Ergot alkaloids and phosphodiesterase; 'in vitro' activities in several rat brain areas[1]

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Summary. The 'in vitro' activity of ergotamine, dihydroergocristine, dihydroergocornine and dihydroergocryptine on the phosphodiesterase system at low and high K_m in several rat brain areas was examined. These drugs were found to exert an inhibitory effect in all the areas examined with regard to both systems, and particularly on low substrate concentration phosphodiesterases.

It is by now well known that cAMP is involved in the mechanism of synaptic transmission[2,3] in the regulation of the rate of neuromediator synthesis, in energy production and in axon transport[4]. Indications about the functional state of the cAMP system are provided by the study of the enzymes of metabolic synthesis, namely adenyl cyclase and phosphodiesterase. Several investigations carried out on the brain and other organs suggest the existence of 2 different forms of phosphodiesterase, one active at low substrate concentrations and membrane-bound, the other active at high substrate concentrations and present in the soluble fraction[5]. Low K_m-phosphodiesterases operate only under normal conditions, while the high K_m ones are triggered only by a significant increase of cAMP levels, consequent, for example, to catecholaminergic stimulation.

Ergot alkaloids have been demonstrated to inhibit the phosphodiesterases more specifically in the brain than in other organs, thus affecting the control system of cAMP levels.

Materials and methods. ^{3}H-cAMP (Radiochemical Centre, Amersham, U.K.); cAMP (Sigma Chem. Co.); Dowex 50-H^+ (Fluka); ergotamine, dihydroergocristine, dihydroergocryptine, dihydroergocornine (Poli Industria Chimica, Milan).

The phosphodiesterase was obtained from Sprague-Dawley Nos male rats weighing 220–250 g, brain areas being removed according to the Glowinski and Iversen's map[6]. The enzyme was prepared according to the method of Schönhöfer et al.[7], as modified by Pagnini et al.[8]. Protein concentration was measured with the method of Lowry et al.[9]. The phosphodiesterase activity was measured according to Schönhöfer et al.[7]: incubation at 35 °C lasted 30 min; the final volume in the tube was 1 ml, containing Tris-HCl 30 mM, pH 7.4, $MgSO_4$ 2 mM, cAMP 2×10^{-4} M (high K_m) 5×10^{-6} M (low K_m). Drugs were dissolved in 0.2 ml of absolute ethanol plus tartaric acid in the same weight as the drug used, and bidistilled water was added to reach a volume of 10 ml and a concentration of 1×10^{-2} M. The employed concentrations were 1×10^{-4} M, 1×10^{-5} M, 1×10^{-6} M.

Results and discussion. In our experiments we studied the effects of 4 ergot alkaloids, a non-hydrogenated one and 3 hydrogenated ones, on the phosphodiesterase system of the cortex, already investigated by other researchers[10]; in addition, we studied 2 other areas, i.e. the corpus striatum, that is a mainly dopaminergic area, with regard to the effects of ergot alkaloids on the dopaminergic system[11] and the hypothalamus, an area involved in neuroendocrine regulation, since alkaloids affect the secretion of several hypothalamic hormones[12].

All the drugs examined exerted an inhibitory effect on both the 'high' and 'low' phosphodiesterase activities; however, the intensity of such effect varied according to the area and the drug considered. The action of tartaric acid plus ethanol alone (diluents) were tested without any significant effect ($\pm2\%$) on the basal activity.

A comparison of the areas examined showed that inhibition was higher on the hypothalamus, especially as far as the low substrate concentration PDE was concerned; at this level, a higher activity was exerted by the hydrogenated drugs, in particular dihydroergocryptine and dihydroergocristine which also exhibited a strong effect at the concentrations of 1×10^{-5} M and 1×10^{-6} M (figs 1 and 2). The most active drug on the cortex 'high' PDEs was dihydroergocristine, even though its inhibitory effect was markedly reduced at the concentrations of 1×10^{-5} M and 1×10^{-6} M (figs 1 and 2).

In the corpus striatum, the non-hydrogenated compound (ergotamine) was poorly effective on the phosphodiesterase activity, its inhibitory effect, which was fairly good at the highest concentration, dropping to almost baseline values with the 2 lower concentrations.

