Biochemical Pharmacology, Vol. 42, No. 7, pp. 1493–1495, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

SHORT COMMUNICATIONS

Changes in plasma lipoprotein metabolism in chicks in response to polychlorinated biphenyls (PCBs)

(*Received* 4 *March* 1991; *accepted* 17 *May* 1991)

Polychlorinated biphenyls (PCBs*) were originally used to insulate and cool transformers and other electrical equipment but are now one of the most widely distributed and persistent pollutants in the ecosystem. They have been identified in almost all life forms and are present in human blood, adipose tissue and milk as a consequence of widespread contamination of the food chain.

Studies on the biochemical and toxicological effects of PCBs and on their mode of action and metabolism have been reviewed in detail by Safe [1]. PCBs cause major changes in hepatic lipid metabolism, including proliferation of the endoplasmic reticulum and the development of a fatty liver [2–4]. The increase in hepatic lipid content on treatment of rats with PCBs was suggested by Sandberg and Glaumann [4] to be due to a reduced rate of very low density lipoprotein (VLDL) secretion from the liver. However PCBs also cause an increase in plasma triglyceride concentration in man [2] and birds [3] and this suggests they may affect lipoprotein clearance from the circulation. Plasma lipoproteins also appear to have a key role in the transport and cellular uptake of PCBs [3].

In previous studies [5] we have developed methods for measuring rates of VLDL secretion and clearance in young chicks and in this paper we describe their application to the study of the effects of PCBs on avian plasma lipoprotein metabolism. In particular, we have tested whether the previously reported PCB-induced hypertriglyceridaemia in birds [3] is due to an increased rate of VLDL secretion and/or a decreased $T_{1/2}$ of VLDL in the circulation.

Materials and Methods

Birds. Day-old chicks from a rapidly growing commercial broiler strain (D.B. Marshall, Newbridge, U.K.) were reared on a standard broiler starter diet and a 14 hr dark : 10 hr light photoperiod. Feed and water were available *ad lib.* At 2 weeks of age, chicks (which then weighed 250–300 g) were injected intraperitoneally with either 1 mL of olive oil/kg of body weight or with 1 mL of olive oil containing 500 mg Aroclor 1254/kg body weight. Blood samples were taken from the wing vein immediately before injection and at intervals thereafter. EDTA was used as anticoagulant. Plasma was prepared by centrifugation at 1000 *g* for 10 min at 4° and stored at −20°.

Plasma triglyceride (TG) and β-hydroxybutyrate concentrations were measured using kits from the Sigma Chemical Co. (Poole, U.K.). Plasma VLDL concentrations was measured using a turbidimetric method [6].

Lipoprotein metabolism. The rate of secretion of triglyceride-rich lipoproteins into the circulation was determined by measuring the initial rate of accumulation of triglyceride in plasma after intravenous injection of sufficient anti-lipoprotein lipase antiserum to block lipoprotein clearance, as described previously [5]. VLDL clearance was measured using biologically labelled VLDL prepared from the plasma of a single donor bird that had received an intravenous injection of 200 μCi of [^{14}C]-palmitate [5]. Post-heparin lipoprotein lipase activity was measured in plasma prepared from blood taken 2 min after intravenous injection of 1000 units heparin/kg body weight. Activated Intralipid was used as substrate [7]. One unit of LPL activity is equivalent to the release of 1 μmol of fatty acid/hr.

Results and Discussion

The time course and magnitude of the hypertriglyceridaemia produced in chicks by intraperitoneal injection of Aroclor 1254 (Fig. 1) was similar to that reported previously in pigeons [3]. There was no significant difference between Aroclor 1254-treated and control birds in rate of triglyceride secretion into the plasma. However, the half-life of normal VLDL in the circulation of treated chicks was longer than in control birds (though not quite significantly so) and post-heparin lipoprotein lipase activity was significantly lower in the chicks injected with Aroclor 1254 (Table 1). Post-heparin lipoprotein lipase activity reflects the sum of the functional activity in all tissues and the difference between treated and control animals was probably sufficient to account for the difference in rate of VLDL and plasma triglyceride concentrations. However, the present results cannot exclude the possibility that the presence of PCBs in circulating VLDL [3] might also influence their susceptibility as substrates for lipoprotein lipase-mediated hydrolysis.

