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Ergot Alkaloids and Cyclic Nucleotides in the CNS

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Abstract. Ergotamine and dihydroergotamine show a higher blocking effect towards the dopamine-induced stimulation of adenylate cyclase activity than to the apomorphine-induced stimulation. This could reflect a possible specificity of the compound to dopaminergic receptor. Bromocriptine blocks in a more competitive way the activation of adenylate cyclase induced *in vitro* by dopamine, but increases the levels of cAMP in rat striatum *in vivo*. This effect is blocked by haloperidol and reserpine when given together with bromocriptine.

On the base of the biochemical observation and various behavioral data the drug has been used in the treatment of parkinsonism with contrasting results and of Huntington's chorea. Moreover, bromocriptine induces a marked decrease of cGMP levels in cerebellum such as haloperidol and chloropromazine while apomorphine and other dopaminergic drugs increase the levels of cGMP. At the dose of 1 mg/kg, bromocriptine as well as DH-ergotoxine markedly reduces the levels of striatal DOPAC.

The mode of action of ergot alkaloids has been poorly understood in the past. Only recently – thanks to the development of new techniques of biochemical pharmacology – some insight has been given to the different mechanisms involved. The first consequence of these studies leads to the conclusion that the various groups of ergot alkaloids act through different mechanisms, involving different physiological structures and that it is difficult to design a common indication for the various modes of action.

LSD has been studied in our laboratory as a stimulant of dopaminergic receptors in striatum, n. accumbens, tuberculum olfactorium, limbic cortex and retina (21, 26). This effect is selectively blocked by neuroleptics. Moreover, 2-Br-LSD, a compound which does not induce hallucinations in man, is not active on the dopaminergic receptors. Ergotamine and DH-ergotamine, on the other hand, reduce in a dose-related fashion the dopamine activation of striatal adenylate cyclase (11). The blocking activity shown by ergotamine and dihydroergotamine towards the dopamine-induced stimulation of adenylate cyclase is less pronounced

Table I. Effect of bromocriptine on motor function and mental performance in 12 patients with Huntington's chorea

	Chorea severity	Gait	Speech	Mental performance
Placebo	3.1 ± 0.30	3.3 ± 0.19	2.7 ± 0.21	3.5 ± 0.33
Bromocriptine	1.2 ± 0.05	1.0 ± 0.09	1.2 ± 0.07	1.8 ± 0.08

Results on a scale of 0 (normal) to 4 (very severely abnormal) are those obtained during placebo and during maximum dose treatment with bromocriptine (5–12.5 mg/day).

than the one towards apomorphine-induced stimulation. This fact may suggest the possible specificity of the compound for a particular type of dopaminergic receptors mediating its pharmacological action.

Recently we demonstrated that another ergot polypeptide derivative, bromocriptine, increases the levels of cyclic adenosine 3′:5′-monophosphate (cAMP) in rat striatum *in vivo,* whereas it blocks in a noncompetitive way the activation of adenylate cyclase induced *in vitro* by dopamine (24).

On the base of these biochemical observations and different behavioral data indicating that the drug induces stereotyped behavior in rats and hypermotility in mice (9, 13), bromocriptine has been used with contrasting results in the treatment of parkinsonism (14, 17, 20).

Moreover, *Frattola et al.* (7) reported that bromocriptine may reduce the intensity and the duration of the on-off attacks induced by long-term treatment with *L*-dopa. On the same line the drug has also been used in the treatment of Huntington's chorea, significantly reducing the abnormal involuntary movements and the disease severity (8, 25, 27) (table I). In the reported trial, bromocriptine has been found at least as useful in the treatment of Huntington's chorea as haloperidol, a drug which is of first choice in the management of this disease. Moreover, the data known on the mechanism of action of bromocriptine may give only a partial explanation of its clinical efficacy (5, 6, 18). The effect of bromocriptine on cAMP concentration in rat striatum is blocked by haloperidol and is dependent on an intact granular storage system: reserpine, in fact, when given together with bromocriptine blocks the increase of cAMP concentration induced by the latter drug (table II).

Table II. Effect of haloperidol and reserpine on the cAMP increase induced by bromocriptine

	cAMP pmoles/mg protein
Controls	5.32 ± 0.29
Bromocriptine, 2 mg/kg	10.25 ± 1.34*
Haloperidol, 2 mg/kg	5.85 ± 0.37
Reserpine, 5 mg/kg	5.40 ± 0.48
Bromocriptine + haloperidol	6.03 ± 0.59
Reserpine + haloperidol	6.49 ± 0.69

Bromocriptine, haloperidol and reserpine have been injected 10, 60 min, and 18 h before killing by a beam of microwave radiation (12). Each number is the mean ± SEM of at least 10 determinations. cAMP has been measured following the method of *Kuo and Greengard* (16).

* $p < 0.01$.

Table III. Cerebellar cGMP concentrations in rat after injection of haloperidol, chloropromazine, apomorphine and bromocriptine

	cGMP pmol/mg protein
Controls	9.31 ± 0.74
Haloperidol, 2 mg/kg	4.53 ± 0.44*
Chloropromazine, 5 mg/kg	3.23 ± 0.33*
Apomorphine, 1 mg/kg	14.07 ± 0.91*
Bromocriptine, 2 mg/kg	5.12 ± 1.05*

Values are the mean ± SEM of at least 12 determinations. Haloperidol, chloropromazine, apomorphine and bromocriptine have been injected 45, 30, 10 and 15 min before killing, respectively. cGMP has been measured following the method described by *Steiner et al.* (23) after extraction and purification from tissue homogenates as indicated by *Mao and Guidotti* (19).

* $p < 0.01$ when compared to control values.

