Dopamine Attenuates the Contractile Response to Angiotensin II in Isolated Rat Glomeruli and Cultured Mesangial Cells

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Recent evidence suggests that dopamine may alter kidney function by actions not only in the renal vasculature but also at the glomerular–mesangial level. We studied this phenomenon by examining the ability of dopamine to antagonize the contractile properties of angiotensin II in isolated rat glomeruli and cultured mesangial cells. In isolated rat glomeruli angiotensin II caused a decrease in the planar surface area, indicating glomerular contraction, an effect that was abolished by coincubation with dopamine. Angiotensin II also mediated shape changes in cultured mesangial cells, which resulted in a decrease in planar areas. Simultaneous addition of dopamine prevented these decreases in cell size. In mesangial cells grown on a flexible silicone rubber support, angiotensin II addition enhanced wrinkling of the mobile surface. This indicated that the angiotensin-II-induced decrease in cell size observed in cells grown on conventional substrata represented contraction. Conversely, dopamine caused a rapid reduction in wrinkling of the surfaces from control cells as well as those previously treated with angiotensin II, actions consistent with cell relaxation. The prostaglandin inhibitor indomethacin did not alter the ability of dopamine to attenuate angiotensin-II-associated reductions in mesangial cell surface area. Direct determination of mesangial cell prostaglandin-E2 production showed that dopamine did not change either basal synthesis or angiotensin-II-stimulated synthesis of prostaglandin. The results demonstrate that dopamine antagonizes the constrictor effect of angiotensin II at the glomerular–mesangial level. This action of dopamine is prostaglandin independent. These findings support a role for dopamine in the regulation of glomerular filtration and may provide a rationale for its use during states of renal vasoconstriction. (Circulation Research 1986;59:529–533)

Dopamine (DA) is an endogenous catecholamine that has positive inotropic properties and vasodilator actions especially in the renal circulation. DA may play a physiological role in regulating renal blood flow. For example, DA decreases renal vascular resistance and antagonizes norepinephrine-mediated vasoconstriction in isolated rabbit efferent and afferent arterioles. DA could also preserve renal blood flow and glomerular filtration rate in clinical conditions characterized by enhanced pressor activity. Kidney tissue is able to synthesize DA and renal cortical slices, proximal tubular cells, and to a lesser extent, isolated glomeruli have been shown to convert L-DOPA to dopamine. Dopaminergic nerves adjacent to the vascular pole of the glomerulus could provide an additional source of DA, especially since dopaminergic receptors have been characterized in isolated rat glomeruli. DA stimulates cAMP production in isolated glomeruli and cultured mesangial cells. Cyclic AMP, in turn, relaxes mesangial cells and antagonizes the shape-change induced by vasopressin. This may be another example of cAMP-induced smooth muscle relaxation.

Vasoactive substances such as angiotensin II (AII) cause renal vascular constriction and decrease the glomerular ultrafiltration coefficient Kf. The latter has been postulated to result in part from diminished effective filtration surface area, possibly as a result of mesangial cell contraction. This hypothesis is supported by the reduction in planar surface area in isolated glomeruli and in cultured mesangial cells following incubation with AII. In order to assess the ability of DA to attenuate the effect of pressor peptides such as AII at the glomerular mesangial level, we examined the direct effects of AII and DA on the surface area of isolated glomeruli and cultured mesangial cells as a reflection of their contractility. To assess a possible role for prostaglandin in mediating the effects of DA experiments were also carried out in the presence of prostaglandin synthesis inhibition. Furthermore, the direct effect of DA on PGE2 synthesis was evaluated. Our results show that DA antagonizes the shape-changes induced by AII in isolated glomeruli and in cultured mesangial cells by a mechanism that is independent of prostaglandin.

Materials and Methods

Dopamine was purchased from Sigma (St. Louis, Mo.). Angiotensin II was obtained from Ciba-Geigy (Summit, N.J.). [3H] PGE2 (130 Ci/mole) was purchased from Amersham (Arlington Heights, Ill.), and

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antibody to PGE₂ was obtained from Institute Pasteur (Paris, France).

