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ACUTE RESERPINE TREATMENT INDUCES DOWN REGULATION OF D-1 DOPAMINE RECEPTOR ASSOCIATED ADENYLYL CYCLASE ACTIVITY IN RAT STRIATUM

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Abstract—Behavioural studies suggest a functional interaction between D-1 and D-2 systems in normal rat striatum to alter motor behaviour and which is disrupted by dopamine depletion induced by acute reserpine treatment. Consequently, we have investigated the effect of acute reserpine treatment on the biochemical interaction between D-1 and D-2 receptors present in rat striatal slices. Twenty-four hours following the administration of reserpine (5 mg/kg i.p.), striatal dopamine content was depleted by more than 73%; the density (B_{max}) of D-1 receptor sites measured by the *in vitro* binding of [^{3}H]SCH 23390 to striatal membranes was increased while the binding of [^{3}H]spiperone to D-2 receptor sites was unaltered. Reserpine treatment had no effect on the affinity (K_d) of [^{3}H]SCH 23390 or [^{3}H]spiperone for D-1 and D-2 sites. Basal levels of cyclic AMP accumulation in striatal slices prepared from reserpine-treated rats were lower than those observed in control slices. In striatal slices prepared from normal rats, dopamine (10–320 μM) and the D-1 agonist SKF 38393 (0.1–3.2 μM) induced concentration-dependent increases in cyclic AMP accumulation. The D-1 antagonist SCH 23390 (10 μM) abolished the accumulation of cyclic AMP produced by dopamine or SKF 38393. The D-2 antagonist (±)-sulpiride (50 μM) enhanced the response to dopamine (10–320 μM) while the D-2 agonist quinpirole (10 μM) abolished the response to SKF 38393 (0.1–3.2 μM). However, 24 hr after reserpine treatment the ability of dopamine (10–320 μM) and SKF 38393 (0.1–3.2 μM) to elicit an increase in cyclic AMP accumulation was markedly reduced in striatal slices. SCH 23390 (10 μM) was able to attenuate the response to dopamine (10–320 μM). However, the inclusion of (±)-sulpiride (50 μM) did not enhance the trend for an increase in cyclic AMP accumulation produced by dopamine. Also, quinpirole (10 μM) did not affect the response to SKF 38393 (0.1–3.2 μM) in striatal slices from reserpine pretreated rats. The data confirm the positive linkage between D-1 receptors and adenylyl cyclase and the inhibitory coupling to D-2 sites in striatal slices from normal, rats. Acute reserpine treatment appears to cause an uncoupling of D-1 receptors associated with adenylyl cyclase.

Dopamine (DA‡) receptors in the brain can be classified into at least two subtypes; the D-1 receptor linked to DA-sensitive adenylyl cyclase (AC) and the D-2 receptor which either has no effect or inhibits the activity of AC [1–4]. D-1 receptor stimulation may modify the intensity and components of motor behaviour induced by D-2 receptor activation. In normal rats the administration of a D-1 receptor agonist does not itself produce stereotyped behaviour, but it synergistically potentiates motor behaviours initiated by the peripheral administration of a D-2 receptor agonist [5–10]. For example, in rats, the D-2 agonist quinpirole induces a dose-dependent hyperactivity (locomotion, sniffing, head movements and rearing), while the D-1 agonist SKF 38393 alone does not [8]. Combined treatment with quinpirole and SKF 38393 intensifies stereotypy to include licking and biting behaviours. The maximal expression of D-2 initiated behaviours appears dependent on additional D-1 receptor "tone" and this may explain why both D-1 and D-2 antagonist drugs are capable of inhibiting behaviour induced by D-2 agonists [11–13]. Similarly, electrophysiological, studies utilizing extracellular single unit recordings from non-DAergic cells in the striatum or substantia nigra have shown that microiontophoretic coadministration of D-1 and D-2 agonists is required to produce a maximal inhibition of spontaneous and glutamate-induced neuronal firing [14, 15].

The involvement of endogenous dopamine in providing D-1 tone in normal animals is apparent following reserpine treatment of mice. In normal mice bromocriptine and quinpirole produce locomotor hyperactivity but this is abolished 3 hr following reserpinization. In reserpine-pretreated mice neither SKF 38393 nor bromocriptine produce motor activity but a combination of these agents causes hyperactivity [16, 17]. Two other events may also occur as a result of reserpine pretreatment. First, in mice treated

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‡ Abbreviations: AC, adenylyl cyclase; cyclic AMP, adenosine cyclic-3'5'-monophosphate; DA, dopamine; DHBA, dihydroxybenzylamine; DOPAC, 3,4-dihydroxyphenylacetic acid; EDTA, ethylenediaminetetracetic acid; HVA, homovanillic acid; IBMX, 3-isobutyl-1-methylxanthine.

