Biochemical Pharmacology, 1963, Vol. 12, pp. 1363–1366. Pergamon Press Ltd., Printed in Great Britain.

N- and O-DEMETHYLATION OF SOME NARCOTIC ANALGESICS BY BRAIN SLICES FROM MALE AND FEMALE LONG-EVANS RATS*

CHRISTIAN ELISON and HENRY W. ELLIOTT

Department of Pharmacology and Experimental Therapeutics, University of California, San Francisco Medical Center San Francisco, Calif., U.S.A.

(*Received* 10 *July* 1963; *accepted* 18 *July* 1963)

Abstract—Under aerobic conditions incubation of brain slices of rat in an appropriate medium to which narcotic analgesics labeled with ^{14}carbon in the O- and N-methyl groups were added resulted in the liberation of $^{14}CO_2$. The amount of $^{14}CO_2$ liberated in 3 hr was not directly proportional to the analgesic potencies of the drugs *in vivo*. The present results suggest that the demethylating enzymes of liver and brain may be identical. Tolerance to morphine does not seem to affect the N-demethylating activity of the brain on morphine as it does in the case of the liver.

IN THE ceaseless effort to elucidate the mechanism of action of morphine and related narcotic analgesics, the biochemical fate of these drugs *in vivo* and *in vitro* has received much attention in recent years. The two best-known metabolic pathways for morphine are conjugation with glucuronic acid[1–3] and N-demethylation.[4–7] In addition, the drug is susceptible to O-methylation both *in vivo* and *in vitro*.[8] On the other hand morphine can be formed in biological systems by O-demethylation of codeine[9] and by N-methylation of normorphine.[10] N-dealkylation of narcotic analgesics has received special attention and close scrutiny. Theories have been developed relating this reaction to the mechanism of analgesic action,[11] and the liver enzyme that catalyses it has been used as a model for brain receptors in formulating a theory for the development of tolerance to these drugs.[12] Unfortunately, findings from experiments using liver microsomal enzymes are difficult to reconcile with these theories. There appears to be no correlation between analgesic potency and susceptibility to enzyme attack,[13,14] and the evidence seems to conflict with the theory that the development of tolerance may be due to inactivation of receptors by continuous interaction with the drug.[12] However, the theories have been judged by data obtained with liver enzymes, and since the validity of extrapolating from liver to brain is open to criticism, the data, no matter how unfavorable to the theories, cannot be considered adequate for rejecting them. Milthers was able recently to demonstrate that rat brain can N-demethylate morphine *in vivo*.[15,16] Her findings agree with observations made in our laboratory with brain slices and using evolved $^{14}CO_2$ as evidence for N-demethylation of morphine-N-$^{14}CH_3$. It seems probable that results obtained with brain would be of greater value in assessing the relationship between N-dealkylation and biologic action

* Supported in part by Research Grant NB 00570 from the National Institutes of Health, Bethesda, Md.

than would results from liver. In the investigations reported here we have extended the study of N-demethylation by brain slices to other narcotic analgesics.

METHODS

The technique employed utilized an incubation mixture identical in every respect with the one used by us in liver work,[14] except that brain slices were substituted for liver enzyme preparations and drugs labeled with ^{14}carbon in the O- and N-methyl groups were used as substrates. Brain tissue was obtained from adult male and female Long–Evans rats, and from male Long–Evans rats which had been made tolerant to morphine by daily injections. The initial dose of 5 mg/kg was enlarged every other day by increments of 5mg/kg to a maximal dose of 100 mg/kg. The animals were stabilized at the highest dose for one week before sacrificing by decaptitation. Slices of uniform thickeness (0·57 mm) were obtained with a McIlwain tissue slicer. Four micromoles of substrate, with specific activities ranging from 0·9 to 2·70 μc/mg, were used per 500 mg of tissue in each experiment. The final volume of the incubation mixture was 10 ml. Incubation was carried out in glass-stoppered 125-ml Erlenmyer flasks provided with two side arms, one of which was connected to a supply of oxygen bubbled through water, and the other to two CO_2 traps in series containing 2 N NaOH. The mixture was shaken at 100 oscillations/min in a Dubnoff shaking metabolic incubator at 38°. At the end of 3 hr the system was inactivated with 3 ml of 15% trichloroacetic acid and the shaking continued for an additional 30 min. The trapped CO_2 was precipitated with saturated barium hydroxide solution, washed twice with ice-cold water, and then dried to constant weight in a 100° oven. The dried precipitate was resuspended in a water:alcohol mixture (2:1) and homogenized. Aliquots were plated on aluminium planchets, dried in a gentle current of warm air, and the radioactivity estimated in a windowless helium–alcohol flow tube with a conventional scaler circuit. Thickness corrections were made from data obtained by plating various weights of similarly processed $Ba^{14}CO_3$.

RESULTS AND DISCUSSION

The results of these experiments are shown in Table 1. It can be seen that the average amounts of morphine demethylated by brain slices from normal and tolerant rats were not appreciably different. These findings indicate that the development of tolerance did not affect the activity of the brain enzymes. The fact that the livers from tolerant rats possess less capacity to N-demethylate narcotic analgesics by virtue of a decreased enzyme concentration[14] must mean that tolerance to these drugs produces changes in some metabolic function of this organ without similarly affecting the organ where the drugs are thought to exert their principal pharmacological actions. The enzymes are therefore, not suitable as models for the receptors of the brain as far as interaction for the production of narcotic analgesia is concerned.