**Glomerular Isolation and Culture of Mesangial Cells**

Male Sprague-Dawley rats (Charles River Breeder, Wilmington, Mass.) 150-250 were maintained on tap water and Purina rat chow ad libitum. Kidneys were removed under pentobarbital anesthesia. The glomeruli were isolated and cultured as previously described.1314 The culture medium consisted of RPMI 1640 (Grand Island Biological Laboratory) supplemented with 10% fetal calf serum, penicillin (0.66 μg/ml) and streptomycin sulfate (60 μg/ml). After mesangial cells had reached confluence (15-20 days after glomerular seeding) they were subcultured according to previously published methods.1314

**Incubation of Mesangial Cell for PG Synthesis**

Experimental incubations were performed on cells 7-10 days after the first subculture. The culture media was discarded, the flask washed twice with 5 ml of buffer (20 mM Tris-HCl, pH 7.4, 5 mM glucose, 135 mM NaCl, 10 mM KCl, 10 mM Na-acetate, 2 mM CaCl₂, and 2 mg/ml of essential fatty acid free bovine serum albumin) and incubated with 3.5 ml of this solution at 37°C. Experimental agents were made up in buffer and added to the incubations after a 10-minute control period to yield the final concentrations indicated in “Results.” Aliquots (0.5 ml) of incubation buffer were removed after an additional 10 minutes and stored at —20°C for subsequent radioimmunoassay of prostaglandin.16

**Direct Microscopical Observation of Mesangial Cells**

**Cells grown on rigid substratum.** Direct observations of mesangial cells grown in conventional plastic flasks (3-7 days after subculture) were carried out at room temperature under phase contrast with an inverted Diaphot-TMD Nikon microscope equipped with a Microphot UFX-11 photographic set (Nikon Kogaku, K.K.). Serial photographs of the cells were taken before and after experimental additions. Photographs of a calibrated micrometer standard were also obtained. Four to 19 individual cells could be evaluated per visual field and experiment. Surface area of individual cells was determined from the photographs in a blinded manner using a #1200 electronic planimeter (Nikon Kogaku, K.K.). Serial photographs of the cells were taken before and after experimental additions. Photographs of a calibrated micrometer standard were also obtained. Surface area under control and experimental conditions were compared using the Bonferroni inequality for the paired t test.13

**Cells grown on flexible substratum.** After subculture, mesangial cells were plated in 35-mm Petri culture dishes containing glass coverslips that had been coated with silicone rubber.15 Briefly, silicone rubber (60,000 centistoke dimethylpolysiloxane) was applied to one surface of a 22 × 22 mm coverslip and heated for 2 seconds over a low Bunsen flame. This formed a thin, flexible skin overlying a viscous fluid base. Resting tension of cells adhered to and growing on this film caused a wrinkling of the silicone rubber surface. Subcultured cells grown for 4-7 days on this surface were observed under conditions described above using phase contrast microscopy and photomicrography. (The wrinkling frequently obscured the cell borders, and we will therefore refer to cell complexes, rather than to individual cells.) The length of wrinkles per cell complex (a composite of elongation of preexistent wrinkles and formation of new ones) was determined in a blinded fashion from the photographs by electronic planimetry, expressed and analyzed as above.

**Determination of Planar Surface Area of Isolated Glomeruli**

Glomeruli isolated by successive sieving,16 were washed three times in a Robinson’s buffer containing 1 mM CaCl₂. Aliquots of glomeruli were incubated with vehicle or experimental agent for 20 minutes at room temperature and fixed in 1% glutaraldehyde. The glomerular planar surface area was determined within several hours using an automated #720 Quantimet Image Analyzer (Cambridge Instruments of Moncie, N.Y.). The planar surface area of 30-60 glomeruli was determined for each experimental maneuver. For group analysis of all experiments, the mean planar surface areas of control and experimental glomeruli in each experiment (i.e., the mean of 30-60 glomeruli was considered as one experiment) were compared using the Bonferroni inequality for the paired t test.13

