Histone H4 stimulates glucose transport activity in rat skeletal muscle

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We investigated the effects of purified histone H4 on glucose transport activity in rat soleus and flexor digitorum brevis muscles. Histone H4, at concentrations up to 11.8 μ M, increased 2-deoxyglucose (2-DG) uptake in a dose-dependent fashion. However, at concentrations higher than 11.8 μ M, H4 caused a decrease in 2-DG uptake from the maximum, suggesting a secondary inhibitory action of this compound. The maximal effect of H4 on 2-DG uptake was not additive to the maximal effect of insulin. Moreover, 2-DG uptake in the presence of both H4 and insulin was significantly lower than the 2-DG uptake in the presence of insulin alone. The maximal effect of H4 on stimulation of 2-DG uptake was neither additive nor inhibitory to the maximal effects of the intracellularly acting insulin mimetics sodium vanadate or H_2O_2 . It was, on the other hand, additive to the maximal effects of muscle contractions. Also, in

contrast with the effects of H4 on insulin-stimulated 2-DG uptake, H4 did not inhibit insulin-like growth factor-I (IGF-I)-stimulated 2-DG uptake, as the maximal effects of H4 and IGF-I were additive. Scatchard analysis of the binding of 125 I-insulin in the absence or presence of histone H4 revealed that H4 increased the specific binding of insulin without affecting receptor affinity. These data suggest that H4 interacts with the insulin, rather than the hypoxia/contraction, pathway for activation of glucose transport in muscle tissue, and that H4 acts either directly or indirectly to increase the number of insulin receptors at the surface of the muscle cell. This interaction does not appear to occur with the similar, although distinct, IGF-I receptor. These studies may provide additional insight into the complex signal-transduction systems of insulin action.

INTRODUCTION

The traditional role of histones in the nucleosome structures of chromatin is well established. However, recent studies have documented that histones have additional functions. For example, histone H1 stimulates phosphorylase phosphatase activity in muscle and kidney cells [1] and inhibits translational activity in muscle cells [2]. Histones H2a and H2b have primary structures identical with those of homeostatic thymus hormones HTH_α and HTH_β respectively [3,4]. Histones have been shown to decrease both aerobic and anaerobic metabolism in leucocytic cells [5]. They have also been shown to interact with several receptors such as the glucocorticoid receptor [6,7], the thyroid receptor [8], the oestrogen receptor [9] and the gonadotropin-releasing-hormone receptor [10]. The C-terminal sequence of histone H4 has opiate-like activity [11], and more recently a 14-amino-acid peptide, called osteogenic growth peptide, has been shown to be identical with the C-terminus of H4 [12].

It has also been reported that the arginine-rich histones H3 and H4 have an insulin-like activity in isolated rat adipocytes [13]. This study demonstrated that histones H3 and H4 were able to stimulate glucose incorporation to the same level as insulin. This insulin-like activity was specific to the histones, since it could not be fully mimicked by either histone fragments or polycations, such as polyarginine or polylysine. Moreover, it appears that histones and insulin share a common pathway of stimulation, since the effects of the co-administration of maximally effective concentrations of both histone and insulin were not additive [13].

However, unlike adipocytes, glucose transport in skeletal muscle is activated by two distinct mechanisms: one that is stimulated by insulin and insulin-like factors, and one that is stimulated by the contractions associated with exercise [14]. In

this context, the purposes of the present study were (1) to characterize the effect of histones on the stimulation of glucose transport activity in skeletal muscle, as assessed by 2-deoxyglucose (2-DG) uptake, (2) to determine whether this effect is mediated via the insulin or the contraction pathway, and (3) to gain insight into a possible mechanism for the stimulation of glucose transport activity by this compound.

We report that histone H4 stimulates glucose transport activity in rat skeletal muscle, but that this stimulation differs dramatically from the effects observed in adipocytes. By the use of additivity studies, we also report that histone H4 stimulates glucose transport activity through the insulin pathway rather than through the contraction-stimulated pathway. Also, 125 I-insulin-binding studies suggest that H4 does not compete for the insulin-binding site, but appears to interact with the insulin receptor to increase the specific binding of insulin.

MATERIALS AND METHODS

Materials

Calf thymus histones (type II-AS), hen egg-white lysozyme, cytochalasin B and [14 C]mannitol were purchased from Sigma (St. Louis, MO, U.S.A.). 2-Deoxy-D-[1,2- 3 H]glucose was purchased from New England Nuclear–DuPont (Boston, MA, U.S.A.) and 125 I-insulin from ICN Biomedical (Costa Mesa, CA, U.S.A.). Sodium vanadate was obtained from Fisher Scientific (Fair Lawn, NJ, U.S.A.) and BSA (fraction V) was purchased from Miles (Kankakee, IL, U.S.A.). Purified histone H4 was generously given by M. C. McCroskey and Dr. J. D. Pearson of the Upjohn Company (Kalamazoo, MI, U.S.A.), and insulin-like growth factor-I (IGF-I) was generously given by Eli Lilly and Co. (Indianapolis, IN, U.S.A.).

