*Br. J. Pharmac.* (1987), **90**, 443–445

# Vasocortin: a novel glucocorticoid-induced anti-inflammatory protein

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The preliminary characterization of 'vasocortin' a novel glucocorticoid-induced anti-inflammatory protein, is described. Vasocortin is released into the rat peritoneal cavity following systemic dexamethasone administration, has an apparent mol. wt. of 100 kD and inhibits rat dextran oedema. Vasocortin is distinct from lipocortin and is likely to be associated with the anti-inflammatory effect of glucocorticoids.

**Introduction** Recently we and others have identified a family of glucocorticoid-induced phospholipase $A_2$ ($PLA_2$) inhibitory proteins (Blackwell *et al.*, 1980; Carnuccio *et al.*, 1980; Hirata *et al.*, 1980; Cloix *et al.*, 1983), collectively called lipocortin (Di Rosa *et al.*, 1984) which are associated with the anti-inflammatory effect of these steroids (Blackwell *et al.*, 1982).

The anti-inflammatory effect of glucocorticoids does not entirely depend on the synthesis of lipocortin since dexamethasone inhibition of rat dextran oedema depends on the induction of a hitherto uncharacterized regulatory protein other than lipocortin (Calignano *et al.*, 1985; Di Rosa *et al.*, 1985) and this model has been used as a tool for discriminating between lipocortin (which is ineffective) and this uncharacterized protein (which is inhibitory).

Now we show that this protein (which we have called 'vasocortin' since it modulates vascular permeability and is induced by corticosteroids) is released into the rat peritoneal cavity following systemic glucocorticoid administration and is associated with the anti-inflammatory effect of these steroids.

**Methods** To generate the protein, male Wistar rats (200–250 g) were injected subcutaneously (s.c.) with saline (1 ml $kg^{-1}$) or dexamethasone sodium phosphate (1 mg $kg^{-1}$). Sometimes actinomycin D (2 mg $kg^{-1}$) was injected s.c. 1 h before dexamethasone. When adrenalectomized or sham-operated animals were used to generate the protein they were subjected to surgery 3 days before the experiment. Two hours after saline or dexamethasone injection animals were killed by exposure to ether and the peritoneal cavity washed with 10 ml of phosphate-buffered saline (50 mM $KH_2PO_4$, 150 mM NaCl). After collection, the fluid was dialysed to equilibrium at 4°C against 3 × 100 vol of 10 mM pH 7.4 ammonium carbonate, lyophilized, suspended in saline and the protein concentration adjusted to 4 mg $ml^{-1}$. Vasocortin activity was assayed in the dextran oedema model in which groups of at least 5 male Wistar rats (140–160 g) were used as previously described (Calignano *et al.*, 1985). Equal volumes of proteins suspended in saline and 6% dextran in saline were mixed so that each rat paw received 0.1 ml of saline containing 3% dextran and 200 μg protein. The oedema was recorded 1 h after the paw injection and results expressed as percentage inhibition of the oedema observed in the absence of any protein.

Partial purification of vasocortin was obtained by precipitating the peritoneal proteins with 60% saturated ammonium sulphate. The precipitated proteins were centrifuged (20,000 *g* for 30 min), suspended in 10 mM pH 7.4 ammonium carbonate, dialyzed to equilibrium at 4°C against the same buffer and applied to a G-200 Sephadex column (see Figure 1). The flow rate was 20 ml $h^{-1}$ and 6 ml fractions were collected and lyophilized.

Aliquots of each fraction were redissolved in saline (vasocortin assay) or in 20 mM pH 8.0 Tris buffer (lipocortin assay). Vasocortin activity was assayed on dextran oedema as described above and lipocortin was assayed as described by Blackwell *et al.* (1982).

**Results** In initial studies we found vasocortin activity in the peritoneal lavage fluid of dexamethasone pretreated male Wistar rats. Dextran oedema in control rats was 1.02 ± 0.04 ml (mean ± s.e.mean, $n = 15$) and there was a significant reduction ($P < 0.05$) of oedema (about 30%) in rat paws injected with 200 μg peritoneal proteins from dexamethasone-treated rats (0.73 ± 0.05 ml, $n = 10$) but proteins from actinomycin D pretreated rats or from adrenalectomized animals were without effect. Proteins collected from saline-treated sham-operated rats exhibited a slight, not significant, inhibition of the oedema (0.86 ± 0.1 ml, $n = 5$) possibly due to endogenous

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corticoid drive. Therefore, the following purification procedures were carried out using adrenalectomized rats in order to eliminate the variation due to the fluctuation of the endocrine state of the animals.

Partial purification of vasocortin was obtained by precipitating the peritoneal proteins with ammonium sulphate. We found that most of the vasocortin activity in the 60% saturation precipitate. A further purification was carried out by gel filtration chromatography of the precipitated proteins (see Figure 1). When these proteins were subjected to molecular sieving on a gel filtration G-200 Sephadex column the bulk of vasocortin activity eluted in a position corresponding to a relative molecular mass ($M_r$) of 100,000 daltons (100 kD) while most of the lipocortin eluted in the zone of 40 kD. Minor vasocortin activity was also found in the low mol. wt. fractions (Nos. 55–65).

Vasocortin seems to be a protein because partially purified material (fractions 48–50 from the Sephadex G 200 column) lost about 90% of its activity after being stirred overnight at 4°C with 5 u $ml^{-1}$ of immobilized trypsin (Sigma).

Heating the above fractions at 70°C for 5 min completely abolished the inhibitory activity on dextran oedema.

Vasocortin is effective following systemic administration since proteins in fractions 48–50 pooled and injected subcutaneously in rats (1 mg $kg^{-1}$ 1 h prior to dextran challenge) reduced the oedema to $0.54 \pm 0.05$ ml ($n = 5$), an inhibition of 47% ($P < 0.01$).

