Further study on the correlation of urinary excretion of a methadone metabolite with methadone metabolism and analgesia in the rat

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Methadone is metabolized in the liver to inactive metabolites (Pohland et al 1971). Many interactions between methadone and other drugs can be explained on the basis of the effects of these drugs on methadone metabolism. Based on the finding that phenobarbitone pretreatment increased the in vitro *N*-demethylation of methadone, Alvares & Kappas (1972) suggested that phenobarbitone pretreatment shortened methadone-induced analgesia by enhancing methadone metabolism. Similarly, the decrease of methadone analgesia by pentobarbitone pretreatment has been attributed to the stimulation of methadone metabolism by pentobarbitone (Ho & Berndt 1976). We have extended the finding of Alvares & Kappas (1972) and shown that the decreased analgesia of methadone following phenobarbitone pretreatment was associated with an increase in methadone metabolism in vivo (Liu et al 1978a). Increased metabolism in vivo was evidenced by a decrease in the concentration of unchanged methadone in brain, liver and urine and a corresponding increase in the concentration of methadone water-soluble metabolites (WSM) in the liver and urine. In a subsequent study, we showed that phenobarbitone pretreatment increased the percentage of total ^{14}C in liver as ^{14}C-WSM and the percentage of total ^{14}C in urine as ^{14}C-WSM 3 h after administration of [^{14}C]methadone (Liu et al 1978b). Our results suggest that determination of a change in the percentage of total ^{14}C in urine as ^{14}C-WSM reflects a change in the rate of methadone metabolism in the liver and provides an indirect measure of a change in methadone metabolism in vivo.

The above mentioned studies from our laboratory were conducted 3 h after administration of methadone when the analgesic response of methadone had decayed. To provide more evidence that measurement of the change in urinary excretion of WSM may truly reflect the change of methadone metabolism in vivo, we investigated further the correlation of a change in the urinary excretion of WSM with a change in the in vivo metabolism of methadone during the peak of methadone analgesia. To facilitate obtaining a wide range of WSM concentrations, phenobarbitone was used to stimulate the metabolism of methadone.

Male Sprague-Dawley rats (120–150 g) purchased from Spartan Research, Haslett, MI, were fed purina laboratory chow and had free access to water. They were housed in an air-conditioned room under a 12-h light-dark cycle. The rats were pretreated with phenobarbitone (90 mg kg^{-1} i.p.) once daily for 1 or 4 days. Control rats received saline injections for 4 days. The rats were used for experiments 1 day after the last injection and were starved with free access to water for 16 h. All the rats were given methadone ([2-^{14}C] heptanone) hydrochloride (5 mg kg^{-1}, 15 μCi kg^{-1} s.c.) and killed 1 h later when the analgesia had been measured by hot plate (TLI-Thermajust Analgesia Meter, Technilab Instrument, Inc., Pequannock, NJ) maintained at 58° ± 0·1 °C as described by Liu & Wang (1975). No reaction within 30 s was considered maximal response. To retain urine in the bladder, before killing, the rat was anaesthetized with ether and its urethra was closed by an arterial clamp. Urine was collected from urinary bladder and metabolic cage 1 h after administration of [^{14}C]methadone as described by Liu et al (1976). The analytical methods of Liu et al (1978a) were used to determine the concentrations of total ^{14}C and ^{14}C-WSM in brain, plasma, liver and urine.

The maximum analgesic effect in all control rats persisted for longer than 60 min (Table 1). Phenobarbitone pretreatment for 4 days decreased the intensity and duration of methadone analgesia. Two of the 4-day phenobarbitone-pretreated rats (PB44 and PB45) never reached the maximum analgesic effect and their reaction times returned to predrug values 60 min after methadone. The reaction time in one 1-day phenobarbitone-pretreated rat (PB15) dropped to 18 s at 60 min.

