

EFFECTS OF MORPHINE ON THE HORMONAL CONTROL OF METABOLISM—IV.

MORPHINE-INDUCED CHANGES IN SENSITIVITY OF THE GLUCOSE-UP TAKE SYSTEM OF MUSCLE TO EXTRACELLULAR MAGNESIUM

M. W. POON, E. O'F. WALSH and M. L. NG

Department of Biochemistry, University of Hong Kong, Hong Kong

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

The effects of morphine, of adrenaline, and of hydrocortisone on normal diaphragm are each dependent upon the presence in the medium of magnesium within a limited range of concentration.

The basic rate of glucose-uptake by chronically morphinized diaphragm, unlike that of normal diaphragm, is not influenced by changes in the magnesium content of the medium and the effect of morphine is not changed by variations in magnesium concentration from 0 to 2.5 mM.

Chronically morphinized diaphragm is insensitive to hydrocortisone (3.85×10^{-6} M) and sensitivity is not restored by changes in magnesium concentration.

Chronically morphinized diaphragm is insensitive to adrenaline (3.85×10^{-6} M) in the standard medium (1.25 mM Mg), but sensitivity is restored by raising the magnesium content of the medium to a level (2.5 mM) which abolishes the effect of the hormone on normal diaphragm.

AS PREVIOUSLY reported,¹⁻³ repeated dosage with morphine induces changes in the hormone-sensitive glucose-uptake system of rat-muscle. Such changes apparently involve modification of membrane properties with effect that *in vitro* responses of the tissue both to the drug and to adrenal hormones by muscle of chronically morphinized animals are different from those of muscle of control animals. It has been suggested^{2, 4} that this property of morphine may in part depend on an ability specifically to interfere with the formation or conformation and function of magnesium complexes that are thought to be involved in the control of membrane-transport systems.

This view is encouraged by the recent observation⁴ that, whereas the rate of glucose-uptake by isolated rat-diaphragm respiring in a phosphate-buffered medium is sensitive to change in magnesium concentration, the rate of uptake by diaphragm from a chronically morphinized rat is not influenced by changes in the magnesium content of the incubating medium.

In the present work, the acute *in vitro* effects of morphine and of adrenal hormones on the rates of glucose-uptake by diaphragm of normal and of chronically morphinized rats are compared in media of different magnesium content.

MATERIALS AND METHODS

The animals used and the experimental procedures were as previously described.¹

The standard incubation medium used in all experiments was an oxygenated phosphate-buffered saline of pH 7.4 containing 130 mM sodium, 5 mM potassium, 2.8 mM calcium, 1.25 mM magnesium, and 10 mM phosphate with added glucose (0.15% w/v). The modified incubation media were of similar composition with the exception of magnesium, the concentration of which was varied from 0 to 5 mM.

EXPERIMENTAL AND RESULTS

Effects of extracellular magnesium on glucose-uptake by isolated rat-diaphragm at different glucose concentrations

In paired technique experiments, the rates of glucose-uptake by rat-diaphragm from oxygenated phosphate-buffered medium with a high magnesium (2.5 mM) content are compared with the rates from the standard medium (1.25 mM Mg) at different glucose concentrations. The results are summarized in Fig. 1.

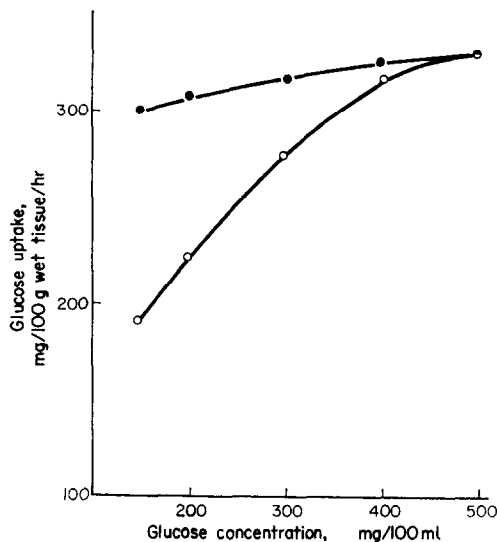


FIG. 1. Effect of doubling the magnesium content of the medium on rate of glucose-uptake by rat diaphragm incubated at pH 7.4 and 37° for 1 hr in oxygenated Krebs-Ringer-phosphate (2.0 ml) at different glucose concentrations. Each point represents the mean of five experiments in which half the excised diaphragm was incubated in the standard medium (○—○) containing 1.25 mM Mg and the other half in the modified medium (●—●) containing 2.5 mM Mg.

