Cerebral vasodilation and prostacyclin

The effects of aspirin and meclofenamate in vitro

Charles E. Chapleau, M.D., Richard P. White, Ph.D., and James T. Robertson, M.D.
Departments of Neurosurgery and Pharmacology, University of Tennessee Center for Health Sciences, Memphis, Tennessee

The effects of aspirin and meclofenamate on the diphasic responses produced by prostacyclin in isolated canine basilar arteries were compared. Meclofenamate enhanced the relaxant action of prostacyclin in low concentrations (10⁻⁷M to 10⁻⁴M) and also significantly inhibited the contractions caused by the highest concentration (10⁻³M) of this prostaglandin. Aspirin had no such effects. The results indicate that some drugs classified as prostaglandin synthetase inhibitors can directly enhance the vasodilator action of prostacyclin on cerebral arteries, and suggest that this enhancement could be of value in the clinical applications of prostacyclin.

Key Words • cerebral artery • prostacyclin • aspirin • meclofenamic acid • vasodilation

Recent reports indicate that prostacyclin (PGI₂) may function as a physiological cerebral vasodilator, and will inhibit platelet aggregation. On the other hand, many prostaglandins (PG's), such as PGF₂α and platelet thromboxane A₂, cause vasoconstriction and may be associated with cerebral vasospasm as well as thrombotic or embolic stroke. Prostaglandin synthesis inhibitors (PSI's), such as aspirin, are frequently used in the treatment of threatened stroke because of their antiplatelet aggregating properties, but high doses of aspirin may cause cerebral thrombosis by inhibiting prostacyclin production in vascular endothelium. Likewise, in selecting therapeutic agents for the treatment of cerebral vasospasm after subarachnoid hemorrhage, it would seem advantageous to use agents that would not interfere with the protective vasodilating prostacyclin system.

Recent animal studies in our laboratory have demonstrated the efficacy of PSI's in vitro and in vivo in treating cerebral arteriospasm. To clarify whether or not PSI's would also inhibit the dilator action of prostacyclin, the following study on isolated basilar arteries was undertaken.

Materials and Methods

Mongrel dogs of both sexes were anesthetized with intravenous sodium pentobarbital (30 mg/kg) and rapidly exsanguinated via the femoral artery. After brain removal, the basilar artery was dissected and placed in a dish of Krebs-Ringer buffer solution at 37°C. A 4-mm segment was taken from the midportion of the vessel and mounted on rigid parallel prongs with the aid of an operating microscope. The bath chamber used was similar to that described by others except that ours contained 25 ml of buffer. Meticulous handling of the vessel and a time interval of less than 20 minutes from sacrifice to mounting were found to be crucial in obtaining a good preparation. Damage to the arterial smooth muscle was minimized by using the arachnoid mater to handle the segment. The chamber was filled with 25 ml of Krebs-Ringer buffer solution (mM concentration): NaCl 120, KCl 4.5, CaCl₂ 2.5, MgSO₄ 1.0, NaHCO₃ 27.0, KH₂PO₄ 1.0, NaEDTA 0.01, and glucose 10.0. A gas mixture of 95% O₂ and 5% CO₂ was continuously bubbled through the bath chamber and buffer reservoir to provide oxygenation, mixing, and calcium.
Prostacyclin and prostaglandin synthetase inhibitors

stability. Temperature was maintained at 37°C ± 0.05°. The vessel was initially washed frequently, and no agents were tested for at least 2 hours. Early in this period, resting tension was set at 1.0 gm for 1½ hours, and then raised to 3 gm, where it remained for the duration of the experiment. Isometric tension was recorded using a Grass polygraph and a Statham strain gauge transducer held by a fine-positioning device to accurately adjust resting tension.*

Test agents and inhibitors were added in 0.025- to 0.25-ml amounts. Serotonin creatinine sulfate (5-HT) was made fresh daily with deionized water, and prostacyclin was prepared just before use by dissolving it in 0.1 M Tris buffer at pH 9.1 and was kept on ice throughout the experiment. Aspirin and meclofenamate were solubilized in water by the incremental addition of 0.1 N NaOH. The final pH of these solutions was approximately 7.5 and 9.6, respectively. The addition of the vehicle alone to the bath chamber did not affect vessel contractility. Several log dose-response curves for 5-HT were obtained in each preparation before the addition of inhibitors, and were repeated periodically between tests. After each trial the vessel was washed 10 times with 60 ml of buffer, and after 2 to 3 minutes of washing the vessel consistently returned to its initial resting tension. A 20-minute delay was allowed between trials to assure full recovery of the vessel. Aspirin was added to the chamber 20 minutes before the addition of 5-HT, because this incubation period was used in a related study; however, although we varied this period from 5 to 45 minutes, it did not alter our findings. Meclofenamate was added 5 to 10 minutes before the agonists.

