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Methadone *N*-oxide in the rhesus monkey

C. M. DAVIS, D. C. FENIMORE, *Texas Research Institute of Mental Sciences, 1300 Moursund Avenue, Houston Texas 77030, U.S.A.*

Conflicting observations have created uncertainty concerning the validity of methadone *N*-oxide as a metabolic product of methadone. Methadone *N*-oxide was first isolated and identified as a metabolite of methadone in hepatic microsomal preparations from several animal species (Beckett, Mitchard & Shihab, 1971). This metabolite has since been isolated from the urine of addicts on methadone maintenance and in patients after a single dose of methadone (Beckett, Vaughan & Essien, 1972). Misra, Mulé & others (1973) and Misra, Bloch & others (1974) found methadone *N*-oxide in the urine of rats and dogs after administration of radiolabelled methadone and suggested that it was a major metabolite of methadone. Other investigators (Baselt & Casarett, 1972; Sullivan & Due, 1973; Änggärd, Gunne & others, 1975) were unable to demonstrate the presence of methadone *N*-oxide as a metabolite of methadone, and one group (Sullivan, Due & McMahon, 1973) suggested that it occurs as an artifact in the urine of man and is probably due to improper storage of urine samples.

In the Rhesus monkey 6 h after intramuscular injection of 0·25 mg kg^{-1} (135 mCi $mmol^{-1}$) of [1-^{3}H]-(—)-methadone HBr (New England Nuclear, Boston, Mass.) a metabolite of methadone was found in all tissues and fluids assayed by t.l.c. (Davis & Fenimore, 1975) which corresponded to the R_F value of a methadone *N*-oxide standard (standard kindly supplied by Prof. A. H. Beckett, London). When [1-^{3}H]-(—)-methadone HBr was added to a tissue homogenate and chromatographed 0·66% ± 0·11 of the radioactivity was assayed to be methadone *N*-oxide.

To minimize the production of methadone *N*-oxide as an artifact of sample preparation, precautions were taken. After dissection, tissues were immediately rinsed with cold physiological saline, blotted dry, sealed in plastic bags and frozen. The frozen samples were crushed in liquid N_2 before homogenization mixed with 4 volumes of water in a cooled glass tube fitted with a Teflon pestle (Arthur Thomas Company, Philadelphia) and homogenized with a variable speed stand mounted motor (Venitron Medical Products, Inc., Carlstadt, N.J.). After extraction with diethyl ether, the organic layer was removed under a stream of N_2 before separation by t.l.c. Because diethyl ether in the presence of air and light produces diethyl peroxides and peroxides form methadone *N*-oxide with methadone, care was taken to avoid using diethyl ether contaminated with peroxides. Newly opened containers of ether were used and peroxide was monitored with potassium iodide-starch test paper.

With these precautions taken, we found a zone of radioactivity appeared on the thin-layer chromatograms which matched the methadone *N*-oxide standard. The percentages of radioactivity in the tissues or fluids which represent methadone *N*-oxide range from insignificant amounts (0·3%) in urine 6 h after drug administration to higher values in spleen (29%), spinal cord (13%), lung (19%), heart (16%), adrenals (22%). To evalulate the possible formation of the *N*-oxide metabolite as an artifact, a kidney homogenate was extracted, dried and redissolved in benzene–methanol (1 : 1 v/v), and periodically for about one and a half months portions of this extract were rechromatographed nine times. The mean percentage of methadone *N*-oxide was 3·46 with a variance of 0·11 when the sample was held at 4° between analysis. When the sample was stored at 30° for 25 days the final analysis showed a twofold increase (7·14%) in the amount of methadone *N*-oxide. Therefore, with adequate safeguards, such as minimizing the oxidative environment, i.e. drying under N_2 and refrigeration, the artifactual formation of methadone *N*-oxide was minimized. While methadone *N*-oxide does occur as an oxidative product of metha-

• Correspondence.

done this does not rule out its metabolic formation as suggested by Beckett & others (1971). Because of our efforts to minimize the possible oxidation of methadone to methadone *N*-oxide during the extraction and assay and because the percentage of methadone *N*-oxide did not increase while the tissue extracts were held at 4°, we have concluded that the methadone *N*-oxide detected in this study is a metabolic product of methadone in the Rhesus monkey.

