Biochemical Pharmacology, Vol. 24, pp. 1127–1130. Pergamon Press, 1975. Printed in Great Britain.

SHORT COMMUNICATIONS

Effect of anesthetic dosages of pentobarbital on the content and turnover of serotonin in brain areas

(*Received* 22 *April* 1974; *accepted* 11 *October* 1974)

The suppression of rapid eye movement (REM) sleep by barbiturates may be related to effects of these agents on serotonergic pathways in the brain [1, 2]. Serotonergic neurons have been implicated in the induction and regulation of REM sleep [3], and evidence is available, although it is somewhat conflicting, to suggest that barbiturates affect brain serotonin (5-HT). Bonnycastle *et al.* [4, 5] and Anderson and Bonnycastle [6] reported an increase in whole brain 5-HT in the rat after treatment with a number of different barbiturates: pentobarbital, hexobarbital, amobarbital or thiopental. On the other hand, Efron and Gessa [7] found no changes in whole brain 5-HT content 30, 60 or 150 min after injections of anesthetic dosages of pentobarbital or phenobarbital.

Although the cell bodies of the neurons which contain 5-HT are localized in the brainstem, their nerve terminals project to areas throughout the brain [8]. It is very conceivable that barbiturates, as well as other drugs, may affect 5-HT differently in various areas of the brain. Small changes in 5-HT content or turnover in discrete areas of the brain might be completely undetected in whole brain studies. Further, whole brain studies cannot differentiate the pentobarbital-induced response of 5-HT containing cell bodies from the response of 5-HT nerve terminals. Due to the importance of pentobarbital as a clinical agent and recently as a drug of abuse and due to the shortage of consistent information concerning the effects of pentobarbital on brain biogenic amines, 5-HT content and turnover were investigated in six discrete areas of the rat brain.

Male Sprague–Dawley rats (150–200 g) were injected intraperitoneally with an anesthetic dosage of pentobarbital (50 mg/kg) or an equivalent volume of saline. In some studies, anesthesia was prolonged by supplemental dosages of pentobarbital (25–30 mg/kg) given 60 and 120 min after the initial injection. Body temperatures of pentobarbital-treated animals were monitored at 15-min intervals. If necessary, an animal's body temperature was maintained with a heating pad. Brain 5-HT was analyzed in six discrete brain areas: the cerebellum, medulla-pons, midbrain, diencephalon (thalamus-hypothalamus), striatum and cerebral cortex. Serotonin was extracted and analyzed according to the method of Maickel *et al.* [9], and 5-hydroxyindoleacetic acid (5-HIAA) was analyzed by the method of Curzon and Green [10].

The rate of synthesis of 5-HT was estimated by measuring 5-HT accumulation after monoamine oxidase (MAO) inhibition by nialamide (100 mg/kg) (Pfizer, Inc.) [11]. The rate of 5-HT degradation was estimated by measuring 5-HIAA accumulation after probenecid (200 mg/kg) (Merck Institute for Therapeutic Research) [11]. In separate studies, nialamide or probenecid was administered 60 min after the initial pentobarbital injection. Groups of pentobarbital-treated and saline-treated rats were sacrificed 0, 30, 60 and 90 min after the nialamide or probenecid.

Five min after the administration of pentobarbital (50 mg/kg), 5-HT content was elevated (20 per cent, $P < 0.005$) in the midbrain of the rat (Fig. 1). By 30 min, 5-HT content in the diencephalon was also significantly elevated (20 per cent, $P < 0.05$) (Fig. 1). No statistically significant elevations in 5-HT content were observed in any other brain area during the first 60 min of pentobarbital anesthesia. By 60 min, 5-HT in the midbrain and diencephalon had returned to control levels.

