Biochemical Pharmacology, Vol. 20, pp. 417–421. Pergamon Press, 1971. Printed in Great Britain

EFFECTS OF MORPHINE ON THE HORMONAL CONTROL OF METABOLISM—VI

EFFECTS *IN VITRO* OF THYROXINE AND ALDOSTERONE ON UPTAKE OF GLUCOSE BY MUSCLE OF NORMAL AND CHRONICALLY MORPHINIZED RATS

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

Department of Biochemistry, University of Hong Kong, Hong Kong

(*Received* 21 *April* 1970; *accepted* 23 *July* 1970)

Abstract—The effects of thyroxine and of aldosterone on uptake of glucose and of oxygen from media of different potassium content by diaphragm of normal and of chronically morphinized rats have been studied *in vitro*.

Thyroxine stimulates glucose-uptake by normal diaphragm when potassium is omitted from the medium and when the potassium concentration of the medium is abnormally high. It depresses glucose-uptake by chronically morphinized diaphragm and this effect is unaffected by potassium in concentrations from 0 to 31 mM. Respiration of both normal and chronically morphinized diaphragm is not influenced by thyroxine under the conditions of experiment.

Aldosterone depresses glucose-uptake by normal diaphragm with maximal effect when the potassium content of the medium is optimal for glucose-uptake. It stimulates glucose-uptake and respiration by chronically morphinized diaphragm and these effects are not influenced by potassium in concentrations from 0 to 31 mM. Respiration of normal diaphragm is also stimulated by aldosterone.

EARLIER studies of the effects of adrenal hormones[1–3] and of insulin[4] on glucose metabolism of isolated diaphragm from normal and from chronically morphinized rats, and the observation that chronic morphinization results in modification of cell membrane properties with loss of sensitivity to extracellular magnesium[5,6] and potassium[7] have led us to suspect that the drug-induced change causes attenuation of a magnesium-potassium-dependent regulatory system that is commonly involved in responses to hormones. A recent finding[8] that intracellular distribution of potassium in skeletal muscle of chronically morphinized rats is different from normal, the potassium content of the microsomal fraction being abnormally high, encourages this view and emphasizes the need for a comparative study with other hormones.

We now report the results of a study of the effects of thyroxine and aldosterone on glucose-uptake and respiration of normal and of chronically morphinized rat-diaphragm.

MATERIALS AND METHODS

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.

The standard incubation medium 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 0·15% w/v glucose.

d-Aldosterone, Sigma and L-thyroxine pentahydrate, Sigma were used.

RESULTS

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

The results of paired experiments (Table 1) show that *in vitro* addition of thyroxine increases the rate of glucose-uptake by diaphragm of normal rats from oxygenated phosphate-buffered saline independently of the presence of potassium in the medium, except when the potassium concentration is such (16·7 mM) that the rate of uptake is already at a maximum.

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

State and no. of rats	K-content of medium	Control	Experiment +Thyroxine	Difference
N (11)	0	127 ± 7	230 ± 10	+103 ± 14 ($P < 0·001$)
Cm (9)	0	225 ± 5	172 ± 3	− 53 ± 5 ($P < 0·001$)
N (12)	5·14 mM	179 ± 5	266 ± 11	+ 87 ± 10 ($P < 0·001$)
Cm (10)	5·14 mM	241 ± 3	217 ± 6	− 24 ± 5 ($P < 0·001$)
N (8)	16·7 mM	321 ± 5	324 ± 10	+ 3 N.S.
Cm (10)	16·7 mM	233 ± 7	199 ± 6	− 34 ± 6 ($P < 0·001$)
N (7)	30·84 mM	128 ± 4	222 ± 8	+ 94 ± 4 ($P < 0·001$)
Cm (10)	30·84 mM	240 ± 7	202 ± 9	− 38 ± 8 ($P < 0·001$)

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 thyroxine (final concentration, $3·85 \times 10^{-5}$ 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; C_m, chronically morphinized.