Animals and muscle preparation

Male Wistar rats (Sasco, Omaha, NE, U.S.A.) weighing approx. 120 g were used in these experiments. After food restriction overnight (4 g per animal), the animals were anaesthetized by an intraperitoneal injection of sodium pentobarbital (5 mg/100 g body wt.) and the soleus and/or the flexor digitorum brevis (FDB) muscles were quickly removed. The FDB (~20 mg) was used intact, whereas the soleus was split as described previously [15] to obtain 20–25 mg strips before the muscle incubations. The viability of these muscle preparations for incubations *in vitro* has been previously documented [16].

2-DG uptake

Soleus muscle strips were placed in stoppered flasks containing 2.0 ml of Krebs–Ringer Hepes (KRH) buffer (125 mM NaCl, 1.25 mM MgSO_4 , 2.5 mM NaH_2PO_4 , 20 mM Hepes, 5.9 mM KCl, 2.5 mM CaCl_2 , pH 7.4) containing 0.5% (w/v) BSA, 2 mM pyruvate and the stimulant as indicated in the Figures and Tables. The flasks, containing the muscle strips, were maintained at 37 °C in a shaking water bath with a gas phase of O_2/CO_2 (19:1) for 40 min. After 40 min, a concentrated radioactive mixture (40-fold) was added to give a final concentration of 1.0 mM 2-[^3H]DG (0.3 $\mu\text{Ci}/\text{ml}$) and 1.0 mM [^{14}C]mannitol (0.02 $\mu\text{Ci}/\text{ml}$) and the incubation was continued for a further 20 min. 2-DG uptake was terminated by removing the muscle, quickly trimming the connective tissue from the ends, and freezing the muscle between blocks of aluminium cooled in liquid nitrogen. The frozen muscle was weighed and solubilized in 0.5 ml of 0.5 M NaOH. After complete solubilization, 5 ml of scintillation fluid was added. The radioactivity was measured in both the ^{14}C and ^3H channels, and the radioactivity in the ^{14}C channel along with the specific radioactivity of the incubation media were used to determine extracellular space. Specific uptake of 2-DG, expressed as nmol/20 min per mg of muscle, was calculated after subtracting the ^3H radioactivity in the extracellular space from the total ^3H radioactivity in each sample.

Stimulation of muscle contractions

One soleus strip was incubated in the KRH buffer solution containing 11.8 μM histone H4, while another strip from the same muscle was incubated in the same buffer without histone H4. After 30 min the distal tendon of the muscle was mounted on a Lucite rod containing two platinum electrodes, and the proximal tendon was clipped to a jeweller's chain that was attached to a Grass model FTO3 force transducer. The mounted muscle was placed in 20 ml of KRH buffer containing 2.0 mM pyruvate and continuously oxygenated with O_2/CO_2 (19:1) at 35 °C. The muscle was electrically stimulated with supramaximal square-wave pulses of 0.2 ms duration by using a Grass S11 stimulator. Ten 10 s tetanic contractions were generated by stimulation at 50 Hz at a rate of 1 contraction/min. Previous research has shown that these conditions are sufficient to stimulate maximally the contraction-dependent glucose transport activity [15]. After electrical stimulation 2-DG uptake was measured as described above.

^{125}I -insulin binding

Insulin binding to soleus muscle strips was determined as previously outlined [17]. Briefly, soleus muscle strips were preincubated for 1 h at 22 °C in KRH buffer containing 0.1% BSA, 5 mM glucose, and 2 mg/ml bacitracin to inhibit insulin degradation. Muscles were then incubated either with or without 6 μM

histone H4 for 4 h at 22 °C in fresh buffer containing 0.1% BSA, 2 mM pyruvate, 2 mg/ml bacitracin, 1 ng/ml ^{125}I -insulin and various concentrations of unlabelled insulin (0.17–32 nM). Non-specific binding was determined by incubation with 8.0 μM unlabelled insulin. After the incubation, the muscles were washed six times (5 min/wash) with ice-cold isotonic saline (140 mM NaCl, 0.1% BSA, 10 mM Hepes, pH 7.6) to remove unbound insulin. Muscles were then solubilized in 0.5 ml of 0.5 M NaOH and counted for radioactivity in a γ -radiation counter.

Statistics

The significance of differences between two groups was assessed by use of a paired or unpaired Student's *t* test. For multiple comparisons, a factorial analysis of variance was used with a *post-hoc* Dunnett or Scheffe *F* test used to locate significant differences. For a group difference to be statistically significant, the 0.05 probability level had to be obtained.

