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STIMULATION OF ADENYLATE CYCLASE ACTIVITY BY BENZAZEPINE D-1 DOPAMINE AGONISTS WITH VARYING EFFICACIES IN THE 6-HYDROXYDOPAMINE LESIONED RAT—RELATIONSHIP TO CIRCLING BEHAVIOUR

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Abstract—The ability of benzazepine D-1 dopamine agonists with varying efficacies in stimulating adenylate cyclase and to induce contralateral circling was investigated in rats with unilateral 6-hydroxydopamine lesions of the medial forebrain bundle. In the 6-hydroxydopamine lesioned rats, the benzazepines SKF 38393 (7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine), SKF 75670 (3-CH_3 analogue), SKF 80723 (6-Br analogue), SKF 83959 (6-Cl, 3-CH_3, 3′-CH_3 analogue), SKF 83565 (6-Cl, 3-CH_3, 3′-Cl analogue) and SKF 82958 (6-Cl, 3-C_3H_5 analogue), all produced contralateral circling. The rank order of efficacies (maximal effect, E_{max}) being, SKF 83565 = SKF 75670 = SKF 83959 = SKF 80723 > SKF 38393 ≫ SKF 82958. In striatal slices from the intact hemisphere, dopamine, SKF 82958, SKF 80723 and SKF 75670 stimulated adenylate cyclase activity. The rank order of efficacies being SKF 82958 (109%) = dopamine (100%) = SKF 80723 (98%) > SKF 75670 (72%). Although, SKF 38393 (67%), SKF 83565 (64%) and SKF 83959 (59%) tended to stimulate adenylate cyclase activity, this effect did not reach statistical significance. In the 6-hydroxydopamine lesioned hemisphere, basal levels of adenylate cyclase activity were lower (−25%) than in the intact hemisphere. The maximal stimulation of adenylate cyclase activity (expressed as % basal levels) produced by dopamine and the benzazepines in the denervated striatum was greater than observed in the intact striatum. The rank order of efficacies in the dopamine denervated striatum being SKF 82958 (124%) > SKF 80723 (109%) = dopamine (100%) > SKF 38393 (82%) = SKF 83959 (77%) = SKF 83565 (70%) > SKF 75670 (55%). Moreover, dopamine stimulated adenylate cyclase activity in the denervated striatum with greater potency than in the intact side. The ability of the benzazepine derivatives to induce circling in the 6-hydroxydopamine lesioned rat is consistent with the general increase in the efficacies of dopamine and benzazepine stimulated adenylate cyclase activity in the dopamine denervated striatum. However, the maximal effects for inducing circling and stimulating adenylate cyclase activity do not correspond (e.g. SKF 82958 and SKF 75670). This discrepancy may reflect the involvement of other factors including a behavioural role for extrastriatal D-1 dopamine receptors and/or transduction systems other than adenylate cyclase.

Key words: adenylate cyclase; D-1 dopamine agonists; benzazepines; rat; 6-hydroxydopamine; behaviour

In the mammalian brain, DA‖ transmission is mediated primarily via the D-1 and D-2 DA receptors, distinguished by their ability to stimulate and inhibit, respectively, the enzyme AC [1, 2]. Although, recent studies have identified other DA receptor subtypes, based on their similarity in structure and function (ability to stimulate AC activity), these may be classified into "D-1 like" (D-1 and D-5 subtypes) and "D-2-like" (D-2, D-3 and D-4 subtypes) DA receptor families [3].

In rats with unilateral 6-OHDA lesions of the nigrostriatal tract, both selective D-1 and D-2 DA agonists induce contralateral circling [4–7]. The behavioural effects of D-2 DA agonists appears to be consistent with the widely reported upregulation in striatal D-2 DA receptor density [8–10]. However, the biochemical correlates for the behavioural effects of D-1 DA agonists in this model are less well established. Indeed, studies investigating biochemical changes in relation to the D-1 DA receptor have reported conflicting findings. Thus, increases, decreases and no changes in striatal D-1 DA receptor density have been observed following 6-OHDA treatment [8, 9, 11–16]. The effect of DA denervation on DA stimulated AC activity is equally uncertain, with reports of increased and unchanged

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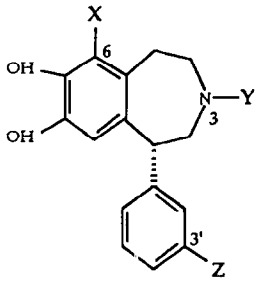
‖ Abbreviations: DA, dopamine; AC, adenylate cyclase; 6-OHDA, 6-hydroxydopamine; SKF 38393, 7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine; SKF 75670, 3-CH_3 analogue; SKF 80723, 6-Br-analogue; SKF 83959, 6-Cl, 3-CH_3, 3′-CH_3 analogue; SKF 83565, 6-Cl, 3-CH_3, 3′-Cl analogue; SKF 82958, 6-Cl, 3-C_3H_5 analogue; IBMX, 3-isobutyl-1-methylxanthine.