Data in Table 1 clearly demonstrate the lack of parallelism between the potency of a narcotic drug and the extent to which it is demethylated by the brain. Both meperidine and codeine were demethylated to a larger extent than the more potent morphine. In addition, brain from female rats possessed less capacity to demethylate morphine than the brain of male rats, although the drug is known to be of equal analgesic potency in the two sexes.[14]

A study of the O-demethylation of codeine was done to provide additional data by

which the activities of the demethylating enzymes from liver and brain may be compared. It has been reported that O-demethylation by liver enzymes occurs to a lesser extent than N-demethylation.[14,17] It has also been shown repeatedly that liver from female rats possesses less capacity to N-dealkylate drugs than liver of male rats,[4,14,18]

TABLE 1. MICROGRAMS OF $^{14}CO_2$ EVOLVED IN THREE HOURS BY AN INCUBATION MIXTURE*

Substrate	Morphine-N-$^{14}CH_3$			Codeine-N-$^{14}CH_3$	Codeine-O-$^{14}CH_3$	Meperidine-N-$^{14}CH_3$
Tissue sources	Male	Female	Tolerant male	Male	Male	Male
Expt.						
1	1·770	0·370	2·100	4·400	1·180	3·170
2	1·770	0·600	4·100	5·170	0·470	8·000
3	2·400	1·000	2·580	3·400	0·650	6·610
4	2·730		1·080	2·810	1·170	5·870
5	2·040		1·830	5·380	0·840	6·680
Means	2·142	0·657	2·338	4·232	0·862	6·066
	(0·049)†	(0·015)	(0·053)	(0·096)	(0·020)	(0·138)
% of dose	1·80	0·55	1·95	3·53	0·72	5·06

* Mixture contains 500 mg brain slices from Long–Evans rats and 4 μmoles narcotic analgesics labeled with ^{14}C in the methyl groups.

† Figures in parentheses are the means expressed in micromoles.

and that meperidine and codeine are demethylated more rapidly than morphine by liver enzymes.[8,14] The present results indicate that the brain exhibits a qualitatively similar behavior toward these drugs. This may mean that the enzymes from the two sources are identical. The much lower activity of the brain may be due to lower enzyme levels, lower accessibility of these drugs to the brain, or both. However, none of the evidence presented constitutes proof of identity. A more definite conclusion in this respect can be reached only when such factors as pH optima, substrate requirements, and the kinetics of these enzymes can be compared. The very low activities of the brain enzymes make it difficult to study these characteristics by available techniques.

The principal question still remains as to whether the N- and O-demethylations demonstrated with brain tissue occur at the receptors where biologic action is exerted. If the answer is yes, then obviously the phenomenon is not correlated with potency. If they may occur elsewhere, then even if the enzymes from liver and brain are identical the results presented here have no more meaning than results obtained with liver preparations.

REFERENCES

1. E. G. GROSS and V. THOMSON, J. *Pharmacol. exp. Ther.* **68**, 413 (1940).
2. L. A. WOODS, *J. Pharmacol. exp. Ther.* **112**, 158 (1954).
3. J. FUJIMOTO and E. L. WAY, *J. Pharmacol. exp. Ther.* **121**, 340 (1957).
4. C. H. MARCH and H. W. ELLIOTT, *Proc. Soc. exp. Biol. (N.Y.)* **86**, 494 (1954).
5. J. AXELROD, *J. Pharmacol. exp. Ther.* **117**, 322 (1956).
6. L. B. MELLETT and L. A. WOODS, *Proc. Soc. exp. Biol. (N.Y.)* **106**, 221 (1961).
7. A. L. MISRA, S. J. MULÉ and L. A. WOODS, *J. Pharmacol. exp. Ther.* **132**, 317 (1961).
8. C. ELISON and H. W. ELLIOTT. In preparation.
9. T. K. ADLER, J. M. FUJIMOTO, E. L. WAY and E. M. BAKER, *J. Pharmacol. exp. Ther.* **114**, 251 (1955).

10. D. H. CLOUET, *Life Sci.* **1**, 31 (1962).
11. A. H. BECKETT, A. F. CASY and N. J. HARPER, *J. Pharm. Pharmacol.* **8**, 874 (1956).
12. J. AXELROD, *Science* **124**, 263 (1956).
13. E. L. WAY and T. K. ADLER, *Pharmacol. Rev.* **12** (4), 383 (1960).
14. C. ELISON, H. W. ELLIOTT, M. LOOK and H. RAPOPORT, *J. med. Pharm. Chem.* **6**, 237 (1963).
15. K. MILTHERS, *Acta pharmacol. (Kbh.)* **18**, 199, (1961).
16. K. MILTHERS, *Acta pharmacol. (Kbh)* **19**, 235 (1962).
17. A. E. TAKEMORI and G. J. MANNERING, *J. Pharmacol. exp. Ther.* **123**, 171 (1958).
18. C. C. LEE, R. C. ANDERSON and K. K. CHEN, *J. Pharmacol. exp. Ther.* **117**, 265 (1956).