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EFFECTS OF MORPHINE ON THE HORMONAL CONTROL OF METABOLISM—VII

MORPHINE-INDUCED CHANGES IN SENSITIVITY OF THE GLUCOSE-UPTAKE SYSTEM OF MUSCLE TO EXTRACELLULAR CALCIUM

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Abstract—The effects of morphine and of hydrocortisone on uptake of glucose from media of different calcium content by diaphragm of normal and of chronically morphinized rats have been studied *in vitro*.

The rate of glucose-uptake by diaphragm from chronically morphinized rats, unlike that by diaphragm of normal rats, is not increased by omission of calcium from a standard medium containing 2·73 mM calcium, but it is depressed by a 2–5 fold increase in the calcium content of the medium.

The stimulant effect of added morphine on glucose-uptake by normal rat-diaphragm is dependent upon the presence of calcium in the medium and is much increased by an increase of calcium in the medium. The depressant effect of morphine on glucose-uptake by chronically morphinized rat-diaphragm is but little affected by changes in calcium concentration of the medium from 0 to 5·46 mM.

The depressant effect of added hydrocortisone on glucose-uptake by normal rat-diaphragm is unchanged by omission of calcium from the medium, but the stimulant effect on chronically morphinized rat-diaphragm is apparently calcium-dependent.

VIRGIN female rats of about 150 g were used. Chronically morphinized rats had received daily injections of morphine sulphate in saline (30 mg/kg body wt.) for 6 weeks: control rats received saline only: none received either food or injection during the 24 hr before experiment. Experimental procedures were as previously described.[1,2]

The standard incubation medium used in these experiments was an oxygenated tris-buffered saline of pH 7·4 containing 128 mM sodium, 5·12 mM potassium, 2·73 mM calcium, 1·28 mM magnesium, 0·15% w/v glucose, and 8 mM tris-(hydroxymethyl) methylamine hydrochloride, in modified media calcium was varied from 0 to 13·7 mM and isotonicity maintained by variation of sodium.

EXPERIMENTAL AND RESULTS

Comparison of rates of glucose-uptake by isolated rat-diaphragm in tris-buffered and phosphate buffered media

In each experiment half an excised diaphragm was incubated at pH 7·4 and 37° for 1 hr in the standard tris-buffered saline, the other half in a standard phosphate-buffered saline as used in previous experiments when potassium and magnesium were varied.[2] No significant difference between the rates of uptake in the two media was observed. In six experiments the mean rate of uptake of glucose, mg/100 g wet tissue/hr

± S.E. mean was 178 ± 13 in the phosphate-buffered medium and 173 ± 14 in the tris-buffered medium. The phosphate-buffered medium cannot be used in experiments when the calcium concentration is increased, since calcium phosphate precipitates.

Effects of variation in calcium concentration on glucose-uptake by isolated diaphragm of normal and of chronically morphinized rats

The results of paired experiments (Table 1) show that while the rate of glucose-uptake by diaphragm from normal rat is much increased by omission of calcium from the incubating medium that by diaphragm of chronically morphinized rat is not significantly affected. When the calcium concentration of the incubating medium is raised to higher levels, on the other hand, depression of the rate of glucose-uptake is similar in diaphragm from normal and from chronically morphinized rat.

TABLE 1. EFFECTS OF EXTRACELLULAR CALCIUM CONCENTRATION ON UPTAKE OF GLUCOSE BY DIAPHRAGM OF NORMAL AND OF CHRONICALLY MORPHINIZED RATS

State and no. of rats	Ratio of Ca, B/A	A, Control (Ca, 2·73 mM)	B, Experiment (Ca, varied)	Difference (B − A)
N (5)	0	192 ± 26	340 ± 45	+148 ± 20 ($P = 0.002$)
Cm (5)	0	243 ± 8	269 ± 5	+26 ± 13
N (5)	2	217 ± 18	152 ± 13	−65 ± 13 ($P < 0.01$)
Cm (6)	2	241 ± 9	214 ± 17	−27 ± 10 ($P < 0.05$)
N (5)	3	202 ± 18	92 ± 16	−110 ± 4 ($P \ll 0.001$)
N (7)	3·5	199 ± 14	61 ± 6	−138 ± 14 ($P \ll 0.001$)
Cm (7)	3·5	240 ± 11	116 ± 11	−124 ± 10 ($P \ll 0.001$)
N (6)	4	206 ± 11	87 ± 7	−119 ± 9 ($P \ll 0.001$)
N (5)	5	195 ± 17	96 ± 8	−99 ± 14 ($P < 0.01$)
Cm (7)	5	217 ± 12	120 ± 10	−97 ± 16 ($P < 0.001$)

Hemi-diaphragms were incubated at pH 7·4 and 37° for 1 hr in oxygenated tris-buffered medium (2·0 ml) containing glucose (0·15%). In each experiment half the excised diaphragm served as a control (A) for the other half (B) in which the calcium content of the medium varied form 0 to 13·7 mM. Mean rates ± S.E. mean are expressed as decrease in glucose content of the medium, mg/100 g wet tissue/hr.

