Vol. 166, No. 1, 1990 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 15, 1990 Pages 238-244

IDENTIFICATION OF A POTENTIALLY NEUROTOXIC PYRIDINIUM METABOLITE OF HALOPERIDOL IN RATS

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Received November 21, 1989

SUMMARY. *In vivo* metabolic studies have revealed that haloperidol is converted to the corresponding pyridinium metabolite which has been characterized in both urine and brain tissues isolated from haloperidol treated rats. Unlike the corresponding conversion of the structurally related Parkinsonian inducing agent MPTP to the ultimate neurotoxic pyridinium metabolite MPP+, the oxidative biotransformation of haloperidol is not catalyzed by MAO-B. Microdialysis studies in the rat indicate that intrastriatal administration of this pyridinium metabolite is about 10% as effective as MPP+ in causing the irreversible depletion of striatal nerve terminal dopamine. The results point to the possibility that some of the neurological disorders observed in experimental animals and man during the course of chronic haloperidol treatment may be mediated by this pyridinium metabolite.

INTRODUCTION. New insights into the molecular events involved in neurodegenerative processes have been gained through recent mechanistic studies on the potent human Parkinsonian inducing neurotoxin MPTP (**1**, Figure 1) (1). MPTP's neurotoxic effects are mediated by its brain MAO-B generated pyridinium metabolite MPP+ (**3**) (2), a non-selective toxin (3) which is formed via the dihydropyridinium intermediate MPDP+ (**2**) and which depletes cellular ATP by inhibiting mitochondrial respiration (4). *In vivo* microdialysis studies (5) and *in vitro* studies on the inhibition of mitochondrial inhibition (6) have shown that a variety of pyridinium derivatives have neurotoxic potential. Therefore, the biotransformation of structurally related piperidine derivatives to pyridinium metabolites may be of neurotoxicological importance.

These considerations have prompted us to pursue studies designed to evaluate the metabolic fate and biochemical toxicological properties of the clinically important piperidinol antipsychotic agent haloperidol (HAL, **4**). Haloperidol is structurally related to MPTP both in terms of its 1,4-disubstitution pattern and the oxidation state of the

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0006-291X/90 $1.50

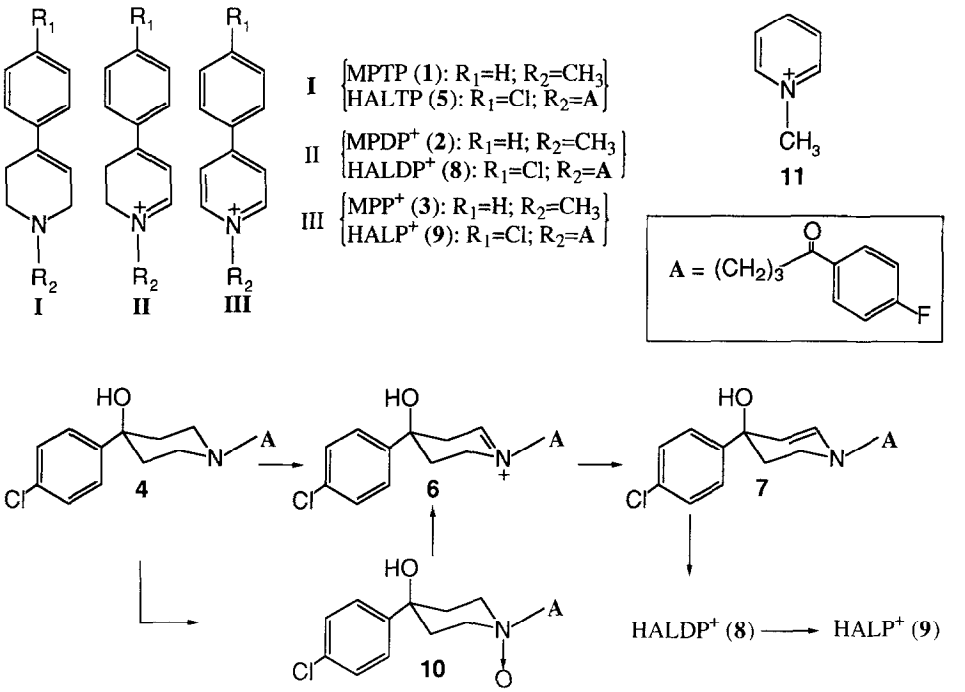

Figure 1. Chemical structures of compounds discussed in the text.

