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Striatally-Mediated Response of Some Structurally Rigid Analogues of Dopamine

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ANTONIAN L J A JOSEPH L R MEYERSON J COUPET D I SCHUSTER, H E KATERINOPOULOS A P S NARULA AND C E RAUH *Striatally-mediated response of some structurally rigid analogues of dopamine* PHARMACOL BIOCHEM BEHAV 24(2) 253–258 1986 —The potency of structurally rigid analogues of dopamine (DA) at striatal dopamine receptors was evaluated in rats using three types of assessments (a) effectiveness in producing rotational and sniffing behaviors by intrastriatal injections (b) inhibition of [3H]-spiroperidol binding and (c) stimulation of adenylate cyclase activity The compounds included apomorphine (APO) and its analogues, (R)-2,10,11-trihydroxyaporphine (R-THA) and (R)-2-hydroxy-10,11-methylenedioxyaporphine (MDO-APO), 2-amino-6,7-dihydroxyaminotetraline (ADTN) and its analogue, exo-2-amino-6 7-dihydroxybenzonorbornene (exo-amine) (R)-THA produced no stereotypy yet it was a potent inhibitor of [3H]-spiroperidol binding and adenylate cyclase activity MDO-APO was quite active in inducing stereotypy and stimulating cyclase activity, but it showed low potency in displacing [3H]-spiroperidol The exo-amine and ADTN were equally potent in enhancing rotation and sniffing intensity, however, the former was completely inactive in biochemical assessments Except for (R)-THA, all DA analogues studied elicited dopaminomimetic behavioral activities of circling and sniffing Relationships between the actions of these drugs in the behavioral and biochemical assessments are discussed

Rigid dopamine analogues Apomorphine ADTN (R)-2,10,11-trihydroxyaporphine Exo-2-amino-6 7-dihydroxy-benzonorbornene Stereotypy 6-OHDA lesion Adenylate cyclase [3H]-spiroperidol

THE development of structurally rigid analogues of dopamine (DA) has greatly assisted in the evaluation of the conformational requirements of DA at the striatal DA receptor (for review see [20]) The most widely studied classes of the dopamine analogues have been the aporphines and the aminotetralines As a structurally rigid analogue, apomorphine (APO) [1] represents the α-conformer while the semi-rigid structure 2-amino-6,7-dihydroxyaminotetraline (ADTN) [11], represents the β-conformer of the trans-anti conformation of DA (Fig 1) Apomorphine has been characterized biochemically as a DA agonist since it stimulates striatal DA-sensitive adenylate cyclase (DSAC) [11], binds with high affinity to the D2 receptor, and induces dose-dependent stereotypy and hyperactivity in behavioral tests [8] ADTN also behaves as an agonist in DSAC assays and has a high affinity for postsynaptic DA receptors in striatal homogenates [7,18] However, data from behavioral evaluations suggest that ADTN has effects on stereotypy primarily when administered directly into the nucleus accumbens of nialamide pretreated rats (see below) Peripheral administration of ADTN [5] fails to produce behavioral effects that are indicative of dopaminergic stimulation Apparently, ADTN does not penetrate the blood-brain barrier or is rapidly metabolized and thus is ineffective when administered peripherally

In attempts to establish structure-activity relationships, a large number of structurally rigid analogues of DA have been tested for their behavioral and biochemical activities at the striatal DA receptor [4,20] In the present study we have selected a few recently synthesized dopamine analogues and a few reference compounds for behavioral and biochemical measurements of dopaminergic activity These compounds include apomorphine, (R)-2,10,11-trihydroxyaporphine (III), and (R)-2-hydroxy-10,11-methylenedioxyaporphine (IV) which constitute the α-rotameric analogues ADTN and exo-2-amino-6,7-dihydroxybenzonorbornene (V), which represent the β-rotomeric analogues of DA were also selected Structurally, compound III differs from apomorphine merely

