be attributed to the combined action of this cytotoxic factor and of endotoxin that is released during growth of the bacteria in culture[7]. The lability of this lesion-producing factor is in marked contrast to the stability of endotoxin. Strains of *E. coli* are known which produce heat stable and heat labile enterotoxins and a cytotoxin that destroys Vero cells in vitro[8-10], but there are few toxins known to produce tissue damage in vivo. Although the cytotoxic agent in CCF has yet to be characterized, the present results suggest that the assumption made by previous investigators that intramammary infusions of endotoxin produce a reaction indistinguishable from *E. coli*[3] is not justified.

1 Permanent address: Department of Veterinary Pathology and Public Health, University of Queensland, Brisbane, Australia.
2 Acknowledgments. We are grateful to Mrs K. Wells, Miss H. Meads and Miss A. Ashford for technical assistance.
3 M.J. Paape, W.D. Schultze, C. Desjardins and R.H. Miller, Proc. Soc. exp. Biol. Med. *145*, 553 (1974).
4 A.J. Frost, A.W. Hill and B.E. Brooker, Proc. R. Soc. *209B*, 431 (1981).
5 A.J. Frost, A.W. Hill and B.E. Brooker, unpublished observations.
6 A.W. Hill, A.L. Shears and K.G. Hibbitt, Infect. Immun. *14*, 257 (1976).
7 S.G. Bradley, A. Rev. Microbiol. *33*, 67 (1979).
8 J. Konowalchuk, J.I. Speirs and S. Stavric, Infect. Immun. *18*, 775 (1977).
9 W.G. Wade, B.T. Thom and N. Evans, Lancet 1235 (1979).
10 S.M. Scotland, N.P. Day and B. Rowe, FEMS Microbiol. Lett. *7*, 15 (1980).

Effects of dihydroergocriptine on mouse and rat resistance to acute anoxia: influence of repetition of treatment

J.M. Legeai, M.M. Benharkate, J.-P. Duverger and R.G. Boulu

Centre de Recherches des Laboratoires Jacques Logeais, rue Denis Papin, F-78190 Z.I. de Trappes-Elancourt (France), and U.E.R. Mécanismes d'Action des Médicaments et des Toxiques, Université René-Descartes, F-75006 Paris (France), 19 June 1980

Summary. The antihypoxic activity of DHEC mesylate, as evaluated in mice and rats submitted to hypobaric hypoxia and to asphyxic anoxia respectively, is noticeably stronger when the compound is administered according to a repeated treatment schedule than when administered only once. The results correlate well with human clinical observations.

For many years, the dihydrogenated alkaloids of rye ergot which belong to the ergotoxine group have been used to treat cerebral diseases caused by senescence or due to vascular insufficiency. In humans, their therapeutic efficacy has been somewhat difficult to assess under some circumstances, but in animals their activity in cerebral areas has been established using objective methods[1,2]. However, the influence of repetition of the administration on the pharmacological effect of the ergot alkaloids does not seem to have been given much attention. In humans, it has been established that several weeks are necessary before the therapeutic effect is apparent in geriatric patients[3,4] and the duration chosen for clinical studies is generally as long as 12 weeks[5]. As the dihydrogenated ergot alkaloids of the ergotoxine group are eliminated rather slowly (plasmatic $t_{1/2}\beta$ of 13 h for dihydroergotoxine, or 17 h for dihydroergocornine[6]) and as their digestive absorption is very poor[7], it seems clear that it would take several days to obtain the effective plasmatic (or tissular) concentration and to observe the therapeutic activity. We have studied the pharmacological effects of dihydroergocriptine (DHEC) after single and repeated administration for 5 days, on 2 simple experimental models of acute anoxia. Previous studies have suggested that among the 3 alkaloids constituting dihydroergotoxine (dihydroergocristine, dihydroergocornine, dihydroergocriptine) DHEC is the most active in counteracting the cerebral effects of acute anoxia in animals[1].

