Mechanism of action of the cisapride-induced vasodilatation in renal vasculature of rat

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Background & objectives: Cisapride is a prokinetic agent with cholinomimetic and 5-HT 4 receptor agonistic properties. It has been proposed that cisapride-induced hypotension is partly mediated by cholinergic system. The aim of this study was to investigate the mechanism of cisapride-induced dilatation in the rat isolated perfused kidney.

Methods: Left kidneys of Wistar rats were isolated and perfused via renal artery and the perfusion pressure was recorded. Cisapride given as bolus injections \(10^{-10} - 10^{-5}\) mol/l produced dose-dependent dilatations. Perfusion of antagonists or inhibitors was started 30 min before the onset of phenylephrine perfusion.

Results: 4-diphenylacetoxy-N-methylpiperidine methiodide (4-DAMP; blocker of M 1 and M 3 muscarinic receptors; \(10^{-7}\) mol/l) inhibited the responses to the lower doses of cisapride while, dextran (\(10^{-7}\) mol/l) and capsaicin (for neuromediator depletion; \(10^{-4}\) mol/l) inhibited those to the higher doses. Dilatations induced by most of the doses of cisapride were inhibited by atropine (non-selective muscarinic receptor antagonist; \(10^{-7}\) mol/l), methylene blue (inhibitor of soluble guanylate cyclase; \(10^{-5}\) mol/l), 1H-[1,2,4] oxadiazolo-[4,3-a] Quinoxalin-1-One (ODQ; inhibitor of soluble guanylate cyclase; \(10^{-5}\) mol/l), and \(\text{N}^\text{G}\)-nitro-\(\text{L}\)-arginine (\(\text{L}\)-NOARG; NO synthase inhibitor; \(10^{-4}\) mol/l). Inhibition induced by \(\text{L}\)-NOARG was reversed by \(\text{L}\)-arginine (\(10^{-3}\) mol/l). The dilatation induced by cisapride was not affected by GR113808 (5-HT 4 receptor antagonist; \(10^{-7}\) mol/l) and indomethacin (cyclooxygenase inhibitor; \(10^{-5}\) mol/l).

Interpretation & conclusion: The findings indicated that cisapride caused vasodilatation through the release of nitric oxide (NO) as a result of the release of a substance acting on muscarinic receptors, in the renal vascular bed of the rat. The role of 5-HT 4 receptors and prostanoids seemed unlikely.

Key words: Cisapride - cholinergic system - isolated perfused kidney - NO - vasodilatation

Cisapride, a substituted benzamide chemically related to metoclopramide, was therapeutically used in various gastrointestinal motility disorders. It has been withdrawn due to its arrhythmogenic potential (QT prolongation). It was thought that the withdrawal of cisapride from the market will present challenges for physicians treating patients with nocturnal heartburn, gastroparesis, and dyspepsia. However, alternatives to this drug exist, and it will continue to be available under a limited - access programme for patients for whom other drug treatments fail. When we started to perform our preliminary experiments, the drug had still been in use for gastrointestinal disorders. The mechanism of prokinetic action of cisapride is ascribed to enhanced release of acetylcholine from postganglionic myenteric nerve endings. However, established prokinetic activity of certain substituted benzamides such as renzapride and
cisapride might be modulated by the 5-hydroxy tryptamine \(_4\) (5-HT\(_4\)) receptor activation\(^9\). The hypotensive effect of cisapride, given intravenously in the rat, is believed to be mediated partly through peripheral muscarinic stimulation\(^10\). This hypotensive effect of cisapride might be, at least, partly related to an effect of the drug on kidney vasculature, since in our preliminary experiments we observed a dose-dependent vasodilatation due to drug in the rat isolated perfused kidney that has all the local control mechanisms without intervention of central sympathetic and humoral regulation. For this reason, the present study was carried out to investigate the mechanism of action of cisapride-induced dilatation and the contribution of muscarinic and serotonergic system, if any, to this action, in the renal vascular bed of the rat. Further considering the involvement of the muscarinic system activation, it was planned to examine whether nitric oxide (NO) played a role in this dilatation.

