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NNC-112 [1], NNC-687 and NNC-756 [1], new selective and highly potent dopamine D_1 receptor antagonists

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The neurochemical properties of three novel benzazepine derivatives NNC-112, NNC-687 and NNC-756 were assessed. These compounds inhibited dopamine D_1 receptor binding in vitro with low nanomolar to picomolar dissociation constants whereas those for the D_2 receptor were in the micromolar range. Contrary to classical neuroleptics, but similar to the atypical neuroleptics, clozapine and fluperlapine, NNC-112, NNC-687 and NNC-756 were relatively more potent in inhibiting dopamine-stimulated adenylyl cyclase than [^{3}H]SCH 23390 binding. Both NNC-112 and NNC-756 had high affinity for the 5-HT_2 receptor whereas NNC-687 had low affinity for this receptor. The affinity for other receptors or neurotransmitter transporters was very low. In vivo, the dopamine D_1 receptor selective profile of NNC-112, NNC-687 and NNC-756 was evident from the potent inhibition of D_1 receptor binding whereas no effect on D_2 receptor binding was apparent. In addition, the compounds blocked D_1 receptor-mediated rotation in unilaterally 6-hydroxydopamine-lesioned rats, but had no effect on D_2-induced rotation. Thus, NNC-112, NNC-687 and NNC-756 are potent and selective dopamine D_1 receptor antagonists that may be useful in the treatment of schizophrenia.

Dopamine D_1 receptors; NNC-112; NNC-687; NNC-756; Binding (in vivo)

1. Introduction

Until recently, dopamine receptors were classified into D_1 and D_2 types (see recent review by Andersen et al., 1990). Previously, D_1 receptors were studied primarily with respect to their function. However, following the identification of SCH 23390 as a D_1-selective ligand (Hyttel, 1983), [^{3}H]SCH 23390 has been used to identify dopamine D_1 receptors both in vitro and in vivo (Billard et al., 1984; Andersen et al., 1985; Andersen and Grønvald, 1986). The use of this radioligand resulted in a number of interesting findings. First, in vitro studies indicated that the D_1 receptor apparently exists in two states or subtypes (Andersen and Braestrup, 1986; see also Seeman et al., 1985). Only one of these subtypes is associated with the adenylyl cyclase whereas the other may be linked to phosphoinositol turnover (Andersen et al., 1990; Monsma et al., 1990). Second, atypical neuroleptics of the clozapine type have some selectivity for blocking the adenylyl cyclase-coupled D_1 receptor, whereas classical neuroleptics are more potent on the other state or subtype (Andersen and Braestrup, 1986). Third, in vivo studies have indicated that the dopamine D_1 receptor is an important target for atypical neuroleptic drugs of the clozapine type and, interestingly, a derivative of clozapine, fluperlapine, appears to be D_1-selective (Andersen et al., 1986, 1988). The importance of the D_1 receptor as an in vivo target for clozapine has been further corroborated by recent PET-scan studies in humans (Farde et al., 1989). Together, these data suggest that, at least in part, the atypical profile of clozapine is due to antagonism of the dopamine D_1 receptor and, perhaps, of a specific subpopulation of D_1 receptors. However, it should also be mentioned that the D_4 receptor has also been found to be an important target for clozapine (Van Tol et al., 1991).

Because of continuing interest in compounds with a clozapine-like dopaminergic profile, we synthesized a number of analogues of SCH 23390. In the present paper, we describe the neurochemical and behavioural profile of three such novel dopamine D_1 receptor

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[1] Previously NO-112 and NO-756, respectively.

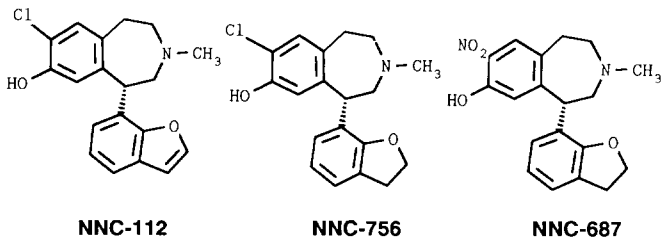

Fig. 1. Structure of NNC-112, NNC-756 and NNC-687.

antagonists, NNC-112, NNC-687 and NNC-756 (previous names, NO-112 and NO-756, respectively) (see fig. 1). Some of these data have been presented at the 18th Annual Neuroscience Meeting (Andersen et al., 1988; Nielsen et al., 1988).

