min to remove undissolved dye. The results (Fig. 1) show a distinctive change in slope at approximately 5 mg ml^{-1} amethocaine and this value was taken as the cmc for amethocaine in this medium.

The influence of CPB or amethocaine on culture turbidity is shown in Fig. 2; while both curves are similar, amethocaine is required at a higher concentration to produce the same increase in turbidity. Both compounds exhibit a diphasic pattern of turbidity increase similar to that described for *N*-alkyl trimethylammonium bromides (Salt 1976). Significant changes in turbidity occurred at concentrations in excess of 15 $\mu g\ ml^{-1}$ CPB and 5 mg ml^{-1} amethocaine, a maximum value being attained at approximately 100 $\mu g\ ml^{-1}$ CPB and 16 mg ml^{-1} amethocaine.

The MIC for amethocaine was 0·6 mg ml^{-1}, which was much less than the concentration that caused turbidity increases in non-growing cells. However, values for bactericidal concentrations of amethocaine and other local anaesthetics have been reported by Schmidt & Rosenkranz (1970) and Weinstein et al (1975) and which, allowing for medium and inoculum variation, approximate to concentrations that are active turbidimetrically.

There is a close correlation between the deduced cmc for amethocaine and that concentration in excess of which large increases in culture turbidity are detected. It is thus possible that the uptake of amethocaine by the cells occurs preferentially when the molecules are in the micellar state. However, it is equally possible that amethocaine is taken up by the cells in significant quantities only as the "non-micellar" molecules approach a maximum concentration at or near the cmc.

The results are consistent with the view that amethocaine has a similar mode of antibacterial action to that of the cationic surfactants.

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Mechanisms of dihydroergotoxine's effect on prolactin release

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Dihydroergotoxine (DHE), a mixture of dihydroergocryptine, dihydroergocrystine and dihydroergocornine, has been used for the treatment of mental function disturbances in the elderly (Hughes, Williams & Currier, 1976). Its mode of action has been attributed to various different mechanisms such as improvement of brain blood flow. Particular interest has been devoted to the possible interference of the drug with the central dopaminergic system since it has been hypothesized that during ageing dopaminergic function is impaired (Finch 1973; Carlsson & Winglad 1976; Samorajski 1977; Cotzias et al 1977). In this context, Govoni et al (1977, 1978) have demonstrated that the function of the dopaminergic receptor system is altered in various brain areas of old rats. In the same group of animals serum prolactin concentrations have been reported to be high (Trabucchi et al, in preparation) suggesting a possible decrease of the inhibitory control exerted by dopamine on prolactin release.

Other ergot derivatives such as bromocriptine have been previously studied with behavioural and biochemical methods (Corrodi et al 1973; Fuxe et al 1975; Johnson et al 1976) for their stimulatory effect on the dopaminergic system and for their capacity to reduce prolactin release (Müller et al 1977). In apparent contrast to these observations, it has been shown that bromocriptine and DHE behave as dopamine antagonists (Trabucchi et al 1976; Spano & Trabucchi 1978; Spano et al 1978), when dopamine-stimulated adenylate cyclase and [^{3}H]spiroperidol binding are used as experimental models. We now report the effect of DHE at very low doses on prolactin secretion and dopamine turnover in the striatum.

Mature male Sprague Dawley (Charles River, Italy) rats, 150–175 g, were kept at constant room temperature and humidity and received a standard diet and had free access to water. DHE was injected intraventricularly under ether anaesthesia according to the method of Noble et al (1967). All rats were decapitated at 3 p.m. and the brains quickly removed. The striata were immediately dissected and frozen on dry ice until dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) concentrations were assayed. The trunk blood was collected in centrifuge tubes, allowed to clot at 4 °C, and centrifuged at 3000 *g*

* Correspondence.

for 10 min. The serum was removed and stored at −20 °C.

Striatal HVA and DOPAC concentrations were assayed fluorimetrically after isolation on Sephadex G-10 according to Westerink & Korf (1976), with minor modifications. In some experiments DOPAC concentrations were also estimated using the radioenzymatic micromethod of Argiolas et al (1977).

Serum prolactin concentrations were measured using the radioimmunoassay kit supplied by the NIAMDD Rat Pituitary Hormone Distribution Program. The assay procedures, a double antibody radioimmunoassay, were the same as recommended by the NIAMDD. The results are expressed in terms of the rat PRL reference standard (NIAMDD Rat-Prolactin-RP-1) in ng ml^{-1} as the mean of all samples in each group of animals. Protein content was measured according to Lowry et al (1951).

The dose of DHE that decreases rat serum prolactin concentration significantly is 10 $\mu g\ kg^{-1}$ which is comparable with that of bromocriptine producing the same effects (Fig. 1). 40 μg of DHE produces maximal inhibition. The decrease of prolactin concentrations induced by DHE lasts for about 6–8 h and returns to normal after 12 h (Table 1). To correlate these results with a specific action on the dopaminergic system, we measured the effects of DHE on the concentrations of dopamine metabolites in rat striatum. DHE, (2·5 mg kg^{-1}, i.p.) decreases striatal DOPAC, while a lower dose is ineffective (Table 2). Moreover intraventricular injection of DHE induces a dose-dependent decrease of DOPAC concentrations, it also reduced HVA concentrations in a manner that paralleled the decrease of DOPAC. This effect is apparent at a dose of 5–10 $\mu g\ kg^{-1}$, (Table 2) which is similar to the dose of DHE effective on prolactin release when the drug is given intraperitoneally. On the other hand, the effect of a intraventricular injection of DHE lasts for about 5–6 h with a time course that may be comparable with the effects of DHE on prolactin release (Table 1).

