J. Pharm. Pharmacol. 1986, 38: 391–393
Communicated October 28, 1985

Transport of phenobarbitone into the intestinal lumen and the biliary tract following i.v. administration to rats

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The exsorption of intravenously administered phenobarbitone into the lumen of the small intestine of rats has been examined by an in-situ single-pass perfusion technique. The levels of the drug in the bile were about twice that in serum. Moreover, it was shown that the drug was appreciably exsorbed into the intestinal lumen. The exsorption rates of the drug into the perfusates were not very different between isotonic phosphate buffers at pH 6·0 and 8·0. The amounts of phenobarbitone exsorbed into the perfusate and excreted into the bile were 6·47 and 0·45% of the dose for isotonic phosphate buffer at pH 6·0, and were 6·09 and 0·54% of the dose for isotonic phosphate buffer at pH 8·0.

The clearance of intravenously administered phenobarbitone (Berg et al 1982) or theophylline (Berlinger et al 1983: Mahutte et al 1983) is increased by orally administered activated charcoal. The possible mechanism has been adsorption of the drug secreted into the gastrointestinal tract. Although there are some reports concerning the transport (exsorption) of intravenously administered drugs into the intestinal lumen (Nogami et al 1963; Kakemi et al 1970; Kimura et al 1971; Nadai et al 1972; Tokunaga et al 1978; Yasuhara et al 1979; Yamamoto et al 1984), few have aimed at the application of intestinal dialysis to the treatment of drug poisoning. Phenobarbitone remains an important drug in clinical toxicology, and if its enteral secretion and reabsorption are extensive, interruption of this circulation by adsorbents such as activated charcoal may increase the rate of its elimination.

The present study was undertaken to elucidate the characteristics of exsorption and/or excretion of phenobarbitone into the small intestinal and the biliary tracts to find the relative importance of exsorption and biliary excretion in the transport of the drug from blood into gastrointestinal tract.

Materials and methods

Materials. Phenobarbitone sodium was obtained from Daiichi Seiyaku Co., Ltd, Tokyo, and other chemicals used were of analytical grade.

Methods. Wistar strain male rats, 320–370 g, fasted overnight with free access to water, were anaesthetized by an intraperitoneal injection of ethyl carbamate (1·2 g kg^{-1}) and the small intestine exposed by a midline abdominal incision. The upper duodenum and the ileocaecal junction were cannulated with polyethylene tubing and the entire small intestine washed with saline maintained at 37 °C. The bile was collected separately every 15 min from the common bile duct also cannulated with polyethylene tubing. Blood samples to determine serum drug concentration were taken from the cannulated left femoral vein, in the middle of the perfusate collection period. Intestinal exsorption experiments were conducted as an in-situ single pass perfusion in which isotonic phosphate buffers (0·1 M, pH 6·0 and 8·0), which had been maintained at 37 °C, were perfused at the rate of approximately 20 ml/15 min from the duodenum through the small intestine to the ileocaecal junction using a glass pump (GM-24, Tokyo Rikakikai). Phenobarbitone sodium at 10 mg ml^{-1} was injected over about 2 min into the right femoral vein at a dose of 10 mg kg^{-1}. After injection, perfusates were collected every 15 min from the ileal outflow in a 25 ml volumetric flask and diluted with buffer to 25 ml before assay.

Analytical method. Phenobarbitone in the perfusate and the bile was determined by high performance liquid chromatography with hexobarbitone as an internal standard. A 2 ml portion of the perfusate or a 50 µl portion of bile was extracted with 5 ml of chloroform containing the internal standard after addition of 1 ml of 1 M hydrochloric acid. Separation was with a LiChrosorb RP-18 column (4·6 mm i.d. × 250 mm) at a flow rate of 1·0 ml min^{-1}. The mobile phase consisted of acetate buffer at pH 4·0 and acetonitrile (70:30). Phenobarbitone was detected at 220 nm. The concentrations of the drug in the serum were measured by a homogenous immunoassay technique (Ames TDA, Ames Co.).

Results and discussion

Fig. 1 shows the concentrations of phenobarbitone in the serum and bile, and the exsorption rate of the drug into the perfusates of isotonic phosphate buffers at pH 6·0 or 8·0 following its intravenous administration (sodium salt) to rats at 10 mg kg^{-1}. The concentrations of the drug in the bile were about twice those in the serum. This observation suggests that phenobarbitone is excreted into the intestinal lumen via the bile tract to an appreciable extent. Moreover, phenobarbitone was also exsorbed into the small intestinal lumen. The exsorption rate–time profile of the drug into the perfusates reflected the serum level profile. Accordingly, the rate of exsorption of the drug into the perfusate seemed to reflect the serum level.

