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# Bromocriptine, Dihydroergotoxine, Methysergide, d-LSD, CF 25-397, and 29-712: Effects on the Metabolism of the Biogenic Amines in the Brain of the Rat

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**Abstract.** The effects of the ergolene derivatives bromocriptine, dihydroergotoxine, methysergide, d-LSD, CF 25-397, and 29-712 on the metabolism of the biogenic amines in the brain of the rat were investigated.

All six ergolene derivatives were found to increase the concentration of 4-hydroxy-3-methoxyphenylethylene glycol sulphate in the brain stem, i.e., to increase the turnover of noradrenaline (NA). Since in brain homogenates the agents inhibited the binding of $^3$H-dihydroergocryptine to α-adrenoceptors, but only weakly inhibited the binding of $^3$H-alprenolol to β-adrenoceptors, it is suggested that the increased turnover of NA may be a consequence of a blockade of α-adrenoceptors by ergolenes.

All of the ergolenes increased the concentration of serotonin (5-HT) in the cortex, but only bromocriptine and 29-712 increased the concentration of 5-hydroxyindoleacetic acid (5-HIAA), the other compounds decreasing the concentration of this metabolite, i.e., inhibiting 5-HT turnover. Reserpine-induced PGO waves in the cat were inhibited by all six compounds, bromocriptine and 29-712 being the least active. Both of these findings suggest that the ergolenes possess serotonergic activity. The increase in the concentration of 5-HIAA after bromocriptine and 29-712 may be secondary to some action on other systems.

The actions of the ergolenes on the metabolism of dopamine (DA) in the striatum are more complex. Bromocriptine, 29-712, and, to a much lesser extent, dihydroergotoxine reduced the concentration of 3,4-dihydroxyphenylacetic acid (DOPAC), i.e., they inhibited DA turnover. These findings are compatible with the proposed dopaminergic activity of the drugs. CF 25-397 caused a slight increase in the DOPAC concentration at high doses, and d-LSD and methysergide caused pronounced increases. At doses below 1 mg/kg i.p., d-LSD decreased the DOPAC concentration. This biphasic effect of d-LSD may be due to interaction with different types of DA receptors or may reflect some secondary action of the compound.

The profiles of activity of the various ergolenes are discussed. Bromocriptine and 29-712, wich have similar profiles of activity, can be clearly differentiated from the other ergolenes. CF 25-397 seems to be a potent and, at low doses, specific serotonergic drug.

**Key words:** Ergolenes – Rat brain – Dopamine – Noradrenaline – Serotonin – PGO waves – Cat.

In most instances the behavioural effects of a drug are the result of its actions on many different neuronal systems, and the usefulness of a therapeutic agent is determined by its relative activities on those systems. These activities may lead to therapeutically desirable effects as well as to adverse reactions. Therefore, it is surprising to note the scarcity in the literature of neurochemical investigations relating effects of drugs on one neuronal system to effects on other (aminergic, cholinergic, etc.) neuronal systems. For example, ergolene derivatives have been shown to exert important effects on the metabolism of the catecholamines (Hökfelt and Fuxe, 1972; Corrodi et al., 1973) and serotonin (Freedman, 1961; Andén et al., 1968) in the brain. From the available data, however, it is difficult to relate the different activity components to each other. Therefore, in the present study the effects of the four well-known ergolenes, d-LSD, bromocriptine, methysergide, and dihydroergotoxine, on the metabolism of brain amines were investigated more systematically. In addition, two new lysergic acid derivatives – CF 25-397 and 29-712 – were also included in the study (Fig. 1).

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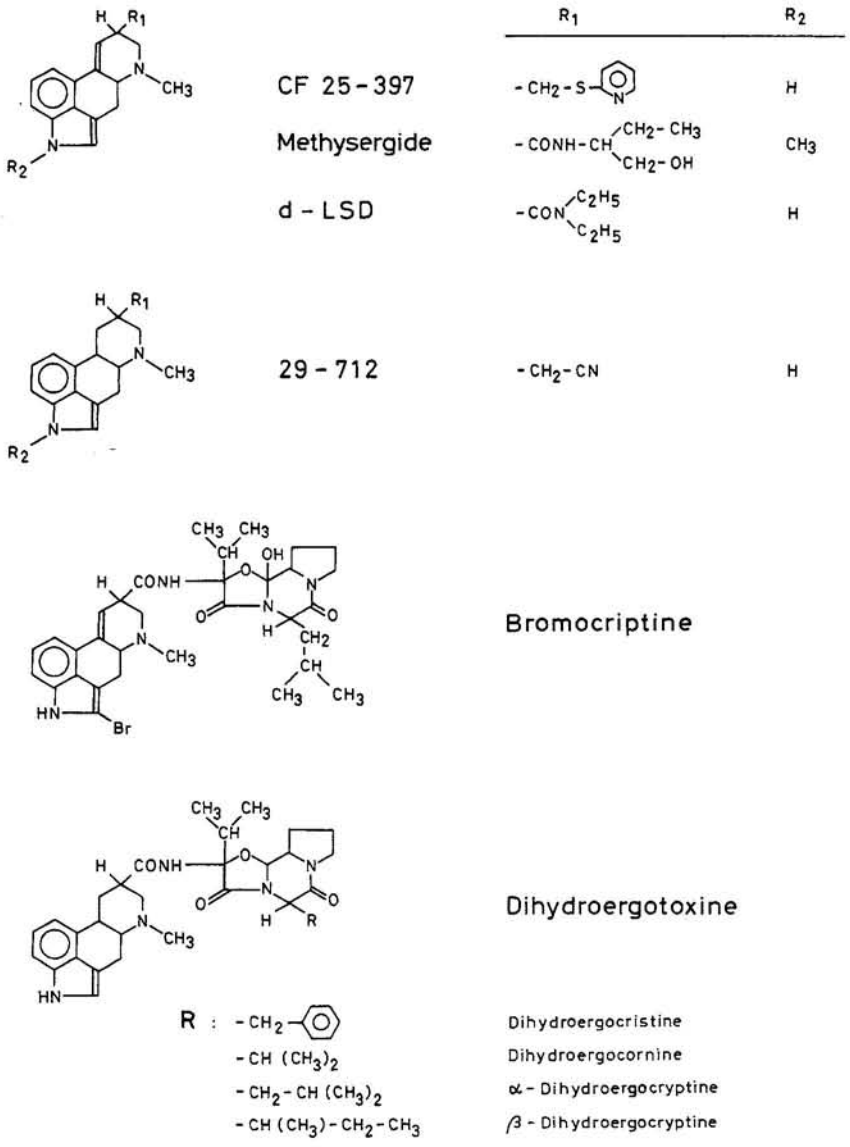


**Fig. 1**
Structural formulae of the ergolene derivatives tested

## Materials and Methods

*Animals.* For the neurochemical assays and the receptor-binding studies, male Sprague-Dawley rats weighing 120–170 g, obtained from Süddeutsche Versuchstierfarm KG, were used. The rats were kept in air-conditioned rooms at 25° C and 50% air humidity and fed with Nafag pellets (Nafag AG) and water ad libitum.

