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Kidney International, Vol. 35 (1989), pp. 1300–1305

Adenosine induces mesangial cell contraction by an A_1-type receptor

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Adenosine induces mesangial cell contraction by an A_1-type receptor. Adenosine is known to decrease renal blood flow and glomerular filtration rate. We have tested the hypothesis that adenosine exerts contractile effects on mesangial cells. Furthermore, we have studied, using selective agonists and antagonists for adenosine, which kind of adenosine receptor, A_1 or A_2, is mainly implicated in this response. We also investigated whether calcium is involved in adenosine-induced mesangial cell contraction. Rat cultured mesangial cells were exposed to adenosine (10^{-7} to 10^{-3} M) and the contraction was measured as changes in planar cell surface area (PCSA). Adenosine induced a time- and dose-dependent reduction of PCSA. This reduction in PCSA was prevented by incubation with the A_1 blocker PD116,948 but not with the A_2 blocker PD115,199. Adenosine-5′-ethylcarboxamide (NECA), an A_2 agonist, did not induce significant changes in PCSA whereas N^6-S-1-methyl-2-phenylethyl adenosine (S-PIA), an A_1 agonist, induced a dose-dependent decrease in PCSA. Adenosine-induced mesangial contraction was prevented by verapamil or by incubation in a calcium-free medium. These results suggest that adenosine induces a specific contraction of cultured rat mesangial cells that seems to be mediated by its binding to the adenosine A_1-type receptor. This contraction seems to be dependent on the influx of extracellular calcium.

Adenosine is present in kidney tissue not only in conditions of ischemia but also in physiological conditions [1]. Intrarenally produced adenosine has been shown to reduce glomerular filtration rate (GFR), decrease renal blood flow (RPF) transiently [2, 3], inhibit renin secretion [4, 5] and decrease renal excretion of sodium and water [6]. Although the importance of adenosine on the intrinsic physiologic control of GFR or RPF is not fully understood, it has been suggested that adenosine mediates tubuloglomerular feedback or to be the link between renal metabolism and GFR regulation [5]. Very little is known about the exact mechanism by which adenosine affects GFR and RPF, but it is widely accepted that it increases preglomerular resistance and lowers efferent arteriolar resistance [7, 8]. Furthermore, angiotensin II appears to be essential in the afferent vasoconstrictor response to adenosine [7].

However, this is not the sole possible mechanism responsible for the adenosine induced changes in GFR. Glomerular mesangial cells are indirectly connected to smooth muscle cells of arterioles establishing a functional syncytium, and it is very likely that a substance that might act on arterioles should also act on mesangial cells, thus influencing the filtration process [9]. Mesangial cells are able to contract when stimulated with several substances and vasoactive peptides [10–12] which control renal function, and this contraction is thought to be related to changes in the K_f, and thus in GFR [13, 14].

We have previously shown that adenosine contracts isolated glomeruli [15] and it is quite probable that mesangial cells are responsible for this contraction. So, we have tested the hypothesis that adenosine has contractile effects on mesangial cells. Furthermore, we have studied, using selective agonists and antagonists for adenosine, which kind of adenosine receptor, A_1 or A_2, [16] is mainly implicated in this response. On the other hand, we investigated whether calcium, the major messenger mediating contractile responses [17], is involved in mesangial cell contraction promoted by adenosine.

Methods

Materials

Adenosine, S-PIA (N^6-S-1-methyl-2-phenylethyl adenosine), NECA (adenosine-5′-ethylcarboxamide), TMB-8 [3,4,5,trimethoxybenzoic acid 8-(diethyl amino) octyl ester hydrochloride], collagenase type I A, from clostridium histolyticum and L-glutamine were purchased from Sigma Chemical Co. (St. Louis, Missouri, USA). Verapamil was provided by Knoll Iberica, Madrid, Spain. PD116,948 (8-ciclopenthyl-1,3-dipropylxantine) and PD 155,199 [N-(2-dimethylaminoethyl)-N-methyl-4-(2,3,6,7-tetrahydro-2,6 dioxo-1,3-dipropyl-1H-purin-8-yl)benzenesulfonamide] were from Warner-Lambert, Parke Davis, Ann Arbor, Michigan, USA. Penicillin was obtained from Laboratories Level SA, Barcelona, Spain. Streptomycin sulfate was obtained from Antibioticos SA, Madrid, Spain. Amphotericin B was obtained from Squibb Industria Farmaceutica SA, Barcelona, Spain. RPMI 1640, Hanks balanced salt solution and fetal calf serum were obtained from Flow Laboratories, Woodcock Hill, United Kingdom.

