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The effect of metyrapone on granuloma induced by carrageenan-impregnated sponges in normal and essential fatty acid deficient rats

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Treatment of normal rats with metyrapone (20 mg kg^{-1} day^{-1}, s.c.) for 5 days, starting on the day before implantation, inhibited the production of granuloma, induced by carrageenan-impregnated sponges, determined 8 days after implantation. Exudate volume and prostaglandin (PG) production were unaffected. In essential fatty acid deficient (EFAD) rats, metyrapone did not alter the already existing adrenal hyperplasia due to EFAD and did not affect either granuloma formation or exudate production. The results are discussed in relation to earlier work using adrenalectomy and with regard to the effect of EFA deficiency on adrenal corticosteroid production. It is suggested that metyrapone is a more useful tool than adrenalectomy in studying the role of endogenous corticosteroids.

The release of adrenal corticosteroids following many different types of stress (Selye, 1950) is now an accepted fact. However, although the release of adrenal corticosteroids, following inflammatory stress, is well documented, the role of these endogenous hormones in modulating the inflammatory response is not so clear (Parnham, 1977). Many early studies on the concentrations of corticosteroids and ACTH in the blood and urine of patients with rheumatic diseases failed to produce any firm conclusions on the modulatory role of corticosteroids (West, 1957; Kelley & Ely, 1960). Furthermore, despite the frequent assumption that endogenous corticosteroids suppress experimental inflammatory responses in animals there are comparatively few findings to support such an assumption. This lack has been discussed recently (Parnham, 1977). Those studies that have been made on animals have mostly involved the investigation of the effects of adrenalectomy on foreign body-induced granuloma formation in the rat. Using a variety of implants, different authors have found that adrenalectomy increases (Atkinson, Jenkins & others, 1962; Maass, Sosnowski & others, 1968), decreases (Taubenhaus & Amromin, 1950; Nakamura & Shimizu, 1974) or has no effect on (Jorgensen, 1962) granuloma formation. Ashford & Penn (1965) have suggested that these contrasting results are due to differences in the timing of the adrenalectomy. One problem associated with this technique is that it also involves the removal of the adrenal medulla and hence adrenal medullary catecholamines. Parnham (1977) has suggested that metyrapone, an inhibitor of the 11 β-hydroxylase step in the biosynthesis of adrenal corticosteroids (Chart & Sheppard, 1959), represents a far more selective tool in studying the role of endogenous corticosteroids in inflammation. However, high doses of metyrapone have also been shown to differentially inhibit the production of prostaglandins (PGs) by the isolated uterus of the pregnant rat, following administration of the drug *in vivo* and *in vitro* (Parnham & Sneddon, 1975; Parnham, 1976). This action may be mediated by inhibition of cytochrome P-450 binding (Cinti & Feinstein, 1976).

In the present investigation we have studied the effect of metyrapone on the production of granuloma induced in the rat by the implantation of carrageenan-impregnated sponges. By using a low dose of metyrapone, possible effects on PG production were avoided. To further rule out possible interference with the PG-system, the effect of metyrapone was also investigated in fatty acid-deficient (EFAD) rats, since essential fatty acid (EFA) deficiency is associated with a marked reduction in PG production (Ziboh, Vanderhoek & Lands, 1974; Bult & Bonta, 1976). However, several workers have shown that adrenal corticosteroidogenesis in the rat is reduced under conditions of EFA deficiency (Hayashida & Portman, 1959; Egwim & Kummerow, 1974). Thus, it was thought likely that the most noticeable effect of EFAD would be interference with the effects of metyrapone on adrenal corticosteroid synthesis rather than

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interference with any slight effects of metyrapone on PG production.

MATERIALS AND METHODS

Animals. Male albino rats of an inbred Wistar strain (Animal Farm TNO, Zeist), 185–220 g, were used and caged on Sol 'Speedi-Dry' (Metallochemie, Ramondt, Holland) (Bonta, Parnham & Adolfs, 1977b). EFA deficiency was achieved by feeding pregnant rats 4% hydrogenated cocos fat for five days before the expected day of delivery and then feeding the pups with the same diet, after weaning, until required (14 weeks old). Fatty acid analyses of diet and erythrocytes and the criterion for EFA deficiency have been described (Vincent, Zijlstra & Bonta, 1975; Bonta, Bult & others, 1977a).

Drug treatment. The experimental procedure was the same for both normal and EFAD rats. However, treatment of the normal rats was totally distinct from that with EFAD animals. In each experiment the animals were divided into 2 groups so that the mean body weights were similar. One group of animals then received saline (1 ml kg^{-1}, s.c.) daily and the other group received metyrapone (CIBA; 20 mg kg^{-1}, s.c., saline) daily. The first injection was given the day before sponge implantation and the doses were repeated for a further 4 days.