To study reserpine-induced ponto-geniculo-occipital (PGO) waves, male adult cats weighing 2.5–3.5 kg were used.

*Drugs.* For i.p. and s.c. administration to rats, the following solutions were used: 9,10-didehydro-6-methyl-8β-[2-pyridylthiomethyl]-ergolene tartrate (CF 25-397) dissolved in N-methylpyrrolidone and lactic acid and diluted with water; 6-methyl-8α-cyanomethyl-ergoline methanesulphonate (29-712) dissolved in N-methylpyrrolidone and lactic acid and diluted with 5% glucose solution; bromocriptine methanesulphonate dissolved in little ethanol and tartaric acid and diluted with 5% glucose solution; dihydroergotoxine methanesulphonate[1] dissolved in 5% glucose solution; d-LSD tartrate and methysergide hydrogen maleinate dissolved in water. Routes of administration and treatment schedules are described in the respective tables.

For i.v. administration to cats, the drugs were dissolved in either saline or aqueous tartaric acid.

$^3$H-Dihydroergocryptine, 9,10-[9,10-$^3$H(N)], S.A. 24.1 $C_i$/mmole, and $^3$H-dihydroalprenolol, levo-[propyl-2,3-$^3$H], S.A. 32.6 $C_i$/mmole, were purchased from New England Nuclear Corporation.

*Neurochemical assays in the rat.* After decapitation of the rats, the brains were dissected and the tissues were homogenized immediately. For the determination of dopamine (DA) and noradrenaline (NA),

[1] Dihydroergotoxine methanesulphonate consists of the mesylates of dihydroergocristine, dihydroergocornine, and dihydroergocryptine in equal proportions. Dihydroergocryptine mesylate is composed of the two isomers dihydro-α-ergocryptine mesylate and dihydro-β-ergocryptine mesylate in 2:1 proportions

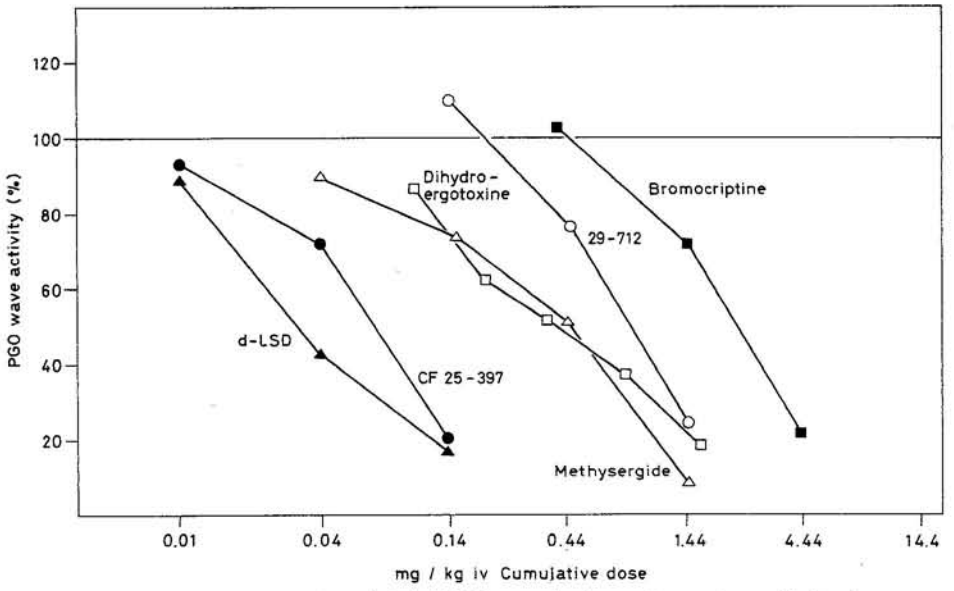


**Fig. 2.** Inhibition of ponto-geniculo-occipital (PGO) waves in the cat by ergolenes. *Each point:* average of at least 3 determinations

the tissues were homogenized in 0.4-N perchloric acid, containing 0.05% sodium metabisulphite and 0.05% EDTA, using a polytron PT 20 OD S homogenizer (Kinematica GmbH, Luzern), and the homogenates were centrifuged at 12800 g for 10 min at 0–4°C. From the perchloric acid supernatants of the pooled striata of 2 rats, DA was determined fluorimetrically after adsorption on aluminium oxide and elution with dilute acetic acid by the method of Laverty and Taylor (1968) with a continuous flow-analyzer as described by Waldmeier and Maître (1973). The turnover of DA was assessed after blockade of the tyrosine hydroxylase with α-methyltyrosine methyl ester hydrochloride (H 44/68) as described by Corrodi and Hanson (1966). H 44/68 (250 mg/kg i.p.) was administered 15 min after the drugs, the rats were killed 2 h later, and the DA content in the striatum was determined. NA was determined fluorimetrically in the pooled brain stems of 2 rats after adsorption from the neutralized perchloric acid extract on aluminium oxide (Weil-Malherbe, 1971) and elution with dilute perchloric acid (Anton and Sayre, 1962), with a continuous flow-analyzer according to Zuspan and Cooley (1969). The turnover rate of NA was assessed after blockade of the dopamine-β-hydroxylase with diethyldithiocarbamate (DDC), as described by Carlsson et al. (1966). DDC (500 mg/kg s.c.) was administered 15 min after the drugs. The rats were killed 2 h later and the NA content in the brain stem was determined.

For the determination of 3,4-dihydroxyphenylacetic acid (DOPAC) and 4-hydroxy-3-methoxyphenylethylene glycol sulphate (MOPEG-$SO_4$), the tissues were homogenized in 0.4-N perchloric acid, using a glass homogenizer with a Teflon pestle (Thomas, Philadelphia), and the homogenates were centrifuged at 12800 g for 10 min at 0–4°C. The supernatant was used for analysis. DOPAC was extracted from the perchloric acid supernatants of the pooled striata of 2 rats with n-butyl acetate and reextracted from the n-butyl acetate phase with ethylenediamine solution for fluorimetric determination according to Spano and Neff (1971). MOPEG-$SO_4$ was isolated from the neutralized perchloric acid supernatants of the pooled brain stems of 2 rats by adsorption onto Dowex 1X4 100–200 mesh ($Cl^-$-form, column height 45 mm, diameter 5 mm), elution with dilute perchloric acid, and determined fluorimetrically according to the method of Meek and Neff (1972).