RESULTS

Effects of histone H4, insulin, and insulin mimickers on glucose transport activity

Preliminary studies looking at the uptake and metabolism of [^{14}C]glucose indicated that both crude histones and purified H4 had a moderate stimulatory effect on the amount of radioactivity that could be isolated in the soleus muscle (results not shown). This prompted us to investigate specifically the effects of purified histone H4 on the glucose transport process, as determined by the uptake of the glucose analogue 2-DG. It has been shown previously that, under the conditions employed here, 2-DG uptake is a valid measure of glucose transport activity [15]. A maximally effective concentration of H4 (11.8 μM) increased 2-DG uptake by 48% (Figure 1). Interestingly, at higher concentrations (17.7 μM), 2-DG uptake was significantly lower than that observed with 11.8 μM .

This stimulation of 2-DG uptake by H4 was mediated specifically by the glucose transporter, since the H4-stimulated uptake, as well as basal uptake, was blocked by cytochalasin B, a fungal metabolite that inhibits glucose transport by binding to glucose-transporter proteins (results not shown). This indicates that the enhanced uptake of glucose in response to H4 was not the result of non-specific uptake of glucose as the result of sarcolemmal

Figure 1 Dose-response of histone H4 on 2-DG uptake in soleus muscle

Soleus muscle strips were incubated with increasing concentrations of H4, and 2-DG uptake was measured as outlined in the Materials and methods section. The means $\pm$ S.E.M. for 21 muscles (0 H4) or 6–18 muscles (5.9–17.7 μM H4) are shown: **P* < 0.05 versus 0 H4; †*P* < 0.05 versus 11.8 μM H4.

Table 1 Effects of histone H4 on insulin-stimulated 2-DG uptake in soleus muscle

Soleus muscle strips were incubated with 47 nM insulin plus increasing concentrations of H4. 2-DG uptake was measured as outlined in the Materials and methods section. Basal uptake (no additions) represents data from Figure 1 and is shown for comparison. Values are means $\pm$ S.E.M., with n representing the number of muscles: * P < 0.05 versus basal; † P < 0.05 versus insulin alone.

Insulin (nM)	H4 (μ M)	n	Uptake (nmol/20 min per mg)
0	0	21	0.193 $\pm$ 0.008
47	0	14	0.670 $\pm$ 0.031*
47	5.9	7	0.646 $\pm$ 0.039*
47	11.8	14	0.489 $\pm$ 0.032*†
47	17.7	7	0.483 $\pm$ 0.033*†

Table 2 Effects of histone H4 and vanadate or H₂O₂ on 2-DG uptake in soleus muscle

Soleus muscle strips were incubated with an insulin mimetic, either 5 mM sodium vanadate or 5 mM H₂O₂, in the presence or absence of 11.8 μ M H4. 2-DG uptake was measured as outlined in the Materials and methods section. Basal uptake (no additions) represents data from Figure 1 and is shown for comparison. Values are means $\pm$ S.E.M., with n representing the number of muscles: * P < 0.05 versus basal.

Insulin mimetic (5 mM)	H4 (μ M)	n	Uptake (nmol/20 min per mg)
None	0	21	0.193 $\pm$ 0.008
Sodium vanadate	0	9	0.309 $\pm$ 0.017*
Sodium vanadate	11.8	9	0.297 $\pm$ 0.014*
H ₂ O ₂	0	12	0.334 $\pm$ 0.021*
H ₂ O ₂	11.8	12	0.335 $\pm$ 0.018*

Table 3 Effects of histone H4 and insulin on 2-DG uptake in the FDB muscle

2-DG uptake was measured in flexor digitorum brevis muscles at basal conditions, with 11.8 μ M H4 present, with 47 nM insulin present, and with both H4 and insulin present, as outlined in the Materials and methods section. Values are means $\pm$ S.E.M., with n representing the number of muscles: * P < 0.05 versus basal; † P < 0.05 versus insulin alone.

Insulin (nM)	H4 (μ M)	n	Uptake (nmol/20 min per mg)
0	0	10	0.242 $\pm$ 0.011
0	11.8	13	0.322 $\pm$ 0.030*
47	0	12	0.503 $\pm$ 0.037*
47	11.8	7	0.396 $\pm$ 0.025*†

damage. In addition, the stimulation was not due simply to the cationic properties of histone H4, since the addition of cationic proteins in the form of additional crude histones or hen egg-white lysozyme did not reproduce the effect of histone H4 (results not shown).

If histone H4 and insulin stimulate 2-DG uptake by the same mechanism, then the addition of H4 to cells already maximally stimulated by insulin should not further increase 2-DG uptake. To test this, various concentrations of H4 were added to soleus muscles in the presence of a maximally effective concentration of insulin (47 nM). As clearly demonstrated in Table 1, the