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DOMPERIDONE, A SPECIFIC *IN VITRO* DOPAMINE ANTAGONIST, DEVOID OF *IN VIVO* CENTRAL DOPAMINERGIC ACTIVITY

PIERRE M. LADURON and JOSÉE E. LEYSEN
Department of Pharmacology, Janssen Pharmaceutica, B-2340 Beerse, Belgium

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Abstract—When tested in different *in vitro* binding assays and in dopamine-sensitive adenylate cyclase, domperidone was found to be a very potent and specific dopamine antagonist. Accordingly, [^{3}H]domperidone binding sites were exclusively detected in homogenates of brain dopaminergic regions. Despite its powerful dopamine antagonism, domperidone did not act centrally in a similar way to neuroleptic drugs. *Ex vivo* and *in vivo* experiments indicated that domperidone is unable to reach dopamine receptors in rat striatum. The very low amount of labelling and atypical distribution found in rat brain after administration of [^{3}H]domperidone is compatible with a lack of penetration into brain structures. Finally, domperidone did not elicit marked HVA increase as is found with classical neuroleptic drugs and metoclopramide. From the present experiments, one may conclude that domperidone, though a very potent and specific dopamine antagonist, cannot readily cross the blood brain barrier, which thus prevents the occurrence of central effects.

Domperidone is a new gastrokinetic and antinauseant drug which has been reported to be effective in functional gastrointestinal disorders such as dyspepsia, gastroesophageal reflux, nausea and vomiting [1]. Pharmacologically, domperidone is a powerful antagonist of apomorphine-induced emesis in dog but in contrast to neuroleptic drugs and metoclopramide, it is practically devoid of central effects [2]. Domperidone only antagonises apomorphine-induced stereotypy and agitation in the rat at very high doses, whilst centrally acting dopamine antagonists show a very good correlation between apomorphine antagonism in rat and apomorphine antagonism in dog [2].

In vitro experiments on isolated guinea-pig stomach showed that domperidone inhibits the relaxation induced by exogenous dopamine [3]. Thus, the gastrokinetic effects of domperidone may be explained by a peripheral interaction with dopamine receptors at the level of the stomach.

The present investigations were undertaken to characterize the specific interactions of domperidone at the receptor level under both *in vitro* and *in vivo* conditions.

MATERIALS AND METHODS

Tissue preparation. Wistar rats (150 ± 10 g) were killed by decapitation and the brains were immediately removed. The striatum and the frontal cortex were dissected out and then homogenized in Tris–HCl buffer (0.05 M, pH 7.6). Most of the *in vitro* experiments were carried out using a total particulate fraction prepared as previously reported [4]. However, a total homogenate, prepared in distilled water, was used for the [^{3}H]dexetimide binding.

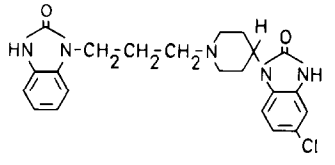

Fig. 1. Chemical structure of domperidone.

In vitro *binding.* For the [^{3}H]domperidone binding assay, the incubation conditions were as follows; a total volume of 2.2 ml contained 2 nM [^{3}H]domperidone and a total particulate fraction corresponding to 25 mg original tissue, suspended in Tris–HCl buffer 0.05 M, pH 7.6 supplemented by: NaCl, 120 mM; KCl, 5 mM; $CaCl_2$, 2 mM; $MgCl_2$, 1 mM; pargyline, 10 μM; and ascorbic acid 0.1%. After 10 min at 37°, the incubation was stopped by rapid filtration under suction through Whatman GF/B glass fiber filters which were washed twice with 5 ml ice-cold buffer. The radioactivity was determined by liquid scintillation counting.

[^{3}H]Haloperidol and [^{3}H]spiperone binding was carried out using total particulate fractions of striatum and frontal cortex respectively according to Creese *et al.* [47] and Leysen *et al.* [5, 6] while [^{3}H]dexetimide was performed using a total homogenate as previously described [7]. IC_{50}-values were calculated as previously described [5].