2. Materials and methods

2.1. Animals

Male Albino Wistar rats (Møllegård's Breeding Labs, Lille Skensved, Denmark), weighing 175 ± 25 g were used. The animals were housed in group cages (four to eight animals/cage) placed in rooms with a 12-h light cycle (06:00-18:00). The room temperature varied between 19–24°C and the relative humidity varied from 30 to 60%.

Male Albino mice (NMRI type, bred at Novo Nordisk or from Møllegård) weighing 20 ± 2 g were also used. They were housed in group cages under the same general conditions as outlined for rats (above). All animals had access to food (standard lab chow) and tap water ad libitum.

2.2. Radioligands

[^{3}H]SCH 23390 (73.4 Ci/mmol), [^{3}H]2-(N,N-di-2,3(n)-propylamino)-8-hydroxy-1,2,3,4-tetrahydronaphtalene([^{3}H]8-OH-DPAT) (176 Ci/mmol), [^{3}H]prazosin (79 Ci/mmol), l-[^{3}H]quinuclidinyl-benzilate (QNB) (39 Ci/mmol), l-[^{3}H]noradrenaline (36.6 Ci/mmol), [^{3}H]serotonin (5-HT) (20 Ci/mmol), [^{3}H]dopamine (49.9 Ci/mmol), [^{3}H]choline (75 Ci/mmol), [^{3}H]RX 781094 (40 Ci/mmol), [^{3}H]pyrilamine (26 Ci/mmol) and [^{3}H]dihydroalprenolol (DHA) (73 Ci/mmol) were obtained from Amersham, U.K., and [^{3}H]muscimol (36.9 Ci/mmol), [^{3}H]diazepam (70 Ci/mmol), [^{3}H]spiperone (29.6 Ci/mmol) and [^{3}H]ketanserin (95 Ci/mmol) were obtained from NEN, Boston, MA, U.S.A.

2.3. Drugs

The following drugs were used: methylphenidate (Ciba-Geigy, Copenhagen, Denmark); pergolide (Eli Lilly, Indianapolis, IN); cis-flupentixol, trans-flupentixol and citalopram (H. Lundbeck A/S, Copenhagen, Denmark); l-propranolol (ICI, Copenhagen, Denmark); haloperidol, spiroperidol, domperidone and ketanserin (Janssen, Beerse, Belgium); apomorphine (Nordisk Droge, Copenhagen, Denmark); (+)-8-chloro-7-hydroxy-3-methyl-5-(7-benzofuranyl)-2,3,4,5-tetrahydro-1H-3-benzazepine (NNC-112) and (−)-8-chloro-7-hydroxy-3-methyl-5-(7-benzofuranyl)-2,3,4,5-tetrahydro-1H-3-benzazepine ((−)-NNC-112), (+)-8-nitro-7-hydroxy-3-methyl-5-(7-(2,3-dihydrobenzofuranyl))-2,3,4,5-tetrahydro-1H-3-benzazepine (NNC-687) and (+)-8-chloro-7-hydroxy-3-methyl-5-(7-(2,3-dihydrobenzofuranyl))-2,3,4,5-tetrahydro-1H-3-benzazepine (NNC-756) were synthesized at Novo Nordisk A/S; prazosin and thiothixene (Pfizer, Copenhagen, Denmark); R- and S-sulpiride (Ravizza Laboratories, Milan, Italy); SKF 38393 (Research Biochemicals, Natick, MA); fluperlapine and clozapine (Sandoz, Copenhagen, Denmark); (+)-8-chloro-7-hydroxy-3-methyl-5-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine (SCH 23390) (Schering Corp., Bloomfield, NJ) and RS 21361193 (Syntex Research Lab., Edinburgh, U.K.). All other drugs and chemicals used were of the best quality commercially available.

