Br. J. Pharmac. (1976), **57**, 559–563

THE POSSIBLE INVOLVEMENT OF Na^+ IONS IN CORTICOSTERONE-INDUCED HYPERCONTRACTILITY IN THE RAT ANOCOCCYGEUS MUSCLE

A. GIBSON[1] & D. POLLOCK
Department of Pharmacology, University of Glasgow, Glasgow G12 8QQ

1 The possibility that corticosterone-induced hypercontractility in the rat anococcygeus muscle might be due to an intramuscular redistribution of Na^+ was investigated.

2 The contractility of the isolated muscle to noradrenaline (NA) was directly dependent on the Na^+ concentration of the Krebs solution.

3 There was a linear relationship between the maximum contractile response of the muscle to NA and the Na^+ concentration of the Krebs solution.

4 Muscle contractility was also increased by the presence of ouabain (10^{-4}M) in the bathing medium.

5 Muscles from rats treated with corticosterone exhibited increases in both total and ouabain-sensitive ATPase activity.

6 The relationship between corticosterone, Na^+ distribution, and muscle contractility is discussed.

Introduction

Administration of corticosterone to rats produces two distinct changes in the sensitivity of the isolated anococcygeus muscle to agonists (Gibson & Pollock, 1975a). These changes are (i) a specific increase in the pD_2 value for acetylcholine (ACh) and (ii) a non-specific increase in the maximum contractile response of the muscle to both ACh and noradrenaline (NA).

The former change appears to result from the steroid-induced reduction in muscle cholinesterase activity (Gibson & Pollock, 1975b), thus explaining the specific increase in sensitivity to ACh. However, the second effect, on muscle contractility is as yet unexplained, and in this paper a possible explanation is presented.

Adrenocorticoids are known to influence electrolyte balance in many tissues (Beck & McGarry, 1962) and glucocorticoids in particular may act to modulate ionic distribution between the intra- and extra-cellular spaces (Brodie, Davies, Hynie, Krishna & Weiss, 1966). In this way the hormones are thought to regulate the responsiveness of the tissue to agonists (Swingle, Da Vanzo, Glenister, Wagle, Osborne & Rowen, 1960). Indeed, the potentiating effects of corticosteroids on vascular reactivity to catecholamines appear to involve an intramuscular redistribution of Na^+ ions (Raab, Humphreys, Makous, De Grandpre & Gigee, 1952; Ross, 1961; Selye, Hall & Rowley, 1943). It seemed possible therefore that Na^+ ions might also be involved in corticosterone-induced hypercontractility in the anococcygeus muscle. In an attempt to test this hypothesis, the following questions were posed. (1) Does variation of the Na^+ content of the Krebs solution affect muscle contractility to NA? (2) Does ouabain, a drug which modifies Na^+ movements by inhibiting the 'Na^+ pump' (Skou, 1965), affect muscle contractility? (3) Does corticosterone administration influence Na/K ATPase activity in the anococcygeus muscle?

[1] Present Address: Department of Pharmacology, Chelsea College, University of London, London, SW3 6LX

Methods

Muscle sensitivity

Animals were killed by stunning and exsanguination. The anococcygeus muscles were dissected by the method of Gillespie (1972).

Isolated muscles were placed in organ baths containing 20 ml Krebs solution (mM: NaCl, 118.1; KCl, 4.7; $MgSO_4$, 1.0; KH_2PO_4, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 25.0; glucose, 11.1) which was maintained at 37°C and gassed continuously with 95% O_2 and 5% CO_2.

A resting tension of 0.2–0.5 g was placed on the muscle and changes in tension were recorded by a Grass FTO3 force-displacement transducer attached to either a Grass Polygraph or a Devices M2 pen-recorder.

Drugs were added to the bath using a calibrated glass syringe in volumes not exceeding 0.4 ml, and were removed by drainage. Responses were measured as the peak rise in tension produced by any dose of agonist, and subsequent doses were not added until the muscle had returned to its resting tension.