Benzazepine	6-X	3-Y	3'-Z
SKF 38393	-H	-H	-H
SKF 80723	-Br	-H	-H
SKF 83565	-Cl	$-CH_3$	-Cl
SKF 83959	-Cl	$-CH_3$	$-CH_3$
SKF 82958	-Cl	$-C_3H_5$	-H
SKF 75670	-H	$-CH_3$	-H

Fig. 1. The molecular structures of the benzazepine derivatives.

striatal AC activity in the 6-OHDA lesioned rat [16–23]. While the reason(s) for this discrepancy is not clear, differences in the methodology involved, including the assay conditions, the use of striatal homogenates versus striatal slices and the extent of denervation of other catecholaminergic neurones, notably noradrenergic, can influence AC activity [17, 23–25].

Analogues of the archetypal benzazepine D-1 DA agonist, SKF 38393 (SKF 81297, SKF 80723, SKF 75670 and SKF 83959, Fig. 1) are all able to induce contralateral circling in the 6-OHDA lesioned rat, with similar maximal effects [4, 26, 27]. Interestingly, in the intact striatum, these benzazepine derivatives stimulate AC activity with full/supramaximal (SKF 81297, SKF 80723 and SKF 82958), partial (SKF 38393 and SKF 75670) and no intrinsic efficacies (SKF 83959) [4, 26–28]. The behavioural effects of the partial efficacy/inefficacious D-1 DA agonists may reflect the high D-1 DA receptor reserve of the rodent brain and/or following DA denervation, an increase in the ability of these benzazepine derivatives to stimulate AC activity [18, 29]. However, with the exception of SKF 38393, the ability of benzazepine D-1 DA agonists to stimulate AC activity in the DA denervated striatum has not been studied. Stimulation of AC by SKF 38393 itself is unaltered in the 6-OHDA lesioned rat [16]. An alternative explanation for the behavioural effects of partial efficacy D-1 DA agonists may be the involvement of transduction systems other than AC. Indeed, in the intact rodent there is increasing biochemical and behavioural evidence for the existence and a behavioural role for D-1 DA receptors not linked to AC [4, 30–33].

In this study, we report the ability of supramaximal/full (SKF 80723 and SKF 82958), partial efficacy (SKF 75670, SKF 38393 and SKF 83565) and inefficacious (SKF 83959) benzazepine D-1 DA agonists to stimulate AC activity in the 6-OHDA lesioned rat. The AC efficacies of the benzazepines are compared to their ability to induce rotation in this rodent model.

MATERIALS AND METHODS

6-Hydroxydopamine lesions in rats. Male Wistar rats weighing 250–350 g were used. Animals were housed in groups of six with free access to food and water, under a 12 hr light/dark cycle. Prior to surgery, the rats were injected with 15 mg/kg (i.p.) desipramine hydrochloride (noradrenaline uptake blocker) and 5 mg/kg pargyline hydrochloride (monoamine oxidase inhibitor). Thirty minutes later and under sodium pentobarbitone (60 mg/kg; i.p.) anaesthesia, the animals were positioned in a Kopf stereotaxic frame with the incisor bar set at 4.5–5.2 mm below the level of the interaural line. A stainless steel cannula (30 gauge), connected to a Hamilton syringe (10 μL) was implanted 1.2 mm posterior to bregma, 9 mm ventral to the skull surface and 1.5 mm lateral to the midline [34]. Unilateral intracranial infusions of 6-OHDA hydrobromide (2 mg/mL in 0.9% saline with 1 mg/mL ascorbic acid as an antioxidant) was made into the right medial forebrain bundle (MFB) (total volume, 4.0 μL at a rate of 0.5 μL/min). Following delivery, the needle was left in place for a further 4–5 min and then withdrawn. The wound was sutured and the animals were treated with ampicillin trihydrate (50 mg/kg as a suspension in 0.9% saline; s.c.).

Behavioural studies in 6-OHDA lesioned rats. Two weeks after surgery, the animals were placed in automated rotometer cages (25 × 23 × 38 cm). Following an acclimatization period of at least 20 min, the extent of the 6-OHDA lesion was evaluated by measuring the circling response to apomorphine hydrochloride (0.5 mg/kg in 0.9% sterile saline with 1 mg/kg ascorbic acid as an antioxidant; i.p.). Rats demonstrating a peak circling rate of 20 or more turns per 5 min were used in behavioural studies and for AC assays. In subsequent studies these animals showed > 95% loss of striatal DA uptake sites, with near total neuronal depletion in the substantia nigra pars compacta (Gnanalingham, unpublished data).

Three weeks post-lesion, the behavioural studies were commenced with various selective D-1 DA agonists: 0.25–8 mg/kg, SKF 38393; 0.09–3 mg/kg, SKF 80723; 0.02–4 mg/kg, SKF 83565; 0.02–2 mg/kg, SKF 83959; 0.125–4 mg/kg, SKF 82958 and 0.015–2 mg/kg, SKF 75670 (dissolved in 1–5% dimethyl sulphoxide and 0.9% saline vehicle, except SKF 82958 which was dissolved in 5% dimethyl sulphoxide and deionized water) [4]. The rotometer

was programmed to count the number of contralateral turns made every 5 min, for a period of 2.5 hr. At least 4 days separated successive drug challenges which were allocated in a random fashion and each animal was used to study one drug only.

cAMP assay—prelabelling with [^{3}H]adenine. The [^{3}H]adenine prelabelling method of Shimizu and co-workers [35], with minor modifications was used to pulse label the ATP in the striatal slices. Approximately 3–4 weeks post-lesion with 6-OHDA, the rats were killed by cervical dislocation and the striata were dissected out and placed in ice-cold Krebs buffer containing NaCl (118 mM), KCl (4.7 mM), $CaCl_2$ (1.3 mM), KH_2PO_4 (1.2 mM), $NaHCO_3$ (25 mM), glucose (11.7 mM), $MgSO_4$ (1.2 mM) and IBMX (a phosphodiesterase inhibitor; 1 mM) and saturated with 95% O_2 and 5% CO_2.

