J. Pharm. Pharmacol. 1984, 36: 373–377
Received August 22, 1983

Investigation on central dopaminergic receptors (D-2) using the antagonistic properties of new benzamides

JEAN-FRANCOIS RUMIGNY*, MARGHERITA STROLIN BENEDETTI AND PHILIPPE DOSTERT

Centre de Recherche Delalande, 10, rue des Carrières, 92500 Rueil-Malmaison, France

The topography of the central dopaminergic receptor (D-2) has been studied using some analogues of tropapride, a new benzamide derivative, and sulpiride and clebopride as reference drugs. The compounds were compared by testing their ability to compete with [^{3}H]spiperone in an in-vitro binding test and by measuring their potency as antagonists of apomorphine-induced climbing in mice. Tropapride was the most active compound, both in-vitro and in-vivo. With the amide group substituted in the 2-position of the tropane ring, the antidopaminergic activity of the compounds was much less than that of the 3-substituted derivatives. The interaction of the tropane derivatives with the D-2 receptor site is stereoselective as the equatorial stereoisomer was much more active than the axial isomer. The ethylene bridge present in the tropane derivatives but not in the corresponding piperidinyl analogues increases the affinity of the tropane derivatives for the D-2 receptor. Interaction with the D-2 receptor was confirmed as being Na^+-dependent. The presence of a benzyl substituent on the basic nitrogen atom seems to be essential in the tropane series emphasizing the important role played in this series by the lipophilic auxiliary binding site postulated in Olson's model. In conclusion, the tropane skeleton may be considered a useful pharmacophoric group in the design of new dopaminergic drugs.

Many studies have been made to elucidate the structural requirements for dopamine receptor activation and/or blockade in different parts of the cns and at the periphery (Horn et al 1975; Goldberg et al 1978; Seeman 1980; Humber 1981; McDermed & Freeman 1981; Olson et al 1981; Pannatier et al 1981; Van de Waterbeemd & Testa 1983).

Because of its involvement in many physiological functions and the implied importance in clinical situations, the D-2 receptor as classified by Kebabian & Calne (1979) has received more attention than the D-1 receptor which is better understood in the laboratory (Calne 1980).

The aim of the present paper was to examine, in the benzamide series, the influence on dopamine-related activity of the relative spatial position of the two nitrogen atoms of the diamino chain and of the number of the CH_2-units between them. Starting from the structure of tropapride (MD 790501, *1*) for which potential high antipsychotic activity has been clearly shown in biochemical and pharmacological tests (Strolin Benedetti et al 1982; Jalfre et al 1983), we have exploited the rigid character conferred by the tropane skeleton on the diamine chain. Therefore, the axial isomer *2* of *1* and the equatorial isomer *3* and axial *4* of the corresponding 2-substituted tropane derivatives have been prepared (Table 1).

In addition, some other derivatives *5, 6, 7, 8, 9*, with structures closely related to that of *1*, clebopride or sulpiride were also examined in order to study the mapping of the D-2 antagonist site.

The compounds were compared by testing their ability to compete with [^{3}H]spiperone in an in-vitro binding test using rat striatum membranes and by measuring their potency as antagonists of apomorphine-induced climbing in mice. As the binding of benzamides is highly sodium-dependent (Theodorou et al 1980; Jenner & Marsden 1981), we also determined the affinity of the tropane derivatives for the D-2 site in the presence or absence of NaCl. The results are discussed in terms of the preferred stereochemistry required to fit the D-2 site and our results compared with those obtained by others (Jenner et al 1978; Jenner & Marsden 1979) with reference benzamides.

