Evidence for Greater Susceptibility of Isolated Dog Cerebral Arteries to Ca Antagonists than Peripheral Arteries

KOICHIRO SHIMIZU, M.D., TOMIO OHTA, M.D., PH.D., AND NOBORU TODA, M.D., PH.D.

SUMMARY In helically-cut strips of dog cerebral, coronary and mesenteric arteries, contracted with prostaglandin (PG) F$_2$, or K$^+$, the addition of verapamil caused a dose-related relaxation. Verapamil-induced relaxations were greater in cerebral than in the other arteries when contracted with PGF$_{2\alpha}$, but did not significantly differ in the arteries contracted with K$^+$. Similar results were obtained with diltiazem and nifedipine. The contractile response to PGF$_{2\alpha}$ was attenuated by pretreatment with verapamil, the attenuation being greater in cerebral than in mesenteric arteries. Nitroglycerin and sodium nitroprusside relaxed cerebral, coronary and mesenteric arteries contracted with PGF$_{2\alpha}$ to a similar extent. It may be concluded that dog cerebral arteries contracted with PGF$_{2\alpha}$, one of endogenous vasospastic substances, are more susceptible to agents which interfere with the influx of Ca$^{++}$ across cell membranes than coronary and mesenteric arteries; these agents may thus be of value in the treatment and prophylaxis of cerebral vasospasm.

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ISOLATED CEREBRAL ARTERIES respond to vasoconstricting and vasodilating agents differently from peripheral arteries. 1,2 Cadmium (Cd$^{++}$) antagonists, including verapamil, nifedipine, diltiazem and Cd$^{++}$, interfere with the influx of Ca$^{++}$ across cell membranes of vascular smooth muscle,3-7 thereby resulting in vasodilatation. Contractile responses to Ca$^{++}$ of cerebral arteries exposed to Ca$^{++}$-free media and depolarized by K$^+$ are attenuated to a greater extent by verapamil and Cd$^{++}$ than those of coronary and mesenteric arteries.8

Nitroglycerin and sodium nitroprusside are potent vasodilators9,10 and cause rapid hypotension. These agents are also expected to release cerebroarterial spasm following subarachnoid hemorrhage.11 No information is available concerning the response of cerebroarterial smooth muscle to these vasodilators.

The present study was thus undertaken to compare quantitatively the response of isolated dog cerebral, coronary and mesenteric arteries to Ca$^{++}$ antagonists, nitroglycerin and sodium nitroprusside. Verapamil prevention of drug-induced contractions was also compared in cerebral and mesenteric arteries.

Methods

Mongrel dogs of both sexes, weighing 7 to 15 kg, were anesthetized with intraperitoneal injections of sodium pentobarbitol 50 mg/kg, and sacrificed by
bleeding from common carotid arteries. The brain and heart were rapidly removed. Basilar and middle cerebral arteries (0.5 to 0.8 mm outside diameter) and ventral interventricular branches of the left coronary artery (0.6 to 0.9 mm) were isolated. Distal portions of the mesenteric artery (0.5 to 0.8 mm) were also removed. The arteries were cut helically into strips approximately 20 mm long. The specimen was vertically fixed between hooks in a muscle bath containing the nutrient solution, which was maintained at 37 ± 0.5°C and aerated with a mixture of 95% O2 and 5% CO2. Hooks anchoring the upper end of the strips were connected to the lever of a force-displacement transducer (Nihonkoden Kogyo Co., Tokyo, Japan). The resting tension was adjusted to 1.5 g, which is optimal for obtaining the maximum contraction. Constituents of the solution were as follows (mM): Na+ 162.1, K+ 5.4, Ca++ 2.2, Mg ++ 1.0, Cl- 159.0, HCO3- 14.9, and dextrose 5.6. The pH of the solution was 7.2 to 7.3. Osmotic adjustment was not made when K+ (up to 30 mM) was added to the bathing media. Before the start of experiments, all preparations were allowed to equilibrate for 90 to 120 min in the control media during which fluids were replaced every 15 to 20 min.

