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Pharmacological characterization and autoradiographic localization of dopamine receptors in the rat adrenal medulla

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Abstract

The pharmacological profile and the anatomical localization of dopamine D_1-like and D_2-like receptors were studied in sections of rat adrenal medulla, with radioligand binding and autoradiographic techniques, respectively. [^{3}H][*R*]-(+)-chloro-2,3,4,5-tetrahydro-5-phenyl-1*H*-3benzazepin-al hemimaleate) (SCH 23390) was used as a ligand for dopamine D_1-like receptors and [^{3}H]spiperone was used as a ligand for dopamine D_2-like receptors. Radioligand binding and light microscope autoradiography did not show specific [^{3}H]SCH 23390 binding in sections of rat adrenal medulla. This suggests that rat adrenal medulla does not express dopamine D_1-like receptors. [^{3}H]Spiperone was specifically bound to sections of rat adrenal medulla. The binding was time-, temperature- and concentration-dependent, with a dissociation constant (K_d) of 1.05 nM and a maximum density of binding sites (B_{max}) of 100.2 ± 3.8 fmol/mg tissue. The pharmacological profile of [^{3}H]spiperone binding to rat adrenal medulla was similar to that displayed by neostriatum, which is known to express dopamine D_2 receptors. Light microscope autoradiography showed the accumulation of specifically bound [^{3}H]spiperone as silver grains within sections of adrenal medulla. Silver grains were found primarily over the cellular membrane of chromaffin cells. The above data indicate that chromaffin cells of the rat adrenal medulla express dopamine receptors belonging to the dopamine D_2 receptor subtype. These receptors are probably involved in the modulation of catecholamine release from chromaffin cells, as documented by functional studies.

Keywords: Dopamine receptor; Adrenal medulla; Radioligand binding; Autoradiography; Chromaffin cell; (Rat)

1. Introduction

Adrenal medullary chromaffin cells are embryologically derived from nervous tissue and are analogous to sympathetic postganglionic neurons (Guyton, 1971). It has been suggested that dopamine is involved in the regulation of catecholamine secretion from chromaffin cells (Kuchel and Racz, 1990) and the adrenal medullary dopaminergic system probably has a role in stress (Kuchel and Racz, 1990; Lokhandwala and Hedge, 1990).

Dopamine D_2-like receptors expressed by adrenal medullary chromaffin cells have been characterized in several radioligand binding studies performed primarily with isolated or cultured adrenal medullary cells (Quik et al., 1987; Bigornia et al., 1990; Ariano et al., 1991; Maroto et al., 1995). The problem of whether there is expression of dopamine D_1-like receptors by adrenal medullary cells has not been clarified yet (Artalejo et al., 1990; Bigornia et al., 1990, Ariano et al., 1991; Maroto et al., 1995). Some biochemical, histofluorescence and functional studies have found dopamine D_1-like receptors in the bovine and cat adrenal medulla (Artalejo et al., 1990, Ariano et al., 1991; Albillos et al., 1992). Other radioligand binding and molecular biology studies did not confirm the expression of dopamine D_1-like receptors by chromaffin cells (Bigornia et al., 1990, Maroto et al., 1995).

Stimulation of chromaffin cell dopamine receptors inhibits catecholamine secretion from the adrenal medulla (Hamilton, 1981; Bigornia et al., 1988, 1990; Kuchel and Racz, 1990; Albillos et al., 1992; Kujacic et al., 1995). This evidence comes primarily from in vitro investigations (Bigornia et al., 1988, 1990; Albillos et al., 1992), whereas there are only few in vivo studies on the topic (Hamilton, 1981; Damase-Michel et al., 1990; Kujacic et al., 1995). Studies in the conscious rat have shown that stimulation of dopamine D_2-like receptors increases catecholamine syn-

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thesis and release in the adrenal gland (Kujacic et al., 1995). The reason for the inconsistent data from in vitro and in vivo studies on catecholamine release is unknown. It has been hypothesized that an interaction with dopamine receptors having different localization may explain the inconsistencies (Kujacic et al., 1995). However, only sparse information is available concerning the biochemical-pharmacological characterization and the localization of dopamine receptors expressed by rat adrenal medulla (Schalling et al., 1990).

