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Dopamine D_2-like receptors in the rat kidney: Effect of denervation

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Abstract

The effects of monolateral denervation induced by renal artery occlusion on dopamine D_2-like receptors were assessed in rat kidney using radioligand binding assay and autoradiographic techniques. [^{3}H]spiperone was used as a ligand. [^{3}H]spiperone was bound specifically to sections of control innervated kidneys with a dissociation constant (K_d) value of 0.07 ± 0.003 nM and a maximum density of binding sites (B_{max}) value of 35.4 ± 0.16 fmol/mg tissue. Light microscope autoradiography showed the accumulation of silver grains both in the arterial tree and in cortical tubules. At the vascular level, [^{3}H]spiperone binding sites were accumulated primarily in the adventitia and in adventitia-media transitional zone. In cortical tubules, the higher density of [^{3}H]spiperone binding sites was noticeable in proximal convoluted tubules. A few binding sites were also found in the glomerular tuft. In denervated kidneys, noradrenaline and dopamine levels were reduced by about 90% and 60% respectively in comparison with control innervated kidneys. Denervation reduced the density of [^{3}H]spiperone binding sites by more than 85%. In denervated kidneys, light microscope autoradiography showed the disappearance of specific vascular binding sites and a remarkable reduction of tubular [^{3}H]spiperone binding sites. The above results indicate that the largest majority of renal dopamine D_2-like receptors labelled by [^{3}H]spiperone is prejunctional in location. © 1997 Elsevier Science B.V.

Keywords: Kidney; Dopamine; D_2-like receptor; Innervation; Radioligand binding assay; Autoradiography; (Rat)

1. Introduction

The kidney is an important target for the actions of dopamine (Lokhandwala and Amenta, 1991). Renal effects of dopamine include enhancement of diuresis, regulation of sodium excretion, vasodilatation and decrease in renal resistance (Bello-Reuss et al., 1982; Bell, 1990; Lokhandwala and Hegde, 1990; Ricci et al., 1993). These properties are common to all mammalian species investigated including humans (Lokhandwala and Amenta, 1991; Cheung and Barrington, 1996).

Biological actions of dopamine are mediated through the interaction with specific dopamine receptors. In the last few years 5 subtypes of dopamine receptors were identified. These receptors belong to the D_1-like and D_2-like receptor families. Dopamine D_1-like receptors include dopamine D_1 (or D_{1A}) and D_5 (or D_{1B}) receptor subtypes. Dopamine D_2-like receptors include D_2 (D_{2L} and D_{2S}), D_3, and D_4 receptor subtypes (Sibley and Monsma, 1992; Gingrich and Caron, 1993; Kebabian and Neumeyer, 1994). The kidney expresses dopamine D_1, D_{2L}, D_3, D_4 and D_5 receptor subtypes (Lokhandwala, 1988; Amenta, 1990a; Amenta, 1990b; Lokhandwala and Amenta, 1991; Ricci et al., 1991b; Siragy et al., 1992; Gao et al., 1994; Matsumoto et al., 1995; Cheung and Barrington, 1996).

Peripheral dopamine D_1-like receptors named formerly vascular dopamine receptors are primarily postjunctional and when stimulated cause vasodilatation (Goldberg and Kohli, 1978; Amenta, 1990a; Clark, 1990a; Lokhandwala and Hegde, 1990). Dopamine D_2-like receptors termed formerly neuronal dopamine receptors are thought to be prejunctional and located on postganglionic sympathetic neuroeffector junctions. Stimulation of dopamine D_2-like receptors causes inhibition of noradrenaline release from sympathetic terminals and decreases vasoconstrictor responses induced by sympathetic nerve stimulation (Goldberg and Kohli, 1978; Amenta, 1990a; Clark, 1990b; Lokhandwala and Hegde, 1990).

