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EFFECT OF NEUROLEPTICS ON INDOLEAMINE-*N*-METHYLTRANSFERASE ACTIVITY AND BRAIN METABOLISM OF BUFOTENIN

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Abstract—A range of neuroleptics, non-neuroleptic phenothiazines and antidepressants were tested for their ability to inhibit purified rabbit lung indoleamine-*N*-methyltransferase (INMT) and to modify the metabolism of [^{3}H]bufotenin administered intraventricularly. Benperidol and droperidol proved to be the most potent inhibitors of INMT among the compounds tested. On the other hand trifluperidol and haloperidol showed no inhibitory activity at concentrations up to 2.5 mM, while chlorpromazine, thioridazine, pericyazine and perphenazine were effective only at 250 μM or 2.5 mM. Oxidative deamination was shown to be an important pathway in normal rat brain for the metabolism of bufotenin. Of the drugs tested only chlorpromazine elevated the brain [^{3}H]bufotenin/[^{3}H]-5-HIAA ratio whereas pimozide and thiothixene caused a decrease. These findings suggest that neuroleptic drugs in general do not exert their anti-psychotic effects by inhibiting the synthesis or accelerating the oxidative deamination of *N,N*-dimethylated indoleamines.

Of the naturally occurring substances which have been implicated in the aetiology of schizophrenia, the hallucinogenic *N,N*-dimethylated indoleamines have attracted much attention. There have been numerous reports that *N,N*-dimethyltryptamine (DMT)† and bufotenin occur more frequently in the blood [1–3] or urine of schizophrenics [1, 4–8] than controls. Other studies have failed to replicate these findings [9–11]. The involvement of this group of compounds in the aetiology of schizophrenia therefore remains controversial.

One of the criteria proposed by Hollister [12] for the evaluation of putative endogenous 'schizotoxins' is that neuroleptics should be capable of inhibiting the synthesis, increasing the metabolism or antagonizing the behavioural effects of these substances. This study reports firstly the influence of a range of anti-psychotic and other drugs upon the activity of purified indoleamine-*N*-methyltransferase (INMT) the enzyme which converts tryptamine and 5-hydroxytryptamine to DMT and bufotenin respectively and which is widely distributed in the tissues of animals and man [13–18]. Secondly, it describes the effect of neuroleptic medication on brain metabolism of bufotenin.

MATERIALS AND METHODS

Preparation and assay of INMT. INMT was extracted from male New Zealand albino rabbit lung and partially purified by ammonium sulphate precipitation and DEAE-cellulose chromatography as previously described [19]. The enzyme was further chromatographed on a column of Sephadex G-150 (fine) to yield a specific activity of 92.1 nmoles DMT formed/mg protein/hour. This amounted to 108-fold purification compared to the original post-microsomal supernatant. INMT activity was assayed using *N*-methyltryptamine (NMT) as substrate [19] and an incubation time of 15 min. Various drugs were added to INMT incubations as indicated in Table 1. In these experiments solvents used to dissolve the drugs were included in blank assays. Protein concentration was determined by the method of Lowry *et al.* [20] using crystalline bovine albumin as standard.

Preparation of [^{3}H]bufotenin. [^{3}H]Bufotenin was prepared by a catalytic exchange in tritiated water by the Radiochemical Centre, Amersham, U.K. On arrival in the laboratory the crude [^{3}H]bufotenin was further purified in the dark by solvent extraction and thin layer chromatography. The radiochemical purity of the purified [^{3}H]bufotenin was 93 per cent as determined by thin layer chromatography on silica gel in methanol–1M NH_4OH (10:3). The specific activity of the purified [^{3}H]bufotenin was calculated to be 3.8 Ci/mmole. Bufotenin was assayed by means of the fluorometric procedure of Narasimhachari and Plaut [21].

Effects of neuroleptics on cerebral metabolism of [^{3}H]bufotenin. Groups each consisting of four male Wistar rats (150 g) were injected intraperitoneally (i.p.) daily for seven days with various doses of chlorpromazine or haloperidol. Control animals received saline. The animals were allowed food and water *ad lib.* [^{3}H]Bufotenin (50 μCi/kg) was administered intraventricularly (i.v.) by the method of Noble *et al.* [22] ten minutes before decapitation

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† Abbreviations used: DEAE, diethylaminoethyl; DMT, *N,N*-dimethyltryptamine; 5-HIAA, 5-hydroxyindoleacetic acid; INMT, indoleamine-*N*-methyltransferase; MAO, monoamine oxidase; NMT, *N*-methyltryptamine.

which was carried out 18 hr after the last injection of neuroleptic drug. Other groups of rats received single doses of the following drugs: chlorpromazine, haloperidol, thiothixene, pimozide and tranylcypromine. After 2 hr these animals were injected i.v. with [^{3}H]bufotenin (50 μCi/kg) and decapitated 10 min later.