For the determination of serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA), the pooled cortices of 2 rats were homogenized in 0.1-N hydrochloric acid containing 0.5% ascorbic acid.

The proteins were precipitated by addition of zinc sulphate and sodium hydroxide, and the reaction mixtures were filtered to yield a clear solution, which was used for the determinations. 5-HIAA was extracted from this solution at pH 1–2 with butyl acetate and reextracted from the butyl acetate phase at pH 7 with phosphate buffer 0.1 M.

5-HT was extracted at pH 10 with n-butanol and reextracted from the butanol after addition of 2 volumes of n-heptane with diluted hydrochloric acid. 5-HT and 5-HIAA were determined fluorimetrically in the hydrochloric acid and phosphate buffer solutions, respectively, sufficient hydrochloric acid being added in each case to give 3-N solutions (Giacalone and Valzelli, 1969).

With each drug-treated group, rats treated with solvent only served as controls. All data were calculated as % ± SD of the respective control groups. The average concentrations ($\bar{x}$ ± SD) of the metabolites of all the controls used in this study follow: 1.13 µg/g ± 0.09 DOPAC (striatum), 0.26 µg/g ± 0.03 MOPEG-$SO_4$ (brain stem), 0.30 µg/g ± 0.04 5-HT (cortex), and 0.20 µg/g ± 0.04 5-HIAA (cortex) ($N$ = 150 in each case); 9.44 µg/g ± 0.83 DA (striatum, $N$ = 29), and 0.72 µg/g ± 0.09 NA (brain stem, $N$ = 20).

Results of the biochemical determinations were corrected for recoveries of internal standards added to the tissue samples. Recoveries ($\bar{x}$ ± SD) of the various metabolites were: 75 ± 2.1% DA ($N$ = 35); 85 ± 5.4% DOPAC ($N$ = 115); 84 ± 2.6% NA ($N$ = 44); 90 ± 6% MOPEG-$SO_4$ ($N$ = 36); 63.9 ± 4.1% 5-HT ($N$ = 23); and 70.2 ± 7.7% 5-HIAA ($N$ = 23). Data were analyzed using a two-tailed $t$-test with a criterion for significance of $P < 0.05$.

*Neurotransmitter Receptor Binding Studies.* After decapitation of the rats, the brains were homogenized in ice-cold 0.32-M sucrose solution, using a glass homogenizer with a Teflon pestle (Thomas, Philadelphia). The homogenates were centrifuged at 3000 g for 3 min. The supernatants were used to assay β-adrenoceptor binding using $^3$H-alprenolol (Bylund and Snyder, 1976), and α-adrenoceptor binding using $^3$H-dihydroergocryptine (Williams et al., 1976).