2.4. Drug dissolution

For all in vitro assays, stock solutions were prepared in water whenever possible, otherwise in methanol. Further dilution was made in water to obtain a maximal concentration of 0.1% methanol in any assay. By itself, this concentration of methanol had no effect in any of the assays.

For in vivo assays, water-soluble compounds were dissolved in 0.9% NaCl and used directly. Water-insoluble compounds were dissolved in 1 M tartaric acid + propyleneglycol (1:1) and brought up to volume with 0.9% saline.

2.5. Tissue preparation for D_1 receptor binding and adenylyl cyclase assays

Rat striatal tissue was processed as described by Andersen et al. (1985). Briefly, the tissue was homogenized gently by hand using a glass-teflon homogenizer (10 strokes) in 100 volumes of 10 mM imidazole-HCl (pH 7.4 at 25°C) containing 2 mM EDTA and centrifuged at 25 000 × *g* for 20 min at 4°C. The pellet was resuspended in 100 volumes of the same buffer and the homogenization/centrifugation step was repeated a total of 3 times. The final pellet was resuspended in 100 volumes of 2 mM imidazole-HCl (pH 7.4 at 25°C) containing 2 mM EDTA and used immediately for binding or adenylyl cyclase assays.

2.6. *In vitro [^{3}H]SCH 23390 binding*

A mixture of 100 μl of the above tissue suspension, 600 μl of 16.67 mM imidazole-HCl (pH 7.4 at 25°C) containing 16.67 mM theophylline, 1 mM EGTA and 10 mM $MgSO_4$, 100 μl [^{3}H]SCH 23390 (final concentration 0.2 nM), and 200 μl of water/test compound/1 μM cis-flupentixol (control/compound testing/non-specific binding) were incubated at 30°C for 60 min followed by rapid filtration through Whatman GF/B filters under vacuum. The filters were immediately washed with 2 × 10 ml ice-cold 0.9% NaCl and transferred to scintillation vials. Four millilitres of scintillation fluid was added and the radioactivity retained on the filters was counted in a conventional scintillation counter.

2.7. *Adenylyl cyclase assay*

A mixture of 50 μl tissue suspension (see section 2.5.), 300 μl of 16.67 mM imidazole-HCl (pH 7.4 at 25°C) containing 16.67 mM theophylline, 1 mM EGTA, and 10 mM $MgSO_4$ was combined with 50 μl of agonist solution and 50 μl of GTP (final concentration 15 μM). This mixture was preincubated for 15 min at 0°C, whereupon 50 μl of ATP (final concentration 1.5 mM) was added and the samples were incubated for 5 min at 30°C. The reaction was terminated by boiling the mixture for 3 min followed by centrifugation at 2800 × *g* for 20 min. To ensure equilibrium, the effects of certain drugs (as indicated in legends) were retested using the same procedure but with a 1-h incubation at 30°C. Identical results were obtained as shown previously (Andersen and Nielsen, 1986), indicating that equilibrium is established even with short incubation times. Cyclic AMP (cAMP) was measured in the clear supernatant as described by Geisler et al. (1977). In brief, 50 μl of the supernatant was incubated for 90 min at 4°C with 50 μl of 1.25 pmol [^{3}H]cAMP, 50 μl of 50 mM Tris-HCl (pH 7.4 at 4°C) containing 30 mM EDTA and 1 mM mercaptoethanol and 50 μl of purified cAMP-dependent protein kinase. One millilitre of 70% $(NH_4)_2SO_4$ was added and the incubation was continued for 15 min at 4°C. The incubation mixtures were then centrifuged at 2800 × *g* for 30 min at 4°C and decanted. After addition of 0.1 ml H_2O and 1.2 ml scintillation fluid, radioactivity was determined by conventional scintillation counting. Standards containing 0–8 pmol cAMP/50 μl were assayed in parallel.

