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Absence of [^{3}H]SCH 23390 binding sites in the rat adrenal gland

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Abstract—The binding of D_2-dopamine receptor ligand [^{3}H]spiperone and selective D_1-ligand [^{3}H]SCH 23390 to the rat adrenal gland and striatum has been compared. [^{3}H]Spiperone showed specific binding in both tissues revealing a B_{max} of 887 fmol mg^{-1} protein and K_D of 0·38 nM, and B of 34 fmol mg^{-1} protein and K_D of 0·66 nM in the striatum and adrenal gland, respectively. On the other hand, [^{3}H]SCH 23390 showed a specific binding to the striatal tissue with B_{max} of 747 fmol mg^{-1} protein and K_D of 0·70 nM, while in the adrenal tissue no specific binding was observed. These results apparently indicated only D_2-dopamine receptor binding sites being present in the rat adrenal gland.

Dopamine-sensitive adenylate cyclase was demonstrated in the rat adrenal gland (Relja & Lacković 1984; Missale et al 1985a, 1986) indicating the presence of D_2-dopamine receptors. Binding studies, on the other hand, indicated the existence of D_2-receptor sites (Dunn & Bosmann 1981; Relja & Lacković 1984; Missale et al 1985b; Lyon et al 1987; Quik et al 1987). However, in these studies non-selective or even D_2-ligands as [^{3}H]haloperidol, [^{3}H]spiperone and [^{3}H]sulpiride were used. Recently, [^{3}H]SCH 23390 has been reported to be a selective ligand of central D_1-receptors (Billard et al 1984; Schulz et al 1985). To evaluate the type of dopamine receptor binding sites in the rat adrenal gland, in the present study the binding of [^{3}H]SCH 23390 and D_1-ligand [^{3}H]spiperone has been compared.

Materials and methods

The following radiolabelled drugs were used: [^{3}H]SCH 23390, (R)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7-ol, 80 Ci mmol^{-1} and [^{3}H]spiperone 21·3 Ci mmol^{-1} (Amersham Laboratories, UK). All other compounds used were purchased from Sigma Co (St Louis, MO, USA).

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The fresh adrenal and striatal tissue of male, adult Wistar rats was homogenized (Ultra Turrax) in 100 vol (w/v) of ice-cold 50 mM Tris buffer. The homogenate was centrifuged at 1000*g* and the supernatant was recentrifuged at 20000*g* for 10 min at 4°C. The resulting pellet was washed twice and finally resuspended in 50 mM Tris buffer containing (mM) NaCl 120, KCl 5, $CaCl_2$ 2, $MgCl_2$1, pargyline 10μM and 0·1% ascorbic acid.

For [^{3}H]spiperone binding, 1 mL aliquots of tissue suspensions were incubated in the presence of [^{3}H]spiperone of various concentrations (0·01–3 nM) for 15 min at 37°C. The specific binding was defined in the presence of 10 μM haloperidol. The final protein concentration was between 200–300 μg mL^{-1}.

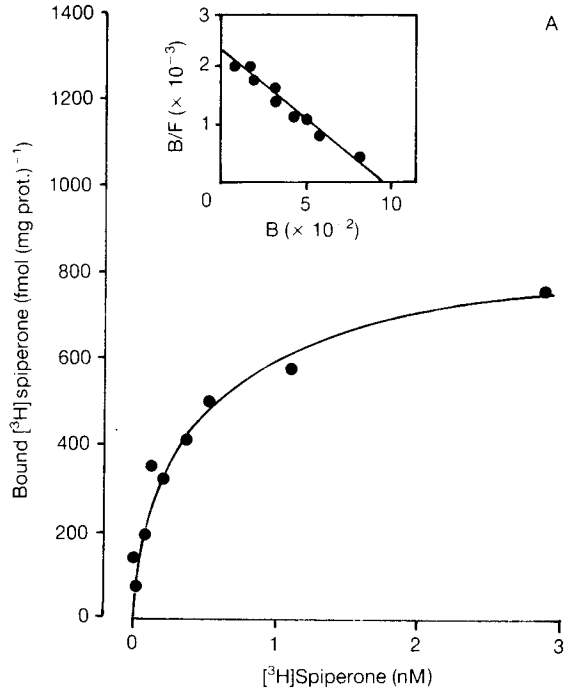
To provide [^{3}H]SCH 23390 binding to the striatal tissue, 1 mL aliquots of the tissue suspensions were incubated in the presence of various concentrations of [^{3}H]SCH 23390 (0·01–6 nM) for 15 min at 37°C. The specific binding was defined in the presence of 1 μM SCH 23390. The protein concentration was 200 μg mL^{-1}.