The experiments show that at lower concentrations of glucose, doubling the magnesium content of the medium raises the rate of glucose-uptake to the same limiting value as attained by increasing the extracellular glucose concentration. This effect of magnesium is not observed in experiments with diaphragm from chronically morphinized rats and, as reported in a preliminary communication,⁴ the rate of glucose-uptake is not affected even by a 4-fold increase in magnesium content of the medium.

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

The effects of added morphine on the rates of glucose-uptake by diaphragm of normal and of chronically morphinized rats in media of different magnesium content are compared in Table 1. A significant effect of added morphine, even in high concentration (7.7×10^{-4} M), on the rate of glucose-uptake by normal diaphragm is observed only in the experiments with the standard medium containing 1.25 mM magnesium. The drug has negligible effect when magnesium is either omitted from the medium or when the concentration is increased to double that of the standard medium. The stimulant effect of morphine on the rate of glucose-uptake by normal diaphragm is thus dependent upon the presence in the incubating medium of magnesium within a limited range of concentration.

TABLE 1. EFFECTS OF MORPHINE ON UPTAKE OF GLUCOSE BY ISOLATED DIAPHRAGM OF NORMAL AND CHRONICALLY MORPHINIZED RATS IN MEDIA OF DIFFERENT MAGNESIUM CONTENT

State and No. of rats	Mg-content of medium	Control	Experiment	Difference
			+ Morphine	
N (8)	0	221 \pm 13	193 \pm 14	— 28 \pm 19
C m (8)	0	212 \pm 10	170 \pm 9	— 42 \pm 8 (P < 0.001)
N (12)	1.25 mM	186 \pm 12	236 \pm 10	+ 50 \pm 12 (P < 0.002)
C m (8)	1.25 mM	234 \pm 10	190 \pm 7	— 44 \pm 6 (P < 0.001)
N (8)	2.5 mM	355 \pm 37	343 \pm 33	— 12 \pm 27
C m (8)	2.5 mM	221 \pm 7	176 \pm 11	— 45 \pm 6 (P < 0.001)

Hemi-diaphragms were incubated at pH 7.4 and 37° for 1 hr in oxygenated Krebs–Ringer-phosphate (2.0 ml) containing glucose (0.15%) \pm added morphine (final concentration, 7.7×10^{-4} M) and in which the magnesium concentration was varied. In each experiment, half the excised diaphragm served as a control for the other half. Mean rates \pm S.E. mean are expressed as decrease in glucose content of the medium, mg/100 g wet tissue/hr.

N—normal; C m—chronically morphinized.

With chronically morphinized diaphragm, on the other hand, morphine depresses the rate of glucose-uptake to a similar extent in all the experiments, this effect being independent of the presence of magnesium in the medium.

Effects of adrenal hormones on glucose-uptake by isolated diaphragm of normal and of chronically morphinized rats in media of different magnesium content

The effects of added adrenaline or of hydrocortisone in final concentrations of the order 10^{-4} M, as used in our earlier experiments,¹⁻³ on the rates of glucose-uptake by hemi-diaphragms of either normal or chronically morphinized rats in an oxygenated phosphate-buffered medium are not significantly affected by doubling the magnesium content of the medium. No effect of either hormone is observed with concentrations of the order 10^{-7} M, but significant effects on normal diaphragm are obtained with concentrations of the order 10^{-5} – 10^{-6} M. A concentration of 3.85×10^{-6} M was therefore chosen to compare the effects of adrenaline on glucose-uptake by normal diaphragm in media of different magnesium content. The results of these experiments,

illustrated in Fig. 2, show that adrenaline in this concentration has maximal effect on normal diaphragm in the standard medium containing 1.25 mM magnesium.

