Metoclopramide Antagonism of 5-Hydroxytryptophan-Induced
"Wet-Dog" Shake Behaviour in the Rat

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Summary. 1. Metoclopramide, a drug with blocking activity at peripheral neuronal receptor sites for 5-hydroxytryptamine (5-HT), was investigated as an antagonist of the 'Wet-Dog' shake response (WDS) to injections of 5-hydroxytryptophan (5-HTP) in rats. Its effects were compared with those of methysergide, an antagonist of 5-HT at non-neuronal receptor sites, and haloperidol, a dopamine receptor antagonist.

2. WDS after 5-HTP and carbidopa, an inhibitor of peripheral aromatic l-amino acid decarboxylase, correlates significantly with the increase in whole brain 5-HT concentrations measured in parallel experiments.

3. Metoclopramide, methysergide and haloperidol inhibited WDS. Metoclopramide, in addition, inhibited the increase in brain 5-HT, but not, apparently, by interfering with the decarboxylase enzyme.

4. Inhibition of the rise in brain 5-HT could not explain inhibition of WDS by metoclopramide, since metoclopramide inhibited 5-HTP-induced shaking once established and also the response to quipazine, a directly-acting agonist at central 5-HT receptors.

5. The mechanism of action of methysergide may involve blockade of post-synaptic receptor sites for 5-HT. Metoclopramide and haloperidol, on the other hand, cause catalepsy at doses which inhibit WDS, presumably through blockade of dopamine receptors in the CNS. The consequent inhibition of the ability of the animals to initiate movement may be the mechanism by which these compounds inhibit WDS.

6. Since agents with cataleptic properties, probably unconnected to brain 5-HT mechanisms, are effective antagonists of the shaking response, WDS after 5-HTP may be less useful than previously supposed as a screen for drugs interacting with 5-HT receptors in the CNS.

Key words: 'Wet-Dog' shake behaviour - 5-Hydroxytryptophan - Quipazine - Methysergide - Metoclopramide - Haloperidol.

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Introduction

Metoclopramide [N-(diethylaminoethyl)-2-methoxy-4-amino-5-chlorobenzamide] is a powerful antiemetic (Justin-Besancón and Laville, 1964) with central dopamine receptor blocking activity (Elliott et al., 1977). It does, however, differ from the 'classical' neuroleptic group of central dopamine antagonists in having no effect on the dopamine-stimulated adenylate cyclase (Peringer et al., 1976) and minimal clinical efficacy as a neuroleptic (Borenstein and Bles, 1965; Nakra et al., 1975). Metoclopramide has recently been shown to be a selective, surmountable antagonist at receptors for 5-hydroxytryptamine (5-HT) on terminal cardiac sympathetic nerves (Fozard and Mobarak Ali, 1978a). It was of interest, therefore, to investigate such a peripheral neuronal antagonist of 5-HT on a central nervous system response mediated by 5-HT. A suitable test is the 'Wet-Dog' shake behaviour (WDS) observed in rats after injection of 5-hydroxytryptophan (5-HTP) or 5-HT agonists (Bédard and Pycock, 1977) which is suggested to result from increased 5-HT receptor activity in the CNS. The effects of metoclopramide were compared with those of methysergide, a selective antagonist of 5-HT at non-neuronal receptors (Douglas, 1975) and with those of haloperidol, a 'classical' neuroleptic drug with central dopamine receptor antagonist properties.

A preliminary account of these experiments was communicated to the British Pharmacological Society in March 1978 (Fozard and Palfreyman, 1978).

Methods

'Wet-Dog' Shake Behaviour. Male Sprague-Dawley rats (Charles River, France; 230–370 g) were used throughout. Shaking was elicited by intraperitoneal (i.p.) injections of 5-HTP, 150 or 300 mg/kg, preceded 30 min before by an i.p. injection of saline or one of the peripheral inhibitors of aromatic L-amino acid decarboxylase, carbidopa (L-D-methyldopahydrazine; MK 486) (Udenfriend et al., 1966) or D-D-difluoromethyldopa (Palfreyman et al., 1978). The
total number of 'Wet-Dog' shakes occurring in the subsequent 140 min was counted. Methysergide, metoclopramide (both given
i.p.) or haloperidol (given subcutaneously, s.c.) were injected with the
saline or the decarboxylase inhibitor, 30 min before administration of
the 5-HTP.
In further experiments, methysergide, metoclopramide or haloperidol were administered 60 min after the injection of 5-HTP at a
time when shaking was established. In these experiments, the
behaviour was quantified as the rate of shaking during the succeeding
80 min observation period.
Shaking was also elicited by quipazine, a putative central 5-HT
agonist drug (Rodriguez et al., 1973). Doses between 2.5 and
10 mg/kg were given i.p. and shaking was recorded during a 140 min
observation period. Modifying agents were injected 30 min prior to
injection of the standard dose of quipazine, 5 mg/kg.