Activation of renal dopamine D_2-like receptors reduces sympathetic vasoconstrictor tone and causes vasodilatation

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(Willems et al., 1985; Bell, 1990; Lokhandwala and Hegde, 1990; Rump and Schollmeyer, 1993; Cheung and Barrington, 1996). However, some studies reported the occurrence of a dopamine D_2-like receptor-mediated vasodilatation persisting after chemical or surgical inactivation of sympathetic nerves (Jose et al., 1986; Felder et al., 1988; Bell, 1990).

The present study was designed to investigate the effect of renal denervation produced by renal artery occlusion (Soares-da-Silva et al., 1992) on the density and microanatomical localization of dopamine D_2-like receptors in the rat kidney.

2. Materials and methods

2.1. Animals and renal denervation

The experiments were conducted in male Wistar rats (Biotério do Instituto Gulbenkian de Ciência, Oeiras) aged 45–60 d and weighing 220–280 g. The rats were housed under alternating 12 h dark/light cycles at a room temperature of 24°C and were given free access to food and tap water. The experiments were all carried out during daytime.

Renal denervation was performed according to the procedures previously described and employing the technique of transient renal artery occlusion (Soares-da-Silva et al., 1992). Briefly, rats were anaesthetized with sodium pentobarbitone (60 mg/kg, i.p.) and the left kidneys were exposed under sterile conditions through an incision of the abdominal wall. Thereafter, the pedicle was isolated and the renal artery clamped for 90 s by application of force clamps. The right kidneys were not manipulated during the ischemic period to which the left kidneys were subjected, and was assumed to reflect the control situation. After surgery, the animals were returned to their home cages and had free access to food and water. On the 5th d after surgery, rats were killed by decapitation under ether anaesthesia, the kidneys were rapidly removed, rinsed in ice-cold saline (0.9% NaCl) solution and divided in two halves. Each half included the upper and the lower lobes, respectively. The upper half was used for the catecholamine assay, the lower half for the radioligand binding assay and light microscope autoradiography. Tissue samples used for determination of catecholamine content were blotted with filter paper, weighed, put in 2 ml of 0.2 M perchloric acid and stored at −20°C until analyzed (usually within 1 week). The lower halves of the kidneys were sliced perpendicularly to their major axis and frozen in isopentane cooled with liquid nitrogen.

In 4 rats the brain was also removed and a forebrain portion corresponding to the coordinates bregma 0.8–1.20 mm was dissected out and frozen in isopentane cooled with liquid nitrogen. Sections of the kidney and of the forebrain were obtained using a −20°C microtome cryostat, mounted on pre-weighted gelatine-coated microscope slides and processed for a dopamine D_2-like receptor assay as detailed below.

2.2. Assay of catecholamines

The assay of dopamine and noradrenaline was performed by high pressure liquid chromatography with electrochemical detection, as previously described (Soares-da-Silva et al., 1992). In brief, aliquots of 500 μl of perchloric acid in which the tissues had been kept were placed in 5 ml conical-based glass vials with 50 mg alumina, and the pH of the sample was immediately adjusted to pH 8.6 by addition of Tris buffer. Mechanical shaking for 10 min was followed by centrifugation, and the supernatant was discarded. Adsorbed catecholamines were then eluted from the alumina with 200 μl of 0.2 M perchloric acid on Spin-X microfilter tubes (Costar, Badhoevedorp). Fifty μl of the eluate were injected into a high pressure liquid chromatography apparatus. The detection was performed electrochemically by means of a thin-layer cell with a glassy carbon working electrode, an Ag/AgCl reference electrode, and an amperometric detector (Gilson model 141, Gilson Medical Electronics, Villiers Le Bell). The detector cell was operated at 0.75 V. The current produced was monitored using the Gilson 712 HPLC integration software connected to a personal computer system. The mobile phase was a degassed solution of 0.1 M citric acid, 0.5 mM sodium octylsulphate, 0.1 M sodium acetate, 0.17 mM ethylenediaminetetraacetic acid, 1 mM dibutylamine, 12% methanol (v/v) (pH 3.5) which was pumped at a rate of 1.0 ml/min. Standard solutions of dopamine, noradrenaline and dihydroxybenzylamine (internal standard) were injected at different concentrations and peak height increased linearly. The lower limits for detection of dopamine and noradrenaline ranged from 350 to 500 fmol.

