There might be other magnesium dependent enzymes which are more sensitive to lithium levels than pyruvate kinase and we are looking at this possibility.

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Tissue variability and some properties of the accumulation of [^{3}H]-corticosterone by isolated organs

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Administration of corticosteroid hormones enhances the responsiveness of a number of tissues to agonist drugs and to nerve stimulation (Brodie, Davies, Hynie, Krishna & Weiss, 1966; Gibson, Pollock & Spence, 1976). This supersensitivity may be due to a redistribution of Na^+ ions within the tissue, probably as a result of hormone-induced enzyme synthesis (Gibson & Pollock, 1976; Scott & Sapirstein, 1975). The first step in the action of corticosteroids on a target organ is entry of the hormone into the cell (Gorski & Gannon, 1976). However, the entry process is poorly defined, and differences in steroid uptake by various tissues have been reported (Jensen & Jacobson, 1960). We therefore began an investigation of the action of corticosteroids on cellular reponsiveness by observing the accumulation of [^{3}H]-corticosterone in various isolated tissues.

Animals were killed by stunning and exsanguination. The tissues were rapidly dissected and incubated at 37°C in Krebs bicarbonate solution containing [^{3}H]-corticosterone (70 nM; 112 Ci/mmole; Amersham). The medium was gassed with 95% O_2 5% CO_2. At various time intervals tissues were removed, blotted, weighed and digested in 1 ml potassium hydroxide (0.5 M; 60°C). The radioactivity in a 0.1 ml aliquot of digestant was then measured using a toluene-Triton scintillation fluid.

Initially accumulation of [^{3}H]-corticosterone was observed in the rat anococcygeus muscle. In this tissue, equilibrium was reached within 30 min, and the tissue/medium ratio after 2 h was 2.2 ± 0.07 ($n=6$). However, there was a marked tissue variability in accumulation, the tissue/medium ratios for other tissues following a 2 h incubation being: rat heart (2.5 ± 0.2); mouse heart (3.7 ± 0.05); rabbit vas deferens (3.4 ± 0.3); mouse vas deferens (7.4 ± 0.3); rat pituitary (5.6 ± 0.3); rat hypothalamus (2.6 ± 0.09). The highest tissue/medium ratio was achieved in the mouse vas deferens and this tissue was used to study some further characteristics of the accumulation process.

Dichloromethane extraction of incubated tissues suggested that 96% of extractable radioactivity was unchanged corticosterone. The accumulated steroid was well retained by the vas, the time of washout being 3 times that of accumulation. The accumulation was temperature sensitive being reduced by 32% at 20°C and by 51% at 4°C. Reduction of the Na^+ content of the medium to 25 mM enhanced accumulation. Rather surprisingly, accumulation was also enhanced by ouabain (10^{-4} M). Preliminary experiments suggest that part of the accumulation is mediated by a specific process since it could be reduced by excess corticosterone (10^{-4} M) but not by hydrocortisone (10^{-4} M).

In conclusion, the accumulation of [^{3}H]-corticosterone exhibited a marked tissue and species variation. Indeed, in the case of cardiac and smooth muscle this variation exhibited a marked similarity to that described for extraneuronal accumulation of catecholamines (Gillespie & Muir, 1970). The mechanisms responsible for the accumulation and its relation to cellular responsiveness is under investigation.

CVS is an M.R.C. Student

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Two separate cholinergic mechanisms for regulation of oxytocin release

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The oxytocinergic neurones of the lactating rat generate every 5–15 min during suckling a synchronized and brief (2–4 s) burst of action potentials (Lincoln & Wakerley, 1975). Each period of accelerated firing (> 40 spikes/s) discharges a pulse of oxytocin (0.5–1.0 mU) from the neurohypophysis; this circulates to the mammary gland and initiates the ejection of milk. The hypothalamic neurones of the supraoptic and paraventricular nuclei, which synthesize and release oxytocin, are excited by iontophoretically applied acetylcholine (Dreifuss & Kelly, 1972) and cholinomimetics stimulate the release of neurohypophysial hormones (Kuhn & McCann, 1971). It is possible, therefore, that acetylcholine may be an essential synaptic transmitter in the pathway for reflex milk ejection. This was examined by the application of cholinolytics to rats that were reflexly milk ejecting, under urethane anaesthesia.

Lactating rats (250–350 g), separated from their young for 20 h at 8–10 days post-partum, were anaesthetized with urethane (1.2 g/kg, i.p.). Intramammary and arterial pressures were recorded, as described elsewhere (Tribollet, Clarke, Dreifuss & Lincoln, 1977). Three hours later, whilst the animal was still anaesthetized, a hungry litter of young were applied to the nipples. The first milk ejection occurred after 20–40 min, and thereafter milk ejections recurred approximately every 6 minutes. Cholinolytics (atropine, hyoscine, mecamylamine and hexamethonium) were injected into the saphenous vein, in a volume of about 0.3 ml. Cholinomimetics (carbachol and bethanecol) were injected into the lateral cerebral ventricle in a volume of 1 µl; the solutions were made isotonic with NaCl.

Atropine and hyoscine, muscarinic antagonists, failed to abolish the milk-ejection reflex of the rat when given in doses up to 100 mg/kg (0/22). Mecamylamine (0.5–2.0 mg/kg) and hexamethonium (5–10 mg/kg), nicotinic antagonists, caused a substantial delay in the recurrence of milk ejection (27/45). This inhibition was dose-dependent and recovery was obtained.

Both the cholinomimetics, carbachol (0.01–0.2 µg) and bethanecol (0.2–4.0 µg), when injected into the lateral ventricle caused a large prolonged release of both oxytocin and vasopressin, as observed through changes in intramammary and blood pressures (36/46). This release was abolished by atropine (0.1–1.0 mg/kg) applied systemically (11/11). Mecamylamine had no effect at 5 mg/kg (0/8).

A direct action of these drugs on the neurohypophysis or mammary glands was unlikely for the responses to intravenous injections of oxytocin and to endogenous oxytocin released by electrical stimulation of the neurohypophysis remained unaltered. Thus, the milk-ejection reflex of the rat appears to contain an excitatory cholinergic relay of the nicotinic type, placed somewhere other than at the level of the oxytocinergic neurone. By contrast, the response to intraventricular cholinomimetics is muscarinic in type.

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