**Material & Methods**

Wistar rats of both sexes (230-300 g) were anaesthetized with urethane (1.25 g/kg; ip). After opening of the peritoneal cavity, left kidney and left renal artery were isolated, removed, and transferred into a warmed plexiglass chamber. Renal artery was cannulated via a polyethylene catheter. The kidney was perfused continuously with warm (37°C) and aerated (95% \(O_2\) and 5% \(CO_2\) gas mixture) Krebs-Henseleit solution using a peristaltic pump (Eyela MP-32; Rikakikai, Tokyo, Japan) delivering a constant flow (8-10 ml/min) throughout the experiment. Drugs were either constantly perfused or given as a bolus injection made into the silicone rubber perfusate tubing close to the kidney. Renal vascular responses were monitored by a pressure transducer (Statham P23 Ac) connected to a polygraph (Grass Model 7, Quincy, MA, USA). The study protocol was approved by the Animal Care Committee of the Hacettepe University.

In control experiments, after an equilibration period of 30 to 40 min, bolus injection of phenylephrine (PE, \(5x10^{-4}\) mol/l) was given to obtain the maximum constrictor response of the individual renal vascular bed. When the perfusion pressure returned to baseline levels, perfusion with PE at a concentration (3x10\(^{-6}\) mol/l) that causes submaximum constriction (60-80% of maximum response; in order to obtain the optimum dilatation due to cisapride) was initiated and continued till the end of the experiment. After the PE-induced vasoconstriction had reached a plateau, subsequent doses of cisapride (10\(^{-10}\)–3x10\(^{-3}\) mol/l) were given by bolus injections and dose-dependent vasodilatations were recorded. In experiments in which antagonists or inhibitors were used, the same protocol was applied except that 30 min before the onset of PE perfusion with antagonists/inhibitors was started and continued throughout the experiment. In each kidney preparation, only one antagonist/inhibitor was tested. The antagonist/inhibitor was applied into the perfusion medium.

In order to test the specificity of antagonists/inhibitors, at the end of the experiments, dose-dependent dilatation induced by papaverine (3x10\(^{-4}\) mol/l) was recorded in the absence (control) and presence of antagonists/inhibitors which inhibited cisapride-induced dilatation.

To remove the endothelium, triton X-100 (TX-100, 0.1 %) was infused for 10 seconds. By testing the response to acetylcholine (ACh) and papaverine 10 min later, it was decided whether endothelial denudation was successful. Then the cisapride was applied at the mentioned doses.

The following drugs were used: atropine sulphate (non-selective muscarinic receptor antagonist; 10\(^{-7}\) mol/l; Sigma, USA), capsaicin (an agent to cause neuromediator release from afferent nerve terminals; 10\(^{-6}\) mol/l; Sigma, USA), cisapride (prokinetic agent and 5-HT\(_4\) receptor agonist; 10\(^{-10}\)–3x10\(^{-5}\) mol/l; Mustafa Nevzat İlaç¸ Sanayi A.Ş., Turkey), 4-diphenylacetoxy-N-methylpiperidine methiodide (4-DAMP) (blocker of M\(_1\) and M\(_3\) muscarinic receptors; 10\(^{-7}\) mol/l; Tocris, Bristol, UK), dextran (10\(^{-7}\) mol/l; Eczacibasi İlaç¸ Sanayi, Turkey), glibenclamide (inhibitor of ATP-sensitive potassium channels; 10\(^{-5}\) mol/l; Fako A.Ş., Istanbul, Turkey), GR113808 (5-HT\(_4\) receptor antagonist; 10\(^{-7}\) mol/l; Glaxo Wellcome, Hertfordshire, UK), indomethacin (cyclooxygenase inhibitor; 10\(^{-5}\) mol/l; Sigma), methylene blue (inhibitor of soluble guanylate cyclase; 10\(^{-5}\) mol/l; Sigma), N\(^\circ\)-nitro-L-arginine (L-NOARG, NO synthase inhibitor; 10\(^{-4}\) mol/l; Sigma), L-arginine (10\(^{-3}\) mol/l; Life Technologies Ltd., Paisley, Scotland), 1H-[1,2,4] oxadiazo[4,3-a] quinoxalin-1-one (ODQ, inhibitor of soluble guanylate cyclase; 10\(^{-5}\) mol/l; Sigma-Aldrich.
Chemie, Steinheim, Germany), papaverine hydrochloride (Ciba-Geigy, Basel, Switzerland), phenylephrine hydrochloride (Sigma-Aldrich Chemie), Triton X-100 (Sigma). The composition of the Krebs–Henseleit solution was as follows (mmol/l): NaCl, 118; KCl, 4.7; CaCl₂, 2.5; KH₂PO₄, 1.2; MgSO₄, 1.2; NaCO₃, 25; glucose, 10.