heterocyclic moiety. Thus simple dehydration of the the 4-piperidinol ring system of haloperidol leads directly to the haloperidol tetrahydropyrdinium derivative HALTP (**5**), a cyclic tertiary allylamine which bears the same 1,2,3,6-tetrahydropyridine moiety present in MPTP. α-Carbon oxidation of haloperidol would be expected to lead to the corresponding haloperidol dihydropyridinium product HALDP+ (**8**) via intermediates **6** and **7** (Figure 1) since aminoenols such as **7** undergo spontaneous dehydration (7). Furthermore, the inherent chemical instability of dihydropyridinium derivatives (8) suggests that compound **8**, if formed, would undergo spontaneous oxidation to the haloperidol pyridinium product HALP+ (**9**). In this communication we summarize the preliminary results obtained from our efforts to characterize the *in vivo* metabolic conversion of haloperidol to the pyridinium species **9** and to evaluate the nigrostriatal toxicity of this MPP+ analog.

MATERIALS AND METHODS. Chemical Studies: Haloperidol {4-(4-chlorophenyl)-1-[4-(4-fluorophenyl]-4-oxobutyl-4-piperidinol, **4**} was obtained from Sigma Chemical Co, St. Louis, MO and MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) and MPP+ (1-methyl-4-phenylpyridinium iodide) from Research Biochemicals, Inc., Natick, MS. The synthesis of 1-methylpyridinium iodide (**11**) was achieved according to the literature (9). The haloperidol tetrahydropyridine analog **5** was obtained by treatment of haloperidol with aqueous HCl. The iodide salt of the haloperidol pyridinium species **9** {4-(4-chlorophenyl)-1-[4-(4-fluorophenyl)-4-oxobutylpyridinium iodide} was prepared by initial oxidation of haloperidol with *m*-chloroperoxybenzoic acid and treatment of the resulting N-oxide **10** with trifluoroacetic anhydride to yield the dihydropyridinium intermediate **8** which underwent subsequent autoxidation to **9**. The details of these

syntheses will be reported separately. All compounds were characterized fully by spectroscopic and elemental analyses. HPLC grade solvents were obtained from Fisher Scientific Co., Springfield, NJ. HPLC-diode array analyses were carried out using a Beckmann dual pump system and a Hewlett-Packard Model 1040A diode array detector. An Alltech 5 μm amino (NH_2) column (25 cm x 4.6 mm) was used with ethanol/acetonitrile (80/20) as the mobile phase which was delivered at a flow rate of 1.0 mL/min. HPLC-EI mass spectral studies employed a Waters 600 MS delivery system interfaced to a VG TRIO-2 mass spectrometer which was operated in the plasmaspray mode with a source temperature of 250 °C, capillary probe temperature of 120 °C and a discharge current of 0 μA. A Perkin Elmer 3 μm Pecosphere C-18CR column (3.3 cm x 4.6 mm) was used with a mobile phase consisting of 50% acetonitrile/0.1 M ammonium acetate (pH 1.8 adjusted with 1% trifluoroacetic acid) which was delivered at a flow rate of 0.7 mL/min. Full scans (450-150 daltons) were taken repetitively at a scan rate of 1 sec/decade.