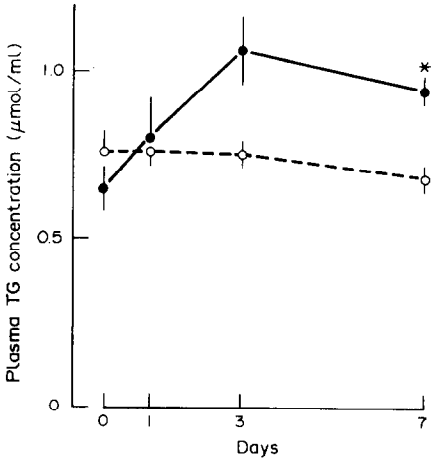

Fig. 1. Effect of PCBs on plasma very low density lipoproteins in the chicken. Young chicks were injected with either olive oil or Aroclor in olive oil as described in Materials and Methods. Results are the means ± SEM of six birds/group. Those for treated birds (solid symbols) that are significantly different from controls (open symbols) are indicated by: * $P < 0.05$.

* Abbreviations: PCBs, polychlorinated biphenyls; VLDL, very low density lipoproteins; LPL, lipoprotein lipase.

Table 1. Effect of PCBs on plasma lipoprotein metabolism in young chickens

	Control	Aroclor-treated	P
Plasma triglyceride (μmol/mL)	1.05 ± 0.05	1.62 ± 0.18	0.039
VLDL secretion rate (μmol TG/mL of plasma/hr)	4.33 ± 0.38	4.77 ± 0.46	NS
$T_{1/2}$ of VLDL in the circulation (min)	4.3 ± 0.3	6.6 ± 0.8	0.057
Post-heparin LPL activity (units/mL of plasma)	241 ± 26	165 ± 17	0.036
Plasma β-hydroxybutyrate μmol/mL)	0.50 ± 0.10	0.13 ± 0.04	0.023

Results are the means ± SEM of 6 treated and 6 control birds 7 days after injection of Aroclor or vehicle.
NS, not significant.

Post-heparin LPL activity in chicks is not particularly responsive to changes in nutritional state and the feed consumptions of control and Aroclor 1254-treated chicks were very similar (data not shown). Post-heparin and tissue LPL activity was markedly reduced by treatment of chicks with bacterial endotoxin [8]. In mammals, this response is thought to be mediated by cytokines such as tumour necrosis-factor-α and interleukin-I [9] and there is indirect evidence that this is also the case in the chicken [10]. It is tempting, therefore, to speculate that the decrease in plasma post-heparin LPL activity and consequent reduction in VLDL clearance observed in the present study was also mediated by cytokines.

PCBs are known to influence the immune response and, like endotoxin, they cause an increase in urea concentration in the plasma [1, 2]. The metabolic responses to endotoxin (and infection) serve to mobilize protein and energy reserves to mount the immune response [9] and a similar cytokine-mediated mobilization may be important in fuelling the hepatic response to PCBs.

The induction of fatty liver by PCBs was suggested by Sandberg and Glaumann [4] to be caused in part by a partial block of hepatic lipoprotein synthesis and assembly. It was a little surprising, therefore, that no significant difference in VLDL secretion rate was found between control and Aroclor 1254-treated chicks in the present study. This result suggests that PCBs alter the distribution of hepatic fatty acids between intracellular storage and lipoprotein synthesis rather than change the absolute rate of VLDL synthesis and secretion. The relatively high concentration of β-hydroxybutyrate in chick plasma [5] indicates that β-oxidation is a significant fate of many fatty acids in chicken liver, even in the fully fed state. The much lower plasma concentrations of β-hydroxybutyrate in chicks treated with Aroclor 1254 suggest that PCBs may be potent inhibitors of β-oxidation in avian liver and this may be an important additional factor in the development of fatty liver.

In summary, the results of the present study strongly suggest that the hypertriglyceridaemia in chicks treated with Aroclor 1254 is a result of reduction in VLDL clearance rather than an increase in rate of VLDL secretion. The decrease in post-heparin LPL activity responsible for reduced VLDL clearance may be mediated by cytokines produced by chronic activation of the immune system.

Acknowledgements—The support given to J.B. by the Cancer Research Campaign of Great Britain is gratefully acknowledged.

* *AFRC Institute of Animal Physiology and Genetics Research*
Roslin, Midlothian EH25 9PS
‡ *Department of Biochemistry and Physiology*
University of Reading
Whiteknights
Reading, RG6 2AJ, U.K.

