J. Neural Transmission 41, 253—264 (1977)

Journal of
Neural Transmission
© by Springer-Verlag 1977

The Influence of Bromocriptine on Serotonin Neurons

J. Maj, L. Gancarczyk, and **A. Rawlów**

Polish Academy of Sciences, Institute of Pharmacology, Kraków, Poland

With 9 Figures

Received May 5, 1977

Summary

Bromocriptine (CB-154) is regarded as a dopamine agonist, hence is used in the treatment of Parkinson's disease. In the paper presented a possibility of the influence of bromocriptine on central serotonin neurons has been studied.

It was demonstrated that CB-154, like tryptophan, 5-hydroxytryptophan, LSD or fenfluramine in previous experiments, potentiates the flexor reflex of the spinal rat, and this effect of CB-154 is prevented by serotonin antagonists—cyproheptadine and danitracen.

CB-154, like fenfluramine used as a comparative serotonergic agent, rises the body temperature in rabbits. The hyperthermic effect of CB-154 is prevented by cyproheptadine, danitracen and mianserin. Haloperidol preventes the hyperthermia caused by a lower dose of CB-154 only, having no influence on the hyperthermic effects of greater doses of CB-154 or on fenfluramine-induced hyperthermia. The results obtained indicate that CB-154, besides a dopaminomimetic action, possesses central serotonin actions as well.

Introduction

Bromocriptine (CB-154), a new antiparkinsonic drug, causes effects which allow to classify it as a dopaminergic agent (*Corrodi et al.*, 1973; *Fuxe et al.*, 1974; *Johnson et al.*, 1974; *Dray* and *Oakley*, 1976; *Johnson* and *Vigouret*, 1976; *Schorderet*, 1976; *Snider et al.*, 1976). CB-154 influences also noradrenaline (NA) neurons in a way suggesting a release of NA or increase of NA turnover (*Corrodi et al.*,

1973; *Fuxe et al.*, 1974; *Snider et al.*, 1976). This action may be an indirect one resulting from the dopamine-noradrenaline interaction, as it was observed for apomorphine (*Persson* and *Waldeck*, 1970; *Maj, Kapturkiewicz* and *Michaluk*, 1976). The influence of CB-154 on 5-hydroxytryptamine (5-HT) neurons has not been widely investigated. *Fuxe et al.* (1974) quote unpublished data that CB-154 does not change the turnover of brain 5-HT. According to *Snider et al.* (1975) CB-154 increases 5-HT levels in brain, decreases 5-hydroxyindoleacetic acid levels not changing the content of tryptophan and the rate of 5-hydroxytryptophan synthesis.

The pharmacological data concerning the influence of CB-154 on 5-HT neurons are lacking. Hence in the paper presented we have decided to study whether CB-154 affects 5-HT neurons employing two models:

(1) the flexor reflex of the hind limb of the spinal rat, which appeared to be a convenient model for demonstrating central serotonergic or serotoninolytic activities (*Maj, Palider* and *Baran*, 1976);

(2) the body temperature in rabbits, in which serotonergic agents induce the temperature rise, antagonized by 5-HT receptor blockers (*Horita et al.*, 1958; *Quock* and *Beal*, 1976; *Quock et al.*, 1976). As 5-HT receptor blockers, according to literature data (see Discussion), were used: cyproheptadine (CH), danitracen (WA-335) and mianserin (MS) and as a serotonergic agent—fenfluramine (FF).

Material and Methods

The Influence on the Flexor Reflex

Experiments were carried out on male, Wistar rats, weighing 180 to 270 g, according to the technic described previously (*Maj, Palider* and *Baran*, 1976). The hind limb of a rat was stimulated electrically and contractions of the muscle tibialis anterior were recorded.

CB-154 with an equivalent amount of tartaric acid and a few drops of ethyl alcohol was dissolved in warm, sterile saline. The solution was prepared immediately before experiments and injected in a volume 1 ml/kg intravenously (femoral vein). WA-335 in a dose 3 mg/kg and CH in a dose 1 mg/kg were suspended in 1 % aqueous solution of Tween 80 and injected in a volume 4 ml/kg intraperitoneally 30 min before CB-154.

