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The possible mechanism of protection induced by dexamethasone against sudden death due to coronary ligation in conscious rats

M. Koltai, I. Leprán, Gy. Nemecz & L. Szekeres

Department of Pharmacology, University Medical School of Szeged and Department of Biochemistry, Biological Research Centre, Szeged, Hungary

Rat isolated peritoneal cells (10^7 cells ml^{-1}) incubated in the presence of dexamethasone (3×10^{-9} M, for 90 min) were shown to release some factor(s), having a mol. wt. of 15 k, as determined by size exclusion chromatography, which inhibited phospholipase A_2 activity and offered significant protection against sudden death due to post-infarction arrhythmias in conscious rats pretreated with actinomycin D (0.5 mg kg^{-1} i.v. 4 h before coronary ligation). This observation suggests that the cardioprotective effect of glucocorticoids in acute myocardial infarction may result from the *de novo* synthesis of macrocortin, an antiphospholipase protein.

Introduction The anti-inflammatory effect of glucocorticoids has recently been shown to be due to the *de novo* synthesis of a 'second messenger' (Blackwell, Carnuccio, Di Rosa, Flower, Langham, Parente, Persico, Russel-Smith & Stone, 1982). This substance has been isolated from guinea-pig lung (Flower & Blackwell, 1979) and from rat peritoneal leucocytes and has been called 'macrocortin' (Di Rosa & Persico, 1979; Blackwell, Carnuccio, Di Rosa, Flower, Parente & Persico, 1980); it has also been isolated from rabbit polymorphonuclear leucocytes and called 'lipomodulin' (Hirata, Schiffman, Venkatasubramanian, Salamon & Axelrod, 1980). These factors inhibit phospholipase A_2 (PLA_2) and therefore prevent the release of arachidonic acid (AA) from the cell membrane.

The products of AA have been shown to be involved in acute myocardial infarction (Needleman, 1976), and some of them, especially thromboxane A_2 (TxA_2), aggravate its symptoms (Coker, Ledingham, Parratt & Zeitlin, 1981). Various anti-inflammatory drugs may produce beneficial effects in experimental myocardial infarction. We have previously demonstrated, in conscious rats using a model developed in our laboratory, that some nonsteroid anti-inflammatory agents (Leprán, Koltai & Szekeres, 1981) and dexamethasone (Leprán, Koltai & Szekeres, 1982) exert prominent cardioprotective effects. The protection offered by the steroid is prevented by actinomycin D (Leprán *et al.*, 1982).

The present study was undertaken to answer the question whether macrocortin is involved in the protection afforded by dexamethasone against sudden death due to early post-infarction arrhythmias.

Methods Randomly-bred male Sprague-Dawley CFY rats weighing 200–230 g were used. The peritoneal cavity was lavaged with heparinised Krebs solution enriched with bovine serum albumin (100 $\mu g\,ml^{-1}$) as described by Di Rosa & Persico (1979). Cell suspensions collected from 50 rats were centrifuged (50 *g*) and pooled. The final suspension contained 10^7 cells ml^{-1}, and 25 ml samples were incubated in the presence or absence of 3×10^{-9} M dexamethasone phosphate (Oradexon, Organon) at 38°C for 90 min, then centrifuged; the supernatants were stored at − 25°C until use.

Partial purification of macrocortin was carried out according to Blackwell *et al.*, (1980). First, the supernatants derived from control (C supernatant) and dexamethasone-treated (D supernatant) cells, were filtered through glass columns (2.9 × 94 cm) filled with Sephadex G-25 and equilibrated with 0.1% ammonium bicarbonate adjusted to pH 7.5. The flow rate was 0.8 ml min^{-1}. Four ml fractions were collected and their absorbance was measured at 215 nm. [^{3}H]-dexamethasone (10^{-12} M, 20 μCi) was used for tracing the corticoid during gel filtration. Only 17.2% of the radioactivity was recovered in the first peak eluted after the void volume (160–200 ml). This was lyophilized, then reconstituted in 4 ml ammonium bicarbonate and applied to columns (1.8 × 68 cm) filled with Sephadex G-50. Elution was carried out with the same buffer and a flow rate of 0.5 ml min^{-1}, and 2 ml fractions were collected. The rest of the radioactivity of D supernatant was recovered in the first peak (42–65 ml). The columns were precalibrated with lysozyme (Sigma, mol. wt. 14.4 k). Its elution peak appeared at 102 ml. The fractions in the range of the marker material, showing a small peak (85–115 ml), were pooled and lyophilized. These were completely devoid of radioactive dex-

amethasone. Before use the 15 k fractions of the C and D supernatants were dissolved in 4 ml isotonic NaCl.

