potentiates dilator effects of adenosine in other vascular beds. Our goals were to determine whether forskolin is a cerebral vasodilator and whether it potentiates cerebral vasodilator responses to adenosine. We measured cerebral blood flow with microspheres in anesthetized rabbits. Forskolin (10 μg/kg per min) increased blood flow (ml/min per 100 gm) from 39 ± 5 (mean ± S.E.) to 56 ± 9 (p < 0.05) in cerebrum, and increased flow to myocardium and kidney despite a decrease in mean arterial pressure. Forskolin did not alter cerebral oxygen consumption, which indicates that the increase in cerebral blood flow is a direct vasodilator effect and is not secondary to increased metabolism.

We also examined effects of forskolin on the response to infusion of adenosine. Cerebral blood flow was measured during infusion of 1–5 μM/min adenosine into one internal carotid artery, under control conditions and during infusion of forskolin at 3 μg/kg per min i.v. Adenosine alone increased ipsilateral cerebral blood flow from 32 ± 3 to 45 ± 5 (p < 0.05). Responses to adenosine were not augmented during infusion of forskolin. We conclude that 1) forskolin is a direct cerebral vasodilator and 2) forskolin does not potentiate cerebral vasodilator responses to adenosine.

FEW DRUGS that are administered intravenously are effective cerebral vasodilators. Forskolin is a potent vasodilator in the coronary bed1 and in other vascular beds.2 Because forskolin is lipid soluble3 and presumably able to cross the blood-brain barrier, we speculated that forskolin may be a cerebral vasodilator.

Forskolin has been reported to increase the intracellular concentrations of cyclic AMP by direct activation of the catalytic subunit of adenylate cyclase4 and/or by interaction with the guanine nucleotide regulatory subunit.5,6 Forskolin stimulates adenylate cyclase in the brain in vitro and, at low concentrations, it potentiates the effect of catecholamines, vasoactive intestinal peptide, PGE2, and adenosine.7 Forskolin has positive inotropic and chronotropic effects in the heart.8 and, in low doses, it also potentiates the direct coronary vasodilator action of adenosine.1 These data suggest that forskolin may be a useful agent to probe the role of cyclic AMP in cerebral vessels.

The purposes of this study were: 1) to determine whether forskolin increases cerebral blood flow independently of cerebral metabolic demands, and 2) to determine whether vasodilator responses to adenosine are potentiated by forskolin in the cerebral circulation, as they are in coronary vessels.1 The effect of forskolin on acetylcholine-induced vasodilation was also studied. Acetylcholine-induced vasodilation may be mediated through cyclic GMP11 while adenosine may cause vasodilation by increasing intracellular cyclic AMP.12 Thus, our hypothesis was that forskolin might potentiate responses to adenosine, but not to acetylcholine.

The study consisted of two protocols. First, we examined effects of intravenous forskolin on regional blood flow, specifically to the cerebrum, myocardium, kidney, and skeletal muscle. Cerebral oxygen consumption also was measured to determine whether effects of forskolin on cerebral blood flow were secondary to changes in cerebral metabolism. Second, we examined effects of adenosine and acetylcholine on cerebral blood flow, with and without simultaneous infusion of forskolin, to determine whether forskolin potentiates the effects of adenosine and acetylcholine.

METHODS

Surgical Approach

New Zealand white rabbits were anesthetized with chloralose (about 80 mg/kg), intubated, and ventilated with room air and supplemental oxygen. Decamethonium (0.3 μg/kg, i.v.) was given for paralysis of skeletal muscle and heparin (500 units/kg, i.v.) was given for anticoagulation. Arterial blood gases and pH were measured frequently during each experiment and maintained at normal levels by adjustment of ventilatory rate and supplemental oxygen, and by injection of small amounts of sodium bicarbonate.

Polyethylene catheters were inserted into both femoral veins for infusion of drugs, through a femoral artery to the aortic arch for monitoring blood pressure, and into both brachial arteries for withdrawal of reference samples during injection of microspheres. After a left thoracotomy, a snare was placed loosely around the descending aorta and a cannula was placed in the left atrium for injection of microspheres.

Measurement of Regional Blood Flow

Regional blood flow was measured with microspheres, as described elsewhere.13 Briefly, between 0.4 and 1.3 million microspheres 15 μm in diameter, labelled with one of six radioactive isotopes, were...
injected into the left atrium over 10 to 20 seconds for each determination of flow. Reference arterial samples were withdrawn at 0.51 or 1.0 ml/min from the brachial arteries, beginning prior to injection of the microspheres and continuing for approximately 2 minutes thereafter. At the end of each experiment, the animal was sacrificed and tissue samples were removed and weighed. Tissue and blood samples were counted in a gamma counter.