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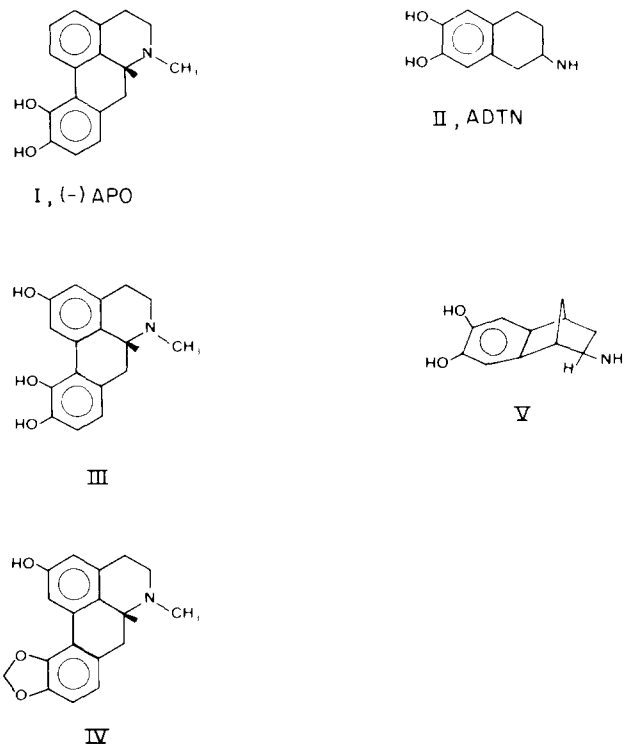

FIG 1 Dopamine agonists grouped according to their alpha- and beta-rotameric conformations

by a hydroxyl group at the 2 position Compound IV is the methylenedioxy ether analogue of compound III Compound V is a tricyclic analogue of ADTN where the amino group is held rigidly in an equatorial position (Fig 1)

In biochemical assessments, (R)-THA (III) has been reported to have lower agonistic potency than APO in D2 binding and D1 cyclase studies [15] Behavioral testing with (R)-THA has not been reported MDO-APO (IV) is a novel APO analogue and, to date, no data have been reported concerning its effectiveness at the DA site It would be speculated that MDO-APO might be active behaviorally since (−)10,11-methylenedioxy-n-propylnoraporphine, which is structurally similar to MDO-APO, has been found to be effective orally and induces a long-lasting D2 agonist stereotypy This finding has led to the suggestion that (−)10,11-methylenedioxy-n-propylnoraporphine may be a prodrug of NPA (n-propylnoraporphine) [3,21] Exo-amine (V) has been shown to be inactive in binding studies against DA agonists and antagonists [19] However, in another study of V a nanomolar affinity of exo-amine against [3H]-ADTN was observed, yet in that study the compound failed to produce stereotypy when injected bilaterally into the nucleus accumbens [1] Thus, contradictory information exists concerning the *in vitro* and *in vivo* potency of compound V

Many inconsistencies are reported in comparing the biological potencies of DA analogues of the apomorphine and aminotetraline classes, when administered into the nucleus accumbens with their biochemical potencies The behavioral manifestations of intra-accumbens injections are either weak or inconsistent [5] Therefore, in the present study we have attempted to quantitatively evaluate striatally mediated DA receptor motor behavior induced by DA, APO, (R)-THA, MDO-APO, ADTN and the exo-amine following their injection into the striatum Two behaviors were examined in this experiment Sniffing behavior was chosen because it is a sensitive indicator of the agonist potencies of various DA-active agents Rotational behavior was also measured because it has been found in numerous experiments to be one of the more specific behavioral assays of striatal DA functioning (e g , see [10, 24, 25]), especially when intrastriatal injections are used These behavioral findings were compared with *in vitro* measures of the D1 cyclase stimulation and D2 receptor binding

METHOD

Animals

Sixty-eight male Wistar rats (350 g, Royal-Hart, New Hampton, NY) were used for the behavioral determinations They were housed two per cage, in 45×15×23 cm polycarbonate cages with sanitized chipped-wood bedding A 12 hr light and dark cycle was maintained in the colony room An additional 24 rats were used for biochemical determinations

