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THE PHARMACOKINETICS OF [^{14}C]-EDROPHONIUM IN NORMAL WISTAR RATS AND HOMOZYGOUS GUNN RATS WITH LIGATED RENAL PEDICLES

D.J. BACK & T.N. CALVEY

Department of Pharmacology and General Therapeutics, University of Liverpool, Liverpool L69 3BX

1 The distribution kinetics of [^{14}C]-edrophonium were studied in both normal Wistar and homozygous Gunn rats with ligated renal pedicles.

2 After intravenous injection the plasma concentration-time curve of [^{14}C]-edrophonium was adequately fitted by a triexponential function in both species of rat and interpreted in terms of a three compartment model.

3 The influence of the route of administration (i.e., systemic venous versus hepatic portal) of [^{14}C]-edrophonium on its concentration in plasma was studied in normal Wistar rats with ligated renal pedicles. There was a marked difference in the area under the plasma concentration-time curve depending on the route of administration.

4 The parameters of the model were determined and compared.

5 The value of the metabolism rate constant was approximately the same in both species of rat. This suggests that the rate of conjugation of [^{14}C]-edrophonium is unaltered in the homozygous Gunn rat despite the genetic lesion in UDP-glucuronyltransferase.

Introduction

Marked differences have been observed between the excretion of the quaternary amine edrophonium in the bile of Wistar and of homozygous Gunn rats. In the Wistar rat this compound is mainly excreted as a glucuronide, although small amounts of the unchanged drug are also detected in bile (Back & Calvey, 1972a). The homozygous Gunn rat, although unable to synthesize conjugated bilirubin because of the genetic lesion in microsomal UDP-glucuronyltransferase, nevertheless shows a qualitatively similar metabolic pattern. However, the rate of biliary excretion of edrophonium glucuronide is approximately 10 times greater in homozygous Gunn rats than in Wistar rats. It has been suggested that such quantitative differences in excretion may be related to competition between endogenous and exogenous glucuronides for transport into bile (Calvey & Back, 1973). The evidence for this view would be strengthened if similar rates of conjugation could be demonstrated.

In the Wistar rat the rapid decline in the concentration of unchanged edrophonium in blood is due to urinary excretion, conjugation in the liver, and uptake into non-hepatic tissues (Back & Calvey, 1972b). The object of the experiments described in the present paper was to study and compare the distribution kinetics of [^{14}C]-edrophonium in normal Wistar and homozygous Gunn rats with ligated renal pedicles. Experimental animals were prepared in this way so that urinary excretion could be eliminated from consideration, thereby making a direct comparison of the removal of edrophonium by conjugation possible.

Methods

Experimental procedure

Wistar rats (200-300 g) of either sex (Carworth Europe, Alconbury, Huntingdonshire), or homozygous Gunn rats (160-230 g) of either sex (bred by crossing homozygous jaundiced males with heterozygous female Gunn rats) were anaesthetized with urethane (14% w/v in distilled water; 10.0 ml/kg, i.p.). The trachea was cannulated and polyethylene catheters were inserted into a femoral vein and a carotid artery. The abdomen was opened by a midline incision and the main

vessels of the rectus sheath were ligated. The abdominal wall was incised laterally (avoiding any visible blood vessels) and both renal pedicles were identified and ligated.

Ethyl [1-^{14}C]dimethyl(3-hydroxyphenyl)-ammonium chloride ([^{14}C]-edrophonium chloride; specific radioactivity 13.7 μCi/μmol) was obtained from the Radiochemical Centre (Amersham, Bucks), dissolved in a solution of 4-chlorocresol (0.25% w/v in deionized water), and stored at -10°C. The dose of the radioactive drug (2.0 μmol/kg in roughly 0.3 ml of 0.9% w/v NaCl solution (saline)) was injected i.v. over a 1 min period. Blood samples (approximately 0.2 ml) were collected from the carotid artery at 1.5, 3, 5, 8, 11, 15, 20, 25, 30, 35, 45, 55, 65 and 75 min after drug administration. Small amounts of heparin (10 mg/ml) were used to prevent coagulation in the carotid arterial cannula. After centrifugation, plasma samples (25 μl) were assayed for total radioactivity by liquid scintillation spectrometry. Unchanged [^{14}C]-edrophonium was separated from its conjugate by descending paper chromatography in the solvent system butan-2-ol-water-ethanol-acetic acid (32 : 12 : 8 : 1, by volume). After resolution for 16 h at room temperature, radioactive zones were detected by directly adding strips (0.5 cm wide) of the chromatogram to scintillation fluid (Back & Calvey, 1972a).