MATERIALS AND METHODS

Binding assay

The method for determining [^{3}H]spiperone binding was adapted from those described by Burt et al (1976) and Creese et al (1977). Male Sprague Dawley rats (Charles River, CD, France) were stunned and decapitated. The corpora striata were

* Correspondence.

rapidly dissected on ice and then homogenized (1 g/100 ml) in ice cold 50 mM Tris-HCl buffer (pH 7·7, at 25 °C) (Potter, 10 strokes at 1200 rev min^{-1}). The homogenate was centrifuged at 47 000*g* for 10 min at 4 °C and the resulting pellets were frozen at −20 °C. On the day of assay, the above procedure was repeated and the final pellet was resuspended (Ultra-turrax, 15 s at setting 3/4) in ice cold Tris-HCl buffer (50 mM, pH 7·4) containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ and 1 mM $MgCl_2$ (Tris ion buffer). As reported by Leff et al (1981), the presence of ascorbic acid was not necessary. In some experiments, sodium ions were omitted and the ionic strength of the buffer was maintained at a constant value by increasing the Tris concentration. In some other experiments GTP (300 μM) was included in the incubation medium containing Na^+. Incubation tubes in triplicate contained 1 ml of tissue homogenate (0·5–0·6 mg protein), 3·5 ml of Tris-HCl buffer, 250 μl of at least 10 different concentrations of each compound and 250 μl of [^{3}H]spiperone (NEN, West Germany, 26·7 Ci $mmol^{-1}$) prepared to obtain a 0·15 nM final concentration, all these solutions being prepared in Tris ion buffer. Samples were incubated for 30 min at 37 °C and rapidly filtered under vacuum through Whatman GF/B filters. After washing twice with 5 ml of cold Tris-HCl (pH 7·7), the filters were transferred to scintillation vials and dried (30 min at 60 °C). 10 ml of scintillation fluid (ACS II Amersham) were added and the vials were counted, after shaking for 1 h in a SL3000 (Intertechnique) liquid scintillation spectrometer.

Specific [^{3}H]spiperone binding was defined as the difference between [^{3}H]spiperone binding in the presence and absence of 1 μM (+)-butaclamol. The concentration that reduced specific binding by 50% (IC50) and the slope factor (apparent Hill number, n) were estimated from indirect Hill plots and linear regression analyses (Weiland & Molinoff 1981) using the formula:

$$\log \frac{(LR)_I}{(LR) - (LR)_I} = -\,n\log(I) + n\log(IC50)$$

where (I) = concentration of competitor ligand, (LR) = concentration of ligand-receptor complex when I = 0, $(LR)_I$ = concentration of ligand-receptor complex in presence of competitor ligand.

In some cases, data from the displacement curves were submitted to a subtype receptor analysis (Molinoff et al 1981) using a Hofstee or a Scatchard-type transformation. Unless otherwise stated, values are means of at least 3 independent determinations.

Antagonism of apomorphine-induced climbing

Potential neuroleptic activity of the compounds was assessed in mice, from the antagonism of climbing induced by apomorphine (1 mg kg^{-1} s.c.) injected 15 min after the intraperitoneal administration of either the compound or the vehicle, as described by Dostert et al (1982). ED50 values, calculated by regression analysis, were determined from at least three different doses with at least 8 animals per dose.

Chemicals

Tropapride, MD 790501, *1*: (exo)-2,3-dimethoxy-*N*-[8-(phenylmethyl)-8-azabicyclo[3.2.1]oct-3-yl]-benzamide (HCl), *2*: (endo)-2,3-dimethoxy-*N*-[8-(phenylmethyl)-8-azabicyclo[3.2.1]oct-3-yl]-benzamide, *3*: (endo)-2,3-dimethoxy-*N*-[8-(phenylmethyl)-8-azabicyclo[3.2.1]oct-2-yl]benzamide (HCl), *4*: (exo)-2,3-dimethoxy-*N*-[8-phenylmethyl)-8-azabicyclo[3.2.1]oct-2-yl]-benzamide (HCl), *5*: (exo)-2,3-dimethoxy-*N*-[8-ethyl-8-azabicylo[3.2.1]-oct-3-yl]benzamide (HCl), *6*: (exo)-2,3-dimethoxy-*N*-[8-azabicyclo[3.2.1]oct-3-yl]-benzamide, *7*: 2,3-dimethoxy-*N*-[1-(phenylmethyl)-4-piperidinyl]-benzamide (HCl), *8*: 2,3-dimethoxy-*N*-[1-(phenylmethyl)-3-piperidinyl]-benzamide (HCl), *9*: 5-(aminosulfonyl)-2-methoxy-*N*-([(1-phenylmethyl)-2-pyrrolidinyl]methyl)-benzamide (CH_3SO_3H), sulpiride, sultopride (HCl) and clebopride (CH_3SO_3H) were synthesized in the Department of Organic Chemistry at the Delalande Research Centre. The two isomers of butaclamol (HCl) were purchased from Ayerst Laboratories; guanosine-5′-triphosphate, disodium salt (GTP) was from Boehringer Mannheim; domperidone, haloperidol and spiperone were obtained as gifts from Janssen Pharmaceutica.

