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The alleged dopamine D_1 receptor agonist SKF 83959 is a dopamine D_1 receptor antagonist in primate cells and interacts with other receptors

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Abstract

So far, no clear correlation has been found between the effects of dopamine D_1 receptor agonists on motor behavior in primate models of Parkinson's disease and their ability to stimulate adenylate cyclase in rats, the benzazepine SKF 83959 (3-methyl-6-chloro-7,8-hydroxy-1-[3-methylphenyl]-2,3,4,5-tetrahydro-]H-3-benzazepine) being the most striking example. Since this discrepancy might be attributed to: (A) the different species used to study these effects or (B) the interaction of SKF 83959 with other catecholamine receptors, the aims of this study were: (1) to study the ability of SKF 83959 to stimulate adenylate cyclase in cultured human and monkey glial cells equipped with dopamine D_1 receptors and (2) to evaluate the affinity for and the functional interaction of SKF 83959 with other catecholamine receptors. Binding studies revealed that SKF 83959 displayed the highest affinity for the dopamine D_1 receptor ($pK_i = 6.72$) and the α_2-adrenoceptor ($pK_i = 6.41$) and moderate affinity for the dopamine D_2 receptor and the noradrenaline transporter. In monkey and human cells, SKF 83959 did not stimulate cyclic adenosine monophosphate (cAMP) formation to a significant extent, but antagonized very potently the dopamine-induced stimulation of cAMP formation in both cell types. The compound stimulated basal dopamine outflow and inhibited depolarization-induced acetylcholine release only at concentrations > 10 μM. Finally, SKF 83959 concentration dependently increased electrically evoked noradrenaline release, indicating that it had α_2-adrenoceptor blocking activity and interfered with the noradrenaline transporter. In conclusion, SKF 83959 is a potent dopamine D_1 receptor and α_2-adrenoceptor antagonist. Thus, the anti-parkinsonian effects of SKF 83959 in primates are not mediated by striatal dopamine D_1 receptors coupled to adenylate cyclase in a stimulatory way.

Keywords: SKF 83959; Benzazepine; Dopamine D_1 receptor agonist; Adenylate cyclase; Parkinson's disease

1. Introduction

Dopamine receptors were originally classified on the basis of their coupling to the adenylate cyclase second messenger system, with activation of dopamine D_1-like and D_2-like receptors having a stimulatory and inhibitory effect on cyclic adenosine monophosphate (cAMP) formation, respectively (Kebabian and Calne, 1979; Stoof and Kebabian, 1981). Whereas dopamine D_2 receptor stimulating agents, such as bromocriptine and pergolide, have already found their way into the clinic to relieve motor symptoms in parkinsonian patients, (Parker et al., 1976; Langtry and Clissold, 1990), the consequences of dopamine D_1 receptor stimulation on parkinsonian symptoms are still under investigation, in particular in animal models of Parkinson's disease.

In the primate model of Parkinson's disease, the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated monkey, the behavioral effects of compounds with high affinity for the dopamine D_1 receptor have been evaluated (Kebabian et al., 1990; Gnanalingham et al., 1995a; Johnson et al., 1995; Goulet et al., 1996; Domino, 1997). Indeed, several of these compounds have been reported to partly restore motor behavior in parkinsonian monkeys. Intriguingly, there appears to be no clear relationship between their therapeutic efficacy on the one hand and their ability to stimulate cAMP formation on the other.

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Some of these so-called 'dopamine D_1 receptor agonists' exert anti-parkinsonian effects in monkeys but do not, or only mildly, stimulate the formation of cAMP in vitro (Andersen and Jansen, 1990; Izenwasser and Katz, 1993; Watts et al., 1993). This discrepancy is most clearly manifested by the benzazepine (3-methyl-6-chloro-7,8-hydroxy-1-[3-methylphenyl]-2,3,4,5-tetrahydro-]H-3-benzazepine) SKF 83959 (Deveney and Waddington, 1995; Gnanalingham et al., 1995c). Compared to other benzazepines which do stimulate cAMP formation, SKF 83959 shows high therapeutic efficacy (Gnanalingham et al., 1995a; Andringa et al., 1998) but does not increase cAMP formation in rat striatal tissue (Arnt et al., 1992; Gnanalingham et al., 1995c). These data strongly suggest that SKF 83959 and perhaps other so-called dopamine D_1 receptor agonists do not use dopamine D_1 receptors coupled to cAMP to exert their behavioral effects in monkeys.

However, it cannot be excluded that methodological factors have played a role in the reported lack of correlation between cAMP formation and stimulation of motor behavior. Thus far, the intrinsic activity of most dopamine D_1 receptor agonists, including SKF 83959, on cAMP formation has been studied in rat striatal tissue, whereas the therapeutic efficacy of these compounds has been studied in primate models. Various studies suggest that the intrinsic activity of dopamine agonists differs between human, monkey and rat dopamine D_1 receptors (Pifl et al., 1991; Izenwasser and Katz, 1993; Watts et al., 1993; Vermeulen et al., 1994). For instance, Vermeulen et al. (1994) reported that the ability of the dopamine D_1 receptor agonists SKF 38393 and SKF 81297 to stimulate D_1 receptors differed between human, monkey and rat tissue. Thus, species differences may underlie the discrepancy between the cAMP formation measured in rat tissue and the behavioral responses observed in monkeys. Consequently, it cannot be excluded that SKF 83959 has a higher intrinsic activity at monkey dopamine D_1 receptors than at rat dopamine D_1 receptors. Therefore, the first aim of the present study was to examine the ability of SKF 83959 to stimulate cAMP formation in both monkey and human cells.

