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STUDIES ON THE METABOLISM OF ERGOLINE DERIVATIVES

METABOLISM OF NICERGOLINE* IN MAN AND IN ANIMALS

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Abstract—Tritium labelled nicergoline is rapidly absorbed from the gut in rat, dog, monkey and man. In the rat radioactivity was present in all tissues examined from 30 min to 12 hr after the administration. In all species tested labelled nicergoline was extensively metabolized in the body and the final products, labelled 1,6-dimethyl-8β-hydroxymethyl-10α-methoxyergoline and 8β-hydroxymethyl-10α-methoxy-6-methylergoline, as well as their glucuronides were excreted mainly by the kidneys. Therefore chemical transformation of the drug in the animal body takes place by three main reactions; hydrolysis of the ester linkage, *N*-demethylation at position 1 and conjugation with glucuronic acid. The last reaction appears to be of minor importance, if any, in man.

NICERGOLINE, 8β-(5-bromonicotinoylhydroxymethyl)-1,6-dimethyl-10α-methoxyergoline (I), shows vasodilating and α-receptor blocking activity.[1] In the perfused brain *in situ*, the drug causes a decrease in cerebral vascular resistance which is associated with an increase in oxygen consumption and an activation of drug metabolizing ability of the tissue.[2] Nicergoline has been proved clinically useful as peripheral vasodilating agent.[3] The present investigation was undertaken in order to determine the metabolism of nicergoline in different animal species and in man.

In the field of the chemically related lysergic acid derivatives it has been shown that ergometrine and lysergic acid diethylamide undergo an aromatic hydroxylation probably at C-12 followed by glucuronide formation. The former shows also a conjugation at the primary alcoholic group when administered at high doses.[4] On the other hand methergine has been found to be the main metabolite of methysergide in man and rabbit.[5]

MATERIALS AND METHODS

Labelled compounds

Randomly tritium-labelled nicergoline ([G-^{3}H]nicergoline, spec. act. 2·15 μc/mg), [17-^{3}H]nicergoline (spec. act. 181·8 μc/mg and 82·9 μc/mg), and [^{14}C,^{3}H]nicergoline (prepared by acylating [17-^{3}H]1,6-dimethyl-8β-hydroxymethyl-10α-methoxyergoline with 5-bromo-[carboxyl-^{14}C]nicotinic acid chloride in pyridine, spec. act. ^{14}C, 49·4 μc/mg and ^{3}H, 54·4 μc/mg) were obtained by Dr. G. Vicario, Istituto Ricerche "G. Donegani", Laboratorio Molecole Marcate, Novara, Italy. Radiochemical purity of

* The trademark of Farmitalia for nicergoline is Sermion.

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the labelled products was found to be greater than 98 per cent by thin layer chromatography. When necessary, the solutions of labelled nicergoline to be used in experiments were appropriately diluted with the non radioactive compound.

Chromatographic analysis

Paper chromatography was carried out on Whatman No. 1 paper using the descending technique with solvent system A, which consisted of the upper phase of the solvent mixture *n*-butanol–acetic acid–water (4:1:5) and solvent system B, upper phase of *n*-butanol–pyridine–water (4:1:5). The dried strips were cut into sections 1 cm in length which were eluted directly in the counting vials with methanol and counted by the liquid scintillation method. For the detection of unlabelled compounds the chromatograms were sprayed with a solution of *p*-dimethylaminobenzaldehyde (2%) and hydrogen chloride (2·5 N) in 80% aqueous ethanol.

Thin-layer chromatography (TLC) was carried out with Silica gel G (Merck, Darmstadt) plates and solvent system C, ethylacetate–dimethylformamide–*n*-butanol–water (4:3:3:1). The quantitative estimation of labelled nicergoline and/or its metabolites involved scraping of chromatogram in sections of the same length, extracting each section with 0·5 ml of methanol in a counting vial and adding 10 ml of the scintillation mixture to the extract.

