EFFECT OF BILIARY EXCRETION ON KETAMINE ANAESTHESIA IN THE RAT

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SUMMARY

Ligation of the common bile duct in rats resulted in a significant prolongation of ketamine sleeping time and a significant increase of plasma concentration of ketamine and its N-demethylated metabolite, when compared with sham-operated control animals. The treated animals also showed significant increases of plasma bilirubin and glutamate pyruvate transaminase. Cannulation of the common bile duct in rats allowed collection of samples of bile which showed high concentrations of ketamine and its N-demethylated metabolite very shortly after i.p. injection of ketamine, the amount of ketamine alone accounting for 10% of the injected dose after 2 h; including the metabolite up to 25% of the injected dose could be accounted for. In view of this we would suggest that ligation of the bile duct in rats produced a prolongation of ketamine anaesthesia by increasing plasma concentrations through interference with a major excretory route for the drug and its main metabolite.

Ketamine is metabolized by the liver (Chang, Dill and Glazko, 1965) and it has been shown that pentobarbitone pretreatment can shorten the duration of anaesthetic action (Piel, Aldrete and Jones, 1969), indicating a role for hepatic metabolism in the duration of action of this drug. Several studies have indicated that tolerance to ketamine may develop in a variety of species, such as man (Bjarnsen and Corssen, 1967; Cronin et al., 1972; Bennett and Bullimore, 1973), monkey (Bree, Feller and Corssen, 1967) and rats (Douglas and Dagirmanjian, 1975), and it has been shown that increased hepatic metabolism plays an important part in the development of tolerance to ketamine (Livingston and Waterman, 1976, 1977, 1978a). As an extension to the studies on hepatic metabolism, a preliminary study on the effects of bile duct ligation on the duration of ketamine anaesthesia (Livingston and Waterman, 1978b) indicated that anaesthesia was significantly prolonged when the ketamine was given only 2 h after the ligation. This observation led to the suggestion that biliary excretion of the drug may be a factor in the termination of its action, particularly since White and others (1976) have detected ketamine in the bile of rats following i.m. injection. This investigation was designed to examine the contribution of biliary excretion to the duration of anaesthetic action of ketamine.

METHODS

Albino Wistar rats (12-week-old females; body weight 190–260 g) from a cross-fostered inbred colony were fed water and a standard diet ad libitum and kept under constant temperature and light conditions.

Bile duct ligation experiments

Groups of rats were lightly anaesthetized with halothane (May and Baker Ltd) and the common bile duct was exposed and ligated (or not, in the case of the sham-operated animals) at the end nearer the duodenum. The incision was closed and the animals left for 1–2 h to recover from anaesthesia. The rats were then injected with ketamine (Ketalar, Parke Davis Ltd) 100 mg kg⁻¹ i.p. and the sleeping time, that is the time between loss and return of the righting reflex, was measured. At the point of recovery the animals were killed by decapitation and a heparinized mixed blood sample was collected and the brain dissected out and weighed. Blood samples were centrifuged to obtain plasma which was frozen until assay, the brains were homogenized in 0.9% saline to give a 10% homogenate, centrifuged at 1500 g for 30 min and the frozen supernatant was stored until assay.

Ketamine and its N-demethylated metabolite were assayed in both plasma and brain samples by the gas–liquid chromatographic method described by Chang and Glazko (1972) using 2-amino-2-(o-bromophenyl)-2-methylamino cyclohexanone as internal standard.
Plasma samples were assayed also for bilirubin (both direct and indirect) by a colorimetric method using a diazo reagent (Sigma kit 605) and serum transaminases (s.g.o.t. and s.g.p.t.) by a colorimetric method using phenylhydrazones of the oxaloacetic or pyruvic acids formed, respectively (Sigma kit 505).

In addition to the recovery experiments, groups of six rats were prepared in the same way and killed at 10, 20, 30, 50 and 70 min after the injection of ketamine and plasma concentrations of ketamine and its N-demethylated metabolite were measured in the test and sham-operated animals. Conjugated bilirubin and transaminases were also measured in the plasma from the animals killed 10 min after injection.

**Bile collection experiments**

Rats were lightly anaesthetized with 1% halothane using open circuit and the common bile duct was exposed and cannulated towards the liver with a fine polyethylene tube. The incision was closed and the cannula was secured to the skin with adhesive tape in a manner which facilitated bile flow along the cannula. The rats were placed on a heated pad and collection of spontaneously flowing bile for 10-min periods was commenced. Bile was collected during halothane anaesthesia for 30 min, the halothane was then withdrawn and a slow i.p. injection of ketamine 100 mg kg$^{-1}$ was given over several minutes; this was necessary to prevent apnoea. Bile fractions were collected for a further 140 min. During the later stages (usually from the 60th min onwards) it was necessary to administer halothane to offset the recovery from ketamine. The bile samples were measured for volume and weight and kept frozen until extraction and assay for ketamine and the N-demethylated metabolites.