24 hr previously with a single administration of reserpine [18, 19], or in rats treated repeatedly with reserpine over a 3–5 day period [20], there is increased behavioural sensitivity to D-2 agonists which now produce stereotypies. Second, there is evidence that there may be a break in the linkage between D-1 and D-2 receptors dependent on the time following reserpinization. Starr *et al.* [18] showed that the D-2 agonists RU 24213 and lisuride evoked slow ponderous walking and head down sniffing that was reduced by both the selective D-1 and D-2 antagonists, SCH 23390 and metoclopramide, 3 hr after reserpine administration. However, 24 hr later, SKF 38393 as well as D-2 agonists promoted rapid locomotion, rearing and grooming directly, but which were only attenuated by selective antagonists for the respective receptors. A similar phenomenon has been shown following repeated reserpine treatment [20, 21].

The ability of reserpine to enhance D-1 and D-2 mediated behaviours and to induce a functional separation of D-1 and D-2 systems might be reflected by changes in the linkage between D-1 and D-2 receptors and AC. For example, following acute reserpine treatment DA- and SKF 38393-stimulated cyclic AMP formation was potentiated when measured in striatal homogenates [21–24]. In addition, increases in the density of striatal D-1 and D-2 receptors have been reported after reserpinization [19, 25]. However, there has been no attempt to date to study the effects of reserpine pretreatment of rats on the coupling of both stimulatory D-1 receptors and inhibitory D-2 receptors to AC.

In the present study, the accumulation of cyclic AMP in rat striatal slice preparations was used to investigate whether a biochemical uncoupling of the D-1 and D-2 receptors parallels the behavioural dissociation and supersensitivity occurring 24 hr after reserpine treatment.

MATERIALS AND METHODS

Reserpine treatment. Female Wistar rats (200–250 g; Bantin and Kingman Ltd, Hull) were treated with reserpine 5 mg/kg, i.p. in a volume of 1.0 mL/kg. Reserpine was initially dissolved in glacial acetic acid then made up to the correct concentration with deionized water and adjusted to pH 4–5 using 1 N NaOH. An identical volume of vehicle was administered to a separate group of rats which acted as controls.

Dissection. Twenty-four hours following reserpine treatment the animals were killed, between 10.00 a.m. and 12.00 p.m. in order to minimize variability due to diurnal fluctuations. The animals were decapitated, the whole brain removed from the skull and the striata dissected out. Tissues for cyclic AMP accumulation and *in vitro* radioligand binding assays were used immediately. Tissues for DA, homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) measurements were immediately frozen over dry ice and then stored at −70°.

Cyclic AMP assay. Cross-chopped (0.35 × 0.35 mm) striatal slices were preincubated in Krebs buffer (NaCl, 118 mM; KCl, 4.7 mM; $CaCl_2$, 1.3 mM; KH_2PO_4, 1.2 mM; $MgSO_4$, 1.2 mM; $NaHCO_3$, 25 mM and glucose, 11.7 mM) containing 1 mM 3-isobutyl-1-methylxanthine (IBMX) as a phosphodiesterase inhibitor and saturated with 95% O_2/5% CO_2 at 37° for 90 min with replacement of buffer every 20 min. Packed slices (20 μL) were incubated in the presence or absence of (±)-sulpiride or SCH 23390 for 5 min and then with DA, SKF 38393 or quinpirole for a further 10 min in a total buffer volume of 300 μL. Basal levels of cyclic AMP accumulation were determined in striatal slices incubated for the total 15 min period in the absence of DA or other drugs. Concentrated HCl (20 μL) was added to each sample and the samples were placed on ice to elute cyclic AMP from the slices. After 20 min the samples were neutralized with 1 N NaOH (200 μL) and centrifuged at 1000 *g* at 4° for 10 min. Duplicate aliquots (50 μL) of the supernatant were analysed for cyclic AMP content using a protein binding assay [26]. The amount of cyclic AMP present in each aliquot was calculated from a standard curve, constructed using 0.125–10 pmol authentic cyclic AMP per tube. The protein content was assayed according to the technique of Lowry *et al.* [27], following sonication of the remaining tissue and the results expressed in pmol cyclic AMP/mg protein.

In vitro radioligand binding. The specific binding of [^{3}H]SCH 23390 (0.06–20 nM) and [^{3}H]spiperone (0.06–2.0 nM) to D-1 and D-2 receptor sites was studied in homogenates obtained from control and reserpine treated rats.

