shorter period than it does in man. Half life estimates in man vary from about 12–22 h (Weiss et al 1979; Dollery & Davies 1980; Kiechel 1980), most estimates being in the upper end of that range. This species difference in half lives has also been reported with clonidine, which has an elimination half life in the rabbit of about 30 min (Reid et al 1980) and a half life in man of about 9 h (Davies et al 1977). Although estimates of half lives vary between workers, it seems that the elimination half lives of these two drugs are about 10 to 20 times longer in man than in rabbit, emphasizing that care is needed in 'between species' comparisons of doses or effects, but showing the validity in this case of between drug comparisons in the same species.

Guanfacine's longer plasma half life is a possible explanation of its longer duration of action in animals and man, and the lower frequency with which a withdrawal syndrome occurs.

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Concentration-dependence of salicylate distribution

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Salicylate shows saturable binding to plasma albumin in man, as evidenced by a concentration-dependent unbound fraction (Shah et al 1974; Borgå et al 1976; Furst et al 1979). Accordingly, the apparent volume of distribution in man is expected to be concentration-dependent. This dependence has been observed in children who have taken an overdose of salicylate (Levy & Yaffe 1974), but no quantitative relationship between volume of distribution and unbound fraction in plasma has yet been established.

Øie & Tozer (1979) developed a model for relating the volume of distribution of a drug to its binding to plasma proteins, distributed both intravascularly and extravascularly, and to other constituents in the body. For a healthy 70 kg man, in whom the extracellular volume minus plasma volume is 12 litres, the plasma volume is 3 litres and the extravascular/intravascular ratio of albumin distribution is 1·4 (Jusko & Gretch 1976), the model can be expressed as:

$$V = 7{\cdot}2 + 7{\cdot}8 \cdot fu + \frac{V_R}{fu_R} \cdot fu \qquad (1)$$

where fu is the fraction of drug unbound in plasma, fu_R is the fraction of drug outside the extracellular fluids which is unbound, and V_R is the cellular fluid space. The units are in litres.

Using data from various published experiments on salicylate kinetics, we have examined whether or not the increase in the volume of distribution associated with an increase in the fraction unbound to albumin at elevated plasma salicylate concentrations can be described by the proposed model by Øie & Tozer (1979).

Method

The average initial volumes of distribution of salicylate after seven different dose sizes (ranging from 0·5 to 20 g, expressed as sodium salicylate) were calculated from literature data (Smith et al 1946; Swintosky 1956; Schachter & Manis 1958; Hollister & Levy 1965; Rowland & Reigelman 1968; Ventafridda & Martino 1976). For five of the seven dose sizes, volume of distribution was determined by dividing the intravenously administered dose by the extrapolated intercept value for the concentration at time zero. The other doses (2 and 4 g) were given orally. Peak concentrations

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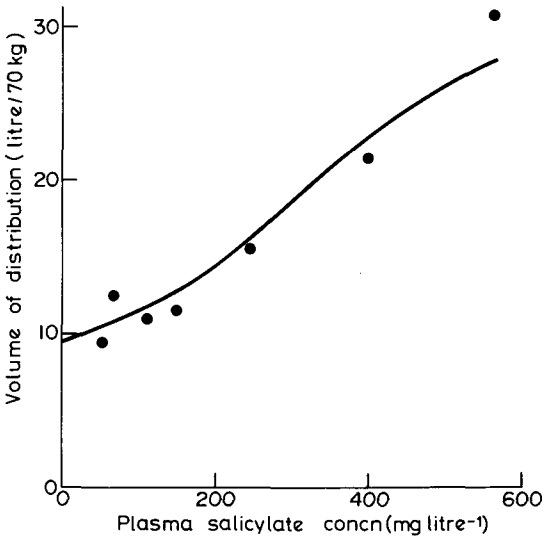

FIG. 1. The apparent volume of distribution of salicylate increases with the concentration of drug. Average data (●) from the literature (see text) following seven different doses. The curve represents the best fit of the volume—unbound fraction data using the model of Øie & Tozer (1979).

here were used as an estimate of the initial concentration. Because salicylate is rapidly and completely absorbed (Hollister & Levy 1965) and because its concentration declines slowly after doses in the 2 to 4 g range, the peak concentration was considered to be a close approximation of the initial extrapolated plasma concentration expected after intravenous administration of the same dose. The volumes of distribution were normalized to 70 kg body weight when the weights were known.

Because the binding of salicylate was not measured in the reported studies from which the apparent volumes of distribution were obtained, the unbound fraction was estimated using binding constants from two other studies (Shah et al 1974; Furst et al 1979). Two different classes of binding sites and corresponding binding parameters on plasma albumin were reported in these studies.

The unbound fraction, fu, was calculated from the relationship:

$$fu = \frac{1}{1+(P_t)\dfrac{n_1 \cdot K_1}{1+K_1 \cdot (D)}+\dfrac{n_2 \cdot K_2}{n_2+K_2 \cdot (D)}} \qquad (2)$$

where (D) and (P_t) are the concentrations of unbound drug and binding protein, respectively; K_1 and n_1 represent the affinity constant and the number of primary binding sites per molecule; and K_2 and n_2 represent the corresponding values for the secondary class of binding sites.

Using the values in the two cited studies, the unbound fraction for each initial concentration was determined for the seven different dose sizes. A normal albumin concentration of 0·65 mM (4·3 g dl^{-1}) was assumed. The values for each dose size were averaged and the model of Øie & Tozer was then fitted to the volume of distribution and unbound fraction data by the method of least squares.

