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EFFECTS OF SOME DERIVATIVES OF 2-AMINOTETRALIN ON DOPAMINE-SENSITIVE ADENYLATE CYCLASE AND ON THE BINDING OF [^{3}H]HALOPERIDOL TO NEUROLEPTIC RECEPTORS IN THE RAT STRIATUM

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Abstract—Putative dopamine agonists from a series of 2-aminotetralin derivatives were assessed for their ability to stimulate dopamine-sensitive adenylate cyclase and to inhibit the binding of [^{3}H]haloperidol to neuroleptic receptors in the rat striatum. Comparisons were made to the ability of these agents to stimulate motor function on intrastriatal injection (stereotyped biting/hyperactivity) or peripheral administration (stereotyped biting). Of the two primary amines, 2-amino-5,6-dihydroxytetralin and 2-amino-6,7-dihydroxytetralin, the 6,7-dihydroxy compound was found to be 20 times more potent than the corresponding 5,6 derivative to stimulate adenylate cyclase. The activities of a series of mono- and di-alkylated substitutes of the two primary amines (methyl, ethyl, propyl, butyl) were determined. Of particular interest, and in contrast with the primary amines, 2-(*N*,*N*-dipropyl)amino-5,6-dihydroxytetralin was shown to be twice as potent as the corresponding 6,7-derivative. Of the compounds which were shown to stimulate adenylate cyclase, little difference was found in their activities. In the second assay procedure, inhibition of [^{3}H]haloperidol binding, 2-(*N*,*N*-dipropyl)amino-5,6-dihydroxytetralin was the most active compound studied (a hundred times activity of dopamine). Similarly to observations with adenylate cyclase, the 6,7- derivative was the more active of the primary amines (times ten) whilst the 5,6- derivative was the more active of the *N*,*N*-dipropyl compounds (times three). General comparisons could be made between the biochemical findings and behavioural observations: those agents most active to induce dopamine-like motor effects on subcutaneous or intrastriatal injection were generally effective to stimulate adenylate cyclase and to inhibit [^{3}H]haloperidol binding, whilst compounds inactive behaviourally were also inactive *in vitro* [*2-(amino)-5,6-dimethoxytetralin* and 2-(*N*, *N*-dimethyl)amino-5,6-dimethoxytetralin]. However, an absolute correlation between *in vivo* and *in vitro* potency could not be found. The most important observation of the present studies is the potency of 2-(*N*,*N*-dipropyl)amino-5,6-dihydroxytetralin in the [^{3}H]haloperidol binding assay which indicates that this 2-aminotetralin derivative may be a useful tool in future studies on dopamine receptors.

Many behavioural studies have indicated that neostriatal dopaminergic mechanisms may modulate motor activity [1, 2]. In biochemical studies *in vitro* using striatal tissue a dopamine sensitive adenylate cyclase has been identified as a potential site of dopamine action [3, 4]. On the other hand binding assays *in vitro* have been described which provide a means for labelling specific sites for neuroleptic agents and this receptor model has also been proposed as a possible site of dopamine action [5, 6]. Recent subcellular fractionation studies have provided evidence that dopamine sensitive adenylate cyclase and the specific neuroleptic binding sites, although both located in the striatum, are two different entities [7, 8].

In a series of behavioural experiments it has been demonstrated that certain *N*-alkylated derivatives of 2-aminotetralin possess potent dopamine-like properties to alter motor function both when administered peripherally and when injected directly into striatal tissue [9, 10]. In the present study we assess the ability of these agents to stimulate striatal adenylate cyclase and to bind to the striatal dopamine receptor.

MATERIALS AND METHODS

For biochemical experiments *in vitro* female Wistar rats (150 g) were used. After decapitation the striata were immediately dissected and cooled at 0°. For the adenylate cyclase measurements striata were homogenized in 20 vol. of Tris–maleate buffer (10 mM, pH 7.4) containing 2.9 mM EGTA using a Duall. For neuroleptic receptor binding the striata were homogenized with an Ultra Turrax in 40 vol. Tris buffer (pH 7.6, 0.05 M). The homogenate was centrifuged at 30,894 *g* for 30 min. The supernatant was discarded and the pellet dissolved in 80 vol. per 1 g original tissue of the Tris buffer containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 0.1% ascorbic acid and 10 μM pargyline.