RESULTS AND DISCUSSION

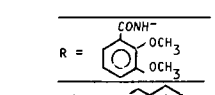
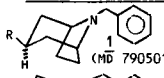
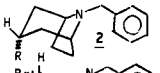
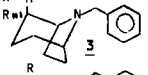
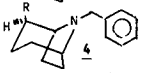
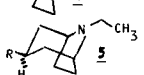
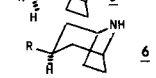
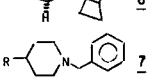
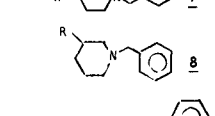
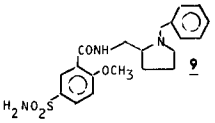
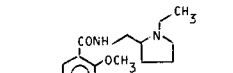
Results of biochemical and pharmacological tests are presented in Table 1.

MD 790501, *1*, is the most active, both in-vitro and in-vivo, of the benzamide derivatives. In particular, *1* exhibits an affinity about 20 times higher for the D-2 receptor than does clebopride the most active of the reference compounds, as shown by comparison of their IC50 value in the [^{3}H]spiperone binding test.

The interaction between *1* and the D-2 receptor seems to be stereoselective as its axial isomer *2* is much less potent, about 4500 and 3000 times in the climbing test and in the binding test, respectively.

When the benzamide moiety of *1* (group R in Table 1) is shifted from position 3 on the tropane skeleton to the 2 position, compounds *3* and *4*, a

Table 1. Effect of benzamide derivatives on the [^{3}H]spiperone binding using rat striatum membranes and in the apomorphine-induced climbing test in mice.

	Antagonism of apomorphine-induced climbing ED50 (mg kg^{-1} i.p.) (95% confidence limits)	[^{3}H]spiperone binding[a]	
		IC50 (nM)	Slope factor (n)
	0·01 (0·009–0·011)	0·64 ± 0·22[b]	0·79 ± 0·09
	30[c]	2,870 ± 350	1·04 ± 0·03
	22·1 (11·5–42·4)	1,030 ± 60	0·92 ± 0·02
	> 60[d]	6,830 ± 2,400	0·94 ± 0·10
	100	8,300 ± 330	0·86 ± 0·02
	> 30[d]	11,700 ± 1,050	1·04 ± 0·01
	0·05 (0·04–0·08)	3·4 ± 0·25	0·87 ± 0·03
	> 100[d]	76,600 ± 7,200	0·87 ± 0·03
	22·6 (18·6–27·4)	100 ± 24	0·80 ± 0·05
 Sulpiride	37·9 (36·8–39)	280 ± 20	0·80 ± 0·04
 Clebopride	0·07 (0·06–0·08)	14 ± 3·5	0·90 ± 0·02

[a] Results are expressed as mean ± s.d.; [b] mean of 7 determinations; [c] toxic at higher doses; [d] at this dose the pharmacological effect was less than 20%.

