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Intravenous kinetics and metabolism of [15,16-^{3}H]naltrexonium methiodide in the rat

A. L. MISRA*, R. B. PONTANI, N. L. VADLAMANI, *New York State Division of Substance Abuse Services, Testing and Research Laboratory, 80 Hanson Place, Brooklyn, New York 11217, State Unviersity of New York, Department of Psychiatry, Health Science Center, Brooklyn, New York 11203, USA*

After a 4 mg kg^{-1} bolus intravenous dose of [15,16-^{3}H]naltrexonium methiodide to the rat, brain to plasma concentration ratios of the compound were 0·031 to 0·228 between 0·25 to 6 h after injection and the $t_{\frac{1}{2}\beta}$ in plasma and brain were 2·92 and 7·61 h, respectively. Ethyl acetate-extracted radioactivity due to metabolites in plasma decayed with $t_{\frac{1}{2}\beta}$ 1·83 h and the ratios of plasma concentration of metabolites to quaternary compound between 0·25 and 6 h were 0·014–0·026. Only unconjugated 7,8-dihydro-14-hydroxynormorphine, naltrexone and traces of 7,8-dihydro-14-hydroxynormorphinone were the metabolites in plasma. Naltrexone (but not normetabolites) was present only in traces in brain up to 0·5 h after injection and not at later times.

Quaternary narcotic antagonists have assumed increasing importance as pharmacological probes for differentiating the central and peripheral components of opiate drug effects and have been used in studies of gastrointestinal motility, ingestive behaviour, antinociception, precipitated opiate withdrawal, blockade of opiate self-administration and discriminative stimulus (Bianchetti et al 1983; Valentino et al 1983; Brown & Goldberg 1985). Quaternary opiate antagonists have low affinity for opiate receptors compared with their non-polar tertiary congeners (Kosterlitz & Waterfield 1975). Thus quaternary naltrexone was 26–77 times less potent than naltrexone in in-vitro assays of displacement of stereospecific etorphine binding in rat brain membranes and inhibition of electrically evoked contraction of the isolated guinea-pig ileum (Valentino et al 1981). It is generally assumed that quaternary opiate antagonists have little or no permeability to the blood-brain barrier relative to their tertiary congeners (Russell et al 1982). In spite of a number of studies, no information is available on the pharmacokinetics and metabolism of these compounds. The present studies were designed to obtain information on the intravenous kinetics and metabolism of [15,16-^{3}H]naltrexonium methiodide in the rat.

Materials and methods

Preparation of [15,16-^{3}H]naltrexonium methiodide. The preparation was an adaptation of an earlier procedure (Koczka & Bernath 1967). [15,16-^{3}H]Naltrexone (obtained from Research Triangle Institute through the courtesy of Dr R. Hawks, NIDA, Rockville, Md.) suitably diluted with non-labelled naltrexone (171 mg), was dissolved in absolute methanol (2 mL) and allowed to react with methyl iodide (0·12 g) at ambient temperature (25 °C) for 2 weeks. The solution was evaporated to dryness under nitrogen and the residue triturated with ether to furnish crude naltrexonium methiodide (42 mg). The unreacted naltrexone from mother liquor was recycled as above with methyl iodide (90 mg) to furnish another crop of the crude quaternary compound (47 mg), which was purified by repeated precipitation from methanol solution with ether and finally by preparative thin layer chromatography (20 × 20 cm silica gel sheets, Gelman Instrument Co., Ann Arbor, Mich.) using ethyl acetate–methanol–conc. ammonia (17 : 2 : 1 v/v) as the developing solvent. The radioactive band of the quaternary compound at the origin was eluted from the sheets by continuous extraction with absolute methanol. The final recrystallization of the quaternary compound (Fig. 1) was achieved using methanol–ether (26 mg, sp. act. 16·2 μCi mg^{-1}). The compound gradually turned brown at 140 °C and did not melt up to 270 °C. The radiochromatographic scan of the purified product (Fig. 2A) showed that it was free