The present experiments were performed to pharmacologically characterize and to localize dopamine receptors of the rat adrenal medulla, using radioligand binding techniques and light microscope autoradiography.

2. Materials and methods

2.1. Animals and tissue preparation

Male Wistar rats (300–350 g body weight, $n = 12$) were obtained from Charles River Italy (Calco, Italy). The animals were killed by decapitation under ether anaesthesia. The brain and the adrenal glands were removed and washed in an ice-cold 0.9% NaCl solution. In 6 rats the striatum and the adrenal medulla were dissected out. Tissues were embedded in a cryoprotectant medium and frozen in a dry-ice-acetone mixture. Dissected striata and adrenal medulla were used for the radioligand binding assay, whereas whole adrenal glands were used for light-microscope autoradiography. Serial sections (8 μm thick) of tissues were obtained with a −20°C microtome cryostat and mounted on pre-weighed gelatine-coated microscope slides.

2.2. Radioligand binding experiments

Sections of striatum and of adrenal medulla were exposed to increasing concentrations of [^{3}H][[*R*](+)-(-chloro-2,3,4,5-tetrahydro-5-phenyl-1,4-benzazepin-al hemimaleate) (SCH 23390) and of [^{3}H]spiperone to label dopamine D_1-like and D_2-like receptors, respectively (Amenta, 1990a; Amenta et al., 1993). Technical details for the radioligand binding assay were reported previously (Amenta, 1990a; Amenta et al., 1993). For dopamine D_1-like receptor binding studies, sections were exposed to increasing concentrations of [^{3}H]SCH 23390 (0.01–5 nM) alone or plus 1 μM (+)-butaclamol to define non-specific binding. For dopamine D_2-like receptor binding studies, sections were exposed to increasing concentrations of [^{3}H]spiperone (0.01–5 nM) alone or plus 1 μM (+)-butaclamol to define non-specific binding. The optimal incubation time (60 min), temperature (25°C) and radioligand concentrations were determined in a series of preliminary experiments according to the procedure detailed elsewhere (Amenta, 1990a; Amenta et al., 1993). At the end of the incubation sections were washed in ice-cold incubation buffer (2 × 5 min) to remove unbound radioligands and were rinsed quickly in distilled water. The sections were then wiped onto Whatman GF-B glass fibre filters and counted by liquid scintillation spectrometry.

The pharmacological specificity of [^{3}H]spiperone binding to sections of rat striatum or adrenal medulla was assessed by incubating some sections with 0.5 nM [^{3}H]spiperone in the presence of increasing concentrations of compounds active at dopamine [apomorphine, bromocriptine, (+)-butaclamol, (−)-butaclamol, clozapine, dopamine, haloperidol, 7-hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin (7-OH-DPAT), quinpirole, SCH 23390 and (−)-sulpiride] and serotonin (ketanserin, methysergide and serotonin) and adrenergic (phentolamine and propranolol) receptors. Incubation with dopamine was performed in the presence and in the absence of a 300 μM guanosine triphosphate (GTP). At the end of the incubation, the sections were washed, transferred into scintillation vials and processed as described above.

2.3. Light microscope autoradiography

Light microscope autoradiography was performed with sections of whole adrenal gland, including the medulla and the cortex. This was done to avoid possible damage to the tissue as a result of removal of the adrenal cortex. For dopamine D_1-like receptor autoradiography, sections were incubated with 1 nM, 2.5 nM or 5 nM [^{3}H]SCH 23390 alone or plus 1 μM (+)-butaclamol to define non-specific binding and processed according to the protocol detailed elsewhere (Amenta, 1990a,b; Amenta et al., 1993). For dopamine D_2-like receptor autoradiography, sections were incubated with 0.5 nM [^{3}H]spiperone in the presence or in the absence of 1 μM (+)-butaclamol to define non-specific binding and were processed as detailed elsewhere (Amenta, 1990a,b; Amenta et al., 1993). The incubation with both radioligands was done at 25°C for 60 min. At the end of the incubation, the 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 adrenal gland sections. After exposure for 4–6 weeks in light-tight boxes, autoradiographs were developed in Kodak D-19, fixed in Agefix Agfa, stained with toluidine blue and viewed under a Zeiss Axiophot light microscope equipped with bright- and dark-field optics.