Adenylate cyclase assay. An incubation mixture of 0.450 ml contained 1 μmole $MgSO_4$, 0.1 μmole EGTA, 2.5 μmole theophylline, 40.5 μmole Tris, 2.5 mg striatal tissue, maleate to obtain pH 7.4 and an appropriate amount of dopamine or tetralin (Cannon) derivative. After incubation for 2.5 hr the reaction was stopped by boiling the mixture for 2.5 hr. The adenosine, 3′,5′ cyclic monophosphate

(cAMP) content was measured using a cAMP assay kit (Radiochemical Centre, Amersham), based on competition of unlabelled cAMP with a fixed quantity of the tritium-labelled compound for binding of a protein which has a high specificity for cAMP[11].

EC_{50}, i.e. 50 per cent activation of basal activity (without dopamine), was graphically estimated from dose–response curves composed of at least seven points (each point being the mean of three determinations) in a concentration range between 10^{-7} and 10^{-3} M.

Neuroleptic receptor binding assay. An incubation mixture of 2.2 ml Tris buffer (0.05 M, pH 7.6 with salts as above) contained 25 mg striatal tissue, 4.4 pmoles [^{3}H]haloperidol (spec. act. 9 Ci/m-mole) (Janssen Pharmaceutica) and either 4.4 m-moles (+)-butaclamol, or 0.44 nmoles (−)-butaclamol or an appropriate amount of tetralin. After incubation for 10 min at 37° the mixture was filtered under suction through Whatman GF/B glass fiber filters. Radioactivity on the filter paper was estimated in a Packard Tri Carb liquid scintillation spectrometer. The IC_{50} values, defined as the concentration producing 50 per cent inhibition of the stereospecific haloperidol binding (i.e. the difference in bound radioassay between assays in the presence of (−)-butaclamol and (+)-butaclamol), were estimated graphically from dose–response curves, composed of six points (each point being the mean of n determinations) between 10^{-9} and 10^{-6} M[12]. The IC_{50} values are the mean of four independently performed determinations.

RESULTS

The compounds under investigation are listed in Table 1. Also indicated are: the EC_{50}-values for adenylate cyclase activation, (2) the IC_{50}-values for stereospecific [^{3}H]haloperidol binding, (3) the minimal dose in nmoles required to induce a consistent motor response on bilateral intrastriatal injection[10], and (4) the minimal dose required to produce a consistent biting response on subcutaneous (S.C.) injection[10].

Certain aminotetralins were found to be as active as dopamine to produce 50 per cent activation of adenylate cyclase activity (EC_{50} in the μmolar range). The primary amine, 2-amino-5,6-dihydroxytetralin, was ten times less active than the corresponding tertiary amines whilst the secondary, *N*-methyl and *N*-butyl derivatives, were inactive. Of the 6,7-dihydroxytetralins tested, the primary amine was shown to be the most active followed by the *N*-methyl and *N,N*-dipropyl derivatives. The monopropyl derivative was one order of magnitude less potent whilst the *N,N*-dimethyl compound was inactive.

As regards activity on neuroleptic receptor binding, certain compounds appeared to be potent inhibitors of stereospecific haloperidol binding (IC_{50} in the 10^{-8} M range). The primary amines were not classified amongst the most potent inhibitors. The *N*-butyl, the methoxylated compounds and the tetralins which lacked the catechol hydroxy functions were virtually inactive.

DISCUSSION

Within the series of *N*-alkylated 2-aminotetralins tested in the present study, certain compounds have been shown to possess potent dopamine-like effects on motor function (as indicated by stereotype induction) after peripheral injection[9, 13]. It is obvious that the possible differing abilities of these drugs to penetrate into the brain, different rates of peripheral metabolism and the potential to activate a number of biochemical systems in many brain areas makes a correlation of the behavioural data resulting from peripheral injection to their activity in biochemical assays *in vitro* using one particular brain area highly speculative. In an attempt to overcome some of these difficulties we have compared the activities *in vitro* of the 2-aminotetralins with their abilities to induce stereotyped behaviour on intrastriatal injection. In the behavioural studies, the most potent compound appeared to be 2-(*N,N*-dipropyl)amino-5,6-dihydroxytetralin: its dopamine-like activity was marked both after peripheral and intrastriatal injection, approximately 10 nmoles producing a definite effect via the latter route. This agent was also extremely active to produce dopamine-like effects in the biochemical assays *in vitro*. Thus, 2-(*N,N*-dipropyl)amino-5,6-dihydroxytetralin was found to be equipotent with dopamine on the adenylate cyclase assay, producing a 50 per cent activation of enzymatic activity at 3×10^{-6} M and, in this test, such compounds which are active in the micromolar range are the most potent so far detected. However, 2-(*N,N*-dipropyl)-amino-5,6-dihydroxytetralin exerted even more marked dopamine-like activity in the neuroleptic receptor binding test. With its IC_{50}-value of 2×10^{-8} M it was more active than any other known dopamine-like agent.

