

CHANGES IN INTRACELLULAR DISTRIBUTION OF POTASSIUM, MAGNESIUM AND CALCIUM IN SKELETAL MUSCLE AS A RESULT OF CHRONIC MORPHINIZATION

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Abstract—The potassium, magnesium, and calcium contents of subcellular fractions of skeletal muscle from chronically morphinized rats and from control rats have been determined. In muscle from chronically morphinized rats abnormally high concentrations of potassium ($3\times$ normal) and of magnesium ($2\times$ normal) are found in the microsomal fraction. Calcium concentration in this fraction is slightly raised. No such changes are observed in the composition of the nuclear and mitochondrial fractions.

It has been suggested¹⁻⁴ that morphine-induced changes in skeletal muscle are confined to the membranes that are concerned with glucose-uptake, that a hormone-sensitive, magnesium-potassium-dependent regulatory system is involved, and that attenuation of this regulatory system may be associated with abnormal local concentrations of potassium, magnesium, and possibly other ions. We now report determinations of the potassium, magnesium, and calcium contents of subcellular fractions of skeletal muscle of normal and of chronically morphinized rats.

METHODS

Virgin female rats of 140-180 g at the time of decapitation for analysis were used. The chronically morphinized animals had received daily intraperitoneal injections of morphine (30 mg/kg body wt.) in saline for 6 weeks. Control animals received only saline injections. All were fed a standard diet without restriction, but none received either food or injection within an 18-24 hr period immediately preceding their decapitation.

Leg muscles, *extensor crucis* and *biceps femoris*, were homogenized in 250 mM sucrose (9 ml/g muscle) at 0° for 10 min using a Sorval microhomogenizer. Homogenates were layered with an equal volume of 340 mM sucrose and then fractionated by differential centrifugation following the procedure of Hogeboom,⁵ whereby nuclear, mitochondrial, and microsomal fractions were obtained.

De-ionized water was used for all solutions and suspensions.

Sonicated suspensions of subcellular particles were analysed for metals using a flame photometer for potassium and an atomic absorption spectrometer for magnesium and calcium. The nitrogen contents of each sample were determined in the evaporated suspension using a Coleman Nitrogen Analyzer.

RESULTS AND DISCUSSION

Distribution of total nitrogen in subcellular fractions of skeletal muscle of normal and of chronically morphinized rats

The results of analyses of the three subcellular fractions of muscle for total nitrogen (Table 1) show no significant difference between normal and chronically morphinized rats in their nitrogen-contents. The results of analyses for metals, each quantitatively expressed in reference to the nitrogen content of the sample, are therefore apposite for comparisons between preparations from normal and chronically morphinized animals.

TABLE 1. DISTRIBUTION OF POTASSIUM, MAGNESIUM, CALCIUM AND OF TOTAL NITROGEN IN SUBCELLULAR FRACTIONS OF SKELETAL MUSCLE OF NORMAL AND OF CHRONICALLY MORPHINIZED RATS

Subcellular fraction	Metal	No. of rats	Metal, mg/g total N (Mean \pm S.E.M.)		
			Control rats	Chronically morphinized rats	Difference
Nuclear	K	(7)	53.8 \pm 2.0	54.2 \pm 2.3	N.S.
Mitochondrial	K	(7)	7.92 \pm 0.40	7.77 \pm 0.71	N.S.
Microsomal	K	(7)	23.9 \pm 1.3	70.7 \pm 12	46.8 \pm 12.6 ($P < 0.01$)
Nuclear	Mg	(9)	1.18 \pm 0.14	1.07 \pm 0.10	N.S.
Mitochondrial	Mg	(9)	1.46 \pm 0.13	1.66 \pm 0.17	N.S.
Microsomal	Mg	(9)	0.67 \pm 0.04	1.26 \pm 0.13	0.59 \pm 0.14 ($P < 0.001$)
Nuclear	Ca	(14)	3.04 \pm 0.23	3.47 \pm 0.28	N.S.
Mitochondrial	Ca	(14)	1.31 \pm 0.07	1.24 \pm 0.78	N.S.
Microsomal	Ca	(14)	4.03 \pm 0.16	5.62 \pm 0.48	1.59 \pm 0.50 ($P < 0.01$)
			Nitrogen, mg/g fresh muscle (Mean \pm S.E.M.)		
Nuclear	N	(30)	20.23 \pm 0.67	18.80 \pm 0.70	N.S.
Mitochondrial	N	(30)	4.20 \pm 0.26	3.77 \pm 0.30	N.S.
Microsomal	N	(30)	1.86 \pm 0.16	1.72 \pm 0.15	N.S.

Distribution of potassium, magnesium, and calcium in subcellular fractions of skeletal muscle of normal and of chronically morphinized rats

The results of analyses of the nuclear and mitochondrial fractions of muscle for metals (Table 1) show no significant difference between normal and chronically morphinized rats in the potassium, magnesium, and calcium concentrations of the nuclei and mitochondria. Very marked differences are found, however, in the composition of the microsomes. In the microsomal fraction from muscle of chronically morphinized rats, the potassium, magnesium, and calcium concentrations are respectively 200, 100 and 40 per cent above the normal.

Such increase in the potassium, magnesium, and calcium concentration in membranes concerned with controlled glucose-uptake could well account for the complete loss of sensitivity of the glucose-uptake system in muscle to changes in

extracellular potassium or magnesium and for the reduction in sensitivity to moderate changes in extracellular calcium, as previously observed in experiments with excised diaphragm of chronically morphinized rats.^{1,2,4}

No comparably drastic abnormality as a result of chronic morphinization has to our knowledge ever been recorded, for it involves a system fundamentally concerned in the control of salt and water balance within the body, in the uptake of metabolites by the musculature, and in the regulation of these processes by the action of hormones.

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