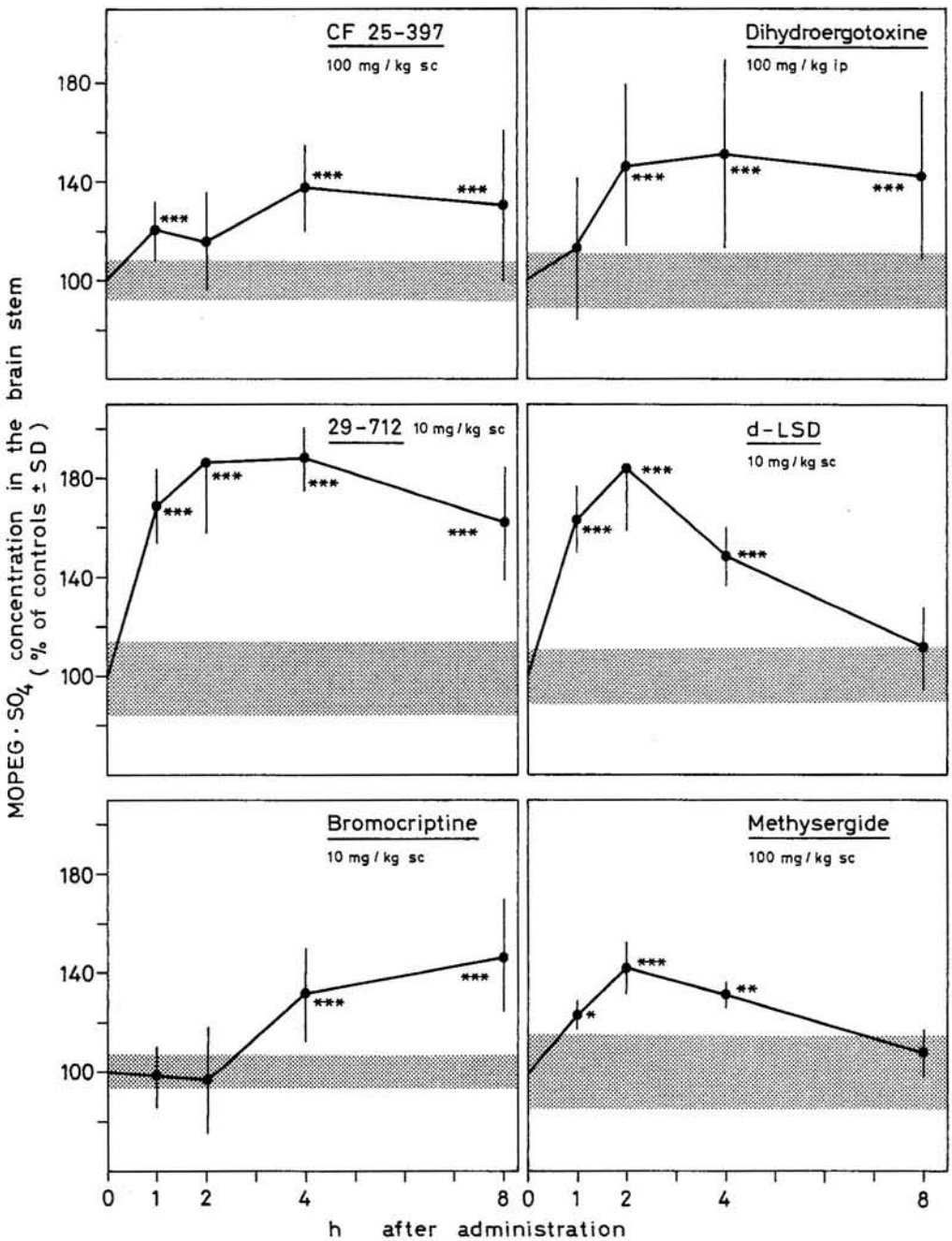


**Fig. 3.** 4-Hydroxy-3-methoxyphenylethylene glycol sulphate content of rat brain stem after treatment with ergolenes. Rats were killed 1–8 h after drug administration. *Vertical bars:* SD of 3–12 determinations. *Stippled areas:* $\bar{x} \pm$ SD of each control group ($N = 10-15$), which were arbitrarily fixed at 100. Statistical comparison between drug-treated and control animals with *t*-test: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Rat brain supernatant (0.7 mg protein in 0.1-ml 0.32-M sucrose) and the test compounds ($10^{-4}-10^{-9}$ M, 0.1 ml) were incubated for 20 min at 27° C with 1.35 pmol $^3$H-dihydroergocryptine or 0.86 pmol $^3$H-dihydroalprenolol in 1 ml of 0.05-M phosphate buffer, pH 7.4. The incubation mixtures were filtered by suction (Whatman GF/B), and the filters washed with ice-cold phosphate buffer and used for scintillation counting. $IC_{50}$ is the concentration of drug which displaces specific $^3$H-ligand binding by 50%. Specific binding is defined as the total binding minus the binding in the presence of 100 μM dihydroergocryptine or pindolol ($^3$H-dihydroalprenolol assay).

*Inhibition of Reserpine-Induced PGO Waves in the Cat.* All studies were carried out with nonanaesthetized, gallamine-immobilized cats under artifical respiration. Reserpine (0.5 mg/kg i.p.) was give 5 h before measuring PGO waves. During the entire experiment, the animals were maintained at 37° C. Under ether anaesthesia, the trachea was cannulated for artifical respiration, and both femoral

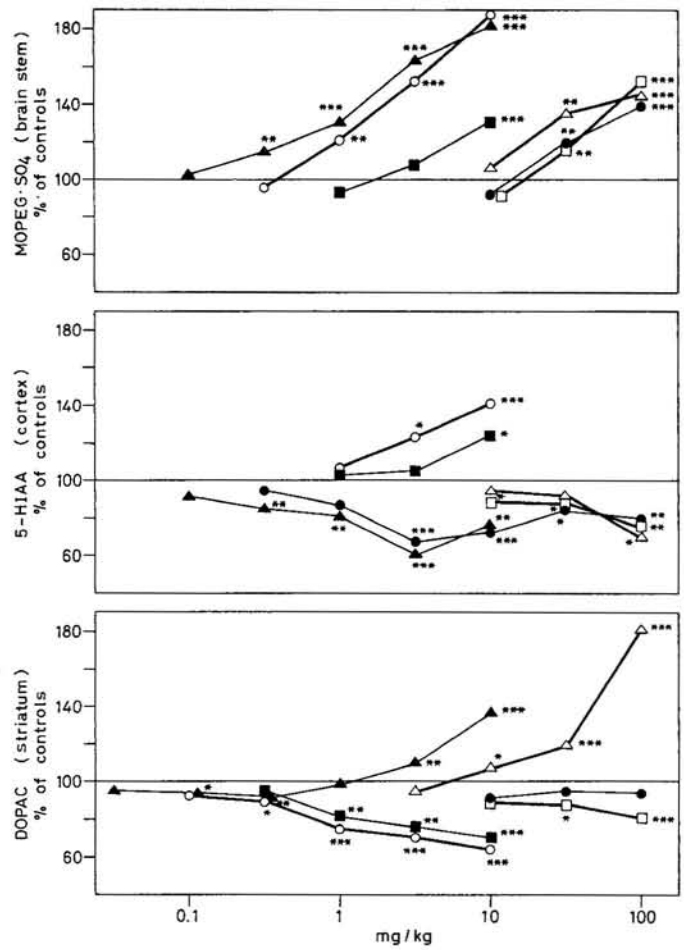


**Fig. 4**
Concentrations of 4-hydroxy-3-methoxyphenylethylene glycol sulphate (brain stem), 5-hydroxyindoleacetic acid (cortex), and 3,4-dihydroxyphenylacetic acid (striatum) after administration of CF 25-397 (●), 29-712 (○), bromocriptine (■), dihydroergotoxine (□), d-LSD (▲), and methysergide (△). Rats were killed 2 h after d-LSD or methysergide, and 4 h after other drugs. Dihydroergotoxine was given i.p., and all other drugs s.c. *Each point:* mean of 3–12 determinations. Statistical comparison with *t*-test: $*P < 0.05$, $**P < 0.01$, $*** P < 0.001$

veins and one femoral artery for i.v. drug application and continuous recording of the blood pressure, respectively. The head was then placed in a stereotactic apparatus; pressure points and wound edges were infiltrated with novocaine. Bipolar concentric electrodes of 0.5-mm diameter were inserted in both lateral geniculate bodies, using the Horsley-Clark coordinates A + 7.0, L ± 10.5, H + 3.5, and adjusted ro receive optimal electrical signals. PGO waves were recorded simultaneously with the electrocorticogram, the blood pressure and the heart rate on a Grass 7 apparatus. PGO waves of more than 100 μV amplitude were counted separately and were printed out as cumulative counts/10 min.

After a control period of 1 h, the test substances were injected i.v. in ascending doses at intervals of 30 min. Changes in the number of PGO waves after administration of the drugs are given as percentages of the number during the control period (Fig. 2).

## Results

*Effects on Noradrenergic Systems.* All of the ergolene derivatives tested increased the concentration of MOPEG-$SO_4$ in the brain stem (Fig. 3). This effect lasted for several hours and, in the case of bromocriptine, ocurred after a delay of about 2 h. The dose-response curves (Fig. 4) reveal that the ability to increase MOPEG-$SO_4$ decreased in the order d-LSD > 29-712 > bromocriptine > methylsergide > dihydroergotoxine = CF 25-397.

To gain further information concerning the mechanism by which these agents increase the concentration of MOPEG-$SO_4$, the concentration and the turnover of NA, measured by the method of synthesis inhibition with DDC, were determined after administration of the two substances that were most potent in increasing MOPEG-$SO_4$, i.e., d-LSD and 29-712. As shown in Table 1, both drugs failed to alter the concentration of NA at a dose of 1 mg/kg, but caused a significant increase in the turnover of NA.