The Influence on the Body Temperature

Experiments were performed on male White Dunish rabbits weighing 1.8—3.5 kg (6—10 animals in each experimental group). The temperature was measured in rectum by means of TE-3 Ellab thermometer. The rabbits

were immobilized and adapted for 2 hours at room temperature of 22 ± 1 °C. Drugs were injected in a volume 0.5 ml/kg into the marginal vein of an ear or intraperitoneally. Results were presented as a mean ± SEM. Statistical significance was controled with Student t-test.

After two preluding measurements of the body temperature taken every 30 min, CB-154 was injected i.v. (dissolved as in experiments on rats) described above. A control group was given the solvent. The body temperature was measured for 10 hours at 30 min intervals.

The same experimental conditions were used in experiments in which FF in a dose 10 mg/kg was injected intravenously. CH in a dose 3 mg/kg, MS in a dose 5 mg/kg, WA-335 in a dose 3 mg/kg and haloperidol (HP) in a dose 0.5 mg/kg were injected intravenously 1 hour before CB-154.

MS, HP, CH and WA-335 were dissolved in sterile redistilled water, the two latter with an addition of a few drops 2 % ascorbic acid. The body temperature was measured at 30, 60, 90, 120, 150 and 180 min after injection of CB-154.

Drugs used:
ascorbic acid (Polfa),
2-bromo-α-ergocriptine methylsulfonate (CB-154, Sandoz),
cyproheptadine hydrochloride (CH, Merck, Sharp and Dohme),
danitracen (9, 10-dihydro-10-[1-methyl-4-piperidylidene]-9-anthrol, WA-335, Dr. K. Thomae),
fenfluramine hydrochloride (FF, Servier),
haloperidol (HP, Gedeon Richter),
mianserin hydrochloride (MS, Organon),
tartaric acid (P.O.Ch).

Results

The Influence on the Flexor Reflex

CB-154 in a dose 4 mg/kg causes an increase of the flexor reflex, which appears at about 5 min after injection reaching a maximal effect at 10 min and lasting 2—3 hours (Fig. 1). The threshold stimulating doses of CB-154 are 2—3 mg/kg. CH in a dose 1 mg/kg or WA-335 in a dose 3 mg/kg prewent the stimulating effect of CB-154 (4 and 8 mg/kg) (Fig. 2).

The Influence on the Body Temperature

CB-154 in doses 1 and 2.5 mg/kg causes a long lasting hyperthermia in rabbits (Fig. 4). The maximal rise of the body temperature amounting 1.7 °C appears at 2.5 hours after injection of CB-154 1 mg/kg. The hyperthermia falls to the control level after 6 hours. The hyperthermic effect caused by a dose 2.5 mg/kg is stronger, reaching the maximal rise of 2.7 °C at 2 hours and falling to the control level at 7 hours after injection of CB-154.

The hyperthermia caused by CB-154 (1 and 2.5 mg/kg) is completely prevented by pretreatment with CH (3 mg/kg), MS (5 mg/kg) and WA-335 (3 mg/kg), Fig. 5 and 6.

5-HT receptor blockers used given alone cause a fall in the body temperature lasting 4 hours (Fig. 7). The fall is maximal at 2 to 2.5 hours after injection and amounts for CH — 1.97 °C, MS — 2 °C, WA-335 — 0.77 °C.

HP (0.5 mg/kg) prevents the hyperthermic effect of CB-154 given in rabbits, in a dose 1 mg/kg, having no influence on the hyperthermia caused by CB-154 in a dose 2.5 mg/kg (Fig. 8).

FF in a dose 10 mg/kg rises the body temperature in rabbits of about 1 °C. The hyperthermic effect of FF is prevented by pretreatment with WA-335 (3 mg/kg) but not with HP (0.5 mg/kg) (Fig. 9). After the combined treatment WA-335 + FF the hypothermia is observed.

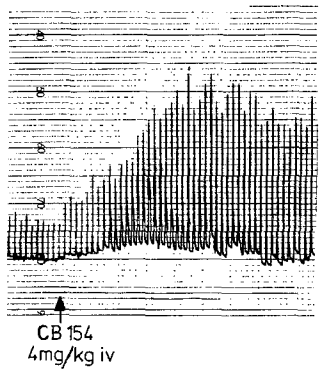

Fig. 1. The influence of CB-154 on the flexor reflex (the muscle tibialis anterior) on the hind limb of the spinal rat. CB-154 was injected into the femoral vein