Surgery

Sixty-eight animals were unilaterally lesioned in the left substantia nigra with 6-hydroxydopamine (6-OHDA) as described previously [10] and allowed to recover for one week Lesion coordinates were as follows 3 2 mm posterior to bregma, 2 4 lateral to the midline of the skull and 8 7 mm ventral to the dura [17] They were then given 1 mg/kg of amphetamine intraperitoneally (IP) and rotational behavior was assessed (see below) for 10 minutes Only animals showing >30 rotations per 10 minutes were used in further assessments These animals were surgically implanted with a chronic cannula (1 4 anterior to Bregma, 3 0 lateral to the midline, 5 0 ventral to the dura [17]) that was placed into the intact striatum All subsequent drug and vehicle injections were delivered via this cannula

Behavioral Experiments

The rats were randomly selected to receive dopamine (N=32) or one of the 5 DA analogues APO(N=9), ADTN (N=15), exo-2-amino-6,7-dihydroxybenzonorbornene (N=12), (R)-2,10,11-trihydroxyaporphine (N=15), and (R)-2-hydroxy-10,11-methylenedioxyaporphine (N=17) All compounds were delivered in 1 μl N2-bubbled distilled H2O (pH 6 5) Compounds other than dopamine were given in doses of 0(vehicle), 1 or 10 μg that were separated by 1 day Dopamine was given in doses of 1,5,25 μg to nialamide-pretreated rats (50 mg/kg IP, 1 hr prior to central injection of dopamine) Rotational behavior (complete left and right rotations) was assessed immediately following the striatal injections as was previously described [10] Sniffing intensity was assessed at the same time using the following rating scale derived from that used by Costall and Naylor [5] as follows 0—no stereotypy, 1—periodic sniffing, 2—continuous sniffing, 3—periodic licking with sniffing, 4—continuous licking and sniffing and periodic jaw movements, 5—all continuous, i e , sniffing, licking, jaw movements

Binding Assay

The striata from male Wistar rats (150–200 g) were prepared according to a previously published procedure [19] The tissue were homogenized in 15 volumes of cold 15 mM

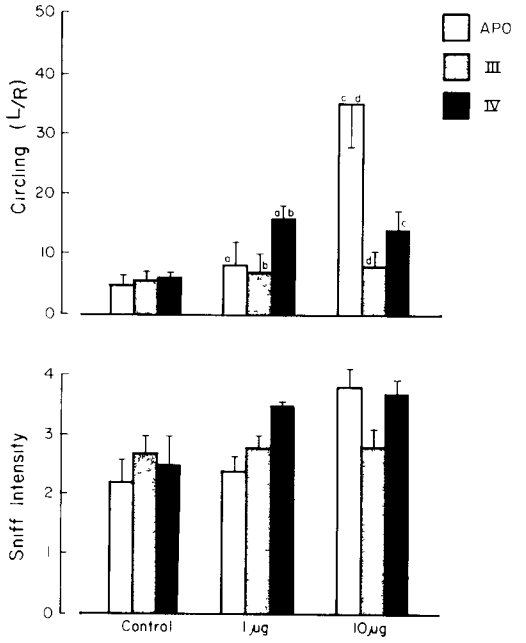

FIG 2 Rotational behavior (top) and sniffing behavior (bottom) elicited by APO, III, and IV "a" denotes significant differences between APO and IV, "b" denotes significant differences between III and IV at the 1 ug dose At the 10 μg dose, "c" denotes differences between APO and IV and "d" denotes differences between APO and III See text for the explanation of the tests

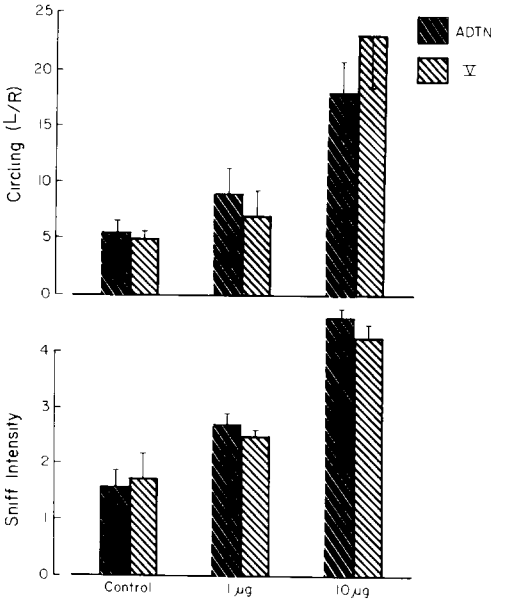

FIG 3 Rotational behavior (top) and sniffing behavior (bottom) elicited by ADTN and V

