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Locomotion and stereotyped behaviours induced by 1-amino-3,5-dimethyladamantane (D 145) and apomorphine in the rat: a comparison

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The locomotion and stereotyped behaviours induced by the two dopamine agonists, 1-amino-3,5-dimethyladamantane (D 145) and apomorphine in rats have been investigated using a holeboard apparatus. Unlike apomorphine, D 145 did not induce compulsive gnawing, but it did induce locomotion dose-dependently. However, the pattern of locomotion was different from that induced by apomorphine and the locomotion itself was potently antagonized by haloperidol but not by sulpiride; this is the opposite to apomorphine-induced locomotion. This indicates that locomotion induced by dopamine agonists is not a unitary phenomenon but that different 'types' of locomotion, with different pharmacological properties, can be induced. Low doses of D 145, given before apomorphine, reduced apomorphine-induced gnawing and enhanced apomorphine-induced locomotion. The long lasting 'priming' effect known to occur after repetitive injections of apomorphine was not mimicked by D 145. Apomorphine and D 145 are considered to be two specific DA agonists; their behavioural-pharmacological profiles, however, are quite different.

One of the pharmacological strategies in the treatment of Parkinson's disease has been to reinstate the function of the dopamine transmission with drugs like L-dopa, bromocriptine or amantadine (Birkmayer & Hornykiewicz 1961; Barbeau et al 1962; Schwab et al 1969; Thorner et al 1980).

The pharmacological and behavioural properties of the amantadine derivative, 1-amino-3,5-dimethyladamantane (D 145), have been investigated (see e.g. Svensson 1973; Maj et al 1974; Costall & Naylor 1975; Randrup & Mogilnicka 1976; Haacke et al 1977; Smialowska 1976; Maj 1977; Menon & Clark 1978) and because of its behavioural–pharmacological effects, it has been claimed to be a DA agonist with properties similar to apomorphine in the central nervous system (Maj et al 1974; Maj 1977).

We have described the development of a behavioural recording technique where eight different components of behaviour can be recorded automatically over time (see Ljungberg & Ungerstedt 1978a) and used the method to characterize the behavioural–pharmacological profile of apomorphine (Ljungberg & Ungerstedt 1977, 1978b; Ljungberg 1979). We also found that neuroleptic drugs which induce a high incidence of extrapyramidal side effects in the clinic (e.g. haloperidol or metoclopramide) potently blocked apomorphine-induced gnawing, while antipsychotic drugs which induce fewer extrapyramidal side effects (e.g. thioridazine, clozapine and sulpiride), blocked apomorphine-induced locomotion (see e.g. Ljungberg & Ungerstedt 1978b; Worms & Lloyd 1979; Worms 1982). We also found that if apomorphine was given repetitively (1–4 h between injections) the induced locomotion was decreased while the induced gnawing was increased after the second injection (Ljungberg 1979).

To characterize further the dopaminergic mechanisms involved in the mediation of the two behavioural patterns—locomotion and stereotyped behaviours—and to investigate further the dopaminergic 'priming' effect, we have been studying other dopamine agonists. The present paper reports results with D 145.

MATERIAL AND METHODS

The experiments were performed on male, Sprague-Dawley rats (Anticimex, Stockholm; 160–210 g) which were kept 5/cage under constant temperature and humidity conditions on a 12 h light/dark schedule (6 am–6 pm) with free access to food. All animals were used only once.