The Na^+ content of the Krebs solution was altered by varying the amount of NaCl. When the Na^+ concentration was reduced appropriate amounts of LiCl were substituted to maintain the tonicity of the solution. However, no compensation was made when the Krebs solution contained excess Na^+ and consequently these solutions were hypertonic.

Ouabain was added to the bath immediately after completion of a control dose-response curve to NA. The effect of ouabain on muscle sensitivity was determined by completion of a second dose-response curve to NA 2 h later. Responses in both curves were calculated as a percentage of the original maximum response.

Assay of adenosinetriphosphatase (ATPase)

Rats were killed by stunning and exsanguination. Both anococcygeus muscles were quickly removed and homogenized in 2 ml sucrose (0.25 M; 0°C) using a ground-glass homogenizer (Jencons; 3 ml) with an electrically-driven glass pestle. ATPase activity was determined by a modification of the method of Hosie (1965).

Reaction tubes were prepared which contained in a volume of 1 ml: NaCl (150 mM); KCl (30 mM); $MgCl_2$ (4 mM); tris-buffer pH = 7.4 (100 mM); and homogenate (20 µl). Ouabain (150 µM) was added to duplicate tubes to inhibit Na^+/K^+ ATPase.

After preincubation at 37°C for 5 min the reaction was started by addition of tris-ATP (3 mM) and terminated after 15 min by 250 µl 25% TCA. To correct for non-enzymatic breakdown of ATP triplicate tubes were treated with TCA prior to addition of ATP.

Inorganic phosphate released from ATP during incubation was then assayed (Fiske & Subarrow, 1925).

The protein content of the homogenate was determined by the method of Lowry, Rosebrough, Farr & Randall (1951).

ATPase activity was calculated as µmoles Pi released/15 min per mg protein.

Animal pretreatment

Corticosterone (10 mg/kg; 5 days; i.p.) was administered as a suspension in ethyl oleate. Control rats received only ethyl oleate.

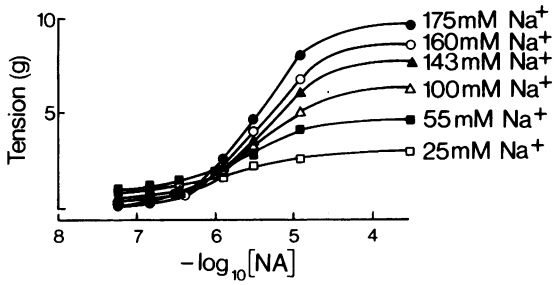

Figure 1 The effect of Na^+ concentration of the Krebs solution on the dose-response curve of the isolated anococcygeus muscle to NA. The s.e. mean values have been omitted to improve clarity, but each point is the mean of at least 6 observations.

Drugs used

Corticosterone (Sigma); ouabain (B.D.H.); noradrenaline bitartrate (Koch-Light).

Results

Effect of varying the Na^+ content of Krebs solution on muscle sensitivity to NA

Variation of the Na^+ content of the Krebs solution over a wide range had little effect on the resting tension of the anococcygeus muscle. However, on reduction of Na^+ to 25 mM the muscle displayed a slow, maintained rise in baseline tension, although this effect could be overcome by repeatedly washing the muscle with fresh bathing solution.

The effect of varying the Na^+ content of the Krebs solution on the dose-response curve to NA is shown in Figure 1. The responses of the muscle to lower doses of NA were little affected by Na^+ variation, but those to high doses (> 2 µM) were markedly dependent on the Na^+ concentration of the bathing medium. Examination of the maximum contractile responses (300 µM NA) revealed a linear relationship ($r = 0.81$; $n = 60$; $P < 0.001$) between muscle contractility and the Na^+ concentrations studied (Figure 2). Indeed, the maximum response could be increased by raising the Na^+ concentration above 'normal' (143 mM).

Effect of ouabain on muscle sensitivity to NA

A second dose-response curve to NA obtained 2 h after completion of the first revealed that muscle sensitivity was unchanged in control conditions (Figure 3a). However, 2 h after addition of ouabain (10^{-4} M) to the bath, muscle sensitivity to NA was increased. The responses to all doses of NA were increased, including the maximum response (Figure 3b).

