*Br. J. Pharmac.* (1975), 55, 381-385

# INHIBITION BY APOMORPHINE OF THE METOCLOPRAMIDE-INDUCED CATALEPSY AND INCREASE IN STRIATAL HOMOVANILLIC ACID CONTENT

LIISA AHTEE[1]

Department of Pharmacology, University of Helsinki, Siltavuorenpenger 10, SF-00170 Helsinki 17, Finland

1 The mechanism of the cataleptic effect of metoclopramide was analyzed by using drugs which alter the activity of dopaminergic or cholinergic neurones or the content of γ-aminobutyric acid in the central nervous system of rats.

2 The cataleptic effect of metoclopramide (20 mg/kg) was antagonized by apomorphine (10 mg/kg) and by atropine (50 mg/kg). Aminooxyacetic acid (AOAA, 25-50 mg/kg) potentiated the catalepsy induced by metoclopramide (5 mg/kg).

3 Metoclopramide alone did not alter the rectal temperature of rats. It did not alter the AOAA-induced hypothermia, but it partially antagonized apomorphine-induced hypothermia.

4 Metoclopramide induced a six-fold increase in striatal homovanillic acid (HVA) concentration, but it did not change the dopamine or noradrenaline content in the brain of rats. Apomorphine decreased the striatal HVA concentration in control and in metoclopramide-treated rats. Atropine and AOAA did not alter the metoclopramide-induced increase in striatal HVA concentration.

5 The results suggest that metoclopramide produces catalepsy by blocking striatal dopamine receptors.

## Introduction

The antiemetic compound, metoclopramide disturbs the extrapyramidal control of motor functions in man (Casteels-Van Daele, Jaeken, van der Schueren, Zimmerman & van den Bon, 1970) and in rats (Costall & Naylor, 1973). Lesions in caudate-putamen and globus pallidus reduce or abolish the cataleptic effect of metoclopramide in rats (Costall & Naylor, 1973). Moreover, in mice the metoclopramide-induced catalepsy is associated with a nearly five-fold elevation of striatal homovanillic acid (HVA) concentration (Ahtee & Buncombe, 1974). Therefore metoclopramide could act, as has been suggested for the neuroleptic compounds (Carlsson & Lindqvist, 1963; Andén, Roos & Werdinius, 1964), by directly blocking the striatal dopamine receptors; the receptor blockade could induce an increased production of dopamine through a neuronal feedback mechanism. In the present experiments the effects of drugs, which alter the activity of dopaminergic or cholinergic neurones or the brain concentration of γ-aminobutyric acid (GABA), on the metoclopramide-induced catalepsy and change in the striatal HVA content were studied. A preliminary communication of this work was given to the British Pharmacological Society (Ahtee, 1975).

## Materials and Methods

Female Wistar rats, weighing 180-220 g, kept on a standard diet and tap water *ad libitum*, were used. During the experiments the rats were kept in individual cages at 20-22° C. Catalepsy was scored as described by Simon, Malatray & Boissier (1970) and by Cashin & Sutton (1973). Four tests were employed: 3 cm high rod, 9 cm high rod, parallel bars and vertical grid. Each test was scored from 0 to 2. The scores of the four tests were added. The rectal temperature was measured with an electric thermocouple (Ellab Instruments, Copenhagen).

[1] Present address: School of Pharmacy, Department of Pharmacology, University of Helsinki, Kirkkokatu 20, SF-00170 Helsinki 17, Finland.

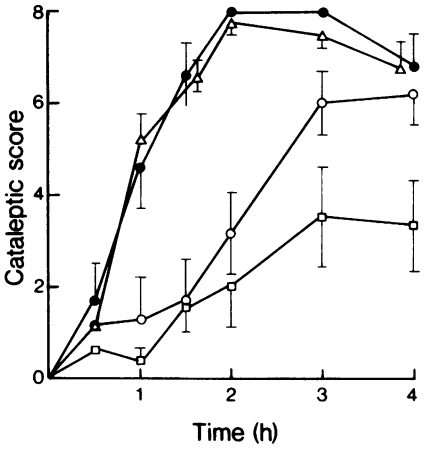


**Figure 1** The cataleptic effect of metoclopramide (20 mg/kg) (s.c.) in rats pretreated with 0.9% NaCl solution (•), apomorphine (○), atropine (□), or aminooxyacetic acid (△). The times given were counted from the injection of metoclopramide. Apomorphine (10 mg/kg, s.c.) and atropine (50 mg/kg, i.p.) were given immediately before metoclopramide and aminooxyacetic acid (25 mg/kg, i.p.) was given 1 h before metoclopramide. Means from 6-8 rats are given; the vertical bars indicate the s.e. of the mean.