No significant difference in plasma concentration of total ^{14}C was found among the groups of control and phenobarbitone-pretreated rats (Table 1). The concentration of total ^{14}C in the brain (mostly as unchanged methadone) of 4-day phenobarbitone-pretreated rats was significantly lower than that in control rats. Unlike the relationship between the analgesic response and plasma concentration of total ^{14}C, there was a positive relationship between the analgesic response and brain concentration of total ^{14}C. The lower the analgesic reaction time, the lower the brain concentration of total ^{14}C (Table 1). This finding is in agreement with previous studies of Miller & Elliot (1955) and Liu & Wang (1975) that the brain concentrations of methadone is one of the primary factors responsible for the duration of methadone analgesia.

Phenobarbitone pretreatment caused an increase in the concentration of ^{14}C-WSM expressed as a percentage of total ^{14}C in liver and a paralleled increase in the percentage of total ^{14}C in urine as ^{14}C-WSM 1 h after administration of ^{14}C-methadone (Fig. 1). In general, pretreatment with phenobarbitone for 4 days produced greater effects than the 1-day pretreatment except with rats PB15 and PB41. Fig. 1 also shows that there was a

* Correspondence.

Table 1. Effect of phenobarbitone (PB) pretreatment on methadone analgesia and concentrations of methadone in plasma and brain. Rats of PB 11–15 and PB 41–45 were pretreated with phenobarbitone (90 mg kg^{-1}, i.p.) once daily for 1–4 days, respectively. Controls (C 01–05) received saline injections for 4 days. 24 h after the last dose of phenobarbitone or saline all rats were given [^{14}C]-methadone (5 mg kg^{-1}, 15 μCi kg^{-1}, s.c.) and killed 1 h later when the analgesic reaction times had been measured.

Pre-treat. and rat No.	Analgesic reaction time(s)			Total ^{14}C (n mol ml^{-1} or g^{-1})	
	Pre-drug	30 min	60 min	Plasma	Brain
C 01	6·1	>30	>30	1·75	6·05
C 02	6·4	>30	>30	1·53	5·93
C 03	4·6	>30	>30	2·71	4·77
C 04	3·1	>30	>30	2·51	6·10
C 05	5·1	>30	>30	3·58	7·24
Mean ± s.e.	5·06 ±0·59	>30	>30	2·42 ±0·366	6·02 ±0·392
PB 11	6·3	>30	>30	1·87	6·76
PB 12	8·2	>30	>30	1·98	5·33
PB 13	7·8	>30	>30	3·30	4·80
PB 14	4·4	>30	>30	2·09	5·81
PB 15	3·5	>30	18·1	1·44	3·28
Mean ± s.e.	6·04 ±0·92	>30	28·0 ±2·38	2·14 ±0·311	5·20 ±0·577
PB 41	5·5	>30	>30	2·17	5·54
PB 42	4·0	>30	>30	3·23	3·65
PB 43	5·3	>30	19·8	2·59	3·11
PB 44	6·0	22·1	4·9	2·46	2·57
PB 45	4·3	17·9	7·3	1·68	2·15
Mean ± s.e.	5·02 ±0·38	26·0 ±2·54	18·4 ±5·37	2·43 ±0·255	3·40 ±0·591*

* Significant difference from control at $P < 0.01$.

