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On the preferred rotameric conformation for dopamine agonist action: an illusory quest?

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Putative dopamine agonists from the 2-aminotetrahydronaphthalene and trans-octahydrobenzo (f) and (g) quinoline series were shown to inhibit the spontaneous locomotor activity of mice. Marked potency differences were observed between the α- and β-rotameric conformations, compounds having the α-rotameric conformation having the greater potency. Thus, 2-di-n-propylmino-5,6-dihydroxytetrahydronaphthalene was 339 times more potent than the 6,7-dihydroxy isomer, and 2-n-propylamino-5,6-dihydroxy-compound was respectively 79 times and 179 times more potent than 6,7-hydroxy- and 7,8-dihydroxycompounds. *trans*-7,8-Dihydroxy-1-n-propyl-1,2,3,4,4a,9,10,10b-octahydrobenzo(f)quinoline was 11 times more potent than the β-rotamer, the 6,7-dihydroxy compound, and within the *trans*-octahydrobenzo(g)quinoline series the α-rotameric *N*-propyl derivative was 467 times more potent than the β-rotamer, and the α-rotameric >N–H analogue was 46 fold more potent than the β-rotamer. Thus, the α-rotamer appears the more potent in causing the present functional dopaminergic change. The dopaminergic nature of the response was indicated by its sensitivity to spiroperidol but not to yohimbine or prazosin. The possibility that a difference in behavioural potency between the α- and β-rotamers may reflect a differential metabolism by catechol-*O*-methyl transferase was assessed by administration of different agonists after pyrogallol pretreatment. This potentiated the activity of 2-di-n-propylamino-6,7-dihydroxytetrahydro-naphthalene but not that of the 5,6-dihydroxy analogue. However, changes in the effects of *N*-propyl derivatives of *trans*-octahydrobenzo(f) and (g)quinoline were not marked and, in all experiments, pyrogallol treatment failed by orders of magnitude to shift the dose-response curves of the β-rotamers to indicate a comparable potency to the α-rotameric forms.

The design of agents having dopamine agonist activity has necessitated detailed investigation of the conformational states of dopamine. The problems of conformational analyses appear threefold. Firstly, does dopamine exist in a *trans* or *gauche* conformation? In reviewing the available evidence it has been concluded that the *trans* conformation is preferred (Horn & Rodgers 1980). Secondly, and more difficult to resolve, is the catechol ring coplanar or perpendicular to the $CH_2.NH_2$ bond? In the dopamine molecule the potential energy differences between the two forms is quite small with crystallographic analysis and molecular orbital studies of n.m.r. coupling constants indicating a preferred perpendicular form (Bergin & Carlstrom 1968; Giessner-Prettre & Pullman 1975). In contrast, dopamine agonists from many chemical series, the aporphines and 2-aminotetrahydronaphthalenes (ATN) for example, with more rigid structures, suggest the importance of a coplanar arrangement. However, accepting that a coplanar arrangement may be preferred by the receptor, a third question arises which has been subject to much debate, what is the preferred orientation of the catechol ring, α- or β-rotameric? Horn & Rodgers (1980) have suggested that the use of the 5,6- and 6,7-dihydroxyATN 'readily provide' an answer to this question, in that these more rigid molecules represent the α- and β-rotameric conformations, and the β-rotameric conformation is consistently more active in a number of biochemical systems. However, Cannon et al (1977) using a series of ATN's have cautiously argued that the greater behavioural potency of the 5,6- as compared with the 6,7-dihydroxy compounds, may indicate a dopamine (striatal) receptor preference for the α-rotameric form.

In the present study we select a behavioural index of dopamine agonist activity, ability to inhibit spontaneous locomotion, and include putative dopamine agonists from the *N*-alkylated ATN series and from the series of *trans*-octahydrobenzo(f) and (g) quinolines, recently synthesized with hydroxyl functions in the α- or β-rotameric conformation, in an attempt to establish the relative importance of these two rotamers for causing functional dopaminergic change.

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MATERIALS AND METHODS

Behavioural testing

Experiments were carried out on male albino mice, B.K.W. strain, 25–40 g, between 09.00 and 18.00 h in a diffusely illuminated room maintained at 22–23 °C. 10 min after injection with a putative dopamine agonist, and after allowing a 30 min pretreatment time for spiroperidol, yohimbine and prazosin and 4 h with pyrogallol, mice were placed in individual screened Perspex cages measuring 10 × 14 × 24 cm each fitted with two photocell units located 2·5 cm above the floor of the cage and 3·0 cm from the sides. Interruptions of the two light beams were recorded separately on electromechanical counters and noted every 5 min: when a mouse exhibited locomotor hyperactivity the numbers of interruptions of the two light beams were approximately equal and these were summed to give a value for spontaneous locomotor activity in counts/5 min. Readings were taken for at least 30 min but the studies clearly showed that a cumulative count/20 min could be used in addition to counts/5 min as an expression of spontaneous locomotion. In addition to noting activity counts every 5 min, mice were also observed for the presence or absence of stereotyped sniffing or biting, climbing behaviour, muscular hypotonia – prostrate appearance, autonomic changes or any other effect which may interfere with the measurement of locomotor activity.