Immediately after decapitation the brain was removed from the skull with a minimum of delay, rinsed in ice-cold physiological saline and immersed into liquid nitrogen where it was stored until analysis. The brains were homogenized in 2 vol. 0.1 M HCl containing 20 μg/ml of each of the following: bufotenin, 5-hydroxyindoleacetic acid (5-HIAA), *N*-methylserotonin and 5-hydroxytryptamine. The homogenate was centrifuged at 20,000 *g* for 20 min at 4°. To 1 ml of supernatant was added 2.7 ml cold absolute ethanol and, after allowing the mixture to stand at 4° for 2 hr, it was centrifuged for 30 min at 32,000 *g*. The supernatant was then transferred to a conical test tube and evaporated to dryness in the dark at 50° under a stream of nitrogen gas.

The residue was taken up in 200 μl ethanol and centrifuged for 4 min at top speed in an Eppendorf Zentrifuge to sediment any insoluble matter. Twenty μl of the clear supernatant was applied to silica gel thin layer plates which were developed in chloroform–methanol–ammonia (28%, w/v) (110:70:4). Spots corresponding to the indoleamines were located by spraying with *o*-pthalaldehyde [21], scraped off the plate and transferred to counting vials. The tritium content was determined by liquid scintillation spectrometry following the addition of 4 ml water and 10 ml Instagel.

Drugs. Drugs used for pretreatment of animals were generously donated by the manufacturers. They were made up as follows: chlorpromazine · 2HCl was dissolved in 0.9% NaCl; haloperidol and thiothixene in a minimal amount of 1 M acetic acid and 0.9% NaCl added to volume; pimozide in a minimal amount of glacial acetic acid and 0.9% NaCl added to volume. Where required the pH of the drug solution was adjusted to between 5.0 and 6.6 with Na_2CO_3 except in the case of pimozide where 1 M NaOH was used. For the study of the action of neuroleptics and antidepressants on the activity of purified rabbit lung INMT the drugs were dissolved in the following solvents: distilled water (ipronazid, amitriptyline, promethazine · 2HCl, thioridazine · HCl, tranylcypromine, trimeprazine tartrate, maprotiline and chlorimipramine), and 0.05 M HCl (tetrabenazine and thiothixene), and 0.5 M acetic acid (nialamide, pericyazine, benperidol, droperidol, trifluperidol, clozapine and perphenazine). Pimozide was dissolved in a minimal amount of glacial acetic acid and made up to volume with distilled water.

Other reagents. *S*-Adenosyl-*L*-[methyl-^{14}C]methionine (58 mCi/mmole) was obtained from Radiochemical Centre, Amersham, U.K. The amines were purchased from Sigma Chemical Company, St. Louis. All other reagents were of high purity analytical grade.

RESULTS

A range of neuroleptics, non-neuroleptic phenothiazines and antidepressants were evaluated for their ability to inhibit purified rabbit lung INMT.

Table 1 shows compounds which at concentrations between 25 μM and 2.5 mM had an inhibitory effect on purified rabbit lung INMT. Benperidol and droperidol were the most potent inhibitors of the enzyme at all concetrations studied. In both cases inhibition was dose-dependent. Other butyrophenone derivatives, viz., trifluperidol and haloperidol showed no inhibitory effect on the enzyme. Of the phenothiazine neuroleptics tested chlorpromazine, thioridazine, pericyazine and perphenazine inhibited INMT activity at high concentration only. Two phenothiazine derivatives which lack neuroleptic actions, promethazine and trimeprazine as well as tetrabenazine, a benzoquinolizine derivative with antipsychotic

Table 1. Drug inhibition of INMT

Drug	Percentage inhibition of INMT 2.5 mM*	250 μM*	25 μM*
Butyrophenone			
Benperidol	89	39	10
Droperidol	85	41	15
Phenothiazine			
Perphenazine	78	0	0
Pericyazine	55	0	0
Chlorpromazine	25	19	0
Thioridazine	10	0	0
Dibenzodiazepine			
Clozapine	44	15	0
Thioxanthene			
Thiothixene	29	2	0
Tricyclic anti-depressant			
Maprotiline	35	0	0
Chlorimipramine	11	3	0

* Drugs at the concentration shown above were added to INMT assays containing purified rabbit lung INMT. Incubation mixtures contained: 100 μl enzyme solution, 840 μM NMT, 78 μM [^{14}C-methyl]-*S*-adenosylmethionine (0.2 μCi) and 68 mM potassium phosphate buffer (pH 7.9) in a final volume of 290 μl. Results are the mean of at least 6 observations.