Mesangial cell culture

Renal glomeruli were isolated from Wistar rats weighing 100 to 150 g, maintained on water and standard rat chow ad libitum. Kidneys were removed under ether anesthesia and glomeruli isolated by successive mechanical sieving (150 and 50 μm) as previously described [18]. Isolated glomeruli were treated with

Received for publication July 11, 1988
and in revised form January 20, 1989
Accepted for publication January 27, 1989

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collagenase, plated in plastic culture flasks and incubated as previously described [19]. The culture medium consisted of RPMI 1640 supplemented with 10% fetal calf serum, L-glutamine (1 mM), penicillin (0.66 μg/ml), streptomycin sulfate (60 μg/ml), amphotericin B (2.5 μg/ml) and buffered with Hepes, pH 7.2. Culture media was changed every two days. Epithelial cells grew rapidly whereas mesangial cells grew slowly, with a peak of mesangial cell density at 18 to 22 days. Studies were performed on day 21 or 22, at which time epithelial cells were no longer detected in the culture flasks. The identity of the cells was confirmed by morphological and functional criteria: under phase contrast microscopy cells appeared large and stellate. By transmission electron microscopy the cells contained numerous bundles of microfilaments, dense patches and elongated nuclei. Mesangial cells showed histochemical evidence of actin fibers and they, unlike endothelial cells, did not stain for factor VIII. In addition, angiotensin converting enzyme activity was negative in the cultures, and no cells with epithelial or endothelial morphological characteristics were seen in the cultures. In all the cultures examined, most of the cells contracted after incubation with angiotensin II or AVP.

Determination of planar surface area of mesangial cells

Before the experiments, cells were washed from the culture media and placed in Tris-glucose buffer (Tris 20 mM, NaCl 130 mM, KCl 10 mM, sodium acetate 10 mM and glucose 5 mM, pH 7.45) containing 2.5 mM Ca^{2+} for 30 minutes. Then adenosine or its agonists (S-PIA or NECA) were added to the media and incubated for 40 minutes. In the experiments in which antagonists of adenosine or calcium antagonists were used, cells were preincubated in the presence of the antagonist (PD115,199, PD116,948, TMB8 or verapamil) for 10 minutes before adding adenosine. Cells incubated in buffer with the solvent of these substances were considered as controls.

Direct observation of mesangial cells grown in conventional plastic culture flasks was carried out at room temperature under phase contrast with an inverted Olympus photomicroscope. Serial photographs of the cells were taken before and after experimental additions. Surface area was determined by a computer-assisted planimetric technique (Cardio-80, Kontron Medical, Munich, FRG). Data of mesangial cell contraction are expressed as mean ± SEM of three different experiments, with 15 to 20 cells measured per experiment.

Statistical methods

Changes with respect to basal values were analyzed by paired Student's *t*-test. Comparisons between means of multiple groups were analyzed by one-way analysis of variance and Scheffé's multiple comparisons test. Time course studies were compared by two-way analysis of variance.

Results

Effects of adenosine on mesangial cell contraction

After 30 minutes of incubation, adenosine decreased mesangial planar cell surface area (PCSA) in a dose-dependent manner. The lowest doses of adenosine tested (10^{-7} M and 10^{-6} M) did not produce any change in mesangial superficial area, but in doses higher than 10^{-6} M induced a statistically significant reduction on PCSA. Figure 1 shows that the addition of adenosine 10^{-4} M induced a change in the cell shape, with decrease in the apparent PCSA of the cells with respect to their shape before adenosine addition. This contraction was dose-dependent (Fig. 2) and time-dependent (Fig. 3). Thus, when mesangial cells were incubated in presence or absence of 10^{-4} M adenosine, it was observed that after 10 minutes of incubation with adenosine, reduction in PCSA was already evident ($P < 0.001$), while no change was observed in control cells.

Effects of adenosine agonists S-PIA (A_1) and NECA (A_2)

S-PIA, an agonist for type A_1 adenosine receptors, had a very similar effect to that of adenosine on mesangial cell contraction. While the lowest doses of S-PIA (10^{-7} and 10^{-6} M) did not have any effect, concentrations higher than 10^{-6} M diminished PCSA (Fig. 4).