Experimental design

To assess the effects of putative dopamine agonists alone on spontaneous locomotor activity 3 groups of 5 mice were used, one receiving the drug, one the vehicle for the drug, whilst the third formed the group of normal, non-treated mice. All experiments were repeated at least once, and the results meaned for construction of the histograms. Preliminary studies for experiments to determine the effects of spiroperidol, yohimbine, prazosin and pyrogallol (interacting drugs) on agonist activity utilized 7 groups of 5 mice which received (a) no treatment, (b) interacting drug, (c) vehicle of interacting drug, (d) dopamine agonist, (e) vehicle of dopamine agonist, (f) interacting drug combined with dopamine agonist, and (g) interacting drug plus vehicle for dopamine agonist. All vehicle effects were established in these preliminary studies, and on no occasion were statistically significant changes recorded. Thus, subsequent drug interaction studies used 4 groups of 5 mice which received (a) no treatment, (b) interacting drug, (c) dopamine agonist, and (d) interacting drug combined with the dopamine agonist. Experiments were repeated at least once.

Biochemical determinations

Five groups of mice treated for 4 h with vehicle for pyrogallol or 25, 50, 100 and 200 mg kg^{-1} i.p. pyrogallol were killed by cervical dislocation. Tissue samples were taken from the liver and from the striatal and mesolimbic areas of the brain (areas of the striatum, nucleus accumbens and tuberculum olfactorium were dissected out and pooled). The tissue was homogenized in 0·25 M sucrose and a 'microsomal vesicle and membrane' fraction prepared according to the method of Revis (1978). Cathechol-*O*-methyl transferase (COMT) activity was determined according to McCaman (1965).

Statistical treatment

Significant differences between treatments was assessed using the Student's *t*-test.

Drugs

The dopamine agonists (see Fig. 1) and pyrogallol (BDH) were prepared immediately before use in nitrogen bubbled distilled water containing 0·1% sodium metabisulphite. Spiroperidol (Janssen) was prepared in the minimum quantity of glacial acetic acid and diluted to volume with distilled water and prazosin. HCl (Pfizer) and yohimbine. HCl (Sigma) were prepared in distilled water. All doses were calculated as the base and injected in a volume of 0·5 ml/100 g, the dopamine agonists by the subcutaneous route, the other agents intraperitoneally.

RESULTS

Modification of spontaneous locomotor activity by N-alkylated derivatives of ATN

Compounds IV and V and the monoalkylated agents I, II and III all caused dose related decreases in spontaneous locomotor activity of mice (Fig. 2). The reduction in locomotor activity caused by the lower doses of these agents was not associated with any obvious autonomic changes, or the induction of climbing or stereotyped behaviour. However, for IV the reduction in spontaneous locomotor activity was followed, as the dose was increased above 6·3 μg kg^{-1} s.c., by a return of spontaneous locomotion, and, at doses in excess of 100 μg kg^{-1} s.c. (not shown), climbing behaviour was observed. Also, the highest dose of V caused stereotyped sniffing behaviour, and muscular hypotonia became apparent.

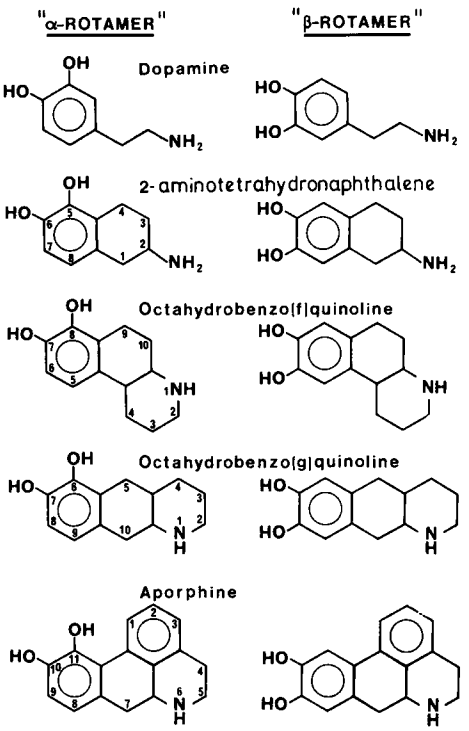

FIG. 1. Carbon skeleton and system of ring numbering of the 'α- and β-rotamers' of dopamine agonists pertinent to the present studies. The compounds used were numbered I to XVII as follows:

I 2-n-Propylamino-5,6-dihydroxytetrahydro naphthalene . HBr
II 2-n-Propylamino-6,7-dihydroxytetrahydro naphthalene . HBr
III 2-n-Propylamino-7,8-dihydroxytetrahydro naphthalene . HBr
IV 2-di-n-Propylamino-5,6-dihydroxytetrahydro naphthalene . HBr
V 2-di-n-Propylamino-6,7-dihydroxytetrahydro naphthalene . HBr
VI cis-7,8-Dihydroxy-1-n-propyl-1,2,3,4,4a,9,10,10b-octahydrobenzo[f]quinoline . HBr
VII *trans*-7,8-Dihydroxy-1-n-propyl-1,2,3,4,4a,9,-10,10b-octahydrobenzo[f]quinoline . HBr
VIII *trans*-7,8-Dihydroxy-1,2,3,4,4a,9,10,10b-octahydrobenzo[f]quinoline . HBr
IX *cis*-6,7-Dihydroxy-1-n-propyl-1,2,3,4,4a,9,10,10b-octahydrobenzo[f]quinoline . HBr
X *trans*-6,7-Dihydroxy-1-n-propyl-1,2,3,4,4a,9,-10,10b-octahydrobenzo[f]quinoline . HBr
XI *trans*-6,7-Dihydroxy-1,2,3,4,4a,9,10,10b-octahydrobenzo[f]quinoline . HBr
XII *cis*-7,8-Dihydroxy-1,2,3,4,4a,9,10,10b-octahydrobenzo[f]quinoline . HBr
XIII *cis*-6,7-Dihydroxy-1,2,3,4,4a,9,10,10b-octahydrobenzo[f]quinoline . HBr
XIV *trans*-1-n-Propyl-6,7-dihydroxy-1,2,3,4,4a,5,10,10a-octahydrobenzo[g]quinoline . HBr
XV *trans*-6,7-Dihydroxy-1,2,3,4,4a,5,10,10a-octahydrobenzo[g]quinoline . HBr
XVI *trans*-1-n-Propyl-7,8-dihydroxy-1,2,3,4,4a,5,10,10a-octahydrobenzo[g]quinoline . HBr
XVII *trans*-7,8-Dihydroxy-1,2,3,4,4a,5,10,10a-octahydrobenzo[g]quinoline . HBr.