All drugs were dissolved in distilled water except indomethacin, glibenclamide, ODQ and 4-DAMP. Indomethacin was dissolved in 6.85 mmol/l Na₂CO₃, glibenclamide in 50 per cent ethanol, capsaicin in 100 per cent ethanol, and ODQ and 4-DAMP were dissolved in dimethyl sulfoxide (DMSO). The solvents have no effect on renal vasculature when used alone.

Vascular responses were measured as the increase or decrease in perfusion pressure, and expressed as percentage of submaximum response to PE (3x10⁻⁶ mol/l). The results were expressed as means±S.E.M. For statistical analysis, two way ANOVA followed by Scheffe post hoc test was used. P<0.05 was considered to be statistically significant.

Results

In isolated perfused rat kidney, under a constant flow of 8-10 ml/min, mean basal perfusion pressure was 93.87±1.65 mmHg (n=60). After the bolus injection of PE that caused maximum constrictor response (5x10⁻⁴ mol/l), a 123.99±2.44 mm Hg (n=60) increase was recorded in basal perfusion pressure. The perfusion of a submaximum dose of PE (3x10⁻⁶ mol/l) caused a 86.94±0.75 mm Hg (n=60) increase in perfusion pressure.

Cisapride (10⁻¹⁰–3x10⁻⁵ mol/l) caused a dose-dependent decrease in perfusion pressure raised by PE (Figure). Maximum dilatation obtained by cisapride was 81.67±7.73 mm Hg (90.96±3.31% of submaximum PE constriction; n=9). Atropine (10⁻⁷ mol/l, n=5) inhibited most of the responses to cisapride (Table), while 4-DAMP (10⁻⁷ mol/l, n=5), and dextran (10⁻⁷ mol/l; not shown, n=5), significantly decreased the responses to the lower and higher doses of cisapride, respectively (Table). L-NOARG (10⁻⁴ mol/l; n=6), methylene blue (10⁻⁵ mol/l; not shown, n=5), and ODQ (10⁻⁵ mol/l; n=4) inhibited almost all of the responses to cisapride (Table). Dilatations inhibited by L-NOARG were reversed by L-arginine (10⁻³ mol/l, n=4). Glibenclamide (10⁻⁵ mol/l, n=4) and capsaicin (10⁻⁶ mol/l, n=3) significantly (P<0.05) decreased the responses to the higher doses of cisapride. On the other hand, indomethacin (10⁻⁵ mol/l; n=3; not shown), and GR 113808 (10⁻⁷ mol/l; n=3; not shown) did not significantly attenuate cisapride-induced dilatation. The antagonists/inhibitors that inhibited cisapride-induced dilatation had no effect on papaverine-induced dilatations (not shown). After endothelial denudation by triton X-100 (TX-100, 0.1%, n=4), cisapride-induced dilatation was abolished (Table).

The vasopressor responses obtained by PE in the absence and presence of the antagonists/inhibitors were not statistically different. Except L-NOARG, methylene blue, DAMP and ODQ, all other antagonists/inhibitors had no effect on basal perfusion pressure. L-NOARG, methylene blue, and DAMP caused transient increases