Isometric contractions and relaxations were recorded on an ink-writing oscillograph (Sanei Sokki Co., Tokyo, Japan). The contractile response to 30 mM K+ was first obtained. Prostaglandin (PG) F2α was added directly to the bathing media in cumulative concentrations, and the contractions relative to those induced by 30 mM K+ are presented. Before the addition of vasodilating agents, arterial strips were contracted with either PGF2α (5 × 10⁻¹⁰ to 2 × 10⁻⁶ M) or K+ (12 to 20 mM). Relaxations induced by 10⁻⁴ M papaverine were taken as 100%.12 and relaxations induced by verapamil, diltiazem, nifedipine, nitroglycerin and sodium nitroprusside relative to those induced by papaverine are presented. Preparations were exposed for 20 min to verapamil, before the dose-response curve for PGF2α was obtained. Experiments with nifedipine were carried out in a dark room illuminated by sodium gas lamp to avoid daylight-induced oxidation.

Results shown in the text, tables and figures represent mean values ± SEM. Statistical analyses were made using the Student’s t-test. Drugs used were verapamil hydrochloride (Eizai Co.), diltiazem hydrochloride (Tanabe Co.), nifedipine (Bayer Co.), nitroglycerin, sodium nitroprusside, PGF2α and papaverine hydrochloride. Nifedipine was dissolved in a mixture of 96% ethanol, polyethylene glycol 400 and distilled water (15:15:70) as a stock solution, which was diluted by distilled water before use.

**Results**

**Responses of Different Arteries to Ca++ Antagonists**

In helically-cut strips of dog cerebral (basilar and middle cerebral), coronary and mesenteric arteries contracted with PGF2α or K+, the addition of verapamil (10⁻⁴ to 2 × 10⁻⁶ M) caused a dose-dependent, persistent relaxation (fig. 1). Verapamil-induced relaxations developed gradually and stabilized within 15 to 30 min. Relaxations of basilar and middle cerebral arteries did not significantly differ. Relaxations induced by verapamil were significantly greater in cerebral arteries than in coronary and mesenteric arteries when contracted with PGF2α (fig. 1, left), while these arteries contracted with K+ relaxed to a similar extent (fig. 1, right).

Typical recordings are presented in fig. 2. Maximum relaxations induced by verapamil as well as ED50's of verapamil in cerebral arteries contracted with PGF2α and K+ did not significantly differ. However, verapamil caused a greater relaxation in coronary and mesenteric arteries contracted with K+ than in the arteries contracted with PGF2α. ED50's of verapamil were less in the K+-contracted arteries (table 1).

Diltiazem (5 × 10⁻⁴ to 5 × 10⁻⁴ M) caused a greater relaxation in cerebral arterial strips contracted with...
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FIGURE 2. Comparison of the responses to verapamil of a basilar and mesenteric arterial strips obtained from the same dog. Horizontal lines just left of each tracing represent the level prior to K⁺ (14 mM) or PGF₂α (10⁻⁸ M). PA = papaverine.

TABLE 1. Maximum Relaxations Induced by Verapamil and ED50's of Verapamil in Cerebral, Coronary and Mesenteric Arterial Strips Contracted with PGF₂α or K⁺

<table>
<thead>
<tr>
<th>Artery</th>
<th>PGF₂α-contrated</th>
<th></th>
<th></th>
<th>K⁺-contrated</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ED50 (10⁻⁸ M)</td>
<td>Max. relax. (%)</td>
<td></td>
<td>ED50 (10⁻⁸ M)</td>
<td>Max. relax. (%)</td>
</tr>
<tr>
<td>Cerebral</td>
<td>1.44 ± 0.32</td>
<td>80.6 ± 3.92</td>
<td>10</td>
<td>1.02 ± 0.14</td>
<td>85.5 ± 2.07</td>
</tr>
<tr>
<td>Mesenteric</td>
<td>6.53 ± 0.88</td>
<td>43.4 ± 4.62</td>
<td>10</td>
<td>2.45 ± 0.28</td>
<td>93.4 ± 2.24</td>
</tr>
<tr>
<td>Coronary</td>
<td>2.43 ± 0.84</td>
<td>56.7 ± 3.10</td>
<td>10</td>
<td>1.53 ± 0.27</td>
<td>87.8 ± 2.45</td>
</tr>
</tbody>
</table>

* = Relaxations relative to those induced by 10⁻⁷ M papaverine. * = Significantly different from values with cerebral arteries. p <0.001. ** = Significantly different from values with PGF₂α-contrated arteries. p <0.001; * = p <0.01. N = Number of preparations used.