2.3. Radioligand binding assay

For dopamine D_2-like receptor assay, sections of control and denervated kidneys were exposed to increasing concentrations of [^{3}H]spiperone (0.01–0.5 nM) alone or plus 1 μM (+)-butaclamol to define non-specific binding. At the end of the incubation, sections were washed in ice-cold incubation buffer (2 × 5 min) to remove unbound radioligand and rinsed quickly in distilled water. The sections were then wiped onto Whatman GF-B glass fibre filters and counted by liquid scintillation spectrometry. Incubation was done for 60 min at 23°C in a HEPES (4-(2-hydroxyethil)-1-piperazine-ethanesulfonic acid) based buffer constituted by 20 mM HEPES, 1 mM ethylenedinitrilo-tetraacetic acid (EDTA), 1 mM ethylenebis(oxyethylenenitrilo)tetraacetic acid (EGTA), and 120 mM NaCl (pH 7.4). This incubation buffer is reported to be the most suitable for labelling the dopamine D_3 receptor using [^{3}H]spiperone as a ligand (Vile et al.,

1995).The incubation times and temperature chosen gave the highest specific:non-specific binding ratio in a series of preliminary experiments. In our incubation conditions, the counts per min were 35/mg of tissue with a 0.25 nM concentration of [^{3}H]spiperone.

The pharmacological specificity of [^{3}H]spiperone binding to sections of rat kidney was evaluated by incubating sections with 0.25 nM [^{3}H]spiperone in the presence of increasing concentrations of compounds active on dopamine (apomorphine, bromocriptine, (+)-butaclamol, (−)-butaclamol, dopamine, fenoldopam, haloperidol, 7-hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin (7-OH-DPAT), quinpirole, SCH 23390 [*R*]-(+)-(chloro-2,3,4,5,-tetrahydro-5-phenyl-1,4,-benzazepin-al hemimaleate), (+)-sulpiride and (−)-sulpiride), serotonin (ketanserin and methysergide) and adrenergic (phentolamine and propranolol) receptors. Competing drugs were used in 5–7 concentrations ranging from 1 nM to 0.1 mM. [^{3}H]spiperone displacement curves by dopamine were performed in the presence or in the absence of 300 μM guanosine triphosphate (GTP). At the end of the incubation, the sections were washed, transferred into scintillation vials and processed as described above.

2.4. Light microscope autoradiography

For light microscope autoradiography sections of kidney and forebrain were incubated in the above buffer for 60 min at 23°C with a 0.25 nM [^{3}H]spiperone concentration in the presence or in the absence of 1 μM (+)-butaclamol to define non-specific binding. At the end of the incubation, sections were washed with ice-cold incubation buffer (2×5 min), rinsed quickly in distilled water and air-dried. Ilford L4 nuclear emulsion (diluted 1:1 with distilled water) coated coverslips were attached to the slides containing renal cortex sections. After exposure for 4–6 weeks in light–tight boxes, autoradiographies were developed in Kodak D-19, fixed in Agefix Agfa, stained with toluidine blue and viewed under a microscope equipped with bright- and dark-field optics.

The number of silver grains developed in the medium sized renal artery branches and in cortical tubules in [^{3}H]spiperone autoradiographies was assessed by quantitative image analysis. Three areas of 500 μm^2 including at least 2 medium sized branches of the renal artery and 3 portions of the renal cortex were randomly selected. Five consecutive sections 20 μm apart per kidney incubated with 0.25 nM [^{3}H]spiperone alone or plus 1 μM (+)-butaclamol to define non-specific binding were examined. Analysis was done using dark-field optics with a $\times 40/1.0$ objective lens to obtain a final magnification of $\times 400$. The images were transferred from the microscope to a Quantimet 500 image analyzer (Leica, Cambridge), connected via a TV camera to the microscope. Silver grains developed in autoradiographies were counted independently for renal artery branches, cortical tubules and glomerular tuft using an automatic program of the image analyzer.