2.4. Data analysis

The data from binding experiments were analyzed by linear regression analysis of Scatchard plots of saturation isotherms. Competitor dissociation constant (K_i) values were determined according to the method of Cheng and Prusoff (1973).

2.5. Chemicals

[^{3}H]SCH 23390 (specific activity 80 Ci/mmol) and [^{3}H]spiperone (specific activity 70 Ci/mmol) were obtained from the Amersham Radiochemical Centre (Buckinghamshire, UK). 7-OH-DPAT, SCH 23390, isomers of butaclamol and sulpiride, haloperidol and quinpirole were purchased from Research Biochemicals (Natick, NJ, USA). Bromocriptine and methysergide were from of Sandoz Pharma (Basle, Switzerland). Ketanserin was obtained from Janssen (Beerse, Belgium). Other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

3. Results

[^{3}H]SCH 23390 was specifically bound to sections of rat neostriatum (Fig. 1), but not of rat adrenal medulla (data not shown). [^{3}H]Spiperone was specifically bound to sections of rat neostriatum (Fig. 2) and adrenal medulla (Fig. 3). In both tissues the binding was time- (data not shown), temperature- (data not shown) and concentration-dependent (Fig. 2 and Fig. 3). [^{3}H]Spiperone was bound to a single class of high affinity sites (Fig. 2 and Fig. 3), with dissociation constant values (K_d) of 0.5 nM in the neostriatum and of 1.05 nM in the adrenal medulla (Fig. 2 and Fig. 3). The maximum density of binding sites (B_{max}) averaged 248 ± 15 fmol/mg tissue in the neostriatum and 100.2 ± 3.8 fmol/mg tissue in the adrenal medulla (Fig. 2 and Fig. 3).

Table 1
Pharmacological specificity of [^{3}H]spiperone binding to sections of rat neostriatum and adrenal medulla

Compound	Neostriatum	Adrenal medulla
Apomorphine	70 ± 6.8	101 ± 7.2
Bromocriptine	11 ± 0.6	10 ± 0.3
(+)-Butaclamol	0.47 ± 0.06	2.5 ± 0.1
(−)-Butaclamol	498 ± 12.2	2601 ± 66.2
Clozapine	1176 ± 0.4	1217 ± 0.2
Dopamine	2800 ± 194	2950 ± 163
Dopamine + 300 μM GTP	3808 ± 215	3953 ± 202
Haloperidol	0.44 ± 0.03	0.48 ± 0.02
Ketanserin	> 5000	> 5000
Methysergide	> 5000	> 5000
7-OH-DPAT	4250 ± 63	3990 ± 81
Phentolamine	> 5000	> 5000
Propranolol	> 5000	> 5000
Quinpirole	4830 ± 81	4050 ± 78
SCH 23390	> 5000	> 5000
Serotonin	> 5000	> 5000
(−)-Sulpiride	38 ± 2.9	50 ± 3.1

Sections of rat striatum and adrenal medulla were incubated with 0.5 nM [^{3}H]spiperone in the presence of 8–10 increasing concentrations of the compounds tested. The values represent the competitor dissociation constant (K_i) expressed in nM and were calculated as indicated in Materials and methods. The data are means ± S.E.M. of experiments performed in triplicate.