In other experiments, [^{14}C]-edrophonium chloride (4.0 μmol/kg in saline) was given by systemic intravenous or intraportal administration to normal Wistar rats with ligated renal pedicles. Intraportal injection was effected by means of a 22 gauge needle attached to a length of fine polyethylene tubing filled with heparin sodium (10 mg/ml) inserted into the portal vein near the hilum of the liver. The other end of the tubing was attached to a needle and syringe. Carotid arterial blood samples were collected at 2, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60 and 75 min after administration of the drug. Plasma samples were assayed for [^{14}C]-edrophonium as described above.

Measurement of radioactivity

Liquid scintillation fluid (10 ml) of the following composition was routinely used: 2,5-diphenyloxazole (6 g), Triton-X-100 (333 ml), toluene (666 ml) and water (80 ml). Radioactivity was counted at an efficiency of approximately 85% in a Unilux Nuclear liquid scintillation spectrometer. Counting efficiency was determined by the channels ratio method using a ^{133}Ba external standard.

Results and Discussion

Distribution kinetics of [^{14}C]-edrophonium

After intravenous administration of [^{14}C]-edrophonium in normal Wistar rats, the concentration of the drug in plasma rapidly decreases between 5 and 55 min, owing to the rapid uptake of the drug by the liver and kidneys (Back & Calvey, 1972b). In rats with ligated renal pedicles, the decline in the plasma level was less rapid, particularly after 25 min (Fig. 1); this difference probably reflects the importance of renal excretion in the elimination of [^{14}C]-edrophonium.

In the present experiments, plasma concentration-time curves were invariably resolved by the method of residuals (Riggs, 1963) into three exponential components. Figure 1 illustrates a characteristic result for a normal Wistar rat; analogous data obtained from a typical homozygous Gunn rat are shown in Figure 2. In both strains, the plasma concentration after a single intravenous injection was described by the equation

$$C_p(t) = Pe^{-\pi t} + Ae^{-\alpha t} + Be^{-\beta t}$$

where $C_p(t)$ is the concentration of the drug at time t; π, α and β are hybrid rate constants; and P, A and B represent extrapolated intercepts at zero time. Values for these constants, and for the 'fast disposition', 'slower disposition', and 'slow disposition' half-lives are shown in Table 1. In homozygous Gunn rats, the half-lives were invariably less than in normal Wistar rats; the differences in 'fast disposition' and 'slow disposition' half-lives in the two strains were statistically significant ($P < 0.05$).

In both strains of animals, the data are consistent with a three compartment open model (Figure 3). A similar three compartment system has been used to explain the influence of the route of administration on the area under the plasma concentration-time curve (Gibaldi & Feldman, 1969). After administration of aspirin into the portal vein, the area under the plasma concentration-time curve was 54-78% of the corresponding area after intracaval injection (Harris & Riegelman, 1969); thus, intraportal administration results in the hepatic metabolism of a considerable part of the dose before it reaches the sampling site. (In contrast, in a two compartment model parameters of distribution and elimination should remain constant despite injection by different vascular routes, since both the kidney and the hepato-portal system are considered to be part of the central compartment.)

Since the plasma concentration-time curves

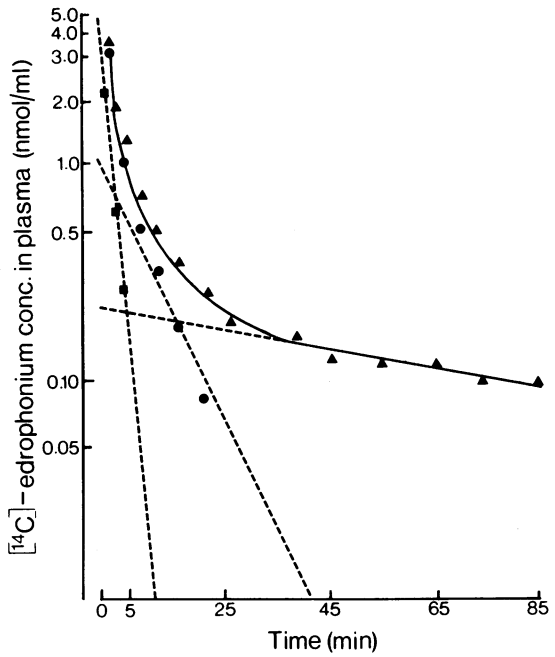

Fig. 1 The concentration of [^{14}C]-edrophonium in the plasma of a normal Wistar rat after intravenous administration of the drug (2.0 μmol/kg). (▲) observed results (the curve was resolved into three exponential components by back extrapolation); (●) first and second exponential terms, after subtraction of the third exponential term; (■) first exponential term, after subtraction of the second and third exponential terms. The data are described by the equation $C_p(t) = 4.8e^{-0.608t} + 1.55e^{-0.149t} + 0.20e^{-0.009t}$

