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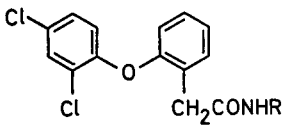
Taurine conjugation of fenclofenac in the dog

Taurine conjugation of bile acids has long been known but it is only recently that conjugation of drugs with this amino-acid has been reported. Quinaldic acid has been shown to be excreted as quinaldylglycyltaurine in the cat (Kaihara & Price, 1961) and James, Smith & Williams (1972a) showed that phenacetyltaurine was present in the urine of a variety of species after dosing with phenylacetic acid, though usually as a minor metabolite in the presence of larger quantities of glycine or glutamine conjugates. James, Smith & others (1972b) suggested that unidentified metabolites found in the urine of several species when dosed with 4-chloro- or 4-nitrophenylacetic acids might be taurine conjugates. Osiyemi & Smith (1972) found a similar pattern of metabolism for indolylacetic acid and suggested that taurine conjugation might be an important metabolic reaction of arylacetic acids in certain species. Case (1973) presented nmr evidence for the conjugation of a substituted propionic acid with taurine though details of the species and structure of the drug were not disclosed. The interest in this route of metabolism prompted us to report our findings on the metabolism, in dog, of fenclofenac, a novel anti-inflammatory agent (Atkinson, Godfrey & others, 1974)*.

Fenclofenac [2-(2,4-dichlorophenoxy)phenylacetic acid] was labelled with tritium in the methylene group of the acetic acid side-chain. Male beagle dogs (4 animals; 10–16 kg) were dosed orally with fenclofenac (10 mg kg^{-1}). Urine and faeces were collected for 3 or 7 days. Analysis for radioactivity showed that the drug was excreted almost equally in urine and faeces. Metabolite identification was performed on samples excreted in the first 24 h after dosing. The taurine, glycine and glutamine conjugates of fenclofenac, which were synthesized by reaction of the arylacetic acid chloride with the sodium salts of the amino-acids, were used as standards for chromatography.



I

Spectral and analytical properties

Fenclofenac taurine conjugate (I; R = $CH_2CH_2SO_3Na$). Found: C, 44·8; H, 3·3; N, 3·0%. $C_{16}H_{14}Cl_2NNaO_5S$ requires C, 45·1; H, 3·3; N, 3·3%. M.p. sodium salt 190–195°; methyl ester 116°. Ultraviolet (sodium salt in ethanol) $\lambda_{max.}$ (nm) 271, 278, 283 sh, 292 sh. ϵ, 1730, 1720, 1470, 910 respectively. Infrared Nujol mull (sodium salt) (cm^{-1}) 3400 broad, 3325 sharp:— bonded and non-bonded –NH. 1635:— > C=O. 1210, 1060, 1050:— $-SO_3$ Na. No absorbtion in the 1650–1800 cm^{-1} region. Nmr (in D_2O, sodium salt) δ 2·81 complex t ($J \approx 6$) 2H $-CH_2SO_3^{\ominus}$,

* Full details of the metabolic studies carried out on fenclofenac will be reported elsewhere.

δ 3·1–3·6 m, δ 3·22 s 4H $-NCH_2-$, $ArCH_2$; δ 6·2–7·5 m, δ 7·21 d (J=2) 7H Ar–*H*, 3′–*H*.

Fenclofenac glutamine conjugate (I; R=$CH(CO_2H)CH_2CH_2CONH_2$). Found C, 53·5, H, 4·3, N, 7·0, Cl, 16·5%. $C_{20}H_{20}Cl_2N_2O_5$ requires C, 53·6, H, 4·3, N, 6·6, Cl, 16·7%. M.p. 163–165°. Infrared—Nujol mull (cm^{-1}). 3380, 3240, 3170:— –NH–, NH_2; 1685:— $-CO_2H$; 1630 (broad):— –CONH–. Nmr (methyl ester in $DMSOd_6$) δ 1·55–2·33 m ≈4H $-(CH_2)_2-$; δ 3·55 s, δ 3·59 s, 5H Ar CH_2-; $-OCH_3$; δ 4·21 q, b (J = 7) ≈1H $-CH(CO_2Me)NH-$; δ 6·5–7·5 m 8H $CONH_2$ + Ar–*H*; δ 7·71 d (J = 2) ≈1H 3′–*H*; δ 8·43 d, b (J = 7) ≈1H CON*H*.

D_2O exchange of the δ 8·43 signal (CON*H*) causes the adjacent methine proton signal (δ 4·21) to simplify from a quartet to a triplet.

Fenclofenac glycine conjugate (I; R= CH_2CO_2H.) Found C, 54·4, H, 3·6, N, 3·8, Cl, 19·7%. $C_{16}H_{13}Cl_2NO_4$ requires C, 54·3, H, 3·7, N, 3·9, Cl, 20·0%. M.p. 188–189°. Infrared—Nujol mull (cm^{-1}) 3270:— –NH–, 1720, 1705:— $-CO_2H$, 1645:— –CONH–. Nmr (in $DMSOd_6$): δ 3·36 s 2H Ar–CH_2; δ 3·71 d (J = 6) 2H NH–CH_2; δ 6·6–7·4 m 6H Ar–*H*; δ 7·46 d (J = 2) ≈1H 3′–*H*; δ 7·94 t, b(J = 6) ≈1H CON*H*.