2.8. *[^{3}H]Spiroperidol binding*

As described previously by Andersen et al. (1985), rat striatal tissue was homogenized in 2 × 10 ml 50 mM Tris-HCl (pH 7.4 at 30°C) containing 120 mM NaCl and 4 mM $MgCl_2$ and centrifuged for 10 min at 40000 × *g*. The pellet was resuspended in 1000 volumes (original wet weight) and used immediately for D_2 receptor binding assay. The assay mixture consisted of 2.5 ml tissue suspension, 25 μl [^{3}H]spiroperidol (final concentration 0.05 nM) and 25 μl H_2O/test compound/100 nM domperidone (control/compound testing/non-specific binding). The incubation was carried out at 30°C for 20 min followed by 10 min on ice. The reaction was terminated by rapid filtration through Whatman GF/B filters followed by a 2 × 10 ml wash with ice-cold 0.9% NaCl. Radioactivity trapped on the filters was measured by conventional scintillation counting.

2.9. *In vivo binding*

Mice were injected i.v. into the tail vein with the radioligand. After 5–20 min the mice were decapitated and the brain (minus the cerebellum) of each mouse was homogenized in 14 ml of 50 mM potassium-phosphate buffer (pH 7.1 at 25°C) or in 50 mM Tris-citrate (pH 7.4 at 30°C) containing 120 mM NaCl and 4 mM $MgCl_2$. For further details see table 1.

For further details concerning D_1 and D_2 binding, see Andersen (1988).

TABLE 1

Conditions for in vivo receptor binding.

	Receptor type		
	D_1	D_2	5-HT_2
Ligand	[^{3}H]SCH 23390	[^{3}H]Raclopride	[^{3}H]Ketanserin
Ci/mouse [a]	4 μCi	4 μCi	4 μCi
Decapitation time after ligand injection	20 min	5 min	10 min
Non-specific binding defined with	20 mg/kg cis-flupentixol i.p. 2 h	20 mg/kg cis-flupentixol i.p. 2 h	100 mg/kg methysergide i.p. 1 h
Level of non-specific binding	5–15%	5–15%	25–35%

[a] I.v. injection volume was 200 μl.

TABLE 2

Effects of NNC-112, NNC-687 and NNC-756 on dopamine-stimulated adenylyl cyclase and D_1, D_2 and 5-HT_2 receptors. A comparison with selected reference compounds.

The results shown are mean values from 2 to 11 separate experiments performed in duplicate, using 2–14 separate concentrations in each experiment; > means less than 10% inhibition of specific binding at the concentration indicated. K_I values calculated from IC_{50} values (see below) and with certain compounds confirmed from Scatchard analysis [c]. K_I [a] values obtained from Schild analysis. [c] agonist; [b] identical K_I values obtained with a 1-h incubation. [d] Pharmacologically inactive isomer. IC_{50} converted to K_I values using $K_I = IC_{50}/(1+[L]/K_D)$, where IC_{50} is the concentration of inhibitor causing 50% inhibition of the specific binding, [L] is the radioligand concentration used and K_D the dissociation constant of the radioligand. Some of these data have been published in Andersen et al. (1988); Andersen and Nielsen (1986); Andersen et al. (1985).

Compound	K_I (nM)			
	Adenylyl cyclase [a]	D_1 ([^{3}H]SCH 23390)	D_2 ([^{3}H]spiperone)	5-HT_2 ([^{3}H]ketanserin)
Benzazepines				
NNC-112	2.8 [b]	0.18 [c]	898 [c]	18 [c]
(−)NNC-112 [d]	NT	170	988	NT
NNC-687	9.1	5.8 [c]	> 10000	355
NNC-756	2.2	0.17 [c]	942 [c]	4.5 [c]
SCH 23390	39.9 [b]	0.14 [c]	895 [c]	37 [c]
SCH 23388 [d]	NT	173	900	> 10000
SKF 38393 [c]	415 (EC_{50})	18	9300	> 10000

2.10. Other in vitro assays

Other neurotransmitter receptor binding assays or neurotransmitter uptake assays used in the present study have been described and characterized in detail in Andersen (1989).

2.11. Inhibition of rotational behaviour in 6-hydroxydopamine (6-OHDA)-lesioned rats

Rats were stereotaxically injected with 9.7 μg of 6-OHDA into the right substantia nigra, using conventional procedures. Upon recovery from surgery, the animals were injected with 0.25 mg/kg s.c. of apomorphine. Only animals exhibiting strong contralateral turning were used in further experiments. In these experiments, rotation dependent upon D_1 receptor stimulation was elicited by 2.5 mg/kg s.c. of SKF 38393. D_2-dependent rotations were produced by s.c. administration of pergolide (0.05 mg/kg).