Apparatus

Animal behaviour was recorded in a text box (69 × 69 cm) designed for the automatic recording of eight components of behaviour. The method, and its routine use have been described by Ljungberg & Ungerstedt (1978a). In short: 'Activity' was defined

as the number of interruptions of 10 photobeams which symmetrically covered the open field area of the hole board. 'Total locomotion' was defined as the number of times the animal walked a fixed distance, defined by the arrangement of the photobeams. 'Forward locomotion' was defined as the number of times the animal walked a distance of the same length but with the requirement that the animal continued from one arm into the next (see Fig. 1). Thirty-two holes (2·5 cm in diameter) were symmetrically distributed over the entire floor. 'Hole count' was defined as the number of interruptions of photobeams positioned 0·5 cm below the surface of the floor, and 'hole time' was the accumulated time of interruption. One vertically directed photobeam was positioned in each corner. 'Corner count' was defined as the number of interruptions and 'corner time' as the accumulated time of interruption. The gnawing of the animal was detected, converted to digital pulses and counted (see Fig. 1).

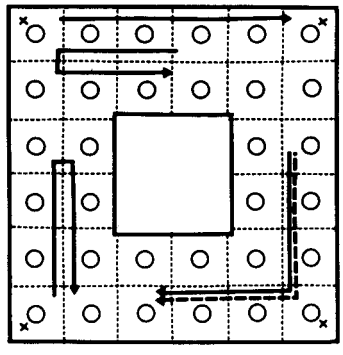

FIG. 1. The holeboard from above schematically. Thin dotted line shows position of photobeams detecting movements of the animals in the open-field. Arrows indicate requirements for 'total' (solid arrow) and 'forward' locomotion counts (dotted arrow). 'X' in the corners shows position of vertical photobeams detecting entries of animal into corner. Circles show position of the holes in the bottom of the test-box.

The data were accumulated in periods of 15 min and analysed and presented either in counts/15 min (Figs 2, 3) or in total counts for the drug effect (i.e. 0–210 min after the D 145 injections, Table 1, and 0–90 min after apomorphine, Table 2).

Drug treatments

D 145 (1-amino-3,5-dimethyladamantane, Merz & Co.) was dissolved in isotonic saline. Metoclopramide (Primperan, Lundbeck), haloperidol (Haldol, Leo) and chlorpromazine (Hibernal, Leo) were obtained as injection ampoules and diluted to volume with isotonic saline. (±)-Sulpiride (Delagrange) was dissolved in a few drops of glacial acetic acid, made up to volume with isotonic saline and adjusted to pH 7 with 1 M NaOH. The doses for D 145 refer to the base while the other doses refer to the above mentioned forms. The injection volume was 5 ml kg^{-1} and the injections were given i.p. Apomorphine was obtained in commercially available 1 ml ampoules (Injectabile Apomorphine 5 mg ml^{-1}, Apoteksbolaget, Sweden with an injection vehicle

Table 1. The different drugs and doses tested, pretreatment times in minutes (min), number of animals used (n) and recorded variables are shown. D 145 was injected i.p. 90 min after the animals were placed in the test box and the data are presented as median total counts for period 0–210 min after the D 145 injection. Only D 145-injected animals are compared with the saline-injected controls, while the neuroleptic-pretreated animals are compared with the D 145 (13 mg kg^{-1})-injected animals.

Treatment	Dose mg kg^{-1}	(min)	(n)	Act	Tot. loc.	For. loc.	Hole c.	Hole t.	Corner c.	Corner t.
NaCl	—	—	8	887	96	47	525	1391	319	8625
D-145	5	—	6	1412	129	76	374	605	328	6022
	10	—	6	3519**	316*	171*	481	246	876*	5952
	13	—	8	5601**	618**	370*	552	242	634	3138
	20	—	5	10234**	1107**	781**	1012	415	1893**	5794
	40	—	6	12716**	1651**	1048**	860	372	2943**	4874
Metoclopramide + D-145	20 13	60 —	5	4706	726	633	1129	760	1299	4747
Haloperidol + D-145	0·05 13	30 —	6	4959	297	158	612	267	807	4504
Haloperidol + D-145	0·2 13	30 —	6	645**	15**	7**	161**	846	46*	1134
Chlorpromazine + D-145 13	20 13	30 —	6	4776	393	349	269	565*	901	5083
Sulpiride + D-145 13	200 13	60 —	7	6174	702	583	395	121	1887	6297

*$P < 0·05$; **$P < 0·01$.

