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The difference in activity between (+)- and (−)-methadone is intrinsic and not due to a difference in metabolism

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The disposition and metabolism of (+)- and (−)-methadone has been compared in rats. At equal molecular doses, somewhat higher plasma levels of (−)-isomer were observed. At equal analgesic doses, brain and plasma concentrations of (+)-methadone were at least 25 times greater than those of (−)-methadone. No qualitative differences were observed between isomers with respect to *in vivo* metabolic pattern or *in vitro* *N*-demethylation rates. The results strongly support the conclusion that the large differences in analgesic potency between the isomers is due to an intrinsic difference in pharmacologic properties and is not related to a difference in disposition or metabolism.

Most, if not all, of the analgesic activity of racemic methadone is due to the (−)-isomer (*cf.* Smits & Myers, 1974, for a recent examination of this question). It is generally thought that differences in analgesic activity between optical isomers are the result of a difference in affinity for a stereoselective analgesic receptor (Beckett & Casy, 1954; Dole, 1970). With methadone isomers this question has been discussed by Way and his associates (Way & Adler, 1962; Sung & Way, 1963; Veatch, Adler & Way, 1964). On the basis of their work and that of others, they have concluded that the many fold difference in analgesic activity between (+)- and (−)-methadone is due to a difference in affinity for the analgesic receptor and is not due to the differential disposition or metabolism of the isomers. This point of view is strongly supported by recent studies in systems in which the effects of metabolism have been minimized. For example in a study in rats utilizing intraventricular injection techniques, only (−)-methadone produced analgesia (Ingoglia & Dole, 1970). Similarly, Nickander, Booher & Miles (1973) found (+)-methadone to be only about 1/50 as effective in depressing the electrically induced twitch of the longitudial muscle of the guinea-pig ileum as (−)-methadone. In other studies, Pert & Snyder (1973) found (−)-methadone to be ten times as effective as (+)-methadone in competition with [^{3}H]-naloxone for binding sites in rat brain homogenate, while Wong & Horng (1973) report that (−)-methadone is 50 times as effective as is (+)-methadone in competition for ^{3}[H]dihydromorphine for binding sites on membranes from rat corpus striatum. Although the studies discussed above seem conclusive, Misra & Mulé (1973) have recently presented the view that the differences in analgesic activity between (+)- and (−)-methadone "could be attributed to (1) the formation of an apparently active metabolite in rat brain with (−)-methadone, but not with (+)-isomer, (2) significant differences in half-lives of these isomers in rat brain and plasma, (3) differential stereoselective *N*-dealkylation pathway being a major route of metabolism with (−)- but not with (+)-isomer". The many *in vivo* and *in vitro* studies summarized above show (−)-methadone to be ten to fifty times as active as (+)-methadone. Thus, rather dramatic differences in disposition would have to exist in order to explain the phar-

macological differences. In this study the comparative disposition and metabolism of the methadone isomers were investigated in order to resolve this issue.

MATERIALS AND METHODS

Materials

The enantiomers of methadone were generously supplied by Dr. A. Pohland and Mr. R. Booher of the Organic Chemistry Division, Lilly Research Laboratories. On the basis of thermal gradient analysis, the (+)-methadone nitrile intermediate was 99·2% optically pure and the (+)-methadone obtained from it was 99·83% optically pure. The hydrochloride salt of (+)-methadone had a specific optical rotation of $[\alpha]_D^{25} = +127{\cdot}4°$ (c = 1·0 in water) while the (−)-methadone hydrochloride had $[\alpha]_D^{25} = -125{\cdot}9°$ (c = 1·1 in water). Both (+)-methadone-[2-^{14}C], $[\alpha]_D^{25} = +128{\cdot}5°$ (H_2O), and (−)-methadone-[2-^{14}C], $[\alpha]_D^{25} = -128{\cdot}7°$ (H_2O), were purchased from New England Nuclear Corp. (Boston). In the studies, drug was dissolved in saline solution for administration; the doses refer to the hydrochloride salts.

