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A role for corticosterone in the *in vivo* regulation of the extraneuronal uptake of [^{3}H]-isoprenaline into rat atria

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1 The role of corticosterone, a potent *in vitro* inhibitor of extraneuronal uptake, in the regulation of the extraneuronal accumulation of [^{3}H]-isoprenaline into rat atria was examined.

2 Procedures which increased plasma corticosterone levels resulted in a decrease in the corticosterone-sensitive component of extraneuronal accumulation of [^{3}H]-isoprenaline (64% after reserpine treatment and 25% after chronic cold exposure).

3 Procedures which decreased levels of plasma corticosterone resulted in an increase in the corticosterone-sensitive component of extraneuronal accumulation of [^{3}H]-isoprenaline (approximately 20% after adrenalectomy and 32% after hypophysectomy). This increase was partially prevented by the *in vivo* administration of dexamethasone (40 $\mu g\,kg^{-1}$).

4 There was a strong inverse correlation between the plasma corticosterone concentration and the level of extraneuronal uptake into atria ($P < 0.01$).

6 Corticosterone appears to play a major role in the regulation of extraneuronal uptake into tissues of the rat.

Introduction

Chronic reserpine treatment resulted in a marked reduction in the extraneuronal uptake of isoprenaline into atria and vasa deferentia (Morton & Mills, 1981; Morton, 1985). This decrease was due to the time-dependent loss of a corticosterone-sensitive component of extraneuronal uptake. However, whilst it is not clear what causes this change, the extraneuronal uptake does not appear to be dependent upon either the presence of the adrenergic neurone or the level of circulating catecholamines (Morton, 1987). Thus some other factor altered by reserpine treatment is implicated in the regulation of the extraneuronal uptake.

One such possible factor is the glucocorticoid, corticosterone. The importance of both the catecholamines and corticosterone in mediating the biological adjustments required to protect an animal from a hostile environment is well established (Chaffee & Roberts, 1971; Gale, 1973; Hodges, 1976), and in recent years a number of studies have suggested a role for corticosterone in the modulation of various adrenergic mechanisms (Pohorecky & Wurtman, 1971; Gibson, 1981; Foster *et al.*, 1983). Further, corticosterone is a potent and specific *in vitro* inhibitor of extraneuronal uptake (Salt, 1972) and the binding of corticosterone to peripheral tissues closely parallels the ability of these tissues to accumulate noradrenaline extraneuronally (Gibson & Street, 1977). Some studies have shown that the total accumulation of catecholamines by adult (Parvez *et al.*, 1979) and developing tissues (Iversen *et al.*, 1967) can be modified by steroids, although in these studies no distinction was made between the accumulation by neuronal or extraneuronal uptake.

In this study, a possible role for corticosterone in the regulation of extraneuronal uptake was examined by measuring the uptake of [^{3}H]-isoprenaline into atria from rats in which the levels of circulating corticosteroids had been altered.

Methods

Animals

Male albino rats (Otago Wistar) were used. All rats were weight-matched at body weights of 225–275 g. Atria were dissected from the hearts of stunned and decapitated rats.

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Extraneuronal uptake studies

Extraneuronal uptake was measured as previously described (Morton, 1987). Briefly, uptake was calculated as the difference between accumulation of [^{3}H]-isoprenaline measured at 37°C and that measured at 0°C, after incubation for up to 120 min. Atria were incubated in modified Liley solution containing [^{3}H]-isoprenaline, 5×10^{-5} M, 200 nCi ml^{-1}; catechol-*O*-methyl transferase (COMT) inhibitor (3,5 dimethoxy,4-hydroxybenzoic acid) 5×10^{-6} M; ascorbic acid 1.14×10^{-4} M, EDTA 2.97×10^{-5} M and bubbled with a mixture of 95% O_2 and 5% CO_2. The medium was continually replaced during incubation. In some experiments corticosterone (100 μM) was used to block extraneuronal uptake, with corticosterone present in the medium throughout the experiment. After incubation, tissues were blotted, weighed and digested in sodium hydroxide (0.5 M) for several days. After this time 0.8 ml of the sample was neutralized and the radioactivity of each sample counted in a Packard Tri-Carb β-liquid scintillation spectrometer. The isoprenaline content was calculated as pmol mg^{-1} tissue wet weight.