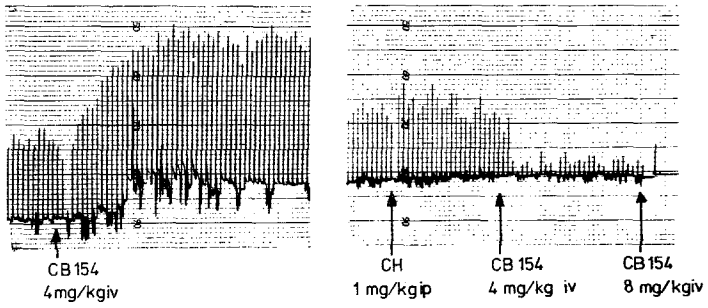

Fig. 2. The influence of CB-154 after pretreatment with CH on the flexor reflex (the muscle tibialis anterior) of the hind limb of the spinal rat. CH was injected intraperitoneally 30 min before the second dose of CB-154

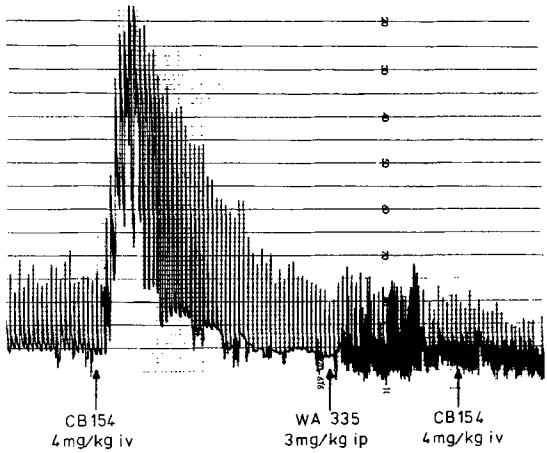

Fig. 3. The influence of CB-154 on the flexor reflex (the muscle tibialis anterior) of the hind limb of the spinal rat after pretreatment with WA-335. WA-335 was injected intraperitoneally 30 min before the second dose of CB-154

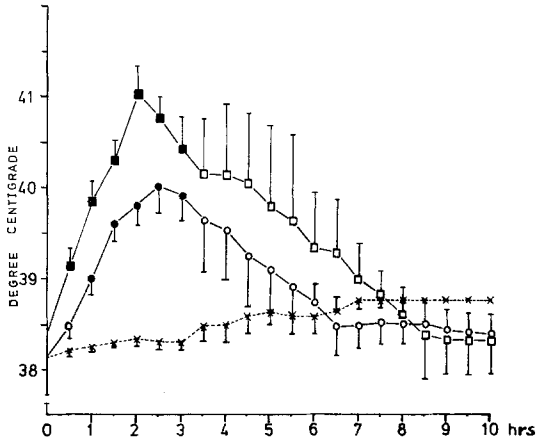

Fig. 4. The influence of CB-154 on the body temperature in the rabbit. CB-154 (dissolved as in experiments on rats) was injected into the ear marginal vein. The results are presented as means ± SEM. Statistical significance was controled by Student t-test. Solid points indicate statistically significant results. x---x solvent; ●—●—○—○ CB-154 1 mg/kg, ● vs x, $p < 0.001$; ■—■—□—□ CB-154 2.5 mg/kg; ■ vs x, $p < 0.001$

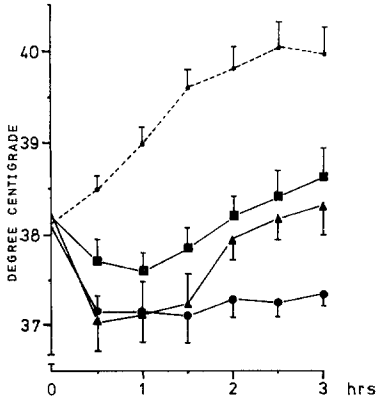

Fig. 5. The influence of CB-154 1 mg/kg on the body temperature in the rabbit after pretreatment with 5-HT antagonists. CH, WA-335, MS were injected into the ear marginal vein 1 hour before CB-154. The results are presented as means ± SEM. Statistical significance was controled by Student t-test. Solid points indicate statistically significant results. •----• CB-154 1 mg/kg; ●—● CH 3 mg/kg + CB-154 1 mg/kg, ● vs •, $p < 0.01$; ■—■ MS 5 mg/kg + CB-154 1 mg/kg, ■ vs •, $p < 0.01$; ▲—▲ WA-335 3 mg/kg + CB-154 1 mg/kg, ▲ vs •, $p < 0.02$