Corrodi et al. (1973) have shown that bromocriptine increases the turnover of NA in rat brain. To explain this effect, they proposed that the drug exerts a weak reserpine-like action on noradrenergic neurons or produces a blockade of NA receptors, which causes an increased NA turnover via a feedback mechanism. In order to decide between these possibilities, the effects of our agents on radioactive affinity labeling of α- and β-adrenoceptors in rat brain homogenate were investigated (Table 2). Binding of $^3$H-dihydroergocryptine,

**Table 1** Effects of d-LSD and 29-712 on the metabolism of noradrenaline in the brain stem

| Drug | Dosage mg/kg | *N* | Noradrenaline content % ± SD | *N* | Noradrenaline content % ± SD after DDC |
|---|---|---|---|---|---|
| None | | 20 | 100 ± 13 | 10 | 43 ± 4 |
| 29-712 | 1 i.p. | 7 | 97 ± 6 | 7 | 35 ± 6* |
| d-LSD | 1 s.c. | 6 | 90 ± 5 | 6 | 26 ± 7** |

Rats were killed 2.25 h after drug administration. DDC (500 mg/kg s.c.) was given 15 min after drugs and 2 h before killing. *N* number of determinations, each performed on pooled homogenates of brain stems from 2 rats. Statistical comparison with *t*-test: * $P < 0.05$, ** $P < 0.001$

**Table 2.** Inhibition of the binding of $^3$H-dihydroergocryptine and $^3$H-alprenolol in whole brain homogenate by ergolenes

| Drug | $^3$H-Dihydro-ergocryptine $IC_{50}$ (μM) | $^3$H-Alprenolol $IC_{50}$ (μM) |
|---|---|---|
| CF 25-397 | 0.2 | 30 |
| 29-712 | 0.3 | 15 |
| Bromocriptine | 0.03 | >100 |
| Dihydroergotoxine | 0.007 | 40 |
| d-LSD | 0.6 | 2 |
| Methysergide | 0.2 | 60 |
| *Miscellaneous compounds* | | |
| l-Adrenaline | 3 | 6 |
| l-Noradrenaline | 4 | 15 |
| l-Isoproterenol | >100 | 1 |
| Serotonin | 4 | 40 |
| (−)Pindolol | 20 | 0.0055 |
| Phentolamine | 0.04 | 55 |

$IC_{50}$ is concentration of drug that displaces specific $^3$H-ligand binding by 50%. Specific binding is total binding minus binding in presence of 100 μM dihydroergocryptine or 100 μM pindolol ($^3$H-alprenolol assay)

which has previously been shown to label specifically α-adrenoceptors in rabbit uterine membranes (Williams et al., 1976), is inhibited by phentolamine, adrenaline, and noradrenaline, but not by isoproterenol and only weakly by the β-adrenoceptor antagonist pindolol. Although these findings suggest selective binding of $^3$H-dihydroergocryptine to α-adrenoceptors in brain tissue, it should be noted that serotonin also displaces $^3$H-dihydroergocryptine binding. All ergolenes tested inhibited $^3$H-dihydroergocryptine binding, which supports the hypothesis that they may be central α-adrenoceptor blockers. The binding of $^3$H-alprenolol to β-adrenoceptors is strongly inhibited by pindolol and, to a much smaller degree, by isoproterenol, adrenaline, and noradrenaline. Surprisingly, d-LSD was as potent as isoproterenol in inhibiting the binding of $^3$H-alprenolol. The other ergolenes were only marginally active in displacing $^3$H-alprenolol.

*Effects on Serotonergic Systems.* The effects of the compounds on the metabolism of 5-HT in the cortex varied considerably. CF 25-397, dihydroergotoxine, d-LSD, and methysergide significantly decreased the concentration of 5-HIAA, which lasted for several hours (Fig. 5). Furthermore, after CF 25-397, d-LSD, and methysergide, significant increases in the concentration of 5-HT were seen. From the dose-response curves (Fig. 4) it is evident that CF 25-397 and d-LSD were most potent in reducing the concentration of 5-HIAA, both drugs being active at doses from 1−3 mg/kg.

Bromocriptine and 29-712, on the other hand, increased the concentration of 5-HT, and increased rather than decreased the concentration of 5-HIAA. After either drug, this effect occurred after a delay of about 2−4 h. The increase in 5-HIAA was dose-dependent (Fig. 4), 29-712 being more active than bromocriptine.

To gain more information about the possible mechanism of action of the ergolenes on the metabolism of 5-HT, the effects of these drugs on reserpine-induced PGO waves in the cat were determined (Fig. 2). It has been proposed that PGO wave activity is controlled by an inhibitory serotonergic input to a pacemaker situated in the locus coeruleus (Jouvet, 1972), and that PGO waves can be suppressed by drugs possessing serotonergic activity. As shown in Figure 2, all of the ergolenes tested suppressed PGO waves in a dose-dependent manner. d-LSD and CF 25-397, which were most potent in decreasing the concentration of 5-HIAA in the rat cortex, were also most potent in suppressing the reserpine-induced PGO waves. Bromocriptine and 29-712, which increased rather than decreased the 5-HIAA concentration, were much less active than d-LSD and CF 25-397 and were also less active than dihydroergotoxine and methysergide. These findings suggest that the inhibition of the turnover of 5-HT by d-LSD, CF 25-397, dihydroergotoxine, and methysergide, which leads to a decrease in the concentration of 5-HIAA, is probably the result of a serotonergic activity of the drugs, the inhibition of the 5-HT turnover being caused by a negative feedback mechanism (Andén et al., 1968). The increase in the concentration of 5-HIAA after bromo-