Another factor that might contribute to the lack of correlation between cAMP formation and therapeutic efficacy is the fact that most of the currently available dopamine D_1 receptor agonists display high affinity, but only moderate selectivity, for the dopamine D_1 receptor (Murray and Waddington, 1990; DeNinno et al., 1991). Many dopamine D_1 receptor agonists are known to have additional affinities for other catecholamine receptors and/or transporters. For instance, CY 208–235 displays significant affinity for dopamine D_2 and 5-HT receptors, whereas A 68930 has moderate affinity for noradrenaline and 5-HT receptor subtypes and dopamine transporters. Since the affinity for other catecholamine receptors and transporters might contribute to the therapeutic effects of SKF 83959, the second goal of our study was to investigate the affinity of SKF 83959 for these receptors and transporters and, more importantly, the effects of this compound on functional paradigms for these entities.

2. Materials and methods

2.1. Chemicals

SKF 83959 (3-methyl-6-chloro-7,8-hydroxy-1-[3-methylphenyl]-2,3,4,5-tetrahydro-]H-3-benzazepine) was provided by Research Biochemicals International (Natick, USA). Tissue culture media and supplements were obtained from Gibco Netherlands (Breda, The Netherlands). 3-Isobutyl-1-methylxanthine was obtained from Aldrich (Brussels, Belgium). [^{125}I]-iodosulpiride, [^{3}H]adenine, [^{3}H]choline, [^{3}H]dopamine and [^{3}H]noradrenaline were from Amersham (Little Chalfont, UK). [^{3}H]nisoxetine, [^{3}H]prazosin, [^{3}H]rauwolscine, [^{3}H]SCH-23390, [^{3}H]WIN-34528 and [^{125}I]-iodocyanopindolol were obtained from New England Nuclear (Du Pont, Dreieich, Germany). All other chemicals were obtained from Sigma (St. Louis, USA).

2.2. Receptor binding assays

For details about assays and assay conditions, the reader is referred to Schotte et al. (1996) and Leysen et al. (1996). The investigated receptors, tissues or cell systems and radioactive ligands are listed in Table 1. Membrane preparations of the tissues or cells were incubated in a volume of 0.5 ml with a low nanomolar concentration of the radioactive ligand in the absence (for total binding) or presence of SKF-83959 for inhibition of radioactive ligand binding. SKF 83959 was first screened at 0.1, 1 and 10 μM. Where activity was observed, inhibition curves were prepared over the concentration range of 0.01 to 10 μM, using eight concentration points. After incubation, the membranes with bound radioactivity were collected on glass fiber filters by filtration under suction, using semi-automated filtration devices. The radioactivity on the filters was counted in a liquid scintillation or gamma spectrophotometer and counts were directly transferred to a personal computer. Data are expressed as percentages of total binding. Inhibition curves, plotting the percent total binding vs. log concentration of SKF 83595, were generated automatically. The sigmoidal inhibition curves were analyzed by computerized curve-fitting, using nonlinear regression analysis (modification of equations described by Oestreicher and Pinto, 1987). The pIC_{50} values ($-\log IC_{50} = -\log$ of the concentration inhibiting 50% of the specific radioligand binding or neurotransmitter uptake) were derived from individual curves. K_i-values were calculated according to the Cheng–Prusoff equation: $K_i = IC_{50}/[1 + [C]/K_D]$, where K_i is the equilibrium inhibition constant of the investigated compound, $[C]$ the concentration and K_D the equilibrium dissociation constant of

Table 1
Receptor binding profile of SKF 83959

Receptor	Radioactive ligand	Tissue	Binding affinity pK_i
Adrenergic α_1	[^3H]prazosin	Rat total cortex	5.1
Adrenergic α_1A	[^3H]prazosin	cloned human 1a CHO cells	5.63
Adrenergic α_2	[^3H]rauwolscine	Rat cortex 2	6.13
Adrenergic α_2A	[^3H]rauwolscine	Cloned human 2a CHO 1B5 cells	6.08
Adrenergic α_2B	[^3H]rauwolscine	Cloned human 2b CHO 3B3 cells	6.41
Adrenergic α_2C	[^3H]rauwolscine	Cloned human 2c CHO 11A9 cells	6.33
Adrenergic-β_1	[^{125}I]-iodocyanopindolol	Human $\beta_1$cloned CHO cells	< 5
Adrenergic-β_2	[^{125}I]-iodocyanopindolol	Human β_2 cloned CHO cells	< 5
Adrenergic-β_3	[^{125}I]-iodocyanopindolol	Human β_3 cloned CHO cells	< 5
Dopamine D_1	[^3H]SCH23390	Rat striatum TP	6.72
Dopamine D_2	[^3H]spiperone	Cloned human DA D_2l CHO cells	5.2
Dopamine D_3	[^{125}I]-iodosulpiride	Cloned human DA D_3-CHO-Kl cells	5.74
Dopamine-D_4	[^3H]spiperone	Cloned human DA D_4-2 CHO cells	< 5
Dopamine transporter	[^3H]WIN35428	Rat striatum	< 5
Noradrenaline transporter	[^3H]nisoxetine	Rat cortex	5.46

Values represent the binding affinity (pK_i) for subtypes of dopaminergic and noradrenergic receptors and transporters.

the labeled ligand (Cheng and Prusoff, 1973). Inhibition curves were determined in two completely independent experiments.