Enzyme assay. Dopamine-sensitive adenylate cyclase was assayed in the presence or absence of 10^{-4}M dopamine according to Kebabian *et al.* [8].

Ex vivo *binding.* Rats were given different doses of domperidone or haloperidol intravenously. Two hours later the rats were decapitated and the striata were removed and homogenized in 20 vol. 0.05 M Tris–HCl buffer pH 7.6. Then the occupancy of receptors was assessed under *in vitro* conditions using the [^{3}H]spiperone binding assay as already reported [9].

In vivo *binding.* [^{3}H]Spiperone (5 μg.kg^{-1}) was injected i.v. into male Wistar rats (200 g). One hour later, animals received i.v. different doses of unlabelled domperidone. Three hours later, the animals were killed and the radioactivity was measured in various brain regions as previously reported [10].

Similarly, the regional distribution of

[^{3}H]domperidone and [^{3}H]clopimozide was determined in the rat brain after intravenous or oral administration.

Homovanillic acid (HVA) determination. Rats were given various doses of drug subcutaneously or orally. At different time after injection, the animals were killed and the whole brain minus cerebellum was removed and homogenized in 6 vol. 0.7% perchloric acid with an ultraturrax homogenizer. After centrifugation, the supernatant was removed and kept at −20°. 4 ml of the supernatant was extracted with 6 ml of freshly distilled ethylacetate. After shaking and centrifuging, 5 ml of the organic phase was extracted with 2.6 ml of Tris–HCl buffer 0.5 M pH 8.5. HVA was measured according to Anden *et al.* [11] using internal standards.

Drug. [^{3}H]Spiperone (sp. act. 6 Ci/m-mole) [^{3}H]dexetimide (sp. act. 17 Ci/m-mole) [^{3}H]haloperidol (sp. act. 8.5 Ci/m-mole) and [3]domperidone (sp. act. 10 Ci/m-mole) were supplied by IRE Fleurus Belgium. [^{3}H]Clopimozide (sp. act. 495 mCi/m-mole) was obtained from the Radiochemical Department, Janssen Pharmaceutica.

RESULTS

In vitro *experiment*

When tested in the [^{3}H]haloperidol binding assay in the striatum, domperidone was found to bind with high affinity to the dopamine antagonist sites (Table 1). Indeed its IC_{50} value was slightly lower than that of haloperidol and 100 times lower than that of chlorpromazine. Domperidone's affinity for serotonergic ([^{3}H]spiperone in frontal cortex [12], and muscarinic ([^{3}H]dexetimide in striatum) receptors was extremely low, which contrasts with the very poor selectivity of chlorpromazine [6]. It is noteworthy that the dissociation between affinities for dopamine and serotonin receptors was greater with domperidone than with haloperidol.

These results were further substantiated by the *in vitro* binding of [^{3}H]domperidone in different brain regions. Figure 2 shows that [^{3}H]domperidone binding sites were only detected in dopaminergic areas such as the striatum, the olfactory tubercle and the nucleus accumbens but not in the frontal cortex or the cerebellum. Interestingly, the blank values obtained by using either (+)-butaclamol (1×10^{-6} M) or domperidone (1×10^{-6} M) were practically similar, whereas (−)-butaclamol did not affect [^{3}H]domperidone binding. Therefore, the saturable sites labelled by domperidone are stereospecific.

The effect of domperidone on the dopamine-sensitive adenylate cyclase was tested. Figure 3 shows that the dopamine-stimulated cAMP formation was inhibited by domperidone but only at very high concentrations.