In a subsequent experiment, anesthesia was prolonged through 120 min by the injection at 60 min of a supplemental dosage of pentobarbital (30 mg/kg). Serotonin content

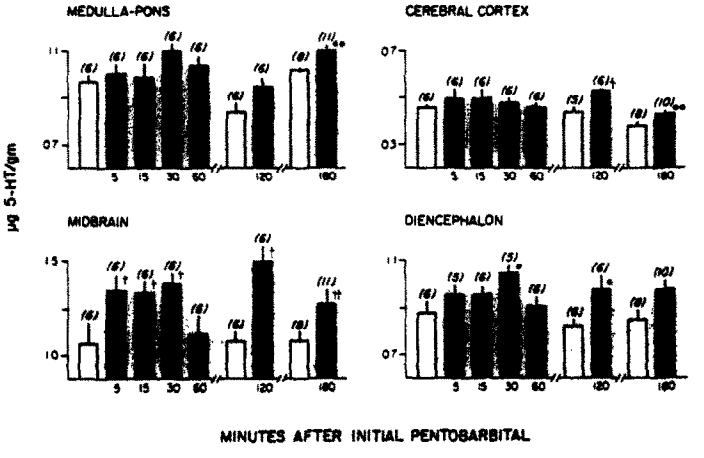

Fig. 1. Effect of anesthetic dosages of pentobarbital on 5-HT content in discrete areas of rat brain. The clear bars represent mean 5-HT content ± standard errors in saline animals, and the black bars represent pentobarbital-treated animals. The 60-, 120- and 180-min results were obtained in three separate studies performed weeks apart, and thus a separate group of saline animals are represented for each study. The number of animals sacrificed are shown for each group by the numbe. in parentheses. Statistical significance was determined by the analysis of variance. Key: * $P < 0.05$; $P < 0.01$; † $P < 0.005$; and †† $P < 0.001$.

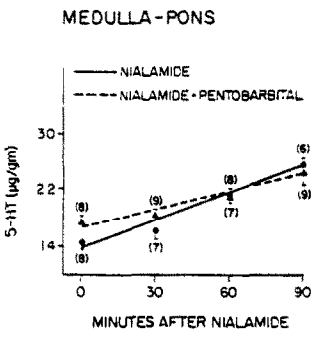

Fig. 2. Effect of anesthetic dosages of pentobarbital on the rate of 5-HT accumulation in the medulla-pons after monoamine oxidase inhibition by nialamide. Mean 5-HT ± standard errors for saline (●) and pentobarbital-treated rats (▲) are shown for 0, 30, 60 and 90 min after nialamide (100 mg/kg). The respective regression lines were determined by the least squares method [12]. The statistical significance of the difference between the slopes of the respective regression lines was determined by a Student *t*-test [13].

was elevated not only in the midbrain (29 per cent, $P < 0.005$) and the diencephalon (20 per cent, $P < 0.05$) but also in the cerebral cortex (20 per cent, $P < 0.005$) (Fig. 1).

When anesthesia was extended to 180 min by giving a second supplemental injection of pentobarbital (25 mg/kg) at 120 min, 5-HT content was significantly elevated in three brain areas: the medulla-pons (8 per cent, $P < 0.01$), the midbrain (13 per cent, $P < 0.001$) and the cerebral cortex (13 per cent, $P < 0.01$) (Fig. 1). Serotonin content was not significantly elevated in the striatum or cerebellum at any time during the first 180 min of pentobarbital anesthesia. No significant changes in 5-HIAA content were observed in any brain area of pentobarbital-treated compared to control animals.

A small but significant decrease ($P < 0.05$) in the rate of accumulation of 5-HT after MAO inhibition was observed in the medulla-pons of pentobarbital-treated rats (Fig. 2), but not in any other brain area. On the other hand, the rate of depletion of 5-HIAA after MAO inhibition was significantly reduced in the medulla-pons ($P < 0.005$), midbrain ($P < 0.001$), diencephalon ($P < 0.05$) and striatum ($P < 0.05$) (Fig. 3). Further, the correlation coefficients (r) for the decrease of 5-HIAA content vs time after nialamide plus pentobarbital were not significantly greater than zero in these same brain areas and also the cerebellum (Fig. 3). The corresponding correlation coefficients in all six brain areas of control nialamide-treated rats were highly significant ($P < 0.01$).

The rate of degradation of 5-HT to 5-HIAA, as measured by the rate of accumulation of 5-HIAA after probenecid, was significantly reduced in all six brain areas of the pentobarbital-treated rats: medulla-pons (47 per cent, $P < 0.005$), midbrain (47 per cent, $P < 0.005$), diencephalon (46 per cent, $P < 0.001$), striatum (38 per cent, $P < 0.05$), cerebral cortex (24 per cent, $P < 0.005$) and cerebellum (50 per cent, $P < 0.01$) (Fig. 4).