**Discussion** Both vasocortin and lipocortin are regulatory proteins induced by glucocorticoids. However, these proteins seem different entities because (i) their estimated molecular weights are different: vasocortin being 100 kD, lipocortin 40 kD; (ii) their biological activities differ: vasocortin inhibits

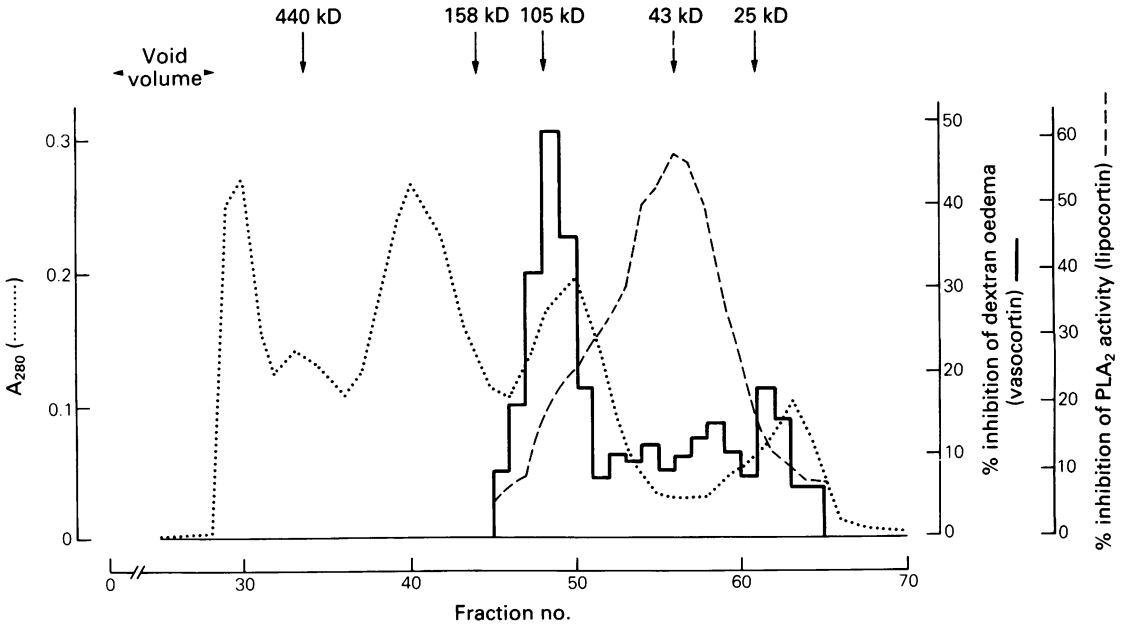


**Figure 1** Partial purification of vasocortin from rat peritoneal lavage fluid by G-200 Sephadex chromatography. Proteins from dexamethasone-treated adrenalectomized rats were precipitated with ammonium sulphate (60% saturation), centrifuged, dialyzed (see Methods) and applied (40 mg) to a G-200 Sephadex column (100 × 2.6 cm) equilibrated and eluted with 10 mM ammonium carbonate adjusted to pH 7.4. The flow rate was 20 ml $h^{-1}$ and 6 ml fractions were collected and lyophilized. Aliquots of each fraction were used for the assay of vasocortin and lipocortin (see Methods). Reported inhibition of dextran oedema (vasocortin activity) is the percentage inhibition (mean value of 5 rats in which s.e.mean was < 10% of the mean) induced by 10 μg proteins injected into the rat paw. The inhibition of $PLA_2$ (lipocortin activity) is referred to 50 μg protein and is expressed as percentage inhibition (mean value of triplicate assays) of the enzyme activity in the absence of any inhibitor.

Locations of standard proteins are as follows: 25 kD, chymotrypsinogen; 43 kD, ovalbumin; 105 kD, collagenase; 158 kD, aldolase; 440 kD, ferritin.

dextran rat oedema while lipocortin is ineffective; (iii) finally they exhibit differing sensitivity to heating: vasocortin activity is abolished by heating at 70°C for 5 min while lipocortin activity remains stable (Blackwell *et al.*, 1980).

Since rat peritoneal lavage fluid contains various proteases it is likely that vasocortin is cleaved into smaller fragments and these could be responsible for the minor activity detected in the low mol. wt. range. We reported that such an event often occurs during lipocortin purification (Blackwell *et al.*, 1982; Hirata *et al.*, 1982).

The mode of action of vasocortin in reducing vascular permeability as well as its relationship to a similar factor derived from Namalva cells (Oyanagui & Suzuki, 1985) are currently being investigated. However, since dextran oedema is mainly sustained by the release of histamine and 5-hydroxytryptamine (Di Rosa & Willoughby, 1971) it is conceivable that vasocortin might interfere with the release and/or the activity of these mediators.

Glucocorticoid-induced anti-inflammatory effects depend on the same molecular mechanism through which they exert physiological effects, i.e. by inducing the synthesis of regulatory proteins (Di Rosa, 1984). However the anti-inflammatory effect of glucocorticoids does not entirely depend on the induction of lipocortin(s) since these proteins, in contrast to the corticosteroids themselves, are unable to inhibit some types of inflammation, e.g. rat dextran oedema.

We have provided the first direct evidence that another glucocorticoid-induced protein (vasocortin) is associated with the anti-inflammatory effect of these steroids. Although it is still hard to identify the individual contribution and the actual role played by each one of these 'second messengers' (lipocortin and vasocortin), our findings support the concept that attributes the widespread action of glucocorticoids through the organism to a variety of induced regulatory proteins.

We thank Ciro Esposito for his helpful technical assistance.

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*(Received October 29, 1986. Accepted December 1, 1986.)*