Animal experiments

Male rats (Sprague–Dawley, 150–250 g), female dogs (Beagle, 9–14 kg), male and female monkeys (*Macaca mulatta*, 2·4–3·0 kg) were used. The drug was administered orally as an aqueous solution of the acid tartrate. The animals were housed in glass and stainless steel metabolic cages and kept on the usual laboratory diet. Rat tissues were homogenized in cold water and extracted twice with hot acetone. Stomach and intestine were voided from the content and washed thoroughly before homogenization. Portions of the combined extracts were evaporated in counting vials, treated with 1 ml of 50% aqueous methanol and counted after the addition of 10 ml of the scintillation mixture. The acetone insoluble materials were assayed for tritium after combustion with hydrogen peroxide and perchloric acid on 20 mg samples.[6] The exhausted solids appeared to contain less than 2 per cent of the radioactivity found in the corresponding extracts. Faeces were treated similarly, and a substantial amount of tritium was also found in the exhausted solids. Urines were diluted with water and counted directly.

Human experiments

[17-^{3}H]nicergoline as the tartaric acid salt was administered to four human subjects. Dosage and description are reported in Table 1. Urines and faeces were collected separately and treated as above. Blood samples were collected in heparinized tubes.

Hydrolysis of metabolites with β-glucuronidase

Was carried out in pH 6·8, 0·1 M phosphate buffer, with Sigma bacterial β-glucuronidase (activity 40·000 U/g). After incubation the solution was evaporated and the residue was dissolved in methanol for analysis.

Measurement of radioactivity

A Packard Tri-Carb liquid scintillation spectrometer Model 3375 and counting

TABLE 1. DESCRIPTION AND DOSING OF HUMAN SUBJECTS

Subject	Sex	Age (yr)	Weight (kg)	Administration route	[17-^{3}H]nicergoline administered	
					mg	μc/mg
M. R.	f	20	62	i.v.	1·16	82·9
F. C.	f	18	49	i.v.	1·10	82·9
G. B.	m	46	60	oral	4·16	11·8
E. A.	m	42	68	oral	5·00	29·5

medium of 100 g naphthalene, 7 g PPO, and 0·3 g POPOP/l. of dioxane were used. The counting efficiency of the samples was determined by the channel ratio method for quenching correction.

Mass spectra

A Perkin Elmer 270 mass spectrometer was used. The sample was introduced directly in the ion source at 190°.

Stability of nicergoline in aqueous solutions and in blood

Stability of nicergoline in rat gastric juice (pH 1·2) in pH 7·3 and 8·0 M/15 phosphate buffer was measured by evaluating the per cent of [17-^{3}H]nicergoline which remained unchanged during storage at 37° of 1 mg/ml solutions.

In order to determine the stability of nicergoline in the blood *in vitro*, 100 μg of [17-^{3}H]nicergoline as the acid tartrate in 0·5 ml of water were added to 9·5 ml of a blood sample collected from a healthy subject. The sample was incubated at 37° and at different intervals a 2·5 ml portion was analysed by reverse isotopic dilution for the presence of unmodified [17-^{3}H]nicergoline. After addition of a known amount of unlabelled nicergoline, each portion was diluted ten-fold with distilled water then extracted at pH 9 thoroughly with chloroform. Nicergoline was isolated from the extract by chromatography on Silica gel column and specific radioactivity of the product was measured.

RESULTS

Tissue distribution and blood levels of radioactivity after the administration of [^{3}H]nicergoline

In the rat about 60 per cent of an oral dose of 20 mg/kg randomly labelled [^{3}H]nicergoline was excreted in the urine in 12 hr and 18 per cent in the faeces (Table 2). High levels of tritium were present in the organs involved in the absorption, such as stomach and intestine, and in the excretion, such as liver and kidney. With the possible exception of testis, skin, hair and fat, which show reasonably low levels of radioactivity, the concentrations of the labelled drug in the tissues, other than stomach and intestine, decreased about ten-fold in the time interval from 30 min to 12 hr after the administration. In another similar experiment in which rats were treated *per os* with 2 mg/kg of [G-^{3}H]nicergoline, tissue concentrations were found to be about one-tenth of those given for the same time values in Table 2.