With diaphragm from chronically morphinized rats, on the other hand, thyroxine decreases the rate of glucose-uptake. This effect is observed at all concentrations of potassium from 0 to 30·84 mM, but it appears to be greatest when potassium is omitted from the medium, the mean difference (−29 ± 7·1) between the effect in the absence of extracellular potassium and that in the standard medium (K = 5·14 mM) being significant ($P < 0·001$).

Effects of thyroxine on uptake of oxygen by isolated diaphragm of normal and of chronically morphinized rats in media of different potassium content

In experiments analogous to those described for aldosterone (Table 3) no significant effect of thyroxine ($3·85 \times 10^{-5}$ M) on the rate of oxygen-uptake of either normal or chronically morphinized rat-diaphragm was recorded within experimental periods of up to 2 hr.

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

State and no. of rats	K-content of medium	Control	Experiment	Difference
			+Aldosterone	
N (8)	0	131 ± 8	113 ± 8	− 19 ± 4 ($P < 0.01$)
Cm (8)	0	232 ± 7	298 ± 12	+ 66 ± 13 ($P < 0.002$)
N (9)	5·14 mM	179 ± 7	133 ± 10	− 46 ± 6 ($P < 0.001$)
Cm (9)	5·14 mM	235 ± 10	300 ± 10	+ 65 ± 8 ($P < 0.001$)
N (9)	16·7 mM	330 ± 11	230 ± 11	−100 ± 12 ($P < 0.001$)
Cm (10)	16·7 mM	220 ± 7	279 ± 9	+ 59 ± 8 ($P < 0.001$)
N (9)	30·84 mM	129 ± 2	114 ± 3	− 15 ± 3 ($P < 0.01$)
Cm (9)	30·84 mM	229 ± 7	277 ± 12	+ 48 ± 7 ($P < 0.001$)

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 aldosterone (final concentration, $3{\cdot}47 \times 10^{-6}$ 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; C_m, chronically morphinized.

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

The results of paired experiments (Table 2) show that *in vitro* addition of aldosterone decreases the rate of glucose-uptake by diaphragm of normal rats from oxygenated phosphate-buffered saline. This effect is maximal when the potassium content of the medium is optimal for glucose-uptake and is small, though significant, when potassium is either omitted from the medium or increased to a concentration (30·84 mM) that reduces the rate of glucose-uptake to a level similar to that observed in the absence of extracellular potassium.

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

State and no. of rats	K-content of medium	Control	Experiment	Difference
			+Aldosterone	
N (9)	0	1358 ± 46	1406 ± 41	+ 48 ± 23 ($P \simeq 0.05$)
Cm (8)	0	1250 ± 39	1358 ± 29	+108 ± 35 ($P < 0.02$)
N (9)	5·14 mM	1261 ± 33	1338 ± 24	+ 77 ± 30 ($P < 0.05$)
Cm (10)	5·14 mM	1341 ± 36	1438 ± 42	+ 97 ± 31 ($P < 0.02$)
N (9)	16·7 mM	1359 ± 28	1461 ± 45	+102 ± 55
Cm (10)	16·7 mM	1137 ± 38	1253 ± 47	+116 ± 36 ($P = 0.01$)
N (9)	30·84 mM	1275 ± 42	1403 ± 36	+128 ± 57 ($P \simeq 0.05$)
Cm (9)	30·84 mM	1178 ± 45	1278 ± 46	+100 ± 28 ($P < 0.01$)

Hemi-diaphragms were incubated with shaking at pH 7·4 and 37° for 1 hr in oxygenated Krebs–Ringer-phosphate (2·0 ml) containing glucose (0·15%) ± added aldosterone (final concentration, $3{\cdot}47 \times 10^{-6}$ M). O_2-uptakes were measured by the Warburg direct method with O_2 as gas phase: the centre wells contained 30% KOH (0·2 ml). In each experiment half the excised diaphragm served as control for the other half. Mean rates ± S.E. mean are expressed as μl O_2/g wet tissue/hr.

N, normal; C_m, chronically morphinized.

With diaphragm from chronically morphinized rats, on the other hand, aldosterone increases the rate of glucose-uptake and this effect is not significantly changed by variation in potassium content of the medium from 0 to 30·84 mM.