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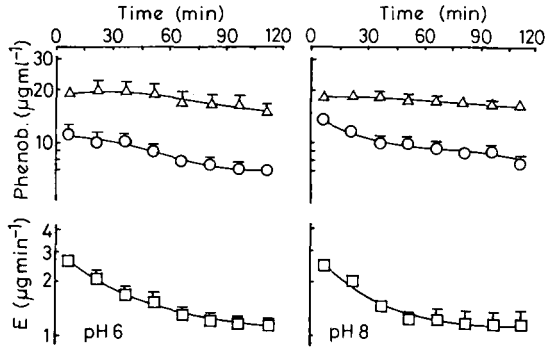

FIG. 1. The concentrations of phenobarbitone in the serum and the bile and the exsorption rate (E) of phenobarbitone into the perfusates of isotonic phosphate buffer at pH 6·0 and pH 8·0 following i.v. administration of phenobarbitone sodium (10 mg kg^{-1}) to rats. Each point represents the mean ± s.e.m. of 3 rats. Key: ○, serum; △, bile.

The pK_a value of phenobarbitone is 7·41 (Suzuki et al 1970). Consequently, it may be expected that the exsorption rates of the drug into the perfusates will vary between two perfusates of different pH because there is the difference in the percentage of the un-ionized form of phenobarbitone between perfusates. However, the exsorption rates of the drug into the perfusates were not very different between isotonic phosphate buffers at pH 6·0 and 8·0. This may be explained by lack of reabsorption under the experimental conditions used since the concentration of the drug in the perfusates was low.

The amounts of phenobarbitone transported into the perfusate and bile in 120 min are shown in Fig. 2. A considerable amount was exsorbed into the intestinal lumen. The average amounts of the drug exsorbed into the perfusate were 6·47 and 6·09% of dose for the buffers at pH 6·0 and 8·0, respectively. This suggests that the intestine has a marked distribution/excretion

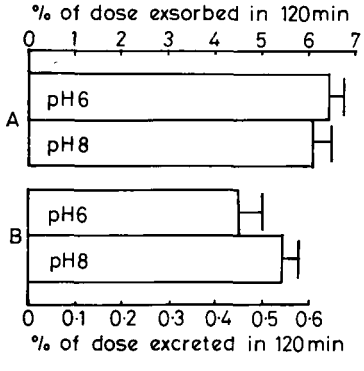

FIG. 2. The amounts of phenobarbitone (A) exsorbed into the perfusate and (B) excreted into the bile in 120 min. The pH of the perfusate is shown within the bars. Each bar represents the mean ± s.e.m. of 3 rats.

function. On the other hand, the amount of the drug excreted into bile was less than the amount exsorbed into the perfusate. The average amounts of the drug excreted into the bile were 0·45 and 0·54% of dose for the experiments using isotonic phosphate buffers at pH 6·0 and 8·0, respectively. Similar observations have been shown for theophylline (Arimori & Nakano 1985) and sulphanilamide (Kitazawa et al 1977). We have previously shown (Arimori & Nakano 1985) that the excretion of theophylline into bile was only about 0·2% of dose in 2 h, while its exsorption into perfusate was 12% in 2 h. Kitazawa et al (1977) have reported that the excretion of sulphanilamide into the bile and the luminal perfusate were 0·5 and 17% of the dose in 3 h, respectively. Their observations suggest that the drug is transported mainly from the blood into the intestinal lumen. On the other hand, Takada et al (1974) have shown that, for bromphenol blue, a half of the dose was secreted into the bile within 30 min. Their observations confirmed the earlier observation that the excretion into bile depends on the chemical characteristics of the drug. Some part of the drug either exsorbed or excreted into the intestinal lumen may be reabsorbed into blood. In the presence of activated charcoal in the lumen, exsorption can be accelerated due to the sink condition created by adsorption of the exsorbed drug by the charcoal, and further reabsorption can be prevented because of the loss of free and absorbable drug in the intestinal tract. We have observed that absorption of orally administered phenobarbitone was reduced in the presence of activated charcoal (unpublished data). From these results, it may be considered that the mechanism by which activated charcoal enhances clearance of phenobarbitone administered intravenously may be by adsorption of the drug excreted into the gastrointestinal tract by the charcoal and prevention of reabsorption.

