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# The effect of pentobarbitone anaesthesia and hypothermia on the hepatic clearance of indocyanine green and *S*(−)-acenocoumarol in the rat

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The effect of pentobarbitone anaesthesia and the effect of hypothermia associated with the anaesthesia on the blood clearance of indocyanine green (ICG) and of *S*(−)-acenocoumarol (AC) was investigated in male Wistar rats using the constant rate infusion technique. During anaesthesia, body temperature was regulated by heat supply. At normothermia (rectal temperature of 37·5 °C), pentobarbitone anaesthesia decreased ICG clearance from 8·0 ± 1·7 ml $min^{-1}$ (mean ± s.e.m., n = 3) in the conscious state to 4·3 ± 0·6 ml $min^{-1}$ and AC clearance from 4·9 ± 0·4 ml $min^{-1}$ to 3·4 ± 0·3 ml $min^{-1}$. Hypothermia further reduced the clearances of the compounds to 0·9 ± 0·1 and 2·5 ± 0·2 ml $min^{-1}$ for ICG and AC, respectively. The effect of hypothermia was reversible. The results show that pharmacokinetic constants obtained in the anaesthetized animal may differ greatly from those in the conscious one. If pharmacokinetic experiments are performed in the anaesthetized animal, body temperature should be controlled and a slight hyperthermia is most favourable. Our pharmacokinetic data on ICG seriously question the use of this drug to estimate liver blood flow in the rat.

It is widely accepted that the pharmacokinetic description of a drug by the physiologically-based or perfusion-limited pharmacokinetic model gives a better understanding of its behaviour in the body than the multicompartment pharmacokinetic model (Gerlowski & Jain 1983). To describe drug disposition in the physiological pharmacokinetic model it is necessary that physiological and pharmacokinetic parameters like flow and intrinsic clearance are known precisely. For this purpose anaesthetized animals are most often used. Anaesthesia, however, could influence organ flow as well as intrinsic clearance. It is known to depress the autonomic nervous reflex system involved in blood pressure regulation (Cox & Bagshaw 1979), and it may cause hypothermia (Birnie & Grayson 1952) which might decrease processes (metabolism, active transport) of drug distribution and of drug elimination (McAllister et al 1978, 1979; Spurr & Dwyer 1972). Also an (inhibitory) interaction of the anaesthetic with the drug metabolizing system might occur (Vermeulen et al 1983). An indication of the pitfalls of estimating physiological parameters during anaesthesia may be found in the deviating data on hepatic blood flow in rats as estimated by the well known indocyanine green (ICG) clearance method. Values between 0·9–1·5 ml $min^{-1}$ $g^{-1}$ liver are reported for anaesthetized as well as for conscious rats (Yokota et al 1976).

To evaluate the influence of anaesthesia in the rat we have investigated the effect of pentobarbitone anaesthesia alone and together with the changes in body temperature, on the hepatic clearance of ICG and of a low clearance drug. For this we used the *S*(−)-enantiomer of the coumarin-derivative acenocoumarol (AC) which is eliminated solely by the liver, having in the rat an extraction ratio of about 0·3 and an elimination half-life of about 20 min (Thijssen et al 1982).

## MATERIALS AND METHODS

*S*(−)-Acenocoumarol (AC) was a gift from Ciba-Geigy, Basel, Switzerland. ICG (Cardiogreen) was received from Brocacef, Heerlen, The Netherlands.

Male inbred Wistar Kyoto rats (TNO Zeist, The Netherlands), 280–330 g were used. The conscious animals had free access to tap water and food. Two days before the experiment, under light ether anaesthesia rats were cannulated (PE-10) in the left femoral artery for blood sampling, in the left femoral vein for drug infusion and in the right femoral vein for the i.v. administration of pentobarbitone. The tip of the femoral artery catheter reached into the aorta abdominalis just below the renal arteries. The catheters were exteriorized in the neck and were

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flushed with 10–50 µl of heparin solution in saline (50 u $ml^{-1}$).