In earlier experiments it was determined that 5-HT- induced contractions were not significantly affected by the previous addition of meclofenamate (1 × 10⁻⁴M) or aspirin (1 × 10⁻⁴M or 1 × 10⁻⁶M). For this reason, these concentrations of the inhibitors were used to determine whether these inhibitors would subsequently modify known responses caused by prostacyclin. Further, it was found that a 1 × 10⁻⁴M concentration of 5-HT produced a maximum response that did not significantly fade over the time required to obtain log dose-responses to prostacyclin. When higher concentrations of 5-HT (1 × 10⁻⁴M) were used, it was found that a much less contracted state was induced (see also Allen et al.²). For these reasons an initial contractile state was induced with 1 × 10⁻⁴M of 5-HT in all preparations. Preliminary observations indicated that meclofenamate in 10⁻⁴M concentrations yielded equivocal effects on the phenomena studied, and that in 10⁻⁵M concentrations it significantly inhibited contractions induced by 5-HT. For these reasons a 10⁻⁵M concentration was selected for this study. When 10⁻⁴M of aspirin yielded negative results, a higher dose was tested on the assumption that it might simply be less potent than meclofenamate. Also, the diphasic effect of prostacyclin reported herein is characteristic of this compound, and therefore the contractions it produced in high concentrations did not depend on the presence of 5-HT. Serotonin was used in this study to produce initial contractions in order that the relaxant properties of prostacyclin could be studied more quantitatively. The Student's t-test was used to determine the level of significance.

It is unknown whether the concentrations of aspirin and meclofenamate used in this study would be present in blood vessels in vivo. However, this is possible because the concentrations of such drugs in different tissues may vary at least eightyfold, and alter independent of blood values. Moreover, the recommended therapeutic blood level for aspirin (MW = 180) in rheumatic fever is 30 mg%. This is greater than a 1 × 10⁻⁴M concentration, which is the highest used in this study (Table 1). Such concentrations are commonly used in pharmacological studies with other inhibitors of isolated vascular smooth muscle (such as papaverine), and are often higher than that required for contractile agonists.

Results

Meclofenamate in 10⁻⁴M concentrations enhanced the relaxation induced by the lower doses of prostacyclin on the contracted basilar artery (Fig. 1 and Table 1). This enhancement was statistically significant at 10⁻⁴M of prostacyclin (p < 0.05), where the relaxation was double that induced before meclofenamate. The enhancement was questionable statistically at 10⁻⁴M to 10⁻⁵M of prostacyclin (p < 0.1) as peak relaxation approached maximum (Fig. 1). However, an analysis by the sum of probabilities indicates that this trend very closely approached significance at p < 0.05, and therefore it is likely that meclofenamate materially increased the relaxation caused by all three lower concentrations of prostacyclin. Although meclofenamate enhanced the relaxation caused by low concentrations of prostacyclin, it significantly inhibited the contractions induced by the highest dose (p < 0.005), as best seen in Fig. 1. Prior to meclofenamate administration, the highest dose of prostacyclin produced a contracted state that was greater than that caused by 5-HT alone (14.5 compared with 13.6 gm), whereas afterward it was only 61% as great. In contrast, the contractions induced by optimum concentrations of 5-HT (10⁻⁴M) were not altered (Table 1). Hence, meclofenamate inhibited the contractile properties of prostacyclin and,


J. Neurosurg. / Volume 53 / August, 1980 189
TABLE 1

Influence of meclofenamate and aspirin on the actions of prostacyclin (PGI₂) upon contractions induced by serotonin (5-HT) in basilar arteries