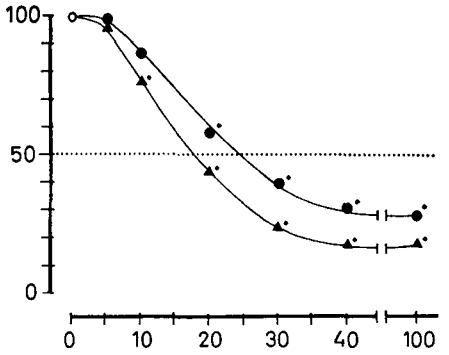

FIG. 1. Effect of dihydroergotoxine (DHE) and bromocriptine on rat serum prolactin concentrations. Animals were treated intraperitoneally with different concentrations of DHE (▲) or bromocriptine (●) and were killed 1 and 2 h after injection respectively. Results are expressed as a percentage of control prolactin concentrations (control values: 18·5 ± 1·7 ng ml^{-1} serum). Values represent the mean of at least three different experiments. $*P<0·01$—The significance has been calculated referring to the absolute value of the controls. Ordinate: % changes (PRL). Abscissa: $\mu g\ kg^{-1}$.

The effect of DHE on prolactin release and on DOPAC and HVA concentrations in striatum may indicate that the drug stimulates the dopaminergic system. Previous behavioural observations suggest that ergot derivatives may have a direct effect on dopaminergic receptors. Clinical data on bromocriptine confirm that it is useful in the treatment of parkinsonism (Calne et al 1974; Parkes et al 1976). More-

Table 1. Time course of striatal DOPAC concentrations and serum prolactin content in rats treated with dihydroergotoxine.

Time (h)	DOPAC (ng mg^{-1} tissue)	Serum prolactin (ng mg^{-1} serum)
—	2·2 ± 0·20	19·1 ± 1·5
0·25	2·0 ± 0·20	15·2 ± 1·1*
0·5	1·8 ± 0·15	10·7 ± 1·3*
1	1·4 ± 0·17*	5·7 ± 0·39*
3	1·3 ± 0·16*	7·6 ± 0·81*
6	1·2 ± 0·15*	11·4 ± 0·90*
12	1·6 ± 0·14*	17·2 ± 1·6
24	1·9 ± 0·23	19·7 ± 1·9

For DOPAC measurements animals were treated intraventricularly with 5 μl of saline containing 10 $\mu g\ kg^{-1}$ of DHE and killed various times thereafter.
For serum prolactin determinations animals were treated i.p. with 40 $\mu g\ kg^{-1}$ of DHE and killed various times thereafter.
Values represent the mean of at least three different experiments.
$*P<0·01$.

Table 2. Effect of intraperitoneal and intraventricular injection of various doses of dihydroergotoxine on 3,4-dihydroxyphenylacetic acid (DOPAC) concentrations in rat striatum.

Intraperitoneal injection		Intraventricular injection	
Dose (mg kg^{-1})	DOPAC (ng mg^{-1} tissue)	Dose ($\mu g\ kg^{-1}$)	DOPAC (ng mg^{-1} tissue)
—	2·2 ± 0·22	—	2·3 ± 0·11
1·25	1·9 ± 0·18	1	1·9 ± 0·10
2·5	1·5 ± 0·21*	5	1·6 ± 0·14*
5	1·3 ± 0·11*	10	1·3 ± 0·15*
10	1·0 ± 0·10*	20	1·3 ± 0·13*

Animals were killed 60 min after drug administration.
Animals treated intraventricularly were treated with 5 μl saline or saline containing DHE.
The values are the mean ± s.e.m. of three different experiments in triplicate.
$*P<0·01$.

over few data are specifically available on the mode of action of DHE.

Our results on prolactin release indicate that the drug is as potent as bromocriptine on this parameter in rats. This effect may be due to various actions of the drug either directly or indirectly on hypothalamic or pituitary dopamine receptors (Moore & Gudelsky 1977; Brown et al 1976; Schmidt & Hill 1977). Moreover the change of DOPAC concentration which possibly reflects a decrease of dopamine release and reuptake by nerve endings (Roth et al 1976) and by dendrites (Korf et al 1976) is a further demonstration that DHE may act through a stimulation of specific dopaminergic receptors.

The different doses of the drug needed to obtain the pharmacological effect after intraperitoneal or intraventricular administration may be explained by the fact that DHE does not cross the blood-brain barrier easily. But when injected directly into the brain DHE exerts a powerful action comparable to that obtained on prolactin release, the regulatory mechanisms of which are supposed to be located outside the barrier (Caron et al 1976). There are different views on how DHE exerts its effect. In particular, Tittler et al (1977) suggest an effect on noradrenergic receptors. This hypothesis has been confirmed by the fact that the IC50 of DHE for dopamine-stimulated adenylate cyclase in the striatum is significantly lower when phentolamine (500 nM) is in the incubation medium (data not shown). On the other hand, preliminary observations in our laboratory suggest a partial agonism of this drug for striatal dopaminergic receptors located on terminals of corticostriatal neurons (Schwarcz et al 1978; McGeer et al 1978).

In conclusion the results indicate that DHE exerts its action in the c.n.s. at least partially through the dopaminergic system.

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