The elevations of 5-HT content observed in the medulla-pons, diencephalon, midbrain and cerebral cortex after pentobarbital administration could have resulted from: (1) an increase in the rate of 5-HT synthesis, (2) a decrease in 5-HT degradation or (3) a combination of these mechanisms. No increase in the rate of 5-HT accumulation after MAO inhibition was observed in any of the six brain areas after pentobarbital-induced anesthesia for 150 min. On the contrary,

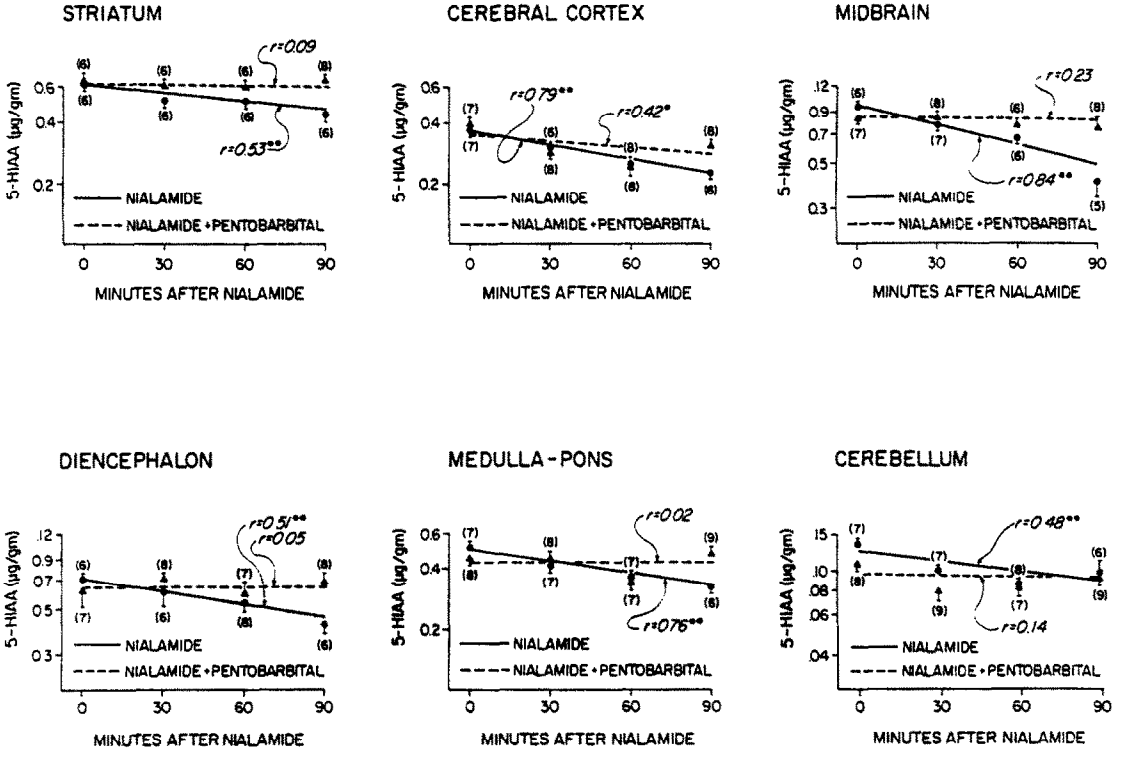

Fig. 3. Effect of anesthetic dosages of pentobarbital on the decline of 5-HIAA content after monoamine oxidase inhibition by nialamide. The mean log concentration of 5-HIAA ± standard error are shown for six discrete areas of the rat brain in both control (●) and pentobarbital-treated animals (▲) at 0, 30, 60 and 90 min after nialamide. The rate of 5-HIAA depletion after nialamide (slope of best fit line) was significantly decreased in the medulla-pons ($P < 0.005$), midbrain ($P < 0.001$), diencephalon ($P < 0.05$) and striatum ($P < 0.05$) in the pentobarbital-treated rats [13]. Key: *$P < 0.05$; **$P < 0.01$.

