Localisation of Dopamine D₁-Like and D₂-Like Receptors in the Pulmonary Vasculature

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The pharmacological profile and the anatomical localisation of dopamine receptor subtypes were investigated in the rabbit pulmonary vascular bed using combined radioligand binding and light microscope autoradiography techniques. Dopamine D₁-like receptor sites, which probably belong to the dopamine D₁ receptor subtype, were characterized in sections of lung using [³H]-SCH 23390 as a ligand. These sites were located within the tunica intima and the tunica media of large sized intrapulmonary artery branches and in the tunica media of medium sized intrapulmonary artery branches. Dopamine D₂-like receptor sites, which probably belong to the dopamine D₂ receptor subtype, were characterized using [³H]-spiroperidol as a ligand. These sites were located within the tunica adventitia of both extra- and intrapulmonary artery branches. Dopamine D₂-like receptor sites were also found in the tunica adventitia of the human pulmonary artery, but not of the rat pulmonary artery. The different anatomical localization of dopamine D₁ and D₂ receptor subtypes in the pulmonary vasculature suggests that these sites are involved in the modulation of pulmonary vascular tone by interacting with different receptors unevenly distributed throughout the pulmonary vascular bed. (*Hypertens Res* 1995; 18 Suppl. I: S153-S156)

Key Words: dopamine receptor subtypes, pulmonary artery, human, rabbit, radioligand binding

Increasing experimental evidence suggests a possible role of the catecholamine dopamine in the regulation of the pulmonary circulation. In fact, high concentrations of dopamine were found in rabbit pulmonary artery (1); a dopamine D₁-like receptor-mediated vasorelaxation was observed in isolated human and rabbit pulmonary arteries (2, 3); the presence of dopamine D₁-like and D₂-like receptors in the pulmonary vasculature was demonstrated by autoradiographic studies (4-6).

Until recently, brain and peripheral dopamine receptors were considered to consist of two receptor subtypes, namely dopamine D₁ and D₂ receptors. The application of molecular biology techniques to dopamine receptor research has identified the occurrence in the brain of at least 5 dopamine receptor subtypes (7). Dopamine D₁ and D₅ receptor subtypes belong to the D₁ (now D₁-like) receptors of the former classification, whereas dopamine D₂, D₃ and D₄ receptor subtypes belong to the D₂ (now D₂-like) receptors of the former classification.

The dopamine receptor subtypes expressed by the pulmonary vascular bed have been sparsely investigated. In the present study, the pharmacological profile and the anatomical localization of dopamine receptor subtypes was investigated in the pulmonary vascular bed using combined radioligand binding and light microscope autoradiography techniques.

Materials and Methods

The main extraparenchymal and intraparenchymal branches of the pulmonary artery were obtained from male albino rabbits. In experiments for the characterization of dopamine D₂-like receptors samples of human pulmonary artery obtained from subjects undergoing partial lobectomies and of the pulmonary artery of male Wistar rats were also used. Tissues were embedded in a cryoprotectant medium, frozen in a dry ice-acetone mixture and cut serially into 10 µm thick sections.

For dopamine D₁-like receptors labelling, sections were incubated for 60 min at room temperature with [³H]-SCH 23390 according to the procedure detailed elsewhere (5). For dopamine D₂-like receptors labelling, sections were incubated for 60 min at room temperature with [³H]-spiroperidol according to the procedure described previously (6). Non-specific binding was defined by incubating sections with the radioligands in presence of 1 µM (+)-butaclamol. The pharmacological specificity of [³H]-SCH 23390 or of [³H]-spiroperidol binding to the different dopamine receptor subtypes was assessed by incubating representative sections of the pulmonary arteries with the radioligands in the presence of increasing concentrations of dopaminergic agents.

At the end of the incubation, slides were washed to remove excess of unbound radioligands and processed for liquid scintillation spectrometry or light

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microscope autoradiography. Sections used for liquid scintillation spectrometry were wiped onto Whatman GF-B glass-fibre filters and put into scintillation vials for radioactivity determination. Sections used for light microscopic autoradiography were air-dried after incubation and processed as described in earlier papers of our group (5, 6).

Results

Sections of extraparenchymal and intraparenchymal branches of rabbit pulmonary artery bound specifically [^3H]-SCH 23390. The binding was time-, temperature- and concentration-dependent (data not shown). The dissociation constant (K_d) and the maximum density of binding sites ($B_{\max}$) are shown in Table 1. Dopamine D_1 -like receptor sites found in sections of rabbit pulmonary artery probably belong to the dopamine D_1 receptor subtype since dopamine competed with [^3H]-SCH 23390 binding in the micromolar range (Table 2) (7).

Light microscope autoradiography revealed the localisation of dopamine D_1 -like receptors in the tunicae intima and media of large intrapulmonary branches (diameter larger than 300 μm) (Fig. 1) as well as in the tunica media, but not in the tunica intima of medium-sized pulmonary artery branches (diameter between 100 μm and 300 μm) (data not shown). No [^3H]-SCH 23390 binding sites were

found in small-sized pulmonary artery branches (diameter less than 100 μm) (data not shown).