Modification of spontaneous locomotor activity by octahydrobenzo(f)-quinoline derivatives

Compounds VIII (7,8-diOH) and XI (6,7-diOH) caused reductions in mouse spontaneous locomotor activity in mg kg^{-1} doses (Fig. 3). There was no clear dose-dependent effect, and piloerection was observed when VIII was used (1·25–20·0 mg kg^{-1} s.c.) although no other disturbances of motor function were apparent. In contrast, the *trans*-n-propyl derivatives VII and X caused dose-related decreases in spontaneous locomotion, and for VII (7,8-di OH) there were no other motor disturbances or autonomic effects. The highest dose of X (6,7-di OH), 1000 μg kg^{-1} s.c., caused muscular hypotonia. Both the cis-n-propyl derivatives VI and IX failed to reduce spontaneous locomotor activity. IX (6,7-di OH) failed to cause any other changes in motor behaviour or autonomic effects, but 125 μg kg^{-1} s.c. of VI (7,8-di OH) caused piloerection, and stereotyped sniffing was observed in the 250–1000 μg kg^{-1} s.c. dose range, with stereotyped biting behaviour developing at 2000–8000 μg kg^{-1} s.c.

Modification of spontaneous locomotor activity by octahydrobenzo(g)-quinoline derivatives

The *trans* compounds XIV, XV and XVI caused dose related decreases in spontaneous locomotor activity of mice which were not associated with other motor or autonomic changes, with the exception of the development of climbing behaviour at 125–500 μg kg^{-1} s.c. of the n-propyl-6,7-dihydroxy analogue (Fig. 4). Only administration of the largest doses, 10 and 20 mg kg^{-1} s.c. of XVII lead to reductions in spontaneous locomotor activity.

Effects of spiroperidol, yohimbine and prazosin on the depression of spontaneous locomotor activity caused by putative dopamine agonists

Doses of spiroperidol (50 μg kg^{-1} i.p.), yohimbine (1·25 mg kg^{-1} i.p.) and prazosin (0·125 mg kg^{-1} i.p.) were selected as the largest doses not themselves causing locomotor depression (see Costall et al 1981). The reduction in spontaneous locomotor activity caused by I, II, III, IV, V, VII, X, XIV, XVI and XVII was specifically antagonised by spiroperidol, yohimbine and prazosin being ineffective (Fig. 5). The motor inhibitory effects of VIII, XI and XV were not significantly modified by any of the three antagonists (Fig. 5).

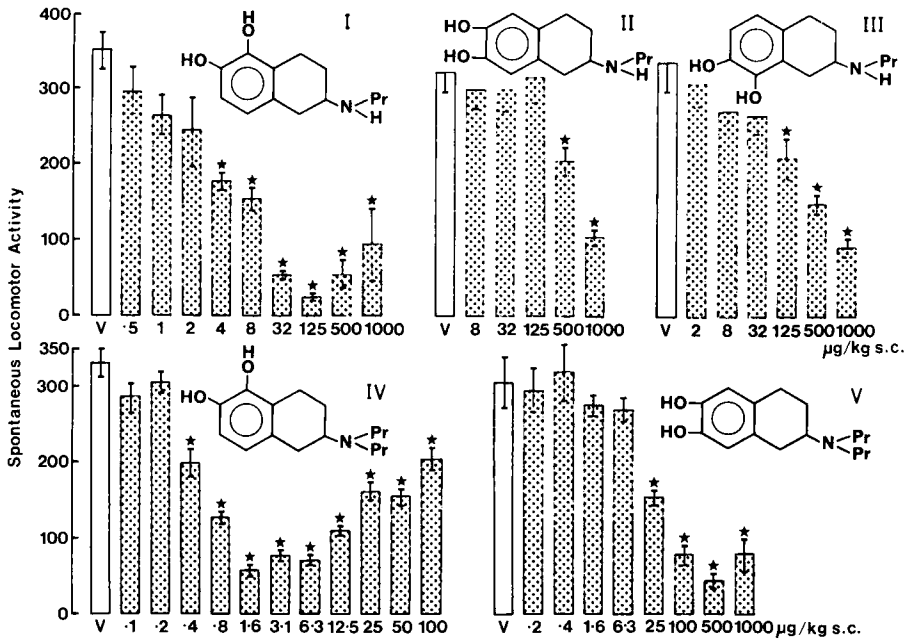

FIG. 2. The reduction in spontaneous locomotor activity caused by various *N*-alkylated TN derivatives I, II, III, IV and V, numbers refer to compounds in Fig. 1. Spontaneous locomotor activity was measured in photocell cages, and is presented in counts/20 min. V represents the response to vehicle. All doses of putative dopamine agonists are given in μg kg^{-1} s.c. n = 10 s.e.m.s are shown. Reductions in spontaneous locomotor activity signficant to * $P < 0{\cdot}01$ – $P < 0{\cdot}001$ (Student's *t*-test).