Briefly, D-1 receptor assays were carried out in 50 mM Tris–HCl buffer (pH 7.4) containing 120 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$ and 1 mM $MgSO_4$. Pooled striatal tissue was homogenized in 50 w/v of buffer using a Brinkman Polytron homogenizer at setting 5 for 10 sec. The homogenate was centrifuged at 48,000 *g* for 10 min at 4°. The resultant tissue pellet was resuspended in 50 w/v of fresh buffer, homogenized again at setting 5 for 10 sec and centrifuged a second time at 48,000 *g* for 10 min at 4°. The final tissue pellet was resuspended in 500 w/v of buffer by homogenization at setting 3 for 10 sec. Aliquots of 0.9 mL of membrane suspension were used directly in binding experiments. Membrane suspension was added to ice-cold glass tubes containing 50 μL deionized water to define total binding or 50 μL *cis*-flupentixol (10^{-5}M) to define non-specific binding. The binding reaction was initiated by addition of [^{3}H]SCH 23390 (80 Ci/mmol, Amersham International plc). Samples were incubated at 37° for 30 min. Bound ligand was separated from free over Whatman GF/C glass filters using rapid vacuum filtration (15–20 mmHg). Filters were rapidly washed with 2 × 5 mL ice-cold buffer and placed into polyethylene scintillation vial inserts. Scintillation fluid (5 mL; OptiPhase "HiSafe" II, LKB Scintillation Products) added to each vial and assessed for radioactivity in a TriCarb 460C counter (Packard), efficiency 45–53% for a 4 min period at 4°.

The procedure for labelling D-2 sites was similar to that described for D-1 binding but employed 50 mM Tris–HCl (pH 7.4) containing 120 mM NaCl as incubation buffer. The final homogenate dilution

was 300–400 w/v. Non-specific binding was defined by (±)-sulpiride (10^{-5}M) and the reaction was initiated by the addition of 50 μL [^{3}H]spiperone (21.8 Ci/mmol, Amersham International plc).

Specifically bound radioligand was defined as the difference between total binding and the binding in the presence of *cis*-flupentixol or (±)-sulpiride for [^{3}H]SCH 23390 and [^{3}H]spiperone respectively. The maximum number of binding sites (B_{max}) and the apparent dissociation constant (K_d) were calculated by the linear regression analysis of Eadie–Hofstee plots corresponding to saturation curves of specific binding.

Measurement of DA and its metabolites. The concentration of DA and its metabolites in striatal tissue was determined by HPLC coupled to electrochemical detection according to a modification of the method described by Weller *et al.* [28]. Briefly, striatal tissue was thawed on ice and sonicated in 10 w/v 0.4 M perchloric acid containing 1 mM ethylenediamietetraacetic acid (EDTA) and 0.5 mM sodium metabisulphite using a Microson tissue disrupter. The resulting homogenate was added to a solution of the internal standard dihydroxybenzylamine (DHBA) in a 9:1 (v/v) ratio to give a final concentration of DHBA of 200 ng/mL. The homogenate was centrifuged at 14,000 *g* for 10 min at 4°. An aliquot (50 μL) of the supernatant was injected onto a 25 cm (0.14 cm i.d.) Spherisorb ODS-2 reverse phase column (Phase Separations) using a Spectra Physics 8780XR automated sampler fitted with a Rheodyne injection valve and 100 μ sample loop. The mobile phase consisted of 0.1 M NaH_2PO_4 in 18% methanol, 6.5 mM octane sulphonic acid, 1 mM EDTA (pH 3.1 adjusted with 3 M phosphoric acid). Chromatographic peaks were measured with a BAS LC-3A amperometric detector with a thin-layer electrochemical cell fitted with a glassy carbon working electrode and Ag/AgCl reference electrode. The working potential was +0.7 V, pressure 2750 psi and the temperature was maintained at 9–10° to prevent sample degradation. Tissue levels of DA and its metabolites were quantified by comparisons with standards of known concentration.

Materials. SCH 23390 (*R*-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine maleate, Schering Bloomfield, NJ, U.S.A.), quinpirole HCl (Lilly Research Laboratories, Indianapolis, IN, U.S.A.), (±)-sulpiride (Delagrange, France) and *cis*-flupentixol dihydrochloride (Lundbeck, Denmark) were gifts from the indicated sources. SKF 38393 (1-phenyl-2,3,4,5-tetrahydro-(1*H*)-3-benzazepine-7,8-diol hydrochloride) was purchased from Semat (St Albans). Other drugs used in this study were purchased from the Sigma Chemical Co. (Poole). All drugs were dissolved in deionized water except for reserpine, which was initially dissolved in glacial acetic acid (50 μL) and then made up to the required concentration with deionized water and adjusted to pH 4–5 with 1 N NaOH. DA, DOPAC and HVA standards for HPLC analysis were dissolved in 0.4 M perchloric acid containing 1 mM EDTA and 0.01% sodium metabisulphite.