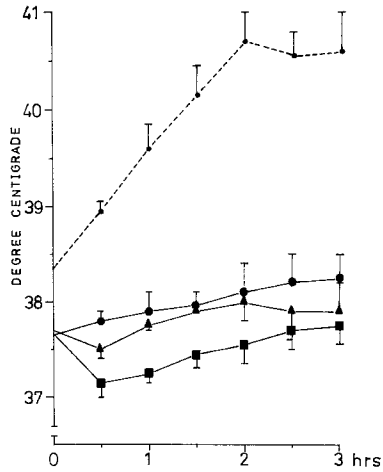

Fig. 6. The influence of CB-154 (2.5 mg/kg) on the body temperature in the rabbit after pretreatment with 5-HT antagonists. The compounds were injected into the ear marginal vein 1 hour before CB-154. The results are presented as means ± SEM. Statistical significance was controled by Student t-test. Solid points indicate statistically significant results. •----• CB-154 2.5 mg/kg; ●—● CH 3 mg/kg + CB-154 2.5 mg/kg, ● vs •, $p < 0.05$; ■—■ MS 5 mg/kg + CB-154 2.5 mg/kg, ■ vs •, $p < 0.001$; ▲—▲ WA-335 3 mg/kg + CB-154 2.5 mg/kg, ▲ vs •, $p < 0.02$

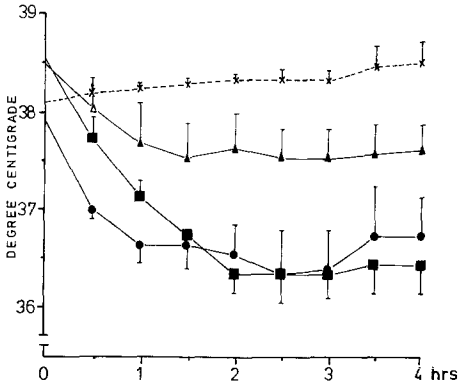

Fig. 7. The influence of 5-HT antagonists of the body temperature in the rabbit. The compounds were injected into the ear marginal vein. The results are presented as means ± SEM. Statistical significance was controled by Student t-test. Solid points indicate statistically sginificant results. x---x saline; ●—● CH 3 mg/kg, ● vs x, $p < 0{,}001$; ■—■ MS 5 mg/kg, ■ vs x, $p < 0.001$; ▲—▲ WA-335 3 mg/kg, ▲ vs x, $p < 0.01$

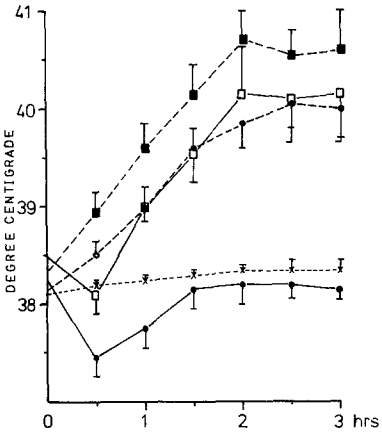

Fig. 8. The influence of CB-154 1 and 2.5 mg/kg on the body temperature in the rabbit after pretreatment with HP 0.5 mg/kg. HP was injected in the ear marginal vein, 1 hour before CB-154. The results are presented as means ± SEM. Statistical significance was controled by Student t-test. Solid points indicate statistically significant results. x---x solvent; ○---○ CB-154 1 mg/kg; ●---● vs x, $p < 0.001$; ■—■ CB-154 2.5 mg/kg, ■---■ vs x, $p < 0.001$; ●—● HP 0.5 mg/kg + CB-154 1 mg/kg, ●—● vs ○---○, $p < 0.01$; □—□ HP 0.5 mg/kg + CB-154 2.5 mg/kg, ■—■ vs □---□, $p < 0.02$