Effects of pretreatment with pyrogallol on the depression of spontaneous locomotor activity caused by putative dopamine agonists

Initial studies investigated the effect of pyrogallol pretreatment alone on spontaneous locomotor activity. The locomotor activity counts/20 min were: normal 285 ± 27, vehicle 281 ± 23, 50 mg kg^{-1} i.p. pyrogallol 252 ± 18, 100 mg kg^{-1} pyrogallol 176 ± 29, 200 mg kg^{-1} pyrogallol 78 ± 24; a dose of 50 mg kg^{-1} was selected as the maximal dose not reducing activity, and a 4 h pretreatment significantly potentiated (2–7·7 fold) the motor inhibitory effects of V. The motor inhibitory effect of IV was not consistently modified (Fig. 5). There was a trend for the motor inhibitory effects of both *trans*-compounds VII and X to be potentiated (2·2 fold) by the pyrogallol pretreatment, although the differences frequently failed to reach significance (Fig. 6). Similar comments would apply to the effects of the pyrogallol pretreatment on the motor inhibitory effects of XIV and XVI.

The effect of pyrogallol on the activity of catechol-O-methyl-transferase

A 4 h pretreatment with pyrogallol at 25, 50, 100 and 200 mg kg^{-1} i.p. inhibited COMT in the liver by 2, 38, 48 and 57% and in the striatal plus mesolimbic brain tissue by 4, 34, 51 and 64%.

DISCUSSION

An ability to inhibit the spontaneous locomotor activity of mice was selected as the behavioural model for the present studies on the basis of its extreme sensitivity to dopamine agonist action (Strombom 1975; Costall et al 1981). This exceptional responsiveness to potent dopamine agonists, effective in nanogram-microgram per kg doses, ensures that the potential motor inhibitory effects of other dopamine agonists having some ten to a thousand fold reduced potency can still be detected at a reasonable dosage and accurate potency ratios established. This flexibility in dosage administration is not available in other behavioural tests for dopamine agonist action. It is also important that the motor inhibitiory effects of a wide range of dopamine agonists from many chemical series are specifically antagonised by neuroleptic drugs (Costall et al 1981), for some actions of a number of 'dopamine agonists' may actually be mediated via α_1 and/or α_2 adrenergic mechanisms (see review by Costall & Naylor 1981). The following analysis is therefore

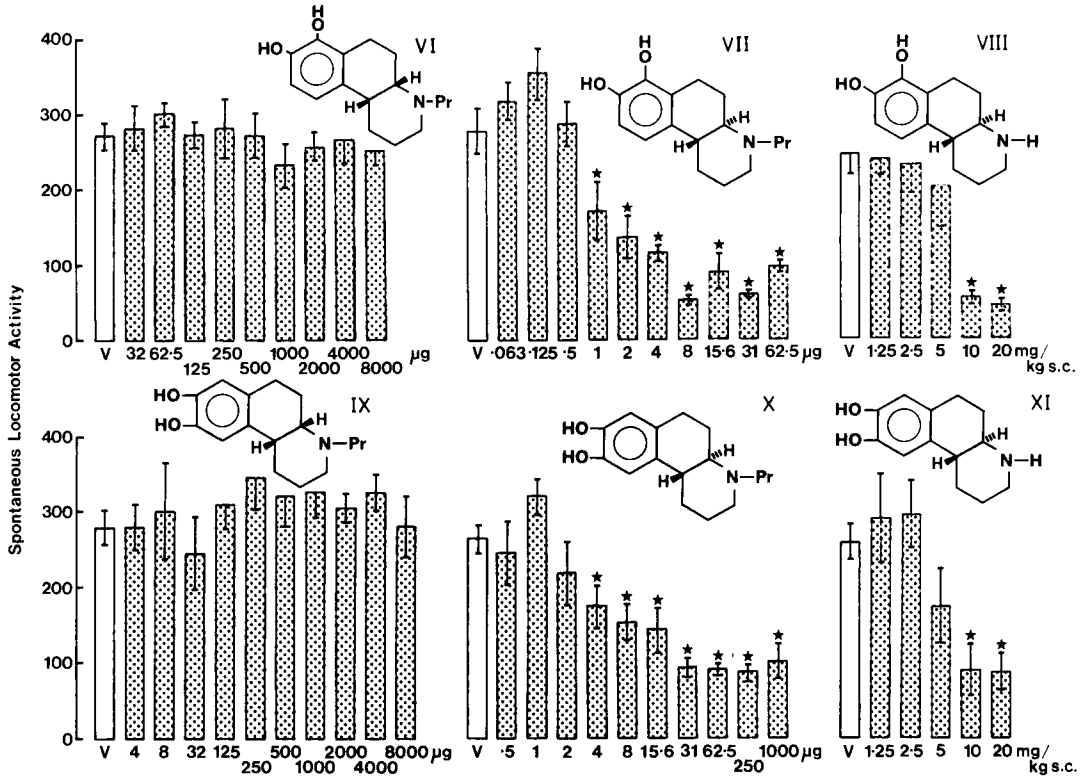

FIG. 3. Effects of octahydrobenzo(f)quinolines on spontaneous locomotor activity of mice. Numbers VI, VII, VIII, IX, X and XI refer to compounds in Fig. 1. Spontaneous locomotor activity was measured in photocell cages, and is presented in counts/20 min. V represents the response to vehicle. Doses of putative dopamine agonists are in µg or mg kg^{-1} s.c. n = 10. s.e.m.s are shown. Reductions in spontaneous locomotor activity significant to * $P < 0{\cdot}01$ – $P < 0{\cdot}001$ (Student's *t*-test).