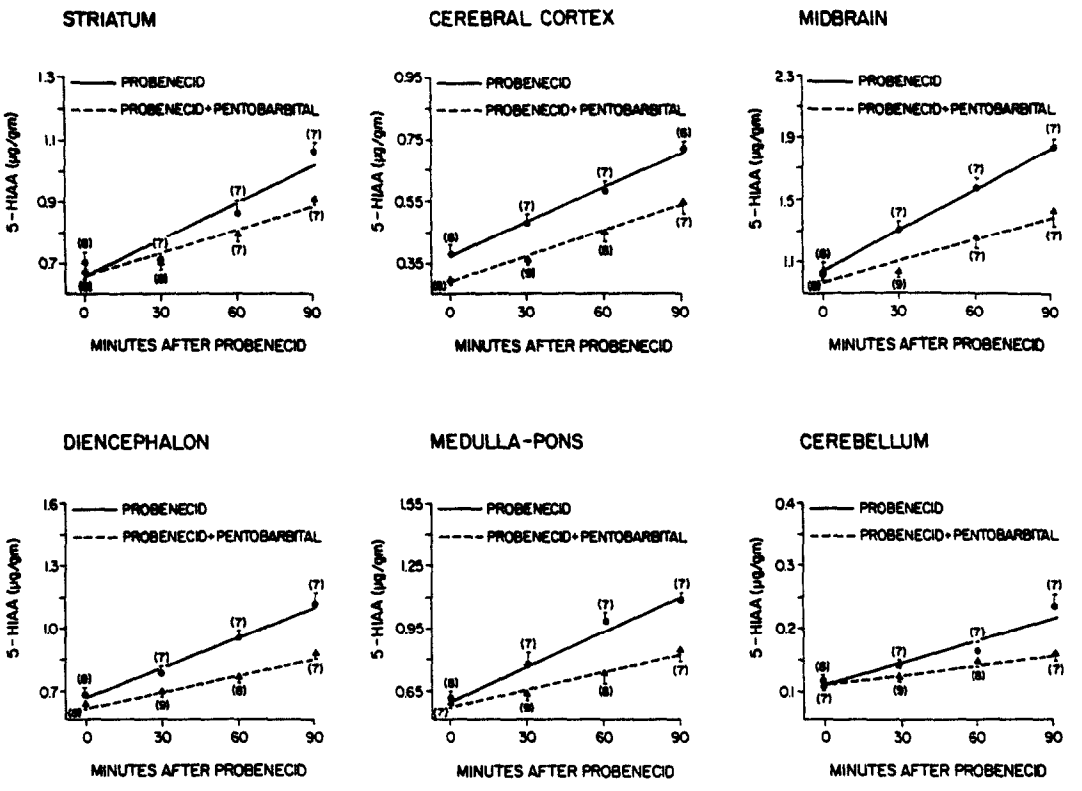

Fig. 4. Effect of anesthetic dosages of pentobarbital on the accumulation of 5-HIAA in six discrete brain areas after the excretion of 5-HIAA has been inhibited by probenecid. The mean 5-HIAA levels ± standard errors are shown for both control (●) and experimental animals (▲) at 0, 30, 60 and 90 min after probenecid (200 mg/kg). The rate of 5-HIAA accumulation after probenecid (slope of best fit line) was significantly reduced in all six brain areas in the pentobarbital plus probenecid-treated rats: medulla-pons, $P < 0.005$; midbrain, $P < 0.005$; diencephalon, $P < 0.001$; striatum, $P < 0.05$; cerebral cortex, $P < 0.005$; and cerebellum, $P < 0.01$ [13].

the rate of 5-HT accumulation after MAO inhibition was either unaffected or slightly decreased by pentobarbital. Thus, it seems unlikely that the increase in 5-HT levels in the various brain areas can be accounted for by an increase in the rate of 5-HT synthesis.

