Pharmacological Characterisation and Autoradiographic Localisation of Dopamine Receptor Subtypes in the Cardiovascular System and in the Kidney

Francesco Amenta, Fabio Ferrante, and Alberto Ricci

Combined radioligand binding and light microscope autoradiography techniques were used for investigating the pharmacological profile and the microanatomical localisation of dopamine receptor subtypes in the cardiovascular system and in the kidney. In superior mesenteric and renal arteries the predominant dopamine D1-like receptor belongs to the D5 (or Dib) subtype. This site is located within smooth muscle of the tunica media. The same receptor subtype predominates in the kidney, where it has a vascular and tubular localisation. The dopamine D2-like receptor subtype expressed by systemic arteries belongs to the D3 receptor subtype. It has a prejunctional and endothelial localisation. In the kidney the predominating dopamine D2-like receptor belongs to the dopamine D3 subtype. Atria but not ventricles express dopamine D2-like receptors belonging to the D4 receptor subtype. The above results suggest that in spite of the emerging complexity of the dopamine receptor profile demonstrated by molecular biology techniques, radioligand binding and autoradiographic techniques, if performed with appropriate radioligands and/or in the presence of compounds active on specific receptor subtypes, may represent a useful tool for better understanding the biological significance of peripheral dopamine receptors. (Hypertens Res 1995; 18 Suppl. I: S23-S27)

Key Words: dopamine receptor, receptor subtype, radioligand binding, light microscope autoradiography

The catecholamine dopamine exerts cardiac, vascular and renal effects mediated by the interaction with specific dopamine receptors localised in the cardiovascular system and in the kidney (1). Cardiac actions of dopamine include increase of myocardial contractility and cardiac output without a concomitant augmentation of heart rate (2). At the vascular level dopamine causes vasodilatation. The vasodilatory activity of dopamine is a direct mechanism, mediated through its interaction with receptors located within arterial smooth muscle and an indirect mechanism. This last consists in the reduction of the sympathetic vasoconstrictor tone, by decreasing noradrenaline release from sympathetic neuroeffector junctions (1). In the kidney dopamine causes natriuresis and vasodilatation (3).

Peripheral dopamine receptors were divided, on the basis of functional and pharmacological evidence, into two main subtypes, namely dopamine DA1 and DA2 receptors (4). These receptors were considered to be similar but not identical to the homologous central dopamine D1 and D2 receptors (4). Although the classification of peripheral dopamine receptors proposed by Goldberg and Kohli (4) was not completely satisfactory for explaining the overall cardiovascular and renal actions of dopamine, it meet the general agreement of investigators and was widely used for defining cardiovascular and renal responses to dopamine (1).

From a biochemical point of view, activation of dopamine DA1 receptors results in stimulation of adenylate cyclase and phospholipase C activities in a number of tissues (1, 3). Activation of dopamine DA2 receptors results in inhibition or lack of effect on adenylate cyclase activity (1, 3).

Our group has been involved in the pharmacological characterisation and microanatomical localisation of dopamine receptors in the cardiovascular system and in the kidney using radioligand binding techniques associated with light microscope autoradiography (1). From these studies, performed both in laboratory animals and in human tissues, we have observed in systemic arteries the localisation of dopamine DA1 receptors within smooth muscle of the tunica media. In contrast, dopamine DA2 receptors showed a double localisation, adventitial (prejunctional) and endothelial (non-prejunctional) (1). The functional significance of endothelial dopamine DA2 sites, if any, has not been established yet (1).

The above classification of peripheral dopamine receptors is not satisfactory at the present. The application of molecular biology techniques to dopamine receptor research has enlarged our knowledge about the subtypes of dopamine receptors. In fact, at least five genes encoding dopamine receptors...
have been identified, which express different receptors so far included in the family of dopamine D₁ (now defined dopamine D₁-like) and of dopamine D₂ (now defined dopamine D₂-like) receptors. The majority of molecular biology studies on the characterisation of dopamine receptor subtypes has been accomplished in the brain, whereas less information is so far available for peripheral dopamine receptors (5). In this paper, our main data on radioligand binding characterisation and microanatomical localisation of peripheral dopamine receptors are detailed.