**RESULTS**

In animals with ligated bile ducts, the experiments to measure sleeping time confirmed the earlier suggestion (Livingston and Waterman, 1978b) that, even after a period of 2 h or less, ligation significantly increased sleeping time after ketamine injection ($P<0.01$) and it was found that, although brain concentrations at recovery were not significantly different between the two groups, the plasma concentration of ketamine ($P<0.01$) and the demethylated metabolite ($P<0.05$) were both significantly greater in the animals with ligated bile ducts at recovery (fig. 1). In the control animals sleeping times were not significantly different from unoperated animals receiving the same dose.

The estimates of bilirubin, s.g.o.t. and s.g.p.t. in the plasma at the time of recovery also showed significant differences between the ligated and sham-operated animals at the level $P<0.001$ in all cases (fig. 2). The estimations of plasma ketamine and demethylated metabolite concentrations at various times after the injection of ketamine in sham-operated and bile duct-ligated animals also showed many significant differences between the two groups (fig. 3) with the increase in concentration of both agents becoming apparent after only 30 min in the bile duct-ligated animals. The measurement of plasma
FIG. 3. Plasma concentrations of ketamine and the N-demethylated metabolite in bile duct-ligated and sham-operated animals at various times after injection of ketamine 100 mg kg\(^{-1}\) i.p. (*P<0.05; **P<0.01; ***P<0.001). Mean ± SEM of three animals.

FIG. 4. Concentration of ketamine and the N-demethylated metabolite in bile of rats at various times after injection of ketamine 100 mg kg\(^{-1}\) i.p. Mean ± SEM of five or six observations.

conjugated bilirubin, g.o.t. and g.p.t. in the animals killed 10 min after the injection of ketamine showed a significant (P<0.05) increase in bilirubin, in g.p.t. (P<0.01) and an increase in g.o.t. which was not significant in the bile duct-ligated group compared with control.

The experiments using animals with cannulated bile ducts to collect bile showed the presence of both ketamine and its N-demethylated metabolite in high concentrations (fig. 4). Following the injection, the concentrations of ketamine increased rapidly, to reach a maximum during the 10–20-min collection period and thereafter declined in an exponential manner for the next 2 h. The concentrations of the demethylated metabolite increased to reach a maximum over the same time period, but then appeared to plateau for the next 2 h before declining, a pattern not dissimilar to that seen in the plasma. However, both agents were found in bile at concentrations about 100 times greater than that found in the plasma at the same time.

The volumes of bile produced were also shown to change during the period of the experiment (fig. 5) with a significant increase in volume of the first collection after the ketamine injection and this volume increase remained significantly increased throughout the period of the experiment. However, the density of the bile (mean 1.1 g ml\(^{-1}\)) did not change significantly during the experiment. These changes in bile volume may well reflect the changes which anaesthesia produced in the peripheral arterial pressure, since a parallel relationship between the two would seem to exist. The initial depression caused by halothane was rapidly reversed on its discontinuation, but the initial depression caused by the ketamine reduced it again, and it was followed by a slow but prolonged increase which persisted until halothane was reintroduced to maintain anaesthesia.
The significant increase in ketamine sleeping time following short-term ligation of the bile duct confirms the earlier work using male rats receiving a lower dose (Livingston and Waterman, 1978b). Although the brain concentrations of ketamine and the N-demethylated metabolite did not differ from those of animals at recovery, there was a significant increase in plasma concentrations of both agents at this time in the treated animals. Marietta and others (1976) have reported that, after the initial redistribution phase, the brain concentrations in control animals declined in parallel with the plasma concentrations, which might suggest that bile duct ligation can affect the distribution of the drug and its major metabolite in a significant manner.

The study of plasma concentrations of ketamine and the N-demethylated metabolite in treated and control animals indicated that the effect of ligation was to increase significantly the concentrations of both agents after a relatively short time. This observation led to the possibility that the effects were caused by a net reduction in the transfer of ketamine and its metabolite from the blood to the bile, although the possibility of conjugation or active secretion could not be ignored. Net transfer could be reduced by an increase in the backflow of bile contents in the blood (Barber-Riley, 1963) and a decrease in bile production (Edlund, 1948). Bilirubin is conjugated in the liver and actively excreted into the bile (Smith, 1966). The observations that plasma bilirubin concentrations in the ligated animals were significantly increased both early in anaesthesia and at recovery would suggest that a similar circumstance involving active secretion might exist with regard to the transfer of ketamine and its metabolite.