Table 2. Disappearance of total radioactivity and metabolism of [^{3}H]bufotenin in rat brain

Time (min)	Total radioactivity (μCi/g brain)	(%)*	[^{3}H]bufotenin / [^{3}H]-5-HIAA
10	1.65 ± 0.16	37.4	2.89 ± 0.12
30	0.93 ± 0.39	21.1	1.29 ± 0.06
60	0.51 ± 0.37	11.5	0.46 ± 0.25
Injection solution			31.8

[^{3}H]Bufotenin (50 μCi/kg body mass) was injected i.v. and the animals sacrificed after different intervals. The [^{3}H]bufotenin and [^{3}H]-5-HIAA content of the brain was determined as described in Materials and Methods. Results are expressed as mean ± S.D. of 4 observations.

* % of injected amount of radioactivity.

activity, showed no inhibitory effect on purified rabbit lung INMT. Clozapine, a neuroleptic belonging to the dibenzodiazapine class and which characteristically elicits few extra-pyramidal side effects [23] caused significant inhibition of INMT from 250 μM. There was no correlation between INMT inhibitory activity and the relative anti-psychotic potency of any on the drugs used in the treatment of schizophrenia [24]. Although the majority of the antidepressants studied showed no inhibitory effect on INMT activity, both maprotiline and chlorimipramine did inhibit the enzyme to a limited extent.

Oxidative deamination appears to be an important pathway in normal rat brain for the metabolism of bufotenin. As shown in Table 2 disappearance of [^{3}H]bufotenin and metabolites following i.v. injection was rapid. Whereas the [^{3}H]bufotenin/[^{3}H]-5-

Table 3. Effect of acute drug treatment on cerebral metabolism of intraventricularly administered [^{3}H]bufotenin in the rat

Drug		Total radioactivity (μCi/g brain)	[^{3}H]Bufotenin / [^{3}H]-5-HIAA
Saline		1.33 ± 0.16	2.89 ± 0.12
Chlorpromazine	(5 mg/kg)	1.69 ± 0.12*	3.70 ± 0.40*
	(20 mg/kg)	1.40 ± 0.18	2.95 ± 0.51
	(100 mg/kg)	1.38 ± 0.26	2.81 ± 0.32
Haloperidol	(0.5 mg/kg)	1.47 ± 0.19	2.86 ± 0.32
	(5 mg/kg)	1.33 ± 0.20	2.95 ± 0.17
	(20 mg/kg)	1.51 ± 0.36	2.76 ± 0.22
Thiothixene	(0.5 mg/kg)	1.23 ± 0.14	2.91 ± 0.34
	(5 mg/kg)	1.47 ± 0.21	2.49 ± 0.21*
	(20 mg/kg)	1.38 ± 0.33	3.24 ± 0.34
Pimozide	(1 mg/kg)	1.30 ± 0.08	2.70 ± 0.03*
	(10 mg/kg)	1.27 ± 0.23	2.56 ± 0.13*
Tranylcypromine	(12 mg/kg)	2.63 ± 0.78*	17.2 ± 1.67†

[^{3}H]Bufotenin (50 μCi/kg body mass) was injected i.v. 10 min before sacrifice which occurred 2 hr after i.p. administration of the drugs. The [^{3}H]bufotenin and [^{3}H]-5-HIAA content of the brain was determined as described in Materials and Methods. Results represent mean ± S.D. (n = 4). P values were determined by Student's t-test.

* $P < 0.02$ compared to saline-treated animals.

† $P < 0.001$ compared to saline-treated animals.

Table 4. Effect of chronic drug treatment on cerebral metabolism of intraventricularly administered [^{3}H]bufotenin in the rat

Drug		Total radioactivity (μCi/g brain)	[^{3}H]Bufotenin / [^{3}H]-5-HIAA
Saline		1.52 ± 0.17	2.93 ± 0.08
Chlorpromazine	(0.1 mg kg)	1.43 ± 0.09	3.11 ± 0.26
	(0.5 mg/kg)	1.38 ± 0.13	3.39 ± 0.35*
	(10 mg/kg)	1.47 ± 0.21	2.72 ± 0.21
Haloperidol	(0.1 mg/kg)	1.37 ± 0.25	2.87 ± 0.29
	(0.5 mg/kg)	1.45 ± 0.18	3.23 ± 0.56
	(2.0 mg/kg)	1.29 ± 0.36	2.69 ± 0.34

Animals (4 in each group) were injected i.p. once daily for one week with the above drugs. [^{3}H]Bufotenin (50 μCi/kg body mass) was injected i.v. 10 min before sacrifice, 18 hr after the last drug injection. The [^{3}H]bufotenin and [^{3}H]-5-HIAA content of the brain was determined as described in Materials and Methods. Results represent mean ± S.D.

