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Lack of Potency of Metoclopramide's Metabolites in Various Dopaminergic Models

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STANLEY, M., D. WAZER, J. VIRGILIO, C. M. KUHN, D. I. BENSON AND L. R. MEYERSON. *Lack of potency of metoclopramide's metabolites in various dopaminergic models.* PHARMACOL BIOCHEM BEHAV **18**(2) 263–266, 1983.—The dopaminergic properties of metoclopramide and four of its metabolites were determined in a series of *in vivo* and *in vitro* tests. *In vivo* measures included changes in dopamine turnover, serum prolactin levels and antagonism of apomorphine-induced stereotyped behavior. In each of these tests the four metabolites were either completely inactive or significantly less potent than the parent compound. The potency of metoclopramide and its metabolites in *in vitro* dopamine/neuroleptic receptor models was compared to the potency of standard reference compounds. *In vitro* results indicate that none of the four metabolites tested had antagonist activity in any of the receptor models in which they were evaluated. These findings will be discussed in light of the current understanding of receptor models as they relate to antipsychotic efficacy.

Dopamine Antipsychotics Metoclopramide Receptor models Dopamine turnover Prolactin elevation

THE anti-dopaminergic properties of antipsychotic drugs is clearly one of their chief identifying characteristics [2]. Most antipsychotic drugs produce predictable syndromes in both animals and man. In animals classical antipsychotic drugs cause increases in serum prolactin, dopamine turnover and inhibit dopamine agonist-induced stereotypic behavior [6, 16, 24]. In man comparable effects are seen. For example, patients treated with neuroleptics have increased levels of serum prolactin [9] and cerebrospinal fluid homovanillic acid [3]. Additionally, antipsychotic agents are capable of antagonizing the behavioral toxicity of amphetamines in man [1].

In addition to their consistent *in vivo* pharmacological effect on dopamine mediated responses, antipsychotic drugs also exert pronounced biochemical effects on *in vitro* dopamine receptor models. Good correlations exist between clinical potencies and the abilities of various antipsychotic drugs to displace tritiated ligands from membrane fragments derived from dopamine-rich areas of calf brains [4,19].

In a series of basic and clinical studies we have examined the dopaminergic properties of the atypical neuroleptic metoclopramide [20, 21, 22]. Metoclopramide has been shown to have a similar pharmacologic profile to many typical antipsychotics. In rats metoclopramide increases dopamine turnover, serum prolactin and antagonizes dopamine agonist-induced stereotypies [20]. When given to man metoclopramide causes extrapyramidal side effects, increases serum prolactin and is an effective antipsychotic agent [22].

Our interest in metoclopramide stems from its lack of activity in the various dopamine receptor models which have been recently proposed. A number of laboratories have reported that metoclopramide is completely inactive in its ability to inhibit the dopamine stimulation of adenylate cyclase in neuronal tissues (D-1) [11,18]. Also, when tested for its potency to displace [^{3}H]-spiroperidol from striatal membrane fractions (D-2) metoclopramide has been shown to be either weak or inactive [4,14].

Metoclopramide's inactivity in the various *in vitro* receptor models might be attributed to the limitations inherent to all in vitro systems, i.e., they do not account for factors such as distribution and metabolism. Therefore, it is possible that the discrepancy between metoclopramide's activity *in vivo* and its *in vitro* inactivity could be accounted for by the need for the parent compound to be metabolized to an active species. In an effort to test this hypothesis we have compared the potency of metoclopramide with several of its common

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metabolites in a series of *in vivo* and *in vitro* dopamine-mediated models.

METHOD

Reagents and drugs

Drugs were obtained from the following sources: Metoclopramide (A.H. Robins, Richmond, VA); haloperidol (McNeil, Spring House, PA); fluphenazine (Squibb, Princeton, NJ); ±6,7-ADTN (Research Biochemicals, Inc., Wayland, MA); metoclopramide metabolites A–D (Beecham Pharmaceuticals, U.K.). [^{3}H]-Spiroperidol, 51 Ci/mmol; [^{3}H]-N-propylnorapomorphine (nPA), 65 Ci/mmol and [^{3}H]-amino 6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN), 29 Ci/mmol were purchased from New England Nuclear Corp., Boston, MA; (+)Butaclamol (Ayerst, Montreal, Canada); Vanillic acid (VA) and homovanillic acid (HVA) were from Regis Chemical Co., Morton Grove, IL. All other reagents were of the highest quality available.