Composition of Liley solution

The modified Liley solution was of the following composition (mM): Na^+ 140.4, Cl 132.5, K^+ 5.0, H_2PO_4 1.0, Ca^{2+} 2.0, HCO_3 17.9, Mg^{2+} 1.0, glucose 11.1; pH 7.2–7.4.

Plasma corticosterone levels

These were measured using the corticosterone binding-globulin assay described by Bassett & Hinks (1969).

Injection program

Intraperitoneal injections of reserpine (1 mg kg^{-1} day^{-1}) or dexamethasone (40 μg kg^{-1} day^{-1}) were given daily for seven days.

Cold acclimatization

Rats were housed individually in wire mesh cages at 4 ± 2°C (relative humidity of 84 ± 1%) for either 7 or 14 days continuously before the uptake studies were done.

Adrenalectomy

Rats were bilaterally adrenalectomized (Ingle & Griffith, 1962) while under ether anaesthesia, and were given NaCl (0.9% w/v) in their drinking water post-operatively. Rats were used for uptake experiments 7 days after their operations. Adrenalectomies were considered to be adequate if adrenaline could not be detected in plasma 4–7 days after the operation (less than 40 pg ml^{-1}).

Hypophysectomy

The operations were performed via the parapharyngeal approach using ether anaesthesia (Ingle & Griffith, 1962). Post-operatively the animals had glucose (5% w/v) added to the drinking water. As evidence of the completeness of pituitary ablation, daily body-weight changes were recorded, and at the time of death the adrenal glands, prostate glands and seminal vesicles were weighed, and the pituitary fossa examined visually.

Statistics

Values are expressed as means ± s.e.mean. A one way analysis of variance was performed on the data and the significance was determined by use of Student's *t* test.

Drugs

Drugs used were: (−)-isoprenaline HCl (Sigma); 3,5 dimethoxy,4-hydroxybenzoic acid (ICI pharmaceuticals Inc.): reserpine (CIBA-GEIGY Ltd); (±)-[7-^{3}H]-isoprenaline HCl, 5–15 Ci mmol^{-1} (Amersham International); [1,2,6,7 ^{3}H]-corticosterone, 75–100 Ci mmol^{-1} (Amersham International); corticosterone and dexamethasone (Sigma).

Results

Reserpine treatment

The accumulation of [^{3}H]-isoprenaline into atria from reserpine-treated rats at 90 min of incubation was significantly lower ($P < 0.01$) than the corresponding control value (Table 1).

Adrenalectomy

The time course of the accumulation of [^{3}H]-isoprenaline into atria from adrenalectomized rats was similar to that into atria from control rats for the first 30 min of incubation. However, after 60 and 90 min of incubation there was an increase in the accumulation of [^{3}H]-isoprenaline into the atria from adrenalectomized rats when compared with that into atria from control rats ($P < 0.05$, 60 min; $P < 0.01$, 90 min) (Figure 1). The increased accumulation of [^{3}H]-isoprenaline measured after 90 min of incubation was sensitive to inhibition by *in vitro* corticosterone. The increase in uptake calculated as the difference in the

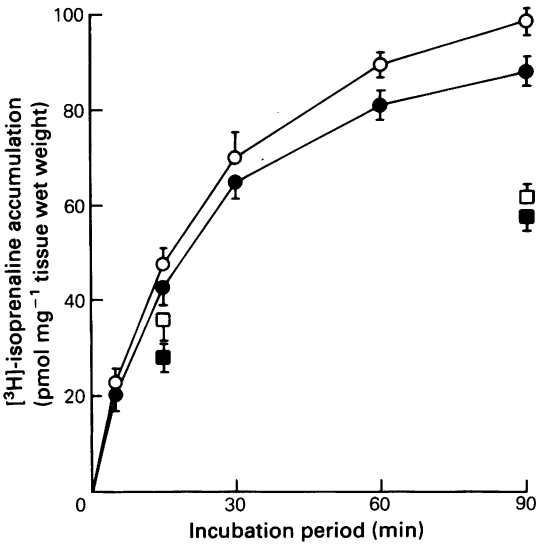

Figure 1 The effect of adrenalectomy on the time course of extraneuronal accumulation of [^{3}H]-isoprenaline into atria. The accumulation was measured in the tissues from intact rats (●, ■), and in tissues from rats bilaterally adrenalectomized 7 days previously (○, □), in the presence (■, □) and absence (●, ○) of corticosterone (100 μM). Each value represents the mean of 6 (corticosterone present) or 12 (no corticosterone present) tissues; vertical lines show s.e.mean.