concerned with dopamine agonist interaction with neuroleptic sensitive mechanisms.

Our use of the *N*-propyl derivatives of the various ATN compounds and octahydrobenzo(f) and (g)quinolines was occasioned by their proven optimal dopamine-like effects in all other behavioural tests (McDermed et al 1975; Cannon et al 1979; Costall et al unpublished data). Only those >N–H derivatives having demonstrable activity following peripheral administration were included: the failure of 2-amino-5,6- and 2-amino-6,7-dihydroxyTN to pass the blood-brain barrier (see Westerink et al 1980) precluded their use in the present studies.

Data obtained showed an entirely consistent and very marked potency difference with respect to the α- and β-rotameric conformations of drugs used. Thus, in order to reduce spontaneous locomotor activity, IV was 339 times more potent than V, and I was respectively 79 times and 179 times more potent than the other catechol isomers II and III. Within the *trans*-octahydrobenzo(f)quinoline series, the α-rotameric *N*-propyl analogue (VII) was 11 times more potent than the β-rotamer (X). (Whilst within this series there was no significant potency difference between the α- and β-rotamers with respect to the >N–H analogues (VIII and XI), very large doses of both agents were required to reduce locomotor activity which could be indicative of non-specific effects, emphasized by concomitant autonomic changes and the failure of spiroperidol to reverse the motor inhibition. These results, therefore, are not considered further). The most marked differences were recorded within the *trans*-octahydrobenzo(g)quinoline series where the α-rotameric *N*-propyl derivative was 467 times more potent than the β-rotamer, and the α-rotameric >N–H analogue was 46 fold more potent than the β-rotamer. These potency ratios are summarized in Table 1 and related to a stereotypic potential. For each measure the relatively greater potency of the α-rotamers is both obvious and marked, and can be extended to the aporphine series. Furthermore, whilst the present experiments have been limited to an analysis of the effects of catechol compounds,

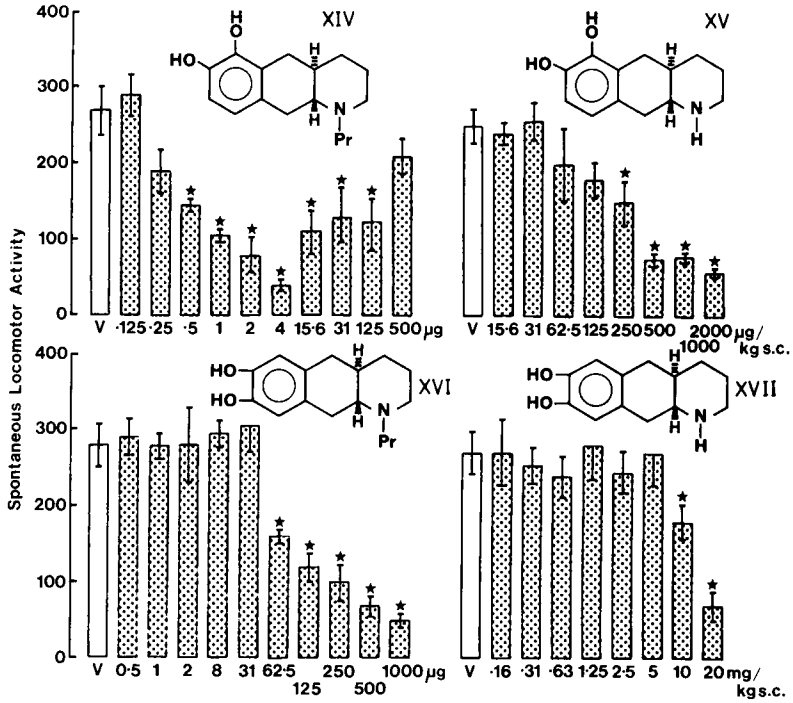

FIG. 4. Effects of octahydrobenzo(g)quinolines on spontaneous locomotor activity in mice. Numbers XIV, XV, XVI and XVII refer to compounds in Fig. 1. Spontaneous locomotor activity was measured in photocell cages, and is presented in counts/20 min. V represents the response to vehicle. Doses of putative dopamine agonists are in µg or mg kg^{-1} s.c.n. = 10. s.e.m.s are shown. Reduction in spontaneous locomotor activity significant to * $P < 0.01$ – * $P < 0.001$ (Student's *t*-test).