A marked inhibition can be observed on low substrate concentration PDEs both in the cortex and in the other 2 areas, corpus striatum and hypothalamus; inhibition is particularly evident in this last area, where a high activity is still observed even with the lowest concentration used, i.e. 1×10^{-6} M (see table and fig. 2).

The table also shows that the inhibitory effect of the drugs tested on high substrate concentration PDEs was much less evident, and decreased to only slightly significant values at the concentration of 1×10^{-6} M.

A comparison of the 3 areas examined (cortex, corpus striatum and hypothalamus) points out that the highest inhibition of both 'high' and 'low' PDEs occurred in the hypothalamus; at this level, the 3 hydrogenated drugs proved more active especially towards 'low' PDEs, thus confirming the different activity of hydrogenated and non-hydrogenated compounds, as well as differences among alkaloids of the same series with regard to the inhibition of the phosphodiesterase activity.

In agreement with the observations of other authors on dihydroergotoxine[13] we can state that the 3 alkaloids pre-

IC_{50}-values were determined by logarithmic probits analysis

	IC_{50} (M) Cortex		Striatum		Hypothalamus	
	High PDE	Low PDE	High PDE	Low PDE	High PDE	Low PDE
Ergotamine	$1.7\cdot10^{-4}$	$5.4\cdot10^{-5}$	$1.8\cdot10^{-4}$	$3.2\cdot10^{-4}$	$7.1\cdot10^{-5}$	$5.7\cdot10^{-6}$
Dihydroergocristine	$5.0\cdot10^{-5}$	$1.2\cdot10^{-4}$	$1.7\cdot10^{-4}$	$7.4\cdot10^{-4}$	$5.0\cdot10^{-5}$	$1.4\cdot10^{-6}$
Dihydroergocornine	$1.7\cdot10^{-4}$	$7.3\cdot10^{-5}$	$1.9\cdot10^{-4}$	$4.1\cdot10^{-4}$	$7.6\cdot10^{-5}$	$2.5\cdot10^{-6}$
Dihydroergocryptine	$1.5\cdot10^{-4}$	$1.7\cdot10^{-4}$	$5.9\cdot10^{-5}$	$8.2\cdot10^{-5}$	$3.5\cdot10^{-5}$	$4.0\cdot10^{-7}$

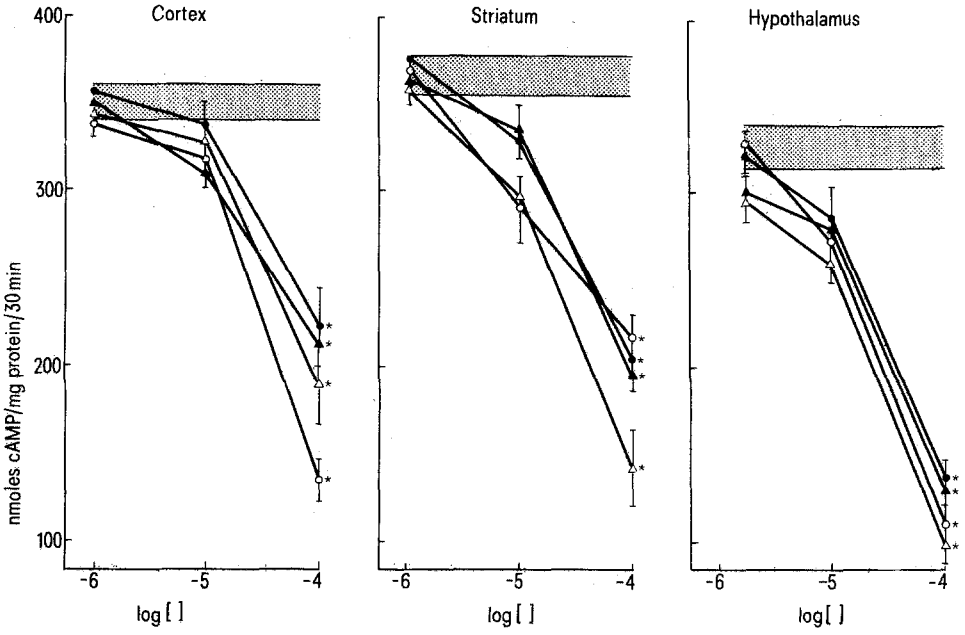

Figure 1. Effects of the 'in vitro' addition of ergotamine (●), dihydroergocristine (○), dihydroergocornine (▲) and dihydroergocryptine (△) on the 'high' phosphodiesterases of some brain areas. Results are the mean of 4 measurements ± SEM. Dotted areas represent basal activity. * $p < 0.005$ in respect to control, by Student's t-test.