*Administration and sampling of acenocoumarol and indocyanine green*

Blood clearance was estimated using the constant rate infusion technique. Infusion was by an external pump device. AC was dissolved in 0·1 M phosphate buffer pH 7·4 and infused at 0·6 µg $min^{-1}$ to give AC blood concentrations sufficiently high (at least 100 ng $ml^{-1}$) that 100 µl blood samples sufficed for analyses. Blood samples were transferred into heparinized vessels and stored at 4 °C. Sample preparation and drug analysis were as described by Thijssen et al (1983).

ICG was dissolved in a 4% bovine albumin solution in saline and infused at 25 µg $min^{-1}$ to give plasma concentrations of approximately 5 µg $ml^{-1}$. Arterial blood samples (100 µl) were transferred into vessels containing 10 µl 0·1 M citrate. ICG plasma concentration was determined spectrophotometrically at 800 nm. Pool plasma was used as reference. Plasma ICG concentration was transformed to blood concentration by correcting for the blood haematocrit (Ht).

*Experimental protocol*

*The effect of anaesthesia*

In preliminary experiments, a fall in body temperature to 32–33 °C occurred within a short time after the induction of pentobarbitone anaesthesia. Therefore to investigate the effect of anaesthesia on the body clearance of both AC and ICG, the temperature of the animals was maintained at normal.

Infusion was started in the conscious animal. Two blood samples (time interval 15 min) were obtained after 60 and 75 min of ICG infusion and after 80 and 95 min of AC infusion. Pilot experiments showed these times suffice to reach steady state conditions. While maintaining infusion of AC or ICG, pentobarbitone anaesthesia was induced by a bolus injection of 0·2 ml Na-pentobarbitone solution (60 mg $ml^{-1}$) and maintained by administration of 0·1 ml of the Na-pentobarbitone solution every 60 min. After animals lost consciousness their body temperature (measured rectally) was maintained at 37·5 °C by a heat lamp and regulator. Two blood samples (time interval 10–15 min) were obtained following the time schedule as before.

*The influence of temperature*

A separate group of rats was anaesthetized with Na-pentobarbitone and the body temperature maintained at 37·5 °C (as above). Infusion of AC or ICG was started and arterial blood was sampled twice at steady state (see time schedule above). During infusion the body temperature was either increased to 39·5 °C by additional heat or cooled to 32·5 °C by stopping the heat and when steady state had been attained, blood samples were obtained according to the previous time schedule.

To complete an experiment, 6 to 8 blood samples were taken from a rat. Each sample was replaced by the same volume of saline (50 µl). The amount of blood (maximum 1 ml/rat) withdrawn had little influence on the blood haematocrit (Ht: 0·38–0·44), therefore, it was not considered necessary to replace the sampled blood volume by donor blood.

*Pharmacokinetic analysis.* Concentration blood = concentration plasma × $(1 - H_T)$. Blood clearance (Cl) was determined from the steady state blood concentration ($C^{ss}$) by: Cl = infusion rate/$C^{ss}$.

*Statistics.* Differences between the various experimental conditions were compared using the paired Student's *t*-test. The 0·05 level of probability was used as the level of significance.

## RESULTS

*The effect of anaesthesia*

The steady-state concentrations during constant rate infusion in the conscious state and under pentobarbitone anaesthesia were, respectively, 0·12 ± 0·04 and 0·17 ± 0·02 for AC (blood) and 5·2 ± 0·7 and 9·6 ± 0·8 µg $ml^{-1}$ for ICG (plasma, mean ± s.e.m.). The estimated blood clearances are in Table 1. Anaesthesia decreased the body clearance of both drugs significantly. Mean AC clearance was reduced by about 30% (from 4·9 to 3·4 ml $min^{-1}$) and ICG clearance by about 45% (from 8·0 to 4·3 ml $min^{-1}$).

