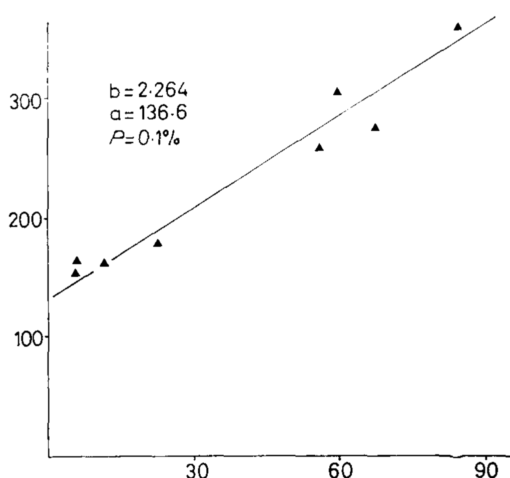

FIG. 2. Plot of C/T (abscissa) versus C (ordinate). T = survival time; C = doses of pentobarbitone (mg kg^{-1}). The analytical constants of the linear correlation (C = a + b C/T) and the probability of casual result are: a = 136·6, b = 2·264 and $P<0·1\%$.

albino mice (~25 g) intraperitoneally or subcutaneously or by stomach tube. The times of death were evaluated with an approximation of 10″.

A sample set of data is reported in the Figures. In Fig. 1 the curve concentration vs survival time of pentobarbitone sodium is given and in Fig. 2 the corresponding linear correlation C/T vs C is given.

The doses (C) were not transformed into log doses according to Clark (1937) and also by Beccari (1949). In any case the method is entirely empirical and from a practical point of view the log transformation of doses seems to be unimportant. For example, the LD50 of pentobarbitone sodium obtained with the log transformation is 134·80 mg kg^{-1} instead of 136·60 mg kg^{-1}; similar results were obtained with the other drugs examined here.

The LD50 values obtained with the method described are compared in Table 1 with data found in the literature and obtained by the traditional method based on percentages of dead animals at various doses.

These results indicate that the method here proposed to evaluate acute toxicity gives reliable results and allows a considerable reduction of the number of animals killed.

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Neuropharmacological studies on the neuroleptic potential of domperidone (R33812)

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Neuroleptic agents are potent antagonists of emesis in both animals and man. This action is thought to involve blockade of dopamine sensitive receptors in the chemoreceptor trigger zone, but is not generally specific in that the same drugs can inhibit the function of other cerebral dopamine systems (Janssen 1970). It was therefore of interest that agents from a series of benzimidazoline derivatives, including domperidone and halopemide, were recently reported to possess potent anti-emetic activity whilst failing to cause certain biochemical or behavioural changes indicative of effect on other cerebral dopamine systems, even though they had the ability to potently displace neuroleptics in in vitro receptor binding assays (Leysen et al 1978; Niemegeers, Laduron, personal communications). Therefore, the present studies were designed to assess the ability of domperidone to exert neuroleptic-like blockade of central dopamine receptor mechanisms.

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The ability of domperidone to modify locomotor hyperactivity responses was assessed both following peripheral and intracerebral administration to male C.F.E. rats. Hyperactivity was measured using individual Perspex cages each equipped with one photocell unit placed off-centre, and interruptions of the light beam were recorded electromechanically in counts per 5 min (see Costall & Naylor 1976, for experimental details). In one series of experiments hyperactivity was induced by 50 μg dopamine injected in 1 μl bilaterally into the nucleus accumbens and by 25 μg dopamine injected in 2 μl bilaterally into the striatum following a nialamide pretreatment, 100 mg kg^{-1}, 2 h (see Costall & Naylor 1976, for stereotaxic techniques and experimental details). The effects of control agents, haloperidol, sulpiride and metoclopramide were assessed at the same time as domperidone. The i.p. administration of haloperidol, 0·025–0·8 mg kg^{-1}, and sulpiride, 2·5–20 mg kg^{-1}, was shown to reduce or abolish the hyperactivity induced from the

caudate-putamen and nucleus accumbens in a dose-dependent manner. Metoclopramide, 5–20 mg kg^{-1} only effectively antagonized the hyperactivity from the caudate-putamen (the data for control agents were indistinguishable from that previously reported, Costall & Naylor 1976), whilst domperidone, 10–80 mg kg^{-1} was inactive in both test procedures (with the exception of a 61% inhibition of the caudate response at 80 mg kg^{-1}). In a second series of experiments domperidone, and fluphenazine as control agent, were injected directly into the nucleus accumbens and the effects on the hyperactivity induced by peripherally administered (+)-amphetamine (1·5 mg kg^{-1} i.p.) or intra-accumbens dopamine (50 μg after nialamide) were determined. The hyperactivity induced by i.p. amphetamine was antagonized dose-dependently by both intra-accumbens fluphenazine and domperidone: the doses required were similar (Table 1). Intra-accumbens injections of fluphenazine and domperidone also antagonized the hyperactivity response to intra-accumbens dopamine, but larger doses were required and a dose-dependency was not demonstrated (Table 1).