Cross-chopped striatal slices (0.26 × 0.26 mm) were incubated in 20 mL of fresh Krebs buffer in a shaking water bath at 37° for 60 min, with replacement of buffer every 20 min. The incubation mixture was centrifuged (700 *g* for 1 min) and the supernatant discarded. Fresh Krebs buffer, containing 5 μCi/mL of [^{3}H]adenine (4 mL/animal), was added to the striatal slices. The centrifuge tube was gassed with 95% O_2/5% CO_2, capped tight and then placed in a shaking water bath at 37° for 40 min. At the end of incubation, the striatal slices were washed in 3 × 20 mL of Krebs buffer and resuspended in fresh buffer.

Procedure for incubation of striatal slices. Gravity packed striatal slices (20 μL) were preincubated for 5 min in Krebs buffer containing the D-2 DA receptor antagonist sulpiride (50 μM, to block the inhibition of AC activity via the D-2 DA receptor) in a shaking water bath (37°). In some studies, the striatal slices were also preincubated with the D-1 DA receptor antagonist SCH 23390 (10 μM; 5 min). DA (in 2.5 mM HCl and distilled water), SKF 82958, SKF 80723, SKF 75670, SKF 38393, SKF 83565 or SKF 83959 (dissolved in 1–5% dimethyl sulphoxide and distilled water) were added and incubations were continued for a further 10 min. The reaction was terminated by the addition of HCl (9 M; 20 μL) to each sample which were then placed on ice for at least 20 min.

The striatal slices were homogenized by ultrasonication and the samples were centrifuged (13,000 *g*; 4 min). Three 20 μL samples of the supernatant were taken. Scintillation fluid (2 mL, Optiphase HiSafe-3) was added to these samples and the total radioactivity incorporated in each assay tube was determined by scintillation spectroscopy (efficiency 40–45%). The incubation mixture was neutralized with concentrated NaOH (8 M; 25 μL). One hundred microlitres each of 3 N barium hydroxide and zinc sulphate were added to each assay tube, to precipitate proteins and non-cyclic nucleotides such as ATP, AMP, ADP and adenine, but not cyclic nucleotides such as cAMP [36]. The samples were left on ice for a further 10 min and were then centrifuged at 13,000 *g* for 5 min. The supernatant was removed and stored at −20°.

Measurement of cAMP by HPLC and liquid scintillation analysis. Separation of cAMP from other tritium labelled nucleotides in the supernatants was achieved via semi-automated HPLC analysis [37]. Supernatant samples (100 μL) were injected into an HPLC system equipped with a C18 ODS2 Ultrasphere 5 μM column (4.6 × 15 mm; Spherisorb, U.K.) and a mobile phase consisting of 0.05 M sodium acetate at pH 4.75 and 10% methanol (flow rate, 2 mL/min). Fractions were collected every 15 sec in scintillation vials and following the addition of 2 mL of scintillation fluid (Optiphase Hisafe-3), the tritium content of the vials were quantified by scintillation spectroscopy (efficiency of 35–45%).

AC activity is expressed as the % conversion of total [^{3}H]adenine to [^{3}H]cAMP in each sample, which correlates with the absolute levels of [^{3}H]-cAMP (unpublished observations [35]). The absolute values for the basal levels and maximal DA stimulated AC activity in striatal slices (from untreated animals) ranged from 0.3 to 0.8 pmol/mg protein and 1.0 to 2.3 pmol/mg protein, respectively.

Statistics. Stimulation of AC activity by DA and the benzazepine derivatives in both the intact and DA denervated striatum was analysed by ANOVA (two-way) and *post hoc* Duncan's multiple range test. The E_{max} and EC_{50} values for AC stimulation in the intact and DA denervated striatum were compared by Student's *t*-test (paired and two-tail). The effect of SCH 23390 pretreatment on DA and benzazepine derivatives stimulated AC activity was analysed by ANOVA (one-way) and *post hoc* Duncan's multiple range test.

Materials. Materials used were supplied by the following companies: [^{3}H]adenine (specific activity, 25–30 Ci/mmole) from Amersham U.K.; desipramine hydrochloride, apomorphine hydrochloride, pargyline hydrochloride, 6-OHDA hydrobromide and IBMX from Sigma, U.K.; SKF 38393 hydrochloride from Research Biochemicals; SKF 80723, SKF 83959, SKF 82958, SKF 83565 (all hydrobromide salts) and SKF 75670 hydrochloride were prepared

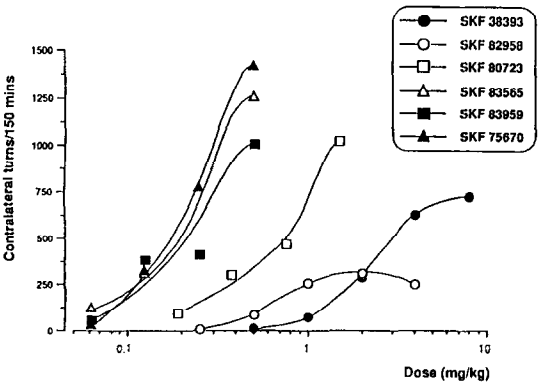


Fig. 2. The dose–response graphs for the contralateral circling induced by benzazepine derivatives in 6-OHDA lesioned rates. SKF 80723, SKF 82958, SKF 75670, SKF 38393, SKF 83565 and SKF 83959 were administered intraperitoneally at doses ranging from 0.015 up to 8 mg/kg (N = 3–6). Cumulative contralateral turns per 150 min are shown. The standard error bars (10–20% of mean values) have been omitted for clarity.