2.3. Culture of human and monkey glial cells

For this study, established cell cultures were used. To study the human D_1 receptor, a human glioma cell line D384, which was derived postmortem from frontal cortex/subcortical white matter, was used. The monkey D_1 receptor was studied using a striatal astrocyte primary culture that was isolated from the brain of a rhesus monkey (*Macaca mulatta*). Cultures of astrocytes were prepared as described previously (Vermeulen et al., 1994).

The cells were grown as monolayer cultures in culture medium consisting of Dulbecco's Modified Eagle's Medium and F-10 nutrient (1:1), 10% fetal calf serum, 2 mM L-glutamine, non-essential amino acids, penicillin (100 IU/ml), and streptomycin (50 μg/ml). They were plated into poly-L-lysine-coated culture flasks. After 2–3 weeks in culture (37°C, 5% CO_2), the cells were trypsinized and subcultured in 12-well dishes (5×10^4 cells per well).

2.4. Adenylate cyclase activity

Experiments were performed with 7-day-old confluent cultures. Adenylate cyclase activity was determined by measuring the conversion of [^{3}H]ATP into [^{3}H]cAMP. Briefly, the cell cultures were washed with phosphate buffer saline (PBS) and exposed for 10 min to a PBS solution containing 1 mM 3-isobutyl-1-methylxanthine (IBMX), 1 μM propanolol and the dopaminergic drugs (10 μM dopamine, SKF 83959 in the concentration range of 0.01–30 μM, or a combination). The reaction was stopped by the addition of 1 ml ice-cold trichloroacetic acid (5%) containing 1 mM of non-tritiated ATP and cAMP. The [^{3}H]cAMP formed was separated from [^{3}H]ATP by sequential chromatography on Dowex and Alumina columns and measured by liquid scintillation counting.

The activity of adenylate cyclase was determined by dividing the amount of [^{3}H]cAMP by the sum of the amounts of [^{3}H]ATP and [^{3}H]cAMP. Finally, the effects of SKF 83959 on adenylate cyclase activity and the effects of SKF 83959 on dopamine-stimulated adenylate cyclase activity were calculated as percentages of the maximal dopamine-induced stimulation of adenylate cyclase activity, in order to compare the intrinsic activity of SKF 83959 with dopamine.

2.5. In vitro acetylcholine, dopamine and noradrenaline release

Male Wistar rats weighing 180–220 g were killed by decapitation. The brains were removed and the striatum (for the measurement of acetylcholine and dopamine release) or frontal cortex (for the measurement of noradrenaline release) was dissected. The dissected tissue was cut in to slices of 300 μm with a McIlwain tissue chopper. The slices were washed and incubated with radiolabeled choline, dopamine or noradrenaline (5 μCi [^{3}H]choline/2 ml, 5 μCi [^{3}H]dopamine/2 ml and 5 μCi [^{3}H]noradenaline/2 ml) in a Krebs–Ringer bicarbonate medium containing (in mM): NaCl 121, KCl 1.87, KH_2PO4 1.17, $MgSO_4$ 1.17, $NaHCO_3$ 25, $CaCl_2$ 1.2 and D(+) glucose 10. The medium was kept under a constant atmosphere of 95% O_2, 5% CO_2 at a temperature of 37°C. After a 15-min incubation period, the slices were washed and aliquots (approximately 5 mg of tissue) were transferred to each of the chambers of a 24-chamber superfusion apparatus. Three to four chambers were used for each experimental condition and each condition was repeated in at least three separate experiments. The chamber volume was 0.2 ml and the superfusion rate was 0.20 ml/min. After 40 min, four 10-min fractions were collected. The first frac-