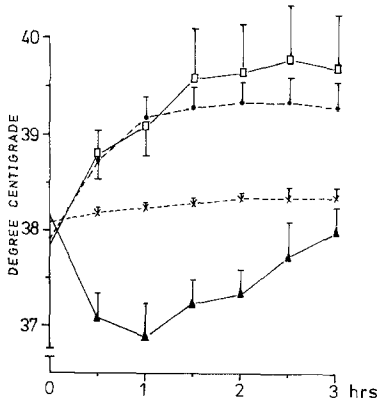

Fig. 9. The influence of FF 10 mg/kg on the body temperature in the rabbit, pretreated with HP 0.5 mg/kg or WA-335 3 mg/kg were injected into the ear marginal vein 1 hour before FF. The results are presented as means ± SEM. Statistical significance was controled by Student t-test. Solid points indicate statistically significant results. x---x saline; ●—● FF 10 mg/kg, ● vs x, $p < 0.01$; □—□ HP 0.5 mg/kg + FF 10 mg/kg, □ vs ●, n.s.; ▲—▲ WA-335 3 mg/kg + FF 10 mg/kg, ▲ vs x, $p < 0.05$

Discussion

The flexor reflex of the hind limb of spinal rat has been one of two models employed to estimate the influence of CB-154 on serotonin neurons. As we demonstrated in previous experiments, serotonergic agents like tryptophan, 5-hydroxytryptophan, LSD, fenfluramine, p-chloroamphetamine (*Maj, Palider* and *Baran*, 1976), quipazine (*Palider* and *Rawłów*, 1977) and mescaline (*Maj, Palider* and *Rawłów*, 1977) potentiate the flexor reflex, and this potentiation is prevented by 5-HT antagonists—cyproheptadine, danitracen, methergoline.

The present experiments indicate that also CB-154 potentiates the flexor reflex. Both 5-HT blockers used: cyproheptadine and danitracen (given in doses estimated previously) prevent this potentiating effect of CB-154. These drugs were employed as 5-HT blockers basing on the aforementioned own experiments and literature data based on other experiments as well (*Stone et al.*, 1961; *Frey* and *Magnussen*, 1968; *Vargaftig et al.*, 1971; *Van Riezen*, 1972; *Engelhardt*, 1975; *Kähling et al.*, 1975; *Maj, Baran, Sowińska* and *Gancarczyk*, 1976). The increase of the flexor reflex can be also induced by stimulation of spinal NA receptor. This kind of stimulation however is not

antagonized by 5-HT receptor blockers (*Maj, Palider* and *Baran,* 1976). Therefore the noradrenergic mechanism of CB-154 action can be excluded.

In some experiments we observed that the second injection of CB-154, preceded by an administration of a 5-HT receptor blocker (CH) induced a depression of the flexor reflex. A cause of this effect, unobserved in a case of serotonergic agents investigated previously (*Maj, Palider* and *Baran,* 1976) is unclear. It does not seem to be a result of the NA receptor blockade since clonidine—a NA agonist given in such experimental conditions retains a stimulating action on the flexor reflex. It is possible that a strong fall of blood pressure caused by CB-154 may, be of some importance (unpublished data).

It is worth of note that flexor reflex preparation in spinal rats seem to be a good model for studying substances which stimulate simultaneously central dopamine and 5-HT receptors. Specific dopamine agonists *e.g.* apomorphine do not influence the flexor reflex whereas agents stimulating both dopamine and 5-HT receptors induce an increase of the flexor reflex antagonized by 5-HT receptors blockers. CB-154 is an example of such agents, the another one is ergometrine (*Antkiewicz-Michaluk,* 1976).

In the second model employed CB-154 also acts like a serotonergic agent. Literature data (*Horita* and *Gogerty,* 1958; *Quock* and *Beal,* 1976; *Quock et al.,* 1976) indicate that serotonergic agents like 5-hydroxytryptophan, LSD, fenfluramine, quipazine elicit hyperthermia in rabbits which is prevented by 5-HT antagonists like CH, cinanserine, methysergide. In the present experiments CB-154 caused the dose-dependent hyperthermia prevented by three 5-HT antagonists used: CH, WA-335 and MS. The latter antagonizes also the potentiation of the flexor reflex induced by 5-hydroxytryptophan, LSD, fenfluramine (*Maj, Baran, Rawłów* and *Sowińska,* 1977) and elicits effects indicating its antiserotonin properties (*Van der Burg et al.,* 1970; *Saxena et al.,* 1971; *Vargaftig et al.,* 1971; *Van Riezen,* 1972).

