Volume 285, number 1, 94–96 FEBS 09880 July 1991

ADONIS 0014579391005870

Beneficial effect of dexamethasone on attenuated hormone-induced uptake of calcium and glycogenolysis by perfused liver of rats infected with *Fasciola hepatica*

Maureen J.E. Hanisch, Carolyn A. Behm and Fyfe L. Bygrave

Division of Biochemistry and Molecular Biology, School of Life Sciences, Australian National University, Canberra ACT 2601, Australia

Received 25 April 1991

Infection of rats with the liver fluke, *Fasciola hepatica*, impaired the responses of the perfused liver to calcium uptake and glucose release induced by the synergistic action of glucagon and vasopressin. Treatment of infected rats with dexamethasone prevented the impairment of each of these two responses.

Liver; Vasopressin; Calcium; Dexamethasone; *Fasciola hepatica*; Glycogenolysis

1. INTRODUCTION

Infection of mammalian liver by the common liver fluke, *Fasciola hepatica*, induces several metabolic disorders of this organ. These include impairment of the detoxification and biotransformation (mixed function oxidase) system in rats [1–3] and in sheep [4]. Moreover, isolated liver mitochondria from experimentally infected laboratory rats exhibit uncoupled oxidative phosphorylation [5–7], increased ATPase activity [6], inhibition of respiration and insensitivity of mitochondrial ATPase activity to oligomycin [7].

Recently we reported that the aberrant respiratory behaviour is seen also in isolated liver mitochondria of fluke-infected sheep [8]. These and the above-mentioned findings led us to consider whether such an aberration would also be observed in metabolic activities normally dependent on mitochondrial function in the intact liver. To this end we have examined the effect of liver fluke infection on the Ca^{2+} uptake and glycogenolytic response by the perfused rat liver induced by the synergistic action of glucagon and vasopressin [9]. Our results show that these responses are attenuated following fluke infection. In addition we report here that such attenuation can be offset by the prior administration of the glucocorticoid dexamethasone to the infected rat.

Correspondence addres: M.J.E. Hanisch, Division of Biochemistry and Molecular Biology, School of Life Sciences, Australian National University, Canberra ACT 2601, Australia.

2. MATERIALS AND METHODS

2.1. *Animals*

Animals were bred and housed in the Animal Care Facility at the Australian National University. They were kept in pairs in plastic cages and allowed a normal diet of rat chow and water. Ambient temperature was kept at about 22°C and 12 h periods of light and dark were maintained. Four to six week-old male white Wistar rats were infected with 30 metacercariae of *F. hepatica* by gastric gavage as described in [7]. Dexamethasone was administered to rats by subcutaneous injection, 2 mg/kg at 48 h intervals. Treatment began on day 35 of the infection and ended about 24 h before the rats were killed. Uninfected controls received either the same treatment with dexamethasone or no treatment.

2.2. *Perfusions*

Rats were anaesthetized by an intra-peritoneal injection of sodium pentobarbitone, 50 mg/kg of body weight. The livers were then perfused in situ in a non-recirculating mode as previously described [10,11]. The perfusate was Krebs–Henseleit buffer [12] with added calcium at physiological concentration (1.28 mM $CaCl_2$). The perfusate buffer was 37°C at the point of entry to the liver and was gassed continuously prior to perfusion with 95% O_2/5% CO_2. The perfusion circuit was set at a constant flow rate of 35 ml/min. The Ca^{2+} concentration of the effluent perfusate was continuously monitored using a Radiometer F2112 Ca^{2+}-selective electrode coupled to a Radiometer K801 (Ag/AgCl) reference electrode via an agarose/KCl salt bridge, as previously described [10,11]. The flow-through electrode chamber was continuously stirred by a magnetic flea. Data were recorded by a Spectra-Physics SP4100 computing integrator which received the translated signal from an Orion model 901 microprocessor ionanalyser connected to the electrodes. Infusion of glucagon (10 nM perfusate concentration) began 15 min after the commencement of the perfusion when a steady baseline had been established. Four minutes later vasopressin (10 nM perfusate concentration) infusion was begun. Both infusions were ended 11 min later. The glucose concentration in the effluent perfusate was measured by the glucose oxidase/peroxidase method (God-Perid test kit, Boehringer).