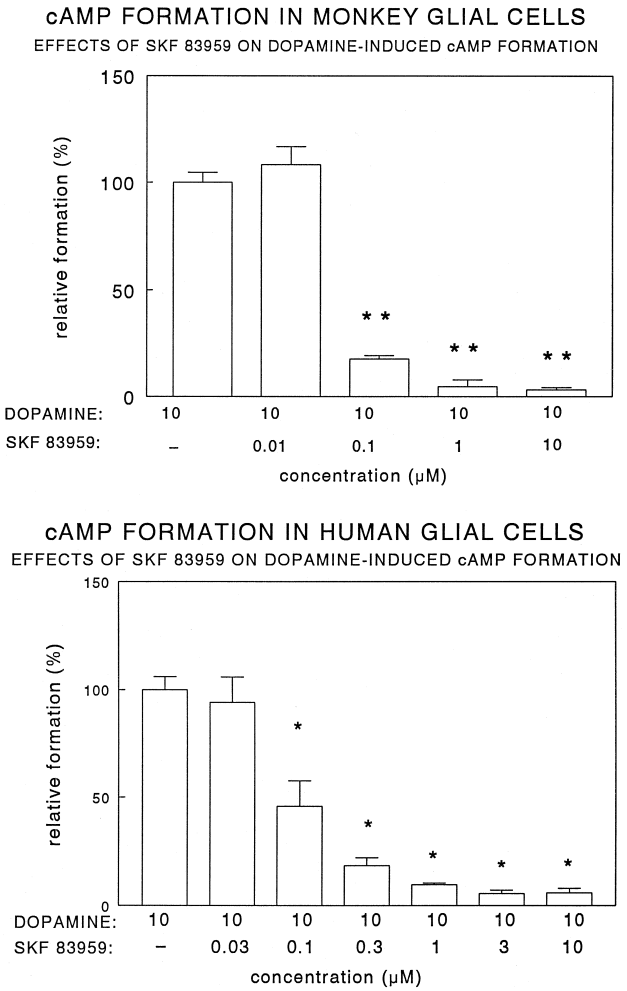

Fig. 1. Effects of SKF 83959 on dopamine-induced cAMP formation in monkey (a) and human (b) cells in culture. The activity of adenylate cyclase was determined by dividing the amount of [^{3}H]cAMP by the sum of the amount of [^{3}H]ATP and [^{3}H]cAMP. The effects of SKF 83959 on dopamine stimulated adenylate cyclase activity are expressed as percentages of the 10 μM dopamine-induced stimulation of cAMP formation. Data are represented as means ± S.E.M. from 9–12 observations obtained in 3–4 independent experiments. In monkey astrocytes, the 10 μM dopamine-stimulated and control adenylate cyclase activity (i.e., the percentage of ATP converted into cAMP) amounted to 0.46 ± 0.01 and 0.06 ± 0.003%, respectively. In the human glial cells, the 10 μM dopamine-stimulated and control adenylate cyclase activity amounted to 0.87 ± 0.03 and 0.05 ± 0.002%, respectively. SKF 83959 strongly inhibited dopamine induced cAMP formation, both in human and monkey cells. Human cells: $F(4,44) = 76.7$, $P < 0.01$: $* P < 0.01$ SKF 83959 (0.1–10 μM) vs. dopamine (10 uM), post-hoc Bonferroni. Monkey astrocytes: $F(7,49) = 35.0$, $P < 0.01$: $* P < 0.01$ SKF 83959 (0.1–10 μM) vs. dopamine (10 uM), post-hoc Bonferroni.

tion ($t = 40–50$ min) was used to determine the basal efflux of radioactivity. With respect to the basal dopamine outflow, in total five fractions were collected. The drugs were added just before the collection of the third fraction, ($t = 60$) and were present until the end of the experiment. With respect to the electrically evoked acetylcholine and noradrenaline release, in total seven fractions were collected. During collection of the second (50–60) and fifth (80–90) fractions release was stimulated electrically by exposing the tissue for 10 min ([^{3}H]acetylcholine) and 5 min ([^{3}H]noradrenaline) to biphasic square wave pulses (1 Hz, 24 mA). The drugs were added to the medium 10 min before the second stimulation, ($t = 70$) and were present until the end of the experiment. At the end of the experiment, the remaining radioactivity was extracted from the tissue. The amount of radioactivity (dpm) present in the collected fractions and tissue extracts was estimated by liquid scintillation counting. For additional information concerning tissue preparation and experimental procedures see Stoof and Kebabian (1982).

The radioactivity present in each superfusion chamber is expressed as the fractional rate, in order to correct for small differences in the amount of tissue present in each chamber. The fractional rate is defined as the amount of radioactivity present in a certain fraction divided by the sum of the amount of radioactivity present in that particular fraction, the subsequent fractions and the extract. The basal outflow of dopamine was calculated as the mean of fractions 4 and 5. With respect to electrically stimulated acetylcholine and noradrenaline release, the amount of radioactivity released as a consequence of electrical stimulation was calculated by correcting the amount of radioactivity released in the stimulated fractions for basal release (through subtraction). The majority of stimulation-induced release occurred in fractions 2 (stimulation 1) and 5 (stimulation 2). Therefore, basal release was calculated as the mean of the fractions 1, 3 and 4 for stimulation 1, and 4, 6 and 7 for stimulation 2. The effects of all drugs were expressed as a percentage of release under control conditions. For further details see Stoof and Kebabian (1982).