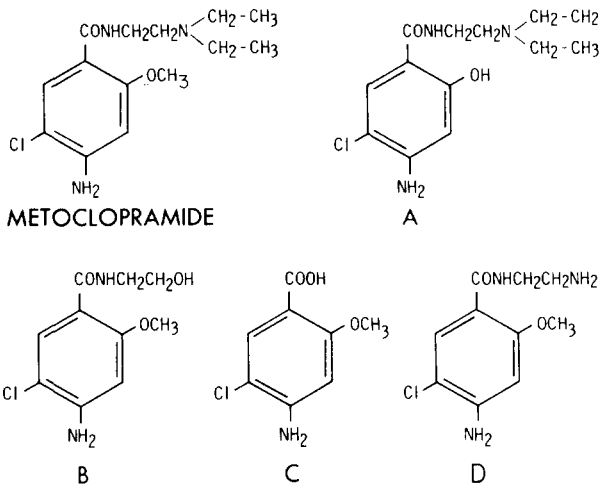

FIG. 1. Structural representation of metoclopramide and its metabolites.

Serum Prolactin

The effects of metoclopramide and its metabolites on serum prolactin were determined. Groups of rats (male Sprague-Dawley) received IP injections of either 3.5 mg/kg of metoclopramide, its metabolites or saline. Rats were decapitated 1 hour following drug administration and trunk blood was collected and allowed to clot. Samples were centrifuged at 5000 ×g for 5 minutes and serum was stored at −80°C until assayed. Serum prolactin was assayed by radioimmunoassay according to the instructions supplied with the NIAMDD kit. Prolactin concentrations are expressed as ng/ml of NIAMDD rat PRL-RP-1. The sensitivity of this assay is approximately 1 ng/ml. All samples were assayed in duplicate and the values reported represent the average of these replications.

Apomorphine-Mediated Stereotypy

Inhibition of apomorphine-induced stereotypic behavior in rats was performed by previously established procedures [6,8].

The intensity of stereotyped behavior was assessed according to the scoring system: 0=behavior indistinguishable from that of control animals; 1=periodic sniffing, repetitive head and limb movements; 2=continuous sniffing, repetitive head and limb movements; 3=periodic gnawing, biting, or licking; 4=continuous gnawing, biting, or licking. Apomorphine was administered by the subcutaneous route and all other agents by the intraperitoneal route. A 1-hr pretreatment time prior to apomorphine challenge was used for metoclopramide and metabolites.

Each value was the mean of at least eight determinations. Standard errors were less than 12% of the means.

HVA Analysis

The effects of acute dosing of metoclopramide and its metabolites on rat striatal homovanillic acid (HVA) levels were determined. Groups of rats (male Sprague-Dawley) were administered a dose (3.5 mg/kg IP) of metoclopramide, metabolite A-D, or saline vehicle as control. Animals were killed 1-hr following drug administration and brains were quickly removed and striata dissected and weighed. Tissue extraction followed by high performance liquid chromatographic (HPLC/LCEC) quantitation of HVA was performed by previously established procedures [10]. Briefly, striatal tissue derived from each group in triplicate was homogenized in 20 vol of 0.1 N perchloric acid. All subsequent steps were conducted at 4°C. Homogenates were centrifuged at 5000 ×g for 10 min and aliquots of 400 μl of the supernatant were transferred to conical, plastic 5.0 ml Savant capped tubes for extraction. Perchloric acid (0.1 N) was added to a final volume of 500 μl. Vanillic acid (10 ng) was added to the tubes as internal standard. Diethyl ether (2.5 ml) was then added to the tubes, closed immediately and vortexed for 30 sec. Following phase separation, 500 μl of the etheral layer was then transferred to a set of 1.5 ml Eppendorf tubes and taken to dryness in a Savant rotary evaporator. The residue was reconstituted in 400 μl of 0.05 M sodium acetate buffer (pH 5.0) and used for chromatographic analysis. HVA and VA were separated on a C_{18} Waters μBondapac steel jacket column with 0.05 M sodium acetate (pH 5.0) as mobile phase. Chromatographic conditions were as follows: flow rate: 2.0 ml/min; pressure 1500 psi; electrochemical detector +0.7 V; temperature 25°C; detector sensitivities ranged between 5 and 10 nA/V. Aliquots (25–75 μl) of the extracted sample were injected for analysis. Calibrated standards of HVA and VA were also extracted and analyzed and were used to calculate the ratios of peak heights. Peak height ratios were employed for accurate quantitation of HVA in the various treatment groups.