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Differences between some biological properties of enantiomers of alkyl *S*-alkyl methylphosphonothioates

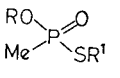
C. RICHARD HALL, THOMAS D. INCH*, ROBERT H. INNS, ALAN W. MUIR, DAVID J. SELLERS, ANDREW P. SMITH,
Chemical Defence Establishment, Porton Down, Salisbury, Wiltshire SP4 0JQ, U.K.

During recent attempts to clarify some aspects of the therapeutic properties of oximes and anti-acetylcholine drugs against poisoning by organophosphorus anticholinesterases, it was observed that whereas a mixture of atropine and pyridine-2-aldoxime methylmethanesulphonate (P2S) provided considerable protection against poisoning by *S*(−)ethyl-*S*-propyl methylphosphonothioate [I(−)], the same mixture provided insignificant protection against the enantiomer, (+)ethyl-*S*-propyl-methylphosphonothioate [I(+)] (see below). This observation prompted a more detailed examination of the series of alkyl-*S*-alkyl methylphosphonothioates listed in Table 1. The following aspects were investigated: (i) inhibition of acetylcholinesterase (EC 3.1.1.7); (ii) reactivation of inhibited acetylcholinesterase by P2S; (iii) blockade of tetanic response of the *in vitro* rat phrenic nerve/diaphragm preparation and its reversal by P2S; (iv) restoration by P2S of neuromuscular function *in vivo* in the gastrocnemius muscle of the rat, previously blocked by administration of 2 LD50's of an anticholinesterase; (v) LD50 values and the protection given by atropine and P2S. The results of these studies are summarized or appropriate examples are given in Table 2 and Figs 1 and 2.

The rank order of the results obtained from the *in vitro* experiments involving measurements of the second order rate constants of inhibition of acetylcholinesterase and of concentrations giving equal degrees

* Correspondence.

Table 1. *Alkyl S-alkyl methylphosphonothioates.* The racemic alkyl-*S*-alkyl methylphosphonothioates were prepared from the appropriate thioacids and alkyl bromides (Gazzard, Sainsbury & others, 1974). The enantiomers were prepared similarly but using optically active thioacids (Boter & Platenburg, 1967). The optical purity of the thioates was checked by the nmr method using a chiral shift reagent (Hall, Inch & others, 1975) or by stereospecific synthesis (Cooper, Hall & Inch, 1975).



Compound No.	R	R¹	Configuration	$[\alpha]_D^0$ (in $CHCl_3$, *c* 2)
I(±)	Et	nPr	Rac	
I(+)	Et	nPr	*R*	+51
I(−)	Et	nPr	*S*	−52
II(±)	Et	iPr	Rac	
II(+)	Et	iPr	*R*	+40
II(−)	Et	iPr	*S*	−40
III(±)	Et	nBu	Rac	
III(+)	Et	nBu	*R*	+51
III(−)	Et	nBu	*S*	−52
IV(±)	Et	nPentyl	Rac	
IV(+)	Et	nPentyl	*R*	+47
IV(−)	Et	nPentyl	*S*	−47
V(±)	cy.Pentyl	Me	Rac	
V(+)	cy.Pentyl	Me	*R*	+70
V(−)	cy.Pentyl	Me	*S*	−68
VI(±)	cy.Pentyl	nPr	Rac	
VI(+)	cy.Pentyl	nPr	*R*	+44
VI(−)	cy.Pentyl	nPr	*S*	−45
VII(±)	Et	$CH_2CH_2NiPr_2$	Rac	
VII(+)	Et	$CH_2CH_2NiPr_2$	*R*	−12
VII(−)	Et	$CH_2CH_2NiPr_2$	*S*	