Test drugs were injected at the indicated times (below) before the agonists. After agonist administration, the animals were attached to a swivel via a Velcro band strapped around the waist and placed in plexi-

TABLE 3

Results from in vitro receptor profiling of NNC-112, NNC-687, NNC-756 and selected reference compounds.

Results are means of two to six separate experiments performed in duplicate with 2–14 different concentrations in each assay. > indicates less than 10% inhibition of binding at the concentration indicated.

Receptor/ligand	IC_{50} (nM)					
	NNC-112	NNC-687	NNC-756	SCH 23390	cis-Flupentixol	Haloperidol
Dopamine receptors						
D_1 [^{3}H]SCH 23390	0.4	12.4	0.4	0.4	0.72	150
D_2 [^{3}H]spiperone	1857	> 15000	1938	1800	0.75	5
Adrenoceptors						
α_1 [^{3}H]prazosin	2300	> 10000	1560	10000	5.2	50
α_2 [^{3}H]RX 781094	369	> 1000	98	891	695	> 10000
β [^{3}H]DHA	> 10000	> 100000	> 10000	> 10000	> 10000	> 10000
Serotonin receptors						
5-HT_{1A} [^{3}H]8-OH-DPAT	1500	> 10000	2600	768	345	1318
5-HT_2 [^{3}H]ketanserin	37	663	7	63	7	175
GABA–benzodiazepine complex						
$GABA_A$ [^{3}H]muscimol	> 10000	> 10000	> 10000	> 10000	> 10000	> 10000
Benzodiazepine [^{3}H]diazepam	> 10000	> 10000	> 10000	> 10000	> 10000	> 10000
Histamine receptors						
H_1 [^{3}H]pyrilamine	1800	1800	1400	1400	963	1400

TABLE 4

Effects of NNC-112, NNC-687, NNC-756 and selected reference compounds on neurotransmitter uptake.

Results are means of two to four separate experiments performed in duplicate with two to eight different concentrations of each compound in each assay. > indicates less than 10% inhibition of uptake at the concentration indicated.

Uptake	IC_{50} (nM)					
	NNC-112	NNC-687	NNC-756	SCH 23390	cis-Flupentixol	Haloperidol
Dopamine	4300	> 10000	> 10000	9600	2200	5900
Noradrenaline	790	2300	> 10000	780	4100	4600
Serotonin	835	> 1000	1500	124	> 1000	> 1000
GABA	> 10000	> 10000	> 10000	> 10000	7500	> 10000

glass bowls (diameter 49 cm). A computer detected rotation-operated photocell interruptions. Three to six animals were tested at each dose of antagonist. The total number of counts were measured for 2 h after agonist treatment.

2.12. Statistical analysis

Computer programmed log-probit methods were used to generate ED_{50} values for rotation data. Regression analysis of the role of cyclase inhibition vs. inhibition of D_1 binding for antagonism of D_1-dependent rotational behaviour was performed with the SAS REG program (SAS Institute, NC, USA).

3. Results

3.1. In vitro profile and mechanism of action

Similar to SCH 23390, NNC-112, NNC-687 and NNC-756 were selective dopamine D_1 receptor antagonists. Thus, the K_i values for inhibition of [^{3}H]SCH 23390 binding to the D_1 receptor were more than a thousand fold lower than the K_i value for displacement of [^{3}H]spiroperidol from the D_2 receptor (see table 2). Other compounds with neuroleptic activity primarily inhibited D_2 receptor binding or were equally potent on both D_1 and D_2 receptor subtypes (e.g. clozapine, fluperlapine and cis-flupentixol). Besides inhibiting D_1 receptor binding, NNC-112 and NNC-756 had fairly high affinity for the 5-HT_2 receptor whereas NNC-687 had low affinity for this receptor. The affinity of these novel benzazepine derivatives for other neurotransmitter receptors was marginal (table 3). Similarly, NNC-112, NNC-687 and NNC-756 had no effect on neurotransmitter transporters. However, SCH 23390 inhibited 5-HT uptake with an IC_{50} of 120 nM (table 4).