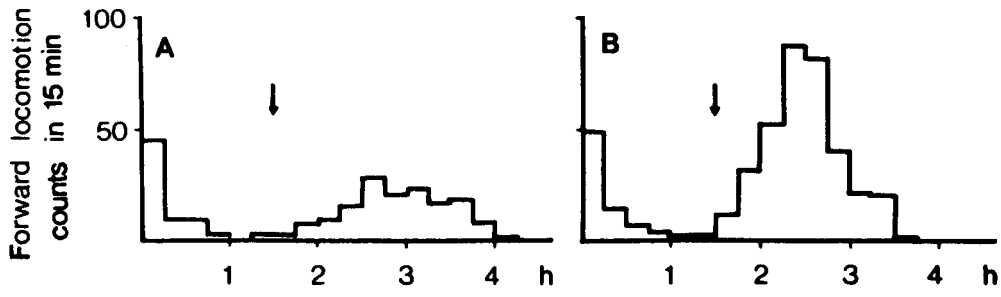

FIG. 2. D 145 was injected i.p. 90 min after the animals were placed in the testbox. The data are presented as median total counts/15 min and show the time course for the D 145 10 mg kg^{-1} (n = 6) and D 145, 13 mg kg^{-1} (n = 8)-induced locomotion.

containing $NaHSO_3$ 1, M HCl 0·9, NaCl 8·23, methyl-*p*-oxybenzoate 2 g and distilled water to 1000 ml) and was injected subcutaneously in the dorsal part of the neck in a volume of 1 ml kg^{-1}. The doses refer to the base. Doses, number of animals and pretreatment times are shown in Tables 1, 2.

Statistics

All the data are presented as medians. The Kruskal–Wallis one-way analysis of variance and the Mann-Whitney U-test were used to test the degree of significance (Siegel 1956).

RESULTS

Controls

In all experiments the animal was habituated to the test-box before drug injection. Saline-injected animals did not show any detectable unspecific activation due to the handling or injection procedure, in agreement with previous studies (Ljungberg & Ungerstedt 1978a). They mainly lay inactive in the corners and only occasionally left the corners for short periods of increased locomotion.

Dose response effects of D 145

Apomorphine is known to induce different behavioural responses when injected at different sites (Puech 1975; Cools et al 1977; Ljungberg & Ungerstedt 1977). Therefore, D 145 was tested to see whether it, too, induced different responses. It was found that the i.p. route gave the strongest activation and the most reproducible results. Because of this, and the fact that the i.p. route has most commonly been used previously with D 145 (see e.g. Svensson 1973; Costall & Naylor 1975; Menon & Clark 1978), it was used in this study.

D 145 caused a dose-related activation of the habituated animals (see Fig. 2, Table 1). The activity consisted mainly of coordinated locomotion with a 'low posture', i.e. with both the belly and the tail touching the ground, and with some sniffing and repetitive head and limb movements. The animals often changed the direction of locomotion, which is reflected in the recordings as a relatively low ratio forward locomotion/total locomotion (Table 1). Even after higher doses no specific compulsive gnawing was induced. After high doses of D 145 (40 mg kg^{-1} and occasionally after 20 mg kg^{-1}) the animals sometimes developed periods with tremor of moderate intensity. There was no specific dose-related increase in head dipping behaviour into the holes (c.f. Boissier et al 1964; Ljungberg & Ungerstedt 1976, 1978a). The KRUWA-analysis showed

Table 2. The different drugs and doses tested, pretreatment times (h), number of animals used (n) and recorded variables are shown. Apomorphine, 5 mg kg^{-1}, was injected s.c. 90 min after the animals were placed in the test box and the data are presented as median total counts for period 0–90 min after the apomorphine injection. The D 145-pretreated animals are compared with the apomorphine-injected animals.