Table 1. Contralateral circling induced by benzazepine derivatives in 6-OHDA lesioned rats

Benzazepine derivatives	E_{max} (turns/150 min)	ED_{50} (mg/kg)
SKF 82958	346 ± 36	0.82 ± 0.26
SKF 80723	1042 ± 318	0.67 ± 0.15
SKF 75670	1194 ± 383	0.24 ± 0.06
SKF 38393	800 ± 229	2.33 ± 0.60
SKF 83565	1359 ± 228	0.27 ± 0.06
SKF 83959	1159 ± 169	0.26 ± 0.05

SKF 80723, SKF 82958, SKF 75670, SKF 38393, SKF 83565 and SKF 83959 were administered (i.p.) at doses ranging from 0.015 up to 8 mg/kg (N = 3–6). The maximal effect (E_{max}) is expressed as the highest cumulative contralateral turns (per 150 min) observed for each D-1 DA agonist. The ED_{50} values were calculated from the dose–response curves shown in Fig. 2.

by Dr D.D. Erol at the Department of Pharmacy, King's College (London, U.K.). The following drugs were gifts from the respective sources: sulpiride from SESIF; SCH 23390 hemimaleate from Schering, U.S.A. All other chemicals were obtained from standard commercial sources.

RESULTS

Behavioural effects in the 6-OHDA lesioned rats

All benzazepine derivatives induced contralateral turning in the 6-OHDA lesioned rat (Fig. 2). The rank order of maximal effects (E_{max}) was SKF 83565 = SKF 75670 = SKF 83959 = SKF 80723 > SKF 38393 ≫ SKF 82958 (Table 1; Fig. 2). The rank order behavioural potencies (ED_{50} values) to induce contralateral circling was SKF 75670 = SKF 83565 = SKF 83959 > SKF 80723 = SKF 82958 > SKF 38393 (Table 1; Fig. 2).

The contralateral circling induced by the benzazepines was almost immediate in onset (within 5 min), with the exception of SKF 38393 for which there was a 10–20 min delay. Moreover, at doses producing maximal effects, contralateral circling was observed throughout the 2.5 hr observation period for SKF 80723, SKF 75670, SKF 38393, SKF 83565 and SKF 83959. In contrast, even at high doses (2–4 mg/kg) the circling response to SKF 82958 lasted only for approximately 1–2 hr. At high doses of the benzazepine derivatives, oral stereotypes (including licking and gnawing) became more apparent and contralateral circling was reduced.

Effects of DA and benzazepine D-1 DA agonists on AC activity

In striatal slices from the intact hemisphere, DA (10–100 μM; $P < 0.01$), SKF 82958 (10 and 100 μM; $P < 0.05$), SKF 80723 (1 and 10 μM; $P < 0.05$) and SKF 75670 (1000 μM; $P < 0.05$) stimulated AC activity in a concentration-dependent manner (Figs 3 and 4). The rank order of maximal AC stimulation (E_{max} was SKF 82958 (109%) = DA (100%) = SKF 80723 (98%) > SKF 75670 (72%) (Table 2). Although SKF 38393 (67%), SKF 83565 (64%) and SKF 83959 (59%) also appeared to stimulate AC activity, these effects were not significant (Fig. 4).

In striatal slices from the DA denervated hemisphere, the basal level of AC activity was reduced in comparison to the intact side (basal levels being 0.31 ± 0.02% and 0.41 ± 0.05% in the denervated and intact hemispheres, respectively; $P < 0.01$; N = 8).

DA (1–100 μM; $P < 0.01$), SKF 82958 (10–1000 μM; $P < 0.01$), SKF 80723 (1–1000 μM; $P < 0.01$), SKF 75670 (10–1000 μM; $P < 0.01$), SKF 38393 (10–1000 μM; $P < 0.01$), SKF 83565 (1–1000 μM; $P < 0.05$) and SKF 83959 (0.1, 10 and 1000 μM; $P < 0.05$) all stimulated AC activity in the DA denervated striatum (Figs 3 and 4). The rank order of maximal AC stimulation (i.e. E_{max}) was, SKF 82958 (124%) > SKF 80723 (109%) = DA (100%) > SKF 38393 (82%) = SKF 83959 (77%) = SKF 83565 (70%) > SKF 75670 (55%) (Table 2). Moreover, the stimulation of AC activity in the denervated striatum was greater than that in the intact striatal slices for DA (1–1000 μM; $P < 0.05$), SKF 82958 (100 and 1000 μM; $P < 0.01$), SKF 80723 (10 and 100 μM; $P < 0.01$), SKF 38393 (10 and 100 μM; $P < 0.05$), SKF 83565 (1000 μM; $P < 0.01$) and SKF 83959 (10 μM; $P < 0.01$) (Figs 3 and 4). Correspondingly, the maximal AC stimulation (E_{max}, expressed as % basal levels) in the denervated striatum was greater than that in the intact side for DA (180% of intact side; $P < 0.01$), SKF 82958 (205%; $P < 0.01$), SKF 80723 (200%; $P < 0.01$), SKF 75670 (139%; $P < 0.05$), SKF 38393 (218%; $P < 0.05$), SKF 83565 (198%; $P < 0.05$) and SKF 83959 (236%; $P < 0.05$) (Table 2). DA (but not the benzazepines) was also approximately 10-fold more potent (lower EC_{50} values) in stimulating AC activity in the DA denervated striatum compared to the intact side ($P < 0.05$; Table 2).