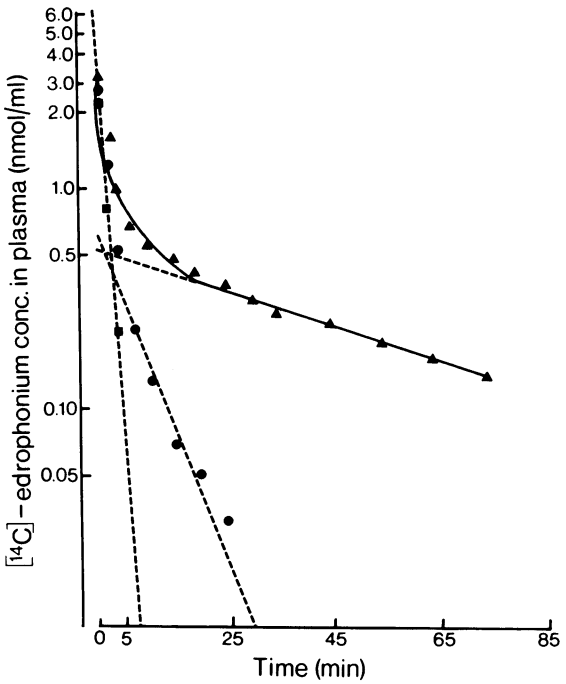

Fig. 2 The concentration of [^{14}C]-edrophonium in the plasma of a homozygous Gunn rat after intravenous administration of the drug (2.0 μmol/kg). (▲) observed results (the curve was resolved into three exponential components by back extrapolation); (●) first and second exponential terms, after subtraction of the third exponential; (■) first exponential term, after subtraction of the second and third exponential terms. The data are described by the equation $C_p(t) = 6.0e^{-0.667t} + 0.55e^{-0.119t} + 0.53e^{-0.019t}$

Table 1 Parameters of the triexponential equation: $C_p(t) = Pe^{-\pi t} + Ae^{-\alpha t} + Be^{-\beta t}$

	Normal Wistar rats	*Homozygous Gunn rats*
P	5.7 ± 0.7	5.9 ± 0.1
A	1.38 ± 0.26	0.51 ± 0.12
B	0.32 ± 0.04	0.41 ± 0.06
π	0.546 ± 0.042	0.817 ± 0.060*
α	0.132 ± 0.012	0.141 ± 0.013
β	0.012 ± 0.002	0.018 ± 0.002*
'Fast disposition' half life (min^{-1})	1.30 ± 0.10	0.87 ± 0.06*
'Slower disposition' half life (min^{-1})	5.46 ± 0.50	5.06 ± 0.34
'Slow disposition' half life (min^{-1})	68.40 ± 11.90	39.60 ± 2.80*

Half lives were calculated from the rate constants π, α and β; values represent mean ± s.e.
* Significant differences between normal Wistar rats and homozygous Gunn rats at $P < 0.05$ (Student's t test).

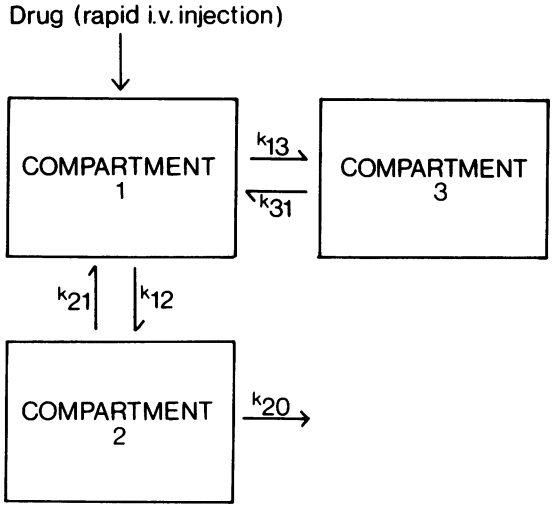

Fig. 3 Three compartment open model.

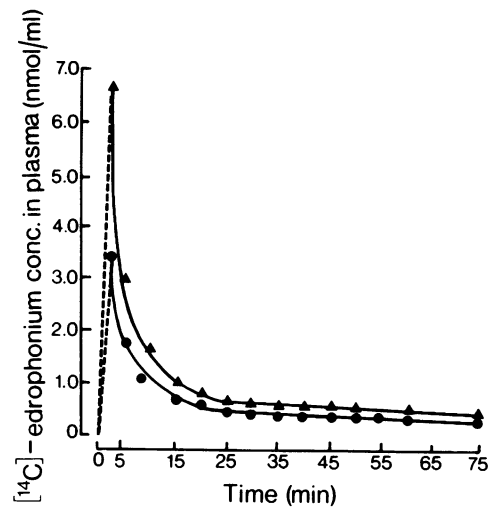

Fig. 4 The concentration of [^{14}C]-edrophonium in plasma after systemic venous (▲) and portal venous (●) administration of the drug (4.0 μmol/kg). Each curve is the mean of two experiments.