Receptor Binding Assays

[^{3}H]-ADTN; [^{3}H]-nPA and [^{3}H]-spiroperidol binding to striatal membrane preparations were performed according to previously outlined procedures [5, 7, 23]. Briefly, rat (male Sprague-Dawley) or calf striata were dissected on ice and either used immediately or kept frozen at −70°C until use. Tissues were homogenized in 40 vol of chilled 50 mM Tris:HCl buffer (pH 7.4) using a Tissumizer homogenizer (Tekmar Co., Cincinnati, OH) and centrifuged at 50,000 ×g for 15 min at 4°C. The resultant pellet was washed once with the same buffer and recentrifuged at 50,000×g for 15 min. The final pellet was dispersed in 40 vol of chilled 50 mM Tris:HCl buffer containing 0.1% ascorbate, 120 mM NaCl, 5 mM KCl,

TABLE 1

THE IN VIVO ANTIDOPAMINERGIC PROPERTIES OF METOCLOPRAMIDE AND ITS METABOLITES

Treatment	Prolactin (ng/ml)	Striatal HVA (μg/g)	Apomorphine Stereotyped Behavior ED_{50} for Inhibition
Saline	8.8 ± 3.4	1.11 ± 0.15	Inactive
Metoclopramide	72.7 ± 10.8*	3.00 ± 0.24‡	2.5 mg/kg
Metabolite A	4.7 ± 0.8	1.76 ± 0.21§	Inactive at 50 mg/kg
Metabolite B	9.4 ± 2.3	1.41 ± 0.20	Inactive at 50 mg/kg
Metabolite C	9.4 ± 2.2	1.56 ± 0.13§	Inactive at 50 mg/kg
Metabolite D	44.8 ± 14.2†	2.04 ± 0.12‡	Inactive at 10 mg/kg

Different from saline controls *$p<0.01$, †$p<0.02$, ‡$p<0.001$, §$p<0.05$.

2 mM $CaCl_2$ and 1 mM $MgCl_2$ to a final pH of 7.2 (at 37°C). For competition studies, triplicate reaction mixtures contained the following components; calf or rat striatal membrane preparation (500 μg protein), [^{3}H]-ligand:[^{3}H]-spiroperidol (0.5 nM), [^{3}H]-ADTN (10 nM) or [^{3}H]-nPA (1 nM), and various concentrations of displacing agents or drugs. Following incubation for 15 min at 37°C the samples were rapidly filtered under vacuum over Whatman GF/B filters and rinsed 3 times rapidly with 5 ml of chilled Tris:HCl buffer to remove unbound radioactive ligand. Corrections were made for nonspecific binding by assaying parallel incubation tubes which contained 1 μM (+) butaclamol for spiroperidol binding, 100 μM apomorphine for nPA binding and 100 μM ADTN for ADTN binding.

Filters were placed in scintillation vials containing 10 ml Beckman Ready Solv-HP scintillation fluid. Radioactivity was determined with a Beckman LS-7500 liquid scintillation spectrometer. Drug IC_{50} values are reported as the concentration that displaces the specific binding of selected [^{3}H]-ligand by 50%.

Protein Determination

Protein concentrations were determined by the method of Lowry [15] using bovine serum albumin as standard.

RESULTS

The results of the *in vivo* antidopaminergic tests are depicted in Table 1.

Serum prolactin levels were significantly increased as a result of metoclopramide treatment (Table 1). The same mg/kg dose of metabolite D also produced a significant increase in prolactin levels. None of the other metabolites influenced prolactin levels.

Metoclopramide produced a highly significant effect on HVA levels (Table 1). HVA was raised to a lesser extent by all of the metabolites except for B which failed to produce a significant increase.

None of the metabolites were active in antagonizing apomorphine-induced stereotypy at the dosages listed in Table 1. Metoclopramide was an effective antagonist of stereotypic behavior (data provided by Dr. David Turner).