On the contrary, NECA, an A_2 agonist, did not show any dose-dependent response and none of the concentrations tested (10^{-7} to 10^{-3} M) induced changes in PCSA.

Effects of the antagonists PD115,199 and PD116,199

Preincubation of cells with PD116,948 (10^{-6} M), an A_1 highly-selective antagonist ligand, completely inhibited adenosine-induced mesangial cell contraction (Fig. 5). However PD115,199 (10^{-5} M), an antagonist ligand of high affinity for A_2 adenosine receptors, was unable to modify adenosine 10^{-4} M induced response. When both A_1 and A_2 antagonists were used, 10^{-4} M adenosine did not contract mesangial cells. Preincubations with either antagonist alone did not significantly modify mesangial cell area after 40 minutes (91 ± 5.3% and 107 ± 4.7% of initial surface area for A_1 and A_2 antagonists, respectively).

Effects of the calcium antagonists verapamil and TMB8

Figure 6 shows that preincubation with verapamil (10^{-5} M) prevented 10^{-4} M adenosine induced contraction, while TMB8 (10^{-4} M) was not able to prevent it. In addition, 10^{-4} M adenosine did not reduce mesangial area when cells were incubated in a calcium free medium. Preincubation with verapamil or TMB8 alone did not modify planar surface area (91.7 ± 4.7% of initial surface area after 40 min in control cells vs. 90 ± 2.7% or 91.7 ± 4.6% in verapamil or TMB8 pretreated cells).

Discussion

Adenosine has been proved to decrease GFR and RBF by modifying arteriolar efferent and afferent resistance [2, 7, 20]. A transient increase of preglomerular vessel resistance appears to explain adenosine-mediated renal transient vasoconstriction. Persistent decrease in GFR which occurs in spite of an increase of RPF after 20 to 30 minutes of adenosine infusion is explained, in addition, by its vasorelaxant effects on efferent arterioles [7]. Here we propose mesangial cell contraction as another mechanism by which adenosine could be able to decrease GFR independently of RPF changes.

Our results demonstrate that adenosine induces a dose- and time-dependent reduction of rat mesangial cell surface area. Mesangial cell area reduction is generally considered as contraction [21, 22, 12]. Adenosine induced reduction of PCSA seems to be mediated by the binding of adenosine to a specific receptor since it can be inhibited by a selective antagonist of A_1 adenosine receptor (PD116,948). The ability of mesangial cells to contract as a specific response [23] to distinct agonists is