(Figs. 1 and 2) were consistent with a three compartment open model, and previous experiments (Back & Calvey, 1972a) suggested that edrophonium was rapidly and extensively metabolized, the influence of the route of administration on the concentration of [^{14}C]-edrophonium in plasma was studied. The results are shown in Figure 4. After intraportal administration, the area under the plasma concentration-time curve was 58% of the corresponding area with systemic venous injection. The discrepancy between the areas subtended by these curves is principally due to a 'first pass effect', and confirms that the hepato-portal system should be treated as a compartment distinct from the vascular site being sampled (Gibaldi & Feldman, 1969).

It is tempting to postulate that compartment 2 represents the hepato-portal system (and possibly other well perfused tissues), and that compartment 3 corresponds to less well perfused tissues from which unchanged [^{14}C]-edrophonium returns to plasma when its concentration in the central compartment falls. Tissue distribution studies (Back & Calvey, unpublished) in both Wistar rats and homozygous Gunn rats are consistent with this interpretation. In both strains more than 30% of the dose was present in the liver after intravenous administration, and well perfused tissues (for instance, cardiac muscle, lung, and intestine) contained a relatively higher concentration of radioactivity than most other tissues and organs. However, there are dangers in attempting to match individual terms of an exponential equation with supposedly corresponding processes or physiological regions of the body (Riggs, 1963).

Kinetic analysis of experimental data

Central compartmental volume (V_c) was calculated from the expression

$$V_c = \frac{\text{Dose}}{P+A+B}$$

Similar results were obtained in the two strains of animals (Table 2). Although the mean values were considerably greater than the plasma volume of the rat, they were consistent with estimates of extracellular fluid space.

In each experiment, the constants P, A, B, π, α and β (Table 1) were used to evaluate the first order rate constants k_{12}, k_{21}, k_{13}, k_{31} and k_{20}, by the method of Nagashima, Levy & O'Reilly (1968). Calculation of the individual parameters was carried out with a digital computer (KDF 9); the results are shown in Table 2. Evaluation of the first order rate constants in Wistar rats revealed that k_{12} was three times the magnitude of k_{21}; k_{13} was seven times the magnitude of k_{31}. In homozygous Gunn rats k_{12} was four times greater than k_{21}, and k_{13} was six times the magnitude of k_{31}. It is evident from a consideration of the individual rate parameters that in the homozygous Gunn rat there is an increased rate of uptake into

Table 2 Individual parameters of an open three compartment model for edrophonium

	Normal Wistar rats	*Homozygous Gunn rats*
Volume of central compartment (V_c)(ml g^{-1})	0.190 ± 0.019	0.189 ± 0.002
First order distribution rate constant (min^{-1})		
k_{12}	0.286 ± 0.023	0.440 ± 0.028
k_{21}	0.104 ± 0.012	0.106 ± 0.006
k_{13}	0.160 ± 0.013	0.281 ± 0.025
k_{31}	0.026 ± 0.003	0.050 ± 0.006
First order metabolism rate constant (min^{-1})		
k_{20}	0.114 ± 0.011	0.099 ± 0.014

Values represent the mean ± s.e. of five experiments.

the peripheral compartments. This phenomenon may be related to the unconjugated hyperbilirubinaemia of the homozygous Gunn rat, and its possible effect on tissue permeability. In addition, a comparison of the values for the half-lives in the two strains suggests that in the homozygous Gunn rat the initial rate of distribution is greater, and that the 'body half-life', $0.693/\beta$, is less.

In the present experiments urinary excretion was eliminated from consideration by renal pedicle ligation, and since direct plasma to bile transfer is only a minor elimination pathway (Back & Calvey, 1972a), k_{20} is solely the metabolism (i.e., glucuronide conjugation) rate constant. A comparison of k_{20} in both strains shows that there is virtually no difference in the value of the rate constant. Thus, the rate of conjugation of [^{14}C]-edrophonium is unaltered in the homozygous Gunn rat despite the genetic lesion in UDP-glucuronyltransferase. Such evidence lends support to the theory (Calvey & Back, 1973) that the vastly increased biliary excretion of [^{14}C]-edrophonium glucuronide in the homozygous Gunn rat is due to an increased rate of transfer across the canalicular membrane, and not to an increased rate of formation of the conjugate.

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