2.5. Data analysis

Data from binding experiments were evaluated by linear regression analysis of Scatchard plots of saturation isotherms. Competitor dissociation constant (K_i) values were determined according to the equation of Cheng and Prusoff (1973). Differences in the density of silver grains developed in normally-innervated and denervated kidneys were analyzed statistically by analysis of variance (ANOVA) followed by the Duncan's multiple range test.

2.6. Chemicals

[^{3}H]spiperone (specific activity 70 Ci/mmol) was purchased from Amersham Radiochemical Centre (Buckinghamshire). 7-OH-DPAT, SCH 23390, isomers of butaclamol and of sulpiride, haloperidol and quinpirole were purchased from Research Biochemicals (Natick, NJ). Bromocriptine and methysergide were obtained from Sandoz Pharma (Basel). Ketanserin was obtained from Janssen (Beerse). Other chemicals were purchased from Sigma (St. Louis, MO).

3. Results

Catecholamine levels were significantly decreased in the kidney submitted to renal artery occlusion in comparison with the controlateral normally-innervated kidney (Table 1). The loss of catecholamines affected primarily noradrenaline, the concentrations of which decreased by 91.2 $\pm$ 6.2% (Table 1). Renal artery occlusion caused a reduction of 56.0 $\pm$ 3.8% of kidney dopamine levels (Table 1).

[^{3}H]spiperone was bound specifically to sections of normally-innervated rat kidney. The binding was time- (data not shown), temperature- (data not shown) and concentration-dependent (Fig. 1A), belonging to a single class of high affinity sites (Fig. 1B). The K_d value was 0.07 $\pm$ 0.003 nM (Fig. 1A) and the B_{max} value 35.4 $\pm$ 0.16 fmol/mg tissue (Fig. 1B). In denervated kidney, B_{max} values were decreased by about 85%, with a value of 5.4 $\pm$ 0.03 fmol/mg tissue (data not shown).

Table 1
Influence of denervation on noradrenaline and dopamine concentrations in rat kidney

Animal group	Dopamine (ng/g)	Noradrenaline (ng/g)
Control ($n = 6$)	3.3 $\pm$ 0.3	188.4 $\pm$ 9.5
Denervated ($n = 6$)	1.4 $\pm$ 0.1 [a]	16.6 $\pm$ 12.1 [a]

Values are the means $\pm$ S.E.M.
[a] $P < 0.01$ versus control group.