**Fig.** Representative tracing of cisapride-induced vasodilatation in the isolated perfused rat kidney. Dots indicate each dose of cisapride (10⁻¹⁰, 3x10⁻⁵ mol/l) given by bolus injection.
<table>
<thead>
<tr>
<th>Cisapride doses (mol/l) (N)</th>
<th>10^{-10}</th>
<th>3x10^{-10}</th>
<th>10^{-9}</th>
<th>3x10^{-9}</th>
<th>10^{-8}</th>
<th>3x10^{-8}</th>
<th>10^{-7}</th>
<th>3x10^{-7}</th>
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<th>3x10^{-6}</th>
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<td>Control (9)</td>
<td>2.78±2.22</td>
<td>5.84±2.71</td>
<td>7.78±2.47</td>
<td>11.00±3.55</td>
<td>12.12±3.41</td>
<td>12.70±3.53</td>
<td>15.30±3.14</td>
<td>22.74±3.73</td>
<td>39.41±4.94</td>
<td>61.05±4.40</td>
<td>76.81±3.43</td>
<td>90.96±3.31</td>
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<td>Atropine (5)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00*</td>
<td>1.00±1.00</td>
<td>3.33±2.10</td>
<td>3.33±2.10*</td>
<td>3.38±1.97*</td>
<td>17.99±3.21*</td>
<td>34.03±5.58*</td>
<td>52.65±7.32*</td>
<td>70.23±9.00*</td>
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</tr>
<tr>
<td>4-DAMP (5)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00*</td>
<td>0.00*</td>
<td>0.00*</td>
<td>0.00*</td>
<td>0.00*</td>
<td>4.19±2.83*</td>
<td>22.53±4.46*</td>
<td>49.01±3.83</td>
<td>71.10±2.88</td>
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<td>0.00</td>
<td>0.52±0.52</td>
<td>1.04±1.04</td>
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<td>1.04±1.04*</td>
<td>1.04±1.04*</td>
<td>0.00*</td>
<td>1.46±1.00*</td>
<td>15.16±3.66*</td>
<td>27.32±5.90*</td>
<td>56.64±7.39*</td>
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<tr>
<td>L-Arginine (4)</td>
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<td>5.10±0.15†</td>
<td>6.80±0.35†</td>
<td>7.20±0.25†</td>
<td>8.90±1.20†</td>
<td>9.10±1.80†</td>
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<td>15.70±0.90†</td>
<td>31.10±3.25†</td>
<td>45.20±4.25†</td>
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<td>76.00±7.90</td>
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<td>0.00*</td>
<td>0.00*</td>
<td>0.00*</td>
<td>1.66±1.66*</td>
<td>6.72±3.58*</td>
<td>16.96±6.81*</td>
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<td>0.56±0.56</td>
<td>1.13±1.13</td>
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<td>6.14±2.07*</td>
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<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00*</td>
<td>1.85±1.85*</td>
<td>12.65±2.74*</td>
<td>33.02±2.94*</td>
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<tr>
<td>Triton X-100 (4)</td>
<td>0.00</td>
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<td>0.00</td>
<td>0.00</td>
<td>0.00*</td>
<td>0.00*</td>
<td>0.00*</td>
<td>0.00*</td>
<td>0.00*</td>
<td>1.14±1.14*</td>
<td>8.29±2.40*</td>
<td>14.80±4.51*</td>
</tr>
</tbody>
</table>

Values of dilatation are given as % of submaximum phenylephrine constriction

*P<0.05 versus control, †P<0.05 versus L-NOARG

4-DAMP - 4-diphenylacetoxy-N-methylpiperidine methiodide

L-NOARG - N^G-nitro-L-arginine

ODQ-1 H-[1,2,4] oxadiazolo-[4, 3-a] quinoxadin-1-one
(27.50 ± 7.50 mmHg, n=6; 59.00 ± 13.82 mmHg, n=5; 59.00 ± 16.31 mmHg, n=5, respectively), while ODQ caused a permanent but a slight increase (15.00 ± 4.56 mmHg, n=4) in the basal perfusion pressure.

The solvents did not affect the dilator/constrictor responses and basal perfusion pressure. There was no significant change in the weight of the kidneys; it was 1.81 ± 0.03 g and 1.88 ± 0.03 g (n=60) before and after the experiment, respectively.

**Discussion**

The more pronounced pharmacodynamic responses were recorded following application of cisapride in doses higher than 10⁻⁷ mol/l. These levels were more than the therapeutic plasma concentrations following administration of cisapride to healthy volunteers²¹. Higher levels of cisapride could be achieved when it was given together with the drugs which inhibited the metabolism of cisapride (i.e., ketocanazole, erythromycin)²². In such cases, cisapride-induced dilatation might be more important.