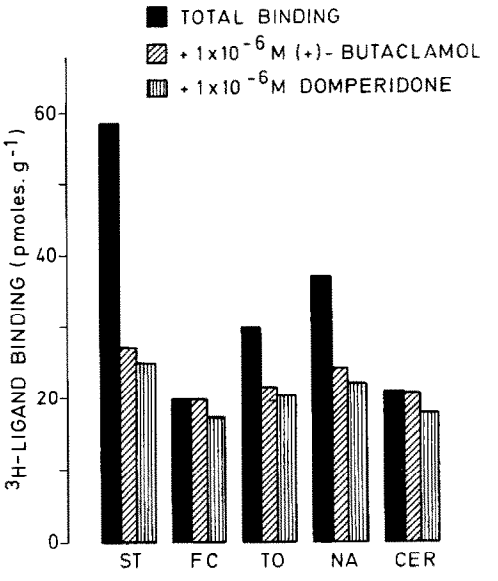

Fig. 2. Binding sites in different brain regions (ST, striatum; FC, frontal cortex; TO, tubercle olfactory; NA, nucleus accumbens; CER, cerebellum) using [^{3}H]domperidone as ligand (see Methods). Stereospecific and saturable domperidone binding sites are given by the difference between total binding and binding obtained in the presence of 1×10^{-6} M of (+)-butaclamol or of domperidone.

Ex vivo *experiment*

The occupancy of [^{3}H]spiperone binding sites was determined under *in vitro* conditions in striata of rats previously treated with various doses of domperidone and haloperidol. Fig. 4 shows that the neuroleptic receptors in the striatum were not occupied when domperidone was injected intravenously even at high dosage (2.5 mg·kg^{-1}). In contrast to this, haloperidol was found to be able to reach the striatal receptors in a dose-dependent manner.

In vivo *binding*

In order to see whether domperidone was able to reach striatal dopamine receptors under *in vivo* conditions, attempts were made to displace [^{3}H]spiperone from its receptors with various doses of domperidone. Table 2 shows that after intravenous injection of quite high doses (2.5 and 10 mg · kg^{-1}), domperidone was unable to displace labelled spiperone accumulated in rat

Table 1. Drug affinity for dopaminergic, serotonergic and muscarinic receptors in rat striatum and frontal cortex

	Receptor binding IC_{50} (M)			Ratio	
	[^{3}H]Haloperidol Striatum (A)	[^{3}H]Spiperone Frontal cortex (B)	[^{3}H]Dexetimide Striatum (C)	B/A	C/A
Domperidone	1.4×10^{-9}	4×10^{-7}	3.5×10^{-5}	285	25,000
Haloperidol	3.6×10^{-9}	1.5×10^{-7}	1.8×10^{-5}	42	5000
Chlorpromazine	1.3×10^{-7}	6.2×10^{-8}	6.6×10^{-7}	0.5	5

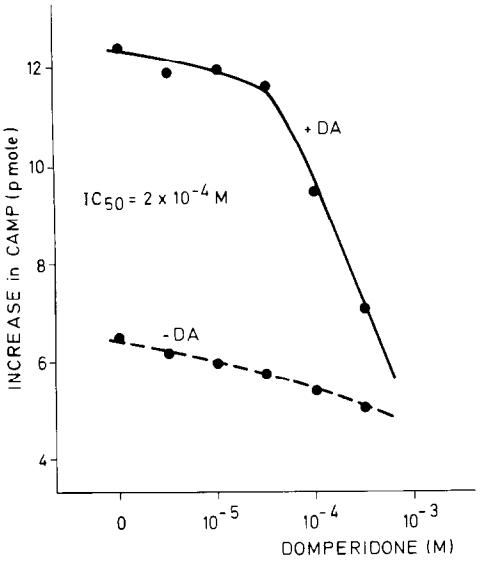

Fig. 3. Adenylate cyclase activity measured in a total homogenate of rat brain striatum in the presence of dopamine (+DA 10^{-4} M) at different concentrations of domperidone. Striata were homogenized in Tris maleate buffer 2 mM pH 7.4 containing 2 mM EGTA.