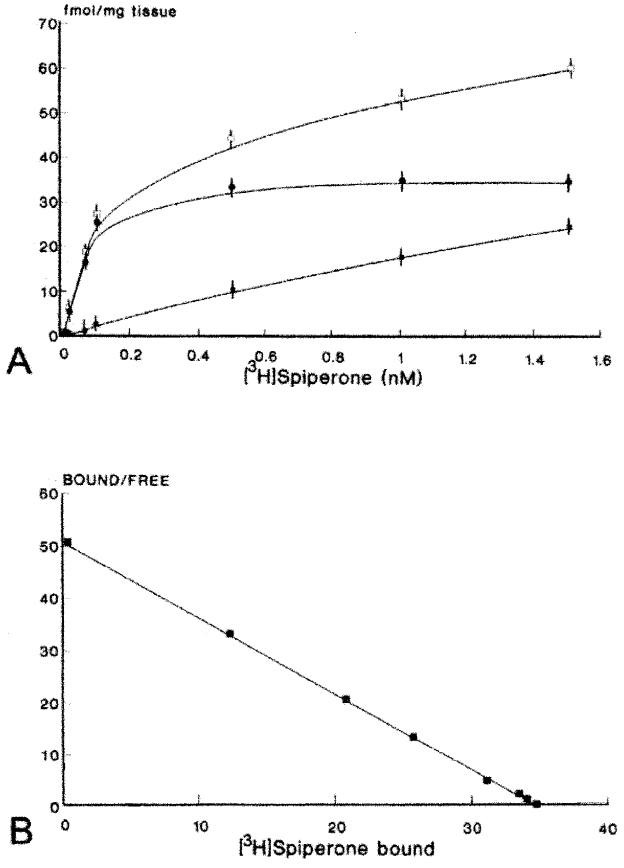


Fig. 1. (A) Saturation curve of [^{3}H]spiperone binding to sections of a normally-innervated rat kidney. Sections were incubated with the radioligand alone (total binding: □) or plus 1 μM (+)-butaclamol to define non-specific binding (■). Specific binding values (●) were obtained by subtracting non-specific from total binding. (B) Scatchard analysis of [^{3}H]spiperone binding to sections of a normally-innervated kidney. Points are the means of 5–7 independent experiments performed in triplicate. Standard error was less than 10%.

Data on the pharmacological specificity of [^{3}H]spiperone binding to sections of rat kidney are summarized in Table 2. The binding profile was consistent with the labelling of a dopamine D_2-like site. In fact, compounds active on dopamine D_2-like receptors (bromocriptine, 7-OH-DPAT, quinpirole and (−)-sulpiride) or on both D_1-like and D_2-like receptors (apomorphine, isomers of butaclamol, dopamine and haloperidol) were the most powerful competitors of [^{3}H]spiperone binding (Table 2). Analysis of [^{3}H]spiperone displacement curves by compounds active at dopamine D_2-like receptors, revealed a competitor activity by the preferential dopamine D_3 receptor agonists 7-OH-DPAT and quinpirole (Felder et al., 1984, Table 2). Moreover, competition curves by dopamine were similar in the presence or in the absence of GTP (Table 2). The [^{3}H]spiperone binding profile was similar in normally-innervated and denervated kidneys (data not shown).

Table 2
Pharmacological profile of [^{3}H]spiperone binding to sections of normally-innervated rat kidney

Displacer	K_i (nM)
Apomorphine	21.8 ± 0.4
Bromocriptine	67.5 ± 2.2
(+)-butaclamol	0.47 ± 0.03
(−)-butaclamol	650 ± 11.4
Dopamine	45 ± 1.6
Dopamine + 300 μM GTP	47 ± 0.9
Haloperidol	12.5 ± 0.7
Ketanserin	3,215 ± 103
Methysergide	3,841 ± 130
7-OH-DPAT	18.3 ± 0.6
Phentolamine	> 10,000
Propranolol	> 10,000
Quinpirole	57.6 ± 2.2
SCH 23390	> 10,000
(+)-sulpiride	> 10,000
(−)-sulpiride	21.6 ± 1.4

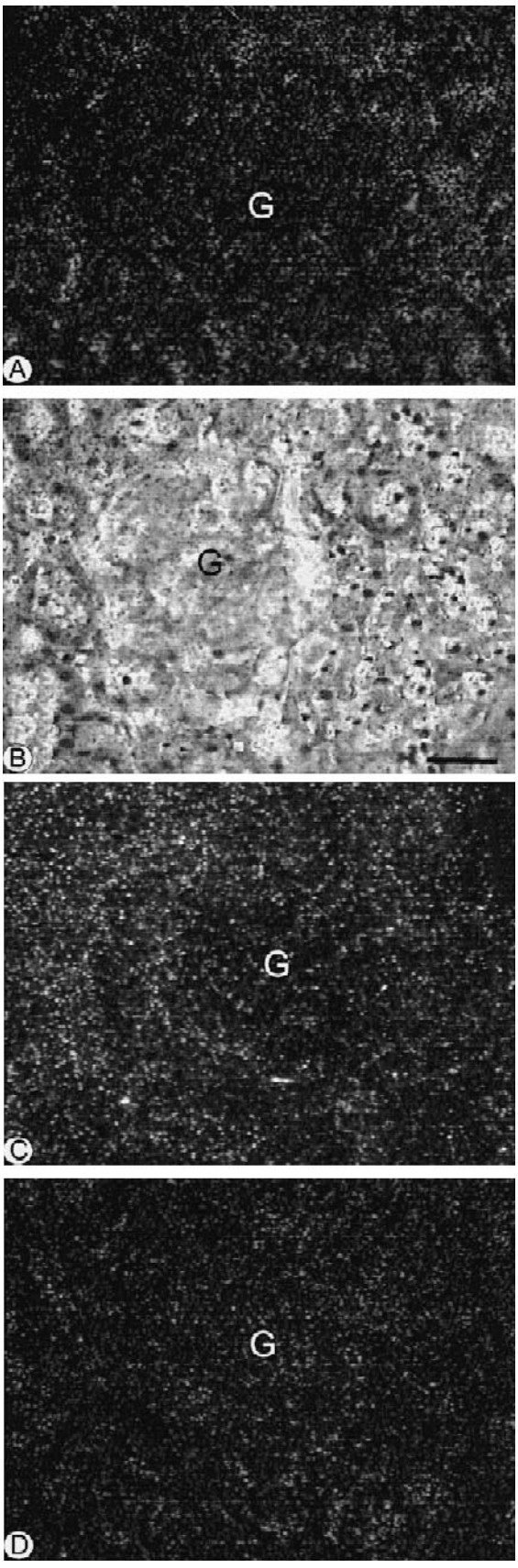
Sections of rat kidney were exposed to a 0.25 nM [^{3}H]-spiperone concentration, in the presence of increasing concentration of the compound tested. The value represent the competitor dissociation constant (K_i) expressed in nM. Data are the means ± S.E.M. of 3–5 experiments performed in triplicate.