Exchange of the CON*H*–proton (δ 7·94) with CD_3OD causes the doublet (δ 3·71) due to the adjacent methylene group to collapse to a singlet.

Examination of the urine by thin-layer chromatography on silica plates in several solvent systems showed a single major metabolite corresponding in R_F value to the synthetic taurine conjugate. Percolation of the urine through a column of Amberlite XAD-2 resin followed by successive washing with water and methanol yielded an alcoholic extract which contained essentially all of the initial activity. Preparative thin-layer chromatography on silica yielded a sample of the metabolite, which was converted to its methyl ester by treatment with ethereal diazomethane. The chromatographic behaviour of this methyl derivative was identical to that of the methyl ester of the authentic taurine conjugate and its infrared spectrum was identical to that of the synthetic material.

The metabolites in urine were also examined by inverse isotope dilution methods which showed that more than 90% of the activity was present as the taurine conjugate (crystallizations were carried out on the sodium salt). Glycine conjugation was virtually undetectable (<1%) and only traces of unconjugated material were found (4%). It is interesting to note that with other arylacetic acids, taurine conjugation has always been accompanied by conjugation with glycine or glutamine (James & others 1972a, b).

The faeces were also examined for metabolites. The freeze dried material was extracted with methanol and the extract was subjected to chromatographic analysis. Most of the radioactivity was present as unchanged fenclofenac (*ca* 90%). Inverse isotope dilution with synthetic taurine conjugate showed the presence of small amounts (9%) of this metabolite. We have no information on the biliary excretion of fenclofenac in dog but the unchanged drug in faeces may arise by hydrolysis of a conjugate by gut bacterial flora.

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Analgesia produced by clonidine in mice and rats

The functional role of noradrenaline in the central nervous system is contradictory. Activation of the noradrenaline neurons may be required to meet situations e.g. involving defence and attack behaviour (for review see Fuxe, Hökfelt & Ungerstedt, 1970). It is not clear whether this is a consequence of a stimulant or an inhibitory function. Clonidine (2-(2,6-dichlorophenylamino)-2-imidazoline HCl), a central noradrenaline receptor stimulating agent, has been shown to possess a sedative effect in man (Brüner & Klein, 1968) and in animals (Delbarre & Schmitt, 1971, 1973; Holman, Shillito & Vogt, 1971). Administration of dihydroxyphenylserine, which is directly decarboxylated to noradrenaline in the brain, produces sleep in the rat (Vogt, 1973). Bolme & Fuxe (1973) have recently suggested that a central inhibitory noradrenergic mechanism exists to control the respiratory rate. Since considerable evidence suggests that central monoaminergic mechanisms play a role in opiate analgesia (for review see Way & Shen, 1971), the effects of clonidine on the threshold for nociceptive stimulation have been investigated in mice and rats.

Male Sprague-Dawley rats (130–190 g) and male NMRI mice (18–20 g) were allowed free access to water but no food 16 h before the test. Nociceptive stimulation was as described by Paalzow (1969a, b) and Paalzow & Paalzow (1973a). By standardized electrical stimulation of the tail of the animals, the changes in the threshold for vocalization were followed in mice, while in rats the threshold for motor response (spinal reflex), vocalization and vocalization afterdischarge (vocalization after withdrawal of stimulus) were studied. The electrodes (injection needles No. 20) were placed and retained intracutaneously in the middle section of the tail. In each animal the individual thresholds were determined before administration of the drug and graded in volts. After injection of the drug, the different thresholds were registered at 15 min intervals and the graded response expressed as a percentage of the pretreatment threshold voltage. For the 100 rats used, the average control thresholds were: motor response $1{\cdot}38 \pm 0{\cdot}03$ V; vocalization $2{\cdot}11 \pm 0{\cdot}08$ V; vocalization afterdischarge $3{\cdot}94 \pm 0{\cdot}14$ V. In mice the corresponding threshold for vocalization was $4{\cdot}26 \pm 0{\cdot}18$V ($n = 45$). The normal thresholds (placebo) of the three responses to nociceptive stimulation remain stable for 3 h or more (Paalzow, 1969a, b; Paalzow & Paalzow, 1973a).

Fig. 1A shows that after subcutanous administration of clonidine to rats there was a dose-dependent increase of the thresholds for vocalization and vocalization after discharge. Clonidine was more potent in the elevation of the threshold for vocalization afterdischarge. An analysis of regression gave significant slopes ($P < 0{\cdot}001$) for the dose-response lines and an analysis of variance showed no significant departure from regression ($P > 0{\cdot}05$).

The threshold for motor response was unaffected by the clonidine treatment in doses from 80–1250 μg kg^{-1}. At a dose of 10 mg kg^{-1} the threshold for a motor response was increased by about 100% during the first 30 min after administration, but at this high