[^3H]-Spiroperidol was specifically bound to sections of both extraparenchymal and intraparenchymal branches of rabbit pulmonary artery as well as of human pulmonary artery (data not shown). K_d and $B_{\max}$ values of [^3H]-spiroperidol binding to sections of the extraparenchymal portion of the rabbit or human pulmonary arteries are shown in Table 1. No specific [^3H]-spiroperidol binding was noticeable in sections of rat pulmonary artery (data not shown). Results of the pharmacological characterization of [^3H]-spiroperidol binding to sections of rabbit and human pulmonary arteries suggest that dopamine D_2 -like receptor sites probably belong to the dopamine D_2 receptor subtype (Table 2) (7). In fact, the dopamine D_3 receptor agonist quinpirole or the dopamine D_4 receptor antagonist clozapine (7) are less potent than other more selective compounds for the dopamine D_2 receptor subtype as competitors of [^3H]-spiroperidol binding (Table 2).

Light microscope autoradiography revealed the localisation of dopamine D_2 -like receptor sites within the tunica adventitia of large- and medium-sized rabbit pulmonary artery branches (Fig. 1) and of human pulmonary artery (data not shown). No specific [^3H]-spiroperidol binding sites were found in sections of rat pulmonary artery (data not shown).

Table 1. Dissociation Constant (K_d) and Maximum Density of Binding Sites ($B_{\max}$) Values in Sections of Pulmonary Artery

	[^3H]-SCH 23390	[^3H]-Spiroperidol	
	Rabbit	Rabbit	Human
K_d	2.05 ± 0.05	2.6 ± 0.07	2.6 ± 0.04
$B_{\max}$ (fmol/mg tissue)	85 ± 4	66 ± 4	86 ± 4

Data are the means $\pm$ SEM. Saturation curves of the radioligands binding to sections of pulmonary artery were obtained from Scatchard plots of saturation isotherms.

Table 2. Pharmacological Specificity of Radioligand Binding to Sections of Rabbit and Human Pulmonary Arteries

	[^3H]-SCH 23390	[^3H]-Spiroperidol	
	Rabbit	Rabbit	Human
Dopamine	$6,400 \pm 305$	$1,650 \pm 83$	$1,312 \pm 8.0$
(+) Butaclamol	10 ± 0.08	12 ± 0.05	9 ± 0.05
Haloperidol	95 ± 4.2	21 ± 0.5	25 ± 0.1
SCH 23390	7 ± 0.0	$> 5,000$	$> 5,000$
Domperidone	$> 10,000$	19 ± 0.7	17 ± 0.8
(-) Sulpiride	$> 10,000$	96 ± 5.2	83 ± 6.9
N-propyl-norapomorphine	n.a.	750 ± 42	630 ± 39
Quinpirole	n.a.	$1,130 \pm 81$	$1,200 \pm 93$
Clozapine	n.a.	220 ± 17	250 ± 18

Data are the means $\pm$ SEM of 3-6 independent experiments performed with 4-8 different concentrations of each displacer and represent the competitor dissociation constant (K_i) expressed in nM. Sections of rabbit pulmonary artery were exposed to a 2 nM [^3H]-SCH 23390 concentration in the presence of increasing concentrations of the compounds tested. Sections of rabbit and human pulmonary arteries were exposed to a 3 nM [^3H]-spiroperidol concentration in the presence of increasing concentrations of the compounds tested.

n.a. = not assayed

Fig. 1. Light microscope autoradiography of [^3H]-SCH 23390 (pictures A-C) and [^3H]-spiroperidol (pictures D-F) binding to sections of large-sized rabbit pulmonary artery branches. Sections were incubated with a 2 nM concentration of [^3H]-SCH 23390 to label dopamine D_1 -like receptors or of 3 nM [^3H]-spiroperidol (total binding, A and D) to label dopamine D_2 -like receptors or with the radioligands plus 10 μM (+)-butaclamol to define non-specific binding (C and F). Pictures A, C, D and F are dark-field micrographs; B and E are bright-field micrographs of pictures A and D stained with toluidine blue to verify microanatomical details. In [^3H]-SCH 23390 autoradiographs silver grains sensitive to (+)-butaclamol displacement are accumulated primarily in the tunica intima and to a lesser extent in the tunica media of the pulmonary artery. In [^3H]-spiroperidol autoradiographs silver grains sensitive to (+)-butaclamol displacement are located within the tunica adventitia. L=lumen of the artery; I=tunica intima; M=tunica media; A=tunica adventitia. ($\times 100$).

Discussion

The above results have shown that rabbit and human pulmonary arteries, but not rat pulmonary artery express dopamine receptors. Different from systemic arteries where dopamine D_1 -like receptors are located within smooth muscle of the tunica media, in large pulmonary arteries dopamine D_1 -like receptors are located primarily within endothe-

lium of the tunica intima and to a lesser extent within the tunica media. These observations are in agreement with functional (4) and autoradiographic investigations (5) performed in the extrapulmonary portion of rabbit pulmonary artery.

In systemic arteries dopamine D_2 -like receptors are prejunctional and involved primarily in reducing the noradrenaline outflow from sympathetic neuroeffector junctions (4). In the pulmonary cir-

ulation the sympathetic plexus is located in the tunica adventitia and in the adventitia-media border (4). Although our investigation has been performed on normally innervated pulmonary arteries, the localisation of dopamine D₂-like receptors in the arterial region where sympathetic neuroeffector junctions are located, suggests their prejunctional localisation. Further work is in progress to clarify this point.

In conclusion, the different anatomical localisation of dopamine D₁-like and D₂-like receptor subtypes in the pulmonary vasculature suggests that these sites are involved in the modulation of pulmonary vascular tone by interacting with different receptors unevenly distributed throughout the pulmonary vasculature.

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