dramatic decrease of activity is observed with the equatorial isomer *3*: (IC50 *3*)/(IC50 *1*) ~ 1600; (ED50 *3*)/(ED50 *1*) ~ 2600 and the axial isomer *4* is practically devoid of activity. With the piperidine analogues *7* and *8*, the same loss of activity is observed when the number of CH_2-units between the two nitrogen atoms is reduced from 3 as in compound *7*, to 2 in compound *8*. Interestingly, the decrease in activity obtained with *8* compared with *7*, corresponds to that observed between compounds *1* and *4*, rather than between *1* and *3*, in spite of the fact that it is difficult to conceive that the substituent R in compound *8* can adopt a pseudo-axial conformation, which would make it structurally closer to *4* than to *3*. It seems more rational to assume that the ethylene bridge present in *3* and not in *8*, affords a better 'fit' with the D-2 receptor for molecule *3* than for molecule *8*, possibly through a lipophilic interaction, as the lipophilicity of *3* is higher than that of *8* as estimated according to Nys & Rekker (1974). Prieto et al (1977), using the antagonism of apomorphine-induced gnawing behaviour in the rat, have also observed a difference between clebopride (minimum effective oral dose: 1·56 mg kg^{-1}) and its 3-piperidyl derivative (no activity at maximal screening dose: 100 mg kg^{-1}). It is, however, interesting that Dostert et al (1982), using the same pharmacological test as we have used here, did not find a marked difference

in activity between the 3- and 4-*N*-benzyl piperidyl derivatives in a series of 2-amino-4-methoxy pyrimidine amides (ED50 ratio ~ 7).

The results in Table 1 allow us to carry out a partial qualitative analysis using Olson's dopamine receptor model (Olson et al 1981). This model is based on three essential binding sites and one lipophilic auxiliary binding site. From the results obtained with our tropane derivatives, the conclusion can be drawn that the lipophilic site seems to play an important role in the interaction of these molecules with the D-2 receptor; accordingly, the substitution of the benzyl group of *1* by an ethyl group or hydrogen atom led to molecules *5* or *6* having respectively IC50 values 13 000 or 18 300 times higher than that of *1*. The pharmacological activity of *5* and *6* compared with *1* is also much decreased [(ED50 *5*)/(ED50 *1*) ~ 10 000)]. The lipophilicity of *1* and *5* is 3·2 and 2 respectively (Nys & Rekker 1974). The degree of the lipophilic character of the substituent borne by the basic nitrogen influences activity in the tropane series but this is not general within the benzamide series, as is evident from a comparison of ED50 values and IC50 values of *9* and sulpiride in Table 1. The substitution of the ethyl group of sulpiride by a benzyl group (*9*) does not modify the pharmacological activity or the affinity for the D-2 receptor to a substantial extent as it does in the tropane series, although the lipophilicity of *9* differs markedly from that of sulpiride. The same conclusion can be drawn from the work of Prieto et al (1977) in which the ratio between the effective dose of the ethyl and benzyl (clebopride) derivatives is only 16.

For compounds *1*, *9* and sulpiride, the slope factor (n) of the displacement curve was slightly less than unity. However, Hofstee or Scatchard-type analysis of the data (Molinoff et al 1981) using the Gauss-Newton iterative procedure suggests that the three compounds interact, under the conditions of our assay, with a single site. The fitting was not significantly improved ($P > 0{\cdot}05$) when a model having two or three sites was used.

A sodium-dependent interaction between reference benzamides and dopamine receptors has been observed (Stefanini et al 1980; Jenner & Marsden 1981; Mizuchi et al 1982; Freedman et al 1982). We therefore thought that it would be interesting to test our tropane derivatives in this respect. Various drugs, including butyrophenone and benzamide derivatives, were tested for their ability to displace [^{3}H]spiperone from its binding site in the presence or absence of sodium ions. As previously described (Stefanini et al 1980; Mizuchi et al 1982), the affinity of butaclamol or butyrophenones for the D-2 receptor was not affected by the absence of Na^+. In contrast, *1*, *2* and the reference benzamides showed a net decrease in their affinity (5 to 20 times) for the site (Table 2). These changes in affinity in a Na^+-free medium are in agreement with those reported by the above authors. In view of these results, it seems that benzamides recognize a sodium-dependent antagonist binding site in the D-2 receptor as suggested by Freedman et al (1982) and Theodorou et al (1983). Some authors, from results obtained with dopaminergic agonists, have proposed that this receptor might exist in two interconvertible conformational states (Makman et al 1982; Battaglia & Titeler 1982) depending on the presence or absence of guanylnucleotides as well as of Na^+. However, when GTP was included in the incubation medium containing Na^+, affinities and slope factors for sulpiride and *1* were not significantly modified (Table 3). Similar

Table 2. Effect of various drugs on the [^{3}H]spiperone binding using rat striatum membranes in the presence (120 mM) or absence of sodium ions. IC50 are expressed in nM.