PGF₂α than in mesenteric arteries (fig. 3, left). However, maximum relaxations induced by 5 × 10⁻⁴ M diltiazem in these arteries did not significantly differ. Greater relaxations of cerebral arteries were also obtained with nifedipine (fig. 3, right). Solvent of nifedipine in a volume equivalent to make the bath concentration of nifedipine 2 × 10⁻⁶ M caused a significant relaxation of mesenteric arteries contracted with PGF₂α (N = 3) but a slight contraction of cerebral arteries (N = 3); therefore, the relaxant effect

FIGURE 3. Dose-response curves for diltiazem (left figure) and nifedipine (right figure) in cerebral and mesenteric arteries contracted with PGF₂α. Relaxations induced by 10⁻⁴ M papaverine were taken as 100%; mean absolute values in cerebral and mesenteric arteries in response to diltiazem were 579 ± 45 mg (N = 9) and 750 ± 90 mg (N = 9), respectively, and those in the arteries in response to nifedipine were 784 ± 181 mg (N = 9) and 747 ± 118 mg (N = 9), respectively. Figures in parentheses indicate the number of preparations used. Vertical bars represent SEM.
of nifedipine was obtained in concentrations up to $5 \times 10^{-7}$ M. ED50's of diltiazem in cerebral and mesenteric arteries were $[2.28 \pm 0.53] \times 10^{-4}$ M ($N = 9$) and $[3.67 \pm 1.46] \times 10^{-4}$ M ($N = 9$), respectively (significantly different, $p < 0.05$), and those of nifedipine were $[9.87 \pm 2.03] \times 10^{-4}$ M ($N = 9$) and $[3.77 \pm 1.07] \times 10^{-4}$ M ($N = 9$), respectively ($p < 0.05$). Relative ED50 values of verapamil, diltiazem and nifedipine were $1:15.8:0.068$ for cerebral arteries and $1:51.6:0.058$ for mesenteric arteries.

**Modification by Verapamil of the Response to PGF$_{2\alpha}$**

The addition of PGF$_{2\alpha}$ ($5 \times 10^{-8}$ to $10^{-6}$ M) elicited a dose-related contraction of cerebral and mesenteric arterial strips. The contractile response to PGF$_{2\alpha}$ was attenuated by prior treatment for 20 min with verapamil ($5 \times 10^{-7}$ and $2 \times 10^{-7}$ M) in a dose-dependent manner (fig. 4). The attenuation was greater in cerebral than in mesenteric arteries; mean values of the inhibition by $5 \times 10^{-7}$ M verapamil in the response to PGF$_{2\alpha}$ in median effective concentrations (ED50's) were 33 and 17%, respectively, and those of the inhibition by $2 \times 10^{-7}$ M verapamil were 55 and 34%, respectively.

**Responses of Different Arteries to Nitroglycerin and Sodium Nitroprusside**

Nitroglycerin and sodium nitroprusside relaxed cerebral, coronary and mesenteric arteries contracted with PGF$_{2\alpha}$ to a similar extent (fig. 5). Relaxations developed rapidly in response to these agents and stabilized within 1 to 2 min. Maximum relaxations of these arteries induced by nitroglycerin and sodium nitroprusside did not differ; however, ED50's of nitroglycerin were significantly less than those of sodium nitroprusside (table 2).

**Discussion**

Verapamil, a Ca++ antagonist, elicited a greater relaxation in arterial strips contracted with K+ than in arteries contracted with PGF$_{2\alpha}$, suggesting that the contraction induced by K+ is dependent to a greater extent upon the influx of Ca++ across cell membranes of arterial smooth muscle than the PGF$_{2\alpha}$-induced contraction. Cerebral arterial strips contracted with...
PGF\textsubscript{2\alpha} relaxed in response to Ca\textsuperscript{++} antagonists, including verapamil, diltiazem and nifedipine, to a greater extent than mesenteric and coronary arteries. Further, prior treatment with verapamil caused a greater inhibition of contractile responses to PGF\textsubscript{2\alpha} of cerebral arteries than of peripheral arteries. Similar results with nifedipine were obtained in cerebral and femoral arteries.\textsuperscript{14} Such a heterogeneity may be specific in the action of Ca\textsuperscript{++} antagonists, since a similar extent of relaxation was induced by norglucerin and sodium nitroprusside in these arteries placed under same experimental conditions. The findings obtained in the present study suggest that contractions of cerebral arteries induced by PGF\textsubscript{2\alpha} are associated to a greater extent with the influx of Ca\textsuperscript{++} than the contractions of peripheral arteries, the Ca\textsuperscript{++} influx in cerebral arteries is more susceptible to Ca\textsuperscript{++} antagonists, or Ca\textsuperscript{++} antagonists possess vasodilating actions other than the interference with Ca\textsuperscript{++} influx, which operate to a greater extent on cerebral arteries. The evidence that verapamil and Cd\textsuperscript{++} produce a greater attenuation of Ca\textsuperscript{++}-induced contractions in dog cerebral arteries exposed to Ca\textsuperscript{++}-free media and depolarized by K\textsuperscript{+} than in mesenteric and coronary arteries\textsuperscript{15} supports the second alternative.