In each experiment and for every drug treatment, cyclic AMP content in striatal slices was assayed in four separate samples. Each experiment was carried out on at least three occasions. DA and SKF 38393 concentration–response curves were analysed using one-way ANOVA. Comparison of the DA and SKF 38393 concentration–response curves in the presence and absence of other drugs were analysed by two-way ANOVA. Statistical analysis of all other changes were performed with Dunn's test for multiple comparisons.

RESULTS

Effects of reserpine treatment on striatal DA, DOPAC and HVA levels

The striatal levels of DA 24 hr after systemic reserpine administration were depleted to 27% of those measured in control tissue (controls: N = 17; 8.25 ± 0.45 μg/g; reserpine pretreated: N = 13; 2.23 ± 0.77 μg/g; $P < 0.05$). The levels of DOPAC were also reduced (by 18%) by reserpine pretreatment (controls: 1.57 ± 0.08 μg/g; reserpine pretreated: 1.29 ± 0.01 μg/g; $P < 0.05$). However, HVA levels were unaffected (controls: 0.83 ± 0.06 μg/g; reserpine pretreated: 0.94 ± 0.11 μg/g; $P > 0.05$). The ratios of DOPAC to DA and HVA to DA were increased following reserpine treatment (DOPAC/DA – controls: 0.19 ± 0.01; reserpine pretreated: 1.79 ± 0.38 mg/g; $P < 0.05$. HVA/DA – controls: 0.11 ± 0.01; reserpine pretreated: 1.46 ± 0.36; $P < 0.05$).

Alterations in striatal [^{3}H]*SCH* 23390 *and* [^{3}H]-*spiperone binding*

Twenty-four hours following reserpine pretreatment the density (B_{max}) of specific [^{3}H]SCH 23390 binding to D-1 sites was increased (31%) in striatal homogenates from reserpine-pretreated rats (controls: N = 6; 74.8 ± 5.6 pmol/g tissue; reserpine pretreated: 98.1 ± 6.3 pmol/g tissue; $P < 0.05$). There was no alteration in the B_{max} values for [^{3}H]-spiperone binding (controls: N = 6; 28.2 ± 0.9 pmol/g tissue; reserpine treated: 28.0 ± 1.9 pmol/g tissue; $P > 0.05$). There was no change in the dissociation constant (K_d) of specific [^{3}H]SCH 23390 (controls: 0.39 ± 0.02 nM; reserpine pretreated: 0.36 ± 0.02 nM; $P > 0.05$) and [^{3}H]spiperone (controls: 0.055 ± 0.009 nM; reserpine pretreated: 0.046 ± 0.004 nM; $P > 0.05$) binding to D-1 and D-2 receptors respectively in rat striatal homogenates when compared to control tissue preparations.

Accumulation of cyclic AMP in striatal slices

Basal levels of cyclic AMP accumulation in striatal slices prepared from rats treated with reserpine were reduced to 80% (29.5 ± 1.4 pmol cyclic AMP/mg protein) of those observed in striatal slices from control animals (36.9 ± 1.3 pmol cyclic AMP/mg protein; $P < 0.05$ compared to reserpine treated rats). In striatal slices from control rats DA (10–320 μM) stimulated cyclic AMP accumulation in a concentration-dependent manner ($F = 2.87_{3,43}$ $P < 0.05$; Fig. 1a). Incubation with SCH 23390 (10 μM) inhibited the concentration-dependent increase in cyclic AMP accumulation induced by DA (10–320 μM; $F = 22.22_{1,81}$ $P < 0.05$).