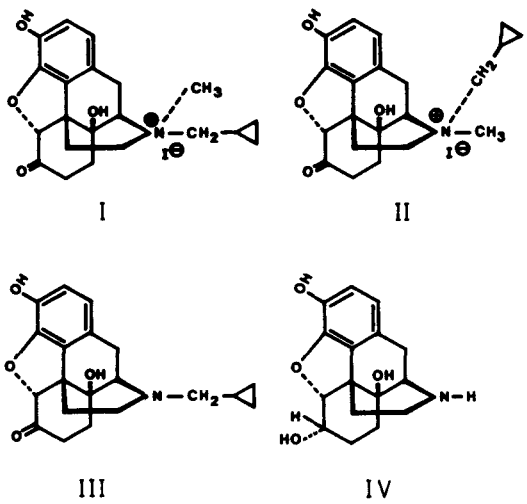

FIG. 1. Structures of (I) naltrexonium methiodide, (II) its stereoisomer and two metabolites, (III) naltrexone and (IV) 7,8-dihydro-14-hydroxynormorphine.

* Correspondence.

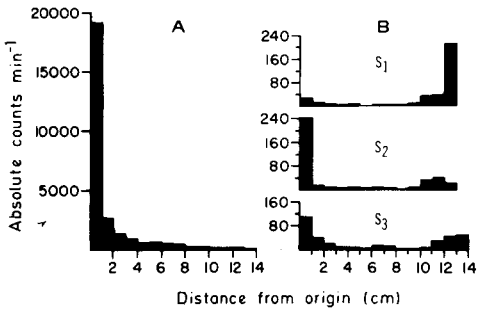

FIG. 2. A. Radioscan of thin layer chromatogram of purified [^{3}H]naltrexonium methiodide used for injection shows freedom from naltrexone (R_F 1·0) in solvent system S_1. B. Radioscans of thin layer chromatograms of ethyl acetate extract of pooled plasma in solvent systems S_1, S_2 and S_3. For details on R_F values of standard compounds and composition of solvent systems see Materials and methods.

from naltrexone (R_F 1·0). The injection solution (4 mg mL^{-1}) was prepared in 0·9% saline.

Estimation of [^{3}H]naltrexonium methiodide. Aliquots (0·2 mL) of plasma diluted (1 : 5) with distilled water or tissue homogenates (20% w/v in 0·5 M HCl) and Protosol tissue solubilizer (1mL, New England Nuclear, Boston, Mass.), were allowed to stand overnight at ambient temperature (25 °C) and the radioactivity counted using 10 mL Ecolite liquid scintillation counting solution (West Chem, San Diego, California) and the counts corrected for quenching using [^{3}H]toluene as an internal standard.

Animal experiments. Male Wistar rats (175–220 g) were injected intravenously with a 4 mg kg^{-1} bolus dose of [^{3}H]naltrexonium methiodide and killed at appropriate times to obtain blood and tissues for analyses.

Estimation of extractable metabolites. Aliquots (2 mL) of diluted plasma or tissue homogenates containing 1 mL non-labelled naltrexone as carrier (500 μg mL^{-1}) were adjusted to pH 9–9·5 with 1 M NaOH, buffered with 2 mL 40% K_2HPO_4 solution and extracted with 15 mL ethyl acetate by shaking for 30 min and centrifugation for 15 min. The aqueous layer was aspirated off and the organic solvent phase washed by shaking with 4 mL 4% K_2HPO_4 solution and centrifugation for 10 min. The aqueous phase was aspirated and 10 mL organic solvent phase transferred to counting vials, and evaporated to dryness on a Fisher Slide Warmer (45 °C). The residue was dissolved in 0·5 mL methanol and radioactivity counted using 10 mL Ecolite solution. In-vitro recovery of quaternary compound by this procedure was 0·75%.