TEAN buffer (15 mM Tris, 5 mM EDTA, 0 02% ascorbic acid, 12 5 mM nialamide) for 10 seconds with a Janke and Kunkle Tissuemizer (setting 45%)

The homogenate was centrifuged at 69,500×g for 10 min The supernatant was discarded, the pellet was resuspended in 15 volumes of buffer The centrifugation and resuspension procedure was repeated a total of 5 times The final pellet was resuspended and homogenized at 10 mg original wet weight per ml of buffer The tissue preparation was stored at −20°C until use

In a total volume of 600 μl, 0 25 nM [3H]-spiroperidol, 100 nM ketanserin (S2 site blocker), the displacing drug, and ~0 2 mg of homogenate protein were incubated at 21°C for 60 minutes Non-specific binding was defined in the presence of 10 uM sulpiride The binding equilibrium was terminated by filtration under vacuum and three 5 ml washes with cold buffer The filters were placed in 10 ml of Beckman Ready Solv HP and radioactivity was determined in a Beckman LS-7500 scintillation counter

Adenylate Cyclase Assay

The adenylate cyclase assay was performed in rat striatal homogenate according to established procedures [6] The amount of c-AMP produced was determined by RIA assay [23] and was expressed as picomoles c-AMP/mg protein min−1 The basal level of cyclase activity was found to be 65 2±4 6 pmoles c-AMP/mg protein min −1 in the experiments The protein content of the tissue was determined by the Lowry procedure [12] The stimulation of the cyclase by DA and the agonists were calculated as the percent change above basal levels

RESULTS

Behavioral Measures

Administration of dopamine, APO, MDO-APO, ADTN and exo-amine increased rotational behavior in a dose dependent manner One-way analyses of variance showed these increases to be significant DA, $F(2,62)=5\ 95$, $p<0\ 005$, ADTN, $F(2,26)=7\ 98$, $p<0\ 005$, exo-amine (V), $F(2,22)=15\ 06$, $p<0\ 001$, apomorphine, $F(2,16)=16\ 53$, $p<0\ 001$, MDO-APO (IV), $F(2,32)=10\ 0$, $p<0\ 001$, (R)-THA (III) did not produce increases in rotational behavior ($F<1$) These findings are illustrated in Figs 2 and 3 (top) for all compounds but DA These figures also show that only compound IV produced increases in rotation at the 1 μg dose Effects were only seen at the 10 μg dose of the other agents However, compound IV did not produce any greater increases in rotations at the 10 μg dose than it did at the 1 μg dose DA produced similar degrees of rotation at both the 5 μg and 50 μg doses (data now shown) These patterns were also seen when sniff intensity was examined They are illustrated in the lower panels of Figs 2 and 3 ADTN, $F(2,32)=7\ 32$, $p<0\ 001$, exo-amine $F(2,22)=17\ 26$, $p<0\ 001$, APO, $F(2,16)=6\ 86$, $p<0\ 01$, (R)-THA, ($F<1$), MDO-APO, $F(2,32)=7\ 95$, $p<0\ 01$

Since structural similarities exist between ADTN (II) and exo-amine (V) and among APO, (R)-THA (III), and MDO-APO (IV), specific comparisons were made within these groupings to determine differences in efficacy for enhancing behavioral responses ADTN and exo-amine did not differ in their ability to enhance rotation ($t<1$) and sniffing intensity ($t<1$) (Fig 3 top and bottom, respectively) Analyses of variance were carried out to determine differences among the effects of APO, (R)-THA and MDO-APO on rotational behavior and sniffing intensity for each dose (1 μg, 10 μg) Significant differences were seen among the three compounds at both the 1 μg and 10 μg doses for both types of behavior rotation, 1 μg, $F(2,37)=3\ 92$, $p<0\ 05$, 10 μg, $F(2,37)=3\ 77$, $p<0\ 05$, sniffing, 1 μg, $F(2,37)=141\ 5$, $p<0\ 001$,

