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In-vitro metabolism of two mono-substituted piperazines using liver homogenates of rats and rabbits

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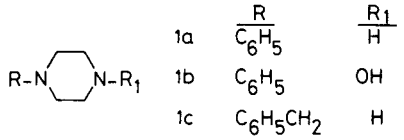
Dealkylations and *N*-oxidation are important pathways for the metabolism of substituted piperazines (Kuntzman et al 1965; Soudijn & Winjngaarden 1968; Breyer 1971; Sugiura et al 1975; Takanashi et al 1975; Reiss 1977; Beckett & Achari 1977 a,b; Achari & Beckett 1981). Oxidation of the α-carbon atoms of the piperazine moiety has been demonstrated in-vivo (Breyer 1972; Gaertner & Breyer 1972; Taylor et al 1977) but not in-vitro. Metabolic *N*-dealkylation and cleavage of the piperazine ring give rise to primary and secondary amines which may undergo further metabolic *N*-oxidation to hydroxylamines. Hydroxylamine formation from piperazine has not been reported and it was of interest to ascertain whether this and ring opening occurred in-vitro. The mono-substituted piperazines Ia and Ic were chosen since unlike the disubstituted compounds metabolism should involve primary attack on the nitrogen and α-carbon atoms.



After incubation of Ia and Ic (10 μ mol) at pH 7·4 (60 min) with fortified 9000 *g* fraction liver homogenates from rats and rabbits, the concentrated ethereal extracts of the incubation mixtures were examined by thin layer chromatography (t.l.c.) (silica gel 60 F_{254}; benzene–diethylamine–methanol 80:10:10; methanol–diethylamine 90:10).

From phenylpiperazine (Ia) ethereal extracts of both alkaline (pH 12) and neutral (pH 7·4) incubation mixtures showed one product (R_F 0·51) under u.v. light other than Ia itself (R_F 0·38). No other products were detected after spraying with ninhydrin, ferric chloride, iodoplatinate, or Dragendorff reagents. The metabolic product (R_F 0·51) gave an immediate black colour with Tollen's reagent. It's mass spectrum (Fig. 1) (direct inlet) recorded on an AEI-MS 9 mass spectrometer (ionization potential, 70 eV), displayed a strong molecular ion at *m/z* 178 which expelled an ȮH radical to form the ion at *m/z* 161. The metastable ion at *m/z* 145·6 supported this direct fragmentation. Such $[M - OH]^+$ fragments have also been reported for other secondary hydroxylamines in which the nitrogen atom is part of a ring system (Coutts et al 1975). Other proposed

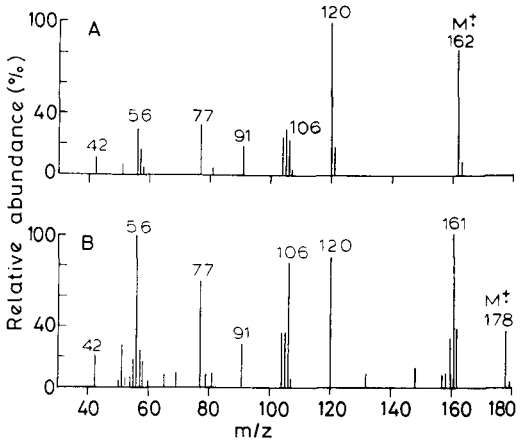


FIG. 1. Direct inlet mass spectra of (A) Phenylpiperazine (Ia) and (B) its metabolite *N*-hydroxyphenylpiperazine (Ib).