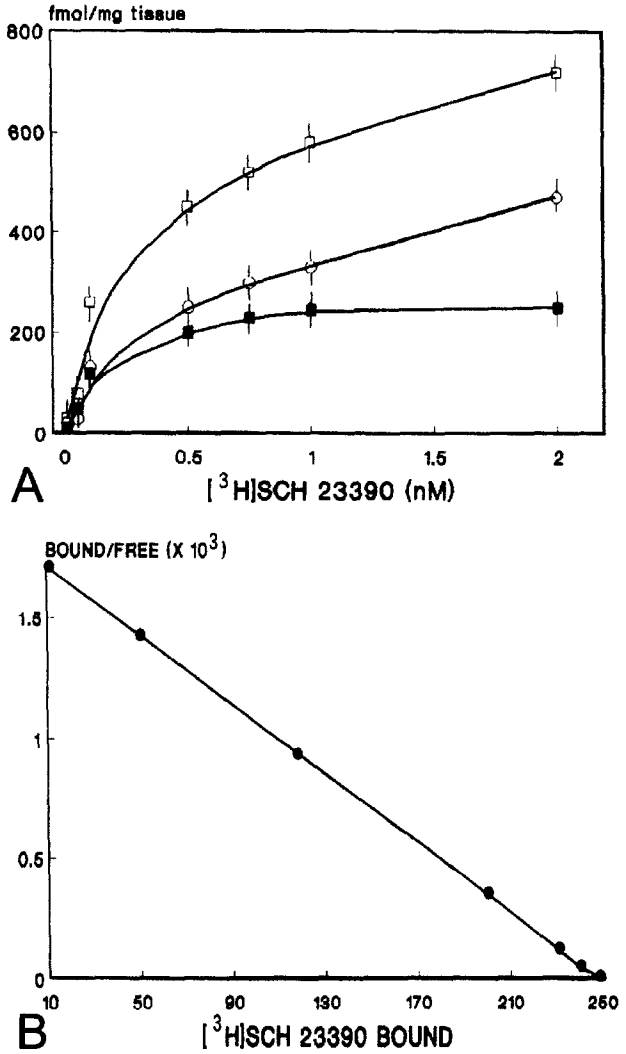

Fig. 1. (A) Saturation curve of [^{3}H]SCH 23390 binding to sections of rat neostriatum. Sections were incubated with the radioligand alone (total binding) (□) or plus 1 μM (+)-butaclamol to define non-specific binding (○). Specific binding (■) was obtained by subtracting non-specific from total binding. Points are the means ± S.E.M. of triplicate determinations. (B) Scatchard analysis of specific [^{3}H] SCH 23390 binding to sections of the rat neostriatum. The B_{max} value was 248 ± 12.6 fmol/mg tissue. Points are the means of triplicate determinations. Standard error was less than 10%.

Data on the pharmacological specificity of [^{3}H]-spiperone binding to sections of rat neostriatum and adrenal medulla are summarized in Table 1. As shown, K_i values were similar in the two tissues investigated. The most powerful displacer of [^{3}H]spiperone binding was haloperidol, followed in descending order by (+)-butaclamol and (−)-sulpiride (Table 1). The most powerful dopamine receptor agonist competing with [^{3}H]spiperone binding in both the neostriatum and the adrenal medulla was bromocriptine followed in descending order by apomorphine and dopamine. The preferential dopamine D_3 receptor agonists, 7-OH-DPAT, and quinpirole displaced [^{3}H]spiperone binding at concentrations significantly higher than the preferential dopamine D_2 receptor agonist,