Results and conclusions

The equation describing the best fit of the volume of distribution-unbound fraction data for the model of Øie & Tozer (1979), using 1/x weighting function, is:

$$V = 7{\cdot}2 + 7{\cdot}8 \cdot fu + 44{\cdot}3 \cdot fu \qquad (3)$$

The relationship between the apparent volume of distribution and the plasma concentration is shown in Fig. 1 together with the average experimental data points. The good fit of the Øie & Tozer model, which incorporates the contribution made by drug binding to albumin in the extravascular fluids, is not surprising. The contribution of extravascular albumin is important at low salicylate concentrations, because a large fraction (about 50–60%) of albumin in the body is located in the extravascular fluids (Jusko & Gretch 1976) and because most of the drug in the body is bound to albumin.

A value of 44 litres for V_R/fu_R is predicted by the model of Øie & Tozer (1979). This value is greater than the volume of intracellular fluid, 27 litres. This suggests that salicylate is either slightly bound to cellular components if evenly distributed in total body water (fu_R is 0·61 when V_R is 27 litres) or that the drug is more extensively bound intracellularly ($fu_R < 0{\cdot}61$) and that the unbound drug does not evenly distribute throughout total body water. Furthermore, the good fit of the data to the model suggests that the value of fu_R changes little over the concentration range studied. A significant change in fu_R, indicative of saturable tissue binding, would tend to counteract the effect of saturable albumin binding on volume, resulting in a situation in which the volume of distribution remains relatively independent of fu changes.

Although the literature data surveyed for salicylate are well-described by the model of Øie & Tozer (1979), it must be kept in mind that the volume-concentration data were obtained from several different sources and that binding constants were estimated from studies unrelated to those in which volumes of distribution were obtained. Furthermore, the unbound fraction depends not only on the salicylate concentration, but also on the plasma albumin concentration. Whether or not the concentration of albumin was the same in all studies could not be ascertained, as there was inadequate information to do so. Nevertheless, equation 3 and Fig. 1 should be helpful in approximating the volume term in subjects with normal albumin concentrations when the plasma salicylate concentration is measured. This prediction has clinical importance in therapeutic drug monitoring and in assessing salicylate ingestion in cases of drug intoxication.

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Distribution of nucleoside transport sites in guinea-pig brain

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Inhibition of the cellular uptake of adenosine may be achieved by impairment of the function of the membrane nucleoside transport mechanism which mediates the entry of adenosine into cells. Such inhibition, which might be expected to potentiate the effects of adenosine deriving from interaction with extracellular adenosine receptors, has been suggested to be involved in the central (Phillis et al 1980) and peripheral (Clanachan & Marshall 1980) actions of a variety of agents including dipyridamole and benzodiazepines (BDZs). The affinities of a series of recognized nucleoside transport inhibitors and BDZs for the nucleoside transport system of human erythrocytes have been estimated through the inhibition of the binding of [G-^{3}H]nitrobenzylthioinosine (NBMPR), a potent and specific inhibitor of nucleoside transport (Hammond et al 1981). Nucleoside transport activity ceases when specific membrane sites, evidently on the nucleoside transporter elements, are occupied by NBMPR, certain NBMPR congeners, or by dipyridamole. NBMPR binding sites appear to be present only on functional nucleoside transport elements (Jarvis & Young 1980; Cass et al 1981). This report describes a study of the regional distribution of nucleoside transport sites, identified by the site-specific binding of NBMPR, in guinea-pig brain.

Method

Female guinea-pigs (250–300 g) were decapitated and brain regions (Table 1) were homogenized in 10 volumes of sucrose (0·32 M) at 4 °C. Membrane fractions (P_2) were prepared by differential centrifugation (Gray & Whittaker 1962). Membranes (0·2–0·4 mg protein) were incubated for 20 min at 22 °C in Krebs-Tris buffer (1 ml final vol) containing [G-^{3}H]NBMPR (0·06–1·25 nM). Binding assays, conducted in 1·5 ml polypropylene centrifuge tubes, were initiated by the addition of membranes and terminated by centrifugation for 2 min in an Eppendorf 5412 microcentrifuge. The pelleted material was washed once with ice-cold Krebs-Tris buffer (1 ml) and dissolved in 0·5 M KOH (250 μl) before the assay of ^{3}H activity by liquid scintillation. Non-specific NBMPR binding was defined as the concentration (fmol mg^{-1} protein) of [G-^{3}H]NBMPR which remained membrane-associated when binding assays were conducted in the presence of a second transport inhibitor, dipyridamole (40 μM). Non-specific binding was never more than 30% of total binding in all brain regions tested. NBMPR binding parameters (K_D and B_{max}) were determined by mass law analysis (Scatchard plot) of the binding data. To determine inhibitor K_i values, membrane preparations were incubated with [G-^{3}H]NBMPR (0·2–0·8 nM) alone and in the presence of two or three concentrations of each inhibitor; data for the site-specific binding of NBMPR were subjected to mass law analysis by the double reciprocal plot method. The protein content of membrane preparations was estimated by the method of Lowry et al (1951).

Results

In each brain area examined, site-specific binding of NBMPR was saturable; in contrast, concentrations of the non-specific component of membrane-associated NBMPR were proportional to NBMPR concentration. Mass law analysis of the binding data indicated that in each brain region, NBMPR molecules were bound to a single class of high affinity sites and dissociation constants (mean ± s.e. mean) for NBMPR (Table 1) bound at these sites ranged from 0·15 ± 0·02 nM (olfactory lobe) to 0·38 ± 0·04 nM (pons/medulla). These differences are probably not attributable to competition with higher extracellular concentrations of adenosine because preincubation of membranes with adenosine deaminase (70 μg ml^{-1}; final concentration) did not influence NBMPR binding constants. It is clear that specific binding sites for NBMPR (expressed as fmol mg^{-1} protein) were not distributed uniformly throughout the brain (Table 1). As has been shown previously (Hammond & Clanachan 1982), dipyridam-

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