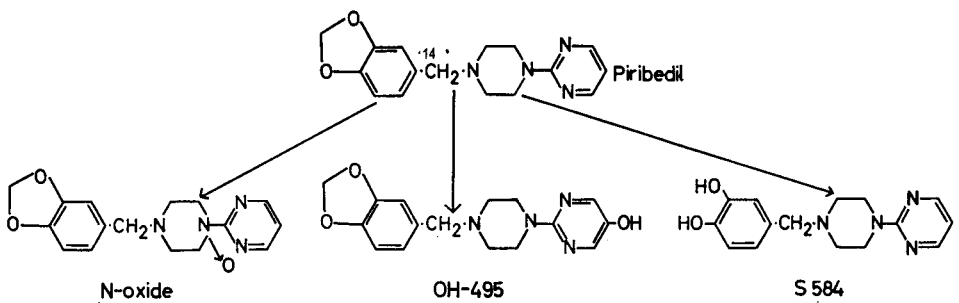
Preliminary investigation of the metabolism of piribedil (ET 495); a new central dopaminergic agonist and potential anti-parkinson agent

Piribedil (ET 495) 1-(3,4-methylenedioxybenzyl)-4-(2-pyrimidyl) piperazine, has been shown to be active as a peripheral vasodilator (Regnier, Canevari & others, 1968). Recently, Corrodi, Fuxe & Ungerstedt (1971) demonstrated that piribedil produced marked and prolonged dopamine receptor stimulation after intraperitoneal administration to rats with central unilateral 6-hydroxydopamine-induced degeneration of the nigro-neostriatal dopamine pathway. This direct dopamine activity was later confirmed by Poignant (1972) and Costall & Naylor (1973), with stereotypic model systems. Piribedil was, however, inactive when administered intra-neostriatally, suggesting that an active metabolite could be responsible for its pharmacological effect. Consequently, we have undertaken to determine the metabolic fate of piribedil in both animals and man.

After oral administration of [^{14}C]piribedil, labelled in the benzyl methylene group, to male Wistar rats (40 mg kg^{-1}; 30 μCi per animal), 45% of radioactivity was recovered in the urine and 30% in the faeces in 72 h. Negligible amounts of $^{14}CO_2$ were found in expired air. Direct solvent extraction against a pH gradient before and after enzymic (suc *Helix pomatia*), acidic hydrolysis and reduction with titanium chloride, removed only 50% of the total radioactivity in both urine and faeces. These results would indicate the presence of significant quantities of highly polar metabolites.

Measurement of solvent extracts, before and after enzymic hydrolysis of urine, indicated that 19% of the dose, equivalent to 84% of the total extractable radioactivity, was present in a conjugated form. In faeces, only 40% of the total extractable radioactivity was conjugated, although examination of bile from fistulated animals showed total conjugation.

Two-way thin-layer chromatography (t.l.c.) (butanol–methanol–water 90:10:100 and pentan-1-ol–ammonia 85:15) of solvent extracts of hydrolysed urine at pH 12, 8 and 1 and subsequent autoradiography showed that only negligible amounts of unchanged material were present. However, three basic (metabolites 1, 2 and 3) and two acidic (metabolites 4 and 5) radioactive metabolites could be detected together with numerous minor components. Isolation of these metabolites by preparative t.l.c. and subsequent examination by direct inlet mass spectrometry (ms), infrared and ultraviolet spectroscopy, gas liquid chromatography (g.l.c.) and comparison with authentic material showed metabolite 1 to be 1-(3,4-dihydroxybenzyl)-4-(2-pyrimidyl) piperazine (S 584), arising by cleavage of the methylenedioxyphenyl bridge as shown for compounds of similar structure by Casida, Engel & others (1966), metabolite 2 to be 1-(3,4-methylenedioxybenzyl)-4-(2-pyrimidyl-5-hydroxy) piperazine, (OH-495), and metabolite 3 to be 1-(3-4-methylenedioxybenzyl)-4-(2-pyrimidyl) piperazine-4-*N*-oxide (495 *N*-oxide). A fourth, non-radioactive, metabolite yet to be conclusively identified



has been isolated and been shown to arise by cleavage of the piperazine ring. By a g.l.c. technique to measure unchanged piribedil and its identified metabolites in urine, it has been demonstrated that after an oral dose of 40 mg kg^{-1}, the rat excretes less than 0·5% of the dose as piribedil or its *N*-oxide and 5·6 and 7·4% as the conjugates of metabolite 1 and metabolite 2 respectively. The identity of the acidic metabolites and the unextractable radioactivity representing highly polar metabolites is at present under investigation.

Similar examination of bile and faeces before and after hydrolysis showed the presence of metabolites 1 and 2, free and conjugated, with smaller amounts of unidentified acidic metabolites 4 and 5.

Investigation of the *in vitro* metabolism of [^{14}C]piribedil in NADP (H) fortified male rat 10 000 *g* hepatic supernatant preparations confirmed the *in vivo* studies, since formation of metabolites 1, 2 and 3 were shown to be major metabolic pathways. Metabolism by hepatic preparations from male rabbits, mice, hamsters and guinea-pigs, showed a qualitatively identical metabolic picture although, using a quantitative g.l.c. procedure, marked species variations in the amounts of each metabolite produced are apparent.

A g.l.c. assay procedure has been developed which can measure piribedil in body fluids at a minimum sensitivity of 2 ng ml^{-1} (to be published). On examination of urine and plasma collected at hourly intervals for the first 8 h and then at periods over 72 h from four healthy volunteers receiving 40 mg piribedil orally in aqueous solution, no unchanged drug could be detected. Conjugates of metabolites 1 and 2 were conclusively identified in urine by g.l.c. and t.l.c. and shown to account for 9·2 $\pm$ 0·7% and 19·1 $\pm$ 1·8% respectively of the administered dose.

Administration of piribedil daily, increasing from 20 to 240 mg over a period of from 7 to 28 days, to patients with Parkinson's disease produced similar 24 h cumulative mean excretion of the conjugates of metabolites 1 and 2, 9·5 $\pm$ 1·4% and 18·8 $\pm$ 0·5% respectively, and were comparable with those found for the healthy human volunteers. This would indicate that neither the disease nor the high and repeated dosage appears to alter the metabolism of piribedil.

These results indicate that extensive metabolism of piribedil occurs in the rat and that its prolonged pharmacological effect (Corrodi & others, 1971) makes the possibility of its action due to an active metabolite a reality. Further, an apparent absence of piribedil in human plasma and urine could suggest a similar mode of action.

Research and Development Department,
Servier Laboratories Limited,
Percival House,
Pinner Road,
Harrow, Middlesex, U.K.

P. JENNER
A. R. TAYLOR
D. B. CAMPBELL

April 19, 1973

REFERENCES

CASIDA, J. E., ENGEL, J. L., ESSAC, E. G., KAMIENSKI, F. X. & KUWATSUKA, S. (1966). *Science.* **153**, 1130–1133.

CORRODI, H., FUXE, K. & UNGERSTEDT, V. (1971). *J. Pharm. Pharmac.*, **23**, 989–991.

COSTALL, B. & NAYLOR, R. J. (1973). *Symposium on the treatment of Parkinsonism*, Raven Press, New York. (To be published).

POIGNANT, J. M., LAUBIE, M., TSCOURIS-KUPFER, D. & SCHMITT, H. (1972). *C.R. Academie Science, Paris*, **275**, 715–717.

REGNIER, G. L., CANEVARI, R. J., LAUBIE, M. & LE DOUREC, J. C. (1968). *J. mednl Chem.*, **11**, 1151–1155.