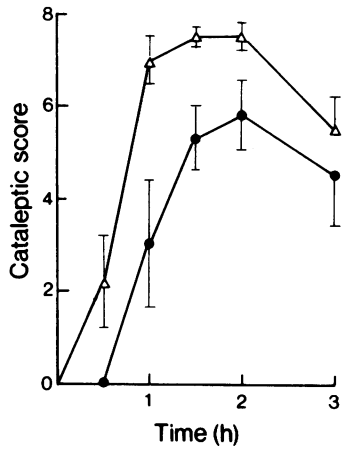


**Figure 2** The cataleptic effect of metoclopramide (5 mg/kg) in rats pretreated with 0.9% NaCl solution (•) or aminooxyacetic acid (△). The times given were counted from the injection of metoclopramide. Aminooxyacetic acid (25 mg/kg, i.p.) was given 1 h before metoclopramide. Means from 6-8 rats are given; the vertical bars indicate the s.e. of the mean.

The rats were killed by decapitation, and the brain was rapidly removed and dissected. The striata from two brains were pooled for the estimation of HVA. The mean weight of tissue in each sample (striata from two rats) was 113 ± 23 mg (mean ± s.d., $n = 45$). HVA was estimated by the method of Portig, Sharman & Vogt (1968). In a few experiments the whole brain dopamine and noradrenaline contents were estimated by the method of Shellenberger & Gordon (1971). Recovery for added HVA was 69 ± 1% (mean ± s.e. mean from 5 estimations), for dopamine 89 ± 1.5% and for noradrenaline 86 ± 1.5% ($n = 4$). Results have been corrected for losses.

The drugs used were metoclopramide hydrochloride (a gift from H. Lundbeck & Co. A/S, Copenhagen), atropine sulphate and apomorphine hydrochloride (Pharmacopoea Nordica) and aminooxyacetic acid (AOAA) hemihydrochloride (Sigma Chemical Company, St Louis, Mo). Apomorphine was dissolved in 0.9% NaCl solution containing 0.1% sodium metabisulphite, AOAA was dissolved in 0.9% NaCl solution and neutralized with 1 N NaOH to pH 6; the other drugs were dissolved in 0.9% NaCl solution. The drugs were injected either s.c. or i.p. in a volume of 0.1 ml/100 g. The doses refer to the base or acid.

## Results

### *Catalepsy and rectal temperature*

Figure 1 shows that in rats metoclopramide (20 mg/kg) induced catalepsy which was maximal at 2-3 h after injection. Apomorphine (10 mg/kg) significantly reduced the cataleptic effect of metoclopramide at 1, 1.5 and 2 h after the metoclopramide injection ($P < 0.01$). Apomorphine alone induced stereotyped behaviour which consisted of sitting or standing in abnormal posture, continuous jerking of head or jaw upwards, backwards locomotion and continuous movement of one or both front legs as well as gnawing of the cage walls. The apomorphine plus metoclopramide-treated rats also showed some stereotypies during the first 2 h after drug injections. Atropine (50 mg/kg) significantly reduced the cataleptic effect of metoclopramide at 1 h ($P < 0.01$), 1.5 h ($P < 0.001$), 2 h ($P < 0.001$), 3 h ($P < 0.001$) and 4 h ($P < 0.01$) after injection.

Aminooxyacetic acid (25 mg/kg) given 1 h before metoclopramide did not change the cataleptic effect of metoclopramide (20 mg/kg; Figure 1). AOAA (25 or 50 mg/kg) alone did not induce catalepsy in the rats. The larger dose of AOAA made the rats fall into a sleep-like state from which they were easily aroused. This dose slightly accelerated the appearance of the

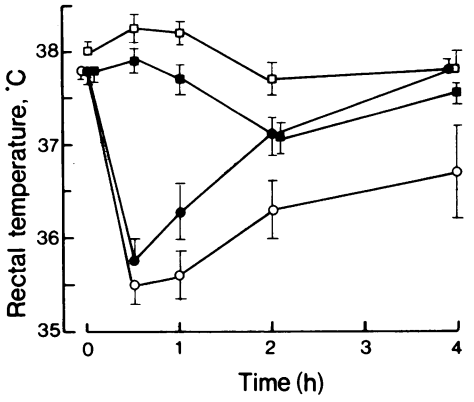


**Figure 3** Rectal temperature in rats treated with 0.9% NaCl solution (□), 20 mg/kg of metoclopramide (■), 10 mg/kg of apomorphine (○) or with both metoclopramide and apomorphine (●). All drugs were injected s.c.; apomorphine was given immediately before metoclopramide. Times given were counted from the injection. Means from 6-8 rats are given; the vertical bars indicate the s.e. of the mean.