Material and methods. Hypobaric hypoxia in mice. These experiments were carried out on SPF male mice of the Swiss strain, weighing between 20 and 22 g (purchased by Iffa-Credo, Les Oncins, France). The methodology used was very close to the one described by Etesse-Carsenti[8]. Each experiment included 3 groups of mice (1 control group + 2 treated groups). The survival time (last visible respiratory or limb movement) of mice submitted to the hypobaric hypoxia (600 mm of mercury barometric depression reached in 100-110 sec) was studied on 3 mice simultaneously, each of them belonging to one of the 3 groups under experimentation. Treatments, dissolved in distilled water, were administered by the oral route (20 ml/kg b.wt). The dose of DHEC mesylate, administered once, that showed the optimum activity under these conditions was first determined (10 animals per group), using a large range of doses (3-3000 µg/kg) and retained for the next experiments. With the aim of studying the influence of the repetition of the treatment, DHEC mesylate has been administered (20 mice per group) either once daily for 5 consecutive days (0.3 mg/kg) or only on the 5th day (only the vehicle being administered on the 4 previous days); control animals were given 5 daily treatments of distilled water. 60 min after the last treatment, the animals were submitted to the hypobaric hypoxia test, using a set of 3 mice (see above). As variation of the body temperature exerts a strong influence on resistance to hypoxia, DHEC effects on this parameter were checked by measuring the rectal temperature before treatment and immediately before the test. Statistical comparisons on survival time and body temperature were established by the Wilcoxon's matched-pair test.

Asphyxic anoxia in rats: cerebral electric activity recording. These experiments have been carried out on SPF male rats of the Sprague-Dawley strain (purchased by Charles River, Saint-Aubin-lès-Elbeuf, France) weighing 300-400 g. 2 cortical electrodes (1 frontal, 1 parietal) made of silver wire were implanted through a hole in the skull of each animal, under diethylether anaesthesia, 3 days before testing. Drugs, dissolved in saline, were injected by the i.p. route (10 ml/kg b.wt, 16 rats per group). DHEC mesylate was injected either once daily for 5 consecutive days (0.3 mg/kg) or only on the 5th day, only the vehicle being administered on the 4 previous days; control rats were given 5 daily treatments of distilled water. The rats were submitted to the asphyxic anoxia 30 min after the last treatment. Under diethylether anaesthesia, they were tracheotomized, i.v. injected with gallamine triioethylate (20 mg/kg b.wt) and artificially ventilated (80 ml/min, 60 strokes/min). The

electrocorticogram (ECoG) was registered on a E&M physiograph recorder. Asphyxic anoxia was obtained by interrupting the artificial ventilation for 105 sec. The following parameters were measured: 1. The delay of ECoG disappearance (DD) following the beginning of the asphyxic anoxia, 2. the delay of reappearance (DR) of a cerebral electric activity following the ending of the asphyxic anoxia, 3. the time duration of isoelectric ECoG (TDi). Statistical comparisons were established by the Mann and Whitney's test.

Results. Hypobaric hypoxia in mice. When administered p.o. only once, DHEC mesylate, starting from the low dose of 30 µg/kg, significantly prolongs the survival time of mice submitted to an hypobaric hypoxia, with the maximum effect being obtained at 300 µg/kg (figure). Under no circumstances did the dosages significantly affect the body temperature (variation observed: from - 0.25 to +0.5 °C; no relation to the dose administered with non statistical significance). In mice receiving DHEC mesylate daily for 5 days at the dose of 0.3 mg/kg, the survival time was markedly more prolonged than in animals that received only 1 administration (see table 1).

Asphyxic anoxia in rats. When animals were given a single administration of DHEC mesylate, the measured parameters DD, DR and TDi were not statistically different from those observed in the animals of the control group (table 2). On the contrary, after 5 daily administrations of DHEC mesylate, the delay of ECoG disappearance was significantly longer ($p < 0.01$) and the delay of ECoG reappearance significantly shorter ($p < 0.01$) in treated animals than in the control group. Similarly, duration of isoelectric ECoG was significantly shorter ($p < 0.01$) after repeated administration of DHEC mesylate, in comparison to the control group (table 2). The statistical comparison established between the results obtained in animals having received a single administration of DHEC mesylate and those submitted to repeated treatment shows that a significant difference exists, whichever of the measured parameters (DD, DR or TDi) is examined ($p < 0.05$).