For the identification of metabolites, pooled samples of diluted plasma and liver homogenates were extracted by the above procedure without the addition of non-labelled carrier and the residues from ethyl acetate extracts submitted to TLC in solvent systems: S_1, ethyl acetate–methanol–conc. ammonia (17 : 2 : 1 v/v); S_2, n-hexane–ethyl acetate–0·15 M ammonia (60 : 40 : 0·1 v/v); S_3, ethyl acetate–methanol–conc. ammonia (96 : 3 : 1 v/v). The R_F values of standard naltrexone, 7,8-dihydro-14-hydroxynormorphinone and 7,8-dihydro-14-hydroxynormorphine in solvent system S_1 were 1·0, 0·96 and 0·89; in S_2, 0·95, 0·04 and 0·0; in S_3, 1·0, 0·50 and 0·34, respectively.

Results

The data on decay of plasma and brain concentration of [^{3}H]naltrexonium methiodide after a 4 mg kg^{-1} i.v. bolus injection in the rat are shown in Fig. 3. The brain to plasma concentration ratios for the quaternary compound at indicated times between 0·25 to 6 h were 0·031, 0·043, 0·075, 0·133, 0·198 and 0·228. Thus, the quaternary compound was able to penetrate the blood–brain barrier in significant concentrations after i.v. injection. The pharmacokinetic parameters calculated from the palsma values were half-lives $t_{\frac{1}{2}}\alpha$ 0·45 h, $t_{\frac{1}{2}}\beta$ 2·92 h; elimination constant Kel 0·86 h^{-1}, apparent volume of distribution Vdβ 0·82 Lkg^{-1}, volume of central compartment Vc 0·23 Lkg^{-1} and total body clearance (plasma) 3·25 mL min^{-1} kg^{-1}. The decay of the quaternary compound from brain occurred with $t_{\frac{1}{2}}\beta$ 7·61 h, implying a slow egress of the compound from brain.

The decay of radioactivity of ethyl acetate-extractable metabolites in plasma (Fig. 4) occurred with $t_{\frac{1}{2}}\alpha$ 0·81 h and $t_{\frac{1}{2}}\beta$ 1·83 h. Thin layer chromatographic experiments and radioscanning of chromatograms (Fig. 2B) provided tentative evidence for the presence in plasma of free 7,8-dihydro-14-hydroxynormorphine, naltrexone, 7,8-dihydro-14-hydroxynormorphinone in the ratios of 63, 32 and 5, respectively. Extraction of pooled plasma or liver samples with ethyl acetate before and after acid

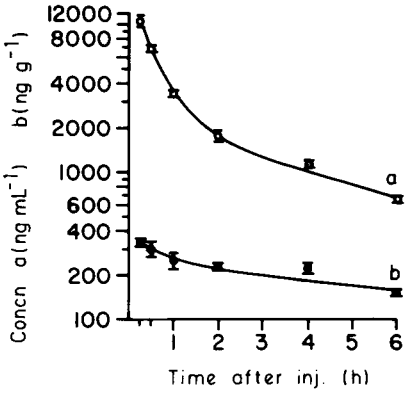

FIG. 3. Decay of concentration of [^{3}H]naltrexonium methiodide in (a) plasma and (b) brain of rats injected with a 4 mg kg^{-1} i.v. bolus dose. Data represent mean ± s.e.m. (ng mL^{-1} plasma or g^{-1} brain) from 3 animals at each time.

or enzymatic hydrolysis with glusulase suggested that no conjugated metabolites were present. The ratios of concentration of metabolites in plasma (as naltrexone equivalents mL^{-1}) to plasma concentration of [^{3}H]naltrexonium methiodide at various times between 0·25 to 6 h (Fig. 4) were 0·014, 0·015, 0·023, 0·026, 0·021 and

