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METABOLISM OF LERGOTRILE TO 13-HYDROXY LERGOTRILE, A POTENT INHIBITOR OF PROLACTIN RELEASE IN VITRO

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Lergotrile mesylate (2-chloro-6-methylergoline-β-acetonitrile, methane sulfonate salt) is a dopamine agonist, and a potent inhibitor of prolactin secretion in vivo and in vitro (1). Lergotrile, whose structure is similar to that of lysergic acid, is metabolized in an analogous manner, i.e. N- demethylation and aromatic hydroxylation (2). A recent report by Siddik et al. (3) stated that lysergic acid diethylamide (LSD) is not hydroxylated in the 12 position as previously suggested (4), but showed that, although the mass spectrum of one of the hydroxy LSD metabolites was identical to 12-hydroxy LSD, the metabolite had different chromatographic characteristics. The two hydroxylated phenolic metabolites have been tentatively assigned the structures of 13-hydroxy and 14-hydroxy LSD.

The data presented in this communication show that in the case of lergotrile the hydroxylated metabolite is 13-hydroxy lergotrile (Fig. 1), which is several times more active than is lergotrile in inhibiting release in vitro.

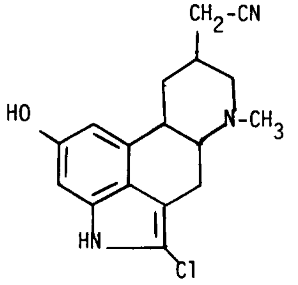

Fig. 1. 13-Hydroxy lergotrile

In order to isolate sufficient quantities of the metabolite for identification purposes and in vitro activity studies, the following in vitro incubation procedures were used. Guinea pigs were dosed with phenobarbital for 4 days prior to preparing a 20% 9000 x g liver supernatant using ice-cold 1.15% KCl and standard differential centrifugation methods. The incubation mixture used has been described previously, but was expanded from

5 ml to 200 ml and the incubation time was 2 hr instead of 30 min (5). The metabolite was isolated from the incubation mixture by the following procedure:

To 200 ml of the enzyme system was added 100 ml of XAD-2 which had been prewashed with MeOH and water and the resulting mixture was swirled gently for 10 min and poured into a glass chromatography column. The column was eluted consecutively with 200 ml of water, 200 ml of 10% MeOH, and 400 ml of 100% MeOH. The metabolite eluted from the column in the first 150-200 ml of the 100% MeOH wash. This fraction was dried down to 7 ml and thin-layered on ITLC-SAF Gelman plates using $CHCl_3$-MeOH- acetone-NH_4OH (63:7:27:2) as the developing solvent. The metabolite was located on the plates by spraying with Van Urk reagent. The metabolite gives a characteristic aqua blue color response with this reagent (Table 1).

TABLE 1. Rf VALUES OF LERGOTRILE AND HYDROXYLATED LERGOTRILE ANALOGUES

		Color Response Spray Reagent		Mass ion+
Compound	R_f*	Van Urk	Nitroaniline	m/e+
Lergotrile	0.79	Gray Green	Lt Yellow	299
12-OH-lergotrile	0.57	Lavender	Magenta	315
13-OH-lergotrile	0.53	Aqua	Purple	315

*The compounds were chromatographed on Merck Silica Gel 60 F-254 plates using $CHCl_3$-MeOH-acetone-NH_4OH (63:7:27:2).

+The mass ion was also the major ion.

After elution with MeOH, the metabolite was further purified by use of high pressure liquid chromatography using a Waters ALC 202/401 high pressure liquid chromatograph containing a 6 ft column packed with Porasil T. The solvent system ($CHCl_3$-MeOH-NH_4OH, 63:7:0.3) was pumped at a flow rate of 0.5 ml/min and the metabolite eluted in the 9-10 ml fraction. The thin-layer chromatography Rf values of lergotrile and hydroxylated analogues of lergotrile are given in Table 1.

The correct structure of the metabolite was ascertained by the following methods:

(1) The mass spectrum of the metabolite was obtained by direct probe mass spectrometry. It has a mass ion of m/e 315, which is 16 more than that of lergotrile.