TABLE 1

Drug	IC_{50} vs [^{3}H]-spiroperidol (nM)	Adenylate Cyclase Stimulation % Increase from Basal	Circling*	Sniffing*
DA	2209 ± 371	34 (@ 10 μM)	+	0
APO	218 ± 7	25 (@ 10 μM)	+	+
(R)-THA	184 ± 23	28 (@ 10 μM)	0	0
MDO-APO	4276 ± 444	42 (@ 10 μM)	+	+
ADTN	109 ± 7	31 (@ 10 μM)	+	+
Exo-norbornene(V)	>10,000	NA† (@ 10 or 100 μM)	+	+

IC_{50} values of [^{3}H]-spiroperidol binding are results of three experiments with their S E M values

*These columns represent qualitative evaluations of the changes in rotational and sniffing behaviors produced by the various agonists They are presented in this form only for the convenience of the reader The actual quantitative data are presented in the figures and text

†NA, not active

10 μg, $F(2,37)=9\ 5$, $p<0\ 001$ At the 10 μg dose both APO and MDO-APO produced greater sniffing than (R)-THA ($p<0\ 005$ in Duncan's test, $df=37$) Rotational behavior was enhanced at the 10 μg dose to a greater extent by APO than either (R)-THA or MDO-APO ($p<0\ 005$ in Duncan's test, $df=37$) At the 1 μg dose MDO-APO had greater effects than either (R)-THA or APO on both behaviors (Fig 2) ($p<0\ 05$ in Duncan's test, $df=37$ for both sniffing and rotation)

Biochemical Measures

All DA agonists were tested in striatal adenylate cyclase assay and were shown to stimulate c-AMP production, with the exception of exo-amine Table 1 shows the percent change in c-AMP from basal level at 10 μM concentration of each drug Optimal changes from the basal level (not shown in Table 1) were obtained with 30 μM DA (77%), with 5 μM (R)-THA (44%) and with 100 μM ADTN (70%) Exo-amine did not stimulate the striatal cyclase either at 10 or 100 μM

APO displaced [3H]-spiroperidol (0 25 nM) from rat striatal homogenates with an IC50 of 218±7 nM (R)-THA was equivalent with APO in this binding assay with an IC50 of 184±23 nM MDO-APO was several fold less active than (R)-THA at [3H]-spiroperidol binding sites yet it was very potent in the stimulation of adenylate cyclase and in behavioral measures (Table 1) (R)-THA has been reported to potently displace [3H]-APO, [3H]-ADTN, and [3H]-spiroperidol, IC50 values of 860 nM and 2600 nM were reported for APO and (R)-THA respectively, when assayed against [3H]-spiroperidol in calf caudate homogenates [15] Our results indicate that (R)-THA is more potent in the rat striatal binding assays compared to those reported in the calf caudate We found an IC50 value for ADTN similar to that reported in the literature [20,22] We found that the exo-amine, (V), was inactive (up to 10 μM) at [3H]-spiroperidol binding sites, as reported elsewhere [1,19]

Thus, among the α-rotomers of DA analogues, (R)-THA was equipotent to APO in D1 and D2 receptors as measured by DSAC and [3H]-spiroperidol binding, MDO-APO was less active than APO at D2 sites, but it was quite potent at the D1 site The β-rotameric analogue, exo-amine, was inactive in all biochemical assessments

DISCUSSION

This study was designed to determine the ability of a select sample of rigid analogues of DA to enhance rotational and sniffing behavior The behavioral results which measured dopaminergic activity were compared with those generated from biochemical assessments which determine the efficacy of these DA analogues in stimulating adenylate cyclase activity and inhibiting [3H]-spiroperidol binding Comparisons were made within the α- (APO, (R)-THA, MDO-APO) and β- (ADTN, exo-amine) rotameric groups