TABLE 2. TISSUE DISTRIBUTION AND EXCRETION OF [G-^{3}H]NICERGOLINE IN MALE RATS*

Tissue	^{3}H as nicergoline (μg/g)				
	30 min	90 min	3 hr	6 hr	12 hr
Blood†	4·3	1·9	1·8	1·1	0·2
Liver	67·3	34·7	34·6	17·6	6·1
Lungs	22·3	11·8	10·3	8·8	4·4
Spleen	10·9	5·3	3·9	3·2	0·7
Kidney	20·8	16·8	11·6	5·8	3·0
Heart	10·1	7·2	5·1	3·5	0·3
Testis	1·5	1·8	2·0	3·1	2·3
Bone (femur)	5·1	2·4	1·7	0·9	0·5
Brain	0·7	0·5	0·4	0·3	0·1
Stomach	223·2	195·0	55·9	20·6	2·0
Intestine (upper segment)	36·8	39·3	46·8	21·7	3·5
Intestine (lower segment)	11·2	5·5	10·6	8·9	12·6
Skin and hair	5·7	5·0	5·1	3·6	2·9
Fat (epididymal)	1·3			1·0	0·6
Carcass	5·5	3·9	2·8	2·2	0·6
Urine (% of dose)	1·9	1·0	9·0	32·0	58·0
Faeces (% of dose)			3·1	4·6	18·6

* The dose of nicergoline was 20 mg/kg, p.o. The data given are the average of determinations obtained on the pooled tissues and excreta of two groups of three rats per group.
† For blood ml instead of *g* are considered.

Blood levels of radioactivity in rat, dog and man after the administration of [17-^{3}H]nicergoline are presented in Fig. 1. Human subjects showed a peak in the range from 1 to 1·5 hr after the administration. The half-life of blood radioactivity could be calculated for subject E.A. and found to be 1 hr 16 min. The peak concentration appeared to be lower in dog than in man, although the dose in the dog was three times greater than in man. The half-life values of blood radioactivity in dog (mean of the three animals) was found to be 8 hr 25 min.

Urinary and faecal excretion of tritium after the oral administration of [^{3}H]nicergoline

The cumulative excretion of radioactivity in experimental animals and in two human subjects after the oral administration of [17-^{3}H]nicergoline is shown in Fig. 2. In the rat, dog, monkey and in man, urinary excretion is the main elimination route of radioactivity after [^{3}H]nicergoline administration.

Urinary metabolites of nicergoline in experimental animals and in man

In the rat experiment recorded in Table 2, paper radiochromatographic analysis in solvent system A of the urines showed that the unconverted drug accounted for less than 1 % of urinary tritium, 99 per cent of radioactivity being distributed in two distinct zones of the chromatograms, corresponding to compounds with increased polarity in respect to nicergoline. Zone I, covering the R_f range (0·65–0·85), represented about 90 % of total urinary tritium, while the remaining appeared in zone II which covered the R_f range (0·10–0·45).

When [^{14}C,^{3}H]nicergoline was administered orally in a dose of 2 mg/kg to the rat, ^{14}C radioactivity was rapidly excreted in the urines, the amount of ^{14}C eliminated

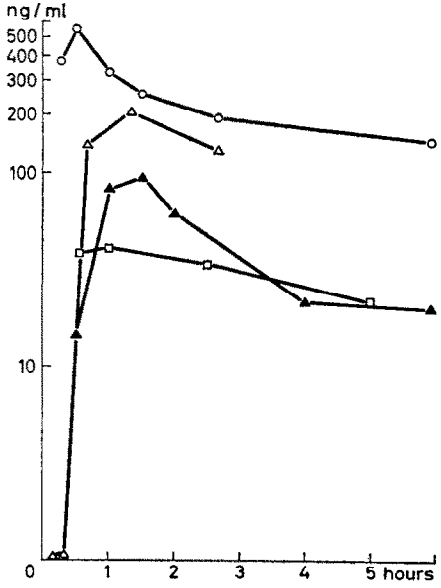


FIG. 1. Concentration of total radioactivity in the blood of rats (○); dogs (□); human subjects G. B. (△) and E. A. (▲), after the oral administration of [17-^{3}H]nicergoline as the acid tartrate. The doses were 2 mg/kg body wt. in rats and 0·2 mg/kg in dogs. Dosage of human subjects is reported in Table 1. Data for rats were the averages of determinations of four individual animals per point. Data for dogs were averages of three animals.