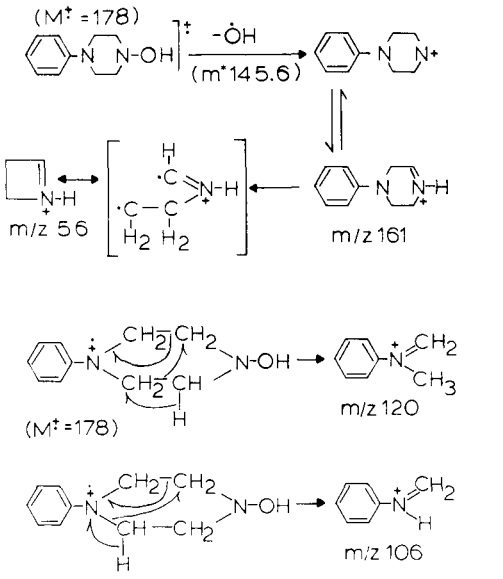


FIG. 2. Proposed mass fragmentation pathways of *N*-hydroxyphenylpiperazine (Ib).

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fragmentation pathways are shown in Fig. 2. The mass spectrum of the metabolic product, which is appreciably different from the parent compound (Ia), suggests that the product was Ib.

With benzylpiperazine (Ic) the original substrate only could be recovered from the incubation mixture.

No 'in-vitro' degradation of the piperazine ring could be demonstrated in the present study and it is of interest that ring degradation products were found only in the tissues of rats after their being given substituted piperazines for 3–7 days (Breyer 1972; Gaertner & Breyer 1972).

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Effect of aprotinin on the rectal delivery of insulin

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Aprotinin is a polypeptide of 58 amino acids obtained from the lungs or parotid glands. Because of its broad spectrum of protease inhibition, aprotinin has been clinically used in the treatment of chronic urticaria (Berova et al 1974) and other disorders associated with increased protease activity (Franceschini 1970; Amris & Scand 1966). Aprotinin has recently been shown to enhance the absorption of a number of polypeptide hormones (Parsons et al 1979; Philippe et al 1979) including insulin (Freidenberg et al 1981) when administered with these compounds either subcutaneously or intramuscularly. However, after subcutaneous or intramuscular injection of aprotinin alone, insulin levels in the blood have been shown to remain unchanged (Berger et al 1980). Another study has demonstrated that aprotinin can inhibit the degradation of insulin in adipose tissue in-vitro (Paulsen et al 1979).

In earlier papers (Nishihata et al 1981a,b; Kamada et al 1981), the enhancing effect of non-surfactant adjuvants such as salicylate, 5-methoxysalicylate and enamine derivatives of amino acids on the rectal and intestinal insulin absorption was reported. The present paper described the use of aprotinin as a protecting agent against insulin deactivation in the rectum after rectal administration.

Method

In a microenema dosage form prepared with distilled water, insulin (Crystalline Porcine Insulin, Lilly) was administered to male Sprague-Dawley rats, 200–251 g (fasted 16 h with water available). During the experiments, rats were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}) and kept on a 38 °C surface. A microenema volume of 1·0 ml kg^{-1} was delivered to the rectum directly via a polyethylene cannula (PE 50). Blood samples were taken from the jugular vein at designated times. Plasma concentrations of glucose were measured by the *o*-toluidine method and plasma insulin levels were measured by a radioimmunoassay (Nishihata et al 1978).

Results

The plasma glucose level after administration of a 1·0 ml kg^{-1} microenema containing 1·0 i.u. of insulin ml^{-1} and 45 mg sodium salicylate ml^{-1} was significantly decreased (Fig. 1). A maximum plasma insulin level of 83·3 ± 20·8 i.u. ml^{-1} (n = 6) was obtained 20 min after microenema administration.

The addition of aprotinin (15 μg ml^{-1} kg^{-1}) to the microenema caused a greater decrease in the plasma glucose level (Fig. 1). The plasma insulin concentration reached a maximum level of 187·4 ± 47·6 i.u. ml^{-1} (n = 6) 20 min after the administration. Aprotinin in an insulin microenema without sodium salicylate caused neither a decrease in plasma glucose levels (normal range indicated by shaded area in Fig. 1) nor an increase in plasma insulin levels which remained below 10 μ i.u. ml^{-1}. This finding indicates that aprotinin does

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