(2) The mass spectrum of the acetylated metabolite obtained by gas chromatography-mass spectrometry analysis was identical to the mass spectrum of chemically synthesized 13-acetoxy lergotrile having as the major ion the mass ion of m/e 357.

(3) The products obtained when the metabolite was methylated with diazomethane had mass ions of m/e 329 and 343 which correspond to those of 13-methoxy and 13-methoxy-1-N-methyl lergotrile.

(4) The metabolite gave a red phenol response when treated with diazotized nitroaniline.

(5) Nuclear magnetic resonance (NMR) analysis on 1 mg of material showed chemical shifts of 6.45 ppm (S, 1, aromatic) and 6.57 ppm (S, 1, aromatic) which is consistent with the assignment of the OH group in the 13 position. If the OH group were in the 12 or 14 position, the aromatic hydrogens would be ortho to each other and one would observe, instead of two singlets, a pair of doublets due to the strong spin-spin coupling between the adjacent protons. NMR spectrums of authentic samples of 13-acetoxy-2-chloro-6-methyl-8-chloromethylergoline and 12-acetoxy lergotrile confirm the assignment of the OH group in the 13 position.

The effect of 13-hydroxy lergotrile on the release of prolactin *in vitro* was determined using a paired pituitary half incubation system and a double antibody radioimmunoassay as described by Clemens *et al.* (1). In this assay the anterior pituitary is hemisected and half the anterior pituitary is used to test the effect of drug on prolactin release. The other half of the same pituitary is used as the control. The results of these experiments are expressed as the mean difference in prolactin release between the control half of pituitary and the drug treated half of the same pituitary and as percent inhibition of prolactin release (Table 2). Thus each pituitary acts as its own control canceling out variations in prolactin release in pituitaries from different rats as well as daily variation in prolactin release from the same pituitary. The results of these experiments, which are summarized in Table 2, show that 13-hydroxy lergotrile is approximately 100 times more active than is lergotrile in inhibiting the release of prolactin *in vitro* from the anterior pituitary. Lergotrile at a concentration of 1.0×10^{-7} M affected a 55 percent inhibition of prolactin release whereas only 1.1×10^{-9} M 13-hydroxy lergotrile was needed to affect essentially the same degree of inhibition, i.e. 56 percent.

Whether or not 13-hydroxy lergotrile is active *in vivo* is not yet known. This metabolite is excreted in guinea pig and rat bile as a glucuronide conjugate. The guinea pig excretes 51 percent of a dose of lergotrile in the bile in 24 hr and rats excrete 22-30 percent in 24 hr. In guinea pigs, approximately 11 percent of the dose is eliminated in the bile as the glucuronide conjugate of 13-hydroxy lergotrile. 13-Hydroxy lergotrile has been found to be a major metabolite in humans*.

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*Rubin, A., Personal Communications.

TABLE 2. EFFECTS OF 13-OH LERGOTRILE (13-OH L) AND LERGOTRILE (L) ON ANTERIOR PITUITARY PROLACTIN RELEASE *IN VITRO*

Treatment*	Conc. of Drug (M)	Mean Difference In Prolactin Release Treatment vs Control	Inhibition
Saline		0.13 ± 0.29†	
13-OH L	1.1×10^{-6}	1.04 ± 0.14‡	78
13-OH L	1.1×10^{-7}	0.60 ± 0.04‡	73
13-OH L	1.1×10^{-8}	0.75 ± 0.13‡	70
13-OH L	1.1×10^{-9}	0.76 ± 0.19§	55
L	1.0×10^{-7}	4.09 ± 0.05§	56
L	2×10^{-8}	0.20 ± 0.05§	40
L	2×10^{-9}	1.72 ± 0.06§	25

*The control was Medium 199 plus saline. The treatment consisted of drug in Medium 199.

†Values are expressed as μg/mg tissue ± S.E. (6 paired pituitary halves/treatment).

§P <0.01.

‡P <0.001.

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