Treatment	Dose (mg kg^{-1})	h	(n)	For. loc.	Gnawing
Apomorphine	5	—	13	148	2492
D-145 + apomorphine	5 5	0·25 —	5	137	767**
D-145 + apomorphine	10 5	0·5 —	7	276**	404**
D-145 + apomorphine	10 5	4 —	4	193	669*
D-145 + apomorphine	10 5	8 —	4	111	2086
D-145 + apomorphine	13 5	8 —	3	115	1715
D-145 + apomorphine	13 5	20 —	6	102	2190
D-145 + apomorphine	20 5	8 —	3	140	2147
D-145 + apomorphine	20 5	20 —	4	106	860

*$P < 0·05$; **$P < 0·01$.

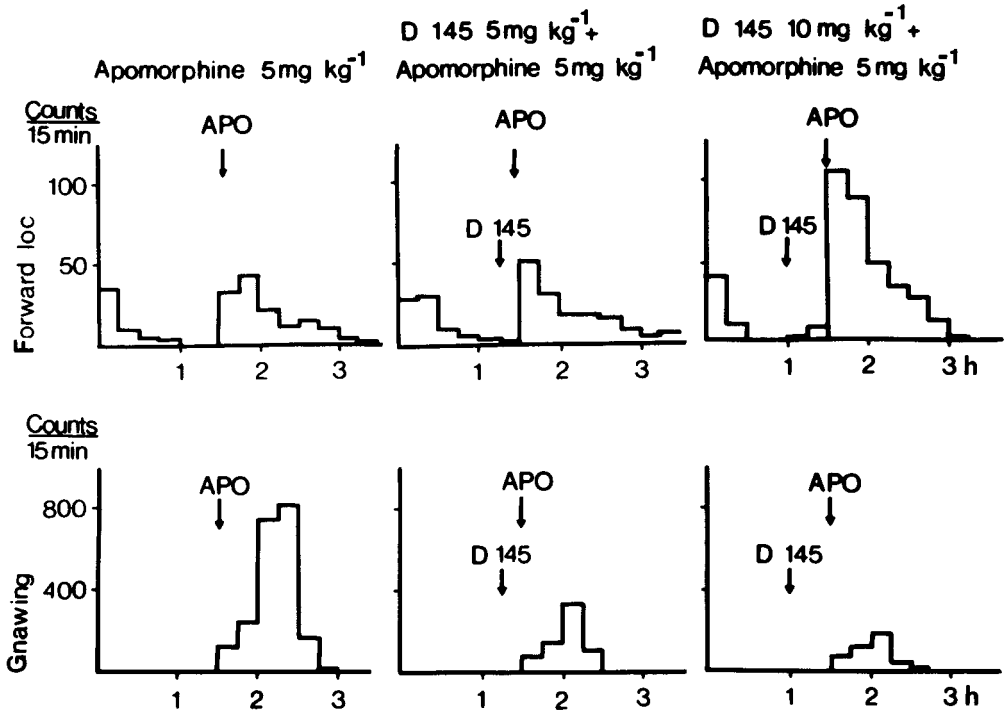

FIG. 3. Apomorphine, 5 mg kg^{-1}, was injected s.c. 90 min after the animals were placed in the testbox. D 145, 5 mg kg^{-1} (n = 5) reduced the apomorphine-induced compulsive gnawing and D 145, 10 mg kg^{-1} (n = 7) both reduced the apomorphine-induced compulsive gnawing and potentiated the apomorphine-induced locomotion. The data are presented as median counts/15 min.

that the recorded variables 'hole counts', 'hole time', 'corner time' and 'gnawing' were not significantly affected. All other recorded variables were significant on the $P < 0{\cdot}001$ level.

Antagonistic effects of neuroleptic drugs

The antagonistic properties of four different neuroleptic drugs were tested. For comparative reasons, the drugs and doses were selected from a previous publication where several neuroleptic drugs were tested for their properties in antagonizing apomorphine (5 mg kg^{-1})-induced behaviour (see Ljungberg & Ungerstedt 1978b).