Degenerative changes have been reported in the livers of rats within 24 h of experimental obstruction of the bile duct (Edlund, 1948), and ketamine has been reported to cause a reversible increase of serum g.o.t. and g.p.t. in dogs (McCarthy et al., 1965). However, in this study the concentrations in the ligated animals were always significantly greater than in the sham-operated controls, which indicated that the ligation, rather than the ketamine injection, was the cause of the increase of enzyme concentrations, although the period was short compared with that in the studies of Edlund (1948). Dogs have a gallbladder but rats do not, and it is possible that any pressure effects on the liver would be more obvious in rats. The studies on the time-course of changes in plasma concentrations and its metabolite would indicate that there was still considerable demethylation of ketamine proceeding, although the concentrations of g.o.t. and g.p.t. were increased.

The concentrations of ketamine and its demethylated metabolite present in the bile were large compared with plasma concentrations. During the 1st h, approximately 6.2% of an average 25-mg injected dose of ketamine was excreted unchanged in the bile, and about 10.3% during the first 2 h. If the amount of demethylated metabolite is included, the values increase to about 14.5% in the 1st h and 26.8% during a 2-h period which represents a considerable proportion of the injected dose. White and others (1976) reported that they could detect only 2.3% of the injected ketamine in the unchanged form, in the bile during the first 1-h period. However, their rats were anaesthetized continuously with halothane and their work clearly demonstrates that halothane does affect the disposition and metabolism of ketamine, and so it is difficult to compare the figures from the two experiments; although the animals were only lightly anaesthetized before our experiments, it is possible that this could have some effect on the results. The figures for plasma and bile concentrations were obtained in two separate experiments and so care must be taken in interpretation, but it would appear that there is about a 100-fold greater concentration in bile than in plasma, which would put ketamine in the "group B" category of Brauer (1959), which contains water-soluble, polar substances with a molecular weight in excess of 300, and includes such compounds as bile salts, bilirubin-glucuronide and conjugates of many drugs and hormones (Smith, 1973). It is not clear, however, if the ketamine and its metabolite are excreted in a free or combined form.

The time-course of bile secretion of the demethylated metabolite was different from that of ketamine, but was close to that seen in the plasma of control animals, suggesting that although the plasma:bile concentration ratio for both drugs was about 1:100, the plasma concentration could influence the pattern of bile concentration.

The bile flow rate during halothane anaesthesia is very similar to the value of 0.124 ± 0.005 ml per 10 min reported by Vanlerenberghe and others (1968) for slightly larger male rats anaesthetized with pentobarbitone, and it is possible that the fluctuations in volume seen are influenced by anaesthetic-induced arterial pressure changes, although these effects are clearly not parallel. Both a reduction in hepatic blood flow and an increase in hepatic venous...
pressure can influence bile formation (Preisig et al., 1963), although the potential osmotic effects of ketamine and its metabolite on bile volume cannot be excluded. However, many other factors are known to affect bile flow, including, of course, the enterohepatic recirculation of bile salts which promote bile secretion (Sperber, 1959). Thus cannulation in these experiments should have caused a reduction in the observed flow.

The observations recorded here, that ligations of the bile duct caused a prolongation of anaesthesia and increased plasma concentrations of both ketamine and its N-demethylated metabolite, would suggest an excretory role for the bile in the metabolism of ketamine, particularly when the rapid appearance and high concentrations of ketamine and its N-demethylated metabolite are considered. These high concentrations would also suggest a process of active secretion of these agents into the bile; however, it is not possible at this stage to state whether ketamine and its metabolite are in a free or conjugated form.

REFERENCES


EFFET DE L'EXCRETION BILIAIRE SUR L'ANESTHESIE A LA KETAMINE CHEZ LE RAT

RESUME

La ligature du canal bilaire chez les rats a eu pour resultat une prolongation significative du temps de sommeil provoque par la ketamine, de meme qu'une augmentation significative des concentrations de ketamine et de son metabolite N-demethyle dans le plasma, par rapport aux animaux temoins ayant subi des operations simulées. Les animaux traites ont aussi accuse des augmentations significatives de bilirubine et de pyruvate transaminase glutamique dans le plasma. La pose d'une canule sur le canal bilaire des rats a permis de ramasser des echantillons de bile montrant de fortes concentrations de ketamine et de son metabolite N-demethylé immediatement apres l' injection i.p. de ketamine, la quantite de ketamine seule representant au bout de 2 h, 10% de la dose injectee; y compris le metabolite jusqu'a 25% de la dose injectee. En raison de ce qui precede nous pouvons annoncer que la ligature du canal bilaire des rats produit une prolongation de l'anesthesie par la ketamine en augmentant ses concentrations dans le plasma, et en entravant de ce fait le chemin excretif le plus important pour le medicament et son principal metabolite.
WIRKUNG VON GALLENAUSSCHEIDUNG AUF DIE KETAMINNARKOSE BEI RATTEN

ZUSAMMENFASSUNG