Table 2
Effects of dopamine and SKF 83959 on cAMP formation in human and monkey glial cells

Drug concentration	cAMP production (expressed as % of dopamine-induced stimulation)	
	Monkey glial cells	Human glial cells
Dopamine 10 μM	100 ± 3.4	100 ± 2.8
SKF 83959 0.01 μM	2.3 ± 2.3	3.4 ± 4.1
SKF 83959 0.1 μM	6.3 ± 2.9	7.6 ± 1.3[a]
SKF 83959 1 μM	4.4 ± 3.5	7.7 ± 2.3[a]
SKF 83959 10 μM	0.1 ± 1.4	9.2 ± 1.8[a]

The effects of the different concentrations of SKF 83959 on adenylate cyclase activity are expressed as percentages of the 10 μM dopamine-induced stimulation of cAMP formation.
The activity of adenylate cyclase was determined by dividing the amount of [^{3}H]cAMP by the sum of the amounts of [^{3}H]ATP and [^{3}H]cAMP.
Data are presented as means ± S.E.M. from 9–12 observations obtained in 3–4 independent experiments.
In the monkey astrocytes, the 10 μM dopamine-stimulated and control adenylate cyclase activity (i.e., the percentage of ATP converted into cAMP) amounted to 0.46 ± 0.01 and 0.06 ± 0.003%, respectively.
In the human glial cells, the 10 μM dopamine-stimulated and control adenylate cyclase activity amounted to 0.87 ± 0.03 and 0.05 ± 0.002%, respectively.
Human glial cells: SKF 83959 0.1 μM, 1 μM and 10 μM vs. control:
[a] $t(8)$, $P < 0.05$, one-sample t-test.

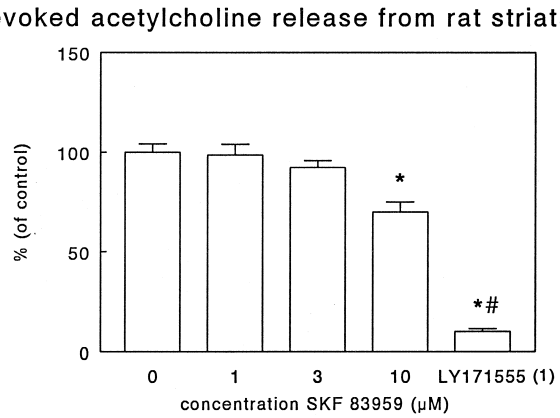

Fig. 2. Effects of SKF 83959 on electrically evoked acetylcholine release from rat striatal tissue. The amount of radioactivity released as a consequence of electrical stimulation was calculated by subtracting the amount of radioactivity released under basal conditions from the amount of radioactivity released under stimulated conditions. Ratios were calculated, whereby the amount of radioactivity released upon the second electrical stimulation was divided by the amount of radioactivity released upon the first stimulation. Under control conditions, this ratio of [^{3}H]acetylcholine release amounted to 0.80 ± 0.05. Ratios in the presence of drugs are expressed as percentages of the control ratio and depicted as such in the figure. Data represent means $\pm$ S.E.M. from 9–12 observations obtained in 3–4 individual experiments. $* P < 0.05$: SKF 83959 (10 μM) vs. control; $\#P < 0.05$: LY 171555 vs. SKF 83959 (10 μM); $* P < 0.01$: LY 171555 vs. control; post-hoc Bonferroni.

2.6. Statistics

The cAMP data were subjected to a one sample *t*-test. With respect to the release data, a One-way analysis of variance (ANOVA) was used, followed by a post-hoc Bonferroni where appropriate. The accepted level of significance was $P < 0.05$.

3. Results

3.1. Receptor affinity

As depicted in Table 1, the benzazepine SKF 83959 displayed the highest affinity ($pK_i = 6.72$) for the dopamine D_1 receptor but also had considerable affinity ($pK_i = 6.41$ and 6.33, respectively) for the α_2B- and α_2C-adrenoceptor subtypes.

Moderate affinity was observed for the dopamine D_2 and D_3 receptors ($pK_i = 5.2$ and 5.74, respectively). Finally, moderate affinity ($pK_i = 5.46$) could be observed for the noradrenergic transporter.

3.2. Adenylate cyclase activity

Dopamine, over the a concentration range 0.01 to 30 μM, stimulated the formation of [^{3}H]cAMP in monkey astrocytes, with the maximal effect occurring at 10 μM (data not shown). This amounted to a mean $\pm$ S.E.M. of $744 \pm 48\%$ of control, as shown in Fig. 1a. SKF 83959, in the range 0.01 to 10 μM, did not stimulate adenylate cyclase activity (Table 2). On the contrary, SKF 83959 concentration dependently antagonized (IC_{50} = between 0.01 and 0.1 μM) adenylate cyclase activity induced by 10 μM dopamine (Fig. 1a).

In human glial cells, dopamine stimulated the formation of [^{3}H]cAMP maximally at 10 μM to a mean $\pm$ S.E.M. of $870 \pm 60\%$ of control (see Fig. 1b). SKF 83959, in a concentration of 0.01 μM, did not significantly stimulate adenylate cyclase activity but in the range 0.1 to 10 μM marginally increased cAMP formation (Table 2). Also in this preparation, SKF 83959, in the range 0.01 to 10 μM, concentration dependently antagonized (IC_{50} = approximately 0.1 μM) adenylate cyclase activity induced by 10 μM dopamine (Fig. 1b).