<table>
<thead>
<tr>
<th>Control Responses</th>
<th>5-HT (10⁻⁶M)</th>
<th>PGI₂ Action as Net Change (gm)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Response (gm)</td>
<td>10⁻⁸M</td>
<td>10⁻⁷M</td>
<td>10⁻⁶M</td>
<td>10⁻⁵M</td>
</tr>
<tr>
<td>13.6 ± 1.2</td>
<td>-3.2</td>
<td>-6.3</td>
<td>-7.5</td>
<td>+0.9</td>
</tr>
<tr>
<td>12.6 ± 1.4</td>
<td>-3.1</td>
<td>-6.9</td>
<td>-8.8</td>
<td>+0.8</td>
</tr>
<tr>
<td>12.3 ± 1.4</td>
<td>-3.3</td>
<td>-7.3</td>
<td>-9.1</td>
<td>+0.7</td>
</tr>
<tr>
<td>*The contraction induced by the optimum concentration of 5-HT was first established, and the changes in this constriction caused by PGI₂ recorded (controls, left). Afterward, the tissue was washed, the prostaglandin synthetase inhibitor (PSI) added to the bath, and the agonists 5-HT and PGI₂ added in that order (right). Each number in the body of the table represents an average of nine observations: the SEM is also shown for 5-HT responses. Meclo = meclofenamate.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PSI Added to Bath</td>
<td>5-HT (10⁻⁶M)</td>
<td>PGI₂ Action as Net Change (gm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Response (gm)</td>
<td>10⁻⁸M</td>
<td>10⁻⁷M</td>
<td>10⁻⁶M</td>
<td>10⁻⁵M</td>
</tr>
<tr>
<td>12.9 ± 0.9</td>
<td>-6.2</td>
<td>-8.5</td>
<td>-10.2</td>
<td>-5.1</td>
</tr>
<tr>
<td>12.8 ± 1.3</td>
<td>-3.2</td>
<td>-7.2</td>
<td>-8.8</td>
<td>+0.3</td>
</tr>
<tr>
<td>13.3 ± 1.2</td>
<td>-2.9</td>
<td>-5.5</td>
<td>-7.9</td>
<td>+0.3</td>
</tr>
</tbody>
</table>

paradoxically, enhanced the relaxation induced by this prostaglandin.

In contrast to meclofenamate, aspirin at both concentrations studied neither enhanced the relaxation nor inhibited the contractions observed with the log dose-responses induced by prostacyclin (Table 1). The responses produced by prostacyclin after 10⁻⁴M concentrations of aspirin were virtually similar to those obtained before. However, in 10⁻³M concentrations aspirin appeared to actually reduce the magnitude of the relaxation produced by the 10⁻⁷M and 10⁻⁶M concentrations of prostacyclin, although this change was not significant statistically. It is evident, therefore, that the pharmacodynamic action of aspirin was completely different from that of meclofenamate.

Discussion

Prostacyclin is the main prostaglandin synthesized by the endothelium of vessels, cerebral arteries synthesize this and other prostaglandins. This synthesis appears essential for the integrity of normal circulation. Prostacyclin is a potent vasodilator and prevents the aggregation of, and even deaggregates, platelets. It is the physiological antagonist of thromboxane A₂, which is synthesized by platelets. Some investigators have postulated that cerebral vasospasm and other vascular diseases may be due to an imbalance in the synthesis of different prostaglandins. The half-life of prostacyclin in blood is longer than that of thromboxane, and it is considered a circulating hormone, the function of which is to restrict the unwarranted spread of thromb.

Since arachidonic acid is a precursor of both thromboxane A₂ and prostacyclin, there is presently much interest in the use of PSI's, which selectively inhibit synthesis of thromboxane, to combat thrombotic stroke. In this regard, the beneficial action of aspirin is related more to low dosage rather than to enzyme specificity. Another therapeutic approach would be to supplement the naturally occurring prostacyclin. For this purpose, prostacyclin is being tested as a treatment for peripheral vascular disease, for thrombotic stroke, for use in coronary bypass surgery, and as an inhibitor of platelet activity during extracorporeal circulation. Prostacyclin may eventually prove efficacious in the management of certain neurosurgical problems, such as carotid endarterectomy, thrombotic stroke, and cerebral vasospasm. It
is, therefore, important to characterize the action of 
this prostaglandin on cerebral vessels and determine 
whether PSI's might pharmacologically alter the 
responses induced. Recent findings showed that in 
threshold concentrations prostacyclin relaxes isolated 
cerebral arteries,\(^{5,4}\) and this appears to be the only 
prostaglandin that has this characteristic.\(^4\) Other 
prostaglandins and arachidonic acid in low concen-
trations produce constriction of the isolated basilar 
artery.\(^{5,6}\) Aspirin and indomethacin reportedly 
reduce slightly (but not enough to be statistically 
significant) the resting tone of some of the 
preparations, it is apparent from the literature that a 
difference in the actions of these drugs is on the 
contractions produced by prostaglandins. This is also 
evident with high concentrations of prostacyclin, in 
which aspirin failed to block this megadose response 
whereas meclofenamate did (Table 1). Whether either 
of these drugs would interfere with the physiological 
vasodilator action of prostacyclin is unknown.