**Results**

**Glomerular Planar Surface Area**

The glomerular planar surface area of 1,053 glomeruli from control incubations in seven experiments was 15,146 ± 641 μ². All (10⁻⁷ M) significantly decreased planar surface area by 11% to 13,500 ± 604 μ² (7 experiments with 304 glomeruli p<0.01 compared to control). Coincubation of glomeruli with AII and DA significantly blunted this effect to values not different from control (14,866 ± 620 μ²; 7 experiments with 282 glomeruli p<0.02 compared to AII only). DA alone had no significant effect on planar surface area (14,948 ± 620 μ²; 7 experiments with 285 glomeruli).

**Planar Surface Area of Mesangial Cells Grown on Rigid Substrates**

Cultured mesangial cells showed the typical stellate appearance with multiple cell extensions using phase contrast microscopy. Under control conditions, cell shape and planar surface area did not appreciably change over a 40-minute period. Addition of AII (10⁻⁷ M) produced a shape change consisting of shortening and narrowing of cell extensions and a decrease in total cell surface area as previously reported.14 At room temperature these changes could be observed as early as 5 minutes after addition of AII and progressed up to at least 20 minutes. A scattergraph of the percent of change observed under the different conditions over a 20-minute period is shown in Figure 1. About 40% of cells showed a decrease in planar size that exceeded 5% of control during AII incubation. DA alone caused...
no significant change, but DA almost completely prevented the decrease in cell planar surface induced by All. A quantitative evaluation of the mean percent of change in planar surface area of all cells under the various conditions is illustrated in Figure 2. All caused an overall mean decrease in planar surface area of 7% with some cells exhibiting minimal changes and others diminishing by more than 20% (see Figure 1). Coincubation of All with DA (10^{-6} M) markedly attenuated the All effect (Figure 2). Addition of DA alone caused no appreciable difference compared to controls. A similar analysis was performed on cells pretreated with indomethacin (10 \mu M) in order to exclude prostaglandin as potential mediator for the DA effect on All-induced shape change. In four series of experiments indomethacin alone, or indomethacin plus DA, caused no appreciable differences in cell size over a 40-minute period. All addition resulted in a significant decrease in surface area by $-463 \pm 63 \mu m^2/cell$ ($p<0.01$), which was attenuated by DA to $-93 \pm 41 \mu m^2/cell$ in spite of inhibition of prostaglandin synthesis.

Mesangial Cells Grown on Flexible Substra

In order to ascertain whether the shape changes observed in mesangial cells were indeed due to contraction, we performed additional experiments with cells grown on a mobile surface.18 In this system, contraction of cells results in increased wrinkling of the mobile film on which the cells are grown, while cell relaxation causes a decrease in wrinkles. Under basal conditions, most cells exhibited some wrinkling of the silicone rubber surface, indicating resting tension (Figure 3). In 14 experiments involving 67 cells, there were no significant changes in the length of wrinkles during a 10-minute control period. Addition of All (10^{-7} M) resulted in a progressive increase in the length of wrinkles from $640 \pm 100 \mu m/cell$ complex to $740 \pm 100 \mu m$ (7 experiments with 23 cell complexes; $p<.005$) during a 20-minute incubation (for example, see Figure 3B), with some effects noted as early as 2 minutes. Addition of DA (10^{-6} M) only (Figure 3A) reduced wrinkle length from $440 \pm 60 \mu m/cell$ complex to $260 \pm 30 \mu m$ (6 experiments with 21 cell complexes; $p<.005$). With sequential addition of experimental agents at 20-minute intervals (Figure 3B), we observed an initial increase in wrinkles from a control of $760 \pm 120 \mu m/cell$ complex to $840 \pm 120 \mu m$ ($p<0.01$) with All followed by a rapid loss of wrinkles with DA to $690 \pm 120 \mu m$, a number not different from control but significantly lower than All only ($p<0.005$; 5 experiments with 17 cell complexes). When DA was added, first cell wrinkles decreased from $300 \pm 50 \mu m/cell$ complex to $200 \pm 40 \mu m$ ($p<0.05$), and subsequent addition of All did not increase wrinkle length (210 $\pm 40 \mu m; 3$ experiments with 12 cell complexes). Thus, DA decreased resting tension of cells and antagonized the All-induced increase in cell tension.