3.3. In vitro electrically evoked acetylcholine release

As shown in Fig. 2, the dopamine D_2 agonist LY 171555, in a concentration of 1 μM, strongly and significantly reduced electrically evoked [^{3}H]acetylcholine re-

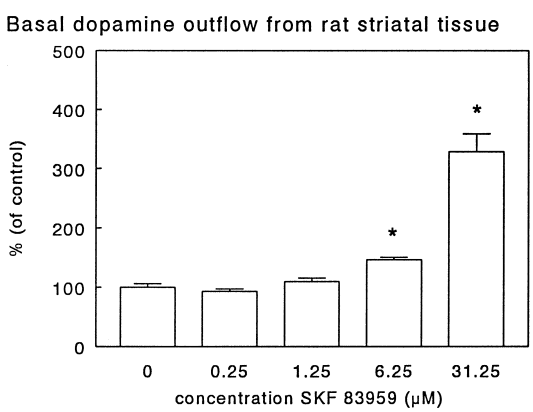

Fig. 3. Effects of SKF 83959 on basal dopamine outflow from rat striatal tissue. The data are expressed as percentages of transmitter outflow under control conditions, i.e., outflow in the absence of drugs. Data represent means $\pm$ S.E.M. from 9–12 observations obtained in 3–4 individual experiments. $F(6,65) = 61.07$, $P < 0.01$: $* P < 0.01$: SKF 83959 (6,25 and 31.25 μM) vs. control; post-hoc Bonferroni.

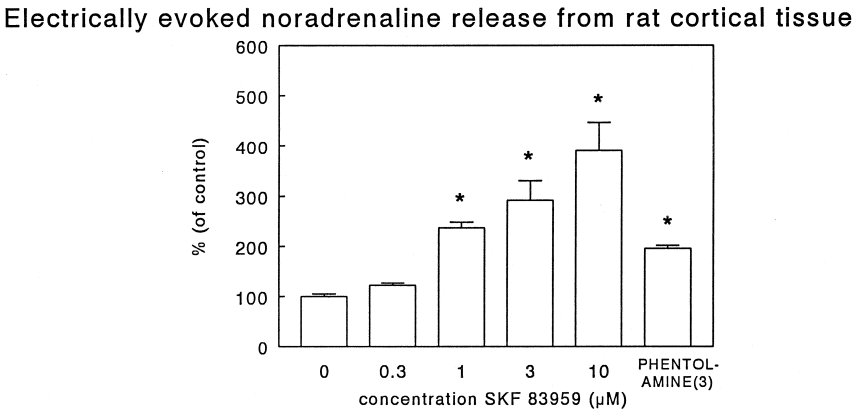

Fig. 4. Effects of SKF 83959 on electrically evoked noradrenaline release from rat cortical tissue. The amount of radioactivity released as a consequence of electrical stimulation was calculated by subtracting the amount of radioactivity released under basal conditions from the amount of radioactivity released under stimulated conditions. Ratios were calculated, whereby the amount of radioactivity released upon the second electrical stimulation was divided by the amount of radioactivity released upon the first stimulation. Under control conditions, this ratio of [^{3}H]noradrenaline release amounted to 0.90 ± 0.03. Ratios in the presence of drugs are expressed as percentages of the control ratio and depicted as such in the figure. Data represent means $\pm$ S.E.M. from 9–12 observations obtained in 3–4 individual experiments. $F(9,81) = 23.2$, $P < 0.01$: $*$ $P < 0.01$: SKF 83959 (0.1–10 μM) vs. control; post-hoc Bonferroni. Phentolamine significantly increased noradrenaline release. $\#P < 0.01$: Phentolamine vs. control; post-hoc Bonferroni.

lease to 10 ± 2% of the control ratio. SKF 83959, in a concentration of 1 and 3 μM, had no effect on [^{3}H]acetylcholine release. However, 10 μM of SKF 83959 significantly inhibited electrically evoked [^{3}H]acetylcholine release to 70 ± 5% of the control ratio.

3.4. In vitro basal dopamine outflow

SKF 83959, in a concentration of 0.25 and 1.25 μM, had no effect on the basal outflow of [^{3}H]dopamine, whereas at 6.3 and 31.3 μM, SKF 83959 significantly increased basal dopamine outflow to 146 ± 4 and 329 ± 30% of the control ratio, respectively (see Fig. 3).

3.5. In vitro electrically evoked noradrenaline release

SKF 83959 concentration dependently increased electrically evoked noradrenaline release (range 0.1–10 μM) to 391 ± 56% of the control ratio (see Fig. 4). The α-adrenoceptor adrenergic antagonist phentolamine, in a supramaximal concentration of 3 μM, increased noradrenaline release to 195 ± 7% of the control ratio.

4. Discussion

Since the majority of dopamine D_1 receptors in the striatum are coupled to the adenylate cyclase second messenger system (Battaglia et al., 1986), dopamine and dopamine agonists were thought to exert their action via this second messenger system (Stoof and Kebabian, 1984). However, with respect to the anti-parkinsonian effects of dopamine D_1 receptor agonists, the incongruity between the behavioral effects and the in vitro effects of SKF 83959 suggests that this assumption is not always correct.

