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The Effect of Naftidrofuryl, a 5-HT_2 Antagonist, on Collateral Vascular Responses to Serotonin and to Platelet Activation

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Summary: Collateral arterial supersensitivity to serotonin has been attributed to a 5-HT_2 receptor mechanism because of the effectiveness of ketanserin in reversing that vasoconstrictor response. To assess that hypothesis we employed a chemically unrelated agent, naftidrofuryl, and assessed the responses of the collateral arterial supply 2 weeks after superficial femoral artery ligation to serotonin or to platelet activation induced by endothelial injury in 25 rabbits. Naftidrofuryl was effective in reversing serotonin-induced vasoconstriction in doses ranging from 0.3 to 3.0 μg/kg/min. Higher doses reduced blood pressure sufficiently that collateral arterial attenuation ensued. When collateral arterial vasoconstriction was induced by endothelial injury, naftidrofuryl in doses of 1.0 and 3.0 μg/kg/min reversed the attenuation ($p < 0.001$) in a dose-dependent fashion. In the absence of vasoconstriction induced by serotonin or platelet activation, naftidrofuryl in these doses did not produce vasodilatation, suggesting that the agent acted as a blocker rather than as a direct vasodilator. The observations strengthen the hypothesis that supersensitivity of collateral arterial vessels to serotonin reflects a 5-HT_2 receptor mechanism.
Key Words: Collateral arteries—Vasospasm—Serotonin—S2 receptors—Blood flow—Ischemia.

Supersensitivity to the vasoconstrictor effects of serotonin have been documented in several species (1–4). Platelet activation provoked by endothelial injury in rabbits with a collateral-dependent arterial supply to the limb released sufficient quantities of vasoactive material to attenuate collateral arterial supply, evidenced by angiography and blood flow measurement (5). When endogenous serotonin is released from platelets, however, the response is complicated by the formation of thromboxane A2, which also contributes to collateral artery vasoconstriction (5–7).

Ketanserin has been effective in reversing completely collateral arterial vasoconstriction induced by serotonin (2–4) and, in part, the vasoconstriction induced by platelet activation (5). In this study, we have ascertained whether a chemically unrelated moiety, naftidrofuryl, exerts a similar action, a study prompted by observations suggesting that this agent has 5-HT_2-blocking properties (8) and opposes 5-HT-induced vasoconstriction (9,10).

METHODS

The studies were performed in 25 rabbits, weighing about 3 kg. Anesthesia was induced and maintained with sodium pentothal (30 mg/kg i.v.). After tracheal intubation, respiration was supported by a Harvard Model 607 respirator. Arterial blood pressure was measured with a Statham strain gauge (model P23) and recorded on a Grass polygraph. To prevent dehydration by osmotic diuresis induced by contrast agents, 0.9% saline was administered i.v. at a rate of 10 ml/kg/h throughout the study.

Two weeks before the protocol, under sterile conditions, the superficial femoral artery was ligated distal to the origin of the profunda femoris and medial and lateral circumflex femoral arteries. The medial and lateral circumflex femoral arteries are visible immediately proximal and medial to the area of ligation (see Fig. 1). The profunda femoris is the major artery proximal to these vessels.