N—normal; Cm—chronically morphinized.

Effects of morphine on glucose-uptake by isolated diaphragm of normal and of chronically morphinized rats in media of different calcium content

The results of paired experiments (Table 2) show that the stimulant effect of added morphine on the rate of glucose-uptake by normal rat-diaphragm is calcium-dependent and is very markedly increased when glucose-uptake is depressed by raising the calcium concentration of the medium to 5·46 mM. The depressant effect of added morphine on the rate of uptake by chronically morphinized diaphragm, on the other hand, is not significantly changed by variation in the calcium concentration of the medium.

Addition of morphine to media of higher calcium concentrations, 8 mM and above, resulted in tissue damage and consistent results could not be obtained.

TABLE 2. EFFECTS OF MORPHINE ON UPTAKE OF GLUCOSE BY ISOLATED DIAPHRAGM OF NORMAL AND OF CHRONICALLY MORPHINIZED RATS IN MEDIA OF DIFFERENT CALCIUM CONTENT

State and no. of rats	Ca content of medium	Control	Experiment	Difference
			+ Morphine	
N (6)	0	330 ± 9	268 ± 22	−62 ± 29
Cm (6)	0	285 ± 15	260 ± 32	−25 ± 19
N (6)	2·73 mM	171 ± 16	208 ± 23	+37 ± 11 ($P < 0.02$)
Cm (7)	2·73 mM	204 ± 16	146 ± 16	−58 ± 8 ($P < 0.001$)
N (6)	5·46 mM	79 ± 13	287 ± 5	+208 ± 12 ($P \ll 0.001$)
Cm (6)	5·46 mM	127 ± 10	87 ± 13	−40 ± 9 ($P < 0.01$)

Hemi-diaphragms were incubated at pH 7·4 and 37° for 1 hr in oxygenated tris-buffered medium (2·0 ml) containing glucose (0·15%) ± added morphine (final concentration, $3{\cdot}85 \times 10^{-4}$M) and in which the calcium concentration was varied. In each experiment, half the excised diaphragm served as a control for the other half. Mean rates ± S.E. mean are expressed as decrease in glucose content of the medium, mg/100 g wet tissue/hr.

N—normal; Cm—chronically morphinized.

TABLE 3. EFFECTS OF HYDROCORTISONE ON UPTAKE OF GLUCOSE BY ISOLATED DIAPHRAGM OF NORMAL AND OF CHRONICALLY MORPHINIZED RATS IN MEDIA OF DIFFERENT CALCIUM CONTENT

State and no. of rats	Ca content of medium	Control	Experiment	Difference
			+ Hydrocortisone ($3{\cdot}85 \times 10^{-6}$M)	
N (7)	0	261 ± 14	191 ± 13	−70 ± 17 ($P < 0.01$)
Cm (7)	0	235 ± 11	199 ± 17	−36 ± 11 ($P < 0.02$)
N (6)	2·73 mM	269 ± 9	182 ± 19	−87 ± 19 ($P < 0.01$)
Cm (8)	2·73 mM	181 ± 6	213 ± 8	+32 ± 7 ($P < 0.01$)
N (5)	5·46 mM	119 ± 14	98 ± 17	−21 ± 5 ($P < 0.02$)
Cm (6)	5·46 mM	129 ± 11	155 ± 11	+26 ± 12
			($3{\cdot}85 \times 10^{-4}$M)	
N (7)	0	238 ± 13	196 ± 10	−42 ± 8 ($P = 0.002$)
Cm (6)	0	213 ± 7	236 ± 6	+23 ± 8 ($P < 0.05$)
N (5)	2·73 mM	195 ± 5	160 ± 7	−35 ± 7 ($P < 0.01$)
Cm (8)	2·74 mM	191 ± 8	223 ± 6	+32 ± 6 ($P < 0.002$)
N (7)	5·46 mM	122 ± 2	143 ± 1	+21 ± 4 ($P = 0.002$)
Cm (10)	5·46 mM	133 ± 8	−139 ± 15	(−273 ± 21) ($P \ll 0.001$)

