The calcium ionophore A23187 increases the tight-junctional permeability in rat liver

Kwok S. KAN and Roger COLEMAN

Department of Biochemistry, University of Birmingham, P.O. Box 363, Edgbaston, Birmingham B15 2TT, U.K.

The effect of an increase in intracellular Ca^{2+} concentration on tight-junctional permeability in rat liver was studied by using the calcium ionophore A23187. Infusion of 100 μl of dimethyl sulphoxide containing various amounts of A23187 over 30 min into isolated perfused livers was followed by a pulse of horseradish peroxidase (HRP) under single-pass conditions. The first biliary HRP peak, a measure of junctional permeability, was increased 4-fold with 100 μg of A23187. There were, however, no significant effects on bile flow or on aspartate aminotransferase leakage as compared with the control at this dosage, and thus the increase in junctional permeability was occurring without evidence of appreciable cholestatic or hepatocellular damage. Higher dosages of A23187, however, caused not only an increase in HRP peak height but also changes in bile flow and increases in aminotransferase leakage, indicating more extensive effects at these higher dosages. A second peak of HRP secretion, occurring 20–25 min after the HRP pulse, was also elevated approx. 3.5-fold; this may indicate that pinocytosis and transcellular movement of HRP are also increased under these conditions.

INTRODUCTION

Tight junctions are the major anatomical barrier to diffusion between blood and bile, and play a vital role in restricting the passage of many materials between plasma and the bile-canalicular lumen. Tight junctions which seal this lumen also serve to retain materials which enter into the canaliculus by hepatocytic secretion. Certain compounds which disrupt the integrity of the tight junctions may destroy the retentive properties of the junctions, and biliary materials may therefore regurgitate back into the plasma (Krell *et al.*, 1982; Lowe *et al.*, 1985; Kan & Coleman, 1986). Tight junctions behave like a molecular sieve, differentially excluding molecules according to molecular size. This view is supported by the observations that (i) the relative amounts of individual plasma proteins in human bile, compared with plasma, bear an inverse relationship to their molecular mass (Mullock *et al.*, 1985), (ii) rats treated with α -naphthylisothiocyanate for different times showed progressive increase in the biliary content of proteins, derived from the perfusion fluid, of increasing molecular mass (Kan & Coleman, 1986). Such observations indicate the involvement of junctional sieving as a contributor to the entry of molecules into bile, in addition to their role in the selective retention of materials in the canalicular lumen.

Some components of hepatic tight junctions have been isolated and purified (Stevenson *et al.*, 1986), but the precise structure of the junctional complexes has yet to be identified. A role of Ca^{2+} in maintaining the stability of the junctional complexes has been reported. Cereijido *et al.* (1978) and Gonzalez-Mariscal *et al.* (1985) noted an increase in the junctional permeability across the MDCK cell junctions when Ca^{2+} was removed from the incubation medium, and Lowe *et al.* (1986) have observed an increase in bile to perfusion-fluid ratio of horseradish

peroxidase (HRP) and [^{14}C]sucrose after lowering extracellular Ca^{2+} concentration in the perfusion medium. An increase in junctional permeability in the perfused liver to both HRP and sucrose was observed (Lowe & Hardison, 1987; Lowe *et al.*, 1988) when α -adrenergic agonists, which elicit a transient increase in intracellular [Ca^{2+}], were infused into the liver; this observation led to the suggestion that hormones at a physiological concentration may have a role in the modulation of junctional permeability.

The possibility that changes in intracellular [Ca^{2+}] may modulate junctional permeability has been studied in the experiments reported here by using the calcium ionophore A23187. Since the treatment of various cell types, especially the liver, with A23187 has been reported to cause an increase in cytosolic Ca^{2+} concentration, it has therefore been used to study the effect of an increase in intracellular [Ca^{2+}] on junctional permeability.

The height of the first biliary peak of a pulse of material, presented as a bolus via the hepatic portal vein, to the perfused liver under single-pass conditions has been ascribed to paracellular permeability resulting from junctional sieving (Lowe *et al.*, 1985; Kan & Coleman, 1986). This peak is elevated by treatments which increase junctional permeability (Kan & Coleman, 1986). This biliary peak height of a pulse of HRP has been used in the present studies to indicate change in junctional permeability after exposure of the liver to A23187.

MATERIALS AND METHODS

Materials

Calcium ionophore A23187, bovine serum albumin, HRP and other fine chemicals were obtained from Sigma Chemical Co., Poole, Dorset, U.K. Aspartate amino-transferase assay kits were supplied by Boehringer Corp.,

Lewes, Sussex, U.K. The plastic cannula tubing (PP10) was purchased from Portex, Hythe, Kent, U.K.