Table IV. Time curve of DOPAC concentration in rat striatum after bromocriptine administration

	Time after injection min	DOPAC μg/g tissue
Controls	–	1.82 ± 0.11
Bromocriptine	30	1.58 ± 0.13
	60	0.84 ± 0.09*
	120	0.79 ± 0.08*
	240	0.91 ± 0.08*
	360	1.65 ± 0.14

Each number is the mean ± SEM of at least 10 determinations. DOPAC concentration has been measured as indicated by *Westerink and Kork* (28).

* $p < 0.01$.

On the other hand, in line with this tentative demonstration on the role of bromocriptine, we analyzed the recent indications suggesting a functional relationship among the dopaminergic system of the striatum and the Purkinje cells layer of the cerebellum, which contains a large amount of cyclic 3′:5′-guanosine monophosphate (cGMP) (1). Bromocriptine administration induces a marked decrease of cGMP levels in cerebellum, such as haloperidol and chloropromazine does; while apomorphine and other dopaminergic drugs increase the levels of cGMP (table III).

These data may receive further support by the demonstration that bromocriptine decreases the levels of striatal 3,4-dihydroxyphenilacetic acid (DOPAC) (table IV). In fact, at the dose of 1 mg/kg bromocriptine markedly reduces the levels of striatal DOPAC for at least 4 h. These changes, indicating that the turnover of dopamine may be reduced, are induced also by DH-ergotoxine. Further studies on the role of ergot alkaloids in the dopaminergic system have been performed measuring the inhibitory action of these drugs on the specific binding of ^{3}H-dopamine in calf caudate membranes (2, 3, 4). Table V shows the concentration of various ergot alkaloids required to inhibit the specific binding of ^{3}H-dopamine by 50%; the amounts are ranging between 40 and 110 n*M* concentration. Table VI presents the inhibitory concentration of 50 various ergot alkaloids for the dopamine-stimulated adenylate cyclase in rat striatum, while the effect exerted by DH-ergocryptine, DH-ergocornine and DH-ergocristine (the various components of DH-ergotoxine) is shown in figure 1. All the ergot alkaloids significantly block the adenylate cyclase activity: some of them at very low concentrations such as DH-ergotamine (5×10^{-7} *M*). For the DH-ergotoxine compounds the pharmacological activity on the dopaminergic receptors is more pronounced at equimolar concentrations for the receptors

Table V. Inhibition of ^{3}H-dopamine binding in calf caudate by ergot alkaloids

Drug	IC_{50}, n*M*
d-LSD	110 ± 30
Bromocriptine	93 ± 18
Ergotamine	35 ± 8
DH-ergocornine	44 ± 11
DH-ergocristine	40 ± 9
DH-ergocryptine	52 ± 14
Metergoline	73 ± 13

IC_{50} values were determinated by long probit analysis, utilizing at least five concentrations of each drug. Values are the means of at least 6 determinations.

^{3}H-binding was measured following the indications of *Burt et al.* (3).

Table VI. Inhibitory concentration (IC_{50}) of various ergot alkaloids for the dopamine stimulated adenylate cyclase in rat striatum

Drug	$\frac{IC_{50}, M}{DA}$
DH-ergocornine	4.5×10^{-6}
DH-ergocryptine	3×10^{-6}
DH-ergocristine	3×10^{-6}
Bromocriptine	7.5×10^{-7}
Ergotamine	7×10^{-7}
DH-ergotamine	5×10^{-7}

IC_{50} values were determined by long probit analysis, utilizing at least five concentrations of each drug. Values are the mean of at least 8 determinations. Adenylate cyclase activity has been measured following the method of *Kebabian et al.* (15).

located in the limbic system in comparison to those of the striatum, indicating a possible preferential effect for the regions of the brain involved in the psychologically integrated functions.

The various activities of ergot alkaloids on different biochemical parameters of the CNS may not directly explain the various central pharmacological effects of these drugs: moreover, the specificity of action on the dopaminergic system indicated by the effects on dopamine receptors, by the decrease of DA turnover, by effects on other neuronal populations depending on the DA system give further support to the possible involvement of these drugs on therapeutic actions.

Recently, we demonstrated an age-related decrease in the sensitivity to dopamine stimulation of adenylate cyclase in striatum, substantia nigra (22) and dopaminergic areas of the limbic system of senescent rats (10). The involvement of the dopaminergic system in the events

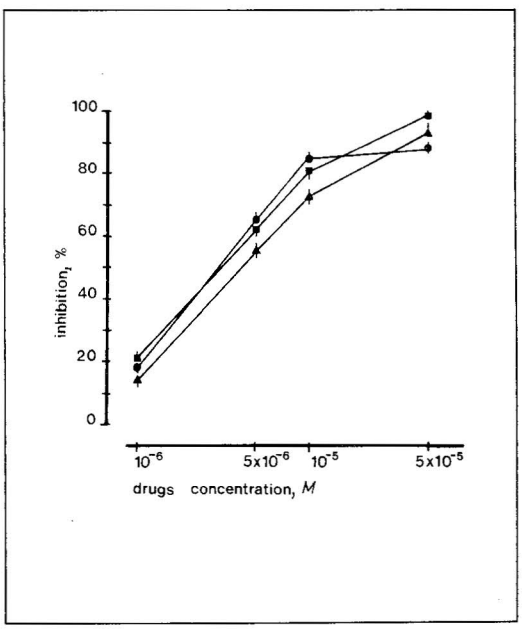

Fig. 1. Inhibitory effect of DH-ergot alkaloids on dopamine (10^{-5} *M*) stimulated adenylate cyclase in rat striatal homogenates. ● = DH-ergocryptine; ▲ = DH-ergocornine; ■ = DH-ergocristine.

characterizing old age is a further indication of the pharmacological relevance of the biochemical changes induced by ergot alkaloids in this system.

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