The IC_{50} values for metoclopramide and its metabolites along with representative standards for displacing [^{3}H]-spiroperidol, [^{3}H]-ADTN, [^{3}H]-nPA are represented in Table 2. Metoclopramide had IC_{50} values of 3000 and 2250 nM for the displacement of [^{3}H]-spiroperidol in calf and rat striatal tissues, respectively. Metoclopramide and all its attendant metabolites produced negligible displacing effects on [^{3}H]-ADTN or [^{3}H]-nPA (IC_{50} value >25 μM) binding in calf striatal tissues. As examples of potent interacting compounds in these receptor binding systems, fluphenazine, haloperidol and ADTN IC_{50} values are given for comparison.

DISCUSSION

The combined results of our *in vivo* and *in vitro* experiments indicate that while some of the metabolites of metoclopramide possess some *in vivo* antidopaminergic properties, none are as potent as the parent compound itself. Thus, our

TABLE 2

DRUG SPECIFICITY OF [^{3}H]-ADTN; [^{3}H]-NPA AND [^{3}H]-SPIROPERIDOL BINDING IN STRIATAL MEMBRANE PREPARATIONS*

	IC_{50} Values (nM)			
	Calf			Rat
Compound	[^{3}H]-ADTN	[^{3}H]-NPA	[^{3}H]-Spiro	[^{3}H]-Spiro
Metoclopramide	>25,000	>25,000	3000	2250
Metabolite A	>25,000	>25,000	>25,000	>10,000
Metabolite B	>25,000	>25,000	>25,000	>10,000
Metabolite C	>25,000	>25,000	>25,000	>10,000
Metabolite D	>25,000	>25,000	>25,000	>10,000
Fluphenazine	90	15	8	3
Haloperidol	150	40	12	5
ADTN	18	60	900	200

*IC_{50} values are equivalent to the concentration of drug necessary to inhibit the specific binding of [^{3}H]-ligand to striatal membrane preparation by 50%. Details of incubations are given in Method section.

findings offer no support for the hypothesis that a metabolite of metoclopramide is responsible for the many antidopaminergic effects observed with metoclopramide itself. In fact, our findings are more suggestive of a hypothesis which seems to indicate that the metabolism of metoclopramide is in the direction of inactivtion. While it is still possible that an as yet untested metabolite might be identified and found to possess the potency necessary to explain the antidopaminergic properties observed, other lines of evidence do not offer support for this hypothesis.

In vitro studies have demonstrated that metoclopramide's ability to reverse the inhibitory effects of apomorphine on tyrosine hydroxylase is as effective as the potent neuroleptic, haloperidol [17]. Further, plasma samples from patients treated with up to 1000 mg/day of metoclopramide were inactive in displacing [^{3}H]-spiroperidol from DA/neuroleptic binding sites even though the same samples had significantly elevated prolactin and metoclopramide levels (as determined by gas chromatography) (Stanley, *et al.*, manuscript in preparation).

Perhaps the most significant conclusion to be drawn from these findings is that the *in vitro* dopamine/neuroleptic receptor models (D_1–D_3) currently in use are inadequate to explain the mechanisms of action of some antipsychotic drugs. Thus, a compound like metoclopramide which is active in numerous behavioral and biochemical tests designed to assess antidopaminergic activity might be judged inactive on the basis of its potency in central dopaminergic receptor models.

The relationship between biochemical and functional receptor subpopulations has not yet been clearly established. The cumulative work with metoclopramide suggests its actions are antagonistic to dopamine at the recognition site or sites mediating most of the biochemical, behavioral and clinical phenomena traditionally associated with this neurotransmitter. Furthermore, unlike classical neuroleptics, it is possible that metoclopramide has a very low affinity for dopamine/neuroleptic binding sites that are not relevant to these effects. Thus, the increase in specific binding we observed following chronic treatment with metoclopramide or other neuroleptics may represent a significant change in a specific but functionally relevant receptor subpopulation.

An additional outgrowth of dopamine receptor studies has been the development of theories purporting to predict the liabilities of drugs to induce tardive dyskinesia. Drugs with low binding affinities for dopamine receptors, such as clozapine, are thought to have a low liability for inducing tardive dyskinesia. It should be noted, however, that metoclopramide, which has a much lower binding affinity than clozapine, induces tardive dyskinesia [12,13].

In conclusion, we believe that metoclopramide, and not an active metabolite, exerts its antidopaminergic effects primarily as a dopaminergic antagonist. The close similarity of metoclopramide's effects with those of other neuroleptics suggests that the receptors sites relevant to these actions are similar.

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