Atropine, a nonspecific antagonist of muscarinic receptors, significantly inhibited the responses to cisapride which suggested the involvement of muscarinic receptor stimulation by the drug in this vascular bed. The inhibition of responses by 4-DAMP, a M₁ and M₃ muscarinic receptor antagonist, and by dextran which allosterically modulates M₂-muscarinic receptor binding properties and decreases the agonistic activity in rat heart²³, confirmed this suggestion. The coupling of M₂-muscarinic receptors to activation of endothelial NO synthase (eNOS) has been reported in Chinese hamster ovary cells²⁴ and cultured rat ventricular myocytes²⁵. Activation of endothelial M₂-muscarinic receptors stimulates NO production and release through eNOS activation, thereby relaxes vascular smooth muscle. In our experimental setting, L-NOARG inhibited cisapride-induced relaxations and L-arginine reversed this inhibition. The result indicated that NO played an important role in vasodilatation induced by cisapride. This finding was further supported by the inhibition caused by methylene blue and more importantly by ODQ, both are the inhibitors of guanylate cyclase which is the target molecule for NO. It could be suggested that NO released by the effect of cisapride, as a result of muscarinic activation, might stimulate soluble guanylate cyclase and cause cGMP formation and finally dilate the renal vascular bed. In a previous study, we have shown that ACh produces a dose-dependent dilatation in the isolated perfused rat kidney²⁶. NO was partly responsible for ACh-induced dilatation²⁶. Similarly, cisapride-induced dilatation of the rat renal vasculature may be through NO release. The finding that removal of the endothelium (by triton X-100) of the vascular bed abolished the dilatation induced by cisapride, supported this suggestion.

Methylene blue, an antimuscarinic agent²⁷ exerts its effects on the muscarinic activated K⁺ current in rat cardiac myocytes that are best explained by the binding of methylene blue to the M₂ subtype of muscarinic receptors²⁸. This property of methylene blue might have contributed to its inhibitory action on the cisapride-induced dilatation of the vascular bed of the rat kidney. Our result with glibenclamide supported the view that ATP-sensitive and M₂-muscarinic receptor-coupled K⁺ channels played a role in the cisapride-induced vasodilatation in this vascular bed.

There is no direct evidence showing that cisapride directly stimulates muscarinic receptors. It is possible that cisapride acts indirectly by evoking the release of any of the vasodilator substance acting on muscarinic receptors. Because capsaicin, a pharmacological tool to deplete neuropeptides, significantly inhibited cisapride-induced dilatation in our experimental setting.

As was observed previously²⁶, L-NOARG and methylene blue transiently increased the basal perfusion pressure in the present study. This finding was in accordance with the study of Radermacher et al²⁹ that demonstrated the importance of basal NO release in determination of the renovascular tone. ODQ also caused a relatively small increase in the basal perfusion pressure. This finding might support the importance of both basal NO and cGMP production in the renovascular tone. As far as the increase in basal perfusion pressure after the administration of 4-DAMP is concerned, the result implied the importance of basal cholinergic influence in determination of the tonus of renal vasculature. At that point, the contribution of antimuscarinic action of methylene blue to the increase in basal perfusion pressure could not be excluded.

Endothelial cells can release vasodilator prostaglandins³⁰. In our study cisapride-induced dilatation was not inhibited by indomethacin. It could thus be suggested that vasodilator prostaglandins did not contribute to the cisapride-induced dilatation of renal vascular bed.
Cisapride has an agonistic activity on 5-HT\textsubscript{4} type serotonergic receptors and causes an inhibition of responses in certain tissues\textsuperscript{6,7}. In the present study, GR 113808, a selective 5-HT\textsubscript{4} receptor antagonist, was tested against the response to cisapride. The lack of any effect of antagonist on the response suggested that the serotonergic component had no role to play in cisapride-induced dilatation.

In conclusion, cisapride induced dilatation in the renal vascular bed through the release of NO, indirectly. Additionally, muscarinic receptor-coupled and ATP-sensitive K\textsuperscript{+} channels contributed to the vasodilatation induced by cisapride in the perfused rat kidney.

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**References**


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