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COMMUNICATIONS

Distribution of antipyrine in the rat liver

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Abstract—The rate and extent of hepatic distribution of antipyrine was examined in the rat isolated perfused liver. Tritiated water and [^{14}C]antipyrine were injected simultaneously into the portal vein as a bolus using either Krebs–Ringer bicarbonate or rat plasma as the perfusate. The effluent profiles of each compound using the two perfusates were superimposable, a finding expected for water and consistent for antipyrine, which was negligibly bound in rat plasma. Although full recovery (97%) of administered material was achieved with both compounds, the fractional output profile for antipyrine peaked at a lower value (0·10 mL^{-1}) and at a later time (24 s) than water (0·14 mL^{-1}, 17·5 s), due to antipyrine having a larger volume of distribution (water 0·61 mL (g liver)$^{-1}$); antipyrine 0·81 mL (g liver)$^{-1}$). This observation is explained by antipyrine binding to, or partitioning into cellular components. Nonetheless, like water, distribution of antipyrine into hepatic cells is perfusion rate limited as evidenced by the superimposition of the dimensionless plots of fractional output vs time normalized to mean residence time.

Antipyrine is widely used as a model compound in drug metabolism and pharmacokinetic studies (Stevenson 1977; Vesell 1979). It has been chosen because it is extensively metabolized, has a low extraction ratio and binds negligibly to plasma proteins. Antipyrine is also said to distribute into total body water space (Soberman et al 1949) and has been used as a marker of this space. However, very limited specific data are available on the distribution of antipyrine in individual organs, particularly the liver, the major organ of elimination for this drug. The present study was conducted in the rat isolated perfused liver to investigate the rate and extent of distribution of antipyrine in comparison with that of [^{3}H]H_2O which distributes into the total aqueous space.

Materials and methods

Animals. Male Sprague-Dawley rats, 340–400 g, fed on normal laboratory rat diet with free access to water, were used.

Materials. All reagents were of analytical grade. [^{3}H]H_2O (5 mCi mL^{-1}; > 99·0% purity) and [*N*-methyl-^{14}C]antipyrine (50 mCi mmol^{-1}; > 98·0% purity) were obtained from Amersham (UK). Opti-Safe Hisafe II scintillant was purchased from LKB (UK). Rat plasma was separated from whole blood after centrifugation of freshly obtained rat blood, which had been collected in tubes containing EDTA.

Protein binding of antipyrine. Rat plasma samples (3 mL) containing 0·02–3·75 μg mL^{-1} antipyrine had [^{14}C]antipyrine added and were then incubated at 37°C for 30 min. After centrifugation (Rotor Type 50 Ti; Beckman, UK) at 166 000 *g*, 37°C for 15 h, an aliquot (in duplicate) of the supernatant (0·25 mL) was added to 5 mL of liquid scintillation fluid (LKB Optiphase Hisafe II, Finland) and [^{14}C]antipyrine was determined in a scintillation counter (Rackbeta Model 1218, LKB) by reference to a ^{14}C quench curve. The value of the fraction unbound was calculated as the ratio of d min^{-1} mL^{-1} in the supernatant to that in the precentrifuged sample.

Liver perfusion experiments. A single-pass isolated perfused in-situ liver system, using male Sprague-Dawley rats (liver weight 12·9–14·5 g) was employed (Pang & Rowland 1977). All experiments were conducted at 37°C. Under peritoneal anaesthesia with sodium pentobarbitone (50 mg kg^{-1}), the bile duct was cannulated with PE-10 tubing. After rapid cannulation of the hepatic portal vein, the liver was perfused at a flow rate of 15 mL min^{-1} with freshly prepared and filtered (0·2 μm) Krebs-Ringer bicarbonate buffer (pH 7·4, 37°C) containing 3 g L^{-1} of glucose saturated with 95% O_2–5% CO_2. Liver effluent was collected via a cannula inserted into the vena cava through the right atrium. After a stabilization period of 15 min, 50 μL of [^{3}H]H_2O (0·5 μCi), as a water space marker, and [^{14}C]antipyrine (0·25 μCi) were injected simultaneously as a bolus into the portal vein. The total effluent from the hepatic vein was collected at 2 s intervals for 114 s using a turntable with 56 sampling holes moving at a constant speed. The Krebs-Ringer bicarbonate buffer was then replaced with rat plasma, saturated with 95% O_2–5% CO_2, and, after a stabilization period of 10 min, the pulse dose experiment with [^{3}H]H_2O and [^{14}C]antipyrine was repeated. The procedure was then repeated using Krebs–Ringer buffer as the perfusate and only when the mean transit time (t), maximum fractional outflow (f_{max}), and the correspondence time of occurrence (t_{max}) after the first and third runs were within 10% of each other, was the liver judged to be stable over the whole experimental period and the results deemed to be usable. At the end of each experiment, the liver was weighed.