Preincubation of the striatal slices with D-1 DA antagonist SCH 23390 (10 μM) inhibited the DA (33 μM; $P < 0.01$), SKF 82958 (100 μM; $P < 0.01$) and SKF 80723 (100 μM; $P < 0.05$) induced increases in AC activity in both the intact and denervated striata. SCH 23390 (10 μM) also inhibited the stimulation of AC activity produced by SKF 75670 (100 μM; $P < 0.05$), SKF 38393 (100 μM; $P < 0.01$), SKF 83565 (100 μM; $P < 0.05$) and SKF 83959 (100 μM; $P < 0.05$) in the DA denervated striatum. In the presence of SCH 23390 (10 μM), DA, SKF 82958, SKF 80723, SKF 75670, SKF 38393, SKF 83565 and SKF 83959 induced AC activity approximated to basal levels of AC activity (data not shown).

DISCUSSION

Although the behavioural effects of D-1 DA agonists in DA denervated rats are well known, the biochemical correlate for this behavioural supersensitivity is less clear [6, 9, 12, 16, 18]. In this study, the relationship between AC stimulation and the induction of contralateral circling produced by a number of benzazepine derivatives was investigated in rats with unilateral 6-OHDA lesions of the nigrostriatal tract.

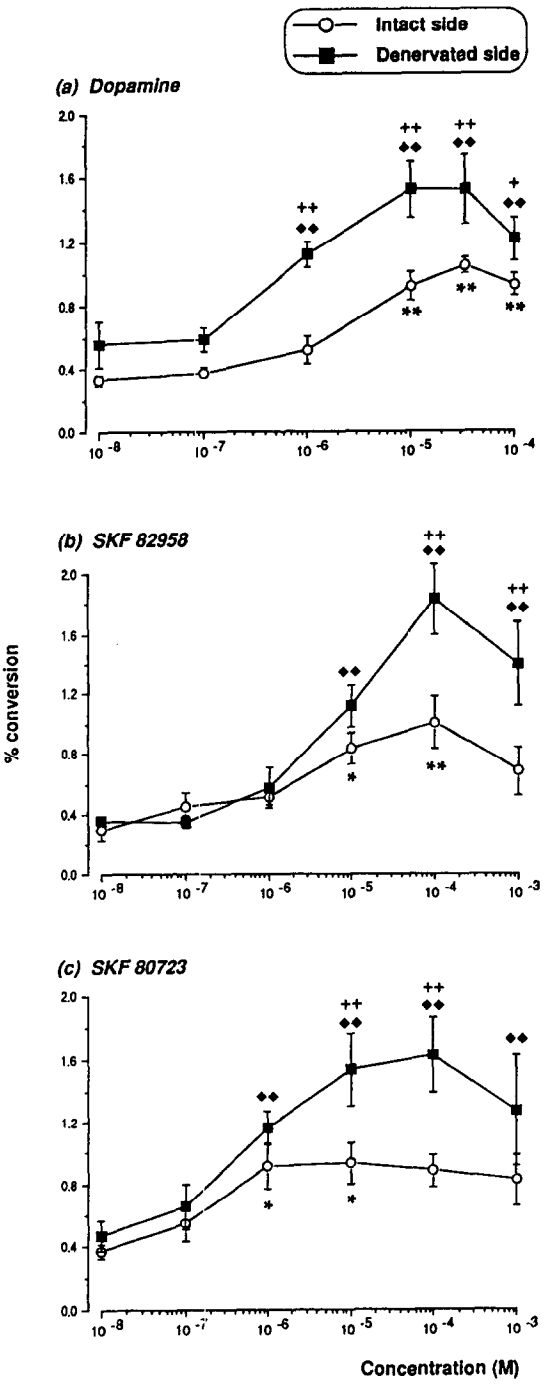


Fig. 3. Stimulation of AC activity by (a) DA, and the benzazepine derivatives (b) SKF 82958, and (c) SKF 80723, in intact and DA denervated striatum of 6-OHDA lesioned rats (N = 4–8). Striatal slices that were preloaded with [^{3}H]-adenine were incubated 10 min with DA, SKF 82958 and SKF 80723 (1×10^{-8} to 1×10^{-3} M). AC activity is expressed as the % conversion of [^{3}H]adenine to [^{3}H]cAMP. The basal AC activity in the intact striatum ranged from 0.39 to 0.42% while that in the DA denervated striatum ranged from 0.32 to 0.34%. *$P < 0.05$; **$P < 0.01$ compared to the basal levels in the intact side; ◆ $P < 0.05$; ◆◆ $P < 0.01$ compared to the basal levels on the DA denervated side; $^+$ $P < 0.05$; $^{++}$ $P < 0.01$ compared to the corresponding AC activity on the intact side. ANOVA (two-way) and *post hoc* Duncan's multiple range test.

