Pargyline: A Glucuronyl Transferase Inducer

In a previous study it has been observed that the analgesia of morphine was significantly decreased in rats that had been treated with pargyline daily for 6 days when morphine was administered 4 and 24 h after the last dose of pargyline¹. This phenomenon was hypothesized as being due to increasing the metabolism of morphine through an induction of glucuronyl transferase. This report presents the results of an investigation designed to examine this hypothesis.

Materials and methods. Holtzman male rats (150–170 g), kept in constant environmental condition and maintained on Wayne Lab-Blox (Allied Mills Inc.) and tap water ad libitum, were treated chronically with 75 mg/kg of pargyline (Abbott Laboratories), i.p. daily for 7 to 8 days. Control animals were injected with the same volume of saline.

24 h after the last injection the rats were sacrificed by a blow on the head and the livers immediately excized, immersed in ice cold 1.15% KCl solution. The livers were blotted dry, weighed, placed in cold 1.15% KCl solution, minced with scissors, and homogenized in cold 1.15% KCl solution with a Potter-Elvehjem homogenizer having a Teflon plunger in the cold to make a 20% homogenate. The homogenate was centrifuged at $10,000 \times g$ for 20 min to remove the mitochondria, nuclei and unbroken cells. The supernatant was then centrifuged at $105,000 \times g$ for 60 min in a Spinco ultracentrifuge. The resultant pellet was resuspended in 1.15% KCl and centrifuged at $105,000 \times g$ for 60 min. Microsomes, equivalent to 1 g (wet weight) of liver, were suspended by gentle homogenization in 1 ml of 1.15% KCl². The protein content of the microsomal suspension was determined according to the method of LOWRY et al.³ with crystalline bovine albumin as standard.

The glucuronide formation of morphine was studied according to the method of AXELROD and INSCOE⁴ except that the concentration of uridine diphosphate glucuronic acid (UDPGA) (Sigma Chemical Co.) was 6.7 μ moles instead of 0.8 μ moles. Incubation mixture contained 0.5 ml of microsomes, $5 \times 10^{-3} M$ $MgCl_2$, $5 \times 10^{-2} M$ *tris* (hydroxymethyl)aminoethane buffer with a pH of 8.0, $2.5 \times 10^{-4} M$ of N-¹⁴C-methyl-morphine with and without 6.7 μ moles (4 mg) of UDPGA and water to make a final volume of 2 ml. Incubations were carried out in 40 ml tubes in duplicate in Dubnoff-shaker at 37°C for 30 min under atmosphere of air. The incubation was terminated by placing

the tubes in a boiling water bath for 10 min and free morphine in the incubated samples were determined according to the procedure described previously⁵. The amount of the glucuronide of morphine formed was calculated from the mean difference between the samples containing UDPGA and those without UDPGA. Enzyme activity was expressed as the amount of the glucuronide formed per 30 min per 1 mg of protein. A total of 7 animals were used, 4 of which had been treated for 7 days and 3 for 8 days with pargyline.

Student's *t*-test was used for comparison of the mean value of the glucuronide of morphine obtained from the liver microsomes of rats treated chronically with pargyline and with saline at $p < 0.05$ as the level of significance⁶.

Results and discussion. The glucuronide formation of morphine was significantly increased by liver microsomes obtained from the rats that had been treated chronically with pargyline (Table). Both the body weight and liver weight were significantly less in the pargyline treated animals than in those receiving saline. However, the liver weight expressed as percent of body weight and the mg protein/g liver were not significantly different. Data obtained in the present study indicate that pargyline is a glucuronyl transferase inducer. The formation of the glucuronide of morphine by microsomes obtained from the pargyline chronic-treated rats was increased about 40% as compared with that obtained from saline control rats. To our knowledge, this is the first demonstration that pargyline is a glucuronyl transferase inducer. The mechanism of reduction of the analgesia of morphine in rats treated chronically with pargyline is probably due to an increased metabolism via glucuronide formation. This finding may well explain the reason that adverse reactions are seldom observed after administration of morphine to patients treated with monoamine oxidase inhibitors^{7–10}.

Zusammenfassung. Nachweis, dass mehrtägige Behandlung von Ratten mit hohen Dosen Pargylin die Glucuronyltransferase-Aktivität der Lebermikrosomen erhöht.

S. Y. YEH¹¹ and C. L. MITCHELL^{12, 13}

The Department of Pharmacology, College of Medicine, University of Iowa, Iowa City (Iowa 52240, USA), 16 August 1971.

Comparison of body and liver (wet) weight, protein content of washed liver microsomes and glucuronyl transferase activity in rats treated chronically with saline or pargyline*

	Treated with	
	Saline	Pargyline
Body wt. g	207.1 $\pm$ 3.91	162.1 ^b $\pm$ 6.25
Liver (wet) wt. (g)	9.45 $\pm$ 0.25	7.10 ^b $\pm$ 0.38
Liver (wet) wt. as % of body wt.	4.56 $\pm$ 0.14	4.35 $\pm$ 0.10
mg protein/g liver	21.07 $\pm$ 0.42	20.51 $\pm$ 0.38
Glucuronyl transferase activity ^c	13.28 $\pm$ 0.273	18.33 ^b $\pm$ 0.362

* Four animals were treated for 7 days and 3 for 8 days with pargyline, 75 mg/kg or saline, 2 ml/kg, daily. The data represent the mean $\pm$ S.E. obtained from 7 animals in each group. ^b Indicates significant difference between pargyline treated rats and that of the saline control rats at $p < 0.05$ level. ^c Morphine glucuronide formed n moles/30 min-mg protein.

¹ S. Y. YEH and C. L. MITCHELL, J. Pharmac. exp. Ther. 179, 642 (1971).

² T. E. ELING, R. D. HARBISON, B. A. BECKER and J. R. FOUTS, J. Pharmac. exp. Ther. 171, 127 (1970).

³ O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. 193, 265 (1951).

⁴ J. AXELROD and J. K. INSCOE, Proc. Soc. exp. Biol. Med. 103, 675 (1960).

⁵ S. Y. YEH and L. A. WOODS, J. Pharm. Sci. 59, 380 (1970).

⁶ R. G. D. STEEL and J. H. TORRIE, *Principle and Procedures of Statistics* (McGraw-Hill Book Co., New York 1960).

⁷ H. PALMER, Br. Med. J. 2, 944 (1960).

⁸ J. C. SHEE, Br. Med. J. 2, 507 (1960).

⁹ D. D. BROWN and D. H. WALDRON, Practitioner 189, 83 (1962).

¹⁰ L. C. JENKINS and H. D. GRAVES, Can Anaesth. Soc. J. 12, 121 (1965).

¹¹ Present address of Dr. S. Y. YEH: Department of Psychiatry, College of Medicine, University of Kentucky, Lexington (Kentucky 40506, USA), to whom reprint requests have to be sent.

¹² This study was supported by U.S. Public Health Service Grant No. NB 02928.

¹³ The authors wish to thank Mr. K. KINGSBURY for excellent technical assistance in this study.