Hemi-diaphragms were incubated at pH 7·4 and 37° for 1 hr in oxygenated tris-buffered medium (2·0 ml) containing glucose (0·15%) ± added hydrocortisone (final concentration, $3{\cdot}85 \times 10^{-4}$ or $\times 10^{-6}$M) and in which the calcium content was varied. In each experiment half the excised diaphragm served as a control for the other half. Mean rates ± S.E. mean are expressed as decrease in glucose content of the medium, mg/100 g wet tissue/hr.

N—normal; Cm—chronically morphinized.

Effects of hydrocortisone on glucose-uptake by isolated diaphragm of normal and of chronically morphinized rats in media of different calcium content

The depressant effect of hydrocortisone on the rate of glucose-uptake by normal rat-diaphragm is independent of the presence of calcium in the incubating medium, though the magnitude of this effect is reduced when glucose-uptake is already depressed by high extracellular calcium (Table 3). In contrast, the stimulant effect of hydrocortisone in a concentration of the order 10^{-6}M on chronically morphinized rat-diaphragm is observed only when calcium is present in the incubating medium. Hydrocortisone in a concentration of the order 10^{-4}M stimulates glucose-uptake by chronically morphinized rat-diaphragm in the calcium free medium; in the medium containing 5·46 mM calcium it apparently disrupts the membrane allowing leakage of reducing substances from the cell.

Addition of hydrocortisone to media of higher calcium concentration (8 mM) results in tissue damage of both normal and chronically morphinized rat-diaphragm.

DISCUSSION

It is well known that calcium within a limited range of concentration is essential for preservation of the integrity and selective permeability of biological membranes, and for the mutual adhesion of cells in tissues. It has been reported that lack of calcium increases permeability of muscle to water,[3,4] to sugars,[5] and to potassium ions.[6,7] Electron-microscopic evidence indicates that larger channels are formed between cells in the absence of calcium.[8]

The present work shows that the rate of glucose-uptake by diaphragm from chronically morphinized rats, unlike that by normal diaphragm, is not significantly affected by omission of calcium from a standard incubating medium. Sensitivity to extracellular calcium is not completely lost, however, and a depression of the rate of glucose-uptake, similar to that observed with normal diaphragm, occurs when calcium concentration is raised to higher levels.

The stimulant effect of morphine on glucose-uptake by normal diaphragm is apparently calcium-dependent: in the absence of calcium, morphine tends to depress rather than stimulate glucose-uptake, and in this respect its effect on normal diaphragm is no different from that on chronically morphinized diaphragm. Considered in relation to this, the results of experiments in which media of higher calcium-content were used are especially revealing: they imply that the stimulant effect of morphine on glucose-uptake is a result of direct antagonism between morphine and calcium. This effect of morphine cannot be explained by assuming that the drug reacts with calcium in solution with effect that calcium ion activity of the medium is reduced: the concentration of morphine is too low in relation to that of calcium, and with chronically morphinized diaphragm, depression of glucose-uptake by raised extracellular calcium is not antagonized by morphine as it would be if the effects were a result simply of calcium–morphine interaction in solution.

It must be assumed that in the normal diaphragm morphine either releases membrane-bound calcium or in some way blocks access of calcium to free calcium-binding sites in the membrane, calcium-saturation of which decreases permeability to glucose. It is presumably by competition for such sites that magnesium and potassium tend to loosen the membrane structure and permit a more rapid glucose-uptake when the concentration of either magnesium or potassium in the medium is increased. As

previously demonstrated,[9] the stimulant effect of morphine on glucose-uptake by normal diaphragm is also magnesium dependent but, in this case, morphine and magnesium appear to act synergistically, which suggests that morphine facilitates displacement of calcium in the membrane by magnesium.

If this be so, it is conceivable that muscle of chronically morphinized animals lacks sensitivity to extracellular magnesium and potassium, but retains a sensitivity to extracellular calcium because the membrane is chronically deficient in calcium and possibly abnormally rich in magnesium and potassium. This would presumably account for the increased fragility of the chronically morphinized tissue that we have observed in the present experiments with morphine and hydrocortisone in media of high calcium concentration.

The intracellular distribution of metal-ions in muscle of normal and chronically morphinized rats is currently being investigated.

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