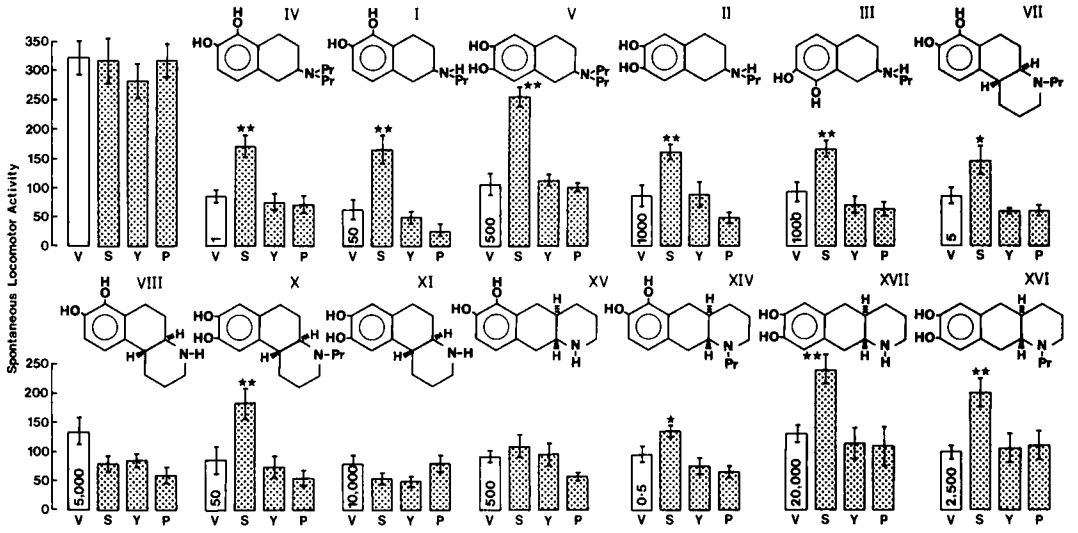

FIG. 5. Abilities of spiroperidol, yohimbine and prazosin to reverse the inhibition of spontaneous locomotor activity of mice caused by putative dopamine agonists, numbered IV, I, V . . . . XVI as in Fig. 1. Spontaneous locomotor activity was measured in photocell cages, and is presented in counts/20 min. V, open column, indicates the mean response of animals given the vehicle for spiroperidol (S), yohimbine (Y) or prazosin (P). V, dose inserted in open column, indicates the mean response of animals given the vehicle for S, Y or P plus the given dose of the appropriate dopamine agonist, µg kg^{-1} s.c. Doses of S, Y and P were 50 µg kg^{-1} i.p., 1·25 mg kg^{-1} i.p. and 0·125 mg kg^{-1} i.p. respectively. n = 10 s.e.m.s are shown. Reversal of the dopamine agonist inhibition by spiroperidol significant to * $P < 0.05$ or ** $P < 0.01$ (Student's *t*-test).

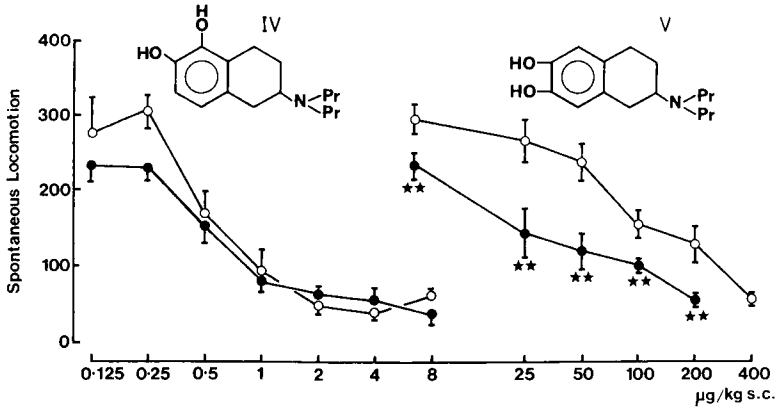

FIG. 6. Effect of pyrogallol pretreatment (50 mg kg^{-1} i.p. 4 h) on the locomotor depression caused by the TN derivatives IV and V (see Fig. 1). Spontaneous locomotor activity was measured in photocell cages, and is presented in counts/20 min. ●—● response of agonist in the presence of pyrogallol, ○—○ response of agonist in animals treated with the solvent for pyrogallol. Difference between these responses significant to ** $P < 0{\cdot}01$ (Student's t-test). s.e.m.s are given. n = 10.

McDermed et al (1976) have used the 5, 6 or 7 monohydroxylated derivatives of 2-di-n-propylTN and shown the 5-hydroxy analogue to be 144 and 256 times more potent to induce stereotypy than the 6 or 7 monohydroxylated derivatives respectively. The conclusion from all these studies is inescapable: to secure a dopaminergic behavioural effect from peripheral drug administration the α-rotamer is the preferred conformation.

