Constriction of Pial Arterioles Produced by Prostaglandin $F_{2\alpha}$

BY WILLIAM I. ROSENBLUM, M.D.

Abstract: Prostaglandin $F_{2\alpha}$ constricted pial arterioles when locally applied to the cerebral surface. Norepinephrine and serotonin each elicited similar contractile effects. The constriction produced by $F_{2\alpha}$, in combination with either biogenic amine was greater than the constriction elicited by $F_{2\alpha}$, or amine acting alone. The effect of one agent on the other was additive rather than potentiating. Since $F_{2\alpha}$, norepinephrine and serotonin are all naturally occurring agents, it is possible that their combined effect is important under pathological circumstances and this combined effect should not be overlooked in the search for single spasmogens of great potency. Before ascribing a pathologically important effect to $F_{2\alpha}$, either alone or in combination, evidence is required showing that doses effective in experiments are similar to the concentrations occurring during disease states and/or that vessels may become hypersensitive to $F_{2\alpha}$ during such states.

Additional Key Words

- microcirculation
- norepinephrine
- serotonin
- cerebral blood vessels
- vasospasm

Prostaglandins (PG) are a group of 20-carbon, unsaturated lipid acids with a wide variety of possible biological actions, some of which are just beginning to be explored. They have been found to produce constriction or relaxation of smooth muscle, including vascular smooth muscle, in vitro and in vivo. The direction of response is dependent upon the type of PG and the test object. Similarly, they have been reported to facilitate or to inhibit adrenergic responses, sometimes by acting on the release of norepinephrine and sometimes by altering the responsivity of the test organ. Prior to this writing, the author is aware of only four reports describing the effect of the PG, known as $F_{2\alpha}$, on cerebral blood vessels. The results of this small number of studies suggested that additional investigation was warranted.

Prior reports indicated that $F_{2\alpha}$ constricts blood vessels somewhere in the cerebral vasculature. Two dealt, at least partly, with surface arteries and arterioles. One of these showed that intracarotid injection caused constriction of pial arteries and arterioles under 200 $\mu$ in diameter, while the other demonstrated that intracisternal $F_{2\alpha}$ produced constriction of large surface arteries. These recent investigations suggested that the action of $F_{2\alpha}$ might be relevant to the clinical problem of vasospasm, especially since prostaglandins, including $F_{2\alpha}$, are present in brain and released by brain into the cerebrospinal fluid (CSF). A relationship between PGs and clinical vasospasm also is suggested by the fact that platelets release PGs, and a platelet factor(s) has been implicated in the vasospasm associated with subarachnoid hemorrhage.

The present investigation focuses attention on the response of smaller pial arterioles, after local application of $F_{2\alpha}$ to the cerebral (i.e., arachnoid) surface. Under such circumstances, the PG will arrive at cerebral surface vessels (pial vessels) after crossing the arachnoid and passing through the CSF. Transport through the CSF also represents a route by which they would arrive at the cerebral blood vessels after release from brain or from platelets in the subarachnoid space. After initiating our studies, an additional report appeared describing constriction of pial arterioles and small arteries in the cat following application of $F_{2\alpha}$ to the cerebral surface. The technique used therein more closely resembles our own than do those in any of the earlier studies.

Methods

Male mice, Swiss strain (Dublin Farms), were used. These weighed 18 to 35 gm and were anesthetized with urethane. A tracheotomy and craniotomy were performed and the dura stripped from the craniotomy site. The brain surface with the exposed pial vessels covered only by the transparent arachnoid was then irrigated continuously with a mock cerebrospinal fluid (Elliott's solution). This solution has the following composition: $Na^+$ 134 mEq/l; $Ca^{++}$ 2.7 mEq/l; $Mg^{++}$ 2.4 mEq/l; $Cl$ 137 mEq/l; $SO_4^{2-}$ 2.4 mEq/l; HPO$_4^{2-}$ 1.5 mEq/l, and dextrose 800 mg/l. In addition the solution contains initially 2.3 mEq/l HCO$_3^-$, and is acidic. The pH rises on standing or mild heating and is readjusted to physiological levels (between 7.3 and 7.4) by bubbling CO$_2$ through the solution.