Some correlation was found between the results of the behavioural studies and the effects of the 2-aminotetralins on adenylate cyclase. Compounds which were very active after intrastriatal injection (i.e. in the order of 10 to 1 nmole) were also found to stimulate adenylate cyclase (e.g. the *N,N*-diethyl and *N,N*-dipropyl derivatives) whilst compounds which were only slightly active or inactive in the test *in vivo* were also unable to stimulate adenylate cyclase (e.g. the *N*-butyl derivative). However, among the active compounds, the activity range in the enzymatic test was very slight and an *absolute* correlation with the potency *in vivo* could not be found[14]. In contrast, the differential activities in the binding test, in which the relative affinity for the neuroleptic receptor was measured, were much more pronounced. The results obtained from this test *in vitro* were in agreement with the observations *in vivo* as regards the differentiation between active and inactive compounds. However, a significant correlation between the receptor affinity and the activity *in vivo* was not found.

As emphasised above, optimal binding affinity was achieved with the *N,N*-dipropyl-5,6-dihydroxy derivative. Monosubstituted *N*-alkyl derivatives, -methyl, -ethyl, and -propyl were approximately equipotent with the corresponding di-methyl and di-ethyl derivatives. The inactivity of the di-methyl derivative after intrastriatal injection is difficult to

Table 1. Effects of some derivatives of 2-aminotetralin on dopamine sensitive adenylate cyclase and on the binding of [^{3}H]haloperidol to neuroleptic receptors in the rat striatum, and behavioural effects induced by the tetralins on intrastriatal and peripheral administration

2-aminotetralin derivative	Stimulation of adenylate cyclase EC_{50}(M)*	Inhibition of haloperidol binding IC_{50}(M)† ± S.E.M.	Induction of stereotypy/ hyper-activity on intra-striatal injection (M)‡	Induction of stereotypy on s.c. injection (mg/kg)‡
2-Amino-5,6-dihydroxytetralin	5.24×10^{-5}	1.9×10^{-6} ± 0.14	8.9×10^{-9}	Inactive (16)
2-(*N*-Methyl)amino-5,6-dihydroxytetralin	Inactive (10^{-5})	3.62×10^{-7} ± 0.46	1.8×10^{-7}	Inactive (16)
2-(*N*-Ethyl)amino-5,6-dihydroxytetralin	N.T.	1.21×10^{-7} ± 0.14	8.0×10^{-9}	2
2-(*N*-Propyl)amino-5,6-dihydroxytetralin	N.T.	1.67×10^{-8} ± 0.15	1.5×10^{-8}	1
2-(*N*-Butyl)amino-5,6-dihydroxytetralin	Inactive (10^{-5})	8.65×10^{-6} ± 3.24	Inactive (1.6×10^{-7})	Inactive (16)
2-(*N*,*N*-Dimethyl)amino-5,6-dihydroxytetralin	N.T.	2.78×10^{-7} ± 0.28	Inactive (1.7×10^{-7})	1
2-(*N*,*N*-Diethyl)amino-5,6-dihydroxytetralin	4.36×10^{-6}	1.01×10^{-7} ± 0.25	9.9×10^{-9}	0.5
2-(*N*,*N*-Dipropyl)amino-5,6-dihydroxytetralin	3.31×10^{-6}	2.01×10^{-8} ± 0.32	1.8×10^{-8}	0.025
2-Amino-6,7-dihydroxytetralin	2.63×10^{-6}	2.63×10^{-7} ± 0.61	3.6×10^{-8}	Inactive (16)
2-(*N*-Methyl)amino-6,7-dihydroxytetralin	5.50×10^{-6}	1.39×10^{-7} ± 0.28	9.2×10^{-8}	Inactive (16)
2-(*N*,Propyl)amino-6,7-dihydroxytetralin	3.02×10^{-5}	4.41×10^{-7} ± 0.77	4.1×10^{-8}	Inactive (16)
2-(*N*,*N*-Dimethyl)amino-6,7-dihydroxytetralin	Inactive (10^{-6})	9.22×10^{-8} ± 2.62	1.3×10^{-7}	Inactive (16)
2-(*N*,*N*-Dipropyl)amino-6,7-dihydroxytetralin	6.30×10^{-6}	7.64×10^{-8} ± 0.88	Inactive (1.4×10^{-7})	4
2-Amino-5,6-dimethoxy-tetralin	N.T.	$>10^{-5}$	Inactive (1.7×10^{-7})	Inactive (16)
2-(*N*,*N*-Dimethyl)amino-5,6-dimethoxytetralin	N.T.	$>10^{-5}$	Inactive (1.5×10^{-7})	Inactive (16)
2-(*N*,*N*-Dimethyl)-amino-tetralin	N.T.	9.13×10^{-6} ± 1.67	Inactive (2.3×10^{-7})	Inactive (16)
2-(*N*,*N*-Diethyl)amino-tetralin	N.T.	4.83×10^{-6} ± 0.86	Inactive (2.1×10^{-7})	Inactive (16)
2-(*N*,*N*-Dipropyl)amino-tetralin	N.T.	2.4×10^{-6} ± 0.56	Inactive (1.9×10^{-7})	16
Dopamine	3.10×10^{-6}	2.99×10^{-6}	6.2×10^{-8}	Inactive (100)