Methods

Throughout the study 220–250 g male Wistar rats were used. The bile duct was cannulated with PP10 tubing during pentobarbital anaesthesia. The liver was isolated *in situ* as described by Hems *et al.* (1966) and perfused with 150 ml of Krebs–Ringer bicarbonate solution (Krebs & Henseleit, 1932) containing 2% albumin, 5 mM-glucose, 2 mM-CaCl₂, a physiological amino acid mixture (see Barnwell & Coleman, 1983) and 20% (v/v) packed human red cells. The solution was continuously gassed with O₂/CO₂ (19:1) and was maintained at 37 °C in a thermostatically controlled cabinet (see Lowe *et al.*, 1985).

At 15 min after the start of the perfusion, 100, 500 or 1000 µg of A23187, dissolved in 100 µl of dimethyl sulphoxide, was infused directly over the course of 30 min into the isolated perfused liver; 100 µl of dimethyl sulphoxide was infused in control experiments. At the end of this infusion, the liver was then converted into a single-pass perfusion, and 1 mg of HRP (5.5 × 10⁵ A units/min) was pulsed into the liver over 1 min: 2 × 2 min and 8 × 5 min samples of bile were collected on ice for the spectrometric assay of HRP as described by Gallati & Pract (1985). Samples of perfusate or bile were assayed for aspartate aminotransferase as an indicator for hepatocellular damage.

RESULTS

After the infusion of 100 µl of dimethyl sulphoxide, 1 mg of HRP (5.5 × 10⁵ A units/min) was pulsed as a bolus into the liver; the subsequent biliary output of HRP is depicted in Fig. 1. Two major peaks are observed in this control. The first major peak occurred at 5 min, with a peak height of about 0.2 A unit/min. The second major peak occurred at 22.5 min, with a peak height of about 0.14 A unit/min (Table 1); a minor peak occurred at 11 min.

Infusion of increasing amounts of A23187 (100 µg and 500 µg in 30 min) caused no decrease in bile flow, but higher rates of infusion (1000 µg in 30 min) resulted in the onset of a cholestasis (Table 1). At the lowest dosage of A23187 (100 µg in 30 min) there was no evidence of increased aspartate aminotransferase leakage into the perfusion fluid, relative to the control, but at higher dosages of A23187 (500 µg and 1000 µg in 30 min) the leakage of aspartate aminotransferase increased with the A23187 concentration. Thus higher concentrations of A23187 (500 µg and 1000 µg in 30 min) were instrumental in causing liver damage, but 100 µg of A23187 (in 30 min) had no detectable adverse effect on either bile volume or enzyme leakage.

Infusion of this low, and relatively non-cytotoxic, concentration of A23187 (100 µg) caused a substantial elevation in biliary HRP peak heights (Fig. 1), i.e. about 4-fold and 3.5-fold increase in the heights of the first and second major peaks respectively; both peaks were significantly higher ($P < 0.05$) than the control. The minor peak at 11 min was lost in the background after A23187 treatment.

Fig. 1. Effect of A23187 on the junctional permeability to HRP

Details of bile-duct cannulation and liver perfusion procedures are described in the Materials and methods section. A 100 µl volume of dimethyl sulphoxide alone (DMSO; ●) or containing 100 µg of A23187 (○) was infused for 30 min into the perfused livers. At the end of the infusion 1 mg of HRP (5.5 × 10⁵ A units/min) was pulsed into the liver, and the biliary output of HRP was measured. The values represent means of four experiments and the error bars represent S.E.M. The peaks at 7 min and at 22.5 min were significantly higher ($P < 0.05$) than the control.

Table 1. Effects of A23187 on biliary and perfusate parameters

After 30 min infusion of 100 µl of dimethyl sulphoxide (DMSO) containing 0, 100, 500 or 1000 µg of A23187 (in the presence of 2 mM extracellular Ca²⁺), 1 mg of HRP (5.5 × 10⁵ A units/min) was then pulsed into the liver within 1 min. Bile samples were collected at 2 min intervals. The bile-flow rates were recorded, and the mean values were taken for the period of 20 min at the point of HRP infusion. The first biliary HRP peak height, expressed as A units of the enzyme per min, was used as a measure of the junctional permeability change. A sample of the perfusate was taken 20 min after the HRP pulse for assay of aspartate aminotransferase (AAT) activity as an indicator of liver cell damage. The AAT activity values given are the total activity of the enzyme released into the perfusate, as a percentage of the total activity in the liver (homogenate). The numbers of observations were: DMSO and 100 µg of A23187, $n = 4$; 500 µg and 1000 µg of A23187, $n = 1$.

Treatment	Bile-flow rates (µl/min)	First HRP peak height (units/min)	AAT released (%)
DMSO (100 µl)	11.9	0.2	< 0.07
A23187 (100 µg)	10.9	0.81	< 0.07
A23187 (500 µg)	13.0	1.1	0.31
A23187 (1000 µg)	8.3	3.12	0.86

DISCUSSION

Enzyme leakage from the liver is observed in the present experiments with the higher amounts of A23187 used, but the lower amount of A23187 caused no appreciable cell damage; this is also supported by the electron-microscopic appearance of cells treated with 100 μg of A23187 (results not shown).