Assay of Brain 5-HT. In parallel experiments, rats were killed by
stunning and decapitation and the whole brain 5-HT concentrations
were estimated the same day by the method of Snyder et al. (1965)
modified to include an extra wash stage with borate buffer, pH 10, to
ensure no interference of administered 5-HTP with the assay method.
Recovery of 5-HT added to the homogenates ranged between 70—
80%. None of the drugs used interfered with the assay procedure.

Statistical Analysis. All mean variations quoted are standard errors,
and Student’s ‘t’ test (2-tailed) was used to assess the significance of a
difference between mean values. n is the number of observations.

Drugs. L-α-Methyldopahydrazine [MK 486; Carbidopa (Merck 1)];
DL-α-difluoromethyldopa (Merrell, Strasbourg); 5-hydroxytrypt-
amine creatinine sulphate (Sigma); DL-5-hydroxytryptophan
(Sigma); methysergide bimaleate (Sandoz 2); metoclopramide hy-
drochloride (Beecham 3); haloperidol (Janssen); quipazine maleate
(Miles 4). All drugs were dissolved in saline. Doses are expressed in
terms of base.

Results

The 'Wet-Dog' Shake Response
and Brain 5-HT Concentrations After 5-HTP Alone
or in Combination with Carbidopa

The data are presented in Fig.1. Injection of 5-HTP,
150 mg/kg, evoked a small shaking response and in-
creased the brain 5-HT concentration from 0.64 ± 0.03,
n = 12 (animals treated with saline) to 1.08 ± 0.09,
n = 7 (μg/g, mean ± SEM). When the inhibitor of pe-

1 To whom thanks are due for generous gifts of drugs.

Effects of Metoclopramide on WDS Behaviour:
Comparison with Methysergide and Haloperidol

The relationship between doses of methysergide, meto-
clopramide or haloperidol and capacity for inhibition
of WDS is presented in Fig.2. WDS was evoked by a
combination of carbidopa, 25 mg/kg followed after
30 min by 5-HTP, 150 mg/kg. A small, but non-
significant reduction in the shaking response was
obtained in animals pretreated with metoclopramide,
0.1 and 1 mg/kg. With 10 and 20 mg/kg, significant
inhibition of shaking was seen; at 40 and 100 mg/kg,
shaking was essentially abolished. With methysergide,
onset of blockade of WDS occurred between 1 and
5 mg/kg and was dose-dependent up to 100 mg/kg.
Haloperidol, 0.125 mg/kg, had no effect on shaking;
0.5 mg/kg and 2 mg/kg inhibited the response in a dose-
dependent manner. After metoclopramide, 10 mg/kg
and higher, and the two highest doses of haloperidol,
some evidence of interference with the normal ability of
the animals to initiate movement was obtained. Thus,
the animals were immobile and slow to respond to an
external stimulus.

Effects of Metoclopramide, Methysergide
and Haloperidol on Whole Brain 5-HT Concentrations;
Correlation with WDS Behaviour

In order to further analyse the mechanism of the
blocking activity against WDS, the whole brain 5-HT
concentrations were measured after various treatment
schedules with methysergide, metoclopramide or haloperidol at times equivalent to the end of the 140 min
observation period. The results obtained are presented in
Table 1. As previously noted (Fig.1A), the combi-
nation of carbidopa, 25 mg/kg, and 5-HTP,
150 mg/kg evoked WDS and an associated increase in
brain 5-HT concentrations. Methysergide at a dose of
10 mg/kg, which caused marked inhibition of WDS,
had no effect on the brain 5-HT concentration
when compared with the appropriate control.
Metoclopramide, on the other hand, at doses which
causen small (10 and 20 mg/kg) or marked (40 mg/kg)
inhibition of WDS, resulted in each case in an
associated decrease in brain 5-HT concentrations when