* $P < 0.05$ compared to saline-treated animals (Student's t-test).

HIAA ratio of the injection solution was 31.8, the figure for brain 10 min after administration was only 2.89. After an hour it was 0.46. By contrast when animals were pretreated with the monoamine oxidase (MAO) inhibitor, tranylcypromine, the ratio at 10 min was 17.2 (Table 2).

Acute or chronic pretreatment of rats with varying doses of haloperidol, chlorpromazine (except a single dose of 5 mg/kg), thiothixene or pimozide had no significant effect on the amount of ^3H in the brain 10 min following i.v. administration of [^3H]bufotenin (50 μCi/kg) (Tables 3 and 4). A single i.p. dose of tranylcypromine (12 mg/kg) given two hours prior to the [^3H]bufotenin injection significantly increased the amount of ^3H in the brain. Chronic pretreatment of rats with chlorpromazine at a daily dose of 0.5 mg/kg followed by i.v. administration of [^3H]bufotenin (50 μCi/kg) 10 min before decapitation significantly elevated the [^3H]bufotenin/[^3H]-5-HIAA ratio. Similar results were obtained with single injections of chlorpromazine at a dose of 5 mg/kg. At other dosages tried this drug had no effect. Haloperidol administered either acutely (0.5 to 20 mg/kg) or chronically (0.1 to 2.0 mg/kg daily) also showed no significant effect. Pimozide and thiothixene (5 mg/kg), however, caused a significant reduction in the [^3H]bufotenin/[^3H]-5-HIAA ratio.

DISCUSSION

While some neuroleptic drugs were found to inhibit INMT activity *in vitro* in agreement with earlier observations on chlorpromazine [14, 25], a number of potent anti-schizophrenic drugs investigated had no inhibitory effect on the enzyme, even at high concentration (2.5 mM). Inhibitory activity was not restricted to any particular type of neuroleptic. Representatives of the phenothiazine, butyrophenone, thioxanthine and benzodiazepine classes featured in the list of active compounds. No correlation was found between INMT inhibitory activity and the anti-psychotic potency of the drugs tested. These findings suggest that drugs effective in the treatment of schizophrenia do not exert their therapeutic influence by inhibiting INMT activity.

The biotransformation of bufotenin is incompletely understood. However, oxidative deamination would appear to be an important pathway in brain, since pretreatment of animals in the present study with an MAO inhibitor prevented the sharp drop in the [^3H]bufotenin/[^3H]-5-HIAA ratio which occurred in untreated animals. Whether bufotenin is a substrate for MAO or whether it is first demethylated to 5-hydroxytryptamine which subsequently undergoes oxidative deamination to 5-HIAA, is not known. 5-HIAA has been shown to be the chief urinary metabolite of bufotenin following intravenous administration of the latter in human volunteers [26].

Chlorpromazine has previously been shown to promote accumulation of DMT in rat brain following administration *in vivo* [27]. This accords with the present findings of significantly higher [^3H]bufotenin/[^3H]-5-HIAA ratios following acute or chronic pretreatment with the drug at certain dosage levels. The mechanism whereby chlorpromazine may influence bufotenin metabolism is unknown. It is likely to involve MAO as it has been reported that this drug does not alter rat brain MAO activity *in vitro* or *in vivo* [28]. Hence some other pathway may well be involved. In this connection it is interesting that plasma of humans and monkeys has been shown to contain high *N*-demethylase activity for bufotenin [29].

Acute pretreatment of animals in the present study with pimozide and thiothixene (5 mg/kg only) significantly lowered the brain [^3H]bufotenin/[^3H]-5-HIAA ratio without altering total radioactivity which suggests that [^3H]bufotenin was metabolized more rapidly. Whether this was accomplished by enhanced MAO activity or some mechanism remains to be investigated.

Domino and co-workers [27] recently reported that administration of large doses of haloperidol to rats led to a decrease in brain DMT concentration. Neither acute nor chronic treatment with haloperidol in the present investigation had any measurable effect on the fate of [^3H]bufotenin in brain. It would seem reasonable to conclude that neuroleptic drugs do not exert their anti-psychotic effects in schizophrenia by influencing the cerebral oxidative deamination of *N*,*N*-dimethylated indoleamines.

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