Table 1. Effects of fluphenazine and domperidone injected into the nucleus accumbens on the hyperactivity induced by peripherally administered amphetamine or intra-accumbens dopamine in the rat. n = 6–9. Values are calculated from maximum effects. S.e.m.s, calculated from values in counts/5 min, were 0–16%. Data given refer to observations made after an initial 2–3 min period of injection artifact (caused by drug or vehicle alone).

Dose μg μl^{-1}	% inhibition hyperactivity induced by: Intraperitoneal amphetamine	Intra-accumbens dopamine
Fluphenazine		
0·2	2	
0·4	17	
0·8	100	0
1·25		0
2·5		100
Domperidone		
0·32	0	
0·63	18	
1·25	68	0
2·5	74	100

A further behavioural index of neuroleptic potential measured after intracerebral injection was the production of asymmetry and circling. These experiments involved the simultaneous administration of 1 mg kg^{-1} s.c. apomorphine and a unilateral intrastriatal injection of the test drug (fluphenazine again used as the control neuroleptic). In the presence of apomorphine both fluphenazine and domperidone were shown to induce a weak asymmetry and a dose-dependent circling behaviour following unilateral intrastriatal injection (Table 2). The direction of circling was ipsilateral to the side of injection. The doses of domperidone and fluphenazine required to produce the described effect were similar. Onset of the effect was notably longer when using domperidone, but the duration of the circling response was in excess of 120 min in all experiments (Table 2).

Table 2. Number of rats responding (I) asymmetry score (II) and circling (III-rev min^{-1}) observed after unilateral intrastriatal injections of fluphenazine and domperidone in the presence of apomorphine in the rat. Mean maximum responses are given. S.e.m.s for circling <12%. Values given were recorded after a 2–3 min period of injection artifact (caused by drug and vehicle) in which animals exhibited periodic asymmetry.

Drug and dose μg/2 μl	Asymmetry/circling: I	II	III	Onset (min)	Duration (min)
Fluphenazine					
0·25	0/6	—	—	—	—
0·5	4/6	1	2·5	5–30	>120
1	6/6	1	4·1	5–10	>120
2	6/6	1	6·0	5	>120
4	6/6	2	4·8	5	>120
Domperidone					
0·5	0/6	—	—	—	—
1	2/6	1	1·8	60–90	>120
2	6/6	1	3·0	60–80	>120
4	6/6	1	6·1	<60	>120

Subsequent experiments assessed the actions of domperidone in more routine screens for neuroleptic action using the peripheral route of administration. Generally, these experiments confirmed only a weak action or an inactivity for domperidone when administered peripherally, in contrast to its action by the intracerebral route. Firstly, in catalepsy tests described by Costall & Naylor (1975), in which catalepsy was scored 1 to 5 according to time of maintenance of an abnormal position, domperidone was virtually inactive: only large (near toxic) doses of domperidone given by the peripheral route caused a very weak inconsistent catalepsy which was not dose-related (responses at 40 and 80 mg kg^{-1} i.p. never exceeded score 1), and which could not be enhanced by combination with either α-methyl-*p*-tyrosine (AMPT) (250 mg kg^{-1} i.p. given as a 6 h pretreatment) or RS86 (5 mg kg^{-1} i.p. given at the same time as domperidone). Haloperidol and metoclopramide, used as control agents under the same test conditions, each induced a dose-dependent catalepsy (score range 1–5 using 0·25–2 mg kg^{-1} haloperidol, 2·5–20 mg kg^{-1} metoclopramide). Threshold doses of both agents (causing a score 1 catalepsy) synergized in the production of catalepsy with AMPT (which alone failed to induce catalepsy), the combination causing score 4–5 catalepsy. Haloperidol (but not metoclopramide) also synergized with RS86 (alone causing a score 1 catalepsy) to cause a score 4 or 5 catalepsy. These data for control agents are similar to those previously

reported (see Costall & Naylor 1975).