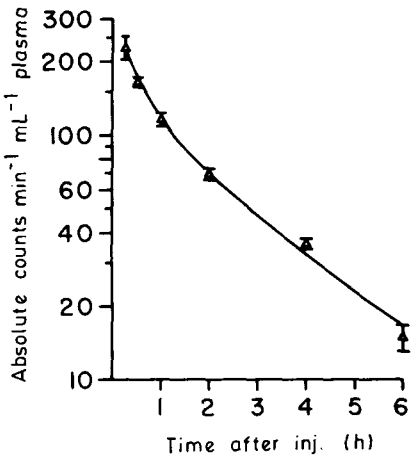

FIG. 4. Decay of radioactivity of ethyl acetate-extracted metabolites from plasma of rats injected with 4 mg kg^{-1} i.v. bolus dose of [^{3}H]naltrexonium methiodide. Data represent absolute counts min^{-1} mL^{-1} plasma (mean ± s.e.m.) from 3 animals at each time.

0·015. Extraction of brain samples with ethyl acetate showed the presence of naltrexone in concentrations of 5·3 ± 0·3 and 4·3 ± 0·3 ng g^{-1} tissue (mean ± s.e.m.) only at 0·25 and 0·5 h after injection of quaternary compound and not at later times. No other metabolites were detected in brain.

Discussion

Previous work (Koczka & Bernath 1967) has shown that during quaternization of the tertiary amine with methyl iodide, the product with the methyl substituent in an axial position was obtained in higher and a stereoisomeric product (Fig. 1) in much lower yield (15–25%). Low brain to plasma concentration ratios of the quaternary compound after i.v. injection indicated the entry of the compound into the central nervous system in small amounts. The penetration of the blood-brain barrier by the quaternary compound will be expected to be even less by the subcutaneous, intraperitoneal or intramuscular routes, as shown by the lack of morphine antagonist activity of this compound even in 100–300 mg kg^{-1} doses intraperitoneally (Russell et al 1982) or its complete ineffectiveness in precipitating withdrawal in morphine-dependent monkeys (Valentino et al 1981), even in doses up to 32 mg kg^{-1} intramuscularly, or 10 000-fold higher potency in reversing catalepsy induced by morphine in rats when given intracerebroventricularly rather than by subcutaneous injection (Brown et al 1983).

Naltrexonium methiodide was metabolized to a small extent by *N*-dealkylation and 6-keto group reduction. Low ratios (0·014–0·026) of the plasma concentration of metabolites to naltrexonium methiodide indicated that only small amounts of drug were metabolised to 7,8-dihydro-14-hydroxynormorphine, naltrexone and 7,8-dihydro-14-hydroxynormorphinone. Oxymorphone was not detected as a metabolite of the quaternary compound. The epimeric nature whether 6α- or 6β-, of 7,8-dihydro-14-hydroxynormorphine is unclear at this time. Only trace amounts of naltrexone were detected in brain at 0·25 and 0·5 h but not at later times after i.v. injection. The normetabolites were not observed in brain and were rapidly eliminated from plasma and liver.

REFERENCES

Bianchetti, A., Nisato, D., Sacilotto, R., Dragonetti, M., Picerno, N., Tarantino, A., Manara, L., Angel, L. M., Simon, E. J. (1983) Life Sci. 33: 415–418

Brown, D. R., Goldberg, L. I. (1985) Neuropharmacology 24: 181–191

Brown, D. R., Robertson, M. J., Goldberg, L. I. (1983) Ibid. 22: 317–321

Koczka, K., Bernath, G. (1967) Acta Chim. Acad. Sci. (Hung.) 51: 393–402

Kosterlitz, H. W., Waterfield, A. A. (1975) Ann. Rev. Pharmacol. Toxicol. 15: 29–47

Russell, J., Bass, P., Goldberg, L. I., Schuster, C. R., Merz, H. (1982) Eur. J. Pharmacol. 78: 255–261

Valentino, R. J., Herling, S., Woods, J. H. Medzihradsky, F., Merz, H. (1981) J. Pharmacol. Exp. Ther. 217: 652–659

Valentino, R. J., Katz, J. L., Medzihradsky, F., Woods, J. H. (1983) Life Sci. 32: 2887–2896