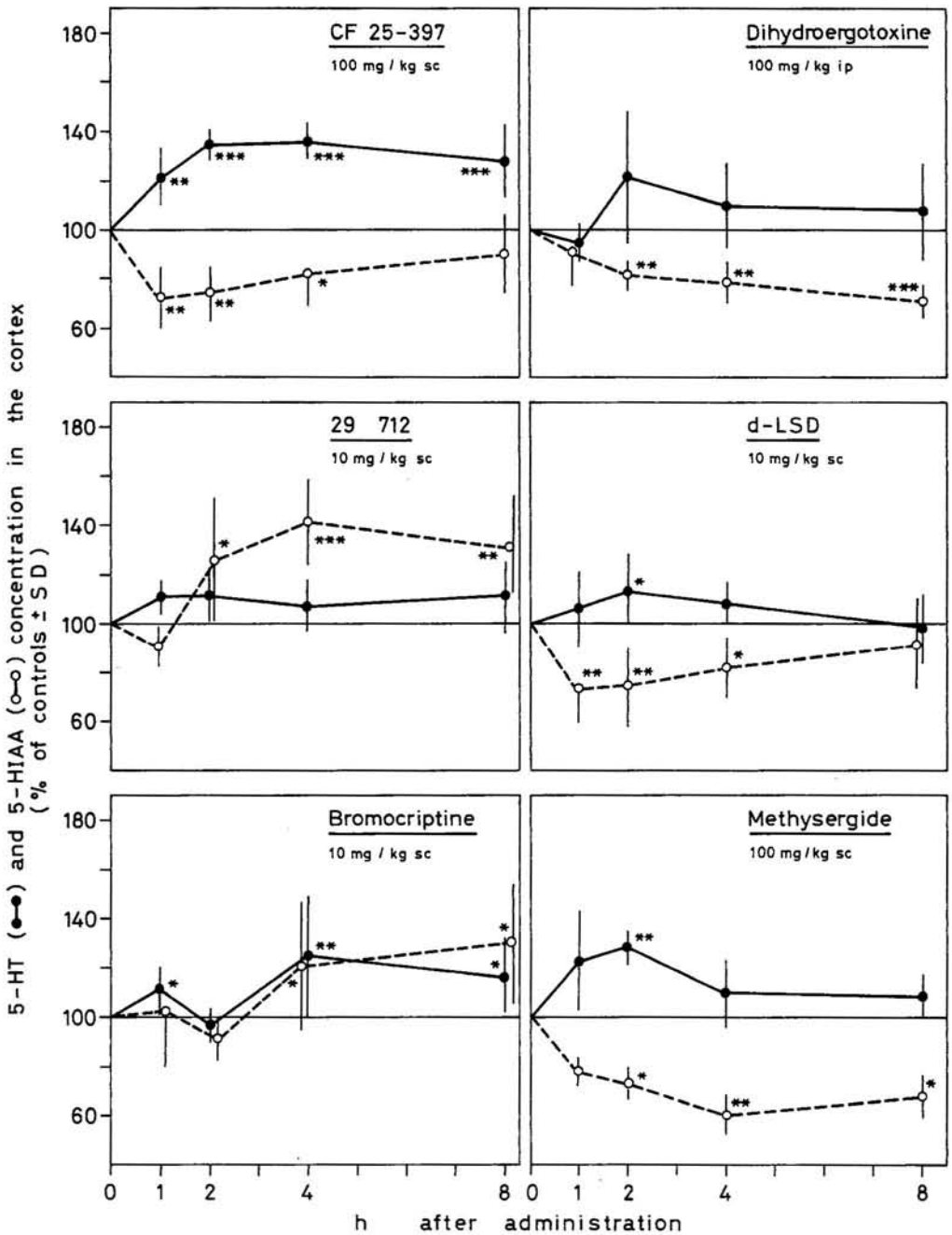


**Fig. 5.** Serotonin and 5-hydroxyindoleacetic acid content of rat cortex after treatment with ergolene derivatives. Rats were killed 1–8 h after drug administration. *Vertical bars:* SD of 3–12 determinations. The mean of each control group ($N = 10-15$) was arbitrarily fixed at 100. Statistical comparison beween drug-treated and control animals with *t*-test: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

criptine and 29-712 could be secondary to some action on other transmitter systems.

*Effects on Dopaminergic Systems.* Bromocriptine, 29-712, and dihydroergotoxine significantly decreased the concentration of DOPAC in the striatum, which lasted for several hours (Fig. 6). The dose-response curves (Fig. 4) indicate that bromocriptine and 29-712 were far more active than dihydroergotoxine. Bromocriptine and 29-712 inhibited the turnover of DA, measured by the method of synthesis inhibition with H 44/68 (Table 3), bromocriptine at 10 mg/kg also causing a significant increase in the concentration of DA. These findings agree with the hypothesis that bromocriptine (Corrodi et al., 1973), 29-712, and, to a much smaller extent, dihydroergotoxine stimulate striatal DA receptors, thus causing feedback inhibition of the turnover of striatal DA.

