

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

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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 $\mu\text{g}/\text{kg}$ per min) increased blood flow (ml/min per 100 gm) from 39 ± 5 (mean \pm 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 $\mu\text{M}/\text{min}$ adenosine into one internal carotid artery, under control conditions and during infusion of forskolin at 3 $\mu\text{g}/\text{kg}$ 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.

Stroke Vol 17, No 6, 1986

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 soluble⁹ 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 cyclase^{4–6} and/or by interaction with the guanine nucleotide regulatory subunit.^{7, 8} 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.⁹ Forskolin has positive inotropic and chronotropic effects in the heart^{2, 10} 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 vasodilatation 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 $\mu\text{g}/\text{kg}$, 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

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This work was supported by a Medical Investigatorship and Research Funds from the Veterans Administration and by National Institute of Health ASCOR HL 14230, Program Project Grant HL 14388, Research Grant HL 16066, and Grant RR59 from the Clinical Research Centers Branch of NIH.

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Received September 17, 1985; revision #1 accepted April 23, 1986.

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 $F_s = (C_s \times 100 \times \text{RBF})/C_r$, 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 $\mu\text{g}/\text{kg}$ 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 for-

skolin and one without. The sequence was alternated so that forskolin was infused first in half of the experiments. Forskolin was infused intravenously at 3 $\mu\text{g}/\text{kg}$ 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 $\mu\text{M}/\text{min}$. Two minutes later the second microsphere injection was made. Adenosine was infused intra-arterially at 5 $\mu\text{M}/\text{min}$ 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 $\mu\text{g}/\text{min}$ and 0.5 $\mu\text{g}/\text{min}$.

Statistics

Control values were compared to subsequent values using the two-tailed t-test, with Bonferoni 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 \pm 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

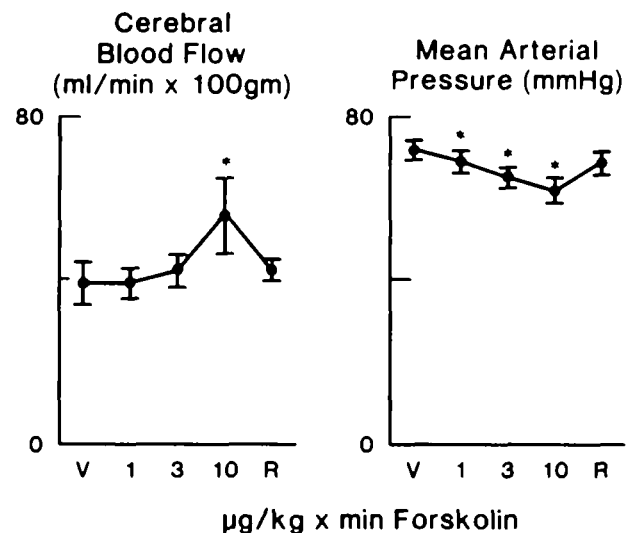


FIGURE 1. Cerebral blood flow and mean arterial pressure during infusion of forskolin. Values are mean \pm S.E. in 11 rabbits. Asterisks indicate a value significantly different from control ($p < 0.05$). V = vehicle, R = recovery.

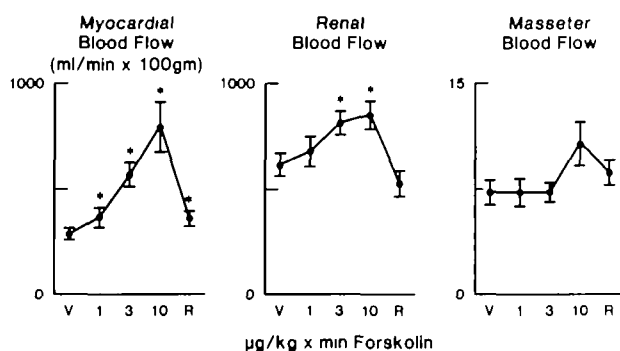


FIGURE 2. Effects of forskolin on myocardial, renal and masseter blood flow. Values are mean \pm S.E. in 11 rabbits. Asterisks indicate a value significantly different from control ($p < 0.05$). V = vehicle, R = recovery.