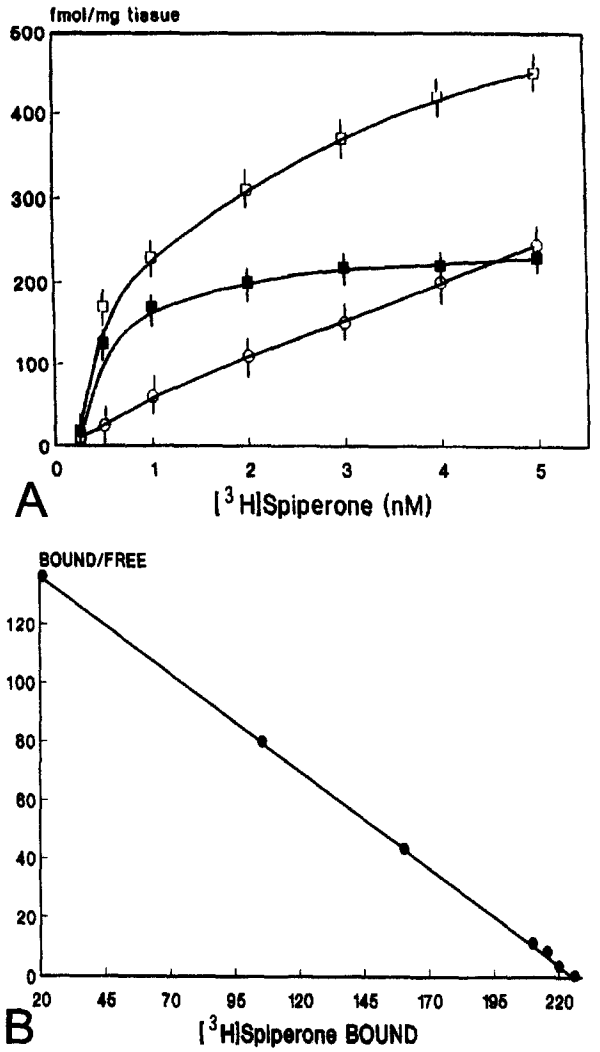

Fig. 2. (A) Saturation curve of [^{3}H]spiperone binding to sections of rat neostriatum. Sections were incubated with the radioligand alone (total binding) (□) or plus 1 μM (+)-butaclamol to define non-specific binding (○). Specific binding (■) was obtained by subtracting non-specific from total binding. Points are the means ± S.E.M. of triplicate determinations. (B) Scatchard analysis of specific [^{3}H]spiperone binding to sections of rat neostriatum. The B_{max} value was 248 ± 15 fmol/mg tissue. Points are the means of triplicate determinations. Standard error was less than 10%.

bromocriptine (Table 1), or dopamine itself (Table 1). The same is true for the putative dopamine D_4 receptor antagonist clozapine, which was markedly less potent than haloperidol, (+)-butaclamol and (−)-sulpiride as displacer of [^{3}H]spiperone binding (Table 1). The serotonergic and adrenergic compounds tested competed with [^{3}H]spiperone binding less potently than compounds active on dopamine receptors (Table 1). The inhibition by dopamine of [^{3}H]spiperone binding was sensitive to GTP in both the neostriatum and the adrenal medulla (Table 1).

Incubation of sections of rat adrenal medulla processed for autoradiography of [^{3}H]SCH 23390 did not allow the development of specific binding within the adrenal medulla (Fig. 4A-C). At the three different concentrations of radioligand used, the number of silver grains developed in light microscope autoradiographs was similar with [^{3}H]SCH 23390 alone or [^{3}H]SCH 23390 plus (+)-butaclamol (non-specific binding) (Fig. 4A-C).

In [^{3}H]spiperone autoradiographs, a dense accumulation of silver grains sensitive to (+)-butaclamol displacement was found in the adrenal medulla (Fig. 4D-F). Bright-field analysis of these autoradiographs at high magnification showed that silver grains were accumulated primarily over the cellular membrane of chromaffin cells (Fig. 5).