This solution was delivered to the brain surface at the rate of 2 ml per minute by a constant perfusion pump which caused the fluid to pass through a heating coil and hypodermic needle. The orifice of the needle was a few millimeters

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above the craniotomy site and solutions emanating from the needle orifice fell in a continuous series of drops to the edge of the craniotomy and spread in a thin film over the cerebral surface, from whence the fluid was led by a cotton wick. The temperature of the fluid at the craniotomy site was 37°C. The pH of the fluid was monitored repeatedly during the observation of each mouse in the study, by sampling drops from the needle orifice. The pH of more than 100 measurements was 7.34 ± 0.03 (M ± SD).

A side arm just proximal to the heating coil permitted delivery of a 1 ml test solution as a bolus interrupting the normal irrigant. This bolus took 2 minutes ± 30 seconds to reach the cerebral surface, at which time its temperature was the same as that of the normal irrigant. The pH of the bolus and that of the irrigant were within 0.05 pH units of each other. The bolus cleared the brain surface in 30 seconds, after which the regular irrigant was once again flowing over the brain.

Solutions of 5% BaCl₂ were used to test the reactivity of the pial arterioles before and after each experiment. All animals entering the study responded to the barium ion with an arteriolar constriction both before and after the application of F₂α. The prostaglandin F₂α used in this study was the tromethamine (THAM) salt, with a molecular weight of 475.6. The free acid has a molecular weight of 354.5. THAM, when used by itself over a dose range exceeding that required and animals not showing such stability were discarded. The pH of the fluid was monitored repeatedly during the bolus study and the pH adjusted to be between 7.3 and 7.4 by adjusting the CO₂ (NOR) was used as the bitartrate and serotonin (5HT) as the creatinine sulfate. Fresh solutions of all agents were made each day in the Elliott solution and the pH adjusted to be within 0.05 pH units of each other. The bolus cleared the brain surface in 30 seconds, after which the regular irrigant was once again flowing over the brain.

The internal diameter of the pial arterioles was measured by using a microscope, image splitter and TV monitor as described by Baez. The diameter of the vessels was represented by a dark column of red cells and thus was underestimated by approximately 4 μ. The diameter of the vessels was monitored for five minutes prior to the time the test solution hit the brain, and continued to be monitored for eight minutes thereafter. Stability of baseline diameter prior to application of test solution was required and animals not showing such stability were discarded. If a change in diameter occurred within the 60 seconds following arrival of test solution at the cerebral surface, the change was counted as a response. In practice, over 90% of the responses occurred during the first 30 seconds.

In these experiments the limiting factor in measuring diameter was the reproducibility with which successive measurements of the same vessel could be made. This depends upon the observer's ability to exactly juxtapose sheared images on the TV monitor and this, in turn, depends upon the clarity of the TV image and the contrast between vessel and background. In our system, which utilizes light reflected from the object being observed rather than transmitted light, we have tested our ability to make reproducible successive measurements of the distance between lines on a stage micrometer, a fixed distance demarcated by highly contrasted lines. These measurements never varied by more than 2 μ, and the reproducibility was independent of the distance between the lines over a range of 20 to 60 μ. We took this 2 μ discrepancy between successive measurements of a fixed object as the maximal "random" error of the method of measurement in our studies and arbitrarily discarded all changes smaller than 2 μ from consideration as true changes. In other words changes of less than 2 μ could not be distinguished from "noise." This change would equal approximately 4% of the mean resting diameter of arterioles in this study. Thus there will be a tendency to underestimate the frequency and magnitude of response to the vasoactive agents, especially at low dose levels.

Results

EFFECTS OF SINGLE DOES OF F₂α ALONE

F₂α constricted pial arterioles as indicated in table 1. No relationship to dose could be seen since the lowest dose in the series, 10 μg per milliliter, where a marked decrement in response was observed. This decrement, however, was due solely to the failure of six of the ten animals to display any recognizable response, thus being counted as 0% constriction. In all other groups 70% to 100% of the animals responded to F₂α. Thus, in each group of ten mice, seven responded to 250 μg per milliliter, eight to 100 μg per milliliter, seven to 50 μg per milliliter, and all ten to 25 μg per milliliter. It is probable that the incidence of response was even higher, since small responses equal to less than 5% of resting diameter would have been dismissed as noise due to either spontaneous change in diameter or observer error in resetting the image splitter to the same null position between successive measurements.