Sponge implantation. Sponge implantation was as described by Bonta & others (1977b) and removal was carried out as described in the second experiment of these authors. Briefly, 2 dried, sodium carrageenan (Marine Colloids, Springfield)-soaked, polyether sponges, were implanted, sub-cutaneously into the back of each rat and removed 8 days later after killing the animals with chloroform. Fluid beneath the surrounding capsule was removed with a syringe and the capsule cut off. The fluid remaining in the sponges was then squeezed out and the total exudate fluid (capsular and sponge) from both sponges in each rat was pooled. This pooled fluid was measured, acidified to pH 3 and extracted with 3 × 1 volume ethyl acetate (Merck) for bioassay of PG-like material. Recovery of 46 000 d min^{-1} ^{3}H-PGE_1 (Radiochemical Centre), through the extraction procedure was 68–80%. After removal of exudate, the capsules and sponges were dried at 80° for 24 h and weighed. Granuloma formation (Δ dry wt) was determined by subtracting the initial (pre-implantation) dry sponge weights from the total dry sponge and capsular weight. While Bonta & others (1977b) expressed granuloma formation as a 'proliferation index' (Δ dry wt/Δ body weight), one of the EFAD rats in the present experiment lost weight over the 8 days implantation, preventing the use of the index, thus the Δ dry wt was expressed in terms of 100 g body weight on day 8. The adrenal glands were also removed on day 8 and both adrenals from each rat weighed together.

Bioassay. PG-like material in exudate extracts was assayed on the rat stomach strip and rat colon against authentic PGE_2 (Upjohn) using the laminar flow cascade superfusion method of Bult, Parnham & Bonta (1977). The superfusing Krebs contained a mixture of antagonists (Gilmore, Vane & Wyllie, 1968) to increase the selectivity of the assay. In a similar (unpublished) experiment using metyrapone in normal rats, exudate extracts were subjected to column chromatography of PGs (Parnham & Sneddon, 1975) before bioassay and were found to contain undetectable amounts of PGF (<1 ng ml^{-1}). Thus, PG-like material was determined as PGE.

RESULTS

Normal animals. The effects of metyrapone on the various parameters of granuloma formation are shown in Table 1. In normal animals metyrapone produced significant adrenal hyperplasia, when compared with controls and this was associated with a significant inhibition of granuloma tissue formation, when compared with controls. Since both saline- and metyrapone-treated animals exhibited similar changes in body weight over the period of the experiment the significance of the fall in granuloma weight following metyrapone treatment was not altered when expressed in terms of 100 g body weight. Although the total exudate volume and the PG-like activity in the exudate both tended to decrease in metyrapone-treated animals, these decreases were not significant. This is in marked contrast to the significant changes in adrenal and granuloma weights obtained in the same rats.

EFAD animals. Metyrapone treatment did not significantly alter adrenal weight in EFAD rats and the barely significant fall in granuloma weight in these drug-treated animals was not significant when expressed in terms of 100 g body weight (Table 1). Similarly, although there was a tendency for PG-like material and total exudate volume to decrease, following metyrapone this was not significant.

DISCUSSION

The results described here show that treatment of normal rats with metyrapone, starting the day

Table 1. *Effect of metyrapone on various parameters of carrageenan sponge-induced granuloma in normal and EFAD rats.* Mean results are given with interquartile ranges (I.R.). The number of rats in each group are shown in brackets. Data on sponge weights were calculated using individual sponge values (n = 10); the remaining data were calculated on the basis of values per rat. Degrees of significance (metyrapone *vs* saline) were determined by one-tailed Mann-Whitney U test; $*P = 0.05$ $***P = 0.001$.