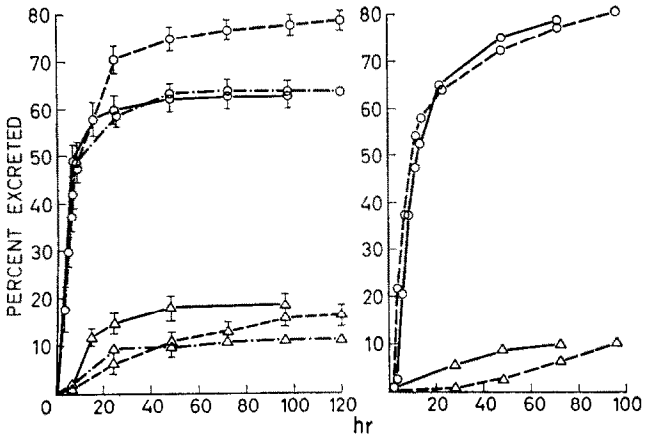


FIG. 2. Urinary (○) and faecal (△) cumulative excretion of radioactivity after the oral administration of [^{3}H]nicergoline to rats (——), dogs (---), a female monkey (·–·–·) (left) and to two human subjects G. B. (——) and E. A. (---) (right). The dose was 20 mg/kg body wt. in rats, 0·2 mg/kg in dogs and 1 mg/kg in the monkey. Dosage and description of human subjects are reported in Table 1. For rats and dogs the data are the averages of determinations on respectively four and three animals per point with standard errors (vertical bars).

being 86 per cent at 15 hr and 97 per cent at 38 hr after the administration. Radiochromatographic analysis of the urine samples is shown in Fig. 3 from which it can be deduced that [^{14}C,^{3}H]nicergoline is completely hydrolysed *in vivo* owing to the observed separation of ^{3}H and ^{14}C in the urinary metabolites.

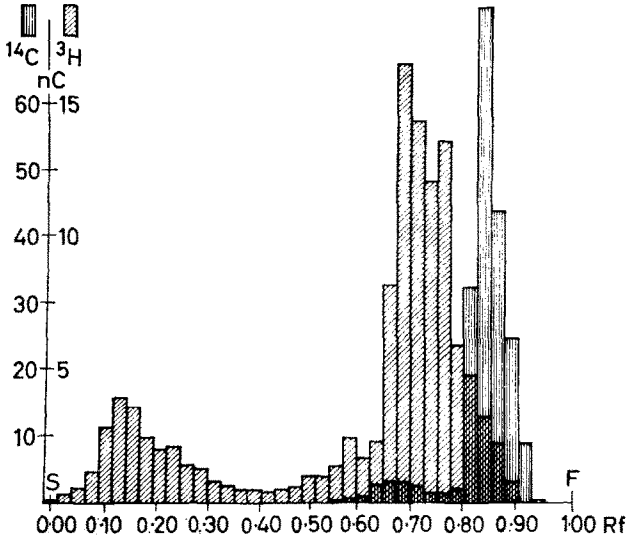


FIG. 3. Chromatograp hic pattern of rat urine in system A after administration of [^{14}C, ^{3}H]nicergoline. Radioactivities of ^{14}C and ^{3}H were counted separately on the same chromatographic strip.

Tritium labelled metabolites could be divided in two fractions each corresponding to a radioactive zone in the chromatogram (zones I and II, see above). In order to identify chemically these metabolites, two rats each weighing 270 g were treated orally with [17-^{3}H]nicergoline in a dose 100 mg/kg. Radiochromatography of the pooled day 1 urines of the two animals, containing an amount of ^{3}H corresponding to 31·5 mg as [17-^{3}H]nicergoline, indicated the presence of nicergoline, (10% of total tritium of the sample) zone I metabolites, (82%) and zone II metabolites (8%). Chloroform extraction at pH 8 of the above mentioned urines allowed the recovery of [17-^{3}H]nicergoline and zone I metabolites. The crude material (31·7 mg) was charged on a Silica gel column and eluted with chloroform in order to remove unconverted [17-^{3}H]nicergoline. Subsequent elution of the column with chloroform containing increasing amounts of ethanol enabled the separation of two main fractions. The first fraction was eluted with 20% ethanol and the amount recovered accounted for 40% of total chloroform extractable tritium. Paper chromatography and TLC behaviour of this fraction was identical to that of 1,6-dimethyl-8β-hydroxy-methyl-10α-methoxy ergoline (II)[7] and the ultraviolet spectrum in methanol indicated a 1-methylergoline derivative. The identity of the metabolite was definitely established by comparison of its mass spectrum (Fig. 4) with that of an authentic sample of 1,6-dimethyl-8β-hydroxymethyl-10α-methoxyergoline.