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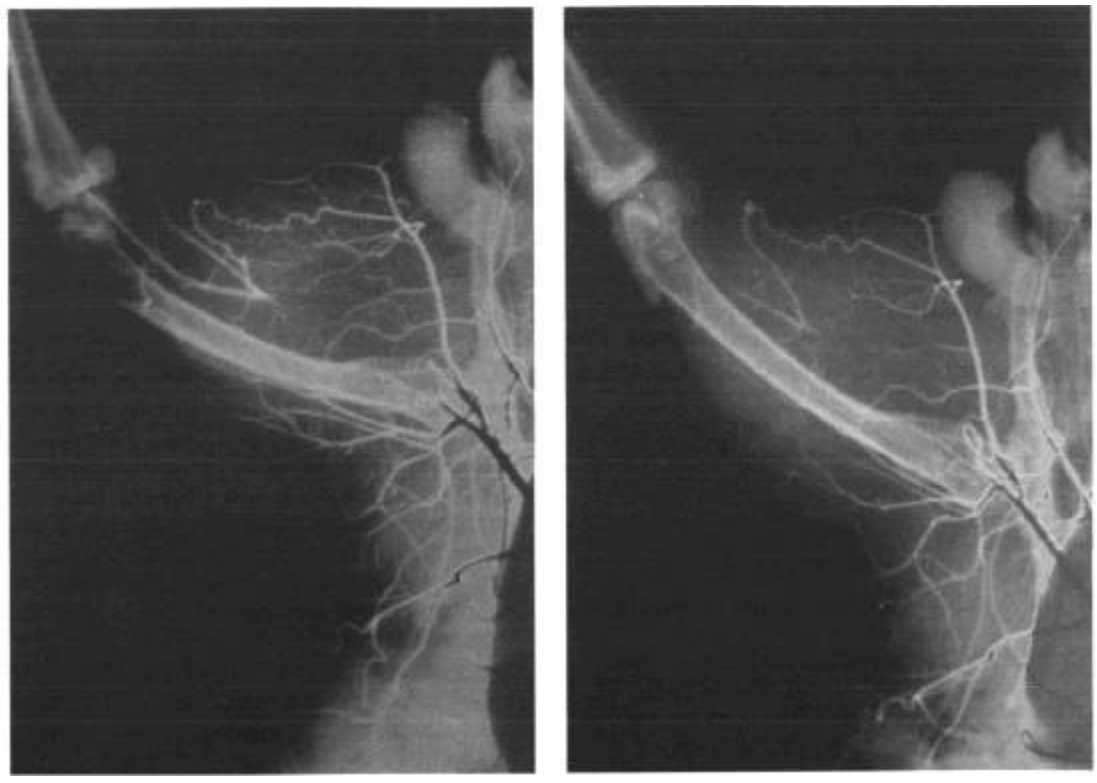

FIG. 1. **A:** An arteriogram obtained 20 min after balloon-catheter injury of the lower aorta. Note vasoconstriction of the collateral arterial tree, evident both at the level of larger stem vessels and the tortuous smaller midzone vessels. **B:** Reversal of attenuation induced by naftidrofuryl (3 μg/kg/min).

At study, a 3F catheter was placed in the lower aorta by way of the left carotid artery and a baseline arteriogram was obtained. In experiments involving endothelial injury, the endothelium of the lower aorta was damaged by insertion of a coronary angioplasty balloon catheter via the carotid artery, and the balloon was advanced to the terminal aorta, inflated, and pulled back to the level of the renal arteries two or three times. The arteriogram was then repeated.

Thereafter, one of several protocols was followed.

(a) Because little information was available on the naftidrofuryl response interaction with serotonin in vivo, we began our studies with an attempt to define that relationship. In four rabbits, serial challenges to serotonin (100 ng/min into the lower aorta), each lasting 7 min, were followed by an arteriogram. This was repeated five times to ascertain the stability of the response.

In an additional four rabbits, an identical protocol was employed but an i.v. infusion of naftidrofuryl was superimposed for the last 5 min of the serotonin infusion. The doses of naftidrofuryl ranged from 0.3 to 100 μg/kg/min.

In two additional rabbits, naftidrofuryl was administered alone to ascertain whether a direct vasodilator response could be documented.

(b) In nine rabbits with a collateral arterial supply, following the baseline arteriogram the endothelium was damaged as described above. Thirty minutes later a follow-up arteriogram was obtained to assess the vasoconstriction induced by the endothelial injury. Thereafter, serial naftidrofuryl doses from 0.3 to 10 μg/min were infused for 5 min each, followed by an arteriogram, as described above.