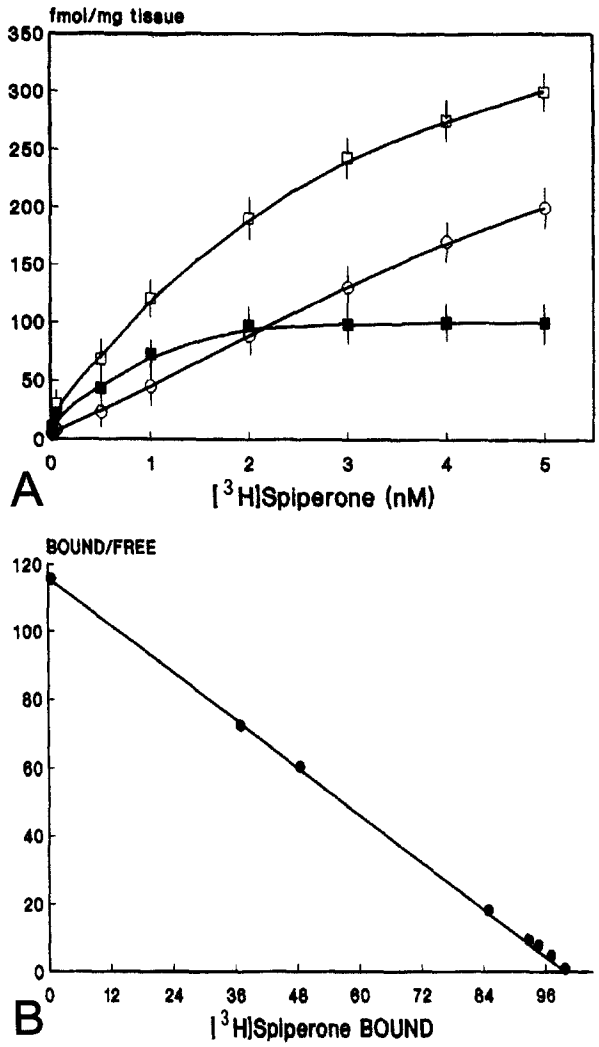

Fig. 3. (A) Saturation curve of [^{3}H]spiperone binding to sections of rat adrenal medulla. Sections were incubated with the radioligand alone (total binding) (□) or plus 1 μM (+)-butaclamol to define non-specific binding (○). Specific binding (■) was obtained by subtracting non-specific from total binding. Points are the means ± S.E.M. of triplicate determinations. (B) Scatchard analysis of specific [^{3}H]spiperone binding to sections of rat adrenal medulla. The B_{max} value was 100.2 ± 3.8 fmol/mg tissue. Points are the means of triplicate determinations. Standard error was less than 10%.

4. Discussion

The present findings provided direct evidence that chromaffin cells of rat adrenal medulla express dopamine D_2-like, but not dopamine D_1-like receptors. As mentioned in the Introduction, the existence of dopamine D_1-like receptors in the chromaffin tissue of the adrenal medulla is still debatable (Artalejo et al., 1990; Bigornia et al., 1990; Ariano et al., 1991; Albillos et al., 1992; Maroto et al., 1995). One demonstration of the occurrence of dopamine D_1-like receptors in the adrenal medulla comes from histofluorescence studies with rhodamine-conjugated dopamine receptor ligands (Artalejo et al., 1990; Ariano et al., 1991). In contrast, radioligand binding studies gave negative results (Bigornia et al., 1990; Maroto et al., 1995). It has been suggested that negative results obtained with radioligand binding techniques were due to the low density of adrenal medulla dopamine D_1-like receptors which did not allow their detection with radioligand assay techniques (Artalejo et al., 1990). To avoid this limitation, in spite of negative results of binding assays, in the present study, we applied autoradiographic analysis. It is known that appropriate light-microscope autoradiography techniques are more sensitive than radioligand binding in the case of low density of receptors or of their expression by small cellular populations (Wharton et al., 1993). In the absence of binding data concerning the concentrations of [^{3}H]SCH 23390 suitable for autoradiographic analysis, our investigations were done with three concentrations, from 1 nM to 5 nM. The 5 nM concentration probably represents the highest used in autoradiographic studies aimed at the characterization of dopamine D_1-like receptors in peripheral tissues (Amenta, 1990a,b; Amenta et al., 1993). At the three concentrations of [^{3}H] SCH 23390 used, no differences were noticeable in the density of silver grains developed in autoradiographs of the adrenal medulla. This indicates that rat adrenal medulla probably does not express dopamine D_1-like receptors.

In agreement with results of radioligand binding and in vitro functional studies performed primarily in bovine adrenal medulla (Quik et al., 1987; Bigornia et al., 1988, 1990; Artalejo et al., 1990; Ariano et al., 1991; Albillos et al., 1992; Maroto et al., 1995), rat adrenal medulla expresses dopamine D_2-like receptors shown by light microscopy to be localized over chromaffin cells. Central and peripheral dopamine receptors have been considered to