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 O₂ consumption may have occurred during forskolin infusion, the increase in cere-

bral 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*

	Cerebral Blood Flow (ml/min per 100 gm)	Cerebral O ₂ Consumption (ml O ₂ /min per 100 gm)	Cerebral Venous O ₂ Saturation (%)
Control	29.6 \pm 3.9	1.66 \pm 0.22	45 \pm 4.4
Forskolin (10 μ g/kg per min)	46.6 \pm 6.6†	1.84 \pm 0.22‡	53 \pm 5.6†
Recovery	37.2 \pm 4.9‡	1.84 \pm 0.24‡	42 \pm 5.0‡

*Values are mean \pm 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**

	Cerebral Blood Flow (ml/min per 100 gm)		
	Ipsilateral to Adenosine	Contralateral to Adenosine	Δ Flow \ddagger
Control	32 \pm 2.9	33 \pm 2.2	-1.3 \pm 1.4
Adenosine 1 μ M/min i.a.	42 \pm 3.6	40 \pm 3.7	+1.7 \pm 2.0
Adenosine 5 μ M/min i.a.	45 \pm 4.5	37 \pm 2.3	+8.6 \pm 2.6§
Control + Forskolin \dagger	40 \pm 3.6¶	40 \pm 1.8	+0.4 \pm 2.7
Adenosine 1 μ M/min i.a. + Forskolin \dagger	41 \pm 2.1	37 \pm 1.9	+3.9 \pm 1.1§
Adenosine 5 μ M/min i.a. + Forskolin \dagger	50 \pm 3.5	41 \pm 1.9	+9.2 \pm 1.9§

*Values are mean \pm S.E. in 9 rabbits.

\dagger Intravenous infusion of forskolin at 3 μ g/kg per minute.

\ddagger 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 ($p = 0.07$).

cerebral blood flow independently of cerebral metabolic requirements. Thus, we speculate that forskolin interacts with vascular muscle membrane to increase the intracellular concentration of cyclic AMP in cerebral vessels *in vivo*. Our data, albeit indirect, suggest that increasing intracellular concentrations of vascular cyclic AMP produce cerebral vasodilation.

Forskolin activates adenylate cyclase at a site distal to membrane surface receptors. Although some aspects are uncertain, it is clear that in some systems activation of adenylate cyclase by forskolin does not require the guanine nucleotide regulatory subunit.^{5,6} From available data, it appears that forskolin may activate adenylate cyclase directly, but that interaction with either stimulatory (N_s) or inhibitory (N_i) forms of the guanine nucleotide regulatory subunit may modulate the net effect.⁵ This possible involvement of the N protein is important, because it may help explain the findings in our study in relation to adenosine.

Adenosine apparently exerts its effects both by interaction with a surface receptor (R) and through a receptor on the cytoplasmic surface termed the P site. The R receptor, whether adenylate cyclase stimulatory (R_s) or inhibitory (R_i), may be blocked by methylxanthines.⁵ There is no known specific blocker of the P site, which is always inhibitory to adenylate cyclase.^{19,20}

In cerebral arteries of the cat *in vitro* adenosine produces relaxation and increases the concentration of cyclic AMP.¹² Both effects are blocked by a methylxanthine.¹² 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,¹² 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_i 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_s receptor coupled with N_s 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**

	Cerebral Blood Flow (ml/min per 100 gm)		
	Ipsilateral	Contralateral	Δ Flow \ddagger
Control	38 \pm 3.3	37 \pm 3.1	1.6 \pm 1.4
Acetylcholine 0.1 μ g/min i.a.	81 \pm 21	46 \pm 4.7	35 \pm 18
Acetylcholine 0.5 μ g/min i.a.	98 \pm 19	51 \pm 5.0	48 \pm 15§
Control + Forskolin \dagger	39 \pm 2.3	38 \pm 4.3	1.7 \pm 1.7
Acetylcholine 0.1 μ g/min + Forskolin \dagger	85 \pm 18	47 \pm 4.7	38 \pm 17§
Acetylcholine 0.5 μ g/min + Forskolin \dagger	112 \pm 18	57 \pm 4.8	54 \pm 17§

*Values are mean \pm S.E. in 7 rabbits.

\dagger Intravenous infusion of forskolin at 3 μ g/kg per min.

\ddagger 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_i input to nullify potentiation of adenylate cyclase by forskolin. This phenomenon has been described 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 conclusions 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 vasodilatation. Because of the evidence that both forskolin and adenosine stimulate cerebral vasodilatation while increasing intracellular cyclic AMP, the failure of forskolin to potentiate responses to adenosine suggests a complex interaction of stimulation and inhibition.

Acknowledgments

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

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