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J. Pharm. Pharmacol. 1986, 38: 393–395
Communicated October 2, 1985

In-vitro release of [^{14}C]glutamate from dentate gyrus is modulated by GABA

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The effect of GABA (10^{-3} and 10^{-4} M) on the release of preloaded [^{14}C]glutamate from slices of rat dentate gyrus, in response to K^+ stimulation, was studied in Ca^{2+}-free and normal Krebs solutions. Release in Ca^{2+}-free solution was significantly enhanced, but there was no change in release in normal Krebs solution. These results imply that Ca^{2+}-dependent (presumably neuronal) release of glutamate from the dentate gyrus is depressed by GABA, while non-neuronal Ca^{2+}-independent release is enhanced.

There is a great deal of evidence available concerning the post-synaptic actions of the inhibitory amino acid, γ-aminobutyric acid (GABA) in the hippocampus (Krnjevic 1976; Alger & Nicoll 1982; Andersen et al 1982). The presynaptic actions of GABA are less well documented, although it has been shown to affect transmitter release in the hippocampus (Baba et al 1983; Fung & Fillenz 1983), as in several other areas of the brain (Bowery et al 1980; Schlicker et al 1984). Our interest in GABA-mediated modulation of transmitter release derives from two recent observations suggesting that long-term potentiation (LTP) in the hippocampus may be under inhibitory control: GABA antagonists reduce the threshold for LTP (Wigström & Gustafsson 1983a, b), and tetanic stimulation of the inhibitory commissural input to the dentate gyrus blocks the induction of LTP in the perforant path (Douglas et al 1982). These results have generally been interpreted in terms of a postsynaptic locus of control over LTP. However, we have recently found that commissural stimulation also blocks the increase in glutamate release which is associated with LTP in the perforant path (Bliss et al 1985), an observation which raises the possibility that the commissural pathway exerts its blocking effect by a presynaptic action. As a first step in the pharmacological analysis of this phenomenon, we have examined the effect of GABA on K^+-induced release of preloaded [^{14}C]glutamate ([^{14}C]glu) from slices of dentate gyrus.

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Methods

Male Sprague Dawley rats (250–300 g) were killed by stunning and decapitation. The brain was rapidly removed and the dentate gyrus separated from the hippocampus proper. Slices (0·2 mm thick) were prepared using a McIlwain tissue chopper, suspended in 2 ml oxygenated Krebs solution containing 20 μl [^{14}C]glu (280 μCi mmol^{-1}, Amersham, UK; final concentration 2×10^{-5} M), and incubated at 37 °C for 20 min. The tissue suspension was divided among 8 tubes, centrifuged at 5000 rev min^{-1} for 4 min at 4 °C and the supernatant discarded. These conditions were employed in all subsequent centrifugation steps. The pellet was resuspended in 2 ml ice-cold normal or Ca^{2+}-free Krebs solution containing 1 mM EGTA, and centrifuged. This rinsing procedure was repeated 4 times, at which stage basal release of radiolabel had reached a steady baseline, as shown in preliminary experiments. The pellet was then resuspended in 2 ml normal or Ca^{2+}-free Krebs solution, incubated at 37 °C for 5 min and centrifuged. Aliquots (0·25 ml) of supernatant were retained for scintillation counting to give an estimate of basal release of [^{14}C]glu. Release was stimulated by repeating the incubation procedure in 2 ml normal or Ca^{2+}-free Krebs solution to which 40 mM K^+ had been added. After the subsequent centrifugation step, 0·25 ml of supernatant was retained for estimating K^+-induced release. Finally the slices were incubated in 2 ml normal or Ca^{2+}-free Krebs solution, centrifuged, and 0·25 ml of supernatant retained to check that [^{14}C]glu release had returned towards baseline. Each aliquot and the drained tissue slices were added to 5 ml scintillant (Beckman, EP) and counted for 5 min. In some experiments GABA (10^{-3} or 10^{-4} M) was added, either alone or in the presence of bicuculline (10^{-4} M), muscimol (10^{-4} M) or baclofen (10^{-4} M), to Krebs solution containing 40 mM K^+. Release was studied in the presence and absence of Ca^{2+}, and the effect of the drugs alone was also examined. Aliquots of supernatant were taken from about half of the experi-