The lack of correlation between the stimulation of cAMP by dopamine D_1 receptor agonists and their effects on motor behavior in animal models of Parkinson's disease can be attributed to several factors. In the present study, we investigated whether or not this disparity is due to a higher intrinsic activity of SKF 83959 at primate dopamine D_1 receptors than at rat D_1 receptors. Moreover, the functional interaction of SKF 83959 with other catecholamine receptors was investigated.

4.1. Intrinsic activity of SKF 83959 at primate dopamine D_1 receptors

Our data show that, as in rat striatal tissue, SKF 83959 was not able to increase cAMP formation significantly in monkey striatal cells and only weakly increased cAMP formation in human cells. Thus, in contrast to other dopamine D_1 receptor agonists, no large species differences appear to exist in the effects of SKF 83959 on cAMP formation. Moreover, SKF 83959 was very potent as a D_1 antagonist in these cells. At this moment, it remains uncertain whether the blockade of D_1 receptors coupled to cAMP can stimulate motor behavior in MPTP-treated primates. However, this is not very likely given the fact that the dopamine D_1 receptor antagonist SCH 23390 blocks the behavioral effects of SKF 83959 (Gnanalingham et al., 1995b). Nevertheless, these data support the view that the behavioral effects of SKF 83959 in monkeys are not mediated via striatal dopamine D_1 receptors coupled to cAMP in a stimulatory way. However, before drawing this conclusion, one should realize that the effects of SKF 83959 on motor behavior are more pronounced in dopamine-depleted animals than to intact animals. Whereas SKF 83959 strongly increases locomotor behavior in MPTP-treated monkeys (Gnanalingham et al., 1995a; An-

dringa et al., 1998) and 6-hydroxydopamine rats (Gnanalingham et al., 1995a,c), it does not in intact animals (Downes and Waddington, 1993). This phenomenon has been attributed to the fact that the MPTP and 6-hydroxydopamine-induced decrease in synaptic dopamine levels changes the interaction between dopamine D_1 receptor agonists and dopamine D_1 receptors. Both dopamine and several dopamine D_1 receptor agonists have an increased intrinsic activity at the dopamine D_1 receptor coupled to adenylate cyclase after dopamine depletion (Mishra et al., 1980; Pifl et al., 1992; Pinna et al., 1997). However, with respect to SKF 83959, this appears not to be the case because SKF 83959 does not significantly increase cAMP formation in dopamine-depleted tissue (Gnanalingham et al., 1995c). Therefore, even when synaptic dopamine levels are low, it is unlikely that the therapeutic effect induced by this drug is mediated via dopamine D_1 receptors coupled to adenylate cyclase in a stimulatory way.

A different type of interaction between SKF 83959 and the dopamine D_1 receptor might play a role in the induction of motor behavior in MPTP-treated animals. SKF 83959 may interact with dopamine D_1 receptors coupled to second messenger systems other than cAMP. Phosphoinositide hydrolysis, resulting in the formation of diacylglycerol and inositol triphosphate (IP_3), is mediated through a site that is sensitive to dopamine D_1 receptor agonists and antagonists (Undie and Friedman, 1990, 1992). The ability of dopamine D_1 receptor agonists to stimulate this site varies, and significant disparities have been demonstrated between the potency of drugs to stimulate cAMP and IP formation (Undie et al., 1994). Therefore, it cannot be excluded that SKF 83959, while having virtually no intrinsic activity on cAMP coupled dopamine D_1 receptors, significantly stimulates IP hydrolysis. Preliminary data from Undie et al. (personal communication) show that 83959 is indeed able to stimulate IP_3 formation. However, it remains to be established how potent SKF 83959 is in this respect and how relevant IP_3 stimulation is for the induction of behavioral effects.

4.2. Functional interaction of SKF 83959 with other catecholamine receptors

Since the majority of currently available dopamine D_1 receptor agonists have additional affinity for other catecholamine receptors, we decided to study the functional interaction of SKF 83959 with other catecholamine receptors and transporters, an interaction which may be involved in the behavioral effects of SKF 83959. From our binding studies, it became apparent that the compound displays significant affinity for the α_2-adrenoceptor, the dopamine D_2 receptor and the noradrenaline transporter. Functional paradigms used to investigate whether a compound behaves as an agonist or antagonist at α_2-adrenoceptors or dopamine D_2 receptors are electrically stimulated noradrenaline (from brain cortex) and acetylcholine (from striatum) release, respectively. Additionally, study of the effects of a compound on dopamine and noradrenaline release may provide insight into re-uptake inhibiting and/or release stimulating effects mediated by the catecholamine transporters.

SKF 83959 mildly stimulated dopamine outflow from rat striatal tissue only at high concentrations. Moreover, only at high concentrations, SKF 83959 reduced acetylcholine release in rat striatal tissue, an effect which is mediated by dopamine D_2 receptor stimulation (Stoof and Kebabian, 1982). As anticipated, the selective dopamine D_2 receptor agonist LY 171555 almost completely inhibited the release of acetylcholine at a concentration of 1 μM. Since the affinity of SKF 83959 for the dopamine D_2 receptor is relatively low (receptor binding experiments of the present study: $pK_i = 5.2$) and the compound releases dopamine, it is suggested that the decrease in acetylcholine release induced by higher concentrations of SKF 83959 is mediated by its dopamine releasing effect.