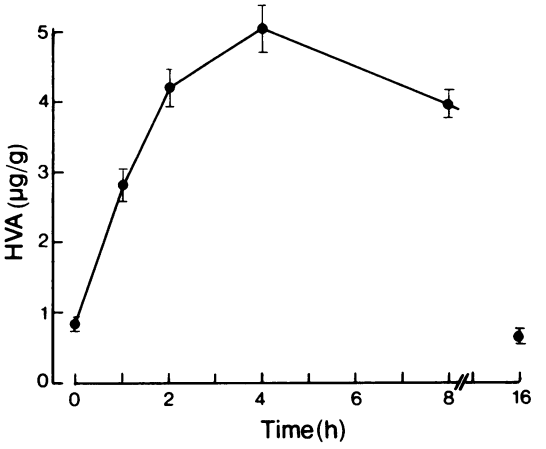


**Figure 4** The effect of metoclopramide (20 mg/kg, s.c.) on the concentration of homovanillic acid (HVA) in the rat striatum. Means from 3-4 estimations (striata from two rats were pooled for each estimation) are given; the vertical bars indicate the s.e. of the mean.

cataleptic effect of metoclopramide (20 mg/kg). AOAA (25 mg/kg) pretreatment potentiated the cataleptic effect of metoclopramide (5 mg/kg) at 1 h ($P < 0.05$) and 1.5 h ($P < 0.02$) after the administration of metoclopramide (Figure 2).

Metoclopramide alone did not significantly change the rectal temperature of rats. Metoclopramide did not alter the AOAA-induced hypothermia, but it partially antagonized the apomorphine-induced hypothermia (Figure 3).

*Striatal homovanillic acid content and cerebral dopamine and noradrenaline content*

Metoclopramide (20 mg/kg) caused a six-fold elevation of striatal HVA content and the HVA content remained elevated at least until 8 h after the injection (Figure 4). The metoclopramide-induced increase of striatal HVA content was dose-dependent (Table 1). Two hours after the injection of metoclopramide (1.25 mg/kg) the

**Table 1** Effect of metoclopramide on the striatal homovanillic acid (HVA) concentration in rats pretreated with 0.9% NaCl solution (saline), atropine or amino-oxyacetic acid (AOAA).

| | *Metoclopramide (mg/kg)* | *HVA (µg/g)* | | |
|---|---|---|---|---|
| | | *Saline* | *Atropine* | *AOAA* |
| 2 h* | 0 | 0.84 ± 0.07 | 0.85 ± 0.02 | 0.93 ± 0.04 |
| | 1.25 | 1.53 ± 0.04 | 1.58 ± 0.18 | 1.73 ± 0.07 |
| | 5 | 3.62 ± 0.32 | 4.35 ± 0.21 | 3.74 ± 0.15 |
| | 20 | 4.21 ± 0.26 | 4.50 ± 0.38 | 4.71 ± 0.19 |
| 4 h* | 0 | 0.75 ± 0.10 | 0.67 ± 0.11 | 0.82 ± 0.04 |
| | 1.25 | 0.81 ± 0.09 | – | 0.93 ± 0.08 |
| | 5 | 3.57 ± 0.23 | – | 3.94 ± 0.14 |
| | 20 | 5.04 ± 0.33 | 5.15 ± 0.29 | 5.80 ± 0.48 |

* The times given were counted from the injection of metoclopramide (s.c.). Atropine (50 mg/kg, i.p.) was given immediately and AOAA (25 mg/kg, i.p.) 1 h before metoclopramide. Means ± s.e. of the mean from 3-4 estimations are given (striata from two rats were pooled for each estimation).