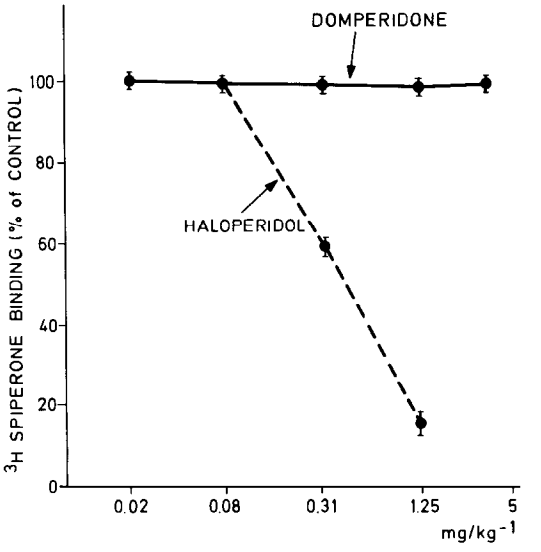

Fig. 4. *Ex vivo* experiment. [^{3}H]Spiperone binding sites were measured in the striatum of rats injected with different doses of domperidone and haloperidol ($n = 3 \pm$ S.E.M.).

striatum. Under the same conditions, the labelling due to the *in vivo* binding of [^{3}H]spiperone in striatum and olfactory tubercle but not in cerebellum was markedly displaced after injection of 0.08 mg · kg^{-1} unlabelled spiperone [9].

The regional distribution of [^{3}H]domperidone in rat brain was studied after intravenous and oral administration, and compared to that of a structurally related compound, [^{3}H]clopimozide. Table 3 shows that after injection of 2.5 mg · kg^{-1} [^{3}H]domperidone, the radioactivity accumulated much more in the cerebellum than in the striatum for instance. In contrast to this, after [^{3}H]clopimozide but more especially after [^{3}H]spiperone or [^{3}H]pimozide as already reported [10], the amount retained was much more elevated in the striatum. The brain regions that accumulated more [^{3}H]domperidone are not of dopaminergic type. In fact, when compared to [^{3}H]clopimozide, the amount of [^{3}H]domperidone recovered in the striatum was ex-

Table 2. *In vivo* displacement of labelled spiperone in rat brain

Region	Labelled spiperone pg · mg^{-1} ($n = 6 \pm$ S.E.M.)		
	Control	Domperidone 2.5 mg · kg^{-1}	Domperidone 10 mg · kg^{-1}
Striatum	3.76 ± 0.15	3.94 ± 0.22	3.73 ± 0.12
Nucleus accumbens	2.78 ± 0.07	2.82 ± 0.19	2.70 ± 0.22
Tuberculum olfactorium	1.70 ± 0.09	1.84 ± 0.23	1.62 ± 0.09
Frontal cortex	0.68 ± 0.13	0.64 + 0.03	0.75 ± 0.03
Cerebellum	0.22 ± 0.01	0.29 ± 0.01	0.22 ± 0.01

Table 3. Regional distribution of [^{3}H]domperidone and of [^{3}H]clopimozide

	pg · mg^{-1}		
	Domperidone 2.5 mg · kg^{-1}		Clopimozide 2.5 mg · kg^{-1}
	i.v.	p.o.	p.o.
Cerebellum	37.1	21	60
Medulla oblongata	28.9	10	62
Thalamus	28.9	14.8	62
Spinal cord	12.3	2.8	56
Striatum	9.9	3.2	101
Parietal cortex	9.2	3.3	67
Hypothalamus	8.7	1.3	66
Tuberculum olfactorium	6.7	2.6	63
Frontal cortex	6.4	0.8	—
Hippocampus	5.9	4.5	71

tremely low (3.2 pg · mg^{-1} when 2.5 mg · kg^{-1} was administrated orally compared to 101 pg for 0.16 mg · kg^{-1} p.o.).

HVA determinations

HVA was measured in rat brain 2 hr after subcutaneous injection of domperidone and metoclopramide or 4 hr after oral administration of haloperidol. Figure 5 shows that even after high dose of domperidone the HVA content was unaffected whereas a dose-dependent increase was observed after metoclopramide. When rats were given a very large dose of domperidone (10 mg · kg^{-1} s.c.) only a slight increase in HVA was observed 6–8 hr after injection (Fig. 6). In contrast, the same dose of metoclopramide caused a pronounced HVA increase peaking 1 hr after injection.