### Table 1

<table>
<thead>
<tr>
<th>Dose F₂α (μg/ml)*</th>
<th>250</th>
<th>100</th>
<th>50</th>
<th>25</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of experiments†</td>
<td>10</td>
<td>16</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Original diameter‡</td>
<td>49 ± 15</td>
<td>47 ± 12</td>
<td>54 ± 13</td>
<td>46 ± 5</td>
<td>45 ± 16</td>
</tr>
<tr>
<td>Percent constriction§</td>
<td>11 ± 11</td>
<td>14 ± 9</td>
<td>12 ± 9</td>
<td>20 ± 7</td>
<td>5 ± 10</td>
</tr>
</tbody>
</table>

*Administered as a 1 ml bolus over a 30-second period.
†Equals number of mice. Only one dose per mouse per experiment.
‡Microns = standard deviation.
§Percent reduction from baseline diameter (mean ± SD).
PROSTAGLANDIN AND PIAL ARTERIOLES

DOSE RESPONSE RELATIONSHIP TO $F_2\alpha$

As indicated above, groups of animals failed to display a relationship between the average response of the group and the dose used in that group. We believe this was due to the great variability in the responses of the mice within each group. However, if successively larger doses were applied to the same animal, allowing the arteriole to recover from one dose before applying the next, greater constrictions were observed in the individual mouse as higher doses were applied to the pial surface of that mouse. This is shown in Figure 1. The figure displays the results in five consecutive animals whose vascular reactivity was unaltered during the course of the experiment. The latter point was checked by comparing the response to BaCl$_2$ before and after the series of $F_{2\alpha}$. In each animal the two responses to BaCl$_2$ were within 10% of each other.

INTERACTION OF $F_{2\alpha}$ WITH SEROTONIN OR NORADRENALINE

In each experiment an effective dose of amine was determined. This dose of amine was given alone, as was a dose of $F_{2\alpha}$, and the response to each was measured. In addition, the same doses were combined and the response to the combination was measured. The order in which these drugs were given was varied, so that in some cases amine was given first, and in others the prostaglandin or the combination. The results were the same, irrespective of the order in which these agents were applied. The responses to $F_{2\alpha}$ were increased if 5HT or NOR were present at the same time. The response to the combination of drugs was equal to the sum of the responses to each of the components in the combination. The data are summarized in Table 2, which displays the average response to the amine alone, to the $F_{2\alpha}$ alone, and to the combination. In these experiments, the dose of NOR was either 0.5 or 5 µg per milliliter and the dose of 5HT was either 5, 10 or 20 µg per milliliter. The dose of $F_{2\alpha}$ was either 5, 25, or 50 µg per milliliter. The reader must remember that in each experiment the doses of amine and $F_{2\alpha}$ used singly were the same as those used in combination. The additive effect of NOR plus $F_{2\alpha}$ or of 5HT plus $F_{2\alpha}$ was observed irrespective of the doses selected.

Discussion

Our data clearly demonstrate constriction of pial arterioles after local application of $F_{2\alpha}$. The results confirm the four previous reports of which we are aware. The effective doses in our study are remarkably similar to those doses locally applied to the brain surface of the cat by Welch et al. in their study of the effect of $F_{2\alpha}$ on pial vessels. The latter is the only published study, of which we are aware, in which $F_{2\alpha}$ was directly applied to the external surface of the brain and the pial vessels. Although we have not used duration of response as a parameter in this particular study, the response peak occurred between ten seconds and one minute following application of the drug, and thus the time course of the response was also similar to the data reported by Welch et al. These investigators did not report dose-response data, nor did they concern themselves with the interaction of $F_{2\alpha}$ and other vasoconstrictors.