Blood flow was computed from the equation $F_s = \left( C_s \times 100 \times RBF \right) / C_r$, where $F_s$ is blood flow to a tissue sample in ml/100 g/min, $C_s$ is counts/gram for the tissue sample, RBF is rate of withdrawal of blood samples from reference arteries, and $C_r$ is the average counts/gram from the two reference blood samples.

**Experimental Protocols**

1. **Effect of forskolin on regional blood flow.** The first injection of microspheres was made during a control period. Vehicle (2% ethanol in saline) then was infused at 0.51 ml/min i.v. for 5 minutes and the second injection of microspheres was made. Forskolin was infused intravenously at 1, 3, and 10 µg/kg per min for 6 minutes, and microspheres were injected 4 minutes after initiation of each dose. Thirty minutes after the last infusion of forskolin, vehicle alone was infused intravenously and a sixth injection of microspheres was made. We minimized hypotension during forskolin infusion by tightening the aortic snare as needed in two rabbits, raising pressure in the upper half of the body. Values for renal blood flow were excluded in these two animals.

2. **Effect of forskolin on cerebral oxygen consumption.** In addition to the surgical approach described above, we inserted a catheter into the dorsal sagittal sinus. A midline scalp incision was made and the cranium was exposed. A burr-hole was made just anterior to the lambdoid suture, exposing the dorsal sagittal sinus. A 25 gauge catheter was inserted into the sinus and held in place by bone wax. Venous blood was withdrawn from this catheter for measurement of oxygen content and calculation of cerebral oxygen consumption.

Venous blood was withdrawn from the dorsal sagittal sinus during control, infusion of the high dose of forskolin, and during the final infusion of vehicle. Arterial blood was collected simultaneously, oxygen content and hematocrit were measured in the samples, and cerebral oxygen consumption was calculated using the formula: $C = (A - V) \times Q/100$, where $C$ is cerebral oxygen consumption in ml/min per 100 grams; $A$ is arterial oxygen content in ml/100 ml of blood, $V$ is venous oxygen content in ml/100 ml of blood, and $Q$ is cerebral blood flow in ml/min/100 grams.

a. **Adenosine and forskolin.** In addition to the surgical approach described above, one external carotid artery was ligated and cannulated with a PE 90 catheter. The catheter was inserted retrogradely to the common carotid artery, and used for unilateral infusion of adenosine.

The protocol consisted of two parts, one with forskolin and one without. The sequence was alternated so that forskolin was infused first in half of the experiments. Forskolin was infused intravenously at 3 µg/kg per min, a dose which has minimal direct cerebral vasodilator effect. The first microsphere injection was made 5 minutes after starting the infusion of forskolin. The forskolin infusion was continued and adenosine was infused into the carotid artery at 1 µM/min. Two minutes later the second microsphere injection was made. Adenosine was infused intra-arterially at 5 µM/min and the third microsphere injection was made. The infusions of forskolin and adenosine were then stopped. About 30 minutes later, the fourth microsphere injection was made. Microspheres were then injected during intra-arterial infusions of low and high doses of adenosine, as described above. Thus, unilateral intracarotid infusion of adenosine was accomplished at two doses, allowing comparison of responses during forskolin and without forskolin.

b. **Acetylcholine and forskolin.** The protocol was identical to the preceding, except that acetylcholine was infused into the carotid artery instead of adenosine. Doses of acetylcholine were 0.1 µg/min and 0.5 µg/min.

**Statistics**

Control values were compared to subsequent values using the two-tailed t-test, with Bonferroni correction for multiple comparisons.

**Results**

**Effect of Forskolin on Cerebral Blood Flow**

Intravenous infusion of forskolin increased blood flow to the brain from 39 ± 5 (mean ± SE) to 56 ± 9 ml/min per 100 gm, despite a small decrease in mean arterial pressure (fig. 1). Blood flow to the myocardium and kidney also increased during forskolin, and flow to the masseter muscle tended to increase but did not.

![Cerebral Blood Flow and Mean Arterial Pressure](image-url)

**FIGURE 1.** Cerebral blood flow and mean arterial pressure during infusion of forskolin. Values are mean ± S.E. in 11 rabbits. Asterisks indicate a value significantly different from control (p < 0.05). $V =$ vehicle, $R =$ recovery.
not achieve statistical significance (fig. 2). Forskolin produced a much greater increase in blood flow to myocardium than to the brain or other organs.