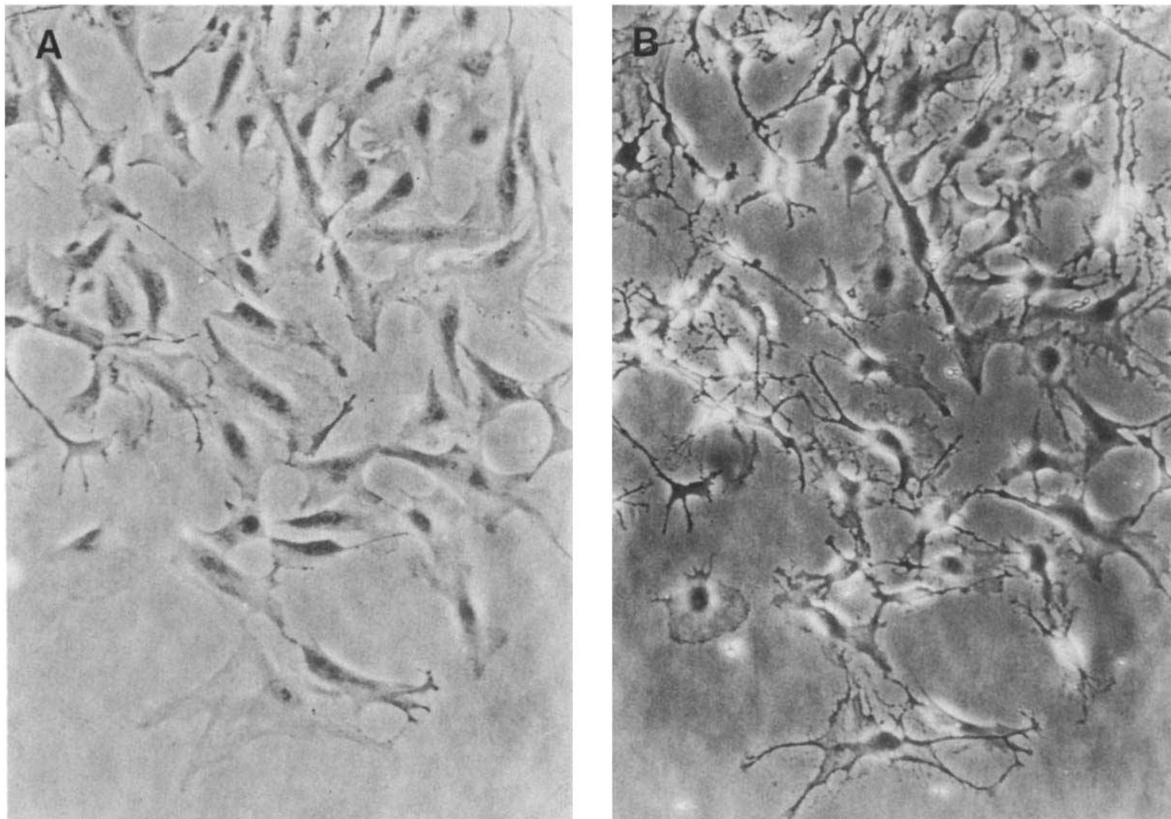

Fig. 1. *Effect of 10^{-4} M adenosine on the shape of cultured mesangial cells.* Figure shows the same photographic field before (**A**) and 30 min after adenosine (**B**). Note the change in cell shape and the decrease in planar cell surface area.

widely considered as a mechanism of reduction of the K_f [12, 24] by which mesangial cells regulate GFR [13, 14, 24]. Thus, adenosine would induce a sustained contraction of these cells that could be responsible, at least in part, for the reduction in GFR induced by adenosine. In agreement with these results we have previously shown that adenosine reduces cross sectional area of isolated glomeruli [15], and probably mesangial cells are the major element which accounts for this effect. To our knowledge, the in vivo effect of adenosine on K_f has been reported only by Oswald, Speilman and Knox [20] and they did not find any change in K_f. However these studies were performed in dogs which probably had an activated renin-angiotensin system because of the surgical stress associated with the micropuncture studies, and the high levels of angiotensin, acting directly on the K_f, could mask the effect of adenosine on K_f. Moreover, in vivo and in vitro experiments are not easily comparable. Renal feedback mechanisms are completely intact in micropuncture experiments whereas only local feedback mechanisms remain active in cultured mesangial cells. In other words, in the micropuncture studies, not only the effect of the injected agonists are observed, but also the effects of other vasoactive systems which is modified by the substance tested. In the case of adenosine, it presumably modifies catecholamine release, renin-angiotensin system activation and prostanoid synthesis, and the effects of this modification are presumably

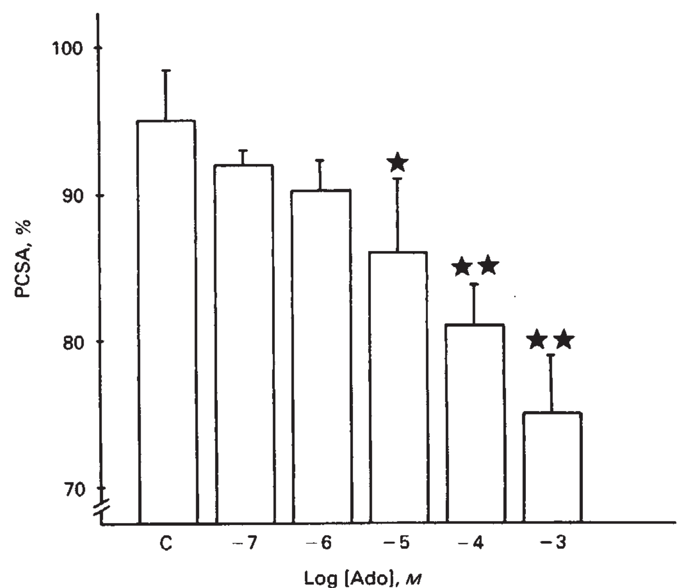

Fig. 2. *Effect of different doses of adenosine (10^{-7} to 10^{-3} M) on the reduction of PCSA after 40 minutes of incubation.* PCSA at minute 40 is expressed as percentage of initial PCSA. Data are mean ± SEM. * Statistically significant differences ($P < 0.05$) with respect to cells incubated in control conditions (C); ** statistically significant differences ($P < 0.01$) with respect to cells incubated in control conditions.