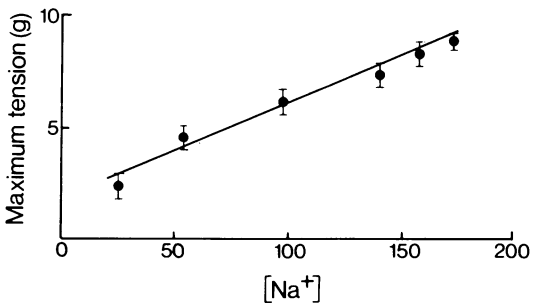

Figure 2 Relationship between maximum contractile response to NA and the Na^+ concentration of the Krebs solution. Each point is the mean of 10 observations and the vertical bars represent the s.e. mean. Regression analysis gives a correlation coefficient of 0.81 ($P < 0.001$).

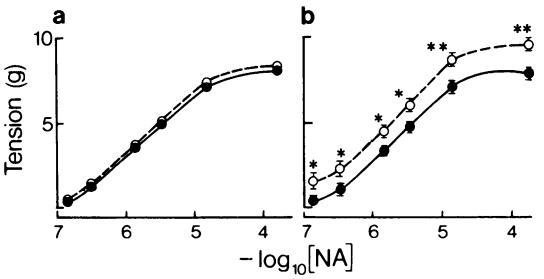

Figure 3 The effect of ouabain (10^{-4}M) on the dose-response curve to NA. In (a) the broken curve (○) was obtained 2 h after completion of the first curve (●). In (b) the broken curve (○) was obtained after soaking the muscle in Krebs solution containing ouabain (10^{-4}M) for 2 hours. Each point on the curves is the mean of 10 observations and the vertical bars represent the s.e. mean.
* $0.05 > P > 0.01$; ** $P < 0.001$.

Effect of corticosterone on muscle ATPase activity

Administration of corticosterone for 5 days produced a 117% increase in the total ATPase activity of muscle homogenates (Table 1). The ouabain-sensitive fraction of the total ATPase activity was also increased, in this case by 96% (Table 1).

Discussion

The results obtained in the present study suggest that Na^+ ions are indeed important in determining the contractile capacity of the isolated anococcygeus muscle. Similar findings have also been reported for aortic strips (Yambayashi & Hamilton, 1959) and isolated myometrium (Marshall, 1964); Bulbring & Kuriyama (1963) found that the electrical and mechanical responses of guinea-pig taenia coli to ACh were greatly enhanced in media containing excess Na^+. Such results therefore support the possibility that Na^+ redistribution may be involved in the hypercontractility induced by corticosterone administration (Gibson & Pollock, 1975).

It is probable that the observed effects on muscle contractility were due to changes in intracellular rather than extracellular Na^+ concentrations. This is suggested by the ability of ouabain to increase muscle contractility to NA. Although ouabain has been shown to inhibit neuronal uptake of NA (Iversen, 1971), it is unlikely that this effect could explain the present results, since, in a previous study cocaine, which also inhibits neuronal uptake, produced a much greater supersensitivity in which the maximum contractile response was unaltered (Gibson & Pollock, 1973). Neither can the potentiation be due to inhibition of extraneuronal uptake of NA, since this mechanism does not appear to be of importance as a route of agonist inactivation in the anococcygeus muscle (Gibson & Pollock, 1973). It is more likely that the mechanism by which ouabain increases muscle contractility is similar to that proposed for the inotropic action of the glycoside on cardiac muscle. Thus, by inhibiting the 'sodium-pump' (Skou, 1965), ouabain produces an increase in intracellular Na^+ levels, which in turn increase the availability of Ca^{++} ions for contraction (Lee & Klaus, 1971; Godfraind, 1973). Further, raising the Na^+ content of the Krebs solution, which increased muscle contractility, would also result in raised intracellular Na^+ levels (Goodford, 1962; Casteels, Droogman & Hendrickx, 1973).

