

## EFFECTS OF MORPHINE ON THE HORMONAL CONTROL OF METABOLISM—V

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

S. C. WONG and E. O'F. WALSH

Department of Biochemistry, University of Hong Kong, Hong Kong

(Received 4 February 1969; accepted 4 April 1969)

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

The basic rate of glucose-uptake by diaphragm from chronically morphinized rats, unlike that of normal diaphragm, is not influenced by changes in the potassium content of the medium from 0 to 50 mM.

The effects of adrenal hormones on glucose-uptake are potassium-dependent in diaphragm of normal rats but not in diaphragm of chronically morphinized rats.

It is suggested that the different effects of hormones on muscle from normal and from a chronically morphinized animal are associated with changes involving a magnesium-potassium-sensitive regulatory system in the membrane.

PREVIOUS work<sup>1, 2</sup> has shown that the rate of glucose-uptake by diaphragm from a chronically morphinized rat, unlike that of normal diaphragm, is not influenced by changes in the magnesium content of the incubating medium; that the acute *in vitro* effects of morphine and of adrenal hormones on glucose-uptake by normal diaphragm are dependent upon the presence in the incubating medium of magnesium within a limited range of concentration, and that such dependence is either abolished or modified as a result of chronic morphinization. In the present work it is shown that chronic morphinization also results in a loss of sensitivity of the glucose-uptake system to extracellular potassium.

#### MATERIALS AND METHODS

The animals used and the experimental procedures were as previously described.<sup>3</sup>

The standard incubation medium used in all experiments was an oxygenated phosphate-buffered saline of pH 7.4 containing 130 mM sodium, 5.14 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 but with potassium varied from 0 to 51.4 mM, increased potassium being balanced by an equivalent decrease of sodium.

## EXPERIMENTAL AND RESULTS

*Effects of varying potassium concentration on glucose-uptake by isolated diaphragm of normal and of chronically morphinized rats*

The results of paired experiments (Table 1) show that omission of potassium from the incubating medium or a 6 to 10-fold increase of the standard concentration (5.14 mM) of potassium lowers the rate of glucose-uptake by normal rat diaphragm

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

State and No. of rats	Ratio of K <sup>+</sup> , B/A	A, Control (K <sup>+</sup> , 5m M)	B, Experiment (K <sup>+</sup> , varied)	Difference (B - A)
N (7)	0	200 ± 14	112 ± 14	-88 ± 15 (P < 0.002)
Cm (7)	0	253 ± 12	242 ± 18	-11 ± 20
N (6)	2	168 ± 14	257 ± 12	+89 ± 6 (P < 0.001)
N (7)	3	185 ± 4	339 ± 10	+154 ± 10 (P < 0.001)
N (6)	3.25	202 ± 13	376 ± 9	+174 ± 22 (P < 0.001)
Cm (12)	3.25	233 ± 8	251 ± 21	+18 ± 17
N (7)	3.5	187 ± 10	344 ± 27	+157 ± 22 (P < 0.001)
N (7)	4	181 ± 11	296 ± 14	+115 ± 16 (P < 0.001)
N (6)	5	179 ± 11	233 ± 14	+54 ± 8 (P < 0.01)
N (6)	6	175 ± 8	108 ± 3	-67 ± 6 (P < 0.001)
Cm (10)	6	233 ± 10	233 ± 11	0 ± 7
N (8)	7	196 ± 10	128 ± 10	-68 ± 6 (P < 0.001)
N (7)	10	190 ± 10	127 ± 12	-63 ± 4 (P < 0.001)
Cm (7)	10	251 ± 15	227 ± 14	-24 ± 11

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%). In each experiment half the excised diaphragm served as a control (A) for the other half (B) in which the potassium content of the medium varied from 0 to 51.4 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.

from an oxygenated phosphate-buffered saline, while a 2 to 5-fold increase has the opposite effect, maximal rate being attained with a potassium concentration of 16.7 mM.

With diaphragm from chronically morphinized rats, on the other hand, the rate of glucose-uptake is not affected by variation in potassium content of the medium from 0 to 51.4 mM.

*Effects of varying magnesium concentration on uptake of glucose by isolated diaphragm of normal rats in media of different potassium content*

The effects of varying the magnesium content of the medium from 0 to 2.5 mM on the rate of glucose-uptake by isolated rat-diaphragm incubated at pH 7.4 and 37° in phosphate-buffered salines of different potassium content (0–51.4 mM) are compared in Table 2. The results show that the effect of raising the magnesium content of the medium is not wholly dependent upon the presence of potassium and that the effect of raising the potassium content of the medium is not dependent upon the presence of magnesium. High rates of glucose-uptake are observed only when potassium is present in the medium.

