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REDUCTION IN THE CHOLINESTERASE ACTIVITY OF THE RAT ANOCOCCYGEUS MUSCLE PRODUCED BY CORTICOSTERONE

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1 Administration of corticosterone caused a 47% reduction in the cholinesterase (ChE) activity of homogenates of the rat anococcygeus muscle.

2 ChE activity was also reduced by morphine withdrawal and this effect was abolished by the corticosteroid synthesis inhibitor, metyrapone.

3 A single dose of reserpine reduced ChE activity in normal but not in adrenalectomized rats.

4 ChE activity was increased by adrenalectomy or by metyrapone treatment.

5 The mechanism of the corticosteroid-induced reduction in ChE activity is discussed.

6 The reduction in ChE activity produced by corticosterone, morphine withdrawal, or a single dose of reserpine might explain the leftward shift of the dose-% response curve to acetylcholine produced by these procedures in the isolated anococcygeus muscle of the rat.

Introduction

Chronic administration of corticosterone produces a characteristic pattern of supersensitivity in the rat anococcygeus muscle (Gibson & Pollock, 1973a, 1974, 1975). A similar type of supersensitivity is also produced by morphine withdrawal and by a single dose of reserpine, and in both these cases the supersensitivity appears to be linked to plasma corticosteroid levels (Gibson & Pollock, 1974, 1975), since the effects can be abolished by metyrapone, which inhibits corticosteroid synthesis, or by adrenalectomy.

Corticosteroid-induced supersensitivity in the rat anococcygeus muscle consists of two distinct components, a specific increase in the pD_2 value for acetylcholine (ACh), and a non-specific increase in the contractile response of the muscle, resulting in increased maximum responses to both noradrenaline and ACh.

The object of the present study was to investigate the mechanism underlying the first of these components. The specificity of the effect on the pD_2 value for ACh suggested that corticosterone might interfere with the metabolism of ACh, and in particular, that it might reduce the activity of cholinesterase (ChE), the enzyme of ACh degradation. Although neostigmine potentiated the response of the rat anococcygeus muscle to ACh (Gillespie & McGrath, 1974), Gillespie (1972) was unable to demonstrate the presence of the enzyme histologically. In this study, therefore, the ChE activity of the muscle was determined colorimetrically, and the effects of corticosterone, morphine withdrawal, or a single dose of reserpine on enzyme activity were investigated. In addition, the effects of the latter two procedures on animals whose adrenal function had been impaired by metyrapone or adrenalectomy respectively were studied.

Methods

Male Wistar rats (200-250 g) were killed by a blow on the head and bled from the neck. The two anococcygeus muscles were quickly dissected (Gillespie, 1972), weighed, and homogenized in 2 ml phosphate buffer (0.1 M, pH 8.0) using a Jencons 3 ml ground glass homogenizer with an electrically driven pestle. ChE activity in the homogenate was then determined by the colorimetric method of Ellman, Courtney, Andres & Featherstone (1961), with a Pye-Unicam SP 8000 dual beam spectrophotometer. All assays were performed at 37° C. The direct effect of corticosterone on ChE was determined by the addition of known amounts of the steroid directly to the assay medium. The hormone was dissolved in 50% alcohol and 32 μl of this solution was added to both test and blank cuvettes. Alcohol itself was found not to affect the assay.

Plasma corticosteroid levels were determined by a modified version of the method of Zenker & Bernstein (1958), which has been described previously (Gibson & Pollock, 1975).

Pretreatments

(i) Corticosterone (20 mg/kg, i.p. daily, for 5 days) was administered as a suspension in ethyl oleate. Control animals received appropriate volumes of ethyl oleate alone. Animals were killed on day 6.

(ii) One group of animals was given morphine (50 mg/kg, i.p. daily, for 8 days); on day 9 these rats were given a saline (0.9% w/v NaCl solution) injection and were killed on day 10. A second group received both morphine (50 mg/kg, i.p. daily, for 8 days) and metyrapone (50 mg/kg, i.p. daily, for 8 days); on day 9 they were given metyrapone alone and were killed on day 10. A third group of rats received metyrapone alone (50 mg/kg, i.p. daily, for 9 days).

(iii) Reserpine was administered in two treatment schedules. In the first, reserpine (1 mg/kg, i.p.) was administered 20 h before death. In the second, reserpine (1 mg/kg, i.p. daily) was administered for 5 days, the animals being killed on day 6.