It is of interest that while ouabain potentiated the responses to all doses of NA, raising the Na^+ concentration affected only the responses to higher doses of the agonist. This probably reflects the rather severe effects of varying the ionic concentration over such a

Table 1 The effect of corticosterone administration on total and ouabain-sensitive ATPase activity in the anococcygeus muscle

	Total ATPase (μmoles P_i/15 min per mg protein)	*n*	*Ouabain-sensitive ATPase (μmoles P_i/15 min per mg protein)*
Control	2.49 ± 0.19	40	0.46 ± 0.08
Corticosterone	5.42 ± 0.38†	28	0.91 ± 0.14*

Values are given as mean ± s.e. mean, *n* = number of observations; * $0.01 > P > 0.001$; $P < 0.001$.

wide range, compared with the more subtle effects of the glycoside on sodium movements.

Nevertheless, increased maximum responses are a rare feature of supersensitive smooth muscle (Fleming, McPhillips & Westfall, 1973) and the ability of both the above treatments to raise the maximum response to NA suggests that the similar effect produced by corticosterone administration may indeed be due to a redistribution of Na^+ ions within the anococcygeus muscle. In vascular smooth muscle, it has been postulated that a relationship exists between raised intracellular Na^+ levels and corticosteroid potentiation of responses to agonists (Raab *et al.*, 1952; Ross, 1961). Certainly the increased Na^+/K^+ ATPase activity observed in muscles from corticosterone treated rats, suggests that the hormone does affect Na^+ distribution within the muscle. However, at this point, an apparent contradiction arises, since although both ouabain and corticosterone raise muscle contractility, the former inhibits while the latter stimulates Na^+/K^+ ATPase activity. One possible explanation is that the hormone does not act directly on Na^+/K^+ ATPase, but rather facilitates entry of Na^+ ions into the cell, which in turn would stimulate the Na^+ pump. In support of this possibility, Sharp & Leaf (1966) have suggested that in the kidney, where corticosterone also stimulates ATPase activity (Chignell & Titus, 1966; Hendler, Torretti, Kupor & Epstein, 1972), the Na^+-retaining property of the corticosteroids is due to synthesis of a specific permease enzyme which increases membrane permeability to Na^+.

In conclusion, the preceding experiments point to a possible involvement of Na^+ ions in the production of corticosterone-induced hypercontractility in the anococcygeus muscle. However, the exact nature of this involvement and the mechanisms involved must await a detailed analysis of the effects of steroids on the movement of Na^+ and other ions between the intra- and extra-cellular spaces in passive and active muscle preparations.

The financial support of the Rankin Fund of Glasgow University is gratefully acknowledged. At the time of this work A.G. was an MRC scholar.

References

BECK, J.C. & McGARRY, E.E., (1962). Physiological importance of cortisol. *Br. med. Bull.*, **18**, 134–140.

BRODIE, B.B., DAVIES, J.I., HYMIE, S., KRISHNA, G. & WEISS, B. (1966). Interrelationships of catecholamines with other endocrine systems. *Pharmac. Rev.*, **18**, 273–289.

BULBRING, E. & KURIYAMA, H. (1963). Effects of changes in ionic environment on the action of acetylcholine and adrenaline on the smooth muscle cells of guinea-pig taenia coli. *J. Physiol., Lond.*, **166**, 59–74.

CASTEELS, R., DROOGMAN, G. & HENDRICKX, H. (1973). Active ion transport and resting potential in smooth muscle cells. *Phil. Trans. R. Soc. Lond. B.*, **265**, 74–56.

CHIGNELL, C.F. & TITUS, E.D. (1966). Effect of adrenal steroids on a Na^+ and K^+ requiring adenosine triphosphatase from rat kidney. *J. biol. Chem.*, **241**, 5083–5089.

FISKE, C.H. & SUBARROW, Y. (1925). The colorimetric determination of phosphate. *J. biol. Chem.*, **66**, 375–400.

FLEMING, W.W., McPHILLIPS, J.J. & WESTFALL, D.P. (1973). Post-junctional supersensitivity and subsensitivity of excitable tissues to drugs. *Ergeb. der Physiol.*, **68**, 55–119.