HARRY GRIFFIN* †
DAWN WINDSOR*
JÜRGEN BORLAKOGLU‡ §

REFERENCES

1. Safe S, Polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs): biochemistry, toxicology and mechanism of action. *CRC Crit Rev Toxicol* **13**: 319–395, 1984.
2. Kimbrough RD, Human health effects of polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PCBs). *Annu Rev Pharmacol Toxicol* **27**: 87–111, 1987.
3. Borlakoglu JT, Welch VA, Edwards-Webb JD and Dils RR, Transport and cellular uptake of polychlorinated biphenyls (PCBs)—II. Changes *in vivo* in plasma lipoproteins and proteins of pigeons in response to PCBs, and a proposed model for the transport and cellular uptake of PCBs. *Biochem Pharmacol* **40**: 273–281, 1990.
4. Sandberg P and Glaumann H, Studies on the cellular toxicity of polychlorinated biphenyls (PCBs). Partial block and alteration on intracellular migration of lipoprotein particles in rat liver. *Exp Mol Pathol* **32**: 1–22, 1980.
5. Griffin H, Acamovic F, Guo K and Peddie J, Plasma lipoprotein metabolism in lean and in fat chickens produced by divergent selection for plasma very low density lipoprotein concentration. *J Lipid Res* **30**: 1243–1250, 1989.
6. Griffin HD and Whitehead CC, Plasma lipoprotein concentration as an indicator of fatness in broilers: development and use of a simple assay for plasma very low density lipoproteins. *Br Poult Sci* **23**: 307–313, 1982.
7. Guo K, Griffin HD and Butterwith SC, Biochemical indicators of fatness in meat-type chickens: lack of correlation between lipoprotein lipase activity in post-heparin plasma and body fat. *Br Poult Sci* **29**: 343–350, 1988.
8. Griffin HD and Butterwith SC, Effect of *Escherichia coli* endotoxin on tissue lipoprotein lipase activities in chickens. *Br Poult Sci* **29**: 371–378, 1988.

† To whom correspondence should be addressed.
§ Present address: Marion Merrill Dow Research Institute, Department of Drug Metabolism and Clinical Biochemistry, 16, rue d'Ankara-B.P. 447 R/9, 67009 Strasbourg Cedex, France.

9. Cerami A, Ikeda Y, Le Trang N, Hotezpj and Beutler B, Weight loss associated with an endotoxin-induced mediator from peritoneal macrophages: the role of cachectin (tumour necrosis factor). *Immunol Lett* **11**: 173–177, 1985.

10. Butterwith SC and Griffin HD, The effects of macrophage-derived cytokines on lipid metabolism in chicken (*Gallus domesticus*) hepatocytes and adipocytes. *Comp Biochem Physiol* **94A**: 721–724, 1989.

Biochemical Pharmacology, Vol. 42, No. 7, pp. 1495–1497, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

P_2-Purinoceptors in a renal epithelial cell line (LLC-PK_1)

(*Received* 26 *November* 1990; *accepted* 16 *May* 1991)

Extracellular ATP and ADP interact with cell surface receptors to regulate a number of physiological processes, which include increases in membrane permeability, relaxation or contraction of various smooth muscles, platelet aggregation, and modulation of neuronal excitability [1, 2]. Two major classes of purinoceptors have been identified: P_1-receptors which are activated by adenosine, and P_2-receptors which are activated by ATP and ADP, but not by AMP or adenosine [3]. In isolated aortic endothelial cells ATP induces a biphasic increase in cytoplasmic free Ca^{2+} concentration ($[Ca^{2+}]_i$), an increase which consists of a rapid and transient and a subsequent sustained phase [4]. The transient increase in $[Ca^{2+}]_i$ is caused by the release of calcium from intracellular stores, while the sustained increase is probably mediated by the activation of Ca^{2+} channels on plasma membranes. The kidney is composed of heterogeneous nephron segments and a variety of cell types, including arterial endothelial cells. Hence, the use of cell culture techniques has permitted the study of a relatively homogeneous preparation and has offered advantages for the examination of many renal cell functions. In the present study we investigated the localization of P_2-purinoceptors in renal non-vascular cells, LLC-PK_1, which have been used as a model for the proximal tubular epithelium [5]. To demonstrate whether LLC-PK_1 cells respond to ATP binding with modulation of intracellular calcium, we examined the effect of ATP on $[Ca^{2+}]_i$.