(iv) Adrenalectomized animals were obtained commercially (Charles River Co., Margate, England), and were given saline to drink.

Drugs

The following drugs were used: acetylthiocholine iodide (B.D.H.); butyrylthiocholine iodide (Sigma); corticosterone (Sigma); metyrapone (Ciba); morphine hydrochloride (Macarthys, Glasgow); reserpine phosphate (Ciba).

Results

In Table 1 the rates of hydrolysis of acetylthiocholine and butyrylthiocholine by homogenates of the rat anococcygeus muscle are compared with those reported previously (Ellman *et al.*, 1961) for other rat tissues. It can be seen that the anococcygeus muscle exhibited a slightly greater enzyme activity than skeletal muscle tissue. Butyrylthiocholine was hydrolysed at 43%, the rate of acetylthiocholine which is in keeping with the previous finding (Ellman *et al.*, 1961). The effects of corticosterone, morphine withdrawal, and of a single dose of reserpine on the ChE activity of the anococcygeus muscle are shown in Table 2a.

Corticosterone

Administration of the steroid produced a 47% reduction in the ChE activity of the muscle.

Morphine withdrawal

ChE activity was also reduced by morphine withdrawal, although the reduction was not as great as that produced by corticosterone. Muscles from animals treated with the corticosteroid synthesis inhibitor metyrapone displayed a greater ChE activity than did controls, and this activity was not significantly altered by morphine withdrawal (Table 2b).

Reserpine

The ChE activity of the anococcygeus muscle was reduced 20 h after administration of reserpine. Muscles from adrenalectomized animals had greater enzyme activity than controls, and reserpine had no significant effect on the ChE content of muscles from adrenalectomized rats (Table 2b). Unlike a single dose, chronic administration of reserpine for 5 days did not alter the ChE activity of the muscle.

Table 1 A comparison of the rates of hydrolysis of acetylthiocholine and butyrylthiocholine in the rat anococcygeus muscles with those reported previously for other rat tissues

Tissue	*Rates (μmol hydrolysed min^{-1} g^{-1} tissue)*	
	Acetylthiocholine	*Butyrylthiocholine*
Lung[1]	1.63	0.54
Liver[1]	1.07	0.65
Muscle[1] (thigh)	1.82	0.12
Kidney[1]	0.28	0.21
Brain[1] (whole)	10.31	0.31
Anococcygeus	2.01	0.87

[1] Values taken from Ellman *et al.* (1961).

Table 2a The effect of various procedures on the cholinesterase (ChE) activity of homogenates of rat anococcygeus muscles

Treatment	*ChE activity ± s.e. (μmol hydrolysed min^{-1} g^{-1} tissue)*	n
Control	2.01 ± 0.08	32
Corticosterone (5 days)	1.07 ± 0.34*	6
Morphine withdrawal (24 h)	1.54 ± 0.21*	7
Reserpine (20 h)	1.34 ± 0.13**	8
Reserpine (5 days)	1.93 ± 0.10	6
Metyrapone (9 days)	2.56 ± 0.19*	6
Adrenalectomy	2.61 ± 0.21*	6

n = No. of observations. s.e. = Standard error of mean.
* $P < 0.05$; ** $0.01 > P > 0.001$—compared to control.

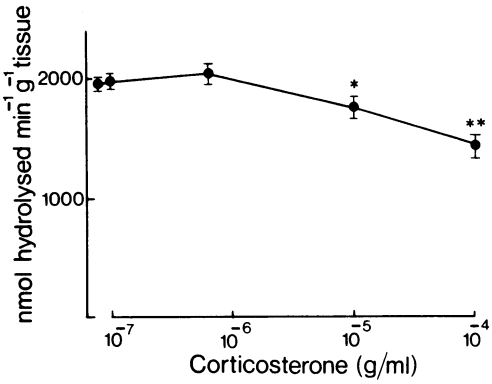

Figure 1 The effect of corticosterone on cholinesterase activity in homogenates of the rat anococcygeus muscle. The hormone was added directly to the assay medium in the concentrations shown. Each point is the mean of 6 observations and the vertical lines represent the standard errors. * $0.05 > P > 0.01$; ** $0.01 > P > 0.001$.

Comparison of plasma corticosteroid levels with concentration of corticosterone required to inhibit cholinesterase in vitro

Corticosterone inhibited the ChE activity of homogenates of anococcygeus muscles *in vitro*, at a concentration of 10 μg/ml or above (Figure 1). Examination of the plasma levels of corticosteroids in animals shown to have a reduced muscle ChE activity revealed that, although levels were raised, on no occasion were they greater than 1 μg/ml (Table 3). Plasma corticosteroids were not elevated in blood from rats treated chronically with reserpine (Table 3).