### Dopamine D₁-like Receptors

The dopamine D₁-like receptor family comprises the dopamine D₁ (or D₁ₐ) and D₅ (or D₁ₖ) sites (6). In general, the sensitivities of the cloned D₁ and D₅ receptors are similar (7), and the characterisation of these subtypes of dopamine receptors is difficult since they are labelled by the same radioligands with a similar degree of affinity (6, 7). The main difference is in the sensitivity of dopamine D₁-like radioligands to dopamine in the micromolar range at the dopamine D₁ receptor and in the submicromolar range at the dopamine D₅ receptor (6, 7).

In our laboratory we have characterised the subtypes of dopamine D₁-like receptors in sections of rat superior mesenteric artery and of human and rat renal artery and kidney using [³H]-SCH 23390 as a ligand. The radioligand was bound to these tissues in a manner consistent with the labelling of dopamine D₁-like receptors (Fig. 1). Analysis of the pharmacological profile of [³H]-SCH 23390 binding to sections of superior mesenteric artery and kidney revealed the displacement of the radioligand by dopamine in the submicromolar range at the dopamine D₁ receptor and in the submicromolar range at the dopamine D₅ receptor (6, 7).

From the above findings it appears that smooth muscle of the tunica media of systemic arteries and epithelial cells of renal cortical tubules express primarily a dopamine D₅ receptor. Further work is in progress to assess, by radioligand binding and autoradiographic techniques, the presence of mixed populations of dopamine D₁ and D₅ receptors in systemic arteries and in the different portions of the nephron.

### Dopamine D₂-like Receptors

The dopamine D₂-like receptor family includes two isoforms of the dopamine D₂ receptor, the so called dopamine D₂S and D₂L sites, and the dopamine D₃ and D₄ receptor subtypes (6, 7). In our laboratory, we have characterised dopamine D₂-like receptors...
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in sections of rat superior mesenteric artery and of human and rat kidney using the non-selective radioligand [3H]-spiroperidol. Both in the superior mesenteric artery and in the kidney, [3H]-spiroperidol was specifically bound in a manner consistent with the labelling of dopamine D2-like receptors (Fig. 3).

Analysis of displacement curves of [3H]-spiroperidol binding to sections of the superior mesenteric arteries showed that quinpirole which has greater affinity for dopamine D3 receptor in the brain is less potent competitor of [3H]-spiroperidol binding than dopamine D2 receptor active compounds such as haloperidol or (-)-sulpiride (Table 2). In contrast, in the kidney, quinpirole had a pharmacological profile consistent with that reported for dopamine D3 receptor in the brain (Table 2). These findings suggest that whereas in the superior mesenteric artery [3H]spiroperidol binds mainly a dopamine D2 receptor, a dopamine D3 site is predominant in the kidney.

To confirm the presence of a dopamine D3 receptor in the kidney, we have further characterised it in another series of experiments using [3H]-7-OH-DPAT as a ligand. This compound is considered the most selective ligand for dopamine D3 receptor available at the present (8). Analysis of [3H]-7-OH-DPAT saturation curve shows that it labels a single population of high affinity sites (Fig. 4). Pharmacological characterisation of [3H]-7-OH-DPAT binding to sections of rat kidney revealed a typical dopamine D3 receptor profile (8), being non-labelled 7-OH-DPAT the most powerful competitor of the radioligand followed by haloperidol, (+)-butaclamol and quinpirole (Table 3). In summary, the above data strongly support the view of expression by rat kidney of a dopamine D3 site is predominant in the kidney.

Fig. 2. Light microscope localisation of dopamine D1-like receptors in sections of human renal artery. Sections were incubated with a 2 nM concentration of [3H]-SCH 23390 alone (A) or plus 1 μM (+)-butaclamol (C) to define non-specific binding. Pictures A and C are dark-field micrographs. Picture B is a bright-field micrograph of A stained with toluidine blue to verify microanatomical details. Specific silver grains sensitive to (+)-butaclamol displacement were accumulated within the tunica media (m) of the renal artery. No differences in the density of silver grains developed in the tunica adventitia (a) or in the tunica intima (i) were noticeable in pictures A and C. This indicates that these grains represent non-specific retention of the radioligand by the tissue. The inset is a high magnification bright-field autoradiograph showing the accumulation of silver grains within smooth muscle cells of the tunica media (asterisks). L = lumen of the artery. Calibration bars: 100 μm.

Fig. 3. Saturation curve (A) and Scatchard analysis (B) of [3H]-spiroperidol binding to sections of rat superior mesenteric artery. Sections were incubated with the radioligand alone (total binding, □) or in the presence of a 1 μM concentration of (+)-butaclamol (○) to define non-specific binding. Specific binding values (●) were calculated by subtracting non-specific from total binding values. Points are means ± SEM of triplicate determinations.