Published by Elsevier Science Publishers B.V.

2.3. *Materials and chemicals*

Dexamethasone (Dexaphos, 5 mg/ml) was from Jurox Pty Ltd., Riverstone, NSW, Australia. Vasopressin and glucagon were from Sigma Chemical Co., St. Louis, MO, USA. Ca^{2+} selectrode membranes (F2002) and filling solution S43316 were from Radiometer, Copenhagen, Denmark.

2.4. *Expression of data*

Data are expressed as the means ± SEM for ≥ 3 determinations for each experimental group.

3. RESULTS

Representative traces of total Ca^{2+} uptake by hormone-stimulated perfused rat livers at 6 weeks post-infection with *F. hepatica* are shown in Fig. 1. The trace obtained for the control (uninfected) animals is similar to those previously obtained in this laboratory (see e.g. [11]) in that immediately following the administration of vasopressin to a perfused liver, into which glucagon already is being perfused, there is a significant influx of Ca^{2+} from the external medium. The amount of Ca^{2+} taken up (from measurements of the area above the curve [13]), was 2250 ± 42 nmol/g of liver (mean of 3 experiments). The features of this phenomenon have been described in detail elsewhere [9]. When the experiment is repeated with livers obtained from rats infected for 6 weeks with *F. hepatica* (trace B in Fig. 1) it is evident that the Ca^{2+} uptake response is markedly attenuated. The amount of Ca^{2+} taken up was 897 ± 87 nmol/g of liver (mean of 5 experiments). Trace C is from an experiment in which 6-week infected rats were treated with dexamethasone for one week prior to liver perfusion. Such treatment results in a normal pattern of uptake of Ca^{2+}; in this instance Ca^{2+} uptake was 2645 ± 511 nmol/g of liver (mean of 4 experiments). Trace D shows that the treatment of control rats with the glucocorticoid did not change the control value for Ca^{2+} uptake which was 2013 ± 120 nmol/g of liver (mean of 5 experiments).