GIBSON, A. & POLLOCK, D. (1973). The effects of drugs on the sensitivity of the rat anococcygeus muscle to agonists. *Br. J. Pharmac.*, **49**, 506–513.

GIBSON, A. & POLLOCK, D. (1957a). The involvement of corticosteroids in the supersensitivity produced in the rat anococcygeus muscle by morphine withdrawal, thyroidectomy, or a single dose of reserpine. *J. Pharmac. exp. Ther.*, **192**, 390–398.

GIBSON, A. & POLLOCK, D. (1975b). Reduction in the cholinesterase activity of the rat anococcygeus muscle produced by corticosterone. *Br. J. Pharmac.*, **55**, 69–72.

GILLESPIE, J.S. (1972). The rat anococcygeus muscle and its response to nerve stimulation and to some drugs. *Br. J. Pharmac.*, **45**, 404–416.

GODFRAIND, T. (1973). Na-Ca interaction and the mode of drug action. In *Pharmacology and the future of man.* Proc. 5th Int. Congr. Pharmacology, San Francisco, 1972, vol. 4, pp. 344–358, Basel: Karger.

GOODFORD, P.J. (1962). The sodium content of the smooth muscle of the guinea-pig taenia coli. *J. Physiol., Lond.*, **163**, 411–422.

HENDLER, E.D., TORRETTI, J., KUPOR, L. & EPSTEIN, F.H. (1972). Effects of adrenalectomy and hormone replacement on Na-K-ATPase in renal tissue. *Amer. J. Physiol.*, **222**, 754–760.

HOSIE, R.J.A. (1965). The localisation of adenosine triphosphatase in morphologically characterised subcellular fractions of guinea-pig ileum. *Biochem. J.*, **96**, 404–412.

IVERSEN, L.L. (1971). Role of transmitter uptake mechanisms in synaptic neurotransmission. *Br. J. Pharmac.*, **41**, 571–591.

LEE, K.S. & KLAUS, W. (1971). The subcellular basis for the mechanism of inotropic action of cardiac glycosides. *Pharmac. Rev.*, **23**, 193–261.

LOWRY, D.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951). Protein measurement with the Folin phenol reagent. *J. biol. Chem.*, **193**, 265–275.

MARSHALL, J.M. (1964). The action of oxytocin on uterine smooth muscle. In *Pharmacology of smooth muscle*, ed. Bulbring, E. pp. 143–153. Pergamon.

RAAB, W., HUMPHREYS, R.J., MAKOUS, N., DE GRANDPRE, R. & GIGEE, W. (1952). Pressor effects of epinephrine, norepinephrine, and desoxycorticosterone acetate (DCA) weakened by sodium deprivation. *Circulation*, **6**, 373–377.

ROSS, E.J. (1961). The adrenal medulla, the adrenal cortex, sodium and blood pressure. *Proc. R. Soc. Med.*, **54**, 1005–1006.

SELYE, H., HALL, C.E. & ROWLEY, E.M. (1943). Malignant hypertension produced by treatment with desoxycorticosterone acetate and sodium chloride. *Can. Med. Ass. J.*, **49**, 88–92.

SHARP, G.W.G. & LEAF, A. (1966). Mechanism of action of aldosterone. *Physiol. Rev.*, **46**, 593–633.

SKOU, J.C. (1965). Enzymatic basis for active transport of Na^+ and K^+ across cell membrane. *Physiol. Rev.*, **45**, 596–617.

SWINGLE, W.W., Da VANZO, J.P., GLENISTER, D., WAGLE, G., OSBORNE, M. & ROWEN, R. (1960). Effects of mineralo- and gluco-corticoids on fasted adrenalectomised dogs subjected to electroshock. *Proc. Soc. exp. Biol. Med.*, **104**, 184–188.

YAMBAYASHI, H. & HAMILTON, W.F. (1959). Effect of sodium ion on contractility of the dogs aortic strip in response to catecholamines. *Amer. J. Physiol.*, **197**, 993–996.

(Received January 28, 1976)