As an in-situ preparation was used, it was not possible to weigh each liver until the end of the experiment. The final liver weight is likely to be a valid estimate of liver weight throughout the experiment, however, for several reasons. Constant visual observations and measurements of bile flow throughout the experiment indicated no oedema. For each liver, the volume of distribution of each solute, [^{3}H]H_2O and antipyrine, remained unchanged throughout the entire experiment and that of [^{3}H]H_2O was close to the known aqueous content of liver.

Radioactivity measurement. Samples (0·4 mL) were counted in Opti-Safe scintillant (5 mL) using a dual quench curve for ^{3}H and ^{14}C. Samples 1 to 20 were counted individually (0·4 mL), samples 21 to 40 were counted as aliquots (0·2 mL) of consecutive pairs and samples 41 to 56 as aliquots (0·1 mL) of four consecutive samples.

In order to compare the curves produced by the two materials, the fractional output per mL (f) of each was calculated by dividing the output radioactivity of each material (normalized to 1·0 mL) by the amount injected.

Calculation of moments and distribution parameters. The mean transit time ($\bar{t}$) of a solute in the whole system (liver and catheter) is obtained from the fractional curve according to the following equation:

$$\bar{t} = \int_0^\infty f \cdot t \cdot dt \qquad (1)$$

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where $\int_0^\infty f \cdot t \cdot dt$ is the total area under the first moment of fractional output time curve.

The value of $\int_0^\infty f \cdot t \cdot dt$ was calculated as follows

$$\int_0^\infty f \cdot t \cdot dt = \int_0^{tn} f \cdot t \cdot dt + \frac{f_n \cdot t_n}{\lambda} + \frac{f_n}{\lambda^2} \quad (2)$$

where t is taken as the midpoint of the collection time, t_n is the corresponding last sampling time, f_n is the last estimated fractional output and λ is the terminal exponential coefficient, estimated by nonlinear regression of the terminal log linear section of the output curve. The area up to t_n was estimated by numerical integration (Charter 1989).

The mean transit time of the solute in the catheter system without liver (estimated to be 3·5 s) was subtracted from that of the whole system (liver and catheter, $\bar{t}$) to obtain the mean transit time of the solute in the isolated perfused liver ($\bar{t}_H$). The maximum fractional outflow (f_{max}) and the time to reach f_{max} (t_{max}) were observed values. The volume of distribution of a non-eliminated solute (V_H) is the product of the perfusate flow rate (Q) and the mean transit time of the solute in the perfused liver ($\bar{t}_H$).

For a protein bound non-eliminated compound, the volume of distribution is related to the fraction unbound in the perfusate (fu) and that in the intracellular compartment (fu_T) of the perfused liver by

$$V = V_I + V_T \frac{fu}{fu_T} \quad (3)$$

where V_I is the extracellular aqueous space of the liver, including the intravascular space and V_T is the intracellular aqueous space to which antipyrine distributes. The volume of V_I was taken to be the volume of distribution of sucrose of approximately 0·16 mL (g liver)$^{-1}$ (Roberts et al 1990) and V_T was taken to be the difference between the volume of distribution of $[^3H]H_2O$ and V_I.

Dimensionless curves for $[^3H]H_2O$ and $[^{14}C]$ antipyrine were obtained after multiplying the outflow fractions of each solute by its volume of distribution and dividing the time, less 3·5 s, by the mean transit time of the solute in the rat isolated perfused liver.