Affinity for dopamine D3 receptor in the brain is less potent competitor of [3H]-spiroperidol binding than dopamine D2 receptor active compounds such as haloperidol or (-)-sulpiride (Table 2). In contrast, in the kidney, quinpirole had a pharmacological profile consistent with that reported for dopamine D3 receptor in the brain (Table 2). These findings suggest that whereas in the superior mesenteric artery [3H]spiroperidol binds mainly a dopamine D2 receptor, a dopamine D3 site is predominant in the kidney.

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Table 2. Pharmacological Specificity of $[^3H]$-Spiroperidol Binding to Sections of Rat Superior Mesenteric Artery and Kidney

<table>
<thead>
<tr>
<th>Compound</th>
<th>Superior mesenteric artery</th>
<th>Kidney</th>
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<tbody>
<tr>
<td>Apomorphine</td>
<td>90 ± 4.2</td>
<td>11.8 ± 0.9</td>
</tr>
<tr>
<td>(+)-Butaclamol</td>
<td>0.28 ± 0.03</td>
<td>0.36 ± 0.02</td>
</tr>
<tr>
<td>Dopamine</td>
<td>1546 ± 41</td>
<td>45 ± 2.4</td>
</tr>
<tr>
<td>Fenoldopam</td>
<td>&gt;10,000</td>
<td>&gt;5,000</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>1.36 ± 0.02</td>
<td>11.38 ± 0.8</td>
</tr>
<tr>
<td>Quinpirole</td>
<td>1132 ± 95.4</td>
<td>57.6 ± 2.3</td>
</tr>
<tr>
<td>SCH 23390</td>
<td>&gt;10,000</td>
<td>&gt;5,000</td>
</tr>
<tr>
<td>(-)-Sulpiride</td>
<td>14 ± 0.78</td>
<td>21.3 ± 1.9</td>
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Values are expressed in nM and represent the competitor dissociation constant ($K_i$) calculated as described in an earlier paper of our group (9). The data are mean values ± SEM of three to five experiments each carried out in triplicate.

The functional nature of this site (vasodilator, natriuretic?) and the localisation (pre-junctional?) should be clarified in future studies.

Light microscope autoradiography of dopamine D$_2$-like receptors in the superior mesenteric artery and in the kidney has shown their adventitial and intimal localisation in different sized arterial branches and a tubular localisation in the kidney (data not shown). Further quantitative autoradiographic analysis is necessary to assess whether other small populations of dopamine D$_2$-like receptor subtypes are expressed by arterial tissue or the kidney.

Our analysis was extended also to dopamine D$_2$-like receptors of the heart. It is known that dopamine is a potent stimulant of certain cardiac functions. The catecholamine as well as dopaminergic compounds have been proposed in the treatment of heart failure (9). Dopamine D$_2$-like receptors have been shown in sections of human heart (9). More recently, the occurrence of a dopamine D$_4$ receptor in the rat heart has been demonstrated with in situ hybridisation techniques (10). We have therefore ana-
lysed the binding of the dopamine D₄ receptor radioligand [³H]-clozapine to sections of rat atria and ventricles.

[³H]-Clozapine was specifically bound to sections of rat atria (Fig. 5), but not of ventricles (data not shown). The binding profile was consistent with the labelling of a dopamine D₄ receptor, being unlabelled haloperidol the most powerful displacer of the radioligand followed by clozapine YM 09151-2, (+)-butaclamol, apomorphine, quinpirole and dopamine (Table 4). Compounds active on dopamine D₁-like receptors such as fenoldopam and SCH 23390 were without effect (Table 4). Light microscope autoradiography revealed the accumulation of silver grains often arranged in cluster areas within atria (data not shown). The resolution limits of light microscope autoradiography and the lack of inclusion of denervated hearts in our experiments did not allow to establish if these sites are prejunctial. Further work is in progress to clarify this point.

Conclusions

In spite of the development of the molecular biology approach for the characterisation of dopamine receptor subtypes, classic radioligand binding techniques associated with light microscope autoradiography may still represent a useful tool for better understanding the biology of dopamine receptors. Of course, this will require the use of rather selective radioligands, alone or in combination with appropriate displacers for the different receptor subtypes. The association of this approach with the quantitative capabilities of light microscope autoradiography can contribute to analyse the density and pattern of peripheral dopamine receptor subtypes in health and disease.

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References