Infusion of high doses of forskolin did not alter cerebral oxygen consumption ($p = 0.57$) (table 1). Cerebral venous oxygen content rose during the infusion and it returned to control values during recovery (table 1).

**Effect of Forskolin on Responses to Adenosine and Acetylcholine**

Unilateral infusion of adenosine produced modest cerebral vasodilatation (table 2). Effects of intra-arterial adenosine were predominantly ipsilateral. Comparison of blood flow to the ipsilateral and contralateral hemispheres were similar with or without intravenous infusion of forskolin.

Acetylcholine also produced cerebral vasodilation, which was not altered by forskolin (table 3). These results indicate that forskolin did not potentiate the response to adenosine or to acetylcholine.

**Discussion**

The important new observations in this study are: 1) forskolin decreases cerebral vascular resistance independently of cerebral metabolic requirements, and 2) forskolin does not potentiate cerebral vasodilator effects of adenosine or acetylcholine. These findings may have implications concerning the role of cyclic AMP in cerebral vasodilatation.

**Consideration of Methods and Design**

Cerebral vasodilators may increase blood flow either by a direct action on vessels or by indirect effects, secondary to increases in cerebral metabolism. We anticipated that forskolin might increase cerebral metabolism because forskolin stimulates cyclic AMP production in cerebrum in vitro. If the increase in cerebral blood flow during forskolin infusion were secondary to increased metabolism, one would expect a proportionate rise in blood flow and oxygen consumption, and cerebral venous oxygen content should not rise. While a small rise in cerebral $O_2$ consumption may have occurred during forskolin infusion, the increase in cerebral blood flow was far out of proportion to this. Thus, our conclusion that forskolin is a direct dilator of cerebral arteries is based on the observations that 1) cerebral oxygen consumption did not change significantly and 2) cerebral venous oxygen content increased.

Recent studies suggest that adenosine is important in regulation of cerebral blood flow. First, brain adenosine concentration rises in response to hypoxia, ischemia, or seizures.14-16 Second, the cerebral vasodilator response to hypoxia can be blocked by adenosine deaminase or by theophylline.14,15 Third, intra-arterial infusion of adenosine causes cerebral vasodilatation.17 In view of these findings, potentiation by forskolin of a cerebral vasodilator response to adenosine might have useful implications in the treatment of cerebral ischemia, where it might augment dilator responses. In contrast to the findings in the coronary circulation,1 however, we did not find that forskolin potentiates the response to adenosine in the cerebral circulation.

Possible explanations for the absence of potentiation by forskolin are that the drugs may act at different sites (e.g., endothelium and vascular muscle), or the blood-brain barrier may limit access of the agonists to the same receptor sites. We can not exclude these possibilities, but because forskolin, acetylcholine, and adenosine all were effective vasodilators when used singly, it is clear that each agonist can penetrate the blood-brain barrier.

If cerebral vessels were maximally dilated by any of the agonists, we would not be able to detect potentiation of vasodilator responses. In order to demonstrate potentiation, doses of acetylcholine and adenosine were chosen which were on the low ends of their respective dose-response curves.17,18 Thus, absence of potentiation of responses by forskolin can not be attributed to examining responses at the high end of the dose-responsive curves.

The explanation for absence of potentiation of responses to adenosine by forskolin may lie in the nature of forskolin's effect on cyclic AMP in arterial smooth muscle. This possibility is discussed in the following section.

**Effect of Forskolin and Adenosine on Adenylate Cyclase**

Forskolin stimulates adenylate cyclase and increases intracellular cyclic AMP levels in several membrane systems.9 We have suggested that forskolin increases

---

**Table 1 Effects of Forskolin on Cerebral Metabolism**

<table>
<thead>
<tr>
<th></th>
<th>Cerebral Blood Flow (ml/min per 100 gm)</th>
<th>Cerebral $O_2$ Consumption (ml $O_2$/min per 100 gm)</th>
<th>Cerebral Venous $O_2$ Saturation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>29.6 ± 3.9</td>
<td>1.66 ± 0.22</td>
<td>45 ± 4.4</td>
</tr>
<tr>
<td>Forskolin</td>
<td>46.6 ± 6.6†</td>
<td>1.84 ± 0.22†</td>
<td>53 ± 5.6†</td>
</tr>
<tr>
<td>(10 $\mu$g/kg per min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recovery</td>
<td>37.2 ± 4.9†</td>
<td>1.84 ± 0.24‡</td>
<td>42 ± 5.0‡</td>
</tr>
</tbody>
</table>

*Values are mean ± S.E. in 5 animals.
†Significantly greater than control ($p < 0.05$) using one-tailed t-test.
‡Not significantly different from control.
TABLE 2  
Effects of Intravenous Forskolin on Cerebral Blood Flow During Infusion of Adenosine into One Carotid Artery*