Compound	Tris + ion buffer IC50 (slope factor)	Sodium-free buffer IC50 (slope factor)
Spiperone	0·17 (0·94)	0·16 (0·97)
Domperidone	1·70 (0·95)	2·07 (0·87)
Haloperidol	5·33 (0·99)	4·11 (0·95)
(+)-Butaclamol	1·53 (1·00)	2·27 (1·04)
(−)-Butaclamol	563 (1·10)	891 (0·97)
Sulpiride	280 (0·80)	5,240 (0·79)
Sultopride	147 (0·78)	745 (0·70)
Clebopride	14·0 (0·90)	217 (0·94)
1	0·64 (0·79)	6·20 (0·94)
2	2,870 (1·04)	23,800 (0·94)
3	1,030 (0·92)	1,440 (0·99)
4	6,830 (0·94)	12,300 (0·87)

Results are means of at least 2 experiments performed in triplicate.

Table 3. Effect of GTP on the binding of tropapride and sulpiride to the D-2 receptor. IC50 are expressed in nM.

Compound	Tris + ion buffer IC50 (slope factor)	Tris + ion + GTP buffer IC50 (slope factor)
1	0·57 (0·88)	0·66 (0·82)
Sulpiride	260 (0·80)	290 (0·82)

Results are mean of two separate experiments performed in triplicate using the same batch of membranes for control buffer or GTP (300 μM) buffer.

results have been observed by Freedman et al (1981): GTP and its stable analogue guanyl-5′-yl imidodiphosphate did not affect the binding of neuroleptics at the [^{3}H]sulpiride site. These results seem to suggest that benzamides do not behave as partial agonists towards the D-2 receptor. Alternatively, some authors have described the benzamide binding site as a new Na^+ dependent D-2 receptor (Mizuchi et al 1982).

Finally, it is also important to note that the binding of the 2-substituted tropane derivatives *3* and *4* was not modified in a sodium free buffer (Table 2). We have no explanation for this.

In conclusion, using a series of new benzamide derivatives, we have shown that use of the tropane skeleton suitably substituted can produce compounds with very high affinity for an antagonistic site in the D-2 receptor. This would appear to account for the potent neuroleptic properties of tropapride, *1*, since the doses effective in pharmacological tests predictive for neuroleptic activity were similar to, or lower than, those of haloperidol and clearly lower than those for reference benzamides (Jalfre et al (1983). These authors have also suggested that *1* possesses a marked ability to penetrate cerebral tissue in contrast to sulpiride. This hypothesis has been confirmed, at least in the rat (Strolin Benedetti et al, unpublished results). However, the increase in affinity does not seem to reduce the selectivity of *1* for the D-2 receptor since this compound only weakly inhibits dopamine-stimulated adenylate cyclase activity (Strolin Benedetti et al 1982) even though, like clebopride (Jenner et al 1978), *1* has a higher inhibitory effect on dopamine stimulated adenylate cyclase than do the other reference benzamides. Thus, the tropane skeleton seems to be a very useful pharmacophoric group in the design of new dopaminergic drugs.

Acknowledgements

The authors wish to thank G. Mocquet for the pharmacological determinations, M. Langlois for useful discussion and C. Gomeni for her help on mathematical analysis of data.