Finally, various doses of domperidone were given subcutaneously or orally and the HVA content was measured after 8 hr (Fig. 7). Here again domperidone caused only a slight HVA elevation when high doses were injected subcutaneously but such an effect was not observed after oral administration.

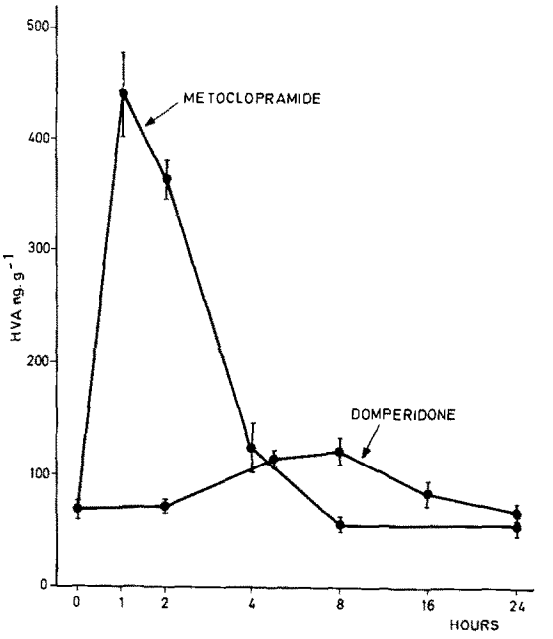

Fig. 6. HVA content in rat brain after subcutaneous injection of domperidone and metoclopramide (10 mg · kg^{-1} s.c.) ($n = 6 \pm$ S.E.M.).

DISCUSSION

The present investigations provide biochemical evidence that *in vitro*, domperidone is a very potent and selective dopamine antagonist. Indeed, it binds with high affinity to the dopamine antagonist sites in the striatum which are labelled by [^{3}H]haloperidol. In contrast to this, it revealed a very low affinity for serotonergic and muscarinic receptors. Domperidone's interference with dopamine receptors, has previously been suggested by pharmacological experiments; namely the antagonism of apomorphine-emesis in dog [2] and of the dopamine-induced relaxation in guinea pig stomach [3]. The fact that domperidone did not stimulate dopamine-sensitive adenylate cyclase, rules out the possibility for them to act as dopamine agonist. From our results, one may conclude that domperidone is one of the most selective dopamine antagonists.

In confirmation of this view, [^{3}H]domperidone binding sites were only detectable in homogenates of dopaminergic areas but not in the frontal cortex or in the cerebellum. Interestingly, the [^{3}H]domperidone binding sites displaceable by unlabelled domperidone were identical to the binding sites displaced by (+)-butacla-

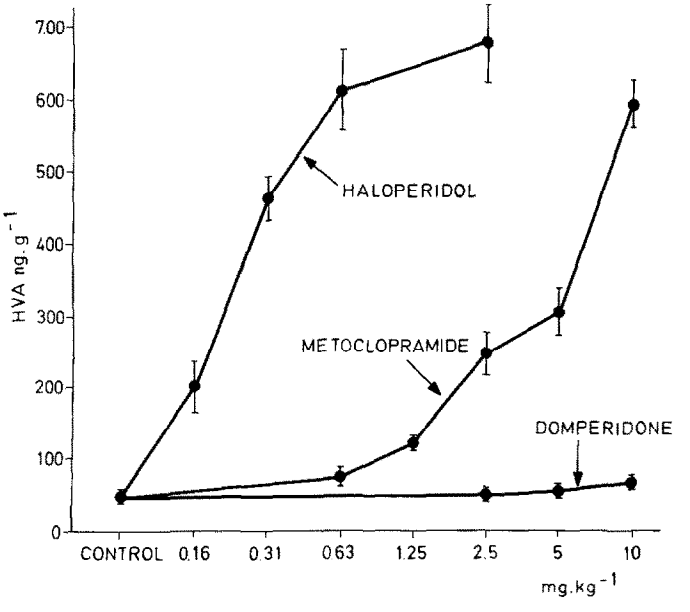

Fig. 5. HVA content in rat brain 2 hr after s.c. injection of domperidone metoclopramide and 4 hr after oral administration of haloperidol ($n = 6 \pm$ S.E.M.).