Methods

Plasma and brain concentrations. In the equi-molar study (+) or (−)-[^{14}C]methadone was administered subcutaneously to male albino rats (Purdue-Wistar strain, 250 g) at a dose of 5 mg kg^{-1}. In the study in which equi-analgesic doses were investigated, unlabeled (+)-methadone was administered at 26 mg kg^{-1} while the (−)-methadone dose was 1·6 mg kg^{-1}. Three rats were killed at each time period. Blood was collected and plasma prepared by centrifugation at 1000 *g* for 10 min. The plasma was extracted in preparation for assay as described earlier (Sullivan & Blake, 1972). Brain was excised immediately after decapitation and homogenized manually in 8 ml of 0·1 N HCl. After precipitation of protein with 35% perchloric acid (2 ml), the homogenate was taken to pH 9·5 and extracted twice with butyl chloride (14 ml). The butyl chloride extract was back washed with base and the methadone then extracted into N HCl (5 ml). The HCl extract was washed with heptane, made basic and then extracted with butyl chloride. The butyl chloride extract was evaporated to dryness under nitrogen in preparation for gas-liquid chromatography (g.l.c.). The g.l.c. assay for methadone used was that of Sullivan & Blake (1972). When applied to rat studies the lower limit of sensitivity for plasma was 50 ng ml^{-1} while the sensitivity for brain extract was 200 ng g^{-1}. In the equi-molar dose study, the radiocarbon content of the plasma samples was determined by standard counting procedures.

Metabolite patterns. The metabolite patterns were determined by analysis of pooled 24 h bile collections for both conjugated and unconjugated metabolites. The details of the procedures used for the identification of all metabolites, except metabolite V (Fig. 1) have been previously described (Pohland, Boaz & Sullivan, 1971; Sullivan, Due & McMahon, 1972a, 1973; Sullivan & Due, 1973; Sullivan, Smits & others, 1972b). In general, pure synthetic standards were available by the courtesty of Dr. Pohland and Mr. Booher for all metabolites except the phenols, III, IV and V (Fig. 1). Metabolites were identified with known compounds on their t.l.c. and g.l.c. behaviour and mass fragmentation spectra. The phenols, all of which were excreted as conjugates, were extracted after hydrolysis and converted to methyl ethers by reaction with diazomethane. The identification of the methyl ethers of III and IV has been reported previously, (Sullivan & others, 1972a). The assignment of the structure of metabolite V as a dihy-

droxy derivative of II was based on its chemical properties, the similarity of the mass spectrum to that of IV (excepting that principal ions were 30 a.m.u. greater showing a second methoxy to be present) and the fact that administration of II to rats led to the excretion of substantial amounts of both IV and V. It is clear that metabolites III and IV from either (+)- or (−)-methadone should occur as a mixture of two diastereoisomers. The t.l.c. and g.l.c. systems used did not separate these isomers, however, and no attempt was made to distinguish them.

In vitro *demethylation.* Livers were removed from decapitated 300 g male rats, immediately homogenized in 0·1 M phosphate buffer (4 ml, pH 7·4) in a Potter-Elvehjem homogenizer at 0° using a Teflon pestle for 1·5 min to obtain maximum activity. The homogenate was then centrifuged at 15 000 *g* for 30 min and the supernatant containing the soluble plus microsomal fraction was decanted.