Metabolic Studies: Male Sprague-Dawley rats (200-250 g) were administered haloperidol (5 mg/day) by daily gavage for 3 days. The animals were housed individually in metabolic cages and urine samples were collected every 8 hours over a 4 day period and were frozen until work-up. Thawed urine samples (10 mL) were extracted with chloroform (3 x 30 mL) and the combined extracts were dried by filtration through a short column of magnesium sulfate and concentrated to 0.5 mL. Aliquots (10 to 50 μL) of this concentrate were analyzed by the HPLC-diode array and HPLC-EI mass spectral procedures. After sacrificing the animals by exposure to CO_2, the entire brains were removed, homogenized in 10 mL 5% aqueous trichloroacetic acid and the resulting homogenates were centrifuged to remove precipitated protein. The supernatent fraction was extracted with 3 equal volumes of chloroform and the chloroform extracts treated in the same manner as described above for the urine extracts.

Microdialysis Studies: *In vivo* brain microdialysis in rats was performed as previously described (5). In brief, male Sprague-Dawley rats (Bantin-Kingman, 220-240 g) were anesthetized with chloral hydrate (450 mg/kg, i.p.) and were placed in a stereotaxic frame. Bilateral cannulas with a U-shaped dialysis membrane were inserted into both striata and the rats were allowed to recover. One day after surgery the striatum was perfused with a Ringer solution at a flow rate of 4.5 μL/min. Dialysate levels of dopamine were measured in 50 μL samples by on-line HPLC and electrochemical detection. After the basal outputs had stabilized, Ringer solutions of the haloperidol pyridinium species **9**, MPP^+ (both at 2 mM) or MP^+ (10 mM) were perfused intrastriatally for various periods (15 min to 24 hours) and the maximum release of dopamine was recorded. One day after drug treatment the effect of a 15 minute challenge perfusion with 10 mM MPP^+ on dopamine output (DA_{MPP^+}) was measured. All results are expressed as percentages of the initial basal dopamine release, which was 4.1 fmoles/min ± 0.48 (n = 24). The extent to which the test compound caused irreversible depletion of dopamine (depression in DA_{MPP^+}) is taken as a measure of nerve terminal damage.

RESULTS AND DISCUSSION. The extraction characteristics and chromatographic properties of the haloperidol pyridinium compound allowed us to develop a moderately sensitive (50 ng/mL detection limit) and highly specific assay for this compound in urine and brain tissue homogenates. Haloperidol and its principal reported urinary metabolites [the corresponding alcohol obtained following reduction of the butyrophenone carbonyl group (10), the C-4 glucuronide derivative and various phenylalkylcarboxylic acid derivatives (11)] do not absorb light above 220 nm and therefore are not detected in this assay. Figure 2 displays the HPLC chromatogram of a chloroform extract obtained from a haloperidol treated rat. The UV diode array spectrum

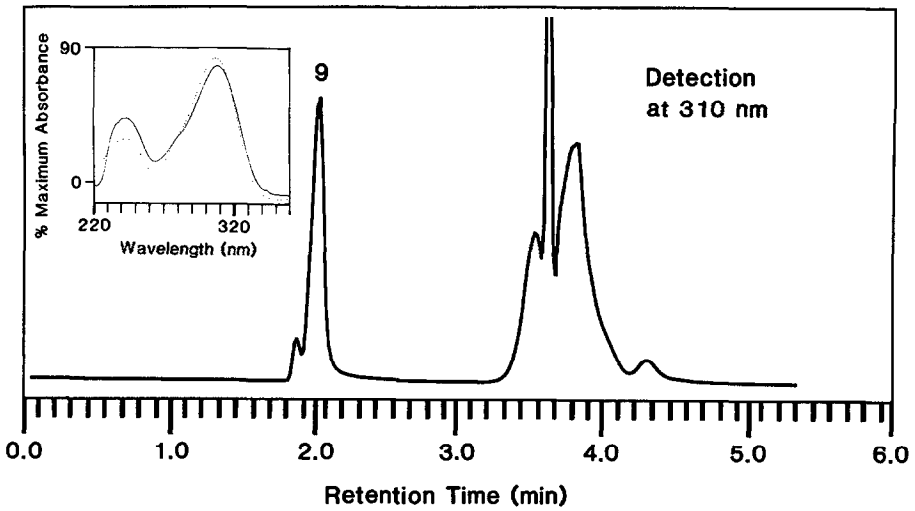

Figure 2. HPLC chromatogram of urine extract obtained from a haloperidol treated rat. The insert displays the UV diode array spectrum of the 2 minute eluting peak (solid line) and the corresponding spectrum obtained from a control urine sample spiked with 200 ng/mL of synthetic **9** (dotted line).