For [^{3}H]SCH 23390 binding to the adrenal tissue, 250 μL aliquots of the tissue suspensions were incubated in the presence of [^{3}H]SCH 23390 of various concentrations (0·5–25 nM) for 15 min at 37°C. The specific binding was defined in the presence of 10 μM SCH 23390. The protein concentration was 1000 μg mL^{-1}.

Incubations were terminated by filtration through Whatman GF/B filters. Radioactivity trapped on filters was determined in a Beckman LC counter (LC 1701) after a 5 mL of Aquasol (NEN, Mass.) had been added.

Results

The saturable specific binding of [^{3}H]spiperone was observed in striatal and adrenal membrane suspensions (Fig. 1). Scatchard



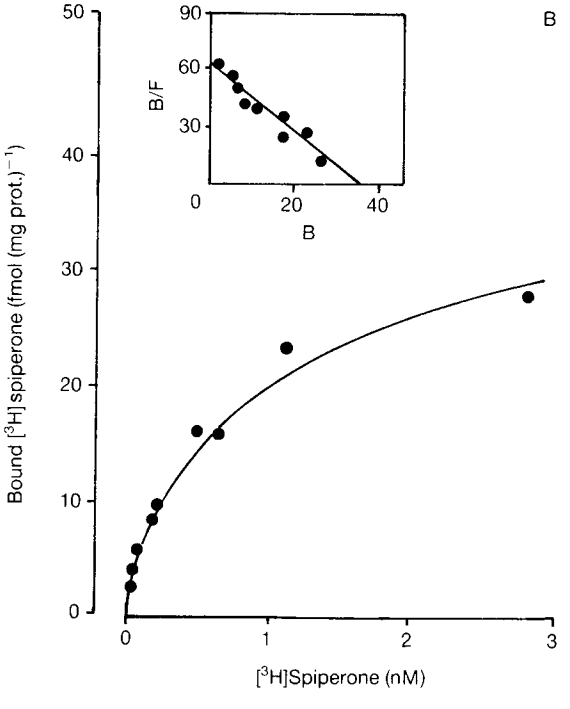
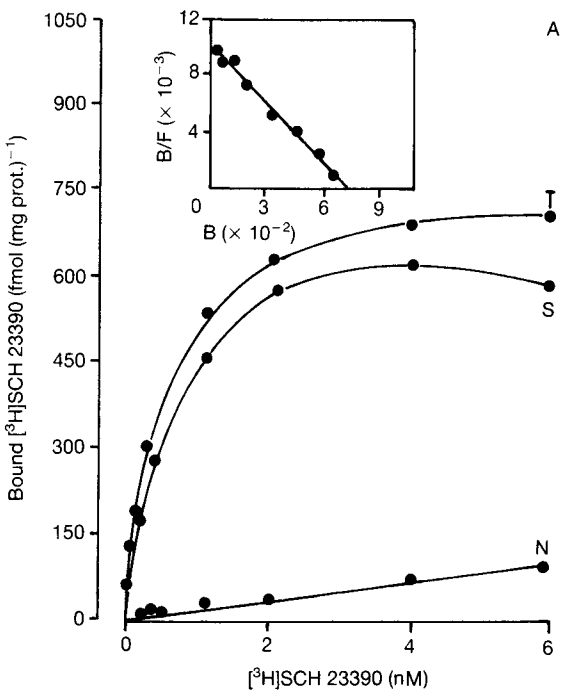


FIG. 1. Saturation of [^{3}H]spiperone specific binding to the rat striatal (A) and adrenal (B) membrane preparations as a function of increasing concentration of [^{3}H]spiperone (0·01–3 nM). The values shown are taken from a typical experiment performed in a triplicate. Inset: Scatchard plot of the same data.