Effects of aldosterone on uptake of oxygen by isolated diaphragm of normal and chronically morphinized rats in media of different potassium content

The results of paired experiments (Table 3) show that *in vitro* addition of aldosterone tends to stimulate respiration of rat-diaphragm, and this is not significantly changed by chronic morphinization. This effect in chronically morphinized diaphragm is independent of variation in extracellular potassium concentration from 0 to 30·84 mM.

DISCUSSION

Reversal of a hormonal effect on cellular metabolism as a result of chronic morphinization was first observed with hydrocortisone[9] which depresses glucose-uptake *in vitro* by normal diaphragm but increases uptake by chronically morphinized diaphragm. The present work shows the effect of aldosterone to be similar to that of hydrocortisone in this respect, and with either hormone the effect is greatest in normal diaphragm when the potassium concentration of the incubating medium is such that glucose-uptake approaches the maximal rate. In both cases, the stimulant effect of the hormone on glucose-uptake by chronically morphinized diaphragm is not influenced by changes in extracellular potassium concentration.

The effect of thyroxine on glucose-uptake is also reversed as a result of chronic morphinization, but in the opposite way. In this respect, the effect of thyroxine is similar to that of morphine itself,[7] the acute *in vitro* effect of which is to increase uptake by normal diaphragm and to depress that by chronically morphinized diaphragm. Here again, the effect on chronically morphinized diaphragm, unlike that of normal diaphragm, is not influenced by changes in extracellular potassium concentration.

It is of interest to note that the effect of aldosterone on respiration is similar to that of adrenaline and opposed to that of hydrocortisone. These effects of the hormones are not changed by chronic morphinization and it may be significant in relation to this that the potassium content of a mitochondrial fraction of skeletal muscle is the same in the normal and in the chronically morphinized rat.[8]

In summary, it appears that the acute *in vitro* effects of morphine and of the hormones, adrenaline, thyroxine, hydrocortisone, and aldosterone on glucose-uptake by normal muscle are influenced by the potassium concentration of the incubating medium and that it is the potassium-sensitive uptake of glucose that is also hormone-sensitive. The hormones which depress uptake by normal diaphragm have their greatest effects when the potassium concentration of the medium favours a high rate of uptake of glucose, while morphine and thyroxine, which have the opposite effect, have their greatest effect when the basal rate of uptake is low, as in the case when potassium is either absent from the medium or is present in abnormally high concentration.

The consistent difference between normal and chronically morphinized diaphragm is that the rate of glucose-uptake by the latter and the effects of all the above mentioned hormones on it are insensitive to changes in extracellular potassium concentration. Such insensitivity might be attributed to an abnormally high potassium concentration

within the membrane itself, as evidenced by the observation[8] that the potassium content of a microsomal fraction of skeletal muscle from a chronically morphinized animal is abnormally high, as much as treble that of normal muscle.

Acknowledgements—We thank the Nuffield Foundation and Hong Kong Government, the University of Hong Kong, and the China Medical Board of New York, Inc. for grants in aid of this work.

REFERENCES

1. C. H. Lee Peng and E. O'F. Walsh, *Biochem. Pharmac.* **12,** 921 (1963).
2. M. L. Ng and E. O'F. Walsh, *Biochem. Pharmac.* **14,** 1003 (1965).
3. M. L. Ng and E. O'F. Walsh, *Biochem. Pharmac.* **15,** 1867 (1966).
4. E. O'F. Walsh, C. H. Lee Peng and S. C. Wong, *Biochem. Pharmac.* **18,** 1529 (1969).
5. E. O'F. Walsh and M. W. Poon, *Nature, Lond.* **215,** 525 (1967).
6. M. W. Poon, E. O'F. Walsh and M. L. Ng, *Biochem. Pharmac.* **17,** 1575 (1968).
7. S. C. Wong and E. O'F. Walsh, *Biochem. Pharmac.* **18,** 1979 (1969).
8. E. O'F. Walsh and S. C. Wong, unpublished data.
9. E. O'F. Walsh, C. H. Lee Peng and M. L. Ng, *Nature, Lond.* **204,** 698 (1964).