PG Production by Cultured Mesangial Cells

The experiments carried out with DA in the presence of indomethacin argued against an effect involving prostaglandins. To verify this, we determined PGE_2 production by mesangial cells under the different conditions. Basal PGE_2 production by cultured mesangial cells was $6.7 \pm 1.3 ng/culture flask$ (results from 11 cultures in 5 different experiments). Incubation with DA (10^{-6} M) did not increase PGE_2 production (7.1 $\pm 0.8 ng$). In contrast, All (10^{-5} M) significantly increased PGE_2 synthesis to $45.6 \pm 7.1 ng$. Coincubation of All with DA resulted in comparable stimulation to $58 \pm 12 ng$, which was not different from All alone.

Discussion

Our results show that DA can antagonize the constrictor effect of All at the glomerular–mesangial level. This is supported by the finding that the All-

![Figure 1. Number of mesangial cells versus percent of change in surface area compared to last preexperimental control. Cell sizes were evaluated after a 20-minute control and a subsequent 20-minute period with experimental agents present. Results are from 5 experiments.](image1)

![Figure 2. Effect of experimental agents on change in planar surface area of mesangial cells. Addition of experimental agents was after a 20-minute control period and cell area was evaluated for another 40-minute incubation at room temperature. Results are means ± SEM of all cells observed in 5 sets of experiments. Numbers of cells evaluated are indicated in brackets. **Indicate p<0.01 or better as compared to 20-minute control.](image2)
FIGURE 3. Representative photographs of the effects of DA (10^{-6} M) and All (10^{-7} M) on cells grown on flexible silicone rubber. A. The wrinkles generated by the resting tension of cells under control conditions (con) markedly decrease 10 minutes after addition of DA, revealing the underlying mesangial cells. B. After 20-minutes All has considerably enhanced wrinkling — indicating increased cell tension — while subsequent addition of DA diminishes that effect at 50 minutes. The bright particle represents an artifact floating in the incubation buffer.

...ated decrease in the planar surface area of isolated glomeruli and cultured mesangial cells was antagonized by coincubation with DA. The results of experiments using flexible silicone rubber substrata further support the idea that the mesangial cell shape change mediated by All represent contraction, while DA causes mesangial cell relaxation. We have previously characterized this system and shown that it permits differentiation of changes in cell shape resulting from contraction from those due to cell detachment or cell relaxation. The exact mechanism of action for DA in the glomerular mesangium has not been defined. Glomeruli contain dopaminergic receptors, and DA enhances synthesis of cAMP in cultured mesangial cells. As the relaxation produced by DA in mesangial cells is similar to that observed with cAMP, it is possible that this effect of DA on glomeruli and mesangial cells is secondary to cAMP generation. On the other hand, our results exclude prostaglandins as mediators of DA action. It has also been reported that pretreatment of rats with indomethacin fails to alter the DA-induced decline in renal vascular resistance. In the present study indomethacin did not attenuate the effect of DA on mesangial cells and DA did not change basal or All-stimulated prostaglandin synthesis. Interestingly, this also shows that All-associated prostaglandin release does not depend on mesangial cell contraction. Edwards has reported direct vasodilatory properties of DA on isolated rabbit efferent and afferent arterioles. Our present results add the glomerulus itself as a target for DA, strengthening the argument for a role of DA in the physiologic regulation of glomerular filtration. Furthermore, these effects of DA may be of particular consequence during the activation of pressor systems. For example, in septic shock, dopamine infusion has been reported to increase glomerular filtration rate without altering renal blood flow.

In summary: DA antagonizes All-mediated contraction of isolated glomeruli and cultured mesangial cells. This action of DA does not depend on prostaglandin. These findings help to explain effects of DA on the regulation of glomerular filtration and may provide a rationale for the use of DA during states of marked renal vasoconstriction.

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