* EC_{50}(M) refers to the concentration required to cause half-maximal stimulation.

† IC_{50}(M) refers to the concentration required to cause 50 per cent inhibition of [^{3}H]haloperidol binding (2×10^{-9} M) to rat striatum total particulate fraction.

‡ Refers to the minimal dose required to produce a consistent motor response. See Cannon and colleagues for details of methodology [10].

N.T.—not tested.

explain, especially in view of the fact that after peripheral administration the compound exerted a moderate stereotypic effect. However, the *in vivo* and *in vitro* tests did correlate as regards a number of observations. Lengthening the alkyl chain to four carbon atoms abolished both *in vivo* and *in vitro* activity, and both situations emphasised the necessity of a catechol group for activity (methoxy derivatives or compounds lacking the hydroxyl groups were virtually inactive). The most marked discrepancy between the potency on neuroleptic receptor binding and the effect on motor function was found with the primary amines. The primary amines, similarly to dopamine, displayed only a moderate affinity for the neuroleptic receptor. On peripheral administration these compounds were also without effect. However, this negative behavioural effect may be explained by the inability of the compounds to cross the blood–brain barrier (Woodruff, personal communication). Indeed, a pronounced locomotor activity was observed after intrastriatal injection, 2-amino-5,6-dihydroxy- and 2-amino-6,7-dihydroxytetralin being respectively seven and two times more active than dopamine. The very low receptor affinity of the primary amines is difficult to interpret in view of their effect *in vivo*. A possible explanation

could be that the primary amines, although showing a lower affinity, introduce a much stronger physiological reaction once they occupy the receptor i.e. they have a greater 'intrinsic' activity. In the neuroleptic receptor binding assay only the *relative* affinity of the compounds for the receptor is measured, and this correlates with the potency *in vivo* of drugs only *within* a given class of compounds. Information on the 'intrinsic' activity, which is involved in the activity *in vivo* of agonists, but which is absent in the effect of antagonists [12], is not obtained in the receptor binding test. It can therefore be questioned as to whether the dopamine-like compounds which display a high receptor affinity may have a partial agonist–antagonist activity. It would be tempting to speculate that the 'intrinsic' activity of the compounds may be better reflected by their relative abilities to stimulate adenylate cyclase. However, the present results failed to lend any support to this hypothesis.

It is also possible that the 'neuroleptic receptor' and 'dopamine receptor' are not entirely the same. Seeman and colleagues have recently shown that a number of 2-aminotetralin derivatives are potent to displace [^{3}H]dopamine and [^{3}H]apomorphine in calf caudate preparations [15, 16] (*also* Tedesco, Bowles, McDermed and Seeman, in preparation). Most interesting is the high affinity of the primary amines (2-amino-5,6- and 2-amino-6,7-dihydroxytetralin) for [^{3}H]dopamine and [^{3}H]apomorphine binding sites in contrast to their low affinity for the [^{3}H]haloperidol sites. It should be considered that agonistic activity *in vivo* may be related to an affinity of the agonists for the [^{3}H]dopamine receptors, notwithstanding that all the behavioural effects induced by the 2-aminotetralin derivatives are specifically antagonised by haloperidol [13].

A general conclusion from the present work is that the striatal adenylate cyclase and the specific neuroleptic binding sites, although responding in a different way, are both targets for agents producing dopamine-like effects on motor function. Of particular importance was the finding that 2-(*N*,*N*-dipropyl)amino-5,6-dihydroxytetralin exhibited an affinity for the neuroleptic receptor which was approximately one hundred times greater than that of dopamine, and this compound is therefore by far the most potent dopamine agonist as yet assessed in this test situation. Hence, this compound may be regarded as the reference dopamine agonist in the biochemical studies and labelled 2-(*N*,*N*-dipropyl)-amino-5,6-dihydroxytetralin may prove to be a most interesting tool for further studies on dopamine receptors.

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