<table>
<thead>
<tr>
<th></th>
<th>Cerebral Blood Flow (ml/min per 100 gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ipsilateral to Adenosine</td>
</tr>
<tr>
<td>Control</td>
<td>32 ± 2.9</td>
</tr>
<tr>
<td>Adenosine 1 μM/min i.a.</td>
<td>42 ± 3.6</td>
</tr>
<tr>
<td>Adenosine 5 μM/min i.a.</td>
<td>45 ± 4.5</td>
</tr>
<tr>
<td>Control + Forskolin†</td>
<td>40 ± 3.6¶</td>
</tr>
<tr>
<td>Adenosine 1 μM/min i.a. + Forskolin†</td>
<td>41 ± 2.1</td>
</tr>
<tr>
<td>Adenosine 5 μM/min i.a. + Forskolin†</td>
<td>50 ± 3.5</td>
</tr>
</tbody>
</table>

*Values are mean ± S.E. in 9 rabbits.
†Intravenous infusion of forskolin at 3 μg/kg per minute.
‡Difference in blood flow to ipsilateral and contralateral cerebral hemispheres.
§Significant (p < 0.05) difference between the 2 hemispheres using two-tailed t-test.
¶Not significantly different from control using two-tailed t-test (p = 0.07).

In cerebral arteries of the cat in vitro adenosine produces relaxation and increases the concentration of cyclic AMP. Both effects are blocked by a methylxanthine. These results are consistent with the hypothesis that adenosine causes relaxation of cerebral arteries by producing an increase in intracellular cyclic AMP.

The failure of forskolin to potentiate responses to adenosine in the cerebral circulation has several possible explanations. First, although adenosine stimulates cyclic AMP production in vascular muscle cells of cats, a similar response has not been demonstrated in rabbits. Thus, we can not exclude the possibility that, in rabbits, adenosine causes cerebral vasodilation through a mechanism independent of cyclic AMP, that it interacts with R, receptors, and that an effect of forskolin in the presence of adenosine is obscured. A second possibility is that adenosine does not stimulate adenylate cyclase in rabbit cerebral arteries, but it does stimulate the inhibitory P site and thereby attenuates cyclic AMP production by forskolin. A third possibility concerns the guanine nucleotide regulatory subunit (N protein). Forskolin alone may stimulate adenylate cyclase directly, and while adenosine alone may likewise stimulate adenylate cyclase through interaction with the R, receptor coupled with N, input, it is possible that forskolin and adenosine together cause suffi-

TABLE 3  
Effects of Intravenous Forskolin on Cerebral Blood Flow During Infusion of Acetylcholine into One Carotid Artery*

<table>
<thead>
<tr>
<th></th>
<th>Cerebral Blood Flow (ml/min per 100 gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ipsilateral</td>
</tr>
<tr>
<td>Control</td>
<td>38 ± 3.3</td>
</tr>
<tr>
<td>Acetylcholine 0.1 μg/min i.a.</td>
<td>81 ± 21</td>
</tr>
<tr>
<td>Acetylcholine 0.5 μg/min i.a.</td>
<td>98 ± 19</td>
</tr>
<tr>
<td>Control + Forskolin†</td>
<td>39 ± 2.3</td>
</tr>
<tr>
<td>Acetylcholine 0.1 μg/min + Forskolin†</td>
<td>85 ± 18</td>
</tr>
<tr>
<td>Acetylcholine 0.5 μg/min + Forskolin†</td>
<td>112 ± 18</td>
</tr>
</tbody>
</table>

*Values are mean ± S.E. in 7 rabbits.
†Intravenous infusion of forskolin at 3 μg/kg per minute.
‡Difference in blood flow to ipsilateral and contralateral cerebral hemisphere.
§Significant (p < 0.05) difference between the 2 hemispheres using two-tailed t-test.
cient N, input to nullify potentiation of adenylate cyclase by forskolin. This phenomenon has been des-
cribed previously.21

Conclusion
In summary, forskolin has been used to examine mechanisms of cerebral vascular effects. Currently available studies of mechanisms that mediate vascular effects of forskolin do not allow unequivocal conclu-
sions about the role of cyclic AMP in cerebral vessels. Nonetheless, our data, which indicate that forskolin is a cerebral vasodilator, provide evidence that activation of adenylate cyclase may contribute to cerebral vasodi-
latation. Because of the evidence that both forskolin and adenosine stimulate cerebral vasodilatation while increasing intracellular cyclic AMP, the failure of for-
skolin to potentiate responses to adenosine suggests a complex interaction of stimulation and inhibition.