Results

The unbound fraction of antipyrine in rat plasma was very high, fu = 0·96 ± 0·004 (s.d.), and constant over the range of antipyrine concentrations studied, 0·02–3·75 μg mL^{-1}.

Mean outflow dilution curves, for $[^3H]H_2O$ and $[^{14}C]$antipyrine, obtained using Krebs–Ringer bicarbonate buffer and rat plasma as the perfusate are shown in Fig. 1A and B, respectively. The perfusate used had no discernible effect on the output profiles of either compound. However, clear differences were observed between these two substances with respect to the time to peak (t_{max}: $[^3H]H_2O$ 17·5 ± 1 s, antipyrine 23·5 ± 2·5 s) and the maximum fractional outflow ($f_{max} \times 100$: $[^3H]H_2O$ 130 ± 24,

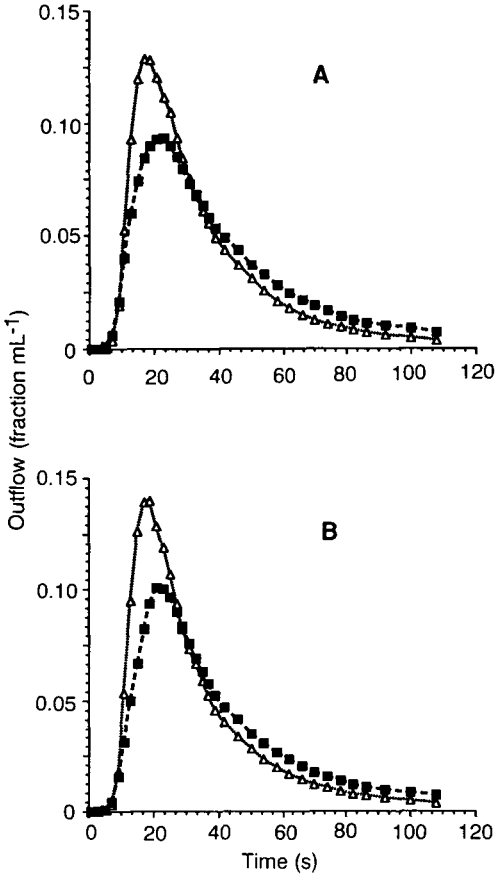

FIG. 1. Mean outflow curves of $[^3H]H_2O$ (△) and $[^{14}C]$antipyrine (■) produced by perfusing the isolated liver with Krebs–Ringer bicarbonate buffer (A) and rat plasma (B) at a flow rate of 15 mL min^{-1} (n = 4).

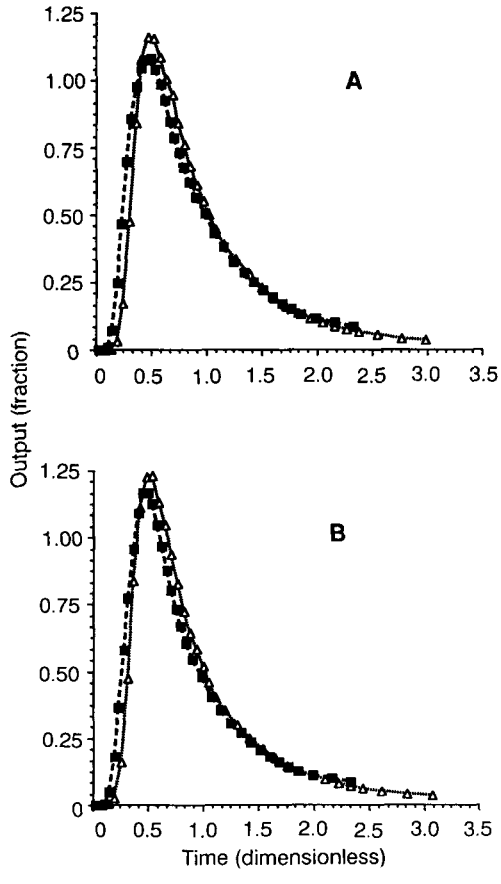

FIG. 2. Mean dimensionless outflow curves of $[^3H]H_2O$ (△) and $[^{14}C]$antipyrine (■) produced by perfusing the isolated liver with Krebs–Ringer bicarbonate buffer (A) and rat plasma (B) at a flow rate of 15 mL min^{-1} (n = 4).