Materials and Methods

Cell culture. The LLC-PK_1 cells, obtained from the American Type Culture Collection (ATCC CRL-1392), were grown to confluence (about 5×10^5 cells/cm^2) in 199-Earle medium supplemented with 10% fetal bovine serum. The cells were subcultured every 5–7 days using 0.02% EDTA and 0.05% trypsin in phosphate-buffered saline. For fluorometric experiments, the cells were grown on glass coverslips (7 × 13 mm; thickness, under 0.12 mm; Matsunami Glass Ind., Ltd., Osaka, Japan).

Binding experiments. LLC-PK_1 monolayers on 24-well tissue culture plates (Corning) were washed twice with binding assay medium [0.1% gelatin, 116 mM NaCl, 5.4 mM KCl, 1.0 mM NaH_2PO_4, 0.2 μM $Fe(NO_3)_3$, 5.6 mM dextrose, 5 mM $NaHCO_3$, and 10 mM Hepes, pH 7.4]. The monolayers were incubated in a final volume of 0.3 mL of binding assay medium. The reaction was initiated by the addition of radioactive-labeled adenine nucleotides. Non-specific binding was defined as the amount of ATP bound in the presence of 10 μM non-radioactive ATP. After different time intervals the reaction was terminated by rapidly aspirating the incubation medium and washing the monolayers three times with 1 mL of the ice-cold binding assay medium. The cells were then disintegrated with 0.3 mL of 0.2 N NaOH, and the radioactivity was measured by liquid scintillation counting.

Intracellular free Ca^{2+} measurements. Intracellular free Ca^{2+} was measured using a fluorescent probe, fura 2, essentially as described [6]. The LLC-PK_1 monolayers were placed into a thermostatted cuvette in a Hitachi F-2000 spectrofluorometer, and incubated with a 2 μM concentration of the acetoxymethyl ester of fura 2 (Dojindo Lab., Kumamoto, Japan) with 0.01% Cremophor EL (a non-cytotoxic detergent) for 30 min at 30° in assay medium [116 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 1.0 mM NaH_2PO_4, 0.2 μM $Fe(NO_3)_3$, 5.6 mM dextrose, 5 mM $NaHCO_3$, and 10 mM Hepes, pH 7.4]. The indicator-loaded monolayers were perfused with assay medium at a flow rate of 0.3 mL/min. After washing the surplus indicator, the Ca^{2+}-dependent emission intensity from the cells was recorded continuously at excitation wavelengths of 340 and 380 nm alternately and an emission wavelength of 510 nm. Calibration of fura 2 fluorescence was performed as previously described [7]. Maximum and minimum fluorescence values were recorded at 10^{-3} and 10^{-9} M Ca^{2+}, respectively, by lysing the cells in 0.1% Triton X-100 followed by chelation of free Ca^{2+} on addition of 5 mM ethyleneglycolbis(aminoethylether)tetra-acetate (EGTA). The values of $[Ca^{2+}]_i$ were calculated using an effective dissociation constant of 224 nM for Ca^{2+} binding to fura 2.

Results and Discussion

The specific binding of [^{3}H]ATP to LLC-PK_1 monolayers was temperature dependent. At a temperature of 30° the specific binding reached an equilibrium value within 15 min and was stable up to 60 min. At 4°, the specific binding did not reach an equilibrium within 60 min, so we performed binding experiments at 30° for 15 min. Moreover all binding experiments were performed in Mg^{2+}- and Ca^{2+}-free medium since the elimination of Mg^{2+} and Ca^{2+} from the medium was beneficial in that it significantly reduced hydrolysis and uptake of adenine nucleotides.

Adenosine and adenine nucleotides inhibited [^{3}H]ATP binding in LLC-PK_1 monolayers in a concentration-dependent manner (Fig. 1a). The order of potency observed was ATP = ADP > AMP > adenosine, which is consistent with that for P_2-purinoceptors. Moreover, a similar inhibition by adenosine and adenine nucleotides with relative potency was also observed in experiments utilizing [^{35}S]adenosine 5′-(β-thio)diphosphate (ADPβS) as a radioligand for binding (Fig. 1b). ADPβS is a selective P_2-agonist and much less sensitive to hydrolysis compared to other ATP and ADP analogs [8]. These data strongly suggest the presence of P_2-purinoceptors in LLC-PK_1 cells. Scatchard analysis of [^{35}S]ADPβS binding to LLC-PK_1 monolayers gave a K_d of 90.5 nM and a B_{max} of 1.14 pmol/10^6 cells (Fig. 2).

Figure 3a shows a typical $[Ca^{2+}]_i$ change experiment observed upon stimulation with 10 μM ATP. Intracellular