Whether the behavioural effect is the consequence of preference for the α-rotamer by the dopamine receptor is subject to debate: whilst data from previous intracerebral injection studies have lead us to conclude that the actual (striatal) dopamine receptor has preference for the α-rotamer (Cannon et al 1977), a number of authors have argued that the biochemical evidence supports exactly the opposite conclusion (Woodruff et al 1977: Horn & Rodgers 1980). Thus, the β-rotamer 2-amino-6,7-dihydroxyTN is 50 times more potent than the α-rotamer 2-amino-5,6-dihydroxyTN to stimulate striatal dopamine sensitive adenylate cyclase (Woodruff et al 1977) and is 17 times more potent to displace [^{3}H]dopamine and [^{3}H]apomorphine in receptor labelling assays (Seeman et al 1977). Horn & Rodgers (1980) have suggested that 'the consistent in vitro data are probably a much better guide to the receptor-site preferred conformation of DA than are the behavioural results'. However, if one accepts that 'in vitro' studies provide a more reliable index of agonist-receptor interaction, and consider the available data for the *N*-propyl derivatives, a different conclusion is inevitable. Thus, IV is twice as potent as V in stimulating striatal dopamine-sensitive adenylate cyclase (Cannon et al 1978) and is 4 times more potent in displacing [^{3}H]haloperidol in binding assays (Cannon et al 1978); I is 26 times more potent than II to displace [^{3}H]haloperidol (Cannon et al 1978). Also, in [^{3}H]apomorphine and [^{3}H]dopamine binding assays, although 2-amino-6,7-dihydroxyTN was 17 times more potent than the 5,6-isomer, *N*-propyl substitution reduces or abolishes this difference such that II was only 3 times as potent as I and IV and V were equipotent (Seeman et al 1977). The potencies of a series of monohydroxylated derivatives of 2-di-n-propylaminoTN to displace [^{3}H]apomorphine is also relevant: 2-di-n-propylamino-5-hydroxyTN has been shown to be 38

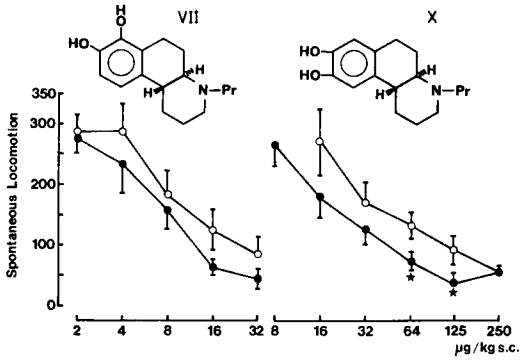

FIG. 7. Effect of pyrogallol pretreatment (50 mg kg^{-1} i.p. 4 h) on the locomotor depression caused by the benzo[f]-quinoline derivatives VII and X (see Fig. 1). Spontaneous locomotor activity was measured in photocell cages, and is presented in counts/20 min. ●—● response of agonist in the presence of pyrogallol, ○—○ response of agonist in animals treated with the solvent for pyrogallol. Differences between the responses significant to * $P < 0{\cdot}05$ (Student's t-tests). s.e.m.s are given. n = 10.

Table 1. Potency ratios of α- and β-rotamers from the ATN and *trans*-octahydrobenzo(f) and (g)quinolines for inhibition of spontaneous locomotor activity (mouse) and stereotypy induction (rat).

Purported dopamine agonist	Motor inhibition ED50 μg kg^{-1} s.c.			Stereotypy induction ED50 μg kg^{-1} s.c.		
	α	β	β/α	α	β	β/α
IV/V	0·62	210	339	12·5[b]	1000[b]	80
I/II	3·8	300	79	500[b]	>16 000[b]	>32
VII/X	2·0	23	11·5	50[a]	>10 000[c]	>200
VIII/XI	7200*	6000*	0·83	5000[a]	>10 000[c]	>2
XVI/XVII	330	15000	46	?	>10 000[c]	?
XIV/XVI	0·45	210	467	6·3[d]	>10 000[c]	>1587
Apomorphine/ isoapomorphine	16[c]	>1000[c]	>63	0·5[a]	>10[d]	>20

[a] Cannon et al 1979.
[b] Cannon et al 1977.
[c] Cannon et al 1980.
[d] Costall et al unpublished data.
[e] Costall et al 1980.
* Non-dopaminergic effect, see text.

Table 2. Potency ratios for α- and β-rotamers of the ATN derivatives to inhibit [^{3}H]apomorphine and [^{3}H]haloperidol binding.

Dopamine agonist	IC50 (nM) α	β	β/α	Ligand
2-Amino-5,6 or -6,7-diOHTN	55	3·3	0·06	[^{3}H]Apomorphine
	1900	263	0·01	[^{3}H]Haloperidol
I	160	50	0·31	[^{3}H]Apomorphine
II	17	441	26	[^{3}H]Haloperidol
IV	12	13	1·1	[^{3}H]Apomorphine
V	20	76	3·8	[^{3}H]Haloperidol
2-Di-n-propylmino-5-OHTN	24			[^{3}H]Apomorphine
6-OHTN	900		38*	[^{3}H]Apomorphine
7-OHTN	520		22*	[^{3}H]Apomorphine

[^{3}H]Apomorphine binding data taken from Seeman et al (1977) and Tedesco et al (1979). [^{3}H]haloperidol binding data taken from Cannon et al (1978), where the IC50 values are taken to indicate the concentration required to displace binding by 50%. * ratios with respect to data obtained using the 5-hydroxyTN.

and 22 times more potent respectively than the corresponding 6-hydroxy and 7-hydroxy derivatives (Tedesco et al 1979). The relevant binding data is summarized in Table 2. One thus concludes that the indication of preferred conformation from biochemical data depends on whether the comparison is made using the primary amines or the *N*-alkylated derivatives. Thus, using the primary amines of the ATN series may indicate a greater potency for 2-amino-6,7-dihydroxyTN and, hence a β-rotametric preference, whilst preference changes in *N*-alkylation, optimal following *NN*-dipropyl substitution and showing IV to have equal or greater potency than V in all biochemical measures, would suggest an α rotameric preference. However, even though both the present behavioural data and that described for biochemical experiments using the *N*-alkylated ATN's is consistently persuasive of an α rotameric