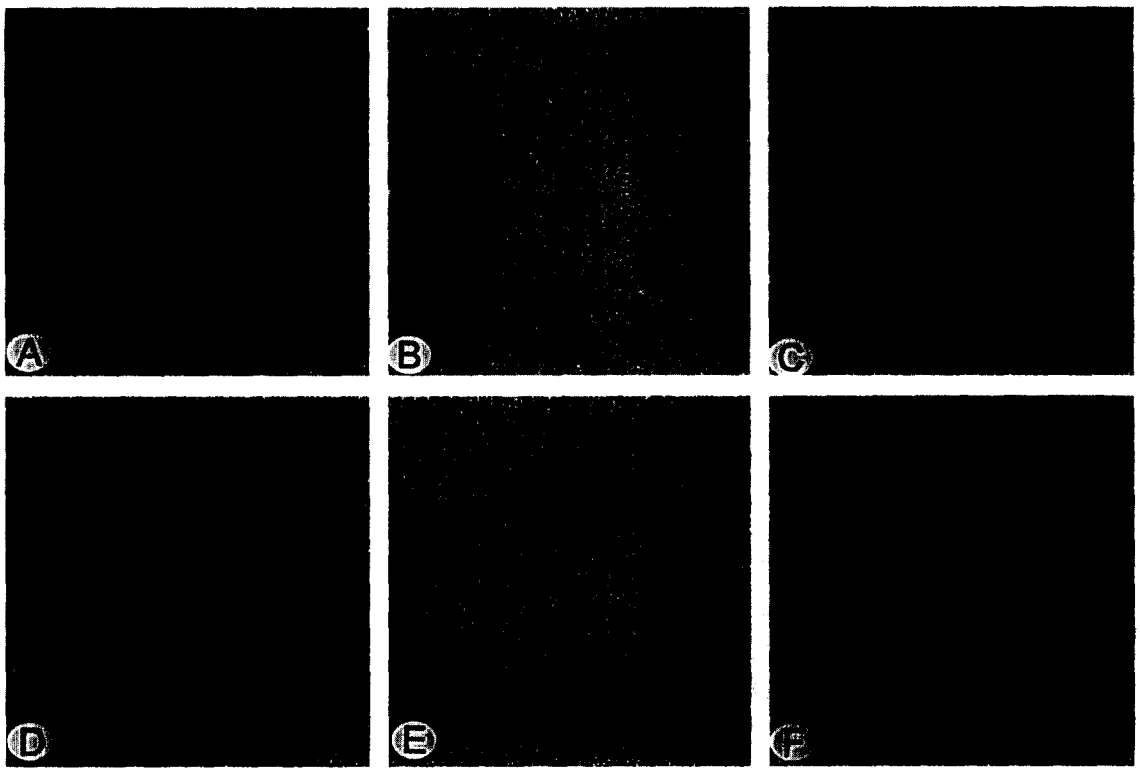

Fig. 4. Light-microscope autoradiographs of [^{3}H]SCH 23390 (pictures A–C) and [^{3}H]spiperone (pictures D–F) binding sites in sections of rat adrenal medulla. Sections were incubated with 2.5 nM [^{3}H]SCH 23390 (picture A) or with 0.5 nM [^{3}H]spiperone alone (picture D) (total binding) or plus 1 μM (+)-butaclamol to define non-specific binding (pictures C and F). Pictures B and E are bright-field micrographs of figures A and D, respectively, stained with toluidine blue to verify microanatomical details. Other micrographs are dark-field pictures. Note that, in sections exposed to [^{3}H]SCH 23390 for labelling dopamine D_1-like receptors (pictures A–C), the density of silver grains was similar in the presence or in the absence of (+)-butaclamol. This indicates the absence of dopamine D_1-like receptors in the rat adrenal medulla. In sections exposed to [^{3}H]spiperone for labelling dopamine D_2-like receptors (pictures D–F), specific silver grains [e.g. sensitive to (+)-butaclamol displacement] were located rather homogeneously within adrenal medullary tissue. Calibration bar: pictures A–C 75 μm; pictures D–F 100 μm.