The second fraction was eluted with 40% ethanol and the amount recovered was 34% of total chloroform extractable tritium. The chromatographic behaviour of this

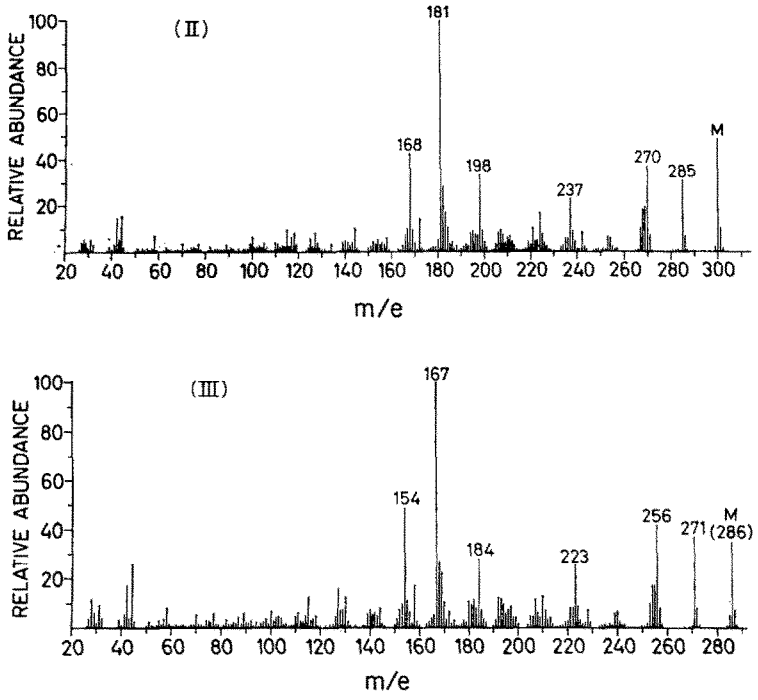
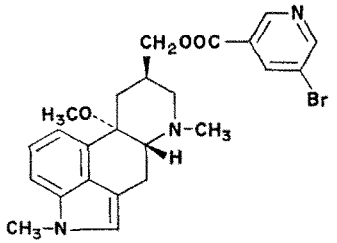


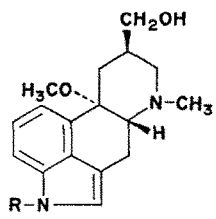
FIG. 4. Mass spectra of nicergoline metabolites.

fraction was identical to that of 8β-hydroxymethyl-10α-methoxy-6-methylergoline (III)[7] and the ultraviolet spectrum indicated the presence of an ergoline derivative. The chemical structure of this metabolite was established on the basis of the identity of its mass spectrum (Fig. 4) with that of (III).

The chloroform-extracted urines contained an amount of radioactivity corresponding to 7·8 mg of [17-^{3}H]nicergoline, essentially due to zone II metabolites. This solution was treated with β-glucuronidase. Radiochromatographic analysis after this treatment showed that more than 90% of tritium now appeared as zone I metabolites. This result indicates that zone II metabolites are glucuronic acid conjugates of [^{3}H]-1,6-dimethyl-8β-hydroxymethyl-10α-methoxyergoline and [^{3}H]8β-hydroxymethyl-10α-methoxy-6-methylergoline.



(I)



(II), R = CH_3
(III), R = H

The excretion of ^{14}C as observed above on administering [^{14}C,^{3}H]nicergoline was compared with that of 5-bromo-[carboxyl-^{14}C]nicotinic acid. To this purpose 0·10 mg (11 μc) of 5-bromo-[carboxyl-^{14}C]nicotinic acid was administered orally in water solution to a rat weighing 200 g. At 16 hr after the administration, urinary excretion of ^{14}C was 95 per cent of total administered radioactivity, total dose being recovered in urines after 36 hr. These results indicate that the rate of excretion of 5-bromo-[carboxyl-^{14}C]nicotinic acid is similar to the excretion of ^{14}C from [^{14}C,^{3}H]nicergoline in agreement with the observed hydrolysis of the drug in the animal body.

Paper chromatographic analysis of the urines of dogs treated orally with [17-^{3}H]nicergoline (0·2 mg/kg body wt.) showed the same radiochromatographic pattern already observed in rats, thus indicating the presence of the same metabolites. A quantitative evaluation carried out on day 1 urines of three animals gave the following results (average values expressed as per cent of total tritium in the urine samples)—unconverted [17-^{3}H]nicergoline, less than 1 per cent, unconjugated metabolites (II) and (III) 73 per cent, conjugates 26 per cent.