Table 1. The effect of pentobarbitone anaesthesia on the blood clearance of acenocoumarol (AC) and indocyanine green (ICG)[a].

| Experimental condition | Blood clearance (ml $min^{-1}$)[b] AC | ICG |
|---|---|---|
| Conscious | 4·9 ± 0·4 | 8·0 ± 1·7 |
| Anaesthesia | 3·4 ± 0·3 | 4·3 ± 0·6 |
| | $P < 0·001$ | $P < 0·01$ |

[a] Experiments under pentobarbitone anaesthesia were at 37·5 °C (rectal temperature).

[b] Mean ± s.e.m., n = 8.

*The effect of body temperature*

The steady-state blood concentration of AC at body temperatures of 37·5 °C (normothermia), 39·5 °C (hyperthermia) and 32·5 °C (hypothermia) were 0·16 ± 0·02, 0·14 ± 0·02 and 0·24 ± 0·03 μg ml$^{-1}$, respectively.

For ICG, the observed plasma concentrations were 7·3 ± 0·6, 9·2 ± 0·5 (7·5 ± 0·5) and 46·6 ± 2·6 μg ml$^{-1}$, respectively. The estimated blood clearances are in Table 2.

Table 2. The effect of body temperature on the blood clearance of AC and ICG during pentobarbitone anaesthesia.

| Body temperature (°C)[a] | Blood clearance (ml min$^{-1}$)[b] AC | ICG[f] |
|---|---|---|
| 37·5 | 3·8 ± 0·4 | 5·7 ± 0·8 |
| 39·5 | 4·4 ± 0·4 | 4·5 ± 0·4 (5·6 ± 0·7)[c] |
| 32·5 | 2·5 ± 0·2[d] | 0·9 ± 0·1[e] |

[a] Body temperature was measured rectally.
[b] Mean ± s.e.m., n = 8. The hyper- and hypothermia values are a compilation of the clearance values obtained in animals that followed either the 37·5–39·5–32·5 (n = 4) or the 37·5–32·5–39·5 (n = 4) temperature schedule.
[c] Mean ± s.e.m., for those animals (n = 4) that followed the 37·5–39·5–32·5 temperature schedule.
[d] $P < 0·01$ vs 37·5 °C.
[e] $P < 0·001$ vs 37·5 °C.

For clarity, the mean blood concentrations and the clearance values at hyper- as well as hypothermia are a compilation of experiments made according to the 37·5–39·5–32·5 and the 37·5–32·5–39·5 temperature schedule (see Methods). In hyperthermia AC clearance was marginally higher than at normothermia (4·4 vs 3·8 ml min$^{-1}$), but the difference was not significant ($P > 0·05$). Hypothermia significantly lowered the AC clearance in comparison with normothermia (2·5 vs 3·8 ml min$^{-1}$).

No difference in ICG clearance from normothermia was observed for those animals where the hyperthermic period (5·6 ml min$^{-1}$) followed on the normothermic control period (5·7 ml min$^{-1}$) (Table 2).

In hypothermia, the clearance of ICG dropped dramatically to 0·9 ml min$^{-1}$ which was only 16% of the normothermic clearance value. This suppression was irrespective of the sequence of the temperature variations.

The time-effect of anaesthesia on the clearance of ICG and AC was investigated with rats in the normothermic state (37·5 °C). No changes in clearances were observed over 360 min (Fig. 1).

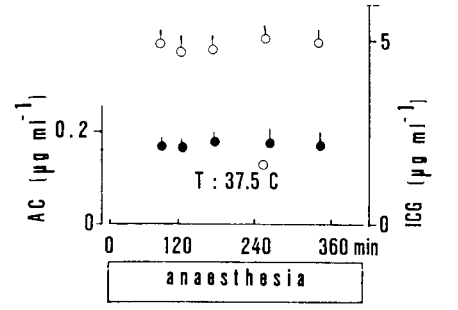


FIG. 1. Arterial blood concentrations (μg ml$^{-1}$) of AC (●) and ICG (○) (mean ± s.e.m., n = 4); following continuous i.v. infusion for 360 min of 0·6 μg min$^{-1}$ AC or 25 μg min$^{-1}$ ICG during anaesthesia, while body temperature was kept at 37·5 °C.