Twenty-four hours after reserpine pretreatment

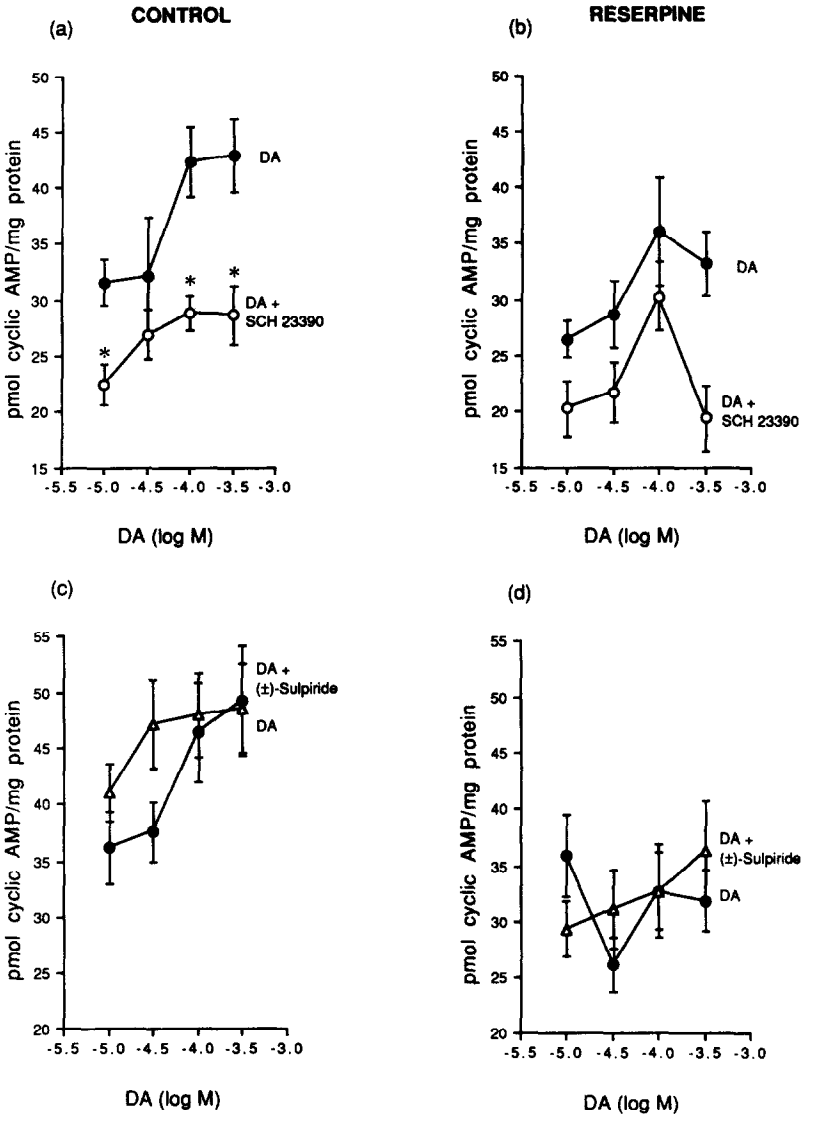

Fig. 1. The intracellular accumulation of cyclic AMP in rat striatal slices elicited by DA (●) and the effect of SCH 23390 (○) or (±)-sulpiride (△) on the DA-induced response in control animals (panels a and c) and in rats treated with reserpine (5 mg/kg i.p.) 24 hr previously (panels b and d). Following a 90 min preincubation in Krebs buffer containing 1 mM IBMX, slices were incubated in the presence and absence of SCH 23390 (10 μM) or (±)-sulpiride (50 μM) for 5 min and then with DA (10–320 μM) for a further 10 min. The results are the means ± SEM of three or individual experiments with N = 3–4 samples for each DA concentration. * $P < 0.05$ (Dunn's test) when compared to the same concentration of DA in the absence of SCH 23390 or (±)-sulpiride.

DA (10–320 μM) did not induce a significant concentration-dependent increase in cyclic AMP accumulation in striatal slices prepared from reserpinized rats ($F = 1.97_{3,41}$ $P > 0.05$; Fig. 1b). However, there was a trend for increasing concentrations of DA to raise the accumulation of cyclic AMP and this response was attenuated by SCH 23390 (10 μM; $F = 13.60_{1,81}$ $P < 0.05$). (±)-Sulpiride (50 μM) enhanced the DA (10–320 μM)-stimulated accumulation of cyclic AMP in striatal slices from control rats ($F = 3,70_{1,104}$ $P < 0.05$; Fig. 1c). The enhancement caused by (±)-sulpiride manifested as a shift in the DA concentration-response curve to the left with no apparent increase in the maximal levels of cyclic AMP accumulation. In contrast, in striatal slices prepared from reserpine-pretreated rats (±)-sulpiride (50 μM) did not alter the non-significant accumulation of cyclic AMP produced by DA ($F = 0.08_{1,110}$ $P > 0.05$; Fig. 1d).

SKF 38393 (0.1–3.2 μM) induced a concentration-dependent increase in cyclic AMP accumulation in striatal slices prepared from control rats ($F = 3.85_{3,35}$ $P < 0.05$; Fig. 2a). In contrast, SKF 38393 did not induce an increase in cyclic AMP accumulation in

