# 1-(*m*-Chlorophenyl)piperazine: a metabolite of trazodone isolated from rat urine

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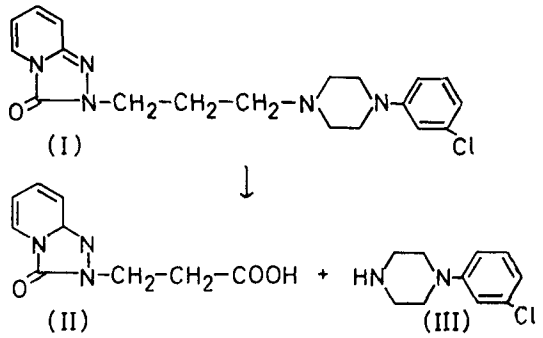
The metabolism of the psychotropic drug trazodone 2-[3-(4-(m-chlorophenyl)1-piperazinyl)-propyl]-s-triazolo-(4,3-a)-pirydin-3-(2H)one(I) has been examined in rats, rabbits and in men (Baiocchi & Frigerio 1974; Jauch et al 1976, Yamato et al 1974a,b, 1976a,b) with trazodone containing labelled carbon atom $^{14}C$ in the triazole ring, and metabolites were isolated by radiochromatographic methods. In the course of these studies β-(3-oxo-s-triazolo(4,3a)pyridin-2-yl)-propionic acid (II,OTPA) and its glucuronide (Yamato et al 1974b) were found in rat urine. The formation of OTPA suggested that, beside hydroxylation of ring systems (as previously found), the trazodone molecule could undergo hydrolysis and oxidation in the aliphatic chain. However, what happened to the remaining fragment of trazodone molecule containing the phenylpiperazine system (1-(*m*-chlorophenyl)piperazine III, CPP) was not known because of the lack of a labelled carbon atom that could be identified by scintillation methods.




Pharmacological studies of trazodone carried out in this Institute (Baran et al in the press, Maj et al 1978a,b, 1979) demonstrated that this drug at low doses had a central anti-5-hydroxytryptaminergic action, while at higher doses it produced a 5-HT-ergic stimulation. The latter effect was observed about 20 min after an intravenous injection of trazodone which suggested that it might be induced not by trazodone itself but by its metabolite. Further investigation showed that OTPA, the trazodone metabolite formerly described, was inactive whereas the hypothetical metabolite CPP, not detected so far, induced 5-HT-ergic stimulation similar to that evoked by high doses of trazodone, but observed immediately after an intravenous injection of CPP.

We set out to find out whether CPP was formed in rats treated with trazodone. The experiments were on male, Wistar rats, deprived of food overnight. Trazodone (Angelini Francesco Roma, 25 mg $kg^{-1}$ i.p.) or CPP (Angelini Francesco Roma, 1 mg $kg^{-1}$ i.p.) were administered in aqueous solution. The rats were housed in metabolic cages and their urine was collected for 24 h. The urine was divided into 1 ml samples, pH was adjusted to 8·0 with 0·05 M $K_2CO_3$ and extracted with 3 ml of ethyl acetate. The acetate layer was separated (fraction F I), pH of inorganic layer was adjusted to 2 with 0·1 M HCl and extracted with acetate (fraction F II). The acidic residue was boiled for 30 min, cooled and extrated again with ethyl acetate (fraction F III). Then the pH of the aqueous phase was adjusted to 8·0 (0·05 M $K_2CO_3$) and extracted with acetate (fraction F IV). Chromatographic study (solvent system methanol—conc. $NH_3$—water 75:5:20) of fractions F I, F II, F III and F IV excluded the presence of free CPP in the urine. However, there was a spot (X) with an $R_F$ value 0·80 that did not correspond to the value of the metabolites previously identified OTPA 0·71, CPP 0·50. The substance in spot X was isolated by preparative t.l.c. (boiling the suitable part of adsorbent with ethyl acetate for 30 min). The product was rechromatographed and its u.v., i.r. and mass spectra were recorded. The results indicated explicitly that the substance examined was 1-*m*-chlorophenylpiperazine (CPP) the compound that was not identified on direct chromatograms of acetate extracts of urine of rats treated with trazodone or CPP. These results suggested that CPP was present most probably in the conjugated form with glucuronic acid, which might be hydrolysed during extraction from silica gel which is an acidic sorbent. To confirm this, CPP *N*-glucuronide was synthesized in vitro (Dutton & Storey 1953) and the product was examined chromatographically as before. The results indicated the identity of CPP *N*-glucuronide with the substance presented in the spot X.