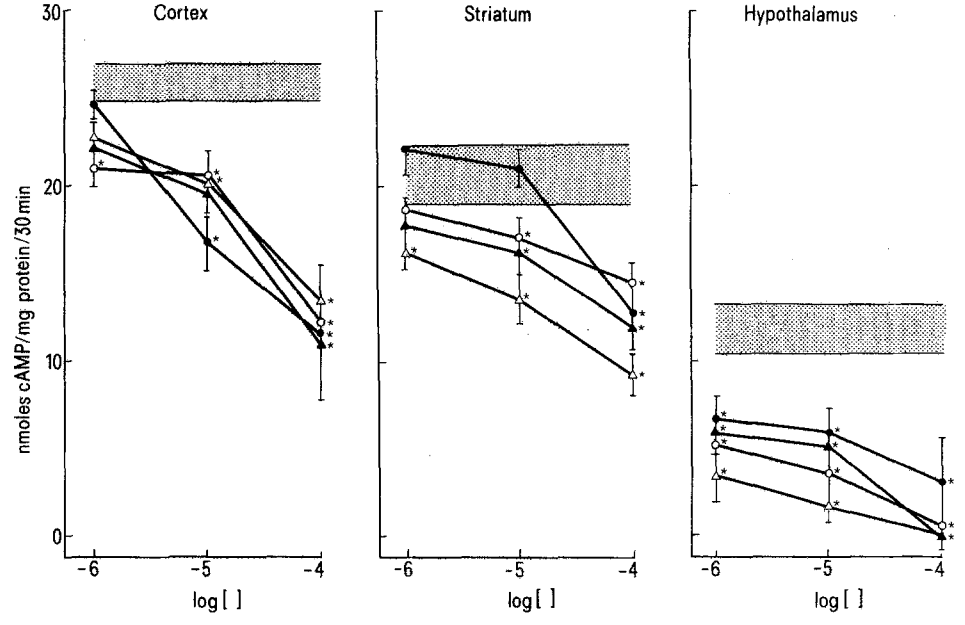

Figure 2. Effects of the 'in vitro' addition of ergotamine (●), dihydroergocristine (○), dihydroergocornine (▲) and dihydroergocryptine (△) on the 'low' phosphodiesterases of some brain areas. Results are the mean of 4 measurements ± SEM. Dotted areas represent basal activity. * $p < 0.005$ in respect to control, by Student's t-test.

sent in it, i.e. dihydroergocristine, dihydroergocornine and dihydroergocryptine, are more active, though to different extents, on the phosphodiesterases that interfere with cAMP at low concentrations, that is on the basal neuronal activity.

Although in our experiments the ergot alkaloids tested were active in the μM range only, it is well documented[14] that the ergot alkaloids are active in 'in vivo' tests in the nM range. This apparent discrepancy could be explained by the multiple sites of action of these drugs. In fact some ergot alkaloids stimulated the adenylate cyclase on 'in vitro' brain slices or after 'in vivo' treatment, but not on 'in vitro' membrane preparations[15].

Such effects on the activity of the phosphodiesterase system might combine with the effects of several neurotransmission systems, since, as is well known, many biogenic amines are capable of controlling hormone secretion by increasing cAMP levels[16].

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Nicotine and ascorbic acid effects on cold-restraint ulcers in rats[1]

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Summary. Rats were orally administered 1-ascorbic acid, nicotine, 1-ascorbic acid and nicotine, or distilled water for 10 days. Following this treatment they were fasted for 24 h and then restrained in a cold environment for 2 h. Nicotine alone produced significantly more gastric ulcers than any other treatment. 1-Ascorbic acid increased ulceration relative to controls. The combined effects of 1-ascorbic acid and nicotine resulted in reduced ulcer incidence and severity. It appears that 1-ascorbic acid and nicotine do not act synergistically to augment stress-induced gastric ulcer.