Behavioural effects of the benzazepine derivatives in the 6-OHDA lesioned rat

A complete 6-OHDA lesion of the nigrostriatal tract, as assessed by contralateral circling in response to the mixed D-1/D-2 DA agonist apomorphine, is associated with a dramatic loss (> 90%) of DA and dopaminergic nerve terminals in the striatum [38, 39]. In this rodent model, the benzazepine derivatives all induced contralateral circling, the rank order of potencies (EC_{50}) being, SKF 75670 = SKF 83565 = SKF 83959 > SKF 80723 = SKF 82957 > SKF 38393. Thus, 2-*N*-methyl substitutions markedly increase behavioural potency, while 6-bromo and 3-*N*-allyl substitutions are associated with more modest increases in potency. Similarly, there were considerable differences in the behavioural efficacies (E_{max}) of the benzazepine D-1 DA agonists, with SKF 83565, SKF 75670, SKF 83959 and SKF 80723, all demonstrating much higher E_{max} values in comparison to SKF 38393 and more especially SKF 82958. Evidently, 3-*N*-methyl and 6-bromo, but not 3-*N*-allyl substitutions are associated with greater maximal contralateral circling.

DA and benzazepine stimulated AC activity in the 6-OHDA lesioned rat

In the intact striatum, DA and the benzazepine derivatives stimulated AC activity to varying extents, the effect being significant with SKF 82958 (109% relative to DA), SKF 80723 (98%) and SKF 75670 (72%). SKF 38393 (67%), SKF 83565 (64%) and SKF 83959 (59%) were less effective. Similar findings have been made by others utilizing rat striatal homogenate tissue, although in some studies SKF 75670 and SKF 83959 totally failed to stimulate AC activity [4, 26–28, 40]. While there may be differences in the sensitivities of the AC assays used in different laboratories [28], these discrepancies may also reflect the use of striatal homogenates.

Following DA denervation, the maximal stimulation of striatal AC activity by DA (+80% relative to intact side), SKF 82958 (+105%), SKF 80723 (+100%), SKF 75670 (+39%), SKF 38393 (+118%), SKF 83565 (+98%) and SKF 83959 (+136%) was increased with respect to the intact hemisphere. This may indicate an increase in D-1 DA receptor density, levels of AC and/or the Gs protein in the denervated striatum. However, in previous studies by our group and others, we have consistently failed to observe striatal D-1 DA receptor upregulation in the 6-OHDA lesioned rat [8, 11, 12, 16]. Nevertheless, in the normal striatum there exists a population of D-1 DA receptors that are not coupled to AC and which account for 40% of the total D-1 DA receptor density [29]. Thus, in the denervated striatum there may be an increase in the coupling of these "spare" non-cyclase linked D-1 DA receptors to AC enzyme (with a pre-existing excess and/or an increase in levels of AC). If so, it would be expected that in the DA denervated striatum, there may be a decrease in D-1 DA receptor reserve, with respect to the intact striatum. Indeed, this is consistent with the observation that the behavioural effects of D-1 DA agonists in the 6-OHDA lesioned rat are more susceptible to D-1 DA receptor inactivation than is the case in the intact animal [41, 42].