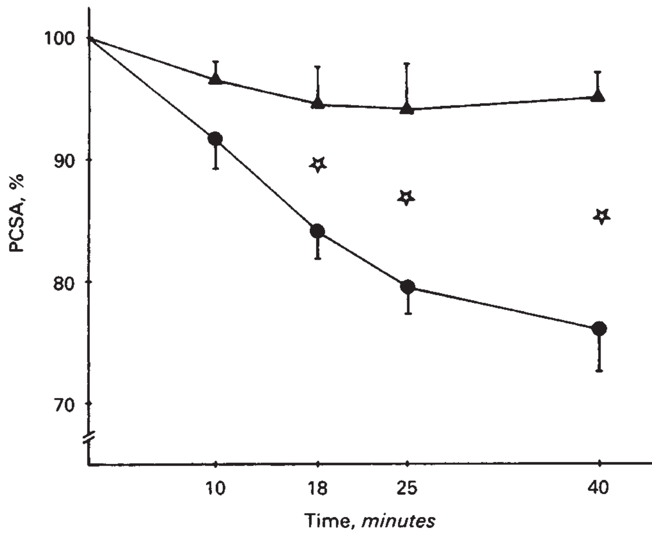

Fig. 3. *Time-course of the effect of adenosine (circles) on the reduction of mesangial PCSA expressed as a percentage of initial PCSA.* Data are mean ± SEM. * Statistically significant differences ($P < 0.01$) with respect to cells incubated in control conditions (triangles).

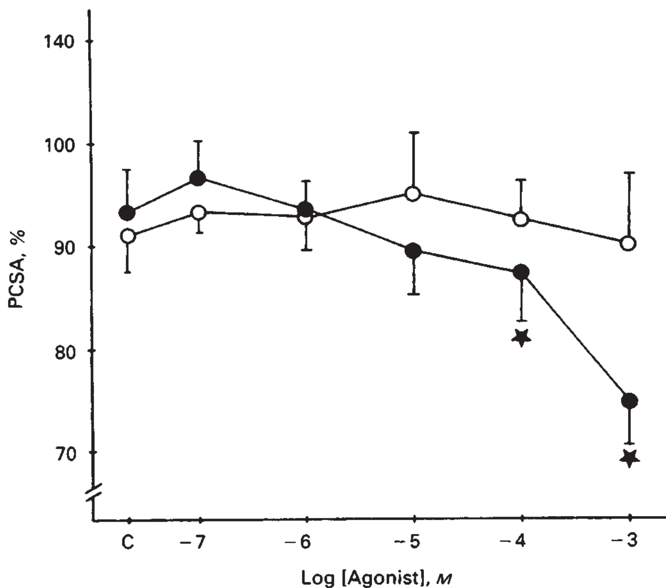

Fig. 4. *Dose-triangle of the A_2 agonist NECA (open circles), and the A_1 agonist S-PIA (filled circles) on the reduction of mesangial PCSA after 40 min of incubation.* Data are mean ± SEM. PCSA at the minute 40 is expressed as a percentage of initial PCSA. * Statistically significant differences ($P < 0.01$) with respect to cells incubated in control conditions (C). The dose-response curve of NECA is statistically different ($P < 0.01$) from that of S-PIA.