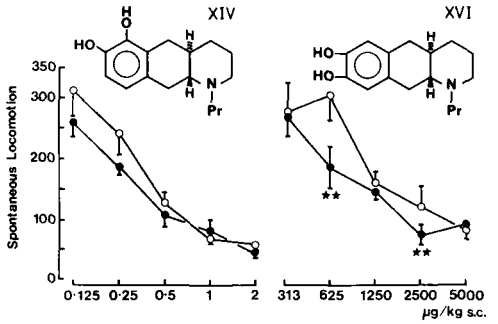

FIG. 8. Effect of pyrogallol pretreatment (50 mg kg^{-1} i.p. 4 h) on the locomotor depression caused by the benzo[g]-quinolines XIV and XVI (see Fig. 1). Spontaneous locomotor activity was measured in photocell cages, and is presented in counts/20 min. ●—● response of agonist in the presence of pyrogallol, ○—○ response of agonist in animals treated with the solvent for pyrogallol. Differences between these responses significant to * $P < 0{\cdot}01$ (Student's *t*-test). s.e.m.s are given. n = 10.

preference, it must be pointed out that the enhanced potency of the *N*-alkylated 5,6-dihydroxyTN analogues in most biochemical tests is small, and unlikely to explain the marked potency differences observed in the behavioural tests.

An additional factor which may influence the relative potencies of α- and β-rotamers to cause behavioural change is differential brain penetration. Detailed studies by Westerink et al (1980) have shown that the brain levels of 2-amino-5,6-dihydroxyTN are 2·5–4·0 fold higher than those achieved by the 6,7-isomer, and the persistence of the brain levels of 2-amino-5,6-dihydroxyTN contrasts with the rapid removal and metabolic degradation of the 6,7-isomer. In a subsequent study the differences in brain concentrations of the isomers were attributed to a difference in susceptibility to metabolism by COMT (Rollema et al 1980). If a difference in behavioural potency between the α- and β-rotameric forms reflects this differential metabolism by COMT, then inhibition of COMT activity would be expected to selectively increase the effects of the β-rotamers. To test this hypothesis the COMT inhibitor pyrogallol was administered in the maximum dose not causing motor depression but which was shown to reduce COMT activity by 34–38%. Such treatment failed to modify the motor inhibitory action of IV whilst potentiating the effects of V 2 to 7·7 fold. This observation would support the hypotheses of Rollema et al (1980) that a more rapid metabolic degradation of the β-rotamer via COMT may be an important factor in deciding the relative potencies of the α- and β-rotamers. However, in the experiments using the *N*-propyl derivatives in the *trans*-octahydrobenzo(f) and (g)quinoline series, whilst there was a clear trend for

the motor inhibitory effects of the β-rotamers to increase after pyrogallol treatment, the differences were not always significant and, possibly more important, were very comparable to shifts in the dose response curves for the α rotameric forms. A more effective (and specific) inhibition of COMT is essential before any firm conclusion can be made as to the importance of *O*-methylation for determining α- and β-rotameric potencies; the use of higher doses of pyrogallol causing more effective enzyme inhibition was precluded by their marked sedative action.

It remains possible that other forms of metabolism may differentially degrade the α- and β-rotameric forms of the various dopamine agonists. For example, it has recently been shown that apomorphine and 2-amino-5,6-dihydroxyTN are respectively 30 and 100 times more potent than the 6,7-isomer in inhibiting monoamine oxidase (Hoffmann et al 1980). Whilst this may partially explain the enhanced behavioural potency of the 5,6-compound, a presynaptic action would appear more relevant to the effects of the 6,7-isomer (Horn et al 1978; Hoffmann et al 1980; Mulder et al 1980) but not to the actions of agents such as IV and V which do not appear to mediate their effects via neurotransmitter release or uptake blockade. Nevertheless, it remains possible that differences in pharmacological potency between some of the compounds may reflect a differential metabolism where, for example, metabolism by monoxygenase enzymes can show stereoselectivity and rotameric discrimination.

Alternatively, it remains possible that the various *N*-alkylated derivatives in the α-rotameric conformation may have a relatively easier passage through the blood-brain barrier. However, there is no evidence presently available to support this hypothesis: the induction of hyperactivity or stereotypy after direct intracerebral injection, into the nucleus accumbens, is marked for the α-rotameric *N*-alkylated derivatives used in the present work but, for the β-rotameric analogues, is absent or so weakly developed as to preclude the estimate of a potency ratio (Costall et al 1977).

Thus, it becomes apparent that attempts to define the preferred rotameric conformation for dopamine agonist action in a single definitive form may be an illusory quest. Although the present studies consistently indicate that the α rotameric conformations within the aporphine, ATN, *trans*-octahydrobenzo(g) and (f)quinoline series demonstrate relatively greater potency, biochemical studies may indicate a preferred α- *or* β-rotameric conformation dependent on the dopamine agonist used and the biochemical index of dopamine agonist action. Further, receptor labelling studies indicate that the preference may change *within* a chemical series, depending on the degree of *N*-alkylation. This data would emphasize the need to include different types of dopamine agonists from the aminotetrahydronaphthlenes and other series, *not* simply the primary amines, in any future studies aiming at an elucidation of the relative importance of the α- and β-rotameric conformations for dopamine agonist activity.

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