Studies on the metabolic fate of 3-methoxy-4-hydroxyphenylethylene glycol (MHPG) in man

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Many drugs are thought to exert their pharmacological effects by altering brain noradrenaline turnover. It would therefore be useful to have a non-invasive technique for the measurement of brain noradrenaline turnover in man. Measurement of noradrenaline levels in urine or plasma has not proved to be a good index of central noradrenaline turnover, as little, if any, neurotransmitter leaves the brain without undergoing metabolism. A solution would be to assay a metabolite of noradrenaline formed only in the brain. Following the reports of Schanberg and co-workers (Schanberg, Schildkraut, Breese & Kopin, 1968a; Schanberg, Breese, Schildkraut, Gordon & Kopin, 1968b) considerable work has led to the belief that in most species, including man, one of the principal metabolites of noradrenaline in the brain is MHPG and further, that the sulphate conjugate of this metabolite has its origin almost exclusively in the brain.

We have therefore developed sensitive, specific stable isotope dilution assays for MHPG and for its sulphate and its glucuronide conjugates, utilizing computerized gas chromatography-mass spectrometry. MHPG and its sulphate conjugate, labelled specifically with deuterium atoms, were synthesized for use as internal standards. MHPG was isolated from 5 ml aliquots of urine by extraction with ethyl acetate while the two conjugates were measured after extraction on Amberlite XAD-2 resin, followed by chromatographic separation on Sephadex LH-20. Both MHPG and its sulphate could be converted directly to the MHPG *tris*-trifluoracetate derivative by treatment with trifluoracetic anhydride. The glucuronide was not cleaved by this reagent and hence did not interfere with determination of the sulphate.

Urinary excretion of the three metabolites in 10 normal volunteers over 24 h was as follows expressed as mg free MHPG (mean $\pm$ s.d.): MHPG 0.08 ± 0.03 mg, MHPG sulphate 1.05 ± 0.38 mg, MHPG glucuronide 1.30 ± 0.40 mg. Thus most MHPG in urine is conjugated, with almost equal quantities of the two conjugates present. Data from 4 patients with adrenal catecholamine-releasing tumours (phaeochromocytoma) revealed elevated urinary levels of all three metabolites. The ratio of glucuronide to sulphate, however, was 0.94–2.16 and thus similar to control values. In contrast, with lumbar CSF from 42 individuals an average of 84% of the MHPG was unconjugated. Preliminary results with pooled samples of CSF indicate that the remainder is present as the glucuronide. This finding is in conflict with previous reports in which conjugated MHPG in CSF was described as the sulphate (Schanberg *et al.*, 1968b).

It is concluded that MHPG can be conjugated with either sulphuric or glucuronic acids in the periphery whereas in the central nervous system conjugation is a minor pathway and appears to occur only with glucuronic acid.

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Studies of the interaction of carbenoxolone sodium and warfarin sodium *in vivo*

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The potentiation of the anti-coagulant activity of warfarin by phenylbutazone is well known (Aggler, O'Reilly, Leong & Kowitz, 1967; O'Reilly & Levy, 1970). Investigations into the mechanism of potentiation both *in vivo* and *in vitro* suggest displacement of warfarin from plasma binding sites as the cause (O'Reilly & Levy, 1970; Jun, Luzzi & Hsu, 1972). We have previously demonstrated a difference in the binding sites on human serum albumin between phenylbutazone, warfarin and carbenoxolone using a technique *in vitro* (Gottfried, Parke, Sacra & Thornton, 1975). We suggested that an interaction, due to displacement from plasma protein binding sites, between carbenoxolone and warfarin, phenylbutazone and other protein-bound drugs was unlikely, having

shown that carbenoxolone was binding at a different class of site to the other drugs.

In the present series of experiments we used a model *in vivo* to demonstrate the potentiation of warfarin anti-coagulant activity by phenylbutazone and the corresponding reduction in plasma half-life of warfarin. This model was then used to investigate any possible interaction of carbenoxolone with warfarin.

Fasted female Biorex Wistar rats (200 ± 10 g) were used in groups of six per treatment for each blood collection. All rats received 10 mg/kg warfarin sodium orally (2.5 ml/kg in distilled water) and at the same time half of the rats received subcutaneously (in 0.9% NaCl, 5 ml/kg) a dose of phenylbutazone (20 mg/kg) or carbenoxolone (40 mg/kg), i.e. at twice the molar equivalent of warfarin. Blood samples (3 ml) were obtained under ether anaesthesia at sacrifice using Hepes/citrate buffer (0.13 M trisodium citrate; 0.05 M *N* - 2 - hydroxyethylpiperazine - *N'* - 2 - ethansulphonic acid) 0.1 ml per ml of blood collected.

A one-stage prothrombin time was determined in duplicate for each rat using rabbit brain thromboplastin (Diagnostic Reagents Ltd) and warfarin concentrations were measured by spectrofluorimetry (Corn & Berberich, 1973).

No changes in prothrombin times from normal (17.0 ± 0.3 s), were seen at 8 h after warfarin treatment but at 24 h the prothrombin time was increased to 79.6 ± 1.7 s and 87.5 ± 3.5 s in the two experiments. Phenylbutazone, at a dose of twice the molar equivalent of warfarin, increased the time to 109.7 ± 7.1 s ($P < 0.005$, t-test) whilst carbenoxolone, at the twice molar equivalent, showed no significant change, 77.2 ± 3.5 seconds. Carbenoxolone and phenylbutazone had no inherent activity on prothrombin times at the dose regimens used.

The plasma half-life of warfarin, in the absence of other drugs, was found to be 8.4 and 8.5 h. Phenylbutazone reduced this to 2.3 h whilst carbenoxolone showed no change at 9.2 hours.

These findings show that carbenoxolone sodium *in vivo* does not potentiate or displace warfarin sodium and are in agreement with our earlier suggestions from studies *in vitro* (Gottfried *et al.*, 1975).

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Rat brain iso-renin

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The components of the renin-angiotensin system have been demonstrated within the brain and they have been shown to be implicated in physiological control of water and sodium balance (Fitzsimons, 1975). Recently a brain iso-renin, distinct from the renal enzyme has been demonstrated in the dog and rat brain (Ganten, Hutchinson, Schelling, Ganten & Fischer, 1976). It has also been tentatively suggested that centrally-acting hypotensive drugs may act via brain iso-renin. In order to test this hypothesis and to study in more detail the physiological role of brain iso-renin, a method has been developed which enables small amounts of enzyme present in rat brain to be measured reliably.

Rat brain extracts were incubated at 37°C in the presence of substrate prepared from nephrectomized dogs according to the method of Hass, Goldblatt, Gipson & La Vera Lewis (1966). The angiotensin produced was adsorbed on to an ion exchange resin, and subsequently eluted from the resin at the end of the incubation period. The activity of this pressor material was estimated against synthetic angiotensin II on the pithed rat blood pressure preparation. The use of (Sar^1, Ala^8)-angiotensin II, a competitive antagonist of angiotensin II (Pals, Masucci, Denning, Sipos & Fessler, 1971), completely abolished the biological activity of the material eluted from the resin. Control experiments showed that the recovery of synthetic angiotensin II amide from the resin after a 24 h incubation period under the experimental