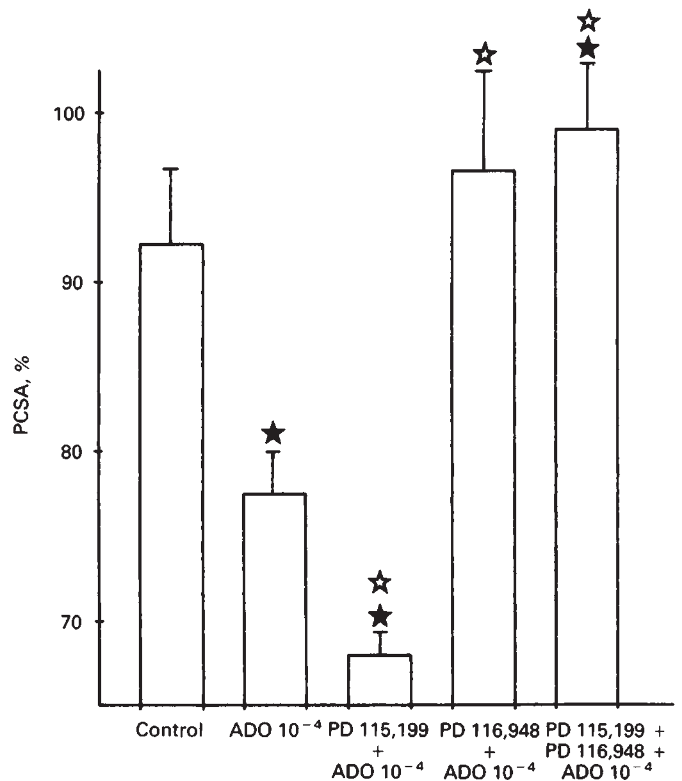

Fig. 5. *Effect of the antagonists of adenosine on the reduction of mesangial PCSA induced by adenosine 10^{-4} M.* Cells were preincubated with the antagonist A_1 (PD116,948) or A_2 (PD115,119) or both for 10 minutes before adding adenosine. PCSA at the minute 40 of incubation with adenosine is expressed as a percentage of initial PCSA. Data are mean ± SEM. ★ Statistically significant differences ($P < 0.01$) with respect to cells incubated in control conditions; ☆ statistically significant differences ($P < 0.01$) with respect to adenosine-treated cells.

measured by the micropuncture techniques. In vitro experiments can clarify these mechanisms.

As mentioned above, adenosine contracts mesangial cells in a concentration dependent fashion. Although the higher concentration tested (10^{-3} M) produced the maximal contraction, these effects were already evident at a dose of 10^{-5} M. However, we are concerned about the dissociation existing between the concentrations of adenosine which exerts its effects in vivo and those that are active contracting mesangial cells "in vitro". We do not have a clear explanation for this discrepancy, but it is possible that differences in temperature could be a reason. The in vitro experiments performed at room temperature (22°C) and in vivo studies performed at 37°C could be a temperature difference influencing both the kinetics of adenosine binding to its receptors, and the intracellular processes leading to cell contraction. In addition, phenotypical characteristics of the cultured mesangial cells could differ from those of cells in vivo in the number of membrane receptors, its affinity for adenosine or the ability to contract. In fact, it has been reported that after multiple culture passages, the cells lose membrane receptors for angiotensin II [25]. Finally, pericellular adenosine concentration in the in vivo studies are not known, and perhaps it could be similar to those in which we found a contractile response.

Two classes of external receptors for adenosine, namely A_1 and A_2 adenosine receptors, have been recognized [6, 16, 26, 27]. In many cell types A_1 receptors couple to adenylate cyclase in an inhibitory manner [16] and A_2 receptors couple to adenylate cyclase in a stimulatory way [28, 29]. The presence of A_1 and A_2 sites in the kidney has been shown by several investigators [26–32]. Autoradiographic visualization of adenosine A_1 receptors in human kidney shows a periglomerular localization of these receptors [31]. Glomeruli have both receptors: A_1,

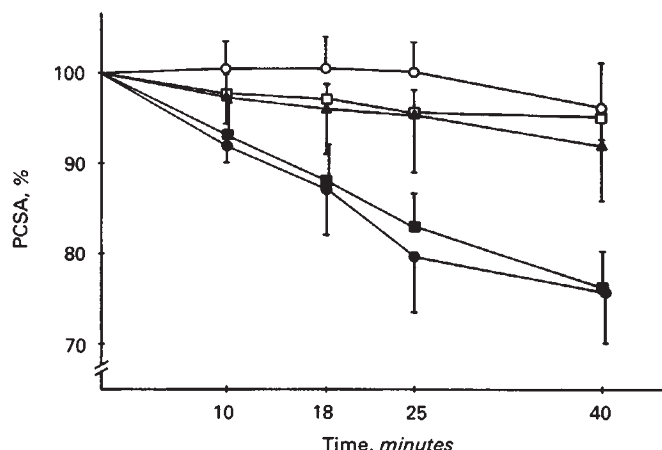

Fig. 6. *Effect of the calcium antagonists on the reduction of mesangial PCSA induced by adenosine 10^{-4} M.* PCSA at each time is expressed as a percentage of initial PCSA. Cells were pretreated with verapamil or TMB8 before adding adenosine. Symbols are: (▲) control cells; (□) verapamil + adenosine; (○) adenosine + calcium-free medium; (■) TMB8 + adenosine; (●) adenosine alone. The curves of adenosine and TMB8 + adenosine treated cells were statistically different ($P < 0.01$) from that of control cells. The curves of adenosine + calcium-free medium and verapamil + adenosine were statistically different ($P < 0.01$) from that of adenosine alone.