In some brain areas, pentobarbital was observed to reduce or block the linear exponential decrease in 5-HIAA content normally observed after MAO inhibition. Pentobarbital could produce this effect (1) by interfering with the inhibitory action of nialamide on MAO or (2) by partially inhibiting the active transport system which removes 5-HIAA from the brain. However, with the exception of the medulla-pons, 5-HT accumulation after MAO inhibition was not significantly different in pentobarbital-treated compared to control animals, and further the correlation coefficients for the accumulation of 5-HT were all significantly greater than zero, indicating that nialamide had effectively inhibited MAO in the pentobarbital-treated animals. Thus, these data best support the hypothesis that pentobarbital interferes with the acid transport system which removes 5-HIAA from the brain.

Since synthesis of 5-HT is not increased by pentobarbital, the elevation in 5-HT content may result from a decrease in the rate of metabolism of 5-HT to 5-HIAA, the primary metabolite of 5-HT in the brain. However, because pentobarbital interferes with the transport of 5-HIAA out of the brain, a significant decrease in 5-HIAA content due to a decrease in 5-HT depletion was not observed after pentobarbital treatment alone. On the other hand, when the rate of degradation of 5-HT to 5-HIAA was determined after probenecid administration, a marked decrease in the accumulation of 5-HIAA was observed in pentobarbital-treated rats, probably resulting from a decrease in 5-HT degradation or turnover. Using different methods, Kuschinsky *et al.* [14] and recently Lidbrink *et al.* [15] have also concluded that pentobarbital produces a suppression of 5-HT turnover. However, alternate explanations must also be considered. Pentobarbital may shunt the degradation of 5-HT into other metabolic pathways in preference to the 5-HIAA pathway. This possibility is unlikely because pentobarbital is not an MAO inhibitor [16], and it does strongly inhibit aldehyde reductase which is responsible for the conversion of 5-HT to 5-hydroxytryptophol [17].

The small magnitude of the increases in 5-HT content observed in certain brain areas after pentobarbital suggest that they are of minor physiological significance, although further studies are needed to determine if the increases of 5-HT are in the functional or the metabolic pool. At present, it appears that the elevations in 5-HT content observed in some brain areas are a reflection of a decrease in 5-HT degradation (turnover) without an equivalent decrease in 5-HT synthesis. As other investigators have suggested [18], measurements of 5-HT turnover may provide a better indication of changes in serotonergic activity than does content. If the turnover of neurohumoral agent is related to the impulse activity of the neuron in which it is contained [19], the decrease in 5-HT turnover observed in the six brain areas may reflect a suppression of serotonergic impulse activity by pentobarbital. The suppression of serotonergic activity could be related to the suppression of REM sleep by barbiturates.

The results presented in this report explain some of the inconsistency in the earlier literature concerning the effects of pentobarbital on the brain serotonergic system. During

the first hour after an anesthetic dosage of pentobarbital. 5-HT was significantly elevated in only two areas of the rat brain. Therefore, it is not surprising that Efron and Gessa [7] found no significant effect of a single dosage of pentobarbital on 5-HT content in the whole rat brain. These data provide a strong argument for the study of the effects of drugs on brain biogenic amines in discrete brain areas rather than in the whole brain.

Acknowledgements—This research was supported by Morrison Trust Foundation Grants, R-A-16 and R-A-36, and Institutional Research Grant RR-05654-04.

Departments of Anatomy and Pharmacology, The University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284, *U.S.A.*