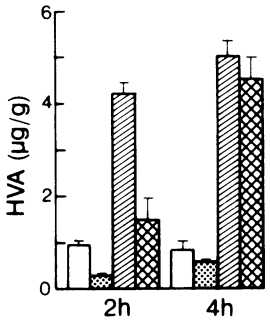


**Figure 5** The effect of apomorphine (10 mg/kg, s.c.) on the metoclopramide (20 mg/kg, s.c.)-induced increase of the homovanillic acid (HVA) content in the rat striatum. The control rats (open column) were injected with 0.9% NaCl solution s.c., apomorphine (stippled) was given immediately before metoclopramide (hatched). Means from 3-4 estimations (striata from two rats were pooled for each estimation) are given; the vertical bars indicate the s.e. of the mean. (Cross hatching represents apomorphine + metoclopramide.)

striatal HVA content was doubled but after 4 h the striatal HVA content of metoclopramide-treated rats was normal again.

Table 1 shows that atropine alone did not cause a statistically significant decrease in the striatal HVA concentration, and it did not alter the metoclopramide-induced increase of striatal HVA concentration at 2 h or 4 h after the injection of the drugs. Atropine did not alter the metoclopramide-induced HVA increase at 1 h after the injections (metoclopramide, 20 mg/kg, alone = 2.80 ± 0.23 µg/g and metoclopramide + atropine = 2.72 ± 0.03 µg/g, mean ± s.e. mean, $n = 3$). As shown in Table 1, AOAA (25 mg/kg) did not alter the striatal HVA concentration in control or metoclopramide-treated rats. AOAA (50 mg/kg) did not alter the striatal HVA concentration in control rats or in rats treated with metoclopramide (20 mg/kg).

From Figure 5, it can be seen that 2 h after the injection of apomorphine (10 mg/kg) the striatal HVA concentration was decreased by 69% ($P < 0.001$). Two hours after the combined treatment with apomorphine and metoclopramide the striatal HVA concentration was 66% ($P < 0.001$) lower than after an injection of metoclopramide alone. Four hours after the injections of the drugs the effects of apomorphine had worn-off in both the control and metoclopramide-injected rats.

Metoclopramide did not change the concentration of dopamine or noradrenaline in the whole brain. The concentration of dopamine in the brain of control rats was 0.92 ± 0.05 µg/g (mean ± s.e. mean, $n = 3$), 3 h after an injection of metoclopramide (20 mg/kg) it was 0.83 ± 0.01 µg/g and 3 h after metoclopramide (40 mg/kg), 0.77 ± 0.04 µg/g. The brain noradrenaline concentrations of the same rats were 0.43 ± 0.02 µg/g (control), 0.42 ± 0.02 µg/g (metoclopramide, 20 mg/kg) and 0.41 ± 0.02 µg/g (metoclopramide, 40 mg/kg), respectively.

## Discussion

Metoclopramide-induced catalepsy in rats was accompanied by a six-fold increase in striatal HVA concentration. This increased striatal HVA concentration without any decrease in brain dopamine concentration most probably indicates that dopamine formation is increased. Apomorphine, a drug which is thought to stimulate the striatal dopamine receptors (Ernst, 1967), reduced the catalepsy and the increase in striatal HVA concentration caused by metoclopramide. This antagonism lasted for about as long as the apomorphine stereotypies in control rats. Similarly to the dopamine receptor blocking neuroleptics (Fuxe & Sjöqvist, 1972; Kruk, 1972) metoclopramide partially antagonized the apomorphine-induced hypothermia. These findings support the suggestion that metoclopramide may block the striatal dopamine receptors.

The acetylcholine receptor blocking agent, atropine, clearly antagonized the metoclopramide-induced catalepsy but it did not antagonize the metoclopramide-induced increase of striatal HVA concentration. This is interesting because atropine antagonizes both the catalepsy and HVA increase produced by neuroleptic agents (O'Keeffe, Sharman & Vogt, 1970; Andén & Bédard, 1971). These results show that the prevention of drug-induced catalepsy by atropine does not simply restrain the overactivity of the dopaminergic neurones. Neuroleptic drugs and metoclopramide could have different effects on the pathways which mediate the feedback information controlling the activity of dopaminergic neurones.

Aminooxyacetic acid (AOAA), a compound which increases the cerebral GABA concentration (Wallach, 1961), antagonizes the effects of chlorpromazine and haloperidol on the striatal dopamine metabolism (Andén, 1974; Lahti & Losey, 1974). In contrast, in the present experiments AOAA did not alter the metoclopramide-induced increase in striatal HVA concentration. AOAA alone did not induce catalepsy in rats. However, it potentiated the

metoclopramide-induced catalepsy. Whether this effect is associated with the AOAA-induced increase of the cerebral GABA content remains to be elucidated.