The data indicate that the process of biotransformation of the piperazine ring results generally in the formation of products in which the piperazine heterocyclic system is maintained, e.g. products of methylation of piperazine ring or its oxidation. In our experiments we did not isolate metabolites of trazodone with the chemical structure mentioned above. The isolation of OTPA as the product of trazodone biotransformation,

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and the identity of the substance in spot X with the *N*-glucuronide of CPP suggest an additional route of trazodone metabolism.

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# The potentiating effects of prostaglandins on bradykinin-induced pain and the effects of various analgesic drugs on prostaglandin $E_1$-potentiated pain in rats

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Ferreira (1972) found prostaglandins (PGs) to potentiate bradykinin-induced pain in man while aspirin did not alter the pain potentiated by $PGE_1$. Ferreira et al (1973) and Moncada et al (1975) reported a similar observation using a method of assessing the pressor reflex as a measure of nociceptive activity in dogs. It has therefore been suggested that non-steroidal anti-inflammatory drugs (NSAIDs) which block the synthesis of PGs (Ferreira et al 1971; Takeguchi & Nih 1972; Flower 1974; Ku & Wasvary 1975; Ziel & Krupp 1975), may exert their analgesic effects by preventing the sensitizing effect of PGs on the pain receptors. We now describe the potentiating effect of PGs on bradykinin-induced pain in rats and the effects of various analgesics on this pain.

Bradykinin, with or without PGs, was injected into the right common carotid artery of unanaesthetized Wistar male rats, 200 to 300 g, following the procedure described by Abe et al (1971). The injections were made through a polyethylene catheter (5·5 mm long; i.d. 0·58 mm; Clay-Adams PE-50), inserted centripetally under light ether anaesthesia into the carotid artery and passed through the subcutaneous tissue to protrude from the back of the animals. On the next day, for each animal, the liminal dose of bradykinin required to provoke both pain responses, that is, dextrorotation of the head and flexion of the right forelimb, was measured. Doses, in 0·2 ml 0·9% NaCl (saline), were 0·125, 0·250, 0·500 and 0·750 μg/animal. The potentiating effects of PGs on the ability of bradykinin to provoke pain responses were tested by the concomitant use of subliminal doses of bradykinin and PGs. In assessing the effects of analgesics on $PGE_1$-potentiated pain, similar results were obtained whether the doses of bradykinin used with $PGE_1$ (1·5 μg/animal) were subliminal or not. The present results were mainly obtained from subliminal doses of bradykinin. Aspirin, phenylbutazone, indomethacin, ibuprofen and amido-

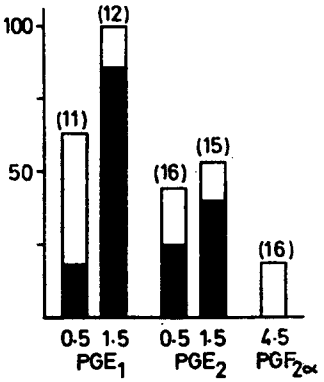


FIG. 1. The potentiating effects of PGs on the pain-provoking ability of the subliminal doses of bradykinin. Closed column: both pain responses, that is, dextrorotation of the head and flexion of the right forelimb were observed. Open column: either pain response was observed. Figures in parentheses indicate the number of animals tested. Abscissa: dose (μg/animal). Ordinate: % potentiation.

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