In that study, we found haloperidol to be 30 times more potent than metoclopramide in inhibiting apomorphine-induced locomotion. In the present study, we tested metoclopramide at 20 mg kg^{-1}, i.e. 100 times the dose of haloperidol that antagonized the locomotion induced by D 145 13 mg kg^{-1}. This dose of metoclopramide has previously been found to block apomorphine, 5 mg kg^{-1}-induced locomotion. We also tested a very high dose of sulpiride, 200 mg kg^{-1}, which previously had been found to antagonize apomorphine, 5 mg kg^{-1}-induced locomotion.

We found that metoclopramide, 20 mg kg^{-1}, given 60 min before the D 145, 13 mg kg^{-1}, did not significantly alter the locomotion elicited by the D 145. Haloperidol, 0·05 mg kg^{-1}, given 30 min before the D 145, 13 mg kg^{-1}, caused a slight reduction of the D 145-induced locomotion and at 0·2 mg kg^{-1} caused blockade of the locomotion. Neither chlorpromazine, 20 mg kg^{-1} (30 min), nor sulpiride, 200 mg kg^{-1} (60 min), antagonized the locomotion induced by D 145, 13 mg kg^{-1} (see Table 1).

Interaction between D 145 and apomorphine

Apomorphine, 5 mg kg^{-1}, markedly activated the habituated animals. During the period 0 to 30 min after the injection the activity consisted of high locomotion on straight legs, with a hunched back posture and with the tail bent upwards. The locomotion was accompanied by sniffing, repetitive head and fore-limb movements and fast biting movements. After about 30 min this pattern changed and the animals instead showed strong compulsive gnawing (see Fig. 3).

After pretreatment with D 145, 5 mg kg^{-1}, 15 min before the apomorphine, 5 mg kg^{-1}, there was a significant reduction of the apomorphine-induced compulsive gnawing while the total apomorphine-induced forward locomotion was not significantly altered. The pretreatment with D 145, 10 mg kg^{-1}, 30 min before apomorphine, further decreased the apomorphine-induced compulsive gnawing but also caused a strong increase in the apomorphine-induced forward locomotion. The KRUWA-analysis showed that the effect on 'total locomotion' was significant at the $P < 0{\cdot}001$ level and the effect on 'gnawing' at the $P < 0{\cdot}01$ level. By comparing the time course for the D 145, 10 mg kg^{-1}, activation shown in Fig. 2 with the apomorphine, 5 mg kg^{-1}, effect and the D 145, 10 mg kg^{-1}, plus apomorphine, 5 mg kg^{-1}, activation shown in Fig. 3, it may be seen that the increase in locomotion by the combined treatment is not caused by an additive effect.

After D 145, 10 mg kg^{-1}, given 4 h before apomorphine, 5 mg kg^{-1}, there was a significant decrease in the apomorphine-induced compulsive gnawing and a slight non-significant increase in apomorphine-induced forward locomotion. This is principally the same response as that caused by D 145, 10 mg kg^{-1}, given 30 min before the apomorphine (see Fig. 3 and Table 2) and this is probably explained by the fact that the initial D 145 effect had not yet worn off. D 145 in different doses given 8 or 20 h before apomorphine, 5 mg kg^{-1}, did not significantly change the apomorphine response. (KRUWA-analysis did not detect any significant effects.)

DISCUSSION

The sterotyped behaviours induced by D 145 consisted of small repetitive movements of the forelegs, up and down movements of the head and some sniffing (Svensson 1973; Maj et al 1974; Costall & Naylor 1975; Randrup & Mogilnicka 1976). It was not possible, even after very high doses of D 145, to induce strong compulsive gnawing, which is a typical behaviour induced by apomorphine (see Table 2, Fig. 3, and c.f. Ernst 1967; Anden et al 1967; Ljungberg & Ungerstedt 1977).