TABLE 2. EFFECTS OF VARYING MAGNESIUM CONCENTRATION ON UPTAKE OF GLUCOSE BY RAT DIAPHRAGM IN MEDIA OF DIFFERENT POTASSIUM CONTENT

No. of rats	K-content of medium	Ratio of Mg, B:A	A, Control (Mg, 1.25 m M)	B, Experiment (Mg, varied)	Difference (B - A)
(7)	0	0	119 ± 8	105 ± 6	-14 ± 3 (P < 0.01)
(8)	0	2	143 ± 6	212 ± 17	+69 ± 15 (P < 0.01)
(7)	10.28 mM	0	242 ± 10	226 ± 16	-16 ± 9
(7)	10.28 mM	2	224 ± 10	287 ± 19	+63 ± 21 (P < 0.05)
(8)	16.7 mM	0	354 ± 18	331 ± 21	-23 ± 12
(7)	16.7 mM	2	347 ± 15	364 ± 12	+17 ± 18
(7)	30.84 mM	0	127 ± 5	115 ± 7	-12 ± 3 (P < 0.01)
(8)	30.84 mM	2	136 ± 10	204 ± 13	+68 ± 8 (P < 0.001)
(8)	51.4 mM	0	127 ± 9	117 ± 4	-10 ± 8
(8)	51.4 mM	2	152 ± 10	222 ± 14	+70 ± 11 (P < 0.001)

Hemi-diaphragms were incubated at pH 7.4 and 37° for 1 hr in oxygenated phosphate-buffered saline (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 magnesium content of the medium differed from that of (A). Mean rates ± S.E. mean are expressed as decrease in glucose content of the medium, mg/100 g wet tissue/hr.

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

The acute effects of morphine on the rates of glucose-uptake by diaphragm of normal and of chronically morphinized rats in media of different potassium content are compared in Table 3. The results imply that the stimulant effect of morphine on glucose-uptake by normal diaphragm and the depressant effect on chronically morphinized diaphragm are independent of the presence of potassium in the medium, though the drug has no effect on normal diaphragm when the potassium concentration is high and the rate of glucose-uptake is maximal.

There is, however, a suggestion that both the normal and chronically morphinized

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

State and No. of rats	K content of medium	Control	Experiment	Difference
			+ Morphine	
N (8)	0	139 ± 10	194 ± 18	+55 ± 14 (P < 0.01)
Cm (8)	0	206 ± 11	167 ± 8	-39 ± 9 (P < 0.002)
N (7)	5.14 mM	181 ± 12	242 ± 15	+61 ± 14 (P < 0.01)
Cm (8)	5.14 mM	239 ± 15	191 ± 5	-49 ± 15 (P < 0.02)
N (7)	16.7 mM	340 ± 18	337 ± 15	-3 ± 15
Cm (7)	16.7 mM	236 ± 8	197 ± 14	-39 ± 10 (P < 0.01)
N (7)	30.84 mM	131 ± 9	221 ± 7	+90 ± 12 (P < 0.001)
Cm (8)	30.84 mM	231 ± 13	138 ± 3	-93 ± 15 (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%) ± added morphine (final concentration,  $7.7 \times 10^{-4}$  M) and in which the potassium 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.

tissues are more sensitive to morphine in the presence of a high potassium concentration (30.84 mM).

These experiments also show that abolition of sensitivity to potassium is not an acute effect of morphine.

*Effects of adrenaline and hydrocortisone on uptake of glucose by isolated diaphragm of normal and of chronically morphinized rats in media of different potassium content*

The effects of adrenaline and of hydrocortisone on the rates of glucose-uptake by diaphragm of normal and of chronically morphinized rats in media of different potassium content are compared in Table 4. With normal diaphragm the potassium-

TABLE 4. EFFECTS OF ADRENALINE AND HYDROCORTISONE ON UPTAKE OF GLUCOSE BY DIAPHRAGM OF NORMAL AND CHRONICALLY MORPHINIZED RATS IN MEDIA OF DIFFERENT POTASSIUM CONTENT