Table 1. Outflow characteristics and distribution parameters (±s.d.) of $[^3H]H_2O$ and [^{14}C]antipyrine in the rat isolated perfused liver, using either Krebs–Ringer bicarbonate buffer or rat plasma as the perfusate (n = 4).

	Krebs–Ringer bicarbonate		Rat plasma	
Parameter	$[^3H]H_2O$	[^{14}C]Antipyrine	$[^3H]H_2O$	[^{14}C]Antipyrine
f_{max} (1000 fraction mL^{-1})	130 ± 24	96 ± 29	141 ± 19	102 ± 29
t_{max} (s)	17·5 ± 1·0	23·5 ± 5·3	18·0 ± 1·2	23·5 ± 2·5
Mean transit time in liver ($\bar{t}_H$; s)	32·6 ± 2·7	42·9 ± 8·9	32·7 ± 5·0	43·9 ± 10·5
V_H (mL (g liver)$^{-1}$	0·60 ± 0·02	0·78 ± 0·12	0·60 ± 0·06	0·80 ± 0·15
fu_T		0·69 ± 0·10		0·67 ± 0·09

antipyrine 96 ± 29). Recovery of both substances was essentially complete at 97·3 ± 5·7 and 97·0 ± 5·8% for $[^3H]H_2O$ and [^{14}C]antipyrine, respectively. Typical dimensionless curves of the two solutes obtained by using Krebs–Ringer bicarbonate and rat plasma as the perfusates are displayed in Fig. 2A and B. As can be seen, such curves are virtually superimposable.

The mean volumes of distribution of $[^3H]H_2O$ and [^{14}C]antipyrine were 0·60 and 0·78 mL (g liver)$^{-1}$, respectively, using Krebs–Ringer bicarbonate buffer, and 0·60 and 0·80 mL (g liver)$^{-1}$, respectively, using rat plasma as the perfusate. The corresponding values for fu_T of [^{14}C]antipyrine were 0·69 and 0·67 (Table 1). For both compounds, no significant difference was found between the values using Krebs–Ringer bicarbonate or rat plasma as the perfusate.

Discussion

Implicit in the analysis of the data is that hepatic ^{14}C-effluent is all unchanged antipyrine. This assumption is reasonable given that, in the rat isolated perfused liver, the hepatic extraction ratio is only 0·05 – 0·07 (Pang & Rowland 1977). Also, strictly the effluent profiles for $[^3H]H_2O$ and antipyrine need to be corrected for dispersion, as well as for mean transit time, as these compounds move through the nonhepatic components of the system. The correction for dispersion is, however, negligible when, as in this case, dispersion in the tubing is small relative to hepatic dispersion (Rowland & Evans 1992).

As anticipated, the effluent profile of $[^3H]H_2O$ was not affected by using rat plasma as the perfusate in comparison with the use of Krebs–Ringer bicarbonate buffer. The superimposable outflow curves of antipyrine produced by the two perfusates is also anticipated with the negligible binding of antipyrine to plasma proteins. However, the outflow curves of the two solutes were not superimposable, with [^{14}C]antipyrine retained longer than $[^3H]H_2O$ in the liver which was consistent with a larger volume of distribution of antipyrine. The permeability of hepatic cells to $[^3H]H_2O$ is extremely high such that its distribution is perfusion rate limited (Goresky 1963). The essential superimposition of the dimensionless plots of antipyrine and $[^3H]H_2O$ signifies that the same condition also prevails with antipyrine. The difference between $[^3H]H_2O$ and antipyrine therefore appears to be intracellular binding of antipyrine, yielding an unbound fraction intracellularly of approximately 0·7. The constituents to which antipyrine binds are as yet unidentified.

In dog and man, antipyrine appears to occupy total body water space (Soberman et al 1949). Pharmacokinetic studies of antipyrine in the rat reveal a similar finding with a volume of distribution of 0·8 L kg^{-1} (Matthew & Houston 1990). The failure of whole body pharmacokinetics to reflect hepatic differences between antipyrine and water may be explained by the liver constituting only 3–4% of body weight. Nonetheless, these data on the liver do raise a question on the use of antipyrine for accurate measurement of tissue water content.

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