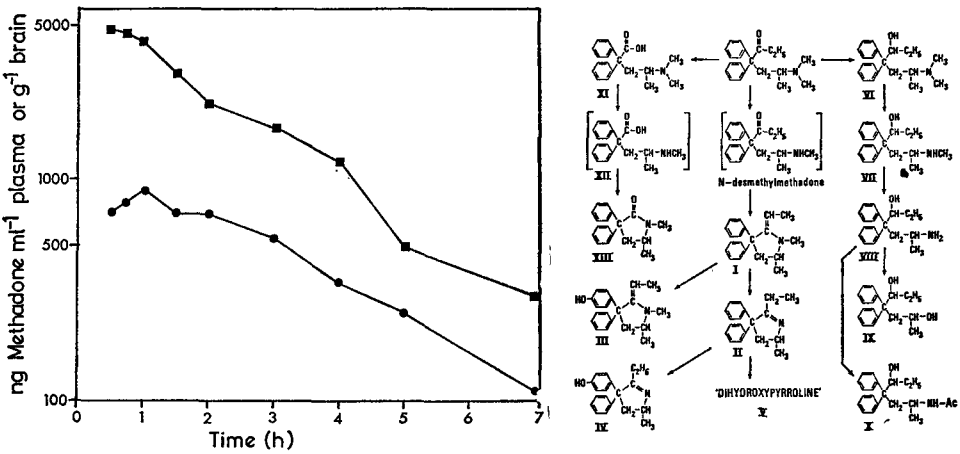

FIG. 1. Brain and plasma concentrations of (+)-methadone in the rat at a subcutaneous dose of 26 mg kg^{-1}.

FIG. 2. The biotransformation of methadone in the rat. Both isomers were metabolized by identical routes. The compounds in brackets are postulated metabolic intermediates which were not isolated. Metabolites III, IV and V were excreted as conjugates in bile. Metabolite V is identical to Metabolite IV except that two aromatic hydroxyls are present.

For the *in vitro* demethylation experiments a mixture of 1 ml of supernatant fraction (200 mg of liver) together with 300 μmol of phosphate buffer, 0·5 μmol of TPN^+, 11 μmol of glucose 6-phosphate, 45 μmol of semicarbazide, 50 μmol of $MgCl_2$ and 2 ml H_2O was added to 5 μmol of substrate. The cofactor concentrations were chosen as those that gave maximum formaldehyde production from butylamine. The reaction mixtures were then incubated in air with shaking at 37° for 30 min. The reactions were terminated by the addition of 4 ml of 10% $ZnCl_2$ and 2 ml of saturated $Ba(OH)_2$ and the amount of formaldehyde formed was determined.

RESULTS AND DISCUSSION

The results of the equi-molar dose study (Table 1), show that the concentrations of (+)-methadone are about 25% lower than those found for (−)-methadone. Plasma concentrations of methadone plus metabolites (i.e. total radiocarbon levels) were also lower for (+)- than for (−)-methadone. This result is consistent with the report of Sung & Way (1963) that tissue concentrations of (+)-methadone are slightly lower. Whether these small differences between the levels of (+)- and (−)-methadone are

Table 1. *Plasma concentrations of total radiocarbon and of methadone in rats receiving (+)- or (−)-[^{14}C]methadone HCl at a dose of 5 mg kg^{-1} s.c. (mean of 3 rats)*

Hours	Methadone concn (g.l.c.) (−) -isomer ng ml^{-1}	(+) -isomer ng ml^{-1}	Total radiocarbon concentrations (in methadone equivalents)	
0·5	233 s.d. 99	179 s.d. 94	650 s.d. 140	470 s.d. 30
1	188 s.d. 190	155 s.d. 127	530 s.d. 40	520 s.d. 400
2	103 s.d. 20	<50	810 s.d. 90	390 s.d. 90
4	<50	<50	370 s.d. 170	350 s.d. 50

due to a different rate of absorption, excretion or metabolism is not known. In any event, the differences in blood concentrations are too small to account for the nearly 25 fold difference in *in vivo* analgesic activity in the rat.

Brain concentrations of methadone were not measured in the above experiment because of the low sensitivity of the g.l.c. method in this tissue (200 ng g^{-1} brain compared to 50 ng ml^{-1} plasma). At a dose of 26 mg kg^{-1} (s.c.) of (+)-methadone however, it was possible to obtain both plasma and brain concentrations. The results (Fig. 1) show that (+)-methadone is readily absorbed after subcutaneous injection and that it readily crosses the blood brain barrier. The brain/plasma ratio at 1 h (4·7) is similar to that reported by Misra & Mulé (1973). Similar data could not be obtained with (−)-methadone since at 26 mg kg^{-1} (s.c.) it was lethal to rats. In the rat tail jerk analgesic assay, a dose of 1·6 mg kg^{-1} (s.c.) of (−)-methadone HCl was equivalent to 26 mg kg^{-1}, (s.c) of (+)-methadone (Smits & Myers, 1974). When rats were given (−)-methadone, 1·6 mg kg^{-1} (s.c.), both the 1 h plasma concentrations (less than 50 ng ml^{-1}) and the 1 h brain concentration (less than 200 ng g^{-1} brain) were below the sensitivity of the analytical method. Thus at equi-analgesic doses (+)-methadone produces brain concentrations of drug at least 25 times as high as those of (−)-methadone (4 200 ng g^{-1} brain *vs* <200 ng g^{-1} brain at 1 h), added proof that (+)-methadone is intrinsically much less active than is (−)-methadone.