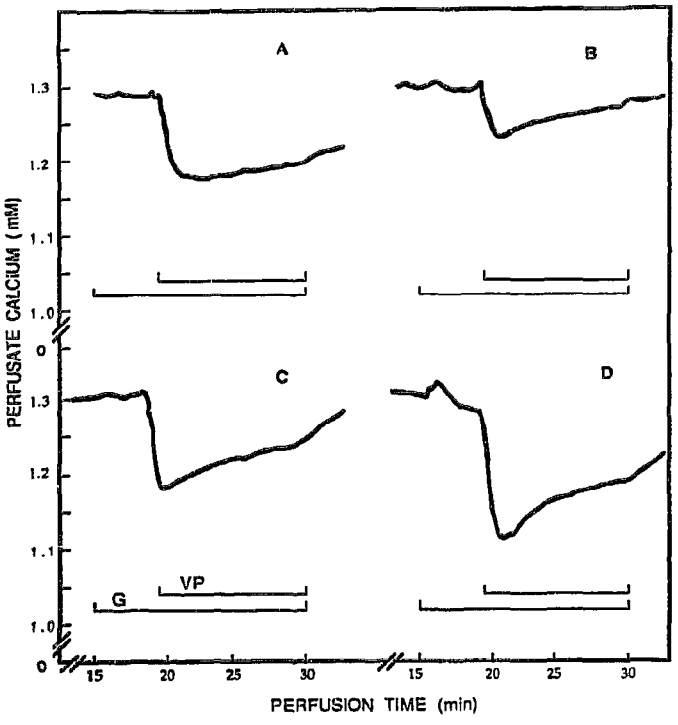

Fig. 1. Representative traces showing the effect of infection with *F. hepatica*, and of dexamethasone treatment of infected rats, on Ca^{2+} mobilisation in the perfused rat liver induced by the co-administration of glucagon and vasopressin. Livers of (A) control (i.e. uninfected) and untreated; (B) 6-week fluke-infected and untreated; (C) 6-week fluke-infected and treated with 2 mg of dexamethasone/kg body wt every 48 h for the last week of the infection, and (D) control and treated with dexamethasone as in (C), were perfused with Krebs-Henseleit buffer with added 1.28 mM Ca^{2+}. The Ca^{2+} concentration in the effluent perfusate was continuously monitored with a Ca^{2+}-selective electrode as described in section 2. At the times indicated by horizontal bars, 10 nM glucagon (G, lower bar) and 10 nM vasopressin (VP, upper bar) were infused via the inflow cannula. Influx of Ca^{2+} into the liver is registered as a downward deflection of the recorder pen.

The patterns of release of glucose in response to the actions of glucagon and vasopressin in the same experiments undertaken in Fig. 1 are presented in Fig. 2. The glycogenolytic response of infected, untreated rat livers (group B) was reduced to 4% of uninfected normal controls (group A). Dexamethasone treatment of infected rats (group C) countered this attenuation to a significant extent but not to the degree seen in the Ca^{2+} uptake response, while dexamethasone treatment alone to control rats had no effect (group D). Consistent with these results, we have found (C.J. Rule, C.A. Behm and F.L. Bygrave, unpublished observations) that glycogenolysis in the perfused rat liver induced by the Ca^{2+}-mobilising agonist phenylephrine, also is markedly attenuated following infection by *F. hepatica*.

4. DISCUSSION

In this paper we have presented evidence that certain aspects of whole liver metabolism are impaired following fluke infection and we have identified additional

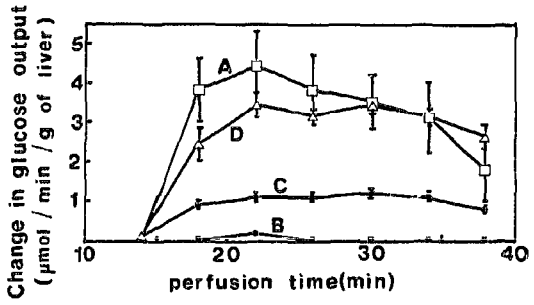

Fig. 2. Glucose release by perfused fluke-infected rat liver in response to glycogenolytic hormones. Livers of 6-week infected animals were perfused exactly as described in Fig. 1. Glucose release was measured in samples of the effluent medium at the times indicated as described in section 2. The designations A-D are identical to those in Fig. 1. Values are the means ± SEM for ≥ 3 determinations.

sites of metabolic impairment in infected livers. We have also shown that administration of the immunosuppressive glucocorticoid, dexamethasone, reverses or ameliorates the metabolic lesions.

Infection leads to attenuation of the characteristic Ca^{2+} uptake response in perfused liver that is induced by the synergistic action of the glycogenolytic hormones glucagon and vasopressin (cf. [13]). Uptake of Ca^{2+} by the mitochondria is considered to be intimately involved in this response [9]. Therefore it appears that attenuation of whole liver Ca^{2+} uptake could well reflect the impairment of mitochondrial energy-linked functions observed previously by others [6] and ourselves [7] in mitochondria from infected rat liver, from sheep liver [8] and in intact hepatic cells and whole liver from rats (M.J.E. Hanisch, F. Topfer, C.A. Behm and F.L. Bygrave, submitted).

Hepatic output of glucose induced by the glycogenolytic hormones glucagon and vasopressin is attenuated also in infected rats. Indeed, there was little discernible output of glucose by infected livers. It is known that the glycogen content of infected rat livers if diminished ([14] and J.E. Millard, C.J. Rule, C.A. Behm and F.L. Bygrave, unpublished observations). Whether the attenuated response is due to this or to impairment of the glycogenolytic response at the enzymatic, receptor or signal transduction levels is under investigation.