of the material present in the peak eluting at 2 minutes is reproduced in the insert (solid line) together with the corresponding spectrum obtained with control rat urine spiked with 200 ng/mL of synthetic pyridinium iodide (dotted line). These spectra are essentially identical to that obtained by scanning a solution of the pyridinium compound in a spectrophotometer. Confirmation of the structure of this haloperidol derived pyridinium metabolite was obtained by HPLC-EI mass spectral analysis. Figure 3 compares the mass spectral tracings obtained from the synthetic standard and the metabolite. Their identity is evident. Haloperidol itself was completely stable when stored in a control urine sample under the conditions used in these experiments. Assuming a quantitative recovery, we estimate the urinary concentration of this metabolite to be about 1 μg/mL. Low levels of this pyridinium metabolite (on the order of 100 ng/g whole brain homogenate) also were detected by HPLC-diode array analysis in extracts of whole brain homogenates prepared from haloperidol treated animals.

The pathway leading to the *in vivo* biotransformation of haloperidol to its pyridinium metabolite remains to be characterized. Attempts to identify the tetrahydropyridine **5** in the urine of haloperidol treated rats have been unsuccessful. Preliminary *in vitro* studies indicate that neither haloperidol nor **5** is a substrate for purified beef MAO-B (data not shown). This is not too surprising since structure-activity studies indicate that compounds bearing N-substituents larger than ethyl are not MAO-B substrates (12). A more likely pathway would involve the reaction sequence summarized in Figure 1 in which the initial oxidative conversion of haloperidol to the iminium intermediate **6** is catalyzed by cytochrome P-450, an enzyme system which is known to catalyze the α-

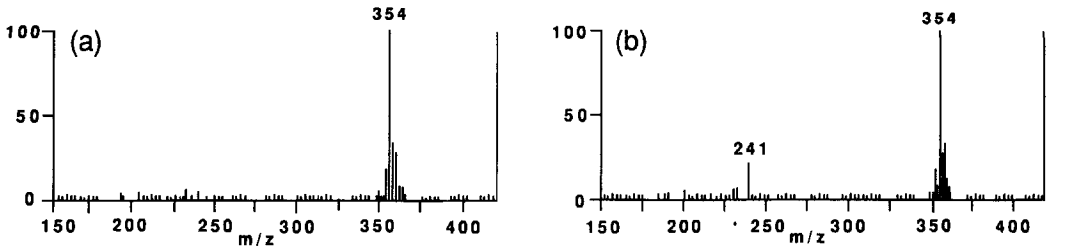

Figure 3. HPLC-EI mass spectra of (a) synthetic haloperidol pyridinium iodide and (b) the metabolite isolated from the urine of a haloperidol treated rat.

carbon oxidation of a variety of cyclic tertiary amines (13). Further *in vitro* studies will be required to address this issue.

The metabolic conversion of haloperidol to the corresponding pyridinium product establishes a novel pathway for compounds containing partially oxidized 6-membered nitrogen heterocyclic moieties. The potential toxicological consequences of this pathway were examined with the aid of an *in vivo* assay using intrastriatal microdialysis (14). This assay allows the intrastriatal administration of the hydrophilic pyridinium species and provides one measure of neuronal toxicity by demonstrating the extent to which dopamine containing nerve terminals are damaged following perfusion with a neurotoxin. Perfusion of the rat striatum with the neurotoxic metabolite MPP^+ (10 mM, 15 minutes) derived from MPTP leads to an initial 20,000% increase in the release of striatal dopamine and to nerve terminal degeneration as demonstrated by the absence of any effect on dopamine levels when the lesioned striatum is perfused with a 10 mM