Light microscope autoradiography revealed in normally-innervated kidneys the accumulation of silver grains over tubules of the renal cortex (Fig. 2) and in adventitia and adventitia-media border of intrarenal arteries (Fig. 3A–C). Denervation caused a remarkable loss of [^{3}H]spiperone binding sites developed in the renal cortex

Table 3
Number of silver grains developed in a 500 μm^2 area of the different portions of rat renal cortex in [^{3}H]spiperone autoradiographies

Control kidney	Total binding	Non-specific binding	Specific binding
Glomerular tuft	155 ± 8.3	55 ± 3.7	100 ± 4.1
Cortical tubules	305 ± 10.3	103 ± 6.9	202 ± 29
Adventitia and adventitia-media of medium sized arteries	430 ± 20.2	130 ± 7.5	300 ± 16.6
Denervated kidney	**Total binding**	**Non-specific binding**	**Specific binding**
Glomerular tuft	125 ± 9.4	45 ± 3.7	80 ± 5.1
Cortical tubules	301 ± 10.1	180 ± 3.6	121 ± 5.8 [a]
Adventitia and adventitia-media of medium sized arteries	160 ± 15.3	150 ± 8.6	undetectable

Sections were incubated with 0.25 nM [^{3}H]spiperone alone (total binding) or plus 1 μM (+)-butaclamol to define non-specific binding. Specific binding values were obtained by subtracting non-specific from total binding. Data are the mean ± S.E.M. of 5 independent measurements per animal ($n = 6$) performed by image analysis.
[a] $P < 0.01$ versus normally-innervated.