The present study clearly shows, therefore, that 
meclofenamic acid enhanced the relaxant effects of 
prostacyclin on the basilar arteries of dogs and 
reduced the constriction obtained with high concentra-
tions. Aspirin had no such effect, and tended to 
facilitate the constrictor action. Such distinct phar-
macological differences among these PSI's may be 
clinically important, as it suggests that patients under 
the influence of meclofenamate might have an ex-
aggerated vasodepressor response to prostacyclin, 
while any constrictor action associated with large 
doses, or overdosage, would be reduced. Since 
the constrictor action of prostacyclin was also 
significantly reduced by meclofenamate, it is possible 
that the enhanced relaxation was due to an unmasking 
of an unknown and unrecordable simultaneous con-
strictor action of prostacyclin that occurs with the lower 
doses. A similar mechanism seems to account 
for the effect of polyphoretin phosphate on the 
vascular actions of other prostaglandins in the intact 
dog,\(^{24}\) and may be operating here. In this regard, it is 
possible that the different effects of meclofenamate 
and aspirin are due to actions independent of 
prostaglandin synthesis. Meclofenamate inhibits such 
synthesis by cerebral arteries,\(^{10}\) and blocks the 
synthesis of these lipids by the heart in response to 
ischemia, and aspirin clearly inhibits platelet cyclo-
xygenase,\(^{7,6}\) but this class of drugs also inhibits the 
flux of calcium in vitro\(^{27}\) and it is possible that only 
meclofenamate exerted such an effect on the cerebral 
arteries. Meclofenamate does not inhibit phospho-
diesterase.\(^1\) Aspirin inhibits many enzyme systems,\(^{20}\) 
but these apparently played no role in the phenomena 
studied. Regardless of the mechanism, the present 
study emphasizes further that the effects of com-
ounds pharmacologically classified as PSI's are com-
plex,\(^1,22\) and that some, but not all, will significantly 
change the pharmacodynamic actions of prostacyclin 
on cerebral arteries. It is possible that in the future 
some PSI's may be used clinically to inhibit platelet 
function while enhancing directly the vasodilator ac-
tion of prostacyclin. Meclofenamic acid could be such 
a drug.

Acknowledgments
The authors wish to thank Dr. John E. Pike of the Upjohn 
Company for providing the prostacyclin and Mrs. Charlotte 
Barker for her technical assistance.

References
postocclusive hyperemia by endogenously synthesized 
prostaglandins in the dog heart. J Clin Invest 55: 
1174–1181, 1975
arterial spasm. Part 1: In vitro contractile activity 
of vasoactive agents on canine basilar and middle cerebral 
human and baboon arteries to prostaglandin endo-
peroxides and biologically generated and synthetic 
prostacyclin: their relevance to cerebral arterial spasm 
4. Chapleau CE, White RP: Effects of prostacyclin on the 
isolated canine basilar artery. Prostaglandins 17: 
573–580, 1979
5. Chapleau CE, White RP, Robertson JT: Cerebral vasos-
spasm: effects of prostaglandin synthetase inhibitors in 
6. Chapleau CE, White RP, Robertson JT: Effects of 
prostaglandin synthetase inhibitors (PSI) on contrac-
tions induced by arachidonate, prostaglandin F\(_2\), 
(Abstract 689)
7. Flower RJ: Drugs which inhibit prostaglandin 
8. Gorman RR: Modulation of human platelet function by 
prostacyclin and thromboxane A\(_2\). Fed Proc 38:83–88, 
1979
as a generator of prostacyclin — hypothesis on 
physiological significance. Arch Pharmacol 304:45–50, 
1978
10. Hagen AA, White RP, Robertson JT: Synthesis of 
prostaglandins and thromboxane B\(_2\) by cerebral 
of thrombosis in patients on hemodialysis by low-dose 
12. Herman AG, Moncada S, Vane JR: Formation of 
prostacyclin (PGI\(_1\)) by different layers of the arterial 
wall. Arch Int Pharmacodyn Ther 227:162–163, 1977
effects of high-dose aspirin in rabbits. J Clin Invest 

This work was supported by NIH Grant USPHS NS06826.

Address reprint requests to: Richard P. White, Ph.D., Department of Pharmacology, University of Tennessee, 800 Madison Avenue, Memphis, Tennessee 38163.