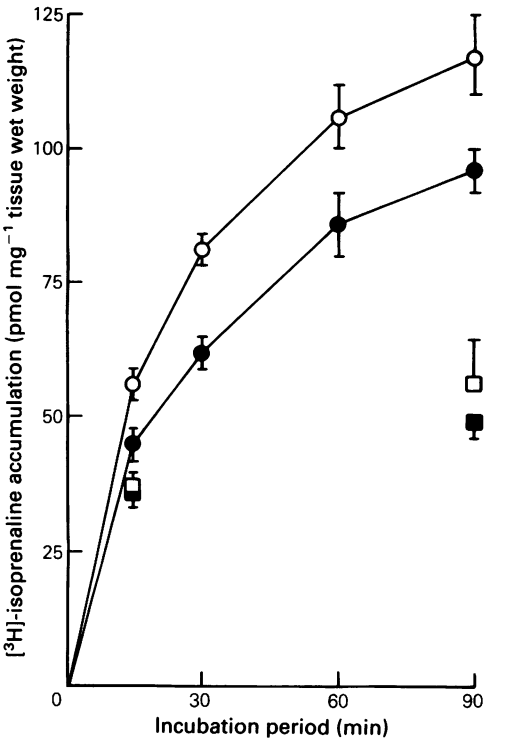

Figure 2 The effect of hypophysectomy on the time course of the extraneuronal accumulation of [^{3}H]-isoprenaline into atria. The accumulation was measured in the tissues from intact rats (■, ●), and in tissues from rats hypophysectomized 7 days previously (□, ○), in the presence (■, □) and absence (●, ○) of corticosterone (100 μM). Each value in the figure represents the mean of 12 tissues, except at 30 and 60 min when 6 tissues were used; vertical lines show s.e.mean.

accumulation measured at 37°C and 0°C was approximately 20% greater than that measured in control tissues.

Hypophysectomy

Seven days after hypophysectomy, the measured accumulation of [^{3}H]-isoprenaline into the atria from hypophysectomized rats was significantly increased at 15 min of incubation and onwards ($P < 0.01$). This increase in accumulation was sensitive to *in vitro* inhibition by corticosterone at 15 and 90 min of incubation (Figure 2). The increase in uptake calculated as the difference in accumulation measured at 37°C and at 0°C was approximately 32% greater than that in control tissues.

Dexamethasone treatment

Dexamethasone was used as an *in vivo* glucocorticoid substitute in adrenalectomized and hypophysectomized rats. A dose of dexamethasone (40 μg kg^{-1} day^{-1}) was chosen such that a small increase in the weight of the adrenal gland of control (intact) rats was seen after 7 consecutive days of administration. *In vitro* incubation of control atria in the presence of 40 μg l^{-1} dexamethasone did not affect the uptake of [^{3}H]-isoprenaline (Table 1). Nor was the accumulation of [^{3}H]-isoprenaline into atria from rats treated *in vivo* with dexamethasone (Table 1) significantly different from that into atria from untreated rats at 7 or 90 min of incubation.

Daily dexamethasone treatment (7 days) of adrenalectomized rats prevented the increase in the accumulation of [^{3}H]-isoprenaline normally seen after adrenalectomy. After 90 min there was no difference between the accumulation into tissues from dexamethasone-treated, adrenalectomized rats and atria from control rats (Table 1d).

The chronic administration of dexamethasone to hypophysectomized rats prevented the hypophysectomy-induced increase in extraneuronal uptake. However, dexamethasone did not completely prevent

Table 1 Accumulation of [^{3}H]-isoprenaline by rat atria

Incubation period	*Accumulation of [^{3}H]-isoprenaline* (pmol mg^{-1} tissue wet weight) *7 min*		*90 min*	
a				
Control (untreated)	26.0 ± 3.2	(12)	101.6 ± 2.6	(12)
Reserpine-treated (1 mg kg^{-1} day^{-1})	28.0 ± 4.6	(12)	58.2 ± 5.2**	(12)
b				
Control (untreated)	29.5 ± 1.6	(6)	88.2 ± 2.3	(6)
Dexamethasone (40 μg l^{-1}) in incubation medium	28.2 ± 3.2	(6)	89.3 ± 2.0	(6)
c				
Control (untreated)	28.3 ± 2.1	(18)	91.0 ± 4.2	(18)
Dexamethasone-treated (40 μg kg^{-1} day^{-1})	26.0 ± 4.2	(18)	84.6 ± 6.0	(18)
d				
Control (sham-operated)	20.5 ± 2.1	(12)	87.5 ± 3.0	(12)
Adrenalectomized (7 days)	22.5 ± 3.4	(12)	98.5 ± 3.5*	(12)
Adrenalectomized + dexamethasone-treated (7 days)	20.5 ± 3.3	(10)	91.2 ± 3.0	(10)