Treatment	Initial dry sponge wt (mg)	Body weight (g) Initial	Body weight (g) Day 8	Δ Dry sponge wt (mg)	Δ Dry sponge wt (mg/100 g)	Total exudate volume (ml)	PGE-like material (ng ml^{-1})	Adrenal wt day 8 (mg/100 g)
Normal rats:								
Saline (n = 5)	30·8 I.R. 6·6	189·8 I.R. 6·0	210·4 I.R. 2·8	379·4 I.R. 183·9	181·2 I.R. 92·8	1·95 I.R. 0·55	32·8 I.R. 18·0	15·3 I.R. 2·2
Metyrapone (n = 5)	29·6 I.R. 11·1	190·2 I.R. 10·5	204·3 I.R. 10·0	253·7 I.R. 136·7***	124·7 I.R. 70·4***	1·80 I.R. 0·40	28·2 I.R. 24·8	18·8 I.R. 2·8***
EFAD rats:								
Saline (n = 5)	27·7 I.R. 1·0	211·4 I.R. 28·0	215·8 I.R. 25·5	251·6 I.R. 38·3	116·8 I.R. 24·3	1·72 I.R. 0·55	3·51 I.R. 3·06	19·8 I.R. 1·1
Metyrapone (n = 5)	27·4 I.R. 0·1	210·0 I.R. 20·0	212·1 I.R. 11·3	234·6 I.R. 112·8*	110·2 I.R. 50·2	1·60 I.R. 0·80	3·09 I.R. 1·38	19·1 I.R. 3·4

before implantation, inhibits the production of carrageenan sponge granulomata. Inhibition of adrenal corticosteroid synthesis by metyrapone was indicated by the fact that the drug-treated animals exhibited marked adrenal hyperplasia. This is an invariable result of the *in vivo* inhibition of any step in corticosterone production in the rat (Temple & Liddle, 1970) and is commonly used as an indicator of the action of metyrapone (e.g. Goldman, 1967). Despite the earlier demonstration that metyrapone may affect PG production, when used at high doses (Parnham & Sneddon, 1975; Parnham, 1976), it is clear that this action was not responsible for the effects of metyrapone in the present study, since PGE-like activity in sponge exudates was unaltered by metyrapone treatment. This effect of metyrapone on granuloma formation supports the results of earlier workers showing that adrenalectomy decreases granuloma formation induced by turpentine (Taubenhaus & Amromin, 1950) and cotton pellets (Ashford & Penn, 1965) and also inhibits the accelerating effect of the implantation of a second cotton pellet (Nakamura & Shimizu, 1974). However, it is in contradiction to the stimulating effect (Atkinson & others, 1962; Maass & others, 1968) and lack of effect (Jorgensen, 1962) of adrenalectomy obtained by others. Ashford & Penn (1965) explained this paradox by showing that adrenalectomy, carried out at the same time as implantation, inhibited granuloma formation, whereas adrenalectomy, carried out 7 days before implantation, potentiated granuloma formation. Adrenalectomy 3 to 5 days before implantation had no effect. In each case corticosterone treatment reversed the effect of adrenalectomy, whether by increasing or decreasing granuloma formation. Since, in our experiment, metyrapone treatment was started only 1 day before implantation, it seems reasonable to conclude, on the basis of the work of Ashford & Penn (1965), that the inhibition of granuloma formation we obtained was due to inhibition of adrenal corticosterone synthesis. As corticosterone has only weak anti-inflammatory activity when administered exogenously (Sayers & Travis, 1971), it is probable that the effect of metyrapone on granuloma formation was due to the removal of the gluconeogenetic activity of corticosterone. This is further indicated by the fact that exudation (an inflammatory phenomenon) was unaffected by metyrapone in normal rats.

There was neither adrenal hyperplasia nor any significant change in granuloma formation or exudate volume in EFAD rats treated with metyrapone, when compared to EFAD, saline-treated controls. Although Hayashida & Portman (1959) found that adrenal weights of rats fed an EFAD diet for 12–14 weeks were reduced, compared to normal animals, feeding the EFAD diet for 15–19 weeks resulted in a marked increase in adrenal weight. In the present study pregnant rats were fed an EFAD diet for 5 days before the expected day of delivery and the offspring were fed the diet for 14 weeks before the experiment which took 8 days, thus the rats had the diet for a total of 16 weeks. Under identical conditions, we have shown previously that, following 8 days sponge implantation, adrenal weights in EFAD rats are significantly higher than in normal controls (Bonta & others, 1977b). Although EFAD and normal animals were treated in two distinct experiments in the present study and thus no direct comparison can be made between them, it is almost certain that the adrenal

weights in EFAD rats were higher than normal. This adrenal hyperplasia in EFAD rats is associated with a marked inhibition of adrenal corticosteroid production (Hayashida & Portman, 1959; Egwim & Kummerow, 1974). It seems likely that metyrapone had little further inhibitory action, thus accounting for the lack of effect on adrenal weight and granuloma and exudate production in EFAD rats.