WILLIAM W. MORGAN
RONALD D. HUFFMAN
LINDA S. MCFADIN
CATHERINE A. YNDO

REFERENCES

1. I. Oswald, *Pharmac. Rev.* **20**, 273 (1968).
2. A. Kales, M. B. Scharf and T. L. Tan, *Fifth In. Congr. Pharmac.*, San Francisco, California, p. 101. S. Karger, New York (1972).
3. M. Jouvet, *Science, N.Y.* **163**, 32 (1969).
4. D. D. Bonnycastle, N. J. Giarman and M. K. Paasonen, *Br. J. Pharmac. Chemother.* **12**, 228 (1957).
5. D. D. Bonnycastle, F. G. Anderson and M. F. Bonnycastle, *Twelfth Int. Congr. Physiol. Sci.*, Buenos Aires, p. 39. Buenos Aires (1959).
6. F. G. Anderson and D. D. Bonnycastle, *J. Pharmac. exp. Ther.* **130**, 138 (1960).
7. D. H. Efron and G. L. Gessa, *Archs int. Pharmacodyn. Ther.* **142** (2), 111 (1963).
8. K. Fuxe, *Acta physiol. scand.* **64**, suppl. Vol. 247, 39 (1965).
9. R. P. Maickel, R. K. Cox, Jr., J. Saillant and F. P. Miller, *Neuropharmacology* **7**, 275 (1968).
10. G. Curzon and A. R. Green, *Br. J. Pharmac. Chemother.* **39**, 653 (1970).
11. N. H. Neff and T. N. Tozer, *Adv. Pharmac.* **6A**, 97 (1968).
12. R. R. Sokal and F. J. Rohlf, in *Biometry*, p. 226. W. H. Freeman, San Francisco (1969).
13. R. G. D. Steel and J. H. Torrie, in *Principles and Procedures of Statistics*, p. 173. McGraw-Hill, New York (1960).
14. K. Kuschinsky, G. Seidel, E. Reetz and C. Mayer-Brugdork, *Experientia* **29**, 826 (1973).
15. P. Lidbrink, H. Corrodi and K. Fuxe, *Eur. J. Pharmac.* **26**, 35 (1974).
16. P. Lidbrink, H. Corrodi, K. Fuxe and L. Olson, *Brain Res. Osaka* **45**, 507 (1972).
17. B. Tabakoff, F. Ungar and S. G. A. Alivisatos, *Nature New Biol.* **238**, 126 (1972).
18. R. C. Lin, E. Costa, N. H. Neff, C. T. Wang and S. H. Ngae, *J. Pharmac. exp. Ther.* **170**, 232 (1969).
19. N. E. Anden, H. Corrodi, A. Dahlstrom, K. Fuxe and T. Hökfelt, *Life Sci.* **5**, 561 (1966).

Biochemical Pharmacology, Vol. 24, pp. 1130–1131. Pergamon Press, 1975. Printed in Great Britain.

Transformation of Δ^1-tetrahydrocannabinol (THC) by rabbit liver microsomes

(*Received* 24 *August* 1974; *accepted* 29 *November* 1974)

The major transformation products of Δ^1-tetrahydrocannabinol (Δ^1-THC) by rabbit liver have been reported by Nilsson *et al.* [1] and Wall [2]. They found that subcellular fractions of rabbit liver readily metabolized Δ^1-THC to 7-hydroxy-Δ^1-THC [1, 2], 6β-hydroxy-Δ^1-THC [2] and 6α,7-dihydroxy-Δ^1-THC [2]. In order to provide a more complete picture of the biotransformations in the rabbit, we have reinvestigated this process with a view toward identifying some of the less abundant metabolites. This report describes the isolation and identification of two pharmacologically active metabolites.

Female albino rabbits approximately 6 months old were used as the tissue source. The livers were homogenized and the microsomal fraction was obtained as previously described by Burstein and Kupfer for the rat [3]. ^{14}C-Δ^1-THC was obtained from the National Institute on Drug

Table 1. Metabolism of Δ^1-THC by rabbit liver microsomes

T.l.c. zone	*Rf*	Assignment*	Retention time (min)	Principal ions† (M/e)
1	0·67	Δ^1-THC acetate		
2	0·40	1,2α-Epoxyhexahydrocannabinol acetate	5·7	372(25), 357(25), 330(50), 315(75), 312(45) 298(100), 288(55), 274(75), 231(8·8)
		6α-Hydroxy-Δ^1-THC diacetate	7·0	372(3·4), 354(100), 339(21), 312(82), 297(65) 295(18)
3	0·30	7-Hydroxy-Δ^1-THC diacetate	7·5	372(3·8), 354(43), 312(100), 297(28), 259(31)
4	0·13	6α,7-Dihydroxy-Δ^1-THC triacetate	11·3	412(34), 397(5), 370(9), 355(12), 337(47) 310(46), 295(100)

* All materials were acetylated prior to t.l.c. with a mixture of acetic anhydride and pyridine. T.l.c. system: Silica gel G, hexane–ether (7:3).

† The spectra were obtained on a Finnegan 1015 at 70 eV. The column conditions were: 2 ft, 2% OV-1; 180–240° (8°/min); carrier gas, He; injector temp., 255°. Numbers in parentheses refer to relative intensities.