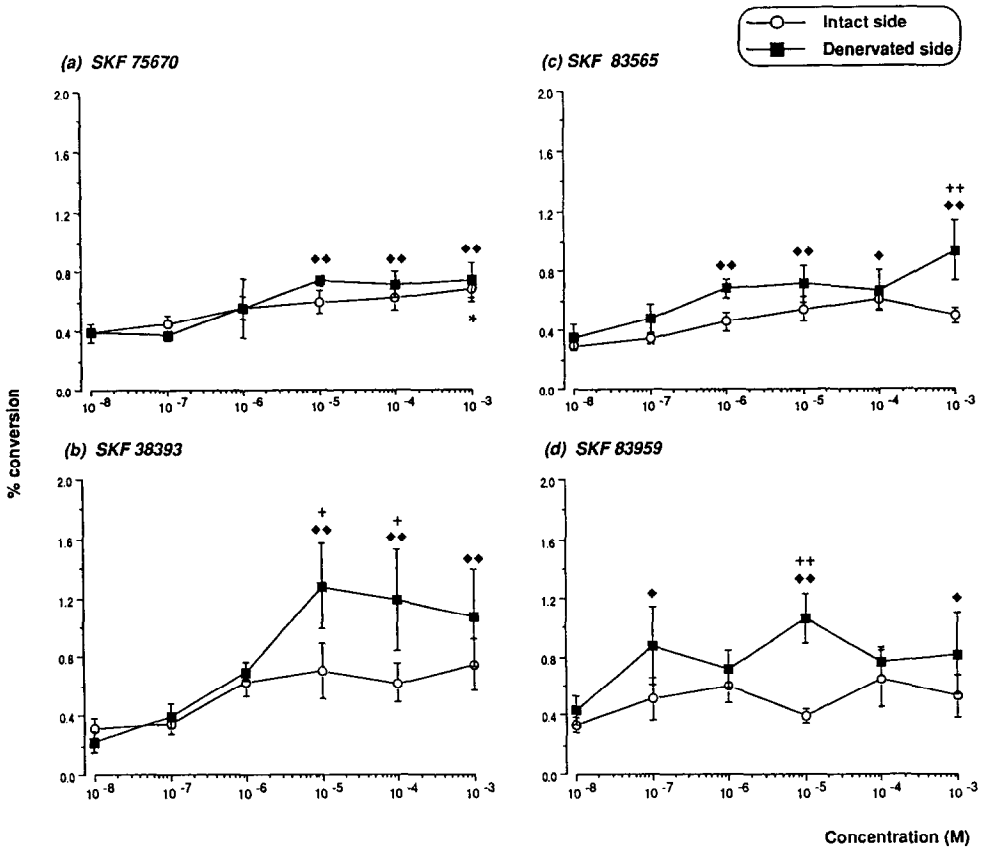


Fig. 4. Stimulation of AC activity by the benzazepine derivatives (a) SKF 75670, (b) SKF 38393, (c) SKF 83565, and (d) SKF 83959 in intact and DA denervated striatum of 6-OHDA lesioned rats (N = 4–8). Striatal slices that were preloaded with [^{3}H]adenine, were incubated for 10 min with the benzazepine derivatives (1×10^{-8}–1×10^{-3} M). AC activity is expressed as the % conversion of [^{3}H]-adenine to [^{3}H]cAMP. The basal AC activity in the intact striatum ranged from 0.39 to 0.46% while that in the DA denervated striatum ranged from 0.31 to 0.36%. * $P < 0.05$ compared to the basal levels in the intact side; ◆ $P < 0.05$; ◆◆ $P < 0.01$ compared to the basal levels on the DA denervated side; $^+$ $P < 0.05$; $^{++}$ $P < 0.01$ compared to the corresponding AC activity on the intact side. ANOVA (two-way) and *post hoc* Duncan's multiple range test.

Table 2. Stimulation of AC activity by DA and benzazepine derivatives in the intact and DA deervated striatum of 6-OHDA lesioned rats

	E_{max} (% basal)				EC_{50} (μM)	
Agonists	Intact side	(% effect of DA)	Denervated side	(% effect of DA)	Intact side	Denervated side
DA	278 ± 18	(100	499 ± 36**	(100)	4.99 ± 1.5	0.53 ± 0.08*
SKF 82958	302 ± 46	(109)	620 ± 74**	(124)	4.00 ± 1.2	5.52 ± 1.5
SKF 80723	271 ± 31	(98)	542 ± 63**	(109)	0.40 ± 0.15	0.31 ± 0.08
SKF 75670	199 ± 21	(72)	276 ± 13*	(55)	2.11 ± 1.49	1.08 ± 0.58
SKF 38393	189 ± 28	(67)	411 ± 28*	(82)	2.54 ± 1.3	0.76 ± 0.27
SKF 83565	176 ± 23	(64)	349 ± 39*	(70)	3.58 ± 1.9	5.15 ± 3.1
SKF 83959	163 ± 14	(59)	384 ± 54*	(77)	1.42 ± 1.0	0.84 ± 0.72

Striatal slices that were preloaded with [^{3}H]adenine were incubated for 10 min with DA, SKF 38393, SKF 80723, SKF 83565, SKF 83959, SKF 82958 and SKF 75670 (1×10^{-8} to 1×10^{-3} M). AC activity is expressed as the % conversion of [^{3}H]adenine to [^{3}H]cAMP. The basal AC activity in the denervated striatum (0.31 ± 0.02) was lower than that in the intact side (0.41 ± 0.05; $P < 0.01$; N = 8). E_{max}, the maximal AC activity is expressed as % basal levels. The EC_{50} values (concentration of agonist producing half maximal response) were calculated from the dose–response curves shown in Figs 3 and 4 (N = 4–8).

* $P < 0.05$; ** $P < 0.01$ compared to inact side; Student's *t*-test (paired).

Moreover, DA was approximately 10-fold more potent in stimulating AC activity in the denervated striatum than in the intact side. While this may indicate a more efficient coupling between D-1 DA receptor occupation and AC enzyme activation, the fact that other full (SKF 82958 and SKF 80723) and partial (SKF 75670, SKF 38393, SKF 83565 and SKF 83959) efficacy benzazepine derivatives did not show similar increases in potency implicates the additional involvement of other mechanisms. The apparent shift to the left in the concentration–response curve for DA may also reflect differences in the re-uptake of DA by DA nerve terminals in the intact and denervated striatal slices [17, 43]. Thus, in the intact striatum re-uptake of DA by nerve terminals may reduce the effective concentration of DA available at the post-synaptic DA receptors. Conversely, in the denervated striatal slices, the destruction of DA nerve terminals may increase the accessibility of exogenously applied DA. In contrast, the benzazepine derivatives, not being substrates for DA uptake sites, are unlikely to be affected in this way.

In the present study, the striatal slices were preincubated with the D-2 DA antagonist sulpiride. Consequently, the increase in efficacy of DA/benzazepine stimulated AC activity and the potency of the DA response in the denervated brain can be assumed to be due to an effect at the D-1 DA receptor and not related to changes in the coupling of D-2 DA receptor to AC in the 6-OHDA lesion rat [16]. Moreover, the stimulation of AC activity by DA and the benzazepine derivatives in both the intact and DA denervated striata was inhibited by the inclusion of the D-1 DA antagonist SCH 23390, further confirming the D-1 DA receptor origin of these effects.