## DISCUSSION

The prime purpose of our experiments was to investigate the effect of anaesthesia and the effect of hypothermia induced by it on the hepatic clearance of two substances, one a drug the other a dyestuff. In the literature few authors discuss the effect of anaesthesia on drug disposition explicitly. Recently, Vermeulen et al (1983) showed that ether anaesthesia almost halved hexobarbitone clearance in rats. Yokota et al (1976) observed the depressant effect of pentobarbitone on ICG clearance in rats and considered hypothermia as a possible cause.

We found pentobarbitone anaesthesia to depress the clearance of both ICG and AC despite the body temperature of the rat being maintained at 37·5 °C or even slightly hyperthermic (i.e. 39·5 °C, which is the temperature of the liver in the conscious rat, Birnie & Grayson 1952). The hypothermia related to anaesthesia further reduced the body clearances, AC clearance being halved and the ICG clearance reduced to one tenth of the conscious values. As AC is a low clearance drug, its body clearance is flow independent and is related directly to the intrinsic capacity of the liver to extract it. Hence the effect of pentobarbitone is to depress that intrinsic capacity. As hypothermia depresses biochemical functions in general, the intrinsic clearance of AC should be lower.

Though it is generally assumed that the ICG clearance is liver flow dependent (Caesar et al 1961; Leevy et al 1962), we doubt that the effects of anaesthesia and hypothermia on ICG clearance in our experiments are reflections of changes in liver blood flow alone. The observed ICG clearance in the conscious rat (about 24 ml min$^{-1}$ kg$^{-1}$) is low compared with liver blood flow (about 80 ml min$^{-1}$ kg$^{-1}$,

Ohnhaus 1979). This indicates that the extraction ratio for ICG is only about 0·3 which, according to the physiological hepatic clearance model (Wilkinson & Shand 1975), suggests a low rather than a high clearance drug. Saturation of ICG elimination during the experimental condition could be a reason for the low extraction ratio. It is well known that ICG elimination is dose-dependent and it has been shown that ICG plasma concentrations above 10 $\mu g\ ml^{-1}$ progressively tend to decrease its clearance (McDevitt et al 1977; Stoeckel et al 1980). However, in our experiments steady state ICG plasma concentrations in the conscious rat were about 5 $\mu g\ ml^{-1}$, and in the anaesthetized normothermic rats about 8 $\mu g\ ml^{-1}$, thus making saturation kinetics a less likely explanation. Yokota et al (1976), using the continuous infusion technique, reported a value of 18 $ml\ min^{-1}\ kg^{-1}$ for the ICG clearance in the conscious rat and this is comparable with our results. Because of the low extraction of ICG and its low clearance, it is not justified to interpret ICG clearance in the rat in terms of liver flow.

Our observation that ICG blood clearance did not return to the normo- or hyperthermic levels following the hypothermic period (Table 2), in all probability is the result of the experimental design. Due to the deterioration of the ICG clearances at hypothermia, ICG plasma concentration rose to levels (i.e. beyond 40 $\mu g\ ml^{-1}$) where deviation from linear ICG pharmacokinetics occurs (Stoeckel 1980). Thus, although the intrinsic liver ICG clearance might be recovered, restoration of ICG elimination rate lags behind because of the initial high ICG plasma concentrations. That is to say the elapsed time before blood samples were taken (i.e. at 60 and 75 min after the hyperthermic state was achieved) was too short for complete recovery of the ICG blood clearance from the hypothermic depression.

In summary, our data have demonstrated clearly that anaesthesia and, in association with it, hypothermia strongly depress the intrinsic capability of the liver to extract xenobiotics from the circulation. Thus, to estimate pharmacokinetic constants, the use of conscious animals is to be preferred. Alternatively, a precise control of the body temperature during anaesthesia is essential. We seriously doubt the accuracy of estimates of liver flow using ICG in the rat.

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