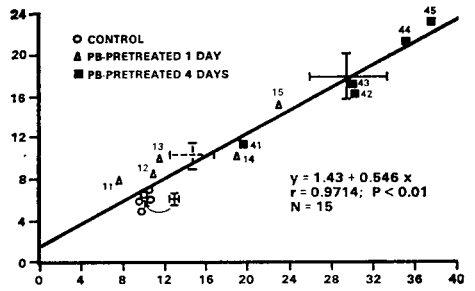

FIG. 1. Relationship of percentage of total ^{14}C as ^{14}C-WSM in liver and urine. Rats were pretreated with phenobarbitone (PB, 90 mg kg^{-1} i.p.) once daily for 1 day (marked Δ11–Δ15) or 4 days (marked 41–45). Control rats (marked 0) received saline injections. [^{14}C]Methadone (5 mg kg^{-1}, 15 μCi kg^{-1} s.c.) was given 24 h after the last dose of phenobarbitone. Rats were killed 1 h after [^{14}C]methadone administration. The concentrations of ^{14}C-WSM in liver and urine were expressed as percentage of total ^{14}C in liver or urine. The horizontal and vertical bars represent mean ± s.e. for the group of rats. Each group consisted of 5 rats. Ordinate: urine ^{14}C-WSM (% of total ^{14}C in urine). Abcissa: liver ^{14}C-WSM (% of total ^{14}C in liver).

highly significant correlation ($r = 0.9714$, $P < 0.01$) between the percentage of total ^{14}C in liver as ^{14}C-WSM and the percentage of total ^{14}C in urine as ^{14}C-WSM. Comparison of the data in Table 1 with Fig. 1, shows that the rats with higher percentages of ^{14}C-WSM in liver and urine had lower brain concentration of ^{14}C and lower analgesic intensity and vice versa.

Methadone is *N*-demethylated followed by spontaneous cyclization to form a pyrrolidine metabolite which metabolizes to form a pyrroline metabolite (Beckett et al 1968; Pohland et al 1971). Further metabolism of the pyrrolidine and pyrroline metabolites results in the formation of WSM (Sullivan et al 1972; Baselt & Bickel 1973). Pohland et al (1971) reported that the pyrrolidine and pyrroline metabolites are analgesically inactive. Thus, *N*-demethylation can be considered as a pathway which terminates analgesic activity of methadone. The fact that the concentrations of ^{14}C-WSM, expressed as percentages of total ^{14}C in liver or urine were much higher than controls indicates that metabolic pathways for formation of WSM from methadone was stimulated by phenobarbitone pretreatment. It is obvious that stimulation of methadone metabolism by phenobarbitone would result in a lower brain concentration of unchanged methadone which, in turn, would decrease the analgesic response of methadone. The 1-h experiment presented in this communication further supports our previous suggestion that there is a positive relationship between the brain concentration of methadone and the intensity of methadone analgesia. Furthermore, the 1-h experiment gives us more substantial evidence that measurement of the change in urinary excretion of WSM may truly reflect the change in liver concentration of WSM which reflects the rate of methadone metabolism in the liver and the concentration of methadone in the brain. In light of the simplicity and rapidity of measuring the percentage of total ^{14}C in urine as ^{14}C-WSM, our results may greatly facilitate the study of drug interactions with methadone by monitoring the changes of urinary excretion of WSM.

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REFERENCES

Alvares, A. P., Kappas, A. (1972) J. Lab. Clin. Med. 79: 439–451

Baselt, R. C., Bickel, M. H. (1973) Biochem. Pharmacol. 22: 3117–3120

Beckett, A. H., Taylor, J. F., Casy, A. F., Hassen, M. M. A. (1968) J. Pharm. Pharmacol. 20: 754–762

Ho, I. K., Berndt, W. O. (1976) Life Sci. 18: 1305–1314

Liu, S. J., Wang, R. I. H. (1975) J. Pharmacol. Exp. Ther. 195: 94–104

Liu, S. J., Chen, K. Z. C., Wang, R. I. H. (1976) Ibid. 198: 308–319

Liu, S. J., Evans, D. B., Wang, R. I. H. (1978a) Ibid. 204: 67–76

Liu, S. J., Evans, D. B., Wang, R. I. H. (1978b). Toxicol. Appl. Pharmacol. 44: 531–538

Miller, J. W., Elliot, H. W. (1955) J. Pharmacol. Exp. Ther. 113: 283–291

Pohland, A., Boaz, H. E., Sullivan, H. R. (1971) J. Med. Chem. 14: 194–197

Sullivan, H. R., Due, S. L., McMahon, R. E. (1972) J. Am. Chem. Soc. 94: 4050–4051