The increase in striatal DA stimulated AC activity following 6-OHDA treatment observed presently, is consistent with the findings of others utilizing both striatal homogenates and slices [18–20, 22, 23]. However, some investigators have also reported no changes in AC activity in the striatal homogenates of 6-OHDA lesioned rats [17, 21]. Indeed, in a previous study by our group, we observed a decrease in the potency and no change in the efficacy of DA in the denervated striatal slices [16].

While the reason(s) for this discrepancy is not entirely clear, it is known that a number of factors, including priming with a D-2 DA agonist, the NaCl content of the incubation buffer and the integrity of the noradrenergic and dopaminergic innervation to the frontal cortex, critically affect AC activity [21, 23–25]. Although, in both the present study and in the study by Thomas *et al.* [16], the 6-OHDA lesioned rats were primed with apomorphine and the assay conditions for the AC assays were similar, in the present study the rats were pretreated with desipramine prior to the intracranial infusion of 6-OHDA. This noradrenaline uptake blocker protects the noradrenergic innervation and also rather surprisingly, the dopaminergic pathway to the frontal cortex and thereby facilitates the development of AC supersensitivity in the DA denervated striatum [24, 25]. This was not the case in the study by Thomas *et al.* [16], in which the rats received no pretreatment prior to the administration of 6-OHDA.

Relationship between the behavioural effects and AC stimulation

Although the general increase in the efficacies of DA and benzazepine stimulated AC activity in the DA denervated striatum appears to be consistent with the ability of the benzazepine derivatives to induce circling in the 6-OHDA lesioned rat, the maximal effects for circling and AC activity do not correspond (see Table 3). Thus, SKF 82958, despite demonstrating the highest efficacy (greater than that of DA) in stimulating AC activity in the denervated striatum, was the least behaviourally efficacious benzazepine D-1 DA agonist. Conversely, the high behavioural efficacy of SKF 75670 fails to relate to its low maximal response to AC activity in the denervated striatum. It is also worth noting that even if the % change in AC activity with respect to the intact side is considered as the measure of AC activity, the discrepancy between behavioural and biochemical efficacies remain.

The behavioural effects of the benzazepines are also dependent upon a number of other factors, including their D-1 DA receptor affinity/selectivity and penetration across the blood–brain barrier. But, perhaps with the exception of SKF 82958, the benzazepine derivatives investigated in this study demonstrate high D-1 DA receptor affinity (low nM range) and selectivity (100-fold or more D-1 DA receptor selective) [4, 44]. SKF 82958 exhibits lower D-1 DA receptor affinity (236 nM) and selectivity (8-fold selective) [4, 32, 44]. Nevertheless, while the low D-1 DA receptor affinity of SKF 82958 may underlie its low behavioural potency, it is unlikely to explain its low behavioural efficacy. Moreover, all benzazepines examined in this study, with the exception of SKF 38393, induce circling within a few minutes of administration. This suggests that these benzazepines readily penetrate across the blood–brain barrier, although measurements of the *in vivo* drug concentrations in the brain, following the systemic administration of the benzazepine D-1 DA agonists would clarify this further. For SKF 38393, there is a delay of approximately 10–20 min, which may indicate poor penetration into the brain and this may explain its low behavioural potency and to some extent behavioural efficacy. In addition, in the 6-OHDA lesioned rat complex interactions between the D-1 DA receptor affinity/selectivity, penetrability across the blood–brain barrier and AC efficacy may underlie the lack of correlation between the behavioural and AC efficacies of the benzazepine derivatives (notably for SKF 82958 and SKF 75670).

Nevertheless, similar discrepancies between AC stimulation and the behavioural effects of benzazepines have been observed in intact and hemitransected rats [4, 30, 32] and more recently in MPTP-treated common marmosets [44]. Such findings increasingly point towards the involvement of multiple D-1 DA receptors and/or transduction systems other than AC in mediating the behavioural effects of D-1 DA agonists. Indeed, recent *in situ* hybridization studies have identified a D-5 DA receptor subtype, with close structural and functional

Table 3. Qualitative comparison of the behavioural and biochemical effects of benzazepine D-1 DA agonists in the 6-OHDA lesioned rat

D-1 DA agonists	Maximal circling (E_{max})	Maximal AC stimulation*	D-1 affinity/selectivity†
SKF 80723	++++	+++	+++
SKF 82958	++	++++	+
SKF 38393	+++	++	+++
SKF 75670	++++	+	+++
SKF 83565	++++	++	+++
SKF 83959	++++	++	+++

* Maximal AC avtivity in the DA denervated striatum (in relation to DA = +++).
† From Refs 1 and 10.
++++, very high; +++, high; ++, moderate; and +, low.

similarities to the existing D-1 subtype, although it also stimulates AC activity [45]. D-1 DA receptor stimulation is also known to affect Ca^{2+} mobilization, inositol phosphate formation, arachidonic acid release and *c-fos* expression [46–49]. However, the behavioural relevance of these transduction mechanisms remains to be established. Furthermore, in the 6-OHDA lesioned rat the D-1 DA receptors of the substantia nigra may play a critical role in the behavioural effects of D-1 DA agonists [50, 51]. Interestingly, the nigral D-1 DA receptors exhibit lower coupling to AC than the striatum [52]. Thus, the evidence to date suggests that the behavioural effects of D-1 DA agonists are mediated via D-1 DA receptors, perhaps situated extrastriatally and linked not only to AC but possibly to other yet undetermined transduction systems.

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