Values are means ± s.e.mean, n = number in parentheses.
* $P<0.05$, ** $P<0.01$ when compared with the value for the corresponding control.
Incubations were conducted at 37°C.

this change, since there was still a significant difference ($P<0.01$) between the accumulation into atria from dexamethasone-treated, hypophysectomized rats compared with that into atria from dexamethasone-treated intact rats (Figure 3).

Plasma corticosterone levels

Corticosterone levels measured in plasma from rats after various treatments are shown in Table 2. Chronic cold exposure (7 days) and reserpine treatment both caused a significant increase in plasma corticosterone, while adrenalectomy and hypophysectomy significantly reduced corticosterone, when compared with the control value. After 14 days of cold exposure, plasma corticosterone levels were significantly lower than after 7 days in the cold, and not significantly different from control values.

Correlation between the change in the extraneuronal uptake into atria after various treatments, and corresponding levels of plasma corticosterone

The coefficient of correlation (Pearson) describing the relationship between the change in the extraneuronal uptake after various treatments and the corresponding levels of plasma corticosterone was calculated using the difference in the accumulation of [^{3}H]-isoprenaline into atria from treated rats and into the corresponding control measured at 90 min of incubation, and the level of corticosterone in plasma sampled in the hour before the uptake studies were done (Table 2). A scattergram illustrating these data is shown in Figure 4. The coefficient of correlation was 0.96, and the correlation between the extraneuronal uptake and the plasma corticosterone levels was statistically significant ($P<0.01$).

Discussion

Chronic treatments which caused a marked decrease in plasma corticosterone (adrenalectomy and hypophysectomy) resulted in an increase in the accumulation of [^{3}H]-isoprenaline measured at 90 min of incubation. This increase was sensitive to *in vitro* inhibition by corticosterone. Conversely, treatments which caused an increase in plasma corticosterone levels (reserpine treatment, 7 day chronic cold-exposure) resulted in a significant decrease in the accumulation of [^{3}H]-isoprenaline. This decrease in accumulation was due to the loss of a corticosterone-sensitive component of extraneuronal uptake.

Statistical analysis of these data showed that there was a strong inverse relationship between the level of corticosterone present in the blood and the level of extraneuronal uptake measured in the atria from rats. The high correlation between the change in extraneuronal uptake after various treatments and the amount of corticosterone measured in the plasma suggests that corticosterone may have a role as an *in vivo* inhibitor of extraneuronal uptake. This suggestion is supported by the finding that the chronic administration of the glucocorticoid substitute dex-

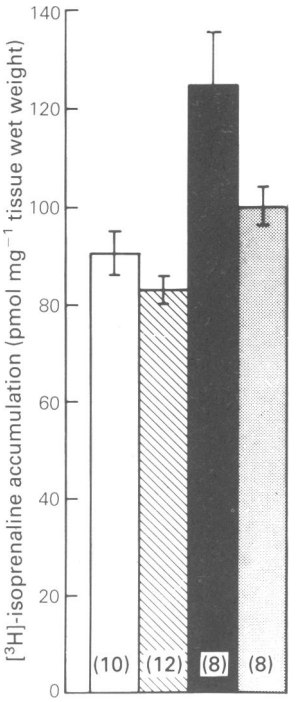

Figure 3 The effect of daily injections of dexamethasone on the accumulation of [^{3}H]-isoprenaline into the atria from rats hypophysectomized 8 days previously. Each column represents the mean accumulation of [^{3}H]-isoprenaline at 90 min of incubation at 37°C; vertical lines show s.e.mean. The number of tissues used is shown in parentheses at the base of each column. Open columns, atria from control rats; hatched column, dexamethasone-treated control rats; solid column, hypophysectomized rats; stippled column, hypophysectomized rats treated with dexamethasone.