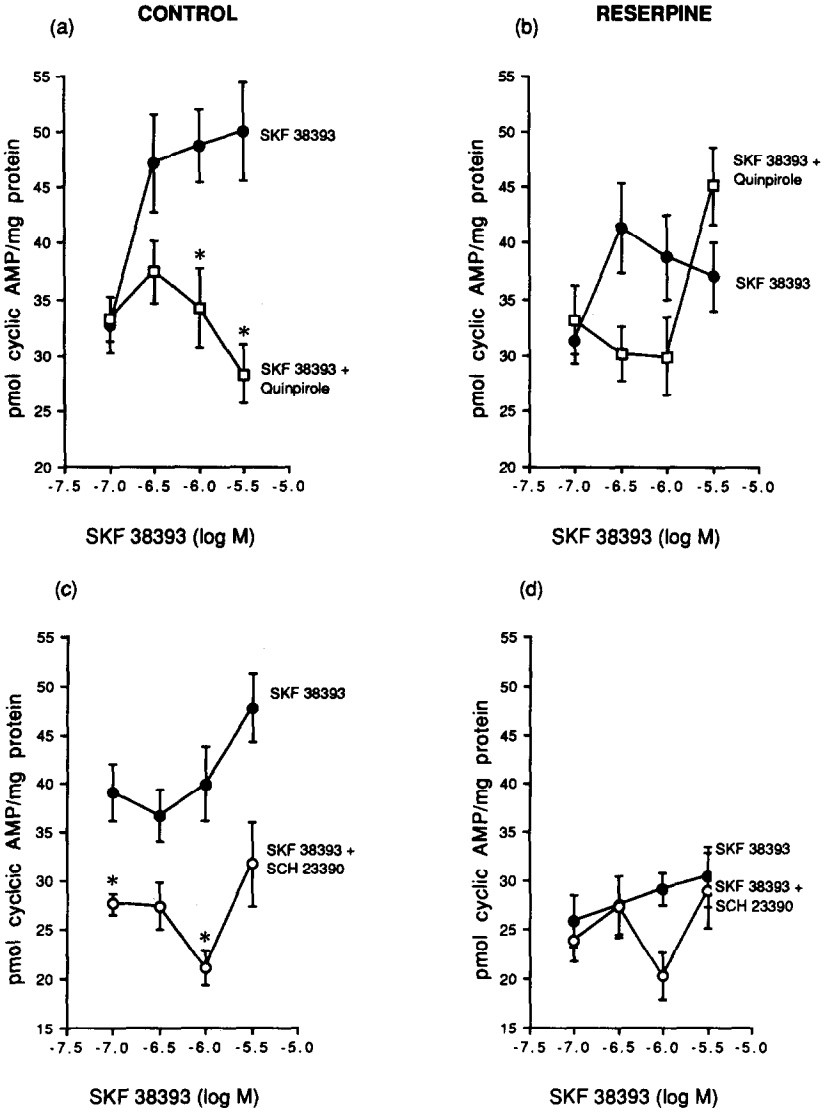

Fig. 2. The intracellular accumulation of cyclic AMP in rat striatal slices elicited by SKF 38393 (●) and the effect of quinpirole (□) and SCH 23390 (○) on the SKF 38393-induced response in control animals (panels a and c) and in rats treated with reserpine (5 mg/kg i.p.) 24 hr previously (panels b and d). Following a 90 min preincubation in Krebs buffer containing 1 mM IBMX, slices were incubated in the presence and absence of SCH 23390 (10 μM) for 5 min and then with SKF 38393 (0.1–3.2 μM) or SKF 38393 plus quinpirole (10 μM) for a further 10 min. The results are the means ± SEM of three individual experiments with N = 3–4 samples for each SKF 38393 concentration. * $P < 0.05$ (Dunn's test) when compared to the same concentration of SKF 38393 in the absence of SCH 23390 or quinpirole.

striatal slices from reserpine-treated rats ($F = 1.73_{3,41}$ $P > 0.05$; Fig. 2b). However, there was a trend to increase cyclic AMP levels at the highest concentrations of SKF 38393 examined in slices from reserpine-treated animals. Quinpirole (10 μM) abolished the SKF 38393-induced increase in cyclic AMP accumulation in slices from control animals ($F = 21.28_{1,70}$ $P < 0.05$; Fig. 2a) but had no effect on the SKF 38393-induced changes in slices from reserpine-pretreated animals ($F = 1.24_{1,78}$ $P > 0.05$; Fig. 2b). Similarly, SCH 23390 (10 μM) completely attenuated the accumulation of cyclic AMP produced by SKF 38393 (0.1–3.2 μM) in control striatal slices ($F = 42.78_{1,81}$ $P < 0.05$; Fig. 2c) but had no effect in slices from rats pretreated with reserpine 24 hr previously ($F = 2.451_{1,83}$ $P > 0.05$; Fig. 2d).

DISCUSSION

The primary action of reserpine is to prevent the normal intraneuronal storage of catecholamines [29, 30]. Indeed, treatment with reserpine reduced striatal DA levels 24 hr after systemic administration. DA synthesis and degradation was largely unaffected

as shown by the levels of the metabolites DOPAC and HVA and there was an increase in DA turnover as shown by the increase in DOPAC/DA and HVA/DA ratios in agreement with earlier findings [31].