The effect of NNC-112, NNC-687 and NNC-756 on the D_1 receptor was investigated in further detail. As observed with SCH 23390, all three compounds inhibited [^{3}H]SCH 23390 binding with Hill slopes close to unity (not shown) (Andersen and Braestrup, 1986). Further, Scatchard analysis indicated a competitive mechanism of action since inclusion of NNC-112, NNC-687 or NNC-756 in the assay affected only the slope of the Scatchard transformation (not shown).

The dopamine D_1 receptor is coupled to adenylyl cyclase in a stimulatory fashion. Inhibition of dopamine-stimulated adenylyl cyclase activity by NNC-112, NNC-687 and NNC-756 occurred in a dose-dependent manner (not shown). Schild transformation indicated a slope close to unity, suggesting that the mechanism behind this inhibition was competitive in nature.

TABLE 5

Effects of NNC-112, NNC-687, NNC-756 and selected reference compounds on D_1, D_2 and 5-HT_2 receptors in vivo.

Results are means from two to three separate experiments with two to six different doses of each compound in each experiment with three mice per dose, [c] means from three mice tested in a single experiment. Drugs were administered [b] s.c. 0.5 h, [c] s.c. 1 h or [d] i.p. 2 h before the animals were killed. > means less than 10% inhibition of the specific binding at the dose indicated. [a] ED_{50} values were always corrected for changes in the cerebral blood flow. Some of these data have been published in Andersen and Grønvald (1986); Andersen and Nielsen (1986); Andersen et al. (1988).

Receptor/ligand	ED_{50} (mg/kg) [a]						
	NNC-112 [b]	NNC-687 [b]	NNC-756 [b]	SCH 23390 [b]	Clozapine [c]	cis-Flupentixol [d]	Haloperidol [d]
Dopamine							
D_1 [^{3}H]SCH 23390	0.015	0.35	0.02	0.017	10	0.4	72.5
D_2 [^{3}H]spiperone	> 10 [e]	> 10	> 10 [e]	13.5	7.5	0.24	0.06
Serotonin							
5-HT_2 [^{3}H]ketanserin	0.9	> 10	0.2	1.5	0.7	0.4	0.72

3.2. *In vivo profile*

The effect of NNC-112, NNC-687 and NNC-756 on the primary in vitro targets was analysed in vivo. As expected, all three NNC compounds were potent and selective inhibitors of D_1 receptor binding in vivo. The effect of NNC-112 and NNC-756 on the 5-HT_2 receptor was seen at substantially higher doses whereas, again as expected, NNC-687 was inactive. NNC-112, NNC-687 and NNC-756 were all inactive in inhibiting [^{3}H]raclopride binding to D_2 receptors (table 5).

The dopamine D_1 receptor selective profile of the NNC compounds was confirmed by the data from the experiments on rotational behaviour in unilaterally 6-OHDA-lesioned rats. Here, the D_1-mediated rotation induced by SKF 38393 was potently blocked by NNC-112, NNC-687, NNC-756 as well as by SCH 23390 and the non-selective dopamine receptor antagonist cis-flupentixol. On the other hand, haloperidol, a D_2-selective antagonist, did not affect D_1 receptor-mediated rotation. Rotation induced by administration of the D_2-selective agonist pergolide was neither affected by NNC-112, NNC-687 or NNC-756 nor by the classical dopamine D_1 receptor antagonist SCH 23390 but was potently antagonized by cis-flupentixol and haloperidol (table 6).

Finally, the role of potency to inhibit cyclase vs. D_1 binding in the ability of the dopamine D_1 receptor antagonists to block D_1-dependent rotational behaviour was examined by regression analysis. The D_1 binding affinity correlated significantly with the blockade of SKF 38393-induced rotations ($r = 0.75$, $P = 0.036$), but not with the blockade of inhibition of adenylyl cyclase ($r = 0.03$, $P < 0.15$).