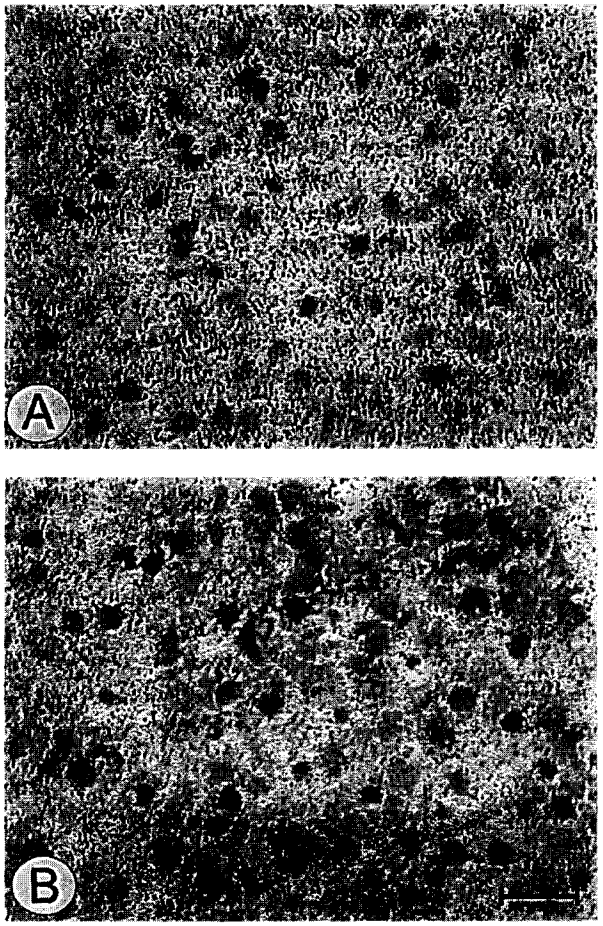

Fig. 5. Bright-field light-microscope autoradiographs of [^{3}H]spiperone binding sites in sections of rat adrenal medulla. Sections were incubated with 0.5 nM [^{3}H]spiperone alone (total binding, picture A) or plus 1 μM (+)-butaclamol to define non-specific binding (picture B). Silver grains were located primarily within the cellular membrane of adrenal chromaffin cells. Calibration bar: 25 μm.

belong mainly to two subtypes, dopamine D_1 and D_2 receptors (Seeman and Grigoriadis, 1987; Goldberg and Kohli, 1989; Lokhandwala and Hedge, 1990; Amenta, 1990a). The application of molecular biology techniques to dopamine receptor research has allowed the identification of five subtypes of dopamine receptors, named D_1, D_2, D_3, D_4, and D_5 receptors (Sibley and Monsma, 1992; Gingrich and Caron, 1993). Dopamine D_1 and D_5 sites belong to the dopamine D_1-like receptor family of the former classification, whereas dopamine D_2, D_3 and D_4 sites belong to the dopamine D_2-like receptor family (Sibley and Monsma, 1992; Gingrich and Caron, 1993).

The radioligand used in this study, [^{3}H]spiperone, is a non-selective compound for the subtypes of the D_2-like receptor family (Sibley and Monsma, 1992; Gingrich and Caron, 1993). However, there is evidence suggesting that the radioligand is specifically bound to the dopamine D_2 receptor in the rat adrenal medulla. In fact, displacement curves showed a higher potency of compounds active on dopamine D_2 receptors, such as (+)-butaclamol, haloperidol, (−)sulpiride and bromocriptine, than of compounds preferentially active at the dopamine D_3 receptor (7-OH-DPAT and quinpirole) or D_4 receptor (clozapine) (Sibley and Monsma, 1992; Gingrich and Caron, 1993). These curves were also consistent with the labelling of one site, indicating that rat adrenal medulla expresses a homogeneous population of dopamine D_2 receptors.

[^{3}H]Spiperone binding sites of rat adrenal medulla were sensitive to guanine nucleotides. This suggests that the site labelled by the radioligand cannot be classified as dopamine D_3 receptor, which is insensitive to guanine nucleotides (Sokoloff et al., 1992). The pharmacological profile of [^{3}H]spiperone binding to sections of rat adrenal medulla is similar to that found in neostriatum, which is known to express mainly dopamine D_2 receptors (Gingrich and Caron, 1993). Finally, molecular biology studies have demonstrated the expression of a dopamine D_2 receptor by rat adrenal medulla (Schalling et al., 1990).

Collectively, the above data suggest that the site we have characterized in rat adrenal medullary chromaffin cells is a putative dopamine D_2 receptor. This receptor has functional relevance in view of the observation that administration of dopamine receptor agonists modifies adrenaline secretion from adrenal chromaffin cells (Kujacic et al., 1995). Light-microscope autoradiography has shown that the putative dopamine D_2 receptor expressed by adrenal chromaffin cells has a widespread localization within adrenal medullary tissue, irrespective of the different type or status of chromaffin cells (Amenta, 1991). This may be the reason for the discrepant data on adrenal medullary catecholamine release caused by dopamine receptor stimulation when it is assessed with in vitro or in vivo techniques (Bigornia et al., 1988, 1990; Artalejo et al., 1990; Huettl et al., 1991; Kujacic et al., 1995).

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