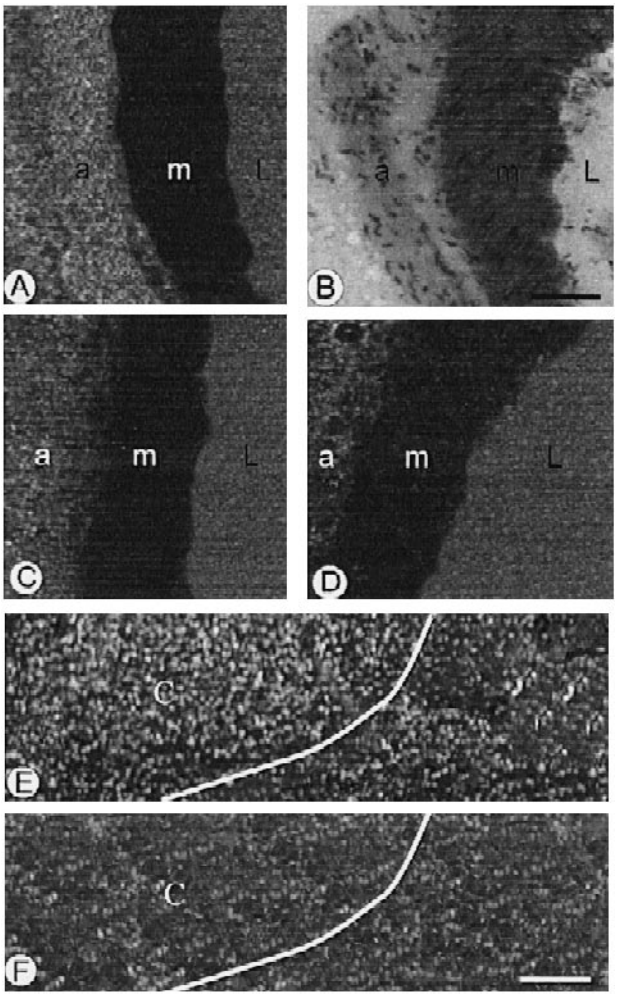


Fig. 3. Light microscope autoradiographies of [^{3}H]spiperone binding to sections of an intraparenchymal branch of renal artery of a normally-innervated kidney (A–C) and denervated kidney (D) and to islands of Calleja (E and F). Micrographs A, C, D, E and F are dark-field pictures of sections exposed to 0.5 nM [^{3}H]spiperone alone (A and E) or plus 1 μM (+)-butaclamol (C and F) to define non-specific binding. Micrograph B is a bright-field picture of A stained with toluidine blue to verify microanatomical details. Specific silver grains (sensitive to (+)-butaclamol displacement) were found in the adventitia (a), including the adventitia-media border. Denervation reduced adventitial silver grains to the same density noticeable in non-specific binding sections. L: lumen of the artery; m: tunica media. The heavy labelling of a forebrain area rich in dopamine D_3 receptor such as islands of Calleja (C, with a border delimited by a white line) with the same protocol used for renal dopamine D_2-like receptors suggests that in our experimental conditions a dopamine D_3 receptors was labelled. Calibration bars: pictures A–D, 75 μm; pictures E–F, 25 μm.

Fig. 2. Light microscope autoradiographies of [^{3}H]spiperone binding to sections of renal cortex of a normally-innervated kidney (A–C) and denervated kidney (D). Micrographs A, C and D are dark-field pictures of sections exposed to 0.5 nM [^{3}H]spiperone alone (A) or plus 1 μM (+)-butaclamol (C) to define non-specific binding. Micrograph B is a bright-field picture of A stained with toluidine blue to verify microanatomical details. Specific silver grains (sensitive to (+)-butaclamol displacement) were found primarily in cortical tubules and to a lesser extent in the glomerular tuft (G). Note the remarkable reduction of tubular silver grains in denervated kidney. Calibration bar: 100 μm.

(Fig. 2 and Table 3) and the complete disappearance of specific vascular silver grains (Fig. 3D and Table 3).

Analysis of sections of the forebrain revealed a heavy accumulation of silver grains in the ventral forebrain, in an area corresponding to the islands of Calleja (Fig. 3E–F).

4. Discussion

Involvement of dopamine D_1-like receptors in the control of renal function was documented by radioligand binding, functional, autoradiographic and molecular biology studies, whereas comparatively less information is available on dopamine D_2-like receptors in the kidney (Lokhandwala and Amenta, 1991; Cheung and Barrington, 1996). The presence of dopamine D_2-like receptors in the renal artery or in the kidney was demonstrated by radioligand binding assay (Brodde and Freistuhler, 1980; Felder et al., 1984; Jose et al., 1986; Huo et al., 1991), light microscope autoradiography (Amenta, 1990a; Amenta, 1990b; Ricci et al., 1991b), measurement of adenylate cyclase (Missale et al., 1985; Amenta et al., 1990; Ricci et al., 1991a) and more recently by molecular biology studies (Huo et al., 1991; Gao et al., 1994). Functional studies have characterized prejunctional dopamine D_2-like receptors modulating noradrenergic neurotransmission in rabbit (Chevillard et al., 1980), dog (Bass and Robie, 1984), rat (Lokhandwala and Steenberg, 1984a; Lokhandwala and Steenberg, 1984b; Rump et al., 1992) and human (Rump et al., 1993) renal nerves. However, some reports have suggested the presence of postjunctional dopamine D_2-like receptors mediating vasodilatation in the renal vasculature (Jose et al., 1986; Felder et al., 1988).