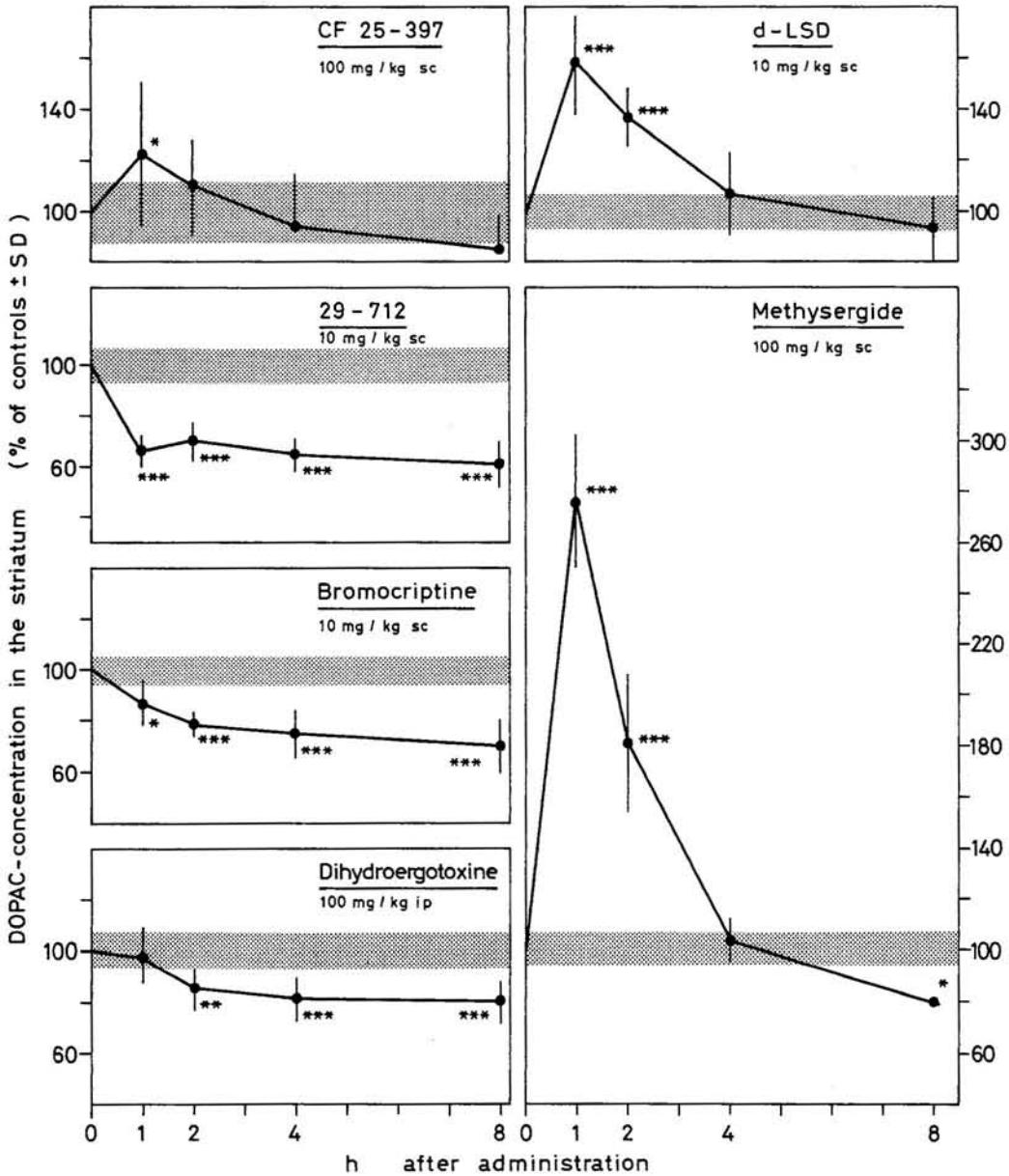


**Fig. 6.** 3,4-Dihydroxyphenylacetic acid content in rat striatum after treatment with ergolenes. Rats were killed 1 – 8 h after drug administration. *Vertical bars:* SD of 3 – 12 determinations. *Stippled areas:* $\bar{x} \pm$ SD of each control group ($N = 10 - 15$), which were arbitrarily fixed at 100. Statistical comparison with *t*-test: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

**Table 3.** Effects of bromocriptine and 29-712 on the metabolism of dopamine in the striatum

| Drug | Dosage mg/kg | *N* | Dopamine content % ± SD | *N* | Dopamine content after H 44/68 % ± SD |
|---|---|---|---|---|---|
| None | | 29 | 100 ± 8 | 10 | 57 ± 6 |
| 29-712 | 1 i.p. | 5 | 104 ± 9 | 5 | 79 ± 7** |
| Bromocriptine | 10 s.c. | 11 | 115 ± 9* | 11 | 68 ± 3** |

Rats were killed 2.25 h after drug administration. H 44/68 (250 mg/kg i.p.) was given 15 min after drugs and 2 h before killing. *N* number of determinations, each performed on pooled homogenates of striatum of 2 rats. Statistical comparison with *t*-test: *$P < 0.01$, ** $P < 0.001$

**Table 4.** Effects of ergolenes on the metabolism of the biogenic amines in rat brain and on PGO waves in cats

| Drug | *T* (h) | DOPAC (striatum) | | MOPEG-$SO_4$ (brain stem) | 5-HIAA (cortex) | | PGO waves |
|---|---|---|---|---|---|---|---|
| | | $ED_{80\%}$ mg/kg | $ED_{120\%}$ mg/kg | $ED_{120\%}$ mg/kg | $ED_{80\%}$ mg/kg | $ED_{120\%}$ mg/kg | $ID_{50\%}$ mg/kg i.v. |
| CF-25-397 | 4 | ineffective | | 34 | 1.5 | | 0.065 |
| 29-712 | 4 | 0.7 | | 1 | | 3 | 0.8 |
| Bromocriptine | 4 | 1.2 | | 6 | | 8 | 2 |
| Dihydroergotoxine | 4 | 100 | | 38 | 90 | | 0.4 |
| d-LSD | 2 | | 5 | 0.5 | 1 | | 0.03 |
| Methysergide | 2 | | 35 | 18 | 60 | | 0.4 |

$ED_{80\%}$ and $ED_{120\%}$ were calculated from data in Figure 4. *T* time between drug administration and killing of rats. Dihydroergotoxine was administered i.p. and all other drugs s.c. $ID_{50\%}$ for inhibition of PGO waves (mg/kg i.v. cumulative dose) was calculated from data in Figure 2

CF 25-397 caused a small but significant increase in the concentration of DOPAC 1 h after administration (Fig. 6). Otherwise, no significant changes in the DOPAC-concentration were seen with any of the doses used (Fig. 4).

The effects of d-LSD and methysergide on the metabolism of striatal DA are more complex. At high doses, both drugs caused pronounced increases in the concentration of DOPAC, which lasted for at least 2 h (Fig. 6). Eight hours after administration of methysergide, the concentration of DOPAC was lower than that of the controls. Furthermore, the dose-response curve (Fig. 4) reveals that low doses (0.1 – 0.3 mg/kg) of d-LSD significantly decreased the concentration of DOPAC; an increase was seen only after doses of 3.2 mg/kg or more. These biphasic effects on the DOPAC concentration were observed only with d-LSD and methysergide.

## Discussion

The neurochemical effects of the six ergolenes have been summarized in Table 4. Since in the controls (see Materials and Methods) the standard deviations of the different metabolites vary between 8% (DOPAC) and 20% (5-HIAA) of the mean, it was arbitrarily decided to calculate from the dose-response curves (Fig. 4) either the $ED_{80\%}$ or $ED_{120\%}$ as a measure of potency. In the case of d-LSD, which exhibits a biphasic effect on the DOPAC concentration of the striatum, only the $ED_{120\%}$ is included in Table 4. The reduction of the DOPAC concentration seen after low doses of this drug, although statistically significant, does not exceed 10% of the controls. Therefore, an $ED_{80\%}$ could not be calculated.

Table 4 shows that the ergolenes influence the metabolism of the brain amines to different degrees. In order to analyze the activity profiles of the drugs, a three-dimensional diagram (Fig. 7) was constructed, using the data of Table 4. In this diagram the $ED_{80\%}$ and $ED_{120\%}$ of DOPAC and 5-HIAA, respectively, are plotted on the horizontal axes in such a way that the more active the drug, the further away from the center it is placed (the center representing absence of significant activity). Similarly, the vertical axis represents the potency to increase MOPEG-$SO_4$. The higher the column, the stronger the effect of a drug in increasing the concentration of MOPEG-$SO_4$.

All of the ergolenes shown in Figure 7 increase the metabolism of NA. Since these drugs inhibit the binding of the α-adrenoceptor label $^3$H-dihydroergocryptine, we suggest that the increase in the metabolism of NA is due to a feedback activation of noradrenergic neurons after blockade of NA receptors by the agents. We recognize, however, that under the experimental conditions used in this study and, in contrast to the experiments with rabbit uterine membranes (Williams et al., 1976), $^3$H-dihydroergocryptine may not be a selective label for α-adrenoceptors, as evidenced by the inhibition of the binding of this ligand by 5-HT (Table 2). In fact, d-LSD and methysergide have been described as weak inhibitors of binding of $^3$H-clonidine and $^3$H-WB-4101 (a benzodioxan derivative α-adrenoceptor antagonist) to postsynaptic α-adrenoceptor sites in rat brain (Greenberg et al., 1976).