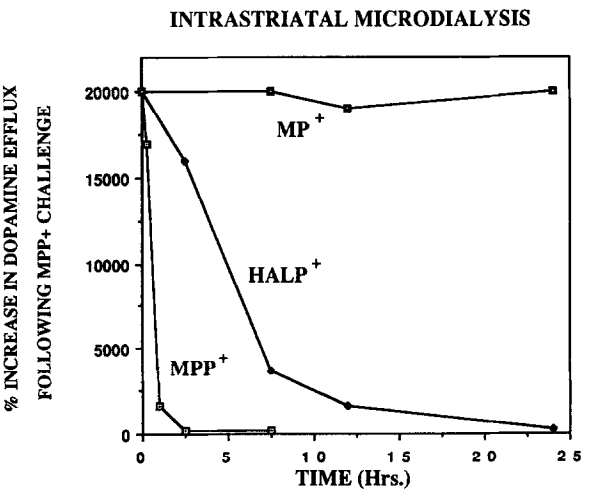

Figure 4. Increase in rat striatal dopamine efflux (% of control) caused by a 15 minute MPP+ challenge perfusion measured 24 hours following perfusion with haloperidol pyridinium derivative **9** (2 mM), MPP+ (2mM) and MP+ (10 mM).

challenge dose of MPP^+ 24 hours later (5, 15). In the present series of experiments, we compared the neurotoxicity of 2 mM solutions of MPP^+ and the pyridinium compound **9**. A 24 hour perfusion with 10 mM 1-methylpyridinium compound MP^+ (**11**) served as a negative control. Perfusion with 2 mM MPP^+ for 2.5 hours leads to a 22,000% increase of extracellular dopamine and to complete blockade of the dopamine releasing effect of the MPP^+ challenge perfusion 24 hours later. The acute dopamine releasing effect of the haloperidol derived pyridinium compound was 2,500% over basal levels; the complete blockade of the MPP^+ challenge induced release of dopamine was achieved after a perfusion time of 10 hours. The lack of any comparable toxic effect following a 24 hour perfusion period with 10 mM MP^+ argues that this blockade of MPP^+'s effect on dopamine release is a consequence of the neurotoxicity of compound **9** and is not due to deterioration of the preparation. **(See Fig. 4.)**

In summary, these results document a novel oxidative metabolic pathway for haloperidol in the rat. Since haloperidol is not a substrate for purified beef liver MAO-B, the formation of this pyridinium species is likely to be dependent on some other oxidase, possible cytochrome P-450. Although the possible toxicological significance of this transformation remains obscure, evidence obtained with the *in vivo* intrastriatal microdialysis assay is consistent with the possibility that compound **9** is neurotoxic. An MPTP-type mechanism has been considered by others in relationship to the drug induced movement disorders observed in haloperidol treated patients and experimental animals (16). Although the current consensus is that these effects are due to the compound's dopamine (D2) antagonist properties and the hypersensitivity that results from the "up-regulation" of D2-receptors (17), recent evidence suggests that no relationship exists between the presence or absence of movement disorders and the number or character of D2 binding sites present in post-mortem putamen samples obtained from patients treated with haloperidol (18). Furthermore, haloperidol failed to alter neostriatal D1-dopamine and D-2 dopamine receptor sensitivity in the rat after one year of chronic treatment. Consequently, a drug induced biochemical lesion of the nigrostriatal system may contribute to the extrapyramidal syndrome observed in patients on chronic haloperidol therapy. Additional studies to characterize the potential neurotoxicological consequences of the formation of the haloperidol pyridinium metabolite **9** are in progress.

ACKNOWLEDGMENTS. Supported by the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) Grant 5-R01-NS23066-02 and NATO grant CRG 890573. The authors thank Susan M. Bjorge for providing the HPLC-EI mass spectra.

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