The α-rotameric analogue APO was used as a reference drug which was active in our biochemical and behavioral assessments as reported previously [8,11] MDO-APO, although it was not very active in inhibiting [3H]-spiroperidol binding, stimulated adenylate cyclase activity as well as sniffing and rotational behaviors Interestingly, MDO-APO produced circling and sniffing behavior at the 1 μg dose and did not produce a dose dependent increase in behavior at the higher dose It might be suggested that the stimulatory effects of MDO-APO on behavior (at 1 mg dose) and cyclase stimulation are mediated through the D1 receptor Perhaps the sustained behavioral effects at the higher dose (which correlate well with the low affinity of MDO-APO at [3H]-spiroperidol sites) are mediated primarily by D2 sites Perhaps at higher doses the effects may be inhibitory Paradoxically, (R)-THA, although equipotent with APO in its activity to stimulate DA cyclase activity and to inhibit [3H]-spiroperidol binding potency, failed to produce any significant effects on rotation and sniffing While stereotypy data have not been previously reported for (R)-THA, binding studies in the calf striatum have demonstrated that (R)-THA has lower potency (IC50=2600 nM) than APO (IC50=860 nM) at [3H]-spiroperidol sites [15] Similarly, (R)-THA is weaker than APO and (−)-2,10,11-trihydroxy-N-n-propylnoraporphine (TNPA) in blocking myoclonic responses (which are mediated via DA mechanisms) to photic stimulation in the baboon [16] Apparently hydroxylation of APO at the 2 position interferes with intrinsic agonistic activity *in vitro* in striatal preparations Our behavioral data support these biochemical findings That (R)-THA was capable of displacing [3H]-spiroperidol binding without inducing any behavioral response may indicate that it can bind

to a dopamine recognition site but cannot initiate a functional response It is unlikely that MDO-APO is a prodrug for (R)-THA since (R)-THA was found to be behaviorally inactive In contrast, compounds such as 10,11-methylenedioxy-N-n-propylnoraporphine [21] and piribedil [14] are thought to be prodrugs for the active metabolites, NPA and S-584, respectively Thus, the order of potency of APO and its analogues in producing dose-dependent rotation was APO>,MDO-APO>(R)-THA The behavioral results (Table 1) suggested that the APO analogue MDO-APO may be operating mainly through the D1 receptor and that the behavioral and the *in vitro* activity of THA did not correlate

ADTN was used as a reference β-rotameric analogue DA The inactivity of the exo-amine (V) at the D2 site and the D1 site, shown here and also reported previously for the D2 binding [1,19], is very interesting, especially in view of its potent effect upon circling and sniffing behavior The exo-amine was equipotent with ADTN in producing rotational behavior following administration of the drug to the intact striatum of lesioned animals Intrastriatal administration of atropine also promotes intense turning behavior [10] by inhibiting striatal cholinergic intraneurons which innervate the striatonigral GABAergic pathways [13] Thus it might be suggested that exo-amine (V) could be operating through cholinergic inhibition However, this is probably not the case since exo-amine (V) failed to inhibit [3H]-QNB binding in the rat striatum (data not shown) It is also possible that exo-amine (V) has α-adrenergic agonistic effects since it has been shown to produce vasoconstriction in the pithed rat preparation *in vivo* [2,9], however, the observed stereotypy in this study has been clearly demonstrated to be dopaminergically mediated [24,25] The present behavioral results differ from previous findings which have failed to demonstrate stereotypy following intra-accumbens administration of the exo-amine [1] Perhaps as suggested elsewhere [5] motor activity is elicited to a greater degree by α-conformers of DA (e g , 5,6-ADTN) that are injected into the accumbens than by β-conformers such as the exo-amine Conversely, in the striatum the β-conformers may be equipotent with the α-conformers in eliciting stereotypy. Thus the *in vitro* and *in vivo* activity of exo-amine do not correlate A possible mode of action of the exo-amine might be to induce the release of DA from the terminals in the intact striatum A second mode of action might be to inhibit striatal DA uptake It is known, for example, that amphetamine, and the non-hydroxylated N-methyl-exo-2-aminobenzonorbornene block α-adrenergic uptake sites [2] Such a mode of action of the exo-amine at the dopamine uptake site could account for the robust stereotypy effects observed in the present study

As more structure-activity relationships are generated through the use of new dopamine agonists, we will be better able to correlate the structural and topographical requirements of the DA agonists with their behavioral and biochemical activities

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