REFERENCES

Battaglia, G., Titeler, M. (1982) Eur. J. Pharmacol. 81: 493–498

Burt, D. R., Creese, I., Snyder, S. H. (1976) Mol. Pharmacol. 12: 800–812

Calne, D. B. (1980) TIPS 412–414

Creese, I., Schneider, R., Snyder, S. H. (1977) Eur. J. Pharmacol. 46: 377–381

Dostert, P., Imbert, T., Ancher, J. F., Langlois, M., Bucher, B., Mocquet, G. (1982) Eur. J. Med. Chem.-Chim. Ther. 17: 437–444

Freedman, S. B., Poat, J. A., Woodruff, G. N. (1981) J. Neurochem. 37: 608–612

Freedman, S. B., Poat, J. A., Woodruff, G. N. (1982) Ibid. 38: 1459–1465

Goldberg, L. I., Kohli, J. D., Kotake, A. N., Volkman, P. H. (1978) Fed. Proc. Fed. Am. Soc. Exp. Biol. 37: 2396–2402

Horn, A. S., Post, M. L., Kennard, O. (1975) J. Pharm. Pharmacol. 27: 553–563

Humber, L. G. (1981) in: De Las Heras, F. G., Vega, S. (eds) Medicinal Chemistry Advances. Pergamon Press, pp 179–190

Jalfre, M., Bucher, B., Dorme, N., Mocquet, G., Porsolt, R. D. (1983) Arch. Int. Pharmacodyn. Ther. 264: 232-256

Jenner, P., Elliot, P. N. C., Clow, A., Reavill, C., Marsden, C. D. (1978) J. Pharm. Pharmacol. 30: 46–48

Jenner, P., Marsden, C. D. (1979) Life Sci. 25: 479–486

Jenner, P., Marsden, C. D. (1981) Neuropharmacology 20: 1285–1293

Kebabian, J. W., Calne, D. B. (1979) Nature (London) 277: 93–96

Leff, S., Sibley, D. R., Hamblin, M., Creese, I. (1981) Life Sci. 29: 2081–2090

Makman, M. H., Dvorkin, B., Klein, P. N. (1982) Proc. Natl. Acad. Sci. 79: 4212–4216

McDermed, J. D., Freeman, H. S. (1981) in: Kohsaka, M., Shomori, T., Tsukada, Y., Woodruff, G. N. (eds) Advances in Dopamine Research. Pergamon Press, pp 179–187

Mizuchi, A., Kitagawa, N., Saruta, S., Miyachi, Y. (1982) Eur. J. Pharmacol. 84: 51–59

Molinoff, P. B., Wolfe, B. B., Weiland, G. A. (1981) Life Sci. 29: 427–443

Nys, G. G., Rekker, R. F. (1974) Eur. J. Med. Chem.-Chim. Ther. 9: 361–375

Olson, G. L., Cheung, H. C., Morgan, K. D., Blount, J. F., Todaro, L., Berger, L., Davidson, A. B., Boff, E. (1981) J. Med. Chem. 24: 1026–1034

Pannatier, A., Anker, L., Testa, B., Carrupt, P. A. (1981) J. Pharm. Pharmacol. 33: 145–149

Prieto, J., Moragues, J., Spickett, R. G., Vega, A., Colombo, M., Salazar, W., Roberts, D. J. (1977) Ibid. 29: 147–152

Seeman, P. (1980) Pharmacol. Rev. 32: 229–313

Stefanini, E., Marchisio, A. M., Devoto, P., Vernaleone, F., Collu, R., Spano, P. F. (1980) Brain Res. 198: 229–233

Strolin Benedetti, M., Cao Danh, H., Guffroy, C. (1982) Abstracts 12th CINP Congress Jerusalem Vol. II p. 701

Theodorou, A. E., Hall, M. D., Jenner, P., Marsden, C. D. (1980) J. Pharm. Pharmacol. 32: 441–444

Theodorou, A. E., Jenner, P., Marsden, C. D. (1983) Life Sci. 32: 1243–1254

Van de Waterbeemd, H., Testa, B. (1983) J. Med. Chem. 26: 203–207

Weiland, G. A., Molinoff, P. B. (1981) Life Sci. 29: 313–330