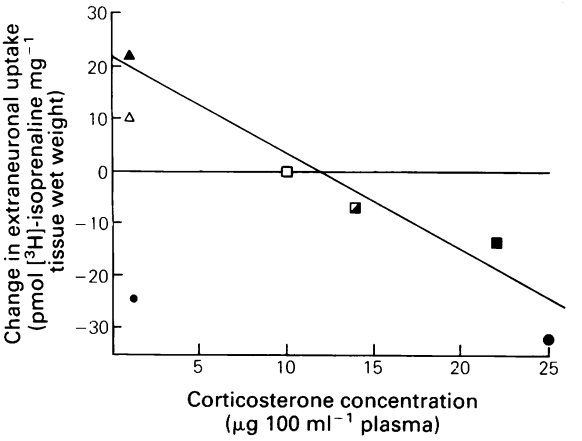

Figure 4 The mean corticosterone level plotted against the corresponding change in the extraneuronal uptake (with respect to control atria (□)) of atria taken from rats after reserpine treatment (●), cold exposure (7 days (■)), cold exposure (14 days) (◪), adrenalectomy (▲), and after hypophysectomy (△).

amethasone to adrenalectomized rats prevented the increase normally seen after adrenalectomy. Chronic administration of dexamethasone also prevented a similar proportion of the increase in extraneuronal uptake usually seen after hypophysectomy, although a significant increase in extraneuronal uptake was still present (compared to control) in dexamethasone-treated hypophysectomized rats. This effect of dexamethasone was unlikely to be due to a direct inhibitory action, since *in vitro* incubation of tissues with dexamethasone did not have an inhibitory effect on extraneuronal uptake. This result supports the work of Salt (1972), who found that dexamethasone did not inhibit extraneuronal uptake at a concentration of $10\,\mu g\,l^{-1}$.

It was particularly interesting that the *in vivo* inhibition of the extraneuronal uptake by corticosterone varied according to the treatment. While both reserpine treatment and cold-exposure (7 days) resulted in a similar increase in plasma corticosterone, the decrease in extraneuronal uptake in atria from reserpine treated rats (64%) was larger than that measured in atria from cold-exposed rats (25%) (Morton, 1987). Similarly, both hypophysectomy and adrenalectomy reduced corticosterone levels to the same extent, yet the increase in extraneuronal uptake seen after hypophysectomy (32%) was greater than that measured after adrenalectomy (20%). Although the high correlation between corticosterone levels and the change in extraneuronal uptake strongly supports the suggestion of a role for *in vivo* corticosterone in the control of extraneuronal uptake, this does not preclude the possible controlling influence of some other factor on the extraneuronal uptake. Since reserpine treatment and hypophysectomy both markedly affect the function of the pituitary, the greater effect of these two treatments on the extraneuronal uptake may be mediated by another pituitary factor independent of the glucocorticoids. The identity of such a factor remains unknown, although recent studies have shown that it is unlikely to be thyroxine (Bryan *et al.*, 1986).

The results presented in this paper indicate that corticosterone appears to play a major role in the regulation of extraneuronal uptake in the rat atria. A dependence of extraneuronal uptake on adrenocortical activity suggests a physiological role for extraneuronal uptake. It has been suggested that extraneuronal uptake may be a major site for inactivation of circulating catecholamines (Gillespie, 1976; Tren-

Table 2 The concentration of plasma corticosterone after various treatments

	Corticosterone concentration ($g\,100\,ml^{-1}$ plasma)
a Untreated	
Decapitated	10 ± 1 (10)
Cannulated	9 ± 2 (6)
b Cold-exposure	
7 days	22 ± 4* (6)
14 days	14 ± 3 (6)
c Dexamethasone-treated	
($40\,\mu g\,kg^{-1}\,day^{-1}$ for 7 days)	3 ± 2* (6)
d Reserpine-treated	
($1\,mg\,kg^{-1}\,day^{-1}$ for 7 days)	25 ± 5* (6)
e Adrenalectomized	
(7 days)	1 ± 1* (4)
f Hypophysectomized (7 days)	
Hypophysectomy only	1 ± 1* (6)
Hypophysectomy plus dexamethasone treatment (as in **C**)	1 ± 1* (4)

Values are means ± s.e.means, n = number in parentheses.
* $P < 0.01$ when compared with the control value.

delenburg, 1980). While the significance of changes in uptake cannot be determined until the physiological importance of extraneuronal uptake has been established, it is possible that under the influence of the glucocorticoid hormones, changes in extraneuronal uptake may mediate the adjustments in circulating catecholamines seen in chronic stress.

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