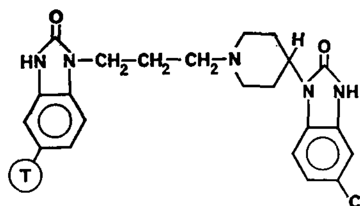
Haloperidol and metoclopramide were also shown to antagonize the stereotypic effects of both amphetamine and apomorphine: these effects were dose-dependent and in each experimental situation 100% inhibition of stereotypy could be achieved. These data were indistinguishable from those previously recorded (see Costall & Naylor 1975): briefly, stereotyped behaviour was induced by 10 mg kg^{-1} i.p. (+)-amphetamine and 2 mg kg^{-1} s.c. apomorphine. Test agents were given as pretreatments and stereotypy was scored as 0—no stereotypy, 1—periodic sniffing and/or repetitive head and limb movements, 2—continuous sniffing and/or repetitive head and limb movements, 3—periodic gnawing, biting or licking, 4—continuous gnawing, biting or licking. In this test situation domperidone again caused only a weak to moderate (not dose related) antagonism of stereotypy (25–50%) but at near toxic doses (20–80 mg kg^{-1} i.p.).

Further experiments to assess neuroleptic potential following peripheral drug administration used male B.K.W. mice. Climbing behaviour was induced by 1 mg kg^{-1} s.c. apomorphine and was measured, using wire-lined cages, as 'the percentage of time spent climbing during the 30 min period following the first climb' (the 'climbing index', see Costall et al 1978; Protais et al 1976). Circling behaviour was induced by 1 mg kg^{-1} s.c. apomorphine in mice with unilateral striatal lesions (induced using a Kopf stereotaxic instrument, rat ear bars and incisor bar, raised 2 mm above the inter-aural line: coordinates 1·0 mm ant. to bregma, 2·3 mm lat. to the mid-line, vert. 3·5 mm from the skull surface: stainless steel electrode, 0·65 mm diameter and insulated except at the tip, current parameters of 1·5 mA for 15 s). Active circling behaviour was assessed in mice as the number of complete revolutions made in one direction only in a 2 min period. Stereotyped biting was induced in mice by 4 mg kg^{-1} s.c. apomorphine; for assessment of drug interactions, stereotypy was indicated as 0—absent, 1—periodic, 2—continuous. Both haloperidol (used as the control neuroleptic in the mouse experiments) and domperidone antagonized the climbing behaviour and circling induced by apomorphine in the mouse. These effects were dose-dependent, but haloperidol was notably more potent than domperidone (at least × 10) (Table 3). Stereotyped behaviour was also antagonized by larger doses of both haloperidol and domperidone (Table 3).

Table 3. Antagonism of climbing behaviour, circling and stereotypy in the mouse by haloperidol and domperidone. Mean maximum effects are given, n = 6–9. S.e.m.s are indicated for climbing and circling. S.e.m.s in stereotypy scores were less than 10%.

Neuroleptic agent	Drug and dose mg kg^{-1} i.p	Climbing index (%)	Circling (rev/2 min)	Stereotypy (% inhib.)
Haloperidol				
	0	71 ± 2	6·7 ± 0·3	
	0·006	66 ± 2	6·9 ± 0·3	
	0·0125	64 ± 3	7·1 ± 0·4	
	0·025	41 ± 9	4·8 ± 0·6	
	0·05	8 ± 4	0	
	0·1	0	0	0
	0·2			4
	0·4			54
	0·8			100
Domperidone				
	0	62 ± 7	6·8 ± 0·2	
	0·16	61 ± 3	4·5 ± 1·0	
	0·31	25 ± 16	4·5 ± 0·7	
	0·63	20 ± 10	2·8 ± 1·0	
	1·25	0	0	0
	5	0	0	40
	10			84
	20			92

All behavioural experiments were carried out between 8·00 am and 6.00 pm in sound proofed, diffusely illuminated rooms maintained at 21 ± 3 °C. For peripheral injection domperidone (R33812, 5-chloro-1-[1-[3-(2-oxo-1-benzimidazolinyl)propyl]-4-piperidyl-2-benzimidazolinone) (Janssen) was prepared in a minimum quantity of *NN*-dimethylformamide made up to volume with distilled water. For intracerebral injection domperidone was used in the injection form prepared by the manufacturers (apropriate vehicle was supplied for control injections).



Since domperidone was clearly as effective as a potent neuroleptic when given intracerebrally, but was only weakly active or inactive when given peripherally, experiments were carried out to assess whether domperidone was reaching cerebral areas after peripheral administration. Groups of rats and mice were njected s.c. with [^{3}H]domperidone (40 μCi kg^{-1} and 400 μCi kg^{-1} respectively) (specific activity of 10 Ci mol^{-1}) and the doses adjusted with unlabelled domperidone to a total of 40 mg and 20 mg kg^{-1}. Doses were selected, as far as possible, on the basis of data obtained from the behavioural studies, but the low weights of the different brain areas of the mouse necessitated the use of a larger dose of labelled compound in this species. Animals were killed by cervical dislocation 1 h after injection and blood samples taken from the inferior vena cava. To ensure removal of blood from cerebral tissue, brains were perfused with ice-cold saline. Brains were rapidly removed and dissected over ice. After weighing, tissue samples were combusted in a Packard Tri-Carb tissue oxidiser (model 306) and counted in a Packard Tri-carb liquid scintillation spectrometer (model 574).