4. Discussion

Although neuroleptic drugs share the ability to antagonize brain dopamine receptors, they also bind to many other neurotransmitter receptors (table 3 and Peroutka and Snyder, 1980). However, NNC-112, NNC-687 and NNC-756 showed a high degree of selectivity for the dopamine D_1 receptor as their affinity constant for this receptor was in the low nanomolar to picomolar range (table 2). In contrast, their affinity for other secondary targets, such as 5-HT_2 and α_2-adrenoceptors, was 25- to 1000-fold lower. The benzazepine SCH 23390 was a relatively potent 5-HT uptake inhibitor. Although the NNC dopamine D_1 receptor antagonists are structurally related to SCH 23390, they failed to block 5-HT uptake.

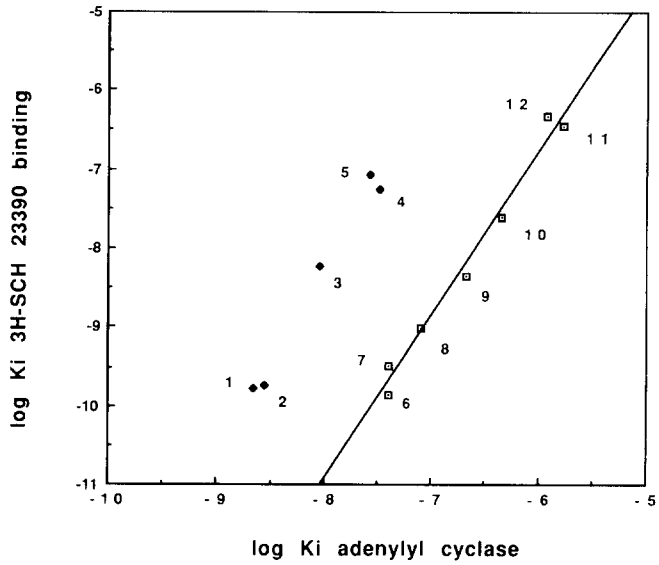

Fig. 2. Correlation between K_i values obtained in [^{3}H]SCH 23390 binding and adenylyl cyclase assays. For details see the legend of table 2. Numbers on graph refer to the following compounds: 1, NNC-112; 2, NNC-756; 3, NNC-687; 4, clozapine; 5, fluperlapine; 6, SCH 23390; 7, cis-flupentixol; 8, (+)-butaclamol; 9, fluphenazine; 10, chlorpromazine; 11, trans-flupentixol; 12, spiroperidol.

NNC-112, NNC-687 and NNC-756 dose dependently inhibited dopamine-stimulated adenylyl cyclase. The Schild analysis suggested a competitive mechanism of inhibition as previously observed with a number of neuroleptics and SCH 23390 (Andersen and Braestrup, 1986). Further, both compounds were relatively more potent in inhibiting dopamine-stimulated adenylyl cyclase activity than [^{3}H]SCH 23390 binding. These characteristics have previously been observed with atypical neuroleptics of the clozapine type (Andersen and Braestrup, 1986). A graphic presentation of the data (from table 2) clearly indicates that, like the clozapine-type compounds the correlation between pK_i values obtained from [^{3}H]SCH 23390 binding and inhibition of dopamine-stimulated adenylyl cyclase for NNC-112, NNC-687 and NNC-756 was shifted to the left (fig. 2).

The selectivity for the dopamine D_1 receptor was further corroborated by the results of in vivo binding experiments. Thus, NNC-112, NNC-687 and NNC-756 inhibited [^{3}H]SCH 23390 binding to the D_1 receptor in

TABLE 6

Effect of NNC-112, NNC-687, NNC-756 and selected reference compounds on D_1 or D_2 receptor-mediated rotation.

Rotation induced by D_1-selective SKF 38393 [a] (2.5 mg/kg) or D_2-selective pergolide [b] (0.05 mg/kg), both administered s.c.

Drug	ED_{50} (mg/kg)	
	D_1 [a]	D_2 [b]
NNC-112 s.c. 0.5 h	0.08	> 2
NNC-687 i.p. 2 h	0.48	> 50
NNC-756 s.c. 0.5 h	0.03	> 1
SCH 23390 s.c. 0.5 h	0.14	> 2
cis-Flupentixol i.p. 2 h	0.28	0.05
Haloperidol i.p. 2 h	> 5	0.1

vivo with ED_{50} values in the $\mu g/kg$ range whereas all three compounds were inactive in inhibiting [^{3}H]raclopride binding to the D_2 receptor. As observed in vitro, NNC-687 was inactive whereas both NNC-112 and NNC-756 inhibited [^{3}H]ketanserin binding to the 5-HT_2 receptor. However, the potencies were 60 and 10 times lower, respectively, than for the D_1 receptor.