A qualitative study of the metabolic pattern of both (+)- and (−)-methadone was also made. Since in the rat most of the metabolites are excreted in bile (90·1% in 24 h for (+)-, 84·2% for (−)-methadone) only those metabolites excreted in the 0–24 h bile were investigated. The metabolic patterns for the two isomers were identical. All of the metabolites shown are inactive as analgesics with the exception that metabolites VII and VIII from (+)-methadone but not (−)-methadone are active and may contribute to the activity of (+)-methadone (Sullivan & others, 1972b; Smits & Myers, 1974). Misra & Mulé (1972) have speculated that (−)-methadone is metabolized in brain to a long-acting metabolite which may be involved in tolerance development. Although no chemical evidence was presented by them, they suggested that this metabolite may be (−)-*p*-hydroxydinormethadol. While brain metabolites were not investigated in the present study no evidence was found for such a metabolite in rat bile. It should also be noted that the optical centre at carbon 6 of (−)-methadone and of (−)-methadol are of opposite configuration (Pohland, Marshall & Carney, 1949; Sullivan & others, 1972b). Therefore methadols formed enzymatically by reduction of the 3-keto group of (−)-methadone cannot have the analgesically active (−)-configuration as proposed by Misra & Mulé. The formation of metabolites XI and XIII involves the loss of carbons 1 and 2 by oxidation. Thus in studies utilizing [1-^{3}H]methadone, $^{3}H_2O$ should appear as a metabolite. As the half-life of $^{3}H_2O$ in the rat is very long it is

possible that the long lived low tritium concentrations observed by Misra & Mulé (1973) may have been due in part to ^3H_2O.

Since the primary *in vivo* route of metabolism of methadone is via *N*-demethylation (Beckett, Taylor & others, 1968; Pohland, Boaz & Sullivan, 1971) one explanation for the somewhat lower blood concentrations of (+)-methadone would be that it was more readily demethylated than (−)-methadone. However in an early study at a single concentration Axelrod (1956) found that (−)-methadone was actually the preferred substrate in rat liver homogenates. Conversely, Beckett, Mitchard & Shihab (1971) found that (+)-methadone was a slightly better substrate for both rat and guinea-pig liver homogenates. More recently Alvares & Kappas (1972), using rat liver microsomes found no difference in the demethylation rates of (+)- and (−)-methadone. Similarly we find the relative V_{max} values for (+)- and (−)-methadone to be 2·49 ± 0·16 μmol g^{-1} liver h^{-1} and 2·76 ± 0·12 μmol g^{-1} liver h^{-1} respectively. The corresponding Km values were 3·24 ± 0·44 × 10^{-4} M and 2·67 ± 0·15 × 10^{-4}M. These results taken together suggest that there is little if any difference in the rate of *N*-demethylation of (+)- and/or (−)-methadone.

The results of our investigation show that the difference in analgesic activity between (+)- and (−)-methadone is not due to differences in disposition or metabolism but rather represents a true difference in intrinsic activity between (+)- and (−)-methadone molecules as suggested by Way and his associates. Recent advances in our understanding of the 'opiate' receptor (Pert & Snyder, 1973; Lowney, Schulz & others 1974; Wong & Horng, 1973; Terenius, 1973) suggest that this difference in intrinsic activity is a consequence of a different affinity for this stereoselective receptor.

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