It is concluded from other investigations (*Hill* and *Horita,* 1971, 1972; *Horita* and *Hamilton,* 1973; *Quock* and *Horita,* 1973) that hyperthermia in rabbits results also from dopaminergic stimulation, induced by *e.g.* apomorphine, amphetamine, L-Dopa. Apomorphine-induced hyperthermia is antagonized by dopamine blockers, but also by 5-HT blockers (*Quock* and *Horita,* 1974; *Przewłocki,* 1975). Since CB-154 is a dopamine agonist, one may suppose that CB-154-induced hyperthermia results from dopaminergic stimulation. But the fact that HP—a dopamine antagonist—does not prevent hyperthermia induced by CB-154 2.5 mg/kg contradicts such a view. Similarly, the hyper-

thermia caused by FF (*Costa et al.*, 1971; *Southgate et al.*, 1971) is not influence by HP but blocked by WA-335, 5-HT antagonist.

The hypothermia induced by lower dose of CB-154 (1 mg/kg) is prevented by HP. It may be concluded that the hyperthermic effect observed after this dose of CB-154 is caused by stimulation of dopamine receptors.

Acknowledgements

The authors thanks for generous supplies of compounds used are due to the donors, particullary to Sandoz for a supply of CB-154.

References

Antkiewicz-Michaluk, L.: The central action of ergometrine. The doctor thesis. Polish Academy of Sciences Institute of Pharmacology, Kraków 1976.

Burg, W. J. van der, Bonta, J. L., Delobelle, J., Ramon, C., Vargaftig, B.: A novel type of substituted piperazine with high antiserotonin potency. J. Med. Chem. *13*, 35—39 (1970).

Corrodi, H., Fuxe, K., Hökfelt, T., Lidbrink, P.: Effect of ergot drugs on central catecholamine neurons: Evidence for a stimulation of central dopamine neurons. J. Pharm. Pharmacol. *25*, 409—411 (1973).

Costa, E., Groppetti, A., Revuelta, A.: Action of fenfluramine on monoamine stores of rat tissue. Br. J. Pharmacol. *41*, 57—64 (1971).

Dray, A., Oakley, N. R.: Bromocriptine and dopamine receptor stimulation. J. Pharm. Pharmacol. *28*, 586—588 (1976).

Engelhardt, G.: Zur Pharmakologie des 9, 10-Dihydro-10-(1-methyl-4-piperidyliden)-9-anthrol (WA-335), eines Histamin- und Serotonin-antagonisten. Arzneim.-Forsch. (Drug Res.) *25*, 1723—1737 (1975).

Frey, M. H., Magnussen, M. P.: Different central medication of the stimulant effects of amphetamine and its p-chloroanalogue. Biochem. Pharmacol. *17*, 1229—1307 (1968).

Fuxe, K., Corrodi, H., Hökfelt, T., Lidbrink, P., Ungerstedt, U.: Ergoconine and 2-Br-α-ergocriptine. Evidence for prolonged dopamine receptor stimulation. Med. Biol. *52*, 121—132 (1974).

Hill, H. F., Horita, A.: Inhibition of (+)-amphetamine hyperthermia by blockade of dopamine receptors in rabbits. J. Pharm. Pharmacol. *23*, 715—717 (1971).

Hill, H. F., Horita, A.: A pimozide sensitive effect of apomorphine on body temperature of the rabbit. J. Pharm. Pharmacol. *24*, 490—491 (1972).

Horita, A., Gogerty, J. H.: The pyretogenic effect of 5-hydroxytryptophan and its comparison with that of LSD. J. Pharmacol. Exp. Ther. *122*, 195—200 (1958).

Horita, A., Hamilton, A. E.: The effects of d, l-α-methyltyrosine and L-DOPA on the hyperthermic and behavioral actions of LSD in rabbits. Neuropharmacol. *12*, 471—476 (1973).

Johnson, A. M., Loew, D. M., Vigouret, J. M.: Stimulant properties of bromocriptine on central dopamine receptors in comparison to apomorphine, (+)-amphetamine and L-DOPA. Br. J. Pharmacol. *56,* 59 to 68 (1976).

Johnson, A. M., Vigouret, J. M., Loew, D. M.: CB-154 (2-Bromo-α-ergocryptine, Bromocriptine), a potential anti-Parkinsonian agent. N.-S. Arch. Pharmacol. *282,* suppl. 40 R (1974).

Kähling, J., Ziegler, H., Balhause, H.: Zentrale Wirkungen von WA-335-BS, einer Substanz mit peripherer Antiserotonin- und Antihistamin-Wirkung. Arzneim.-Forsch. (Drug Res.) *25,* 1737—1744 (1975).