Like apomorphine (see e.g. Ljungberg & Ungerstedt 1977), D 145 induced locomotion in habituated animals in a dose-dependent manner, but the pattern of locomotion was very different. We further found that the D 145-induced locomotion was antagonized in a manner different from that induced by apomorphine, e.g. the D 145-induced locomotion was potently blocked by the dopamine antagonist haloperidol (previously also found by Costall & Naylor 1975), but not by sulpiride; this is opposite to apomorphine-induced locomotion (see Table 1 and Ljungberg & Ungerstedt 1978b).

Locomotion induced by dopamine agonists can therefore not be considered a unitary phenomenon. Different 'types' of locomotion, which show different pharmacological properties, can be induced. This finding has a wide implication for pharmacological experiments where the transmitter mechanisms regulating 'locomotion' in rodents are studied, as the results obtained will only be relevant for the specific 'type' of drug-induced locomotion studied (for references see Ljungberg & Ungerstedt 1985).

It has previously been found that amantadine can antagonize apomorphine-induced 'compulsive gnawing' (Hackman et al 1973) or apomorphine-induced stereotypies (Cox & Tha 1973; Randrup & Mogilnicka 1976). This has also been found to occur after pretreatment with D 145 (Costall & Naylor 1975; Randrup & Mogilnicka 1976). In our experiments D 145 dose-dependently reduced apomorphine-induced compulsive gnawing (see Table 2, Fig. 3). However, we also found that pretreatment with D 145 caused a strong increase in apomorphine-induced locomotion. It thus seems that pretreatment with D 145 itself does not antagonize apomorphine-induced behaviour, but causes a 'shift' from gnawing to locomotion (see Ljungberg & Ungerstedt 1977; Ljungberg 1979).

It has also previously been found that various 'stereotyped behaviours' induced by apomorphine are increased by previous repetitive injections of DA agonists (Klawans & Margolin 1975; Baudray et al 1977; Martres et al 1977; Ljungberg 1979), while apomorphine-induced locomotion is decreased by repetitive injections (Ljungberg 1979). This priming effect has also been shown to occur after repetitive injections of amantadine or D 145 (Randrup & Mogilnicka 1976). Westermann (1982), however, did not find this priming effect after pretreatment with amantadine. In our study, we could not find a priming effect after pretreatment with D 145 (see Table 2).

There are several possible explanations for these differences in action between D 145 and apomorphine. D 145 has been proposed to possess both DA-releasing and DA-stimulating properties in the central nervous system (Svensson 1973; Maj et al 1974; Costall & Naylor 1975; Smialowska 1976; Haacke et al 1977; Menon & Clark 1978), while apomorphine has been considered mainly a DA receptor stimulating agent (see Ljungberg & Unger-

stedt 1977; Seeman 1980). D 145 and apomorphine might also differ in their effect on the presynaptic level and thus interact differently in the feed-back mechanisms (Carlsson 1975) or have different affinities for different populations of postsynaptic DA-receptors, i.e. D_1 and D_2 receptors (for a review and discussion, see Seeman 1980). Furthermore, the locomotion induced by D 145 and apomorphine might be differently related to and influenced by other transmitter systems, e.g. the GABA system (Menon & Clark 1978; Tilley & Kramer 1981).

The results obtained in the present study show that the behavioural patterns induced and the pharmacological properties of D 145 are quite different from those of apomorphine; mechanisms explaining the difference have yet to be found.