The reaction set for PLA_2 assay comprised the following: the enzyme was derived from *Vipera Russelli* (1 u cleaved 1 μM min^{-1} of substrate; the substrate was α-palmitoyl, β-[^{3}H]-oleyl-phosphatidylcholine (specific activity 100 nCi μM^{-1}). The incubation medium contained 20 mM Tris-HCl buffer (pH 6.5), 10 mM $CaCl_2$, 0.3 u ml^{-1} enzyme, 1 μM substrate, and 50 μl fractions of C or D supernatant (each containing 60 $\mu g\ ml^{-1}$ protein as determined by the method of Lowry, Rosebrough, Farr & Randall (1951)). Incubation was at 37°C for 60 min, and the effect of the fraction of D supernatant as compared to that of C supernatant was expressed as %.

Coronary occlusion was produced in conscious rats by tightening a loose silk loop placed around the left main coronary artery one week earlier. Full details are given elsewhere (Leprán *et al.*, 1981). The survival rate was determined and the incidence as well as the length of postocclusion arrhythmias were assessed during the first 20 min. In the surviving animals, the infarct size was measured by nitroblue tetrazolium staining (Nachlas & Shnitka, 1963). The animals were treated with actinomycin D (Merck, Sharpe and Dohme) 0.5 mg kg^{-1} intravenously 4 h, then 2 groups were given an intravenous bolus of 0.2 ml fractions of C and D supernatants 10 min before occlusion. The statistical analysis was made by the Chi-squared method.

Results Fractions eluted from Sephadex G-50 gels in the range of 15 k were tested for anti-PLA_2 activity. The factor obtained from D supernatant inhibited the enzyme-induced cleavage of radiolabelled substrate by 80%, while that isolated from C supernatant had no such an effect.

The effect of these fractions was also examined in conscious rats subjected to coronary occlusion. As the formation of macrocortin was found to be inhibited by actinomycin D (Flower & Blackwell, 1979; Di Rosa & Persico, 1979), the rats were pretreated with 0.5 mg kg^{-1} of the antibiotic 4 h before coronary ligation in order to prevent the *in vivo* formation of macrocortin. This intervention has been shown previously to have hardly any influence on the outcome of acute myocardial infarction in this model (Leprán, Koltai & Szekeres, 1982). The 15 k fraction of the C supernatant had no effect on any parameter studied when the results were compared to the data obtained in the actinomycin-treated group. The same fraction obtained from D supernatant offered a statistically significant protection against sudden death and the occurrence of arrhythmias (Table 1). In the rats that survived the acute period, the appearance of arrhythmias was significantly delayed (from 4.44 ± 0.21 to 5.90 ± 0.81 min, means ± s.e.) and the length of ventricular tachycardia was markedly shortened (from 61.7 ± 13.5 to 18.0 ± 9.5 s, means ± s.e.). The size of the infarcted area was 23.4 ± 2.6% in the control group and was not altered by macrocortin.

Discussion The cardioprotective effect of glucocorticoids in acute myocardial infarction has previously been described (Libby, Maroko, Bloor, Sobel & Braunwald, 1973; Spath, Lane & Lefer, 1974). This finding was confirmed in our laboratory in coronary-ligated, conscious rats (Leprán *et al.*, 1982). In the present study, we have found that macrocortin, a 15 k fraction of the supernatant of dexamethasone-treated isolated peritoneal cells of the rat offers a beneficial effect in the early phase of acute myocardial infarction. As macrocortin, like lipomodulin (Hirata *et al.*, 1980), has anti-phospholipase activity (thereby preventing AA release from the cell membrane) it appears likely that the cardioprotective effects of glucocorticoids are related to inhibition of early TxA_2 release due to ischaemia (Coker *et al.*, 1981).

Table 1 Effect of macrocortin on the acute phase of myocardial infarction in conscious rats

Groups	n	*Survival rate* (%)	*Occurrence of arrhythmias* (%) *None*	*Ventricular fibrillation*	*Ventricular tachycardia*	*Other*
(1) Actinomycin D	25	24	0	80	76	24
(2) Actinomycin D + 15 k fraction of C supernatant	15	27	0	93	80	33
(3) Actinomycin D + 15 k fraction of D supernatant	16	62[a,b]	12	56	62[b]	31

[a] Significantly different from group (1), [b] significantly different from group (2) at the level of $P < 0.05$.

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