In an earlier study we found that granuloma formation in EFAD rats was increased, compared to normal animals, when calculated on the basis of the change in body weight (Bonta & others, 1977b). This effect was attributed to the marked reduction in PG production in these animals (also seen in the present study). In the experiments described here we have not been able to confirm this effect of EFAD on granuloma formation since the normal and EFAD animals were treated in two totally distinct experiments, though the effect has been confirmed in two other (unpublished) experiments. Prolonged removal of adrenal corticosteroid production also results in increased granuloma formation (Atkinson & others, 1962; Ashford & Penn, 1965). Since adrenal corticosteroid synthesis is inhibited in EFAD rats (Hayashida & Portman, 1959; Egwim & Kummerow, 1974) and since metyrapone has no effect on granuloma formation in EFAD rats (present study) it is possible that inhibition of adrenal corticosteroid synthesis, either directly or indirectly, may account for the increased granuloma formation, observed by Bonta & others (1977b) in EFAD rats. On the basis of this reasoning, prolonged pretreatment of rats with metyrapone would be expected to produce the same effect as EFAD on subsequent granuloma formation.

In conclusion, metyrapone has been shown to exert similar effects to adrenalectomy on granuloma formation in the rat, without the problems of surgery, adrenal catecholamine removal and drastic changes in electrolyte balance. Bearing in mind the effects of metyrapone on PG production and possibly on electrolyte balance (Parnham, 1977), it is suggested that this drug could be used much more widely as an experimental tool.

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REFERENCES

ASHFORD, A. & PENN, G. B. (1965). *J. Pharm. Pharmac.*, **17**, 10–14.
ATKINSON, R. M., JENKINS, L., TOMICH, E. G. & WOOLLETT, E. A. (1962). *J. Endocr.*, **25**, 87–93.
BONTA, I. L., BULT, H., VINCENT, J. E. & ZIJLSTRA, F. J. (1977a). *J. Pharm. Pharmac.*, **29**, 1–7.
BONTA, I. L., PARNHAM, M. J. & ADOLFS, M. J. P. (1977b). *Prostaglandins*, in the press.
BULT, H. & BONTA, I. L. (1976). *Nature*, **264**, 449–451.
BULT, H., PARNHAM, M. J. & BONTA, I. L. (1977). *J. Pharm. Pharmac.*, **29**, 369–370.
CHART, J. J. & SHEPPARD, H. (1959). *J. med. pharm. Chem.*, **1**, 407–441.
CINTI, D. L. & FEINSTEIN, M. B. (1976). *Biochem. biophys. Res. Commun.*, **73**, 171–179.
EGWIM, P. O. & KUMMEROW, F. A. (1974). *Lipids*, **9**, 191–195.
GILMORE, N. J., VANE, J. R. & WYLLIE, J. H. (1968). *Nature*, **218**, 1135–1140.
GOLDMAN, A. S. (1967). *J. clin. Endocr.*, **27**, 1390–1394.
HAYASHIDA, T. & PORTMAN, O. W. (1959). *Am. J. Physiol.*, **197**, 893–896.
JORGENSEN, O. (1962). *Acta pharmac. tox.*, **19**, 101–106.
KELLEY, V. C. & ELY, R. S. (1960). *Ann. N.Y. Acad. Sci.*, **86**, 1115–1128.
MAASS, A. R., SOSNOWSKI, G., WIEBELHAUS, V. D. & WEINSTOCK, J. (1968). *J. Pharmac. exp. Ther.*, **163**, 239–249
NAKAMURA, H. & SHIMIZU, M. (1974). *Eur. J. Pharmac.*, **27**, 198–205.
PARNHAM, M. J. (1976). *Ibid.*, **40**, 285–290.
PARNHAM, M. J. (1977). *Agents and Actions, Suppl.* **2**, 85–97.
PARNHAM, M. J. & SNEDDON, J. M. (1975). *Br. J. Pharmac.*, **55**, 535–539.
SAYERS, G. & TRAVIS, R. H. (1971). In: *The Pharmacological Basis of Therapeutics*, pp. 1604–1642. Editors: Goodman, L. S. and Gilman, A., New York: MacMillan.
SELYE, H. (1950). *The Physiology and Pathology of Stress*. Montreal: Acta Inc.
TAUBENHAUS, M. & AMROMIN, G. D. (1950). *J. Lab. clin. Med.*, **36**, 7–18.
TEMPLE, T. E. & LIDDLE, G. W. (1970). *Ann. Rev. Pharmac.*, **10**, 199–218.
VINCENT, J. E., ZIJLSTRA, F. J. & BONTA, I. L. (1975). *Prostaglandins*, **10**, 899–911.
WEST, H. F. (1957). *Ann. rheum. Dis.*, **16**, 173–181.
ZIBOH, V. A., VANDERHOEK, J. Y. & LANDS, W. E. M. (1974). *Prostaglandins*, **5**, 233–240.