The present results suggest that metoclopramide, like the neuroleptic compounds, acts directly on the striatal dopamine receptors. However, biochemically and/or anatomically, the metoclopramide-induced catalepsy may differ from the catalepsy induced by neuroleptic compounds.

This work was supported by the Medical Research Council of the Academy of Finland. I would like to thank Mrs P. Oikkonen and Mr G. Buncombe for skilful assistance.

## References

AHTEE, L. (1975). Effect of drugs on metoclopramide-induced catalepsy and increase in striatal homovanillic acid content. *Br. J. Pharmac.*, **53**, 460P.

AHTEE, L. & BUNCOMBE, G. (1974). Metoclopramide induces catalepsy and increases striatal homovanillic acid content in mice. *Acta pharmac. tox.*, **35**, 429-432.

ANDÉN, N.-E. (1974). Effects of aminooxyacetic acid and gammahydroxybutyric acid on the brain dopamine. *Acta pharmac. tox.*, **35**, Suppl. 1, 16.

ANDÉN, N.-E. & BÉDARD, P. (1971). Influences of cholinergic mechanisms on the function and turnover of brain dopamine. *J. Pharm. Pharmac.*, **23**, 460-462.

ANDÉN, N.-E., ROOS, B.-E. & WERDINIUS, B. (1964). Effects of chlorpromazine, haloperidol and reserpine on the levels of phenolic acids in rabbit corpus striatum. *Life Sci.*, **3**, 149-158.

CARLSSON, A. & LINDQVIST, M. (1963). Effect of chlorpromazine or haloperidol on formation of 3-methoxytyramine and normetanephrine in mouse brain. *Acta pharmac. tox.*, **20**, 140-144.

CASHIN, C.H. & SUTTON, S. (1973). The effect of anti-Parkinson drugs on catalepsy induced by α-methyl-p-tyrosine in rats pretreated with intraventricular 6-hydroxy-dopamine. *Br. J. Pharmac.*, **47**, 658P-659P.

CASTEELS-VAN DAELE, M., JAEKEN, J., VAN DER SCHUEREN, P., ZIMMERMAN, A. & VAN DEN BON, P. (1970). Dystonic reactions in children caused by metoclopramide. *Arch. Dis. Child.*, **45**, 130-133.

COSTALL, B. & NAYLOR, R.J. (1973). Neuroleptic and non-neuroleptic catalepsy. *Arzneimittel-Forsch.*, **23**, 674-683.

ERNST, A.M. (1967). Mode of action of apomorphine and dexamphetamine on gnawing compulsion rats. *Psychopharmacologia*, **10**, 316-323.

FUXE, K. & SJÖQVIST, F. (1972). Hypothermic effect of apomorphine in the mouse. *J. Pharm. Pharmac.*, **24**, 702-705.

KRUK, Z.L. (1972). The effect of drugs acting on dopamine receptors on the body temperature of the rat. *Life Sci.*, (I) **11**, 845-850.

LAHTI, R.A. & LOSEY, E.G. (1974). Antagonism of the effects of chlorpromazine and morphine on dopamine metabolism by GABA. *Res. Comm. Chem. Pathol. Pharmac.*, **7**, 31-40.

O'KEEFFE, R., SHARMAN, D.F. & VOGT, M. (1970). Effect of drugs used in psychoses on cerebral dopamine metabolism. *Br. J. Pharmac.*, **38**, 287-304.

PORTIG, P.J., SHARMAN, D.F. & VOGT, M. (1968). Release by tubocurarine of dopamine and homovanillic acid from the superfused caudate nucleus. *J. Physiol., Lond.*, **194**, 565-572.

SHELLENBERGER, M.K. & GORDON, J.H. (1971). A rapid, simplified procedure for simultaneous assay of norepinephrine, dopamine and 5-hydroxytryptamine from discrete brain areas. *Anal. Biochem.*, **39**, 356-372.

SIMON, P., MALATRAY, J. & BOISSIER, J.R. (1970). Antagonism by amantadine of prochlorpemazine-induced catalepsy. *J. Pharm. Pharmac.*, **22**, 546-547.

WALLACH, D.P. (1961). Studies on the GABA pathway-I. The inhibition of γ-aminobutyric acid-α-ketoglutaric acid transaminase *in vitro* and *in vivo* by U-7524 (amino-oxyacetic acid). *Biochem. Pharmac.*, **5**, 323-331.

*(Received April 28, 1975)*