In Table 2 the effects of adrenaline and of hydrocortisone (3.85×10^{-6} M) in this standard medium on glucose-uptake by diaphragm of normal and of chronically morphinized rats are compared with their effects in media containing either no magnesium or double the standard concentration of magnesium. The results of these

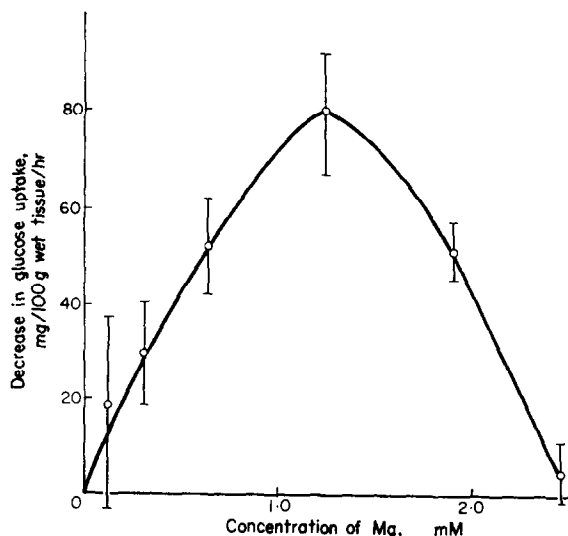


FIG. 2. Effect of adrenaline on rate of glucose-uptake by rat diaphragm incubated at 37° and pH 7.4 for 1 hr in oxygenated Krebs-Ringer-phosphate (2.0 ml) containing glucose (0.15%) at different magnesium concentrations. In each experiment, half the excised diaphragm served as a control for the other half + adrenaline (final concentration, 3.85×10^{-6} M). Each point represents the mean difference \pm S.E.M. for 5 experiments.

experiments show that either hormone has a significant effect on normal diaphragm only when the standard medium was used. The chronically morphinized diaphragm, on the other hand, is insensitive to either hormone when presented in the standard medium, but sensitivity to adrenaline is restored by doubling the magnesium concentration.

DISCUSSION

Evidence is accumulating that certain effects of several hormones and drugs may be attributed, at least in part, to an ability specifically to interfere with ion-transporting mechanisms of membranes, and with the formation or conformation and function of metal ion complexes that are involved in membrane activity and the control of cellular metabolism. The present work supports the view² that morphine is a drug within this category.

It is now demonstrated that the acute, *in vitro* effect of added morphine on the rate of glucose-uptake by normal rat-diaphragm is dependent upon the presence in the incubating medium of magnesium within a limited range of concentration. The drug has no effect when presented in the medium of high magnesium content, presumably because the rate of glucose-uptake is maximal under these conditions and addition of

morphine cannot increase it further. On the other hand, the observation that omission of magnesium from the medium also abolishes the effect of morphine implies that magnesium is essential for morphine to exert an effect. Morphine inhibits glucose-uptake by chronically morphinized diaphragm and this effect of the drug is the same in the absence as in the presence of magnesium.

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

State and No. of rats	Mg-content of medium	Control	Experiment	Difference
			+ Adrenaline	
<i>N</i> (8)	0	193 \pm 8	196 \pm 10	+ 3 \pm 14
<i>C m</i> (7)	0	201 \pm 11	178 \pm 11	— 23 \pm 6 ($P < 0.01$)
<i>N</i> (9)	1.25 mM	197 \pm 9	122 \pm 13	— 75 \pm 14 ($P < 0.001$)
<i>C m</i> (6)	1.25 mM	217 \pm 20	179 \pm 14	— 38 \pm 23
<i>N</i> (8)	2.5 mM	244 \pm 10	239 \pm 9	— 5 \pm 17
<i>C m</i> (8)	2.5 mM	213 \pm 11	143 \pm 11	— 70 \pm 12 ($P < 0.001$)
			+ Hydrocortisone	
<i>N</i> (7)	0	184 \pm 10	164 \pm 20	— 20 \pm 17
<i>C m</i> (7)	0	178 \pm 10	170 \pm 9	— 8 \pm 15
<i>N</i> (10)	1.25 mM	164 \pm 7	223 \pm 13	+ 59 \pm 9 ($P \ll 0.001$)
<i>C m</i> (7)	1.25 mM	161 \pm 9	160 \pm 4	— 1 \pm 7
<i>N</i> (7)	2.5 mM	250 \pm 12	244 \pm 20	— 6 \pm 13
<i>C m</i> (7)	2.5 mM	177 \pm 7	158 \pm 13	— 19 \pm 15