Arteriography was performed as described earlier (4,5). In brief, meglumine diatrizoate (76%) was injected with a Med Rad Mark II power injector at a controlled rate, to deliver 6 ml over 3 s. A 0.13-mm focal spot was used with a 40-in. focal film distance for magnification. A Franklin role film changer was used to change films at a rate of 1/s for 6 s after the contrast injection.

The arteriograms were assessed systematically on a coded basis. The global assessment included evaluation of smaller cognate arteries and the tortuous smaller midzone collateral arteries. The velocity of contrast transit through the arterial bed was also assessed as a rough index of blood flow. In our ordinal system, 0 indicated no change or worsening from the baseline attenuation induced by vascular injury or serotonin; 3+ indicated an unambiguous and global reversal of the collateral arterial attenuation; 2+ indicated probable but less striking and less complete improvement; 1+ indicated ambiguous but probable improvement.

Nonparametric tests (χ^2 and Fisher Exact tests) were used to assess probability for the arteriographic studies. Analysis of variance was used to assess changes in blood pressure. The null hypothesis was rejected when p was less than 0.05.

RESULTS

Serotonin and naftidrofuryl

In the four rabbits that received serotonin at 100 ng/min, an unequivocal vasoconstrictor response that was well sustained over the five infusions occurred in every animal. In the four rabbits in which an identical protocol was employed, but with superimposed naftidrofuryl infusion, the results were complex.

Naftidrofuryl doses from 0.3 to 3 μg/kg/min produced a dose-related reversal of the serotonin-induced response, which was best seen at the 3 μg/kg/min dose. Because this dose produced a minimal change in blood pressure (-2.8 ± 2.9 mm Hg), this was considered the optimal dose for assessing the response to endothelial injury. At doses of 10 μg/kg/min or greater, a blood pressure fall of 22–38 mm Hg routinely occurred, reducing mean arterial blood pressure to the 40–52 mm Hg range. At these doses, a positive influence of naftidrofuryl on collateral arterial attenuation induced by serotonin could not be identified.

In two rabbits without endothelial injury, the intrinsic vasodilator activity of naftidrofuryl for the collateral arterial supply was assessed. In neither rabbit was there evidence of vasodilatation.

Endothelial injury

Clear collateral arterial spasm occurred in each of the nine rabbits following endothelial injury. No rabbit showed a response (2+ or 3+) to the 0.3 μg/kg/min naftidrofuryl dose. Four of nine rabbits showed a response to the 1 μg/kg/min dose, which was striking (3+) in three of the rabbits. Five rabbits showed a response to the 3 μg/kg/min dose, which was 3+ in an additional two rabbits (see Fig. 1). Only three of nine rabbits showed no response at any dose, and a clear response occurred in the other six.

In the six "time control" studies following endothelial injury, an identical protocol was employed except that the naftidrofuryl infusion was replaced by a placebo saline infusion. In the 2- to 3-h protocol, none of the six rabbits showed spontaneous reversal of the vascular attenuation. By the Fisher Exact Test, the difference between the naftidrofuryl-treated and placebo-treated groups was statistically different by animal ($p < 0.25$). In this study (Table 1) the difference between groups was more striking ($p < 0.001$).

TABLE 1. *Between-group differences using the Fisher Exact Test*

	Number of rabbits	Number of studies	Response			
			0	+	++	+++
Naftidrofuryl[a]	9	17	7	2	3	5
Placebo[b]	6	27	22	5	0	0

[a] Naftidrofuryl doses 1.0–3.0 μg/kg/min.
[b] Placebo vs. naftidrofuryl, $\chi^2 < 0.001$.