Discussion. When administered p.o. only once, DHEC mesylate, starting from the low dose of 30 µg/kg, increased the survival time of mice submitted to a hypobaric hypoxia. The maximum effect was obtained at the dose of 300 µg/kg, the antihypoxic activity observed decreasing thereafter at the upper dose levels tested. Boismare and Micheli[1] previously described the same lessening of the antihypoxic activity of ergot alkaloids at high dose levels. After repeated daily administration of 0.3 mg/kg, the antihypoxic activity of DHEC mesylate was considerably and significantly increased in mice. The repetition of treatment similarly increased the antihypoxic activity of DHEC mesylate in rats, since cerebral electric activity is more resistant to anoxia in animals that received repeated treatment. These results show that the pharmacological evaluation of antihypoxic agents, and particularly of ergot alkaloids, should be performed after a single and also after repeated dosage regimen, in order to take into account the effect obtained when blood and tissue concentrations come close to a steady-state equilibrium. In the case of the dihydrogenated ergot alkaloids of the ergotoxine group, the plasma levels obtained after a single administration are very low and decrease rapidly during the distribution phase ($t\,{}^{1}/_{2}\,a$ in man equals 90-150 min[6]), due to a very active hepatic, renal, splenic and muscular removal. So, it can be understood why DHEC mesylate presented just a weak anti-hypoxic activity in rats after a single treatment. Our results suggest that treatment repetition increases the cerebral tissular concentration of DHEC mesylate and consequently its pharmacological activity; they are in good agreement with the human therapeutic observations.

Table 1. Mouse survival time to hypoxic hypoxia after single or repeated (5 days) administration of DHEC mesylate

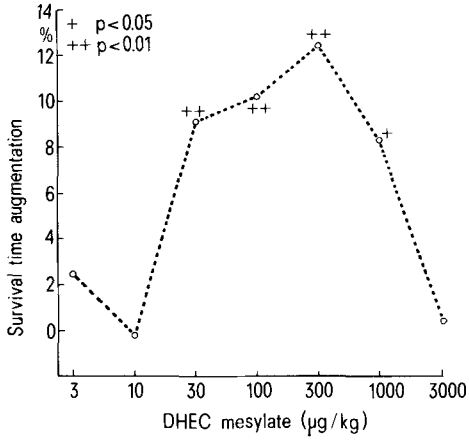
Treatment	Distilled water	DHEC mesylate (0.3 mg/kg)	DHEC mesylate (5×0.3 mg/kg)
Mean survival time (sec)	108	120[a]	134[a.b]

Wilcoxon's test, [a] comparison with controls: $p < 0.01$, [b] comparison with single administration group: $p < 0.01$.

Table 2. Rat cerebral resistance to asphyxic anoxia, after single or repeated (5 days) administration of DHEC mesylate

Treatment		Distilled water	DHEC mesylate (0.3 mg/kg)	DHEC mesylate (5×0.3 mg/kg)
Delay of ECoG disappearance (DD)	A	70	76	84
	B		+ 9%	+ 20%[b]
	C			+ 11%[a]
Delay of ECoG reappearance (DR)	A	16.5	14	7.5
	B		− 15%	− 55%[b]
	C			− 46%[a]
Time duration of isoelectric ECoG (TD_i)	A	51	43	28
	B		− 16%	− 45%[b]
	C			− 35%[a]

A, mean values (sec); B, percentage deviation from controls; C, percentage deviation between treated groups. Mann-Whitney's U-test, [a] $p < 0.05$, [b] $p < 0.01$.



Augmentation of survival time to hypobaric hypoxia by DHEC mesylate in mice, after single administration.

1 F. Boismare and L. Micheli, J. Pharmac., Paris *5*, 221 (1974).
2 W. Meier-Ruge, H. Emmenegger, A. Enz, P. Gygax, P. Iwangoff and N. Wiernsperger, Pharmacology *16*, suppl. 1, 45 (1978).
3 C. Giove and N. Silva, Xth Congr. int. Neurology, Barcelona 1973.
4 J. Bargheon, Nouv. Presse méd. *2*, 2053 (1973).
5 R. Venn, in: Ergot alkaloids and related compounds, p. 533. Ed. B. Berde and H. Schild. Springer, Berlin 1978.
6 W. Aellig and E. Nüesch, Int. J. clin. Pharmac. *15*, 106 (1977).
7 P. Loddo, P. Spano and M. Trabucchi, Boll. Chim. Farm. *115*, 570 (1976).
8 H. Etesse-Carsenti, Thesis D. Pharm., Paris 1976.
9 H. Eckert, J.R. Kiechel, J. Rosenthaler, R. Schmidt and E. Schreier, in: Ergot alkaloids and related compounds, p. 719. Ed. B. Berde and H. Schild. Springer, Berlin 1978.