There was an increase in the density of D-1 receptors in the striatum, with no change in binding affinity, 24 hr after reserpine pretreatment, as judged by [^{3}H]SCH 23390 binding. No alteration was observed in either the density or the affinity of striatal D-2 receptors identified by [^{3}H]spiperone. The increase in D-1 receptor numbers may reflect an adaptative response to a reduction in striatal DA tone 24 hr after reserpine treatment. These results are in agreement with those of Chipkin *et al.* [19] who found an increased density of D-1 but not D-2 receptors 24 hr after 30 mg/kg (i.p.) reserpine treatment. However, the density of striatal D-1 receptors was unaltered after subchronic (5 days) administration of a low dose (1 mg/kg, i.p.) of reserpine [24] and following longer (30 days) treatment with reserpine increases in both D-1 [32] and D-2 [33] receptors were observed. Apparently, D-1 receptors are acutely more susceptible to upregulation than D-2 receptors following striatal DA depletion with reserpine.

In striatal slices from vehicle-treated animals DA and SKF 38393 induced a concentration-dependent increase in cyclic AMP accumulation. This appears due to a direct action on D-1 receptors since the D-1 antagonist SCH 23390 inhibited both the DA- and SKF 38393-initiated responses. In control animals the D-2 receptor was also able to modulate cyclic AMP accumulation in striatal slices. Thus, (±)-sulpiride potentiated the DA-induced response, and stimulation of the D-2 receptor by quinpirole attenuated the accumulation of cyclic AMP induced by SKF 38393. Thus, these results are in agreement with published reports [2, 34], showing that the D-2 receptor is negatively coupled to striatal AC.

In striatal slices prepared from reserpine treated rats, DA and SKF 38393 failed to consistently stimulate significant increases in the accumulation of cyclic AMP. There was, however, a trend for increasing concentrations of DA and SKF 38393 to stimulate cyclic AMP accumulation. The attenuation of the DA- and SKF 38393-mediated response after reserpine treatment may be a consequence of two factors. First, there may be a decrease in the coupling of the D-1 receptor via the stimulatory G protein (Gs) to AC. A decrease in the coupling of the D-1 receptor to AC may be envisaged as a reduction in the functional or active receptor reserve after reserpine pretreatment. As such, the reduced maximal response to D-1 receptor activation may be indicative of a decrease in AC-linked receptors, since a population of non AC-linked D-1 receptors has been identified in the striatum [35, 36]. Paradoxically, the density of D-1 receptors specifically labelled with [^{3}H]SCH 23390 was increased following reserpine pretreatment. This may reflect a compensatory response resulting in a selective upregulation of non-functional or non-cyclase-linked D-1 receptors. Second, reserpine pretreatment may initiate an increase in the agonist-induced coupling of the D-2 receptor via the inhibitory G protein (Gi) to AC. Thus, incubation with a D-2 antagonist would be expected to inhibit the action of DA at the D-2 receptor and cyclic AMP accumulation would be stimulated due to the selective action of DA at the D-1 receptor. However, (±)-sulpiride was unable to "release" cyclic AMP production under these conditions. Moreover, SKF 38393 which possesses essentially no D-2 receptor activity, did not increase cyclic AMP levels above basal levels in reserpine pretreated striatal slices. Therefore, it seems unlikely that the decline in responsiveness of AC to selective D-1 stimulation is due to an increased interaction of the inhibitory D-2 receptor to Gi.

Recent reports have described a molecular diversity within the D-1 and D-2 classes of DA receptors. Cloning studies have shown that in addition to D-1 receptors [37–39], a D-1 like receptor, D-5 [40], exists which shares a similar pharmacology and brain distribution to the D-1 receptor, but is distinguished by its 10-fold higher affinity for DA. A third receptor in this family has been cloned [41]. This receptor, D-1B, has a fractionally higher affinity for SCH 23390 than the D-1 receptor and the increase in the density of striatal [^{3}H]SCH 23390 binding observed in the present investigation may reflect a preferential interaction of the antagonist with the D-1B receptor following acute reserpine treatment. However, the expression of D-1B mRNA is very low in the striatum. It could be postulated that the down-regulation of D-1-associated AC activity seen after reserpine may be due to an alteration in the association of D-1 agonists from D-1 to D-1B/D-5 receptors. This hypothesis is not consistent with the evidence to date that all classes of D-1-like receptors are closely linked to the activation of AC, at least in the cell lines in which the cloned receptors have been transfected.