The effect of dexamethasone in ameliorating the observed metabolic lesions is interesting in that it sheds new light on how the lesions are induced. Dexamethasone has a large number of effects on a variety of aspects of mammalian metabolism and also on the cellular immune system. It was shown, for example, that incubation with glucocorticoid prevented the antigen-induced rise in free intracellular calcium in rat basophilic leukemia cells [15]. Particularly relevant for this work may be the observations that dexamethasone impairs the inflammatory response by killing lymphocytes [16,17] and eosinophils [18] as well as by inhibiting the production of inflammatory cytokines [19] and eicosanoids [20]. Therefore, our observations provide some evidence suggestive of a role for the cellular immune response in the aetiology of the metabolic lesions observed in fluke-infected rat livers. Further experimental examination of this question is in progress in our laboratory.

Acknowledgements: We are grateful to Dr J.C. Boray for the provision of metacercariae, to Mr Ari Karjalainen for assistance with the perfused liver technique and to the Australian Research Council for financial support of this work.

REFERENCES

[1] Facino, R.M., Carini, M., Bertuletti, C.G. and Malchiodi, A. (1981) Pharmacol. Res. Commun. 13, 731–742.
[2] Galtier, P., Eeckhoutte, C. and Larrieu, G. (1987) Exp. Parasitol. 63, 189–194.
[3] Tekwani, B., Shulka, O. and Ghatak, S. (1988) Parasitol. Today 4, 4–10.
[4] Galtier, P., Larrieu, G., Tufenkji, A.E. and Franc, M. (1986) Drug Metab. and Disposition 14, 137–141.
[5] Van den Bossche, H., Verhoeven, H. and Lauwers, H. (1980) in: The Host Invader Interplay. (Van den Bossche, H. ed.) pp. 699–704, Elsevier, Amsterdam.
[6] Van den Bossche, H., Verhoeven, H. and Arnouts, D. (1983) Contr. Microbiol. Immunol. 7, 30–38.
[7] Rule, C.J., Behm, C.A. and Bygrave, F.L. (1989) Biochem. J. 260, 517–523.
[8] Rule, C.J., Hanisch, M.J.E., Behm, C.A. and Bygrave, F.L. (1991) Int. J. Parasitol. (in press).
[9] Altin, J.G. and Bygrave, F.L. (1988) Biol. Rev. 63, 551–611.
[10] Reinhart, P.H., Taylor, W.M. and Bygrave, F.L. (1982) Biochem. J. 208, 619–630.
[11] Altin, J.G. and Bygrave, F.L. (1985) Biochem. J. 232, 911–917.
[12] Krebs, H.A. and Henseleit, K. (1932) Hoppe-Seyler's Z. Physiol. Chem. 210, 33–66.
[13] Altin, J.G. and Bygrave, F.L. (1986) Biochem. J. 238, 653–661.
[14] Gameel, A.A. (1982) Z. Parasitenk. 68, 181–184.
[15] Her, E., Weissman, B.A. and Zor, U. (1990) Biochim. Biophys. Acta 1051, 203–206.
[16] Munck, A. and Crabtree, G.R. (1981) in: Cell Death in Biology and Pathology (Bowen, I.D. and Lockshin, R.A. eds) pp. 329–363, Chapman and Hall, London.
[17] Tzavaras, T.J., Tsawdaroglou, N.H. and Sekeris, C.E. (1989) FEBS Lett. 250, 30–34.
[18] Kawabori, S., Soda, K., Perdue, M.H. and Bienenstock, J. (1991) Lab. Invest. 64, 224–233.
[19] Kawakami, M., Ikeda, Y., Le Trang, N., Vine, W. and Cerami, A. (1984) in: Proceedings of the International Union of Pharmacology (Patton, W. ed.) pp. 377–384, Macmillan Press, London.
[20] Flower, R.J. and Blackwell, G.J. (1979) Nature 278, 456–459.