PGF\textsubscript{2\alpha} is one of endogenous vasoconstricting substances. PGF\textsubscript{2\alpha} and other vasoconstricting PG's, including PGE\textsubscript{2} and H\textsubscript{2}, are postulated to share the same sites of excitatory action.\textsuperscript{14, 15} It has been suggested that vasoconstricting PG's are involved in the cerebral vasospasm following subarachnoid hemorrhage (Toda and Shimizu, unpublished data).\textsuperscript{15} Thus, the greater susceptibility of cerebroarterial contractions to Ca\textsuperscript{++} antagonists may indicate the usefulness of these agents for the treatment and prophylaxis of cerebral vasospasm in doses insufficient to induce vasodilatation in peripheral vasculatures. Effectiveness of nifedipine on experimentally-induced cerebral vasospasm in dogs has recently been reported.\textsuperscript{26}

The mechanism of vasodilating action of nitroglycerin and sodium nitroprusside has not been clarified. Cerebral and peripheral arteries of a similar size, contracted with PGF\textsubscript{2\alpha}, responded with a similar extent of relaxations to nitroglycerin or sodium nitroprusside, suggesting that these agents and Ca\textsuperscript{++} antagonists do not necessarily share the same mechanism of vasodilating action. The similar conclusion has been drawn by Hester et al.\textsuperscript{31} from the finding that D 600, the methoxy derivative of verapamil, and sodium nitroprusside differently affect \textsuperscript{45}Ca\textsuperscript{++} fluxes in isolated dog renal arteries.

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INCREASED MEDIA THICKNESS in arteries of hypertensive individuals has generally been considered to imply an adaptation of arterial design to increased intraluminal pressure. It has been shown that arterial vessels of the rat adapt structurally during renal hypertension by increasing their media thickness. However, according to Friedman et al. a sustained hypertensive state does not necessarily result in hypertrophy of the arterial wall. Although the arterial media has been reported to be thicker during established hypertension, there exists no morphological proof that the aberration in these cases implies an adaptation to increased intraluminal pressure. To study structural adaptation, information is needed not only about arterial morphology during established hypertension, but also about arterial structure during early hypertension or a pre-hypertensive stage. In order to clarify the question of arterial structural adaptation in SHR an investigation of different vascular beds of 15- and 200-day-old SHR as well as normotensive controls was carried out. Preliminary results have previously been reported. The final results and statistical evaluation of cerebral arterial vessels and internal carotid arteries are presented in this paper.

**Methods**

**Animals: 15 Days.** Cerebral vessels were measured in non stroke-prone SHR and Wistar Kyoto rats (WKY), each group consisting of 4 males and 4 females. Internal carotid arteries were collected from a different population, comprising 7 sex- and weight matched rats of either kind. Since the controls of this last study had slightly lower body weight, they were compared with 13-day-old SHR.

**200 Days.** This study comprised non stroke-prone spontaneously hypertensive rats (SHR) Wistar Kyoto rats (WKY) and local Wistar rats (LWR), each group consisting of 4 male rats.

**Blood Pressure Measurements.** In the 200-day-old rats mean arterial pressure (MAP) was measured in the aorta through a catheter from the femoral artery during diazepam anesthesia. In 15-day-old rats MAP was measured through a specially designed needle in the femoral artery. Initially, a small dose of methohexitol (Bretital®), was given intraperitoneally, whereupon the animals were maintained on N₂O and O₂ in the proportion of 2:1. For technical reasons, other 15-day-old animals were used than were used in the morphometric study. Each group consisted of 4 males and 4 females. The mean value of 6 recordings was calculated for each animal. Student's t-test was used for statistical evaluation. In the 15-day-old rats of the
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