demonstrated by binding studies, and A_2, functionally identified [32]. Our results suggest that adenosine-induced mesangial cell contraction is mediated by an A_1 adenosine receptor for the following reasons: The A1 agonist S-PIA reproduced the dose-dependent adenosine response, whereas the more potent A_2 agonist NECA did not induce mesangial cell contraction. A_1 selective antagonist PD 116,948 abolished the response elicited by ADO, in contrast to the A_2 antagonist PD 115,199, which did not modify it. Although the existence of an A_1 receptor type involved in mesangial contraction can be deduced from these data, the characterization of this receptor by binding studies would be necessary for direct evidence.

The lack of a role for A_2 receptors in adenosine induced contraction of glomerular mesangial cells is supported by the fact that NECA infusion did not modify GFR in anesthetized rats [8]. In addition, NECA increased GFR in perfused kidneys by relaxing the efferent arteriole [33]. Although NECA has some affinity for A_1 receptors, its physiological effects seem to be related to relaxation and cAMP increments by acting on A_2 receptors. Another result supporting the lack of involvement of A_2 receptors in mesangial contraction is the lack of effect of the A_2 antagonist PD115,199 to prevent adenosine-induced mesangial cell contraction.

Our results also suggest that adenosine induced contraction of mesangial cells is dependent on the influx of calcium from extracellular medium since it did not occur in a calcium-free medium or in the presence of the calcium entry blocker, verapamil. In agreement with these results, dogs pretreated with verapamil showed neither the vasoconstrictor effect of adenosine nor the adenosine induced decrease in GFR [4, 34]. In addition, verapamil inhibited the contraction of rat isolated glomeruli induced by adenosine [15]. An alternative explanation for the effect of verapamil on the response of mesangial cells to adenosine would be that the calcium antagonist inhibits the binding of adenosine to its receptor as suggested by Marangos, Deckert and Bisserbe [35]; however, the non-dihydropyridine calcium channel blockers such as verapamil have a very low effect on modifying the binding of adenosine to its receptors [35]. We have also tested the possible role of the intracellular calcium pools in the adenosine induced contraction. For that purpose, we used a relatively selective blocker of the calcium release from the intracellular pools, TMB-8 [36]. Pretreatment with this compound did not modify the contractile response of the mesangial cells to adenosine, thus suggesting the lack of a major role for the intracellular pools in this contraction. However, these results must be considered cautiously, because the cellular effects of TMB-8 are not completely known.

In summary, adenosine acts on mesangial cells reducing planar cell surface area. This reduction may be considered as a contraction of mesangial cells as previously reported [12] and may be related to a decrease in the K_f and therefore in the GFR. Thus, the reduction in GFR after adenosine infusion may be mediated, at least in part, by its effects on mesangial cells. However, in the present study, the adenosine induced contraction has been observed only at pharmacological concentrations, thus rendering the direct application of these results difficult to the physiological conditions. This contractile effect seems to be mediated by a specific A_1 adenosine receptor. In addition, calcium entry is necessary for the decrease in PCSA induced by adenosine. However, additional experiments are necessary for the complete elucidation of the cellular mechanism of action on mesangial cells.

Acknowledgments

These results have been presented in part to the Xth International Congress of Nephrology, London, 1987, and the VII Latinoamerican Congress of Nephrology, Caracas, 1988 and were published in abstract form. The work has been partially supported by grants from the National Institute of Health of Spain (FIS 1873/88), CAICYT (590/84). A. Olivera was a fellow of the Plan de Formación de Personal Investigador, Spanish Ministerio de Educación y Ciencia. We especially acknowledge Warner Lambert Research Laboratories, Michigan, USA, and Dr F. Anton, Medical Director, Parke-Davis Spain, Barcelona, for the gift of the adenosine antagonists. We also thank the Department of Cardiology for allowing us the use of the computer-assisted planimeter, and the staff of the Medical Media Department, Jimenez Diaz Foundation, for their help with the photographic work.

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