Structures outside the blood-brain barrier, the pineal gland and pituitary gland, were shown to accumulate relatively large concentrations of domperidone in both

the rat and the mouse. The concentrations of domperidone in other brain areas of the rat were considerably less than the blood content, although in the mesolimbic areas of this species, the nucleus accumbens and tuberculum olfactorium, and in the globus pallidus domperidone accumulated in concentrations approximately equivalent to or greater than those of the blood. Generally, the levels of domperidone measured in the mouse brain were lower than those in the rat (Table 4).

Table 4. Distribution of domperidone in the rat and mouse brain. Values are expressed as a percentage of the concentration of radioactivity (d min^{-1} wet weight) within the blood, and each is the mean of 4 to 6 determinations ± s.e.m. Concentration of [^{3}H]domperidone: [a]20·7 ± 0·8 d min^{-1} mg^{-1}; [b]225·4 ± 81·4 d min^{-1} mg^{-1}.

Tissue	Percentage of blood counts mg^{-1}	
	Rat	Mouse
Blood	100[a]	100[b]
Pineal gland	987·1 ± 129·8	1033·4 ± 289·8
Pituitary gland	305·6 ± 73·6	236·7 ± 52·4
Cerebral cortex	18·6 ± 2·8	11·4 ± 2·3
Pons and medulla	26·2 ± 2·5	18·7 ± 3·8
Cerebellum	15·7 ± 2·3	10·7 ± 1·2
Tuberculum olfactorium	126·0 ± 31·7	22·1 ± 4·3 (Tuberculum olfactorium and Nucleus accumbens)
Nucleus accumbens	399·9 ± 87·2	
Hypothalamus	39·2 ± 6·9	15·3 ± 3·0
Thalamus	67·2 ± 18·0	13·3 ± 3·4
Globus pallidus	114·1 ± 40·5	12·3 ± 1·7 (Globus pallidus and Caudate-putamen)
Caudate-putamen	19·8 ± 5·1	
Hippocampus	19·2 ± 3·3	14·3 ± 2·8

The test agent domperidone, and the control neuroleptic and related agents used in this study, all exert potent anti-emetic effects, but domperidone, in contrast to the control agents, failed to induce other effects indicative of cerebral dopamine blockade when given peripherally to the rat. Thus, domperidone failed to induce a consistent cataleptic response, even when catecholamine function was reduced (AMPT) or cholinergic function enhanced (RS86), conditions which facilitate a cataleptic potential (Costall & Naylor 1973). Similarly, the anti-stereotypic effects of domperidone were weak. Catalepsy induction and stereotypy antagonism probably involve an inhibitory neuroleptic effect on striatal and mesolimbic dopamine systems (Costall & Naylor 1974a,b; Pijnenburg et al 1975). It is clear that the *peripheral* administration of domperidone in the rat fails to satisfactorily block these dopamine systems. This conclusion is supported by the failure of peripherally administered domperidone to effectively antagonize the hyperactivity responses to intra-accumbens and intrastriatal dopamine. Domperidone was, however, more active in tests for neuroleptic potential following peripheral administration in the mouse, although still 10 to 20 times less active than haloperidol. Nevertheless, the low activity or inactivity recorded after peripheral administration of domperidone contrasted markedly with its potency on intracerebral injection. Intra-accumbens domperidone was virtually equipotent with fluphenazine to antagonize amphetamine hyperactivity, or the hyperactivity induced by intra-accumbens dopamine. Similarly, on unilateral intrastriatal injection domperidone and fluphenazine were virtually equipotent to induce asymmetry/circling in the presence of apomorphine (see Elliott et al 1977). It is suggested that the low activity/inactivity of peripherally administered domperidone to influence cerebral dopamine systems may reflect an inability to effectively penetrate cerebral tissue. The low activity of tritiated domperidone (and possibly metabolite(s)) which were found in cerebral tissue after peripheral injection may support this suggestion. The powerful anti-emetic activity in the dog (PD50 value 0·006 mg kg^{-1}, Niemegeers) is readily explained as a potent effect on the dopamine receptors in the area postrema, a mechanism outside the blood-brain barrier. Domperidone may similarly be expected to affect other dopamine systems outside the blood-brain barrier, for example, those in the stomach (Baeyens et al 1978). Thus, domperidone should prove to be a useful experimental tool, and an anti-emetic potential in the clinic, in the absence of side effects attributable to a blockade of cerebral dopamine systems, has already been realised (Nogarède et al 1978).

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