Maj, J., Baran, L., Rawłów, A., Sowińska, H.: Central effects of mianserin and danitracen—new antidepressant of unknown mechanism of action. Pol. J. Pharmacol. Pharm. *29,* 213—214 (1977).

Maj, J., Baran, L., Sowińska, H., Gancarczyk, L.: Central action of WA-335. Arch. Immunol. Ther. Exp. *24,* 205—222 (1976).

Maj, J., Kapturkiewicz, Z., Michaluk, J.: The central action of nomifensine. Pol. J. Pharmacol. Pharm. *28,* 557—562 (1976).

Maj, J., Palider, W., Baran, L.: The effect of serotoninergic and antiserotoninergic drugs on the flexor-reflex of spinal rat: a proposed model to evaluate the action on the central serotonin receptor. J. Neural Transm. *38,* 131—147 (1976).

Maj, J., Palider, W., Rawłów, A.: The influence of mescaline on the flexor-reflex of the hind limb of the spinal rat. J. Pharm. Pharmacol. *29,* 177—178 (1977).

Palider, W., Rawłów, A.: Effect of serotonin uptake blocking agents on the flexor-reflex of the hind limb of the spinal rat. Pol. J. Pharmacol. Pharm. *29* (1977, in press).

Persson, T., Waldeck, B.: Is there an interaction between dopamine and noradrenaline containing neurons in the brain? Acta Physiol. Scand. *78,* 142—144 (1970).

Przewłocki, R.: The influence of the central dopaminergic neurons stimulation on the activity of serotonin neurons. The doctor thesis. Polish Academy of Sciences, Institute of Pharmacology, Kraków 1975.

Quock, R. M., Beal, G. A.: Fenfluramine-induced hyperthermia and stimulation in the rabbit. Res. Com. Chem. Path. Pharmacol. *13,* 401—409 (1976).

Quock, R. M., Beal, G. A., Chan, E. L. F.: Quipazine: a serotoninergic hyperthermic agent in the rabbit. J. Pharm. Pharmacol. *28,* 170—172 (1976).

Quock, R. M., Horita, A.: The interaction of naloxone with apomorphine induced hyperthermia in rabbits. Proc. West. Pharmacol. Soc. *16,* 68—69 (1973).

Quock, R. M., Horita, A.: Apomorphine: modification of its hyperthermic effect in rabbits by p-chlorophenylalanine. Science *183,* 539—540 (1974).

Riezen, H. van: Different central effects of 5-HT antagonists mianserin and cyproheptadine. Arch. Int. Pharmacodyn. Ther. *198,* 256—269 (1972).

Saxena, P. R., Houwellingen, P. van, Bonta, I. L.: The effects of mianserin hydrochloride on the vascular responses evoked by 5-hydroxytryptamine and related vasoactive substances. Eur. J. Pharmacol. *13*, 295—305 (1971).

Schorderet, M.: Direct evidence for the stimulation of rabbit retina dopamine receptors by ergot alkaloids. Neurosci. Lett. *2*, 87—91 (1976).

Snider, S. R., Hutt, Ch., Stein, B., Fahn, S.: Increase in brain serotonin produced by bromocriptine. Neurosci. Lett. *1*, 237—241 (1975).

Snider, S. R., Hutt, Ch., Stein, B., Prasad, A. L. N., Fahn, S.: Correlation of behavioural inhibition or excitation produced by bromocriptine with changes in brain catecholamine turnover. J. Pharm. Pharmacol. *28*, 563—566 (1976).

Southgate, P. J., Mayer, S. R., Boxall, E., Wilson, A. B.: Some 5-hydroxytryptamine-like actions of fenfluramine: a comparison with (+)-amphetamine and diethylpropion. J. Pharm. Pharmac. *23*, 600—605 (1971).

Stone, S. A., Wenger, H. C., Luden, C. I., Stavorski, J. M., Ross, C. A.: Antiserotonin, antihistaminic properties of cyproheptadine. J. Pharmacol. Exp. Ther. *131*, 73—84 (1961).

Vargaftig, B. B., Coignet, J. L., De Vos, C. J., Grijsen, H., Bonta, J. L.: Mianserin hydrochloride: peripheral and central effects in relation to antagonism against—5-hydroxytryptamine and tryptamine. Eur. J. Pharmacol. *16*, 336—346 (1971).

Authors' address: Dr. *J. Maj*, Polish Academy of Sciences, Institute of Pharmacology, 12 Smetna Str., PL-31343 Kraków, Poland.