The neurochemical activity profiles of the ergolenes in Figure 7 are mainly determined by their relative activities on dopaminergic and serotonergic systems. Accordingly, distinct groups can be formed of agents possessing similar activities.

Bromocriptine and 29-712, which lower the concentration of DOPAC in the striatum and increase the 5-HIAA concentration in the cortex, are situated in area A. In pharmacological tests, i.e., induction of stereotyped behavior in rats and induction of rotational behavior in rats with 6-hydroxydopamine-induced nigral lesions (Ungerstedt model), both drugs show actions typical of postsynaptic DA-receptor stimulants (Vigouret et al., 1978). The fact that bromocrip-

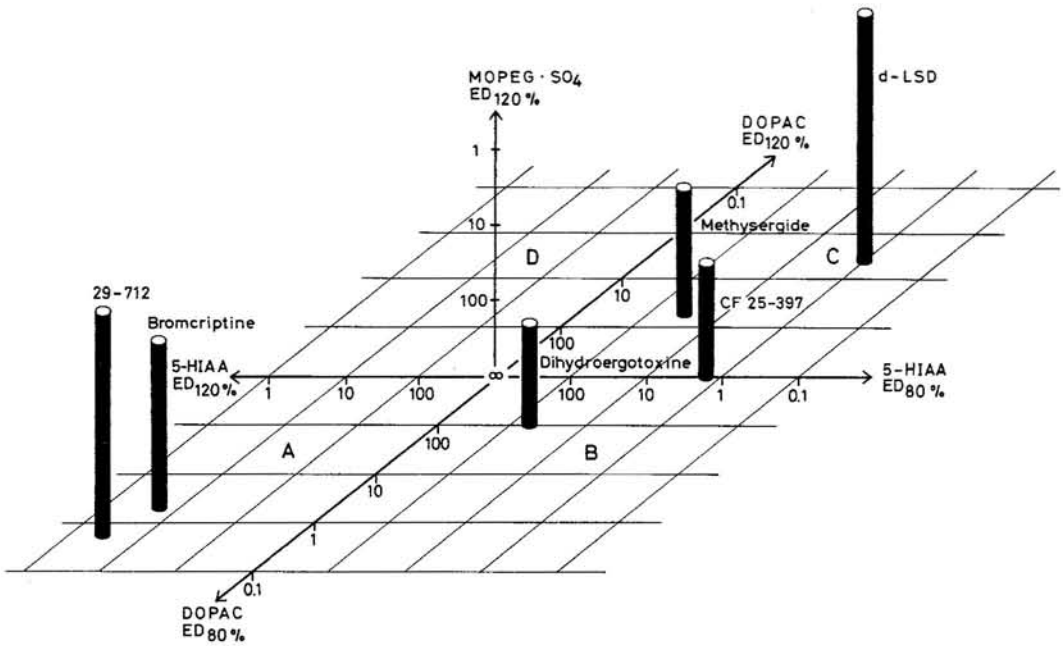


**Fig. 7.** Profiles of activity of ergolenes tested (for explanation see Discussion)

tine is useful in the treatment of parkinsonism (Calne et al., 1974, 1976; Lieberman, 1976) is further evidence for a powerful dopaminergic action of this drug. On the other hand, the relatively weak action of these drugs on reserpine-induced PGO waves and their increasing rather than decreasing action on the 5-HIAA concentration suggest that the serotonergic activity is a minor constituent of the profiles of activity of these drugs. In fact, the increase in 5-HIAA after bromocriptine and 29-712 may even be due to some peripheral effect causing an increased transport of tryptophan into the brain and an increased synthesis and metabolism of 5-HT, as has been reported for a variety of drugs (Tagliamonte et al., 1971, 1973).

Dihydroergotoxine, located in area B, exerts both dopaminergic and serotonergic activities, as indicated by the reductions of the concentrations of DOPAC and 5-HIAA. In functional tests, too, dihydroergotoxine has shown actions typical of 5-HT and DA-receptor stimulants (Loew et al., 1976).

d-LSD and methysergide are found in area C of Figure 7. Both drugs reduce the concentration of 5-HIAA and suppress PGO waves. When applied microiontophoretically, d-LSD suppresses the rate of firing of raphe neurons (for discussion see Aghajanian, 1972). Thus, the activity profile of these drugs is dominated by a central serotonergic component. In addition to this activity, there is a complex interaction with striatal dopaminergic mechanisms, which causes the concentration of DOPAC to increase at high doses of the drugs. After methysergide, the initial increase of the DOPAC concentration is followed by a reduction of the concentration of this metabolite. In low doses, d-LSD reduces the DOPAC concentration and, in functional tests, acts like a DA-receptor stimulant (Da Prada et al., 1975; Pieri et al., 1974). In in vitro experiments designed to study $^3$H-ligand receptor binding in the caudate it was found that $^3$H-LSD binding involved postsynaptic DA receptors displaying high and low affinity components (Burt et al., 1976). It has also been shown that d-LSD tends to increase adenylate cyclase activity in the brain, but blocks the stimulation of the enzyme by DA (Hungen et al., 1974). Therefore, the biphasic effects of d-LSD and methysergide on the DOPAC concentration may be caused by the interactions of these drugs with different types of DA receptors. Alternatively, the increase in the DOPAC concentration after high doses of these drugs could be secondary to some action on other transmitter systems.

Of great interest are the findings with the new lysergic acid derivative CF 25-397. This drug elicits contralateral turning in the Ungerstedt model and counteracts reserpine-akinesia in mice (Jaton et al., 1975). Using histofluorescence techniques, Fuxe et al. (unpublished data) found that CF 25-397 selectively inhibited the turnover of DA in the 'islandic system' of the striatum. CF 25-397, however, differs clearly from bromocriptine with respect to its effects on the concentration of DOPAC in the striatum. Bromo-

criptine causes a strong and prolonged reduction in the DOPAC content, and CF 25-397 a weak increase of short duration. In contrast to its doubtful dopaminergic activity, CF 25-397 is remarkably active on the serotonergic system. Both the reduction of the 5-HIAA concentration and the inhibition of the reserpine-induced PGO waves are evidence for a serotonergic activity of the drug. In fact, from the biochemical data it is suggested that in low doses CF 25-397 may act exclusively on 5-HT receptors. Therefore, this drug may prove valuable in the investigation of the physiological role of serotonergic systems.

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