Paracellular diffusion of materials into bile from blood or perfusion fluid is affected not only by the properties of the tight junction, but also by bile-flow rates, i.e. the diffusion rate is higher at lower bile-flow rates. On infusion of A23187 at 1000 μg the bile-flow rate is appreciably decreased, but at 500 μg it is increased, and therefore may contribute in both a negative and a positive way, respectively, to the apparent change in permeability at these dosages.

With 100 μg of A23187, however, bile-flow rate is not significantly different from that of the dimethyl sulphoxide control (Table 1) and from that with saline (results not shown), whereas the peak height of HRP was elevated by 4-fold. This increase in peak height can therefore be attributed to an increase in junctional permeability, i.e. a consequence of a change in the junctional function rather than in bile-flow rate.

This change in junctional permeability after exposure to the calcium ionophore (in the presence of Ca^{2+} ions) is complementary to the observations of Lowe *et al.* (1988), who have demonstrated that exposure to α -adrenergic agonists, which can cause an increase in intracellular Ca^{2+} concentration, results in an increase in hepatic junctional permeability.

Although an increase in intracellular Ca^{2+} concentration therefore appears to cause an increase in junctional permeability, the exact mechanism bringing this about is not yet clear. A change in cytosolic Ca^{2+} may directly destabilize the tight junctions, leading to a change in permeability by a direct effect of $[\text{Ca}^{2+}]$ itself initiating a primary effect, which then affects the junctions in a secondary fashion.

Studies with isolated intestinal brush borders (Burgess, 1982; Keller & Mooseker, 1982) showed that tight-junctional complexes were associated with cytoskeletal microfilaments; these observations led to the suggestion that Ca^{2+} -dependent contraction of the microfilaments may act *in vivo* to alter tight-junction permeability.

In Fig. 1 an increase in the second HRP peak (20–25 min) is observed after A23187 treatment, and implies that the transcellular movement of HRP in vesicles is also increased. In the report of Lowe *et al.* (1988), no change in this second peak was observed after exposure of the liver to vasopressin. The discrepancy with the effects of A23187 seen in Fig. 1 may possibly be related to the difference in chemically induced and hormonally induced Ca^{2+} changes, in that the physiological effect of the hormone may be to provoke a more transient change in Ca^{2+} concentration, whereas that produced by A23187 may be more prolonged.

REFERENCES

- Barnwell, S. G. & Coleman, R. (1983) *Biochem. J.* **216**, 409–414
- Burgess, D. R. (1982) *J. Cell Biol.* **95**, 853–863
- Cereijido, M., Robbins, E. S., Dolan, W. J., Rotunno, C. A. & Sabatini, D. D. (1978) *J. Cell Biol.* **77**, 853–880
- Gallati, V. H. & Pracht, I. (1985) *J. Clin. Chem. Clin. Biochem.* **23**, 453–460
- Gonzalez-Mariscal, L., Ramirez, B. C. D. & Cereijido, M. (1985) *J. Membr. Biol.* **86**, 113–125
- Hems, R., Ross, B. D., Berry, M. N. & Krebs, H. A. (1966) *Biochem. J.* **101**, 284–292
- Kan, K. S. & Coleman, R. (1986) *Biochem. J.* **238**, 323–328
- Keller, T. C. S. & Mooseker, M. S. (1982) *J. Cell Biol.* **95**, 943–959
- Krebs, H. A. & Henseleit, K. (1932) Hoppe-Seyler's *Z. Physiol. Chem.* **210**, 33–36
- Krell, H., Hoke, H. & Pfaff, E. (1982) *Gastroenterology* **82**, 507–514
- Lowe, P. J. & Hardison, W. G. (1987) *Gastroenterology* **92**, 1752
- Lowe, P. J., Kan, K. S., Barnwell, S. G., Sharma, R. K. & Coleman, R. (1985) *Biochem. J.* **229**, 529–537
- Lowe, P. J., Hardison, W. G., Dawali, K. & Miyai, K. (1986) *Hepatology* **6**, 225
- Lowe, P. J., Miyai, K., Steinbach, J. H. & Hardison, W. G. M. (1988) *Am. J. Physiol.*, in the press
- Mullock, B. M., Shaw, L. J., Fitzharris, B., Peppard, J., Hamilton, M. J. R., Simpson, M. T., Hunt, T. M. & Hinton, R. H. (1985) *Gut* **26**, 500–509
- Stevenson, B. R., Silician, J. D., Mooseker, M. S. & Goodenough, D. A. (1986) *J. Cell Biol.* **103**, 755–766

Received 22 August 1988/7 October 1988; accepted 13 October 1988