Although D_2 receptor stimulation relieves motor symptoms in MPTP-treated monkeys, it is unlikely that the modest dopamine outflow is completely responsible for the therapeutic effects of SKF 83959, for the following reasons. First, SKF 83959 is able to stimulate motor behavior in low dosages (0.1 mg/kg) whereas the effects on dopamine and acetylcholine release are only induced in the high micromolar range. Second, since MPTP- and 6-hydroxydopamine-treated animals have low brain dopamine levels, it remains to be established to what extent SKF 83959 is able to elevate dopamine levels in these animals. Indeed, the effects of SKF 83959 on turning behavior in unilaterally 6-hydroxydopamine-treated rats suggest that its dopamine-releasing ability is not relevant to its behavioral effect. Turning behavior is induced due to an asymmetry of dopaminergic activity between the two hemispheres: animals turn away from the side with the highest dopaminergic activity. Indeed, 6-hydroxydopamine-treated animals treated with amphetamine turn away from the intact side, since the highest amount of dopamine is released from this side (Ungerstedt and Arbuthnott, 1970). However, SKF 83959-treated animals turn away from the lesioned side, suggesting that the dopaminergic activity is not higher in the intact side (Gnanalingham et al., 1995a). Taken together, it is unlikely that the dopamine-releasing effect of SKF 83959 is responsible for the therapeutic effects induced by SKF 83959.

As observed in our binding studies, SKF 83959 had high and moderate affinity for the α_2-adrenoceptors and the noradrenergic transporter, respectively. In accordance with these data are our findings which show that, in rat cortical areas, SKF 83959 increased noradrenaline release. Apparently, two actions of the compound are responsible for this phenomenon. First, the increased noradrenaline release may be induced by blockade of presynaptically located α_2-adrenoceptors. This hypothesis is strengthened by the observation that the α_2-adrenoceptor antagonist

phentolamine, in a supramaximal concentration of 3 μM, increased noradrenaline release to approximately 200% of control. As has been demonstrated previously in our laboratory (Wemer et al., 1979) noradrenaline, under the presented stimulation conditions, inhibits its own release. This inhibition is abolished by phentolamine. However SKF 83959, in concentrations of 3 and 10 μM, induced a much higher noradrenaline than did phentolamine (Fig. 4) and, moreover, the release was not completely blocked by the α_2-adrenoceptor agonist oxymetazoline (data not shown). These data strongly suggest that the interaction of SKF 83959 with α_2-adrenoceptors is, at least not completely, responsible for the high noradrenaline release. Since SKF 83959 also has micromolar affinity for the noradrenergic transporter, it is suggested that the additional increase in noradrenaline release is due to the interaction of SKF 83959 with the noradrenergic transporter.

The effects of noradrenergic stimulation on motor behavior in animal models of Parkinson's disease have been studied because in Parkinson's disease there is degeneration not only of dopaminergic neurons, but also of noradrenergic neurons, especially in the locus coeruleus and cortical areas (Fahn et al., 1971; Alvord et al., 1974). However, the noradrenergic system appears to have only a subtle, modulating role in the control of locomotor behavior (Dickinson et al., 1988). In reserpine-treated animals, it appears that, besides dopaminergic stimulation, noradrenergic stimulation is required to give a full restoration of motor behavior (Andén et al., 1973; Dolphin et al., 1977). Moreover, α_2-adrenoceptor antagonists and α_1-adrenoceptor agonists have been reported in some studies to potentiate dopamine receptor agonist-induced motor behavior in these monoamine-depleted rodents (Pichler and Pifl, 1989; Starr and Starr, 1994). A few studies suggest a role for noradrenergic agents in diminishing motor symptoms in other animal models for Parkinson's disease. For instance, in the unilaterally 6-hydroxydopamine-treated rat, an increase and decrease of noradrenergic tone via α_1- and α_2-adrenoceptor stimulation is claimed to facilitate and inhibit amphetamine-induced rotation, respectively (Madrivis et al., 1991). One study reports a therapeutic effect of the α_2-adrenoceptor R 62 561 in one monkey treated with MPTP (Colpaert et al., 1991). Thus, although the evidence for anti-parkinsonian effects of α_2-adrenoceptor antagonists is not very strong, it cannot be completely ruled out that the α_2-adrenoceptor antagonistic effect of SKF 83959 plays a role in the anti-parkinsonian effect of SKF 83959.

4.3. General conclusion

It is concluded that the reported anti-parkinsonian effects of SKF 83959 are unlikely to be mediated by striatal dopamine D_1 receptors coupled to adenylate cyclase in a stimulatory way, especially since the compound behaves as a potent antagonist of this receptor subtype. Moreover, SKF 83959 interacts with other catecholamine receptors, stimulating the release of noradrenaline and to a lesser extent dopamine.

Whether blockade of the adenylate cyclase-coupled dopamine D_1 receptor or blockade of a dopamine D_1-like receptor linked to a different transduction mechanism is involved in the anti-parkinsonian effects of SKF 83959 or whether these effects are mediated via other catecholamine receptors requires further study.

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