Unconverted [17-^{3}H]nicergoline was also absent in the urines of two monkeys (a male and a female) which had received an oral dose of the labelled drug (1 mg/kg body wt.). Radiochromatographic analysis showed that approximately 60% of urinary tritium was accounted for by labelled metabolites (II) and (III), the remaining 40% being located in zone II R_f range (glucuronides). The same excretion pattern was observed in two other monkeys heavily pretreated with unlabelled nicergoline (50 mg/kg body wt. daily/6 weeks).

Human subjects M. R. and F. C., treated with single i.v. doses (Table 1), excreted two-thirds of the dose in the urines during three days after the administration. Radiochromatographic analysis on paper (solvent system A) and on thin layer plates (solvent system C) of the said urines showed that from 88 to 91% of the excreted tritium behaved as zone I rat metabolites, i.e. as compounds (II) and (III). On the other hand, the radioactivity classified as zone II metabolites (conjugates) was evaluated as being of the order of few units per cent of urinary tritium. Unconverted [17-^{3}H]nicergoline was present in small amounts, never exceeding 6–7% of total excreted tritium. Similarly, analysis of day 1 urines of subjects G. B. and E. A. (Table 1) revealed the absence of unconverted labelled nicergoline, nearly all radioactivity (more than 90 per cent) being accounted for by the fraction containing metabolites (II) and (III). Radioactivity which could be ascribed to slower moving compounds in the chromatograms was very low, never greater than 5% of total tritium in the urine samples.

Stability of [17-^{3}H]nicergoline in rat gastric juice, in phosphate buffer and in human blood is shown in Table 3.

TABLE 3. STABILITY OF NICERGOLINE *in vitro* PER CENT OF [17-^{3}H]NICERGOLINE UNCHANGED AT DIFFERENT TIMES DURING STORAGE AT 37°

Medium	1 hr	3 hr	6 hr	8 hr	96 hr
Rat gastric juice	98·1	96·4	93·8		88·6
M/15 Phosphate buffer, pH 7·3	98·7	96·2	93·0		55·8
M/15 Phosphate buffer, pH 8·0	96·8	93·6	91·0		31·8
Human blood	81·3	60·0		31·1	

DISCUSSION

Our results clearly show that all species so far tested handle nicergoline in a similar way in regard to absorption from the gut, chemical transformation in the body and elimination through the kidney.

Maximal blood levels of tritium were reached within 1 hr after oral dosing. It can be deduced that nicergoline is rapidly absorbed from the gut, may be in the stomach, and that the absorption is almost complete, owing to the high recoveries of tritium in the urines. The drug disappeared rapidly from the blood and was concentrated in different organs, at least in the rat, the only animal species in which a tissue distribution study was made.

Nicergoline appears to be actively transformed in all species tested by three different metabolic reactions.

The first is the hydrolysis of the ester function with formation of the ergoline derivative containing a hydroxymethyl group at C-8 and 5-bromonicotinic acid. This reaction appears to be of enzymatic nature because of the stability of the aqueous solutions of the drug, when a time period of hr is considered. As shown, this reaction can take place in blood, but probably it could take place also in other tissues, owing to the known presence of esterases in animal tissues. This hydrolysis is apparently rapid because detectable amount of unchanged drug appeared to be excreted in the urines by the rat only when doses of 100 mg/kg were used. Moreover the rate of excretion of the 5-bromonicotinic acid residue from nicergoline is close to that of 5-bromonicotinic acid itself when administered alone.

The second metabolic reaction is demethylation at position 1 of the ergoline nucleus, and both the unchanged drug and 1,6-dimethyl-8β-hydroxymethyl-10α-methoxyergoline could act as substrate. The fact that 1-demethylated nicergoline could never be detected is probably related to the high rate of the hydrolysis. *N*-Demethylation at the indole nitrogen is a metabolic reaction described also for other 1-methylergoline derivative such as methysergide[5] and for metergoline.[8]

The third reaction appears to be the glucuronic acid conjugation of the free alcohols formed by the hydrolysis and apparently it involves the C-17 alcoholic function. It is of minor importance, if any, in man, but noticeable in the other species particularly in the monkey. Conjugation of a primary alcoholic function with glucuronic acid was already observed in the field of ergoline derivatives in the case of ergometrine.[4]

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