The D_1 selectivity of the NNC benzazepines was confirmed in the behavioural experiments. Thus, in unilaterally 6-OHDA-lesioned rats, NNC-112, NNC-687, NNC-756 and SCH 23390 antagonized only D_1 receptor-induced rotational behaviour. The compounds were without effect on D_2 receptor-mediated rotation, which was blocked by the D_2-selective dopamine receptor antagonist haloperidol. The non-selective dopamine D_1/D_2 receptor antagonist cis-flupentixol antagonized both the D_1- and D_2-mediated rotation.

Interestingly, the inhibitory potency of the dopamine D_1 receptor antagonists at the D_1 receptor binding site, but not on the inhibition of cyclase correlated with their antagonistic potency in blocking D_1-dependent rotations. The significance of the differential ability of some compounds (e.g. clozapine) to block the dopamine-sensitive adenylate cyclase is presently not known. However, enhanced dopamine-dependent cyclase sensitivity is observed in post-mortem striatal tissue from schizophrenics, suggesting that inhibition of cyclase may be important in the antipsychotic effect of clozapine (e.g. Memo et al., 1983).

Except for clozapine, antipsychotic drugs induce extrapyramidal side-effects and increase plasma prolactin levels (e.g. Andersen and Nielsen, 1991). Can these side-effects be expected with NNC-112, NNC-687 and NNC-756 which, unlike classical neuroleptics, are highly selective dopamine D_1 receptor antagonists? First, conflicting data have been reported concerning the ability of SCH 23390 to induce catalepsy in rodents (a putative animal counterpart of human extrapyramidal side-effects) (Iorio et al., 1983; Christensen et al., 1984; Morelli and DiChiara, 1985; Barnett, 1986). In our laboratory, dopamine D_1 receptor antagonists readily induce a cataleptic syndrome which is indistinguishable from that induced by either D_2 selective or non-selective dopamine D_1, D_2 receptor antagonists (see Nielsen et al., 1992). However, the propensity of dopamine D_1 receptor antagonists to induce extrapyramidal side-effects in patients may be lower than that observed with dopamine D_2 receptor antagonists. Thus, recent studies indicate that, in contrast to dopamine D_2 receptor antagonists, dopamine D_1 receptor antagonists are incapable of inducing classical extrapyramidal side-effects in *drug-naive* primates (Coffin et al., 1989). This has been confirmed in primates with NNC-756 in doses up to 1 mg/kg s.c. (Hansen and Gerlach, 1991). It should be noted that the D_1 antagonist SCH 23390 readily induces extrapyramidal side-effects in primates that have *previously* received D_2 haloperidol (Kistrup and Gerlach, 1987). However, i.m. injected (but not p.o. injected) SCH 23390 was found by Lawrence et al. (1991) to produce signs of 'parkinsonism' in drug-naive primates (mainly increased sedation, decreased blink rate and, at high doses, tremor). Thus, the route of administration and species may play a role in the expression of motor side-effects by SCH 23390. Further, there may be a difference in the symptomatology of 'Parkinsonism' (e.g. as defined by Lawrence et al.) and extrapyramidal side-effects (as defined by Coffin et al., 1989). Second, since the dopamine D_1 receptor antagonists are devoid of D_2 affinity, they should be without effect in tissues containing only this receptor subtype, e.g. the mammotrophic cells of the pituitary. Consistent with this, dopamine D_1 receptor antagonists have little effect on plasma prolactin levels in animal experiments (Iorio et al., 1983; Rovescalli et al., 1987).

NNC-112, NNC-687 and NNC-756 are new dopamine D_1 receptor-selective antagonists with a neurochemical profile suggestive of antipsychotic potential. Since D_1-selective antagonists may have a lower propensity to induce extrapyramidal symptoms, such compounds hold great clinical promise.

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