Table 2b The effect of morphine withdrawal and a single dose of reserpine on the cholinesterase (ChE) activity of muscles excised from metyrapone-treated rats or adrenalectomized rats respectively

Treatment	*ChE activity ± s.e. (μmol hydrolysed min^{-1} g^{-1} tissue)*	n
Metyrapone (9 days)	2.56 ± 0.19	6
Morphine withdrawal (24 h) + metyrapone (9 days)	2.78 ± 0.35	6
Adrenalectomy	2.61 ± 0.21	6
Reserpine (20 h) + adrenalectomy	3.19 ± 0.46	6

n = No. of observations. s.e. = Standard error of mean.

Discussion

The present results indicate that the rat anococcygeus muscle does contain a significant amount of ChE activity, thus explaining the enhancement of the responses to ACh produced by neostigmine (Gillespie & McGrath, 1974). The rat anococcygeus muscle, therefore, provides an example of a tissue which responds to ACh and contains the enzyme of ACh degradation, but which does not appear to receive a cholinergic innervation (Gillespie, 1972).

The ChE activity of the muscle was reduced by corticosterone and was increased both by adrenalectomy and by the corticosteroid synthesis inhibitor, metyrapone. It has been suggested that the corticosteroid hormones exert a regulatory influence over the enzymes of catecholamine synthesis and degradation (Wurtman, Axelrod, Vessel & Ross, 1968; Sumathy & Clarke, 1972; Parvez & Parvez, 1972). The present results suggest that the steroids may also be important in regulating the activity of the enzyme of ACh degradation.

During morphine withdrawal, the ChE activity of the anococcygeus muscle was decreased. The involvement of corticosteroids in this reduction was indicated by the lack of effect of morphine withdrawal on the ChE activity of muscles taken from animals treated with metyrapone. This provides further indirect evidence for the possible importance of these hormones in the production of at least some of the phenomena associated with the withdrawal state (Gibson & Pollock, 1974, 1975).

The reduction of ChE activity produced by a single dose of reserpine also appeared to be due to release of excess corticosteroids since the effect was absent in adrenalectomized animals. A single dose of reserpine increases the secretion of adrenocorticotrophic hormone (ACTH) from the pituitary, probably by stimulating the release of brain monoamines (Westermann, 1965). However,

Table 3 Plasma corticosterone levels in rats treated as shown

Treatment	*Plasma corticosterone (μg/100 ml)*	n
Control	29.7 ± 2.1	13
Corticosterone (5 days)	41.6 ± 2.7*	6
Morphine withdrawal (24 h)	45.6 ± 3.8*	6
Reserpine (20 h)	38.4 ± 3.2*	6
Reserpine (5 days)	31.1 ± 2.8	6

n = No. of observations. Values are given ± s.e. mean.
* = $P < 0.05$.

continued administration of the drug leads to a depletion of these monoamines, and hence subsequent doses of reserpine no longer stimulate ACTH release (Wells, Briggs & Munson, 1956). Indeed, it was found that chronic administration of reserpine neither raised plasma corticosteroid levels, nor reduced muscle ChE activity. Further, chronic administration of reserpine produced a pattern of supersensitivity in the anococcygeus muscle which was different from that produced by a single dose of reserpine (Gibson & Pollock, 1973b).

It is unlikely that the reduction of ChE activity was due to a direct inhibition of the enzyme by the corticosteroids, since ChE was inhibited only at high concentrations of corticosterone, which were more than ten times higher than those observed in the plasma of treated rats. The inhibition produced by high concentrations is probably due to the non-specific surface active properties of the steroids (Munck, 1957). Corticosteroid-induced reduction of enzyme activity *in vivo* is probably related to the effect of the hormones on protein synthesis and degradation (White, Blecker & Jedeikin, 1961; Parvez & Parvez, 1972).

Finally, the reduction in ChE activity of the rat anococcygeus muscle produced by corticosterone, morphine withdrawal, or a single dose of reserpine provides an explanation for the specific leftward shift of the dose-% response curve to ACh produced by these procedures (Gibson & Pollock, 1975). However, the second feature of corticosteroid-induced supersensitivity, namely the ability to increase the maximum response to agonists cannot be explained by inhibition of ChE activity, and the mechanism is at present under investigation.

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