Our data show a simple, additive effect between

<table>
<thead>
<tr>
<th>Interaction of $F_{2\alpha}$ With Vasoactive Amines</th>
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</thead>
<tbody>
<tr>
<td>% Constriction $\pm$ standard deviation</td>
</tr>
<tr>
<td>Norepinephrine</td>
</tr>
<tr>
<td>Prostaglandin $F_{2\alpha}$</td>
</tr>
<tr>
<td>Combination</td>
</tr>
<tr>
<td>Serotonin</td>
</tr>
<tr>
<td>Prostaglandin $F_{2\alpha}$</td>
</tr>
<tr>
<td>Combination</td>
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*Mean = standard deviation. Constriction as a percent of resting diameter.

†N = number of experiments.
the response to F<sub>2a</sub> and the response to NOR or 5HT. These results contrast with those reported for isolated extracerebral vessels<sup>16</sup>,<sup>17</sup> where F<sub>2a</sub> was shown to potentiate the responses to 5HT and other contractile stimuli rather than produce a simple additive response. However, in the latter experiments much lower concentrations of F<sub>2a</sub> were used, concentrations which themselves had no effect on vascular tone, and of course cerebral vessels were not tested. Our own results may be of some interest, since they suggest that marked constriction of cerebral arterioles may follow contact with a combination of agents, any one of which might produce only modest constriction if acting alone. Thus the search for a single, elusive “spasmogen”<sup>18</sup>-<sup>20</sup> to account for clinically important vasospasm may be missing an important point, namely the additive effect of several agents released by platelets and brain tissue into the CSF and subarachnoid space.

One must make further mention of the arteriolar constriction induced by either NOR or 5HT in these experiments. In earlier publications by this author the contractile response of pial arterioles to NOR was described as unreliable<sup>21</sup> or nonexistent<sup>22</sup> and negative data also were reported with respect to a contractile effect of 5HT.<sup>23</sup> More recently, we have reported a reliable, dose-related constriction elicited by NOR.<sup>24</sup> This is confirmed in the present experiments, and a constriction induced by 5HT is also described. Several differences in methodology have been introduced since the earlier negative studies. These include better control of pH and temperature; a shift from pentobarbital to urethane anesthesia; avoidance of applying to the same preparation multiple doses of vasoactive drug in maximal strength; and the use of the TV microscope and image splitter to provide reliable, sensitive measurements of diameter, and a written record of diameter changes during the experiment. We cannot say which of these factors may have contributed to the change in our results. We have tried pentobarbital anesthesia in conjunction with the new recording system and we continue to get constriction like that observed with urethane. Thus far, the reliability of the recording system seems extremely important, since in the past small transient constrictions induced by single applications of NOR or 5HT might have been overlooked or dismissed as illusory when viewed by peering directly down the microscope aided only by an ocular micrometer. However, extremely small changes were consistently recorded with the more primitive technique when other vasoactive drugs were used.<sup>24</sup>(Table 1)

Whatever the explanation for our previous inconsistent or negative data with biogenic amines, the present studies have been markedly consistent, provide evidence not only for a contractile effect of these agents but also for F<sub>2a</sub>, and indicate an additive effect of the latter with either NOR or 5HT. Unfortunately, in order to solidly implicate F<sub>2a</sub> as a contractile stimulus of clinical importance, or for that matter to implicate NOR or 5HT, one must show that their concentrations in these and other experiments are comparable to that occurring in disease states. This is not the case, and in the present study, as well as the studies of other workers, each of these vasoactive agents is effective only in concentrations greater than those known to occur even during pathological conditions.<sup>25</sup>,<sup>26</sup> However, the concentration of these materials during pathological states is still relatively unexplored and for PG is virtually unknown with respect to the central nervous system and cerebrospinal fluid. Moreover, the sensitivity of pial vessels may be enhanced during periods of microcirculatory derangement.<sup>27</sup>-<sup>29</sup> Thus it will be important to test responses to F<sub>2a</sub> alone and in the presence of other vasoactive agents, when such derangements are present, in order to see whether marked constriction can be elicited by doses lower than those used here.

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