DISCUSSION

Our goal of this study was to ascertain whether an agent that was chemically unrelated to ketanserin but which shared 5-HT_2-blocking characteristics would mimic the influence of ketanserin on a collateral arterial supply. This hypothesis was confirmed in several ways. First, when employed alone in animals receiving neither serotonin nor following endothelial artery injury, naftidrofuryl did not induce a recognizable response. Second, when serotonin was employed as the provocative maneuver, naftidrofuryl was capable of reversing the vasoconstriction thus induced completely. Third, when platelet activation following endothelial injury was employed as the stimulus, naftidrofuryl resembled ketanserin in producing a partial response, as in our earlier study (5). Similar to the findings in the earlier study, the reversal of platelet activation–induced collateral arterial attenuation was incomplete. In our earlier study, the combination of ketanserin and a thromboxane antagonist or thromboxane synthetase inhibitor was found to be substantially greater than either alone (5). No attempt was made to replicate that observation in this study.

Naftidrofuryl differed from ketanserin in one way. The rabbit demonstrated little dose-related blood pressure fall when high doses of ketanserin were employed, despite the fact that at high doses it becomes an α-adrenergic blocking agent. Increasing doses of naftidrofuryl, on the other hand, produced a dose-related blood pressure fall that reversed the angiographic response in this study. No explanation for the blood pressure fall is available from the data in this study.

Reversal of the collateral arterial response to serotonin and to activated platelets by ketanserin has been interpreted as indicating that a 5-HT_2 receptor mechanism contributed to the supersensitivity to serotonin demonstrated by growing arterial collateral vessels (2–5). A similar response to a chemically unrelated 5-HT_2 antagonist provides strong support for that conclusion.

REFERENCES

1. Schaub RG, Meyers KM, Sande R. Serotonin as a factor in depression of collateral blood flow following experimental arterial thrombosis. *J Lab Clin Med* 1977;90:645–53.
2. Verheyen A, Vlaminckx E, Lauwers F, VanDen Broeck C, Wouters L. Serotonin-induced blood flow changes in the rat hindlegs after unilateral ligation of the femoral artery. Inhibition by the S2 receptor antagonist ketanserin. *Arch Int Pharmacol Ther* 1984;270:280–98.
3. Nevelsteen A, DeClerck F, Loots W, DeGryse A. Restoration of post-thrombotic peripheral collateral circulation in

the cat by ketanserin, a selective 5HT-2 receptor antagonist. *Arch Int Pharmacodyn Ther* 1984;270:268–79.
4. Orlandi C, Blackshear JL, Hollenberg NK. Specific increase in sensitivity to serotonin of the canine hindlimb collateral arterial tree via the 5-hydroxytryptamine-2 receptor. *Microvasc Res* 1986;32:121–30.
5. Hollenberg NK, Monteiro K, Sandor T. Endothelial injury provokes collateral arterial spasm: role of thromboxane and serotonin. *J Pharmacol Exp Ther* 1988;244:1164–8.
6. Hamberg M, Svensson J, Samuelsson B. Thromboxanes: a new group of biologically active compounds derived from prostaglandin endoperoxides. *Proc Natl Acad Sci USA* 1975;72:2994–8.
7. Ellis EF, Oelz O, Roberts LJ, et al. Coronary arterial smooth muscle contraction by a substance released from platelets: evidence that it is thromboxane A2. *Science* 1976;193:1135–7.
8. Maloteaux JM, Haiech J, DeCampaneere D, Berta P, Vidal N. Biochemical and physiological evidences for antiserotonergic properties of naftidrofuryl. *Arzneimittelforschung* 1986;36:1194–8.
9. Rosenblaum WI. Inhibition of constriction of cerebral arterioles in vivo by naftidrofuryl. *Arzneimittelforschung* 1987;37:495–7.
10. Zander JF, Aarhus LL, Katusic ZWS, Rubanyi GM, Vanhoutte PM. Effects of naftidrofuryl on adrenergic nerves, endothelium and smooth muscle in isolated canine blood vessels. *J Exp Pharmacol Ther* 1986;239:760–7.