FEW DRUGS that are administered intravenously are effective cerebral vasodilators. Forskolin is a potent vasodilator in the coronary bed and in other vascular beds. Because forskolin is lipid soluble 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 and/or by interaction with the guanine nucleotide regulatory subunit. Forskolin stimulates adenylate cyclase in the brain in vitro and, at low concentrations, it potentiates the effect of catecholamines, vasoactive intestinal peptide, PGE$_2$, and adenosine. Forskolin has positive inotropic and chronotropic effects in the heart and, in low doses, it also potentiates the direct coronary vasodilator action of adenosine. 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. The effect of forskolin on acetylcholine-induced vasodilatation was also studied. Acetylcholine-induced vasodilatation may be mediated through cyclic GMP while adenosine may cause vasodilatation by increasing intracellular cyclic AMP. Thus, our hypothesis was that forskolin might potentiates 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. Briefly, between 0.4 and 1.3 million microspheres 15 µm in diameter, labelled with one of six radioactive isotopes, were
were withdrawn at 0.51 or 1.0 ml/min from the bra-
chiual arteries, beginning prior to injection of the mi-
crospheres and continuing for approximately 2 min-
utes thereafter. At the end of each experiment, the
animal was sacrificed and tissue samples were re-
moved 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 con-
trol period. Vehicle (2% ethanol in saline) then was
infused at 0.51 ml/min i.v. for 5 minutes and the sec-
ond injection of microspheres was made. Forskolin
was infused intravenously at 1, 3, and 10 \( \mu \)g/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 micros-
pheres 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 consump-
tion.** 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 cra-
nium 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 oxy-
gen content and calculation of cerebral oxygen con-
sumption.

Venous blood was withdrawn from the dorsal sagi-
tal 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 cere-
bral 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 surgi-
cal approach described above, one external carotid
artery was ligated and cannulated with a PE 90 cath-
eter. The catheter was inserted retrogradely to the com-
mon 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 experi-
ments. Forskolin was infused intravenously at 3 \( \mu \)g/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 \( \mu \)M/min. Two
minutes later the second microsphere injection was
made. Adenosine was infused intra-arterially at 5
\( \mu \)M/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 ac-
complished at two doses, allowing comparison of re-
sponses 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 ade-
osine. Doses of acetylcholine were 0.1 \( \mu \)g/min and 0.5
\( \mu \)g/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 ± SE) to 56 ± 9
ml/min per 100 gm, despite a small decrease in mean
arterial pressure (fig. 1). Blood flow to the myocardii-
um and kidney also increased during forskolin, and
flow to the masseter muscle tended to increase but di
d
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. Second, the cerebral vasodilator response to hypoxia can be blocked by adenosine deaminase or by theophylline. Third, intra-arterial infusion of adenosine causes cerebral vasodilatation. 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, 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. 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. We have suggested that forskolin increases
TABLE 2  Effects of Intravenous Forskolin on Cerebral Blood Flow During Infusion of Adenosine into One Carotid Artery*

|               | Ipsilateral to Adenosine | Contralateral to Adenosine | Δ Flow‡  
|---------------|--------------------------|-----------------------------|----------
| Control       | 32 ± 2.9                 | 33 ± 2.2                    | -1.3 ± 1.4 |
| Adenosine 1 μM/min i.a. | 42 ± 3.6                 | 40 ± 3.7                    | +1.7 ± 2.0 |
| Adenosine 5 μM/min i.a. | 45 ± 4.5                 | 37 ± 2.3                    | +8.6 ± 2.6$ |
| Control + Forskolin† | 40 ± 3.6†                | 40 ± 1.8                    | +0.4 ± 2.7 |
| Adenosine 1 μM/min i.a. + Forskolin† | 41 ± 2.1                 | 37 ± 1.9                    | +3.9 ± 1.1$ |
| Adenosine 5 μM/min i.a. + Forskolin† | 50 ± 3.5                 | 41 ± 1.9                    | +9.2 ± 1.9$ |

*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.

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-
cient N, 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.

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