Acknowledgments
We thank Keith Breese and Justine Messier for their technical assis-
tance; Dr. Leon Burmeister for assistance with statistical analyses; Karla Taber and Sara Jedlicka for secretarial assistance; and Drs. Ross Feldman and Alyyn Mark for their critical review of the manuscript.

References
1. Kusachi S, Bugni WJ, Olsson RA: Forskolin potentiates the coro-
nary vasoreactivity of adenosine in the open-chest dog. Circ Res 55:
116–119, 1984
2. Lindner E, Dohadwalla AN, Bhattacharya BK: Positive inotropic and blood pressure lowering activity of a diterpene derivative iso-
lated from coleus forskohli: forskolin. Drug Res 28:
284–289, 1978
33–35, 1982
4. Seamon K, Daly JW: Activation of adenylate cyclase by the di-
terpene forskolin does not require the guanine nucleotide regulatory protein. J Biol Chem 256:
9799–9801, 1981
5. Daly JW: Forskolin, adenylate cyclase, and cell physiology: an overview. Adv Cyclic Nucleotide Res 17:
81–89, 1984
6. Meisler S, Reisine T: Forskolin stimulates adenylate cyclase activ-
ity, cyclic AMP accumulation, and adrenocorticotropin secretion from mouse anterior pituitary tumor cells. J Neurochem 42:
1659–1666, 1984
7. Stengel D, Guenet L, Desmier M, Insel P, Manoune J: Forskolin requires more than the catalytic unit to activate adenylate cyclase. Mol Cell Endocrinol 28:
681–690, 1982
8. Krall JF: A kinetic analysis of activation of smooth muscle adenyl-
ate cyclase by forskolin. Arch Biochem Biophys 29:
492–497, 1984
9. Daly JW, Padgett W, Seamon KB: Activation of cyclic AMP-
generating systems in brain membranes and slices by the diterpene forskolin: augmentation of receptor-mediated responses. J Neuro-
chem 38:
532–544, 1982
212–223, 1984
11. Rapoport RM, Draznin MB, Mund F: Endothelium-dependent re-
laxation in rat aorta may be mediated through cyclic GMP-depen-
dent protein phosphorylation. Nature 306:
174–176, 1983
12. Edvinsson L, Fredholm BB: Characterization of adenosine recep-
tors in isolated cerebral arteries in cat. Br J Pharmacol 90:
631–637, 1983
13. Marcus ML, Heistad DD, Ehrhardt JC, Abboud FM: Total and re-
gional blood flow measurement with 7–10 μm, 15 μm, 25 μm, and 50 μm microspheres. J Appl Physiol 46:
501–507, 1976
14. Emonon Jr TE, Raymond RM: Involvement of adenosine in cere-
bral hypoxic hyperemia in the dog. Am J Physiol 241:
H134–H138, 1981
15. Morri S, Winn HR, Berne RM: Effect of theophylline, an adeno-
sine receptor blocker, on cerebral blood flow (CBF) during rest and transient hypoxia. J Cereb Blood Flow Metab 3 (Suppl 1):
S480–S481, 1983
16. Wei EP, Kontos HA: Role of adenosine in cerebral arterial dilata-
tion from arterial hypoxia. J Cereb Blood Flow Metab 1:
S395–S396, 1981
240:
H775–H780, 1981
18. Heistad DD, Marcus ML, Said SI, Gross PM: Effect of acetylcho-
line and vasoactive intestinal peptide on cerebral blood flow. Am J Physiol 239:
H73–H80, 1980
H737–H744, 1985
20. Wolf J, Londos C, Cooper DMF: Adenosine receptors and the regulation of adenylate cyclase. Adv Cyclic Nucleotide Res 14:
199–214, 1981
21. Seamon KB, Daly JW: Guanosine 5’ (β, α-imido) triphosphate inhibition of forskolin-activated adenylate cyclase is mediated by the putative inhibitory guanine nucleotide regulatory protein. J Biol Chem 257:
11591–11596, 1982
Effects of forskolin on cerebral blood flow: implications for a role of adenylate cyclase.
D G Wysham, A F Brotherton and D D Heistad

Stroke. 1986;17:1299-1303
doi: 10.1161/01.STR.17.6.1299

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1986 American Heart Association, Inc. All rights reserved.
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:
http://stroke.ahajournals.org/content/17/6/1299

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in
Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office.
Once the online version of the published article for which permission is being requested is located, click Request
Permissions in the middle column of the Web page under Services. Further information about this process is
available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org/subscriptions/