Hemi-diaphragms were incubated at pH 7.4 and 37° for 1 hr in oxygenated Krebs-Ringer-phosphate (2.0 ml) containing glucose (0.15%) \pm added hormone (final concentration, 3.85×10^{-6} M) and in which the magnesium content was varied. In each experiment, half the excised diaphragm served as a control for the other half. Mean rates \pm S.E. mean are expressed as decrease in glucose content of the medium, mg/100 g wet tissue/hr.

N—normal; *C m*—chronically morphinized.

As previously reported,⁴ the rate of glucose-uptake by chronically morphinized diaphragm, unlike that of normal diaphragm, is not affected even by drastic changes in the magnesium content of the incubating medium. This observation and the results of the present experiments with added morphine imply that sensitivity to extracellular magnesium has either been abolished as a result of chronic morphinization, or so changed that it is not always detectable in these experiments in which the extracellular concentrations of calcium, sodium, and potassium are not varied. The results of experiments with adrenaline suggest this latter interpretation.

Herman and Ramey^{5, 6} observed that the *in vitro* effects of adrenaline and of hydrocortisone on the rate of glucose-uptake by rat-diaphragm incubated in phosphate-buffered saline are abolished by doubling the magnesium content of the medium. The present work confirms their findings and shows that the effects of these hormones are also abolished by omitting magnesium from the medium. The evidence suggests that maximal effect of a hormone on the glucose-uptake system is attained only when there is a precise quantitative relationship between the concentration of the hormone and the concentrations of the various metal ions which influence the system.

In our experiments with hydrocortisone, the hormone in concentrations of the order 10^{-5} – 10^{-6} M increases the rate of glucose-uptake by normal diaphragm, the lower concentration having the greater effect, but a concentration of the order 10^{-4} M depresses the rate.² When a hormone has a stimulant effect at one concentration and a depressant effect at another, there is presumably an intermediate concentration at which it will have no effect. The level of such intermediate concentration has apparently been changed as a result of chronic morphinization. This is implied by our observations that the effect of a hormone on chronically morphinized tissue is different from and in some cases the reverse of that of the same concentration of the hormone on normal tissue, and that to achieve the same effect on chronically morphinized tissue as on normal tissue different concentrations of the hormone are required. The *in vitro* effect of hydrocortisone in a concentration of the order 10^{-4} M on chronically morphinized diaphragm,⁷ for example, is the reverse of that on normal diaphragm, but similar to that with a concentration of the order 10^{-5} – 10^{-6} M on normal diaphragm. This chronic effect of morphine could be the result of a change in the molecular structure or conformation of the membrane system, or it could be the result of a change in one or more of the several variables that affect the system, a change in local ion-concentration ratios for example.

In the experiments with adrenaline it is demonstrated that to produce an effect on chronically morphinized diaphragm similar to that on normal diaphragm a different concentration ratio between the hormone and extra-cellular magnesium is required.

Colburn and Maas^{8, 9} have studied the formation of co-ordination complexes of adrenaline with magnesium and have reviewed the evidence that magnesium complexes play a functional rôle in hormone-sensitive mechanisms associated with membrane activity. Assuming that such complexes are involved and that adrenaline influences the rate of glucose-uptake by co-ordinating with magnesium at specific membrane sites, it would appear that chronic morphinization has resulted in some modification of such sites with effect that a higher extracellular magnesium concentration is required to maintain their sensitivity to adrenaline.

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