State and No. of rats	K content of medium	Control	Experiment	Difference
+ Adrenaline				
N (7)	0	116 ± 6	97 ± 4	-19 ± 5 (P < 0.01)
Cm (8)	0	229 ± 7	168 ± 7	-61 ± 9 (P < 0.001)
N (17)	5.14 mM	192 ± 8	128 ± 10	-63 ± 10 (P < 0.001)
Cm (17)	5.14 mM	239 ± 6	194 ± 8	-45 ± 9 (P < 0.001)
N (8)	16.7 mM	351 ± 10	234 ± 7	-117 ± 13 (P < 0.001)
Cm (8)	16.7 mM	247 ± 10	178 ± 13	-69 ± 12 (P < 0.001)
N (8)	30.84 mM	124 ± 4	109 ± 3	-15 ± 4 (P < 0.01)
Cm (8)	30.84 mM	257 ± 5	188 ± 8	-69 ± 7 (P < 0.001)
+ Hydrocortisone				
N (7)	0	125 ± 4	104 ± 6	-21 ± 8
Cm (8)	0	226 ± 9	259 ± 12	+33 ± 7 (P < 0.01)
N (8)	5.14 mM	188 ± 5	134 ± 10	-54 ± 7 (P < 0.001)
Cm (8)	5.14 mM	236 ± 8	272 ± 15	+36 ± 12 (P = 0.02)
N (8)	16.7 mM	330 ± 13	238 ± 9	-92 ± 16 (P < 0.001)
Cm (7)	16.7 mM	253 ± 8	315 ± 21	+62 ± 14 (P < 0.01)
N (7)	30.84 mM	124 ± 5	95 ± 6	-29 ± 7 (P < 0.01)
Cm (7)	30.84 mM	207 ± 11	246 ± 10	+39 ± 13 (P = 0.02)

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%) ± added hormone (final concentration,  $3.85 \times 10^{-6}$  M) and in which the potassium 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.

stimulated glucose-uptake is especially sensitive both to adrenaline and to hydrocortisone. These hormones have relatively little effect on glucose-uptake in the absence of potassium or when the potassium concentration is abnormally high (30.84 mM). With chronically morphinized diaphragm on the other hand, the effects of the hormones are apparently independent of the presence of potassium.

The effects of hydrocortisone on both normal and chronically morphinized rats observed in these experiments are similar to those previously reported<sup>4, 5</sup> with higher concentrations of hormone but different from those obtained by Poon *et al.*<sup>2</sup> with a low concentration. We are unable to confirm that a low concentration of hydrocortisone stimulates glucose-uptake by normal diaphragm.

## DISCUSSION

The rate of glucose-uptake by isolated rat-diaphragm from a standard phosphate-buffered medium of physiologically normal cationic composition and glucose content is increased by raising either the magnesium<sup>2</sup> or the potassium content of the medium, maximal rate being approached either by doubling the magnesium or trebling the potassium concentration.

It has previously been shown that diaphragm from a chronically morphinized rat has completely lost such sensitivity to extracellular magnesium. It is of interest to find that there is a similar loss of sensitivity to extracellular potassium. It might, therefore, be expected that the chronically morphinized diaphragm would be insensitive to hormones or drugs, the effects of which on normal diaphragm are dependent upon the presence in the medium of either or both magnesium and potassium. Our experiments show this not to be the case: the chronically morphinized diaphragm still possesses a sensitivity to adrenal hormones but such sensitivity, unlike that of normal diaphragm, is not potassium-dependent. The potassium-stimulated glucose-uptake of normal diaphragm is especially sensitive to adrenal hormones.

Since chronic morphinization does not abolish sensitivity to adrenal hormones it would seem that specific hormone-receptor sites of the membrane have survived chronic morphinization. Yet changes, presumably in the membrane-structure, have occurred since, as acute experiments with added morphine show, the mere presence of morphine does not oppose the stimulant effects of a raised extracellular potassium or magnesium,<sup>3</sup> though chronic morphinization completely abolishes such sensitivity. We, therefore, suspect that the drug-induced changes in some way attenuate a magnesium-potassium-sensitive regulatory system within the membrane that is commonly involved in responses to hormones which influence glucose-uptake.

*Acknowledgements*—The authors thank the Nuffield Foundation and Hong Kong Government, the University of Hong Kong, and the China Medical Board of New York, Inc. for their contributions in support of this work.

## REFERENCES

1. E. O'F. WALSH and M. W. POON, *Nature, Lond.* **215**, 525 (1967).
2. M. W. POON, E. O'F. WALSH and M. L. NG, *Biochem. Pharmac.* **17**, 1575 (1968).
3. C. H. LEE PENG and E. O'F. WALSH, *Biochem. Pharmac.* **12**, 921 (1963).
4. E. O'F. WALSH, C. H. LEE PENG and M. L. NG, *Nature, Lond.* **204**, 698 (1964).
5. M. L. NG and E. O'F. WALSH, *Biochem. Pharmac.* **14**, 1003 (1965).