The hypothesis of postjunctional vasodilator dopamine D_2-like receptors was not unequivocally confirmed (Horn and Kohli, 1991). The present study has therefore evaluated the influence of denervation on the density and pattern of dopamine D_2-like receptors in the kidney. The procedure used in this study offers the advantage to have in the same animal one kidney denervated and the other one with a normal pattern of innervation (Soares-da-Silva et al., 1992). The almost complete disappearance of noradrenaline after denervation indicates that the majority of the catecholamine is contained in sympathetic nerves (Soares-da-Silva et al., 1992). The dopamine pool insensitive to denervation, represents the amount of catecholamine contained in renal tubules (Soares-da-Silva, 1988; Siragy et al., 1989; Soares-da-Silva, 1992).

Radioligand binding data are in agreement with former studies indicative of the expression of dopamine D_2-like receptors in the kidney (Felder et al., 1984; Jose et al., 1986; Amenta, 1990a; Amenta, 1990b; Huo et al., 1991; Ricci et al., 1991b). In comparison with previous investigations on the subject (Felder et al., 1984; Jose et al., 1986; Amenta, 1990a; Amenta, 1990b; Huo et al., 1991; Ricci et al., 1991b), we have used low concentrations of [^{3}H]spiperone (0.01–0.5 nM) to label renal D_2-like receptors. This allowed us to obtain, in sections of the kidney, K_d values similar to these found in the central nervous system (Seeman and Van Tol, 1994). The use of low concentrations of radioligand allowed the development of higher specific:non-specific binding ratios than in previous studies of our group (Amenta, 1990a; Amenta, 1990b; Ricci et al., 1991b). This is probably the reason of the identification of the specific binding of [^{3}H]spiperone in the glomerular tuft which we were unable to assess in previous investigations (Amenta, 1990a; Amenta, 1990b; Ricci et al., 1991b). This observation is consistent with molecular biology studies demonstrating the expression of dopamine D_2-like receptors in rat renal glomerulus (Gao et al., 1994).

Analysis of the pharmacological profile of [^{3}H]spiperone binding, with compounds preferentially active on dopamine D_3 receptors such as 7-OH-DPAT and quinpirole (Felder et al., 1984) being effective competitors of [^{3}H]spiperone, suggests that the site we have labelled belongs mainly to a dopamine D_3 receptor subtype. This hypothesis is supported by the demonstration of no effect of guanine nucleotides (Sokoloff et al., 1990; Sokoloff et al., 1992) on [^{3}H]spiperone binding to sections of rat kidney and by the observation that using the same experimental conditions [^{3}H]spiperone was bound to the islands of Calleja, a forebrain area rich in dopamine D_3 receptors (Lévesque et al., 1992; Levant and De Souza, 1993)

Analysis of the influence of denervation on renal dopamine D_2-like receptors, revealed that vascular binding sites and the majority of tubular binding sites are prejunctional since they are sensitive to denervation. A small portion of tubular binding sites and glomerular binding sites are not prejunctional and unaffected by denervation. The presence of both innervated and non-innervated dopamine D_2-like receptors is the most probable reason of the inconsistent results (Chevillard et al., 1980; Bass and Robie, 1984; Lokhandwala and Steenberg, 1984a; Lokhandwala and Steenberg, 1984b; Jose et al., 1986; Felder et al., 1988; Rump et al., 1992; Rump et al., 1993) concerning the presence or not of postjunctional dopamine D_2-like receptors in the kidney.

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