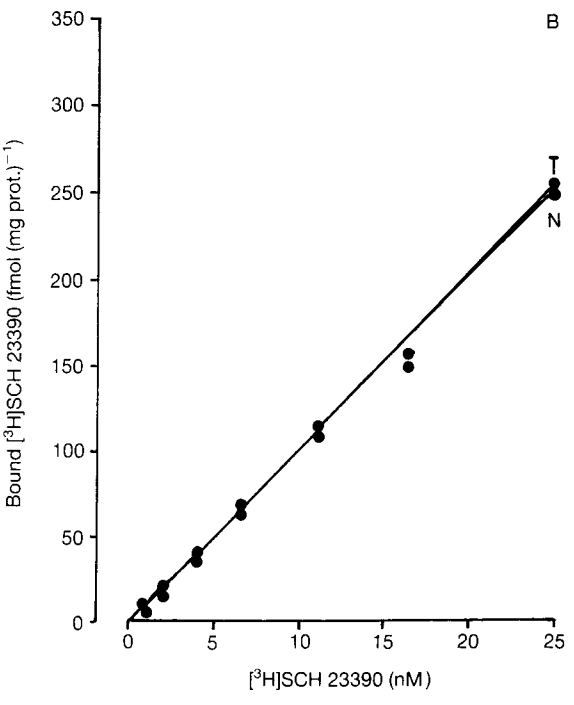


FIG. 2. Saturation curves of [^{3}H]SCH 23390 binding to the rat striatal (A) and adrenal (B) membrane preparation as a function of increasing concentration of [^{3}H]SCH 23390 (0·01–6 nM striatum; 0·5–25 nM adrenal gland). The non-specific binding (N) subtracted from the total binding (T) gives the specific binding (S). The non-specific binding is defined in the presence of 1 (striatum) or 10 μM SCH 23390 (adrenal gland). The values shown are taken from a typical experiment performed in triplicate. Inset (2A): Scatchard plot of the same data.

analysis of [^{3}H]spiperone binding to striatal membrane preparations showed a K_D value of $0{\cdot}38 \pm 0{\cdot}05$ nM and B_{max} of 887 ± 80 fmol mg^{-1} protein (n = 3). Scatchard analysis of a binding to the adrenal gland showed a K_D value of $0{\cdot}66 \pm 0{\cdot}06$ nM and B_{max} of 34 ± 6 fmol mg^{-1} protein (n = 3).

No saturable specific binding of [^{3}H]SCH 23390 was detected in the adrenal gland (Fig. 2B). On the contrary, under almost the same experimental conditions, the high affinity, saturable specific binding of [^{3}H]SCH 23390 to the striatal membrane was observed with the K_D value of $0{\cdot}70 \pm 0{\cdot}05$ nM and a B_{max} of 747 ± 60 fmol mg^{-1} protein (n = 3) (Fig. 2A).

Discussion

The present study demonstrated that the specific binding of D_2-receptor ligand [^{3}H]spiperone existed in membrane preparations of the adrenal gland. In comparison, the striatal tissue was studied under almost identical conditions. Saturation (B_{max} = 887 fmol mg^{-1} protein) and affinity (K_D = 0·38 nM) of [^{3}H]spiperone binding to that tissue was within extreme values being published up to now (Leysen et al 1978; Andron & Maguire 1980; Howlett & Nahorski 1980; Naber et al 1980). In comparison to the striatum, in the adrenal gland we found a 26 times lower number of [^{3}H]spiperone binding sites (B_{max} = 34 fmol mg^{-1} protein), whereas the affinity of that binding to membranes from the adrenal (K_D = 0·66 nM) and striatal tissue was similar, indicating similarities between these two binding sites. The observation was in agreement with several studies in which the presence of adrenal D_2-receptors had been postulated (Dunn & Bosmann 1981; Relja & Lacković 1984; Missale et al 1985b; Lyon et al 1987; Quik et al 1987).

In contrast to the [^{3}H]spiperone binding, the present study demonstrated that no specific binding sites for a selective D_2-receptor antagonist, [^{3}H]SCH 23390, could be detected in the rat adrenal gland even when 25 nM of [^{3}H]SCH 23390 was added. Under almost the same experimental procedure, specific saturable binding of [^{3}H]SCH 23390 was observed in the striatum revealing K_D and B_{max} values similar to those described by others (Billard et al 1984; Schulz et al 1985; Rovescalli et al 1987). In addition, the specific binding of [^{3}H]SCH 23390 to the kidney tissue had been observed recently (Zdilar & Lacković to be published). Thus, it seemed that [^{3}H]SCH 23390 and the methodological procedure employed were suitable for characterization of peripheral D_1-binding sites.

The presence of dopamine-stimulated adenylate cyclase in a particular tissue was used as a keystone to classify a particular dopaminergic receptor into the D_1-category (Kebabian & Calne 1979; Stoof & Kebabian 1984). On the other hand, in the search for specific D_1-and D_2-receptor ligands, SCH 23390 was believed to be, both in the brain (Iorio et al 1983; Hyttel 1983) and in the periphery (Goldberg et al 1984; Hilditch et al 1984) and the most selective D_1-receptor antagonist so far synthesized. Thus, the presence of dopamine stimulated adenylate cyclase (Relja & Lacković 1984; Missale et al 1985a, 1986) and the absence of [^{3}H]SCH 23390 binding sites in the adrenal gland created a problem, the solution to which might have a fundamental importance in the further research of dopaminergic receptor subclassification.

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