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REFERENCES

Anden, N.-E., Rubenson, A., Fuxe, K., Hökfelt, T. (1967) J. Pharm. Pharmacol. 19: 627–629

Barbeau, A., Sourkes, T. L., Murphy, G. F. (1962) in: de Ajuriaguerra J. (ed.) Monoamines et systeme nerveux centrale. Masson, Paris, pp 247–262

Baudray, M., Costentin, I., Marcais, M., Martres, M. P., Protais, P., Schwartz, J. B. (1977) Neurosci. Lett. 4: 203–207

Birkmayer, W., Hornykiewicz, O. (1961) Wien. Klin. Wocheschr. 73: 787–788

Boissier, J.-R., Simon, P., Lwoff, J.-M. (1964) Therapie 19: 571–589

Carlsson, A. (1975) in: Usdin, E., Bunney, W. E. (eds) Pre- and postsynaptic receptors. Marcel Dekker, New York, pp 49–63

Cools, A. R., Broekkamp, C. L. E., van Rossum, J. M. (1977) Pharmacol. Biochem. Behav. 6: 705–708

Costall, B., Naylor, R. J. (1975) Psychopharmacology 43: 53–61

Cox, B., Tha, S. J. (1973) Eur. J. Pharmacol. 24: 96–100

Ernst, A. M. (1967) Psychopharmacology 10: 316–323

Haacke, U. von, Sturm, G., Suwer, V., Wesemann, W., Wildenhahn, G. (1977) Arzeneimittel-Forsch./Drug Res. 27 (II) 7: 1481–1483

Hackman, R., Pentikäinen, P., Neuroven, R. J., Vapaatalo, H. (1973) Experientia 29: 1524–1525

Klawans, H. L., Margolin, D. I. (1975) Arch. Gen. Psychiatry 32: 725–732

Ljungberg, T. (1979) Neuropharmacology 18: 327–334

Ljungberg, T., Ungerstedt, U. (1976) Eur. J. Pharmacol. 36: 181–188

Ljungberg, T., Ungerstedt, U. (1977) Ibid. 46: 41–50

Ljungberg, T., Ungerstedt, U. (1978a) Pharmacol. Biochem. Behav. 8: 483–489

Ljungberg, T., Ungerstedt, U. (1978b) Psychopharmacology 56: 239–247

Ljungberg, T., Ungerstedt, U. (1985) Pharmacol. Biochem. Behav. 23: 479–485

Maj, J. (1977) Naunyn-Schmiedebergs Arch. Pharmacol. 297: S53–S54

Maj, J., Sowinska, H., Baran, L., Sarnek, J. (1974) Eur. J. Pharmacol. 26: 9–14

Martres, M. P., Costentin, J., Baudray, M., Marcais, H., Protais, P., Schwartz, J. C. (1977) Brain Res. 136: 319–337

Menon, M. K., Clark, W. G. (1978) Neuropharmacology 17: 1049–1052

Puech, A. (1975) These Med. (Paris)

Randrup, A., Mogilnicka, E. (1976) Pol. J. Pharmacol. Pharm. 28: 551–556

Schwab, R. S., England, A. C., Jr., Poskanzer, D. C., Young, R. R. (1969) J. Am. Med. Assoc. 208: 1168–1170

Seeman, P. (1980) Pharmacol. Rev. 32 (3): 229–313

Siegel, S. (1956) Nonparametric statistics for the behavioural sciences. McGraw-Hill, Kogakusha Ltd

Smialowska, M. (1976) Pol. J. Pharmacol. Pharm. 28: 259–267

Svensson, T. H. (1973) Eur. J. Pharmacol. 23: 232–238

Thorner, M. O., Fluckiger, E., Calne, D. B. (1980) Bromocriptine. A clinical and pharmacological review. Raven Press, New York

Tilley, J. W., Kramer, M. J. (1981) Prog. Med. Chem. 18: 1–44

Westermann, K. H. (1982) Acta Physiol. Pharmacol. Bulg. 8(1–2): 58–63

Worms, P. (1982) in: Rotrosen, J., Stanley, M. (eds) The benzamides: pharmacology, neurobiology and clinical aspects. Raven Press, New York, pp 7–16

Worms, P., Lloyd, K. G. (1979) Pharmacol. Ther. 5: 445–450