The effect of nicotine on gastric and secretion and gastric ulcer formation is unclear. Some investigators suggested that nicotine augments gastric secretion[3,4], while others report an inhibitory effect of nicotine on gastric function[5,6]. Pare[7] observed that nicotine augmented the formation of activity-stress ulcers in rats, but not significantly. It has been suggested[8,9] that nicotine 'sensitizes' or predisposes the gut to the ulcerogenic effects of gastric secretion or even to the effects of other substances. A similar sensitizing effect was reported to occur with 1-ascorbic acid administration. It was reported that rats pre-treated with 1-ascorbic acid displayed increased gastric damage produced by restraint-cold[10]. It is of interest to examine the synergistic effects of commonly used combinations of substances. Aspirin and large doses of 1-ascorbic acid have been shown to act synergistically to potentiate ulcer formation[11], however, the combination of nicotine and ascorbic acid has received little attention in the literature. This study focussed on the effects of pre-treatment with nicotine alone, 1-ascorbic acid alone or combined nicotine and 1-ascorbic acid on the subsequent development of restraint-cold-induced gastric lesions in rats.

Methods. 40 male Wistar rats (200± 10 g at the start of the study) were used. Rats were randomly divided into 4 equally sized groups. One group of 10 rats was treated for 10 days with 1-ascorbic acid (Fisher Chemical Co.) in their drinking water (30 g/l; pH = 3.2) available ad libitum. A 2nd group of 10 rats was treated with nicotine (BDH Chemical Ltd) in their drinking water (500 µg%; pH = 9.9) available ad libitum. A 3rd group of 10 rats was treated for 10 days with a combination of 1-ascorbic acid (30 g/l) and nicotine (500 µg%; pH = 4.9) in their drinking water available ad libitum. A 4th group of 10 rats was untreated and given distilled water (pH = 5.4) ad libitum. Liquid intake was recorded daily. Following the 10 day treatment period, all rats were starved for 24 h and then restrained in the supine position for 2 h in a cold (4–6 °C) environment[12]. Previous data have shown that 12–24 h of pre-restraint starvation and 2 h of restraint are optimal durations for producing a reliable degree of gastric ulcers in rats[13]. Following the period of restraint, all rats were sacrificed with chloroform, their stomachs excised and examined for ulcer disease with a dissecting microscope. The location (rumenal or glandular), number, and cumulative length in millimetres of the ulcers were recorded.

Results. The table shows ulcer incidence, frequency and severity (cumulative length of ulcers) for the 4 treatment groups. Nicotine-treated rats developed significantly more frequent ($F(3,36) = 16.02$; $p < 0.01$; Tukey HSD-test) and significantly more severe ($F(3,36) = 4.18$; $p < 0.05$; Tukey HSD-test) gastric ulcers than rats in the other groups. 1-Ascorbic acid treatment increased ulceration relative to that seen in control animals (Tukey HSD-test; $p < 0.05$). Control rats (given only distilled water) exhibited a small number of glandular ulcers in response to the restraint procedure. This is a typical observation. Regardless of other treatment, animals subjected to restraint will exhibit some gastric damage. The combination of nicotine and 1-ascorbic acid resulted in a level of ulceration not significantly different from that seen in control rats. Correlations between amount of solution consumed and ulcer severity were: $r = 0.98$ for 1-ascorbic acid alone; $r = 0.29$ for nicotine alone; and $r = 0.10$ for nicotine and 1-ascorbic acid.

Summary of stomach pathology* for the 4 treatment groups

Group	No. of tested rats	No. of rats with ulcers	Mean No. of ulcers (± SE)	Mean cumulative lenght of ulcers in mm (± SE)
Nicotine	10	10	5.70±0.81	13.80±4.31
1-Ascorbic acid	10	10	2.70±1.30	3.70±1.20
Nicotine+ 1-ascorbic acid	10	10	1.20±0.43	2.00±0.73
Distilled water	10	10	1.57±0.31	2.50±1.31

*Glandular ulcers only.