The decrease in the responsiveness of AC stimulation, mediated by the D-1 receptor in striatal slices following reserpine pretreatment may be similar to the desensitization of D-1-coupled AC activity in striatal homogenates following acute amphetamine administration [42, 43]. Amphetamine acts as an indirect DAergic agonist by enhancing endogenous DA release in the striatum [44] which in turn stimulates postsynaptic DA receptors in this brain region [45]. Reserpine treatment decreases DA storage but increases transmitter synthesis and release [46] which may therefore be responsible for the, apparent desensitization of D-1 receptors observed 24 hr after reserpine treatment. Furthermore, prolonged incubation of striatal slices with DA, apomorphine and SKF 38393 has been shown to induce subsensitivity of AC [47]. Desensitization can be homologous involving a single receptor subtype or heterologous involving all stimulatory types present on the cell [48]. Homologous and heterologous desensitization of the β-adreno-receptor-associated AC involves the phosphorylation and subcellular redistribution of one or more components of the AC macromolecular complex, typically sequestration of the receptor itself [49]. Indeed, persistent stimulation of DA sensitive AC in striatal slices results in the phosphorylation of specific membrane components [50].

The reduced sensitivity of DA-associated AC

following reserpine treatment reported here conflicts with earlier reports by other workers. Thus, increased AC activity was reported following both acute [22, 23] and subchronic [24, 51] reserpine pretreatment. This discrepancy is unlikely to be due to the dose of reserpine employed or the time interval between reserpine administration and assay. In the acute studies the dose of reserpine used was of the same order of magnitude as that used in the current study and produced a similar depletion in striatal DA [22]. The interval between reserpine pretreatment and AC assay was approximately the same in our study and other acute experiments. In fact, Vasse and Protais [23] showed that AC supersensitivity became apparent at 4 hr and was maximal 18 hr following reserpine administration. However, the major difference between the present study and other reports is that this investigation measured the intracellular cyclic AMP accumulation in striatal slice preparations whilst previous investigations measured AC activity in striatal homogenates. This may be important if reserpine-induced desensitization involves the internalization of cyclase linked D-1 receptors.

It has been suggested by others that the increased responsiveness of AC to DA stimulation was responsible for the enhanced behavioural response to D-1 and D-2 agonists following reserpine treatment. The time courses for the potentiation of apomorphine-induced stereotypy and SKF 38393-induced AC activity was highly correlated following reserpine administration [23]. However, since our results show a subsensitivity of DA-associated AC to agonists after reserpine pretreatment such a premise seems unlikely. Recent studies using the irreversible receptor alkylating agent *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline to inactivate DA receptors [52], showed that the reduction in apomorphine-induced sniffing in normal rats paralleled the reduction in striatal D-2 receptors but not D-1 receptors [53] or AC activity [54]. Thus, in normal rats apomorphine-induced behaviour is mediated through non-cyclase-linked D-2 receptors and this may also hold in DA depleted animals.

As previously discussed, a "behavioural" uncoupling of D-1 and D-2 receptors has been demonstrated in rodents 24 hr after acute and subchronic systemic reserpine administration [18–21]. The time-dependent shift in the role of the D-1 receptor from that of modifying D-2 receptor-mediated behaviours observed in normal animals to one of independent behavioural expression indicates an alteration in the functional coupling of the D-1 to AC after reserpine pretreatment. This functional change may reflect uncoupling of the D-1 receptor from AC as observed in this study. The behavioural response to SKF 38393 24 hr after acute reserpine administration may be a consequence of the agonist acting at non-cyclase-linked D-1 receptors. In addition to the down regulation of D-1-associated AC activity the results of this study also indicate that the D-2 receptor may become uncoupled from AC after acute reserpine treatment in rats since (±)-sulpiride no longer potentiated the accumulation of cyclic AMP elicited by DA and quinpirole did not inhibit the response to SKF 38393 as observed in striatal slices from vehicle-treated animals. The conclusion that the D-2 receptor becomes uncoupled from AC following reserpinization should be treated with caution since DA and SKF 38393 failed to elicit a significant increase in cyclic AMP accumulation in striatal slices from reserpine-treated rats. Indeed it is known that D-1 receptor stimulation is a prerequisite for the expression of D-2-mediated inhibition of AC in the striata of normal rats. However, a decrease in the coupling between the D-2 receptor and AC may contribute to the alteration in the behavioural responses to D-1 and D-2 agonists after reserpine treatment.

In conclusion, the results demonstrate that 24 hr after acute reserpine pretreatment there was a reduction in the ability of a D-1 receptor agonist to stimulate cyclic AMP production despite an increase in D-1 receptor numbers. This may result from an uncoupling of the D-1 receptor from its effector system. The biochemical uncoupling observed following acute reserpine treatment may be related to the loss of the permissive role of the D-1 receptor on D-2 receptor-mediated behaviours observed in normal rats.

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