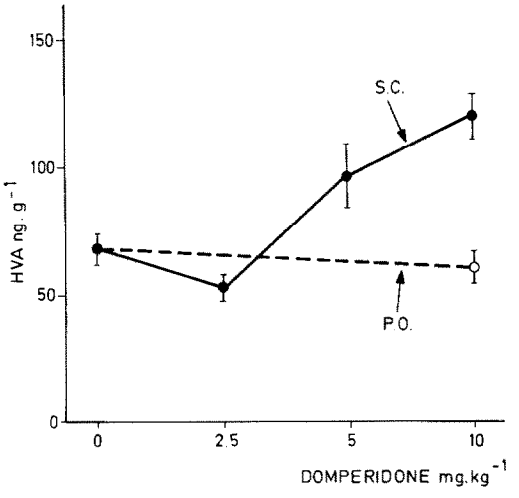

Fig. 7. HVA content in rat brain 8 hr after 10 mg · kg^{-1} domperidone injected subcutaneously (s.c.) or administered orally (p.o.) ($n = 6 \pm$ S.E.M.).

mol. For these reasons, domperidone may also be considered as an appropriate ligand for labelling dopamine receptors.

In contrast to this, domperidone was practically inactive towards dopamine sensitive adenylate cyclase. Its IC_{50} value was more than 13 and 250 times higher than that of pimozide and haloperidol respectively [13]. For these two neuroleptic drugs, the IC_{50} values were already abnormally high in comparison with those obtained in the binding assay. The fact that various drugs such as domperidone, halopemide and sulpiride are potent dopamine antagonists in the binding assay but are practically inactive inhibitors of the dopamine sensitive adenylate cyclase (unpublished results) further supports the view, originating from our subcellular fractionation study [14], that the neuroleptic binding site and the dopamine sensitive adenylate cyclase are two different entities not related to each other.Therefore it is not surprising that no correlation has been found between the inhibition of dopamine sensitive adenylate cyclase and the clinical and pharmacological potencies of neuroleptic drugs [15] while such a correlation exists with the [^{3}H]haloperidol or [^{3}H]spiperone binding [6, 15].

Although *in vitro*, domperidone is a very potent and specific dopamine antagonist it does not reach brain dopamine receptors under *in vivo* conditions. Four lines of evidence were provided to demonstrate that domperidone does not act centrally in the same way as classical neuroleptic drugs.

First, *ex vivo* experiments indicated that domperidone did not occupy dopamine receptors in rat striatum while such is the case for haloperidol, pimozide or spiperone [9]. Secondly even at high doses, domperidone was unable to displace [^{3}H]spiperone under *in vivo* conditions. In the same conditions, haloperidol, pimozide, spiperone and pipamperone even at a dose more than 100 times lower than that of domperidone were found to displace [^{3}H]spiperone either from dopaminergic or serotonergic receptors [6, 9, 10]. Thirdly, the amount of labelling recovered in the brain after administration of [^{3}H]domperidone was extremely low and not compatible with central activity. Moreover, its regional distribution profile was quite different from that found with a neuroleptic drug which is accumulated in dopaminergic areas [9, 10]. The fact that the concentrations of [^{3}H]domperidone in brain were 6 times lower than the corresponding plasma levels [16] is quite in accordance with the present results.

Finally, after injection of high doses of domperidone, there was practically no change in the HVA content in rat brain. In contrast, neuroleptic drugs and metoclopramide are known to elicit marked HVA increase even at low dosage. Therefore, domperidone did not display the characteristic effects of a neuroleptic drug on dopamine turnover.

From the foregoing experiments one may conclude that despite its pronounced ability to bind to dopamine receptors in *in vitro* conditions, domperidone is not able to reach such receptors under *in vivo* conditions. These observations may be explained if one assumes that domperidone does not readily cross the blood brain barrier. In addition, the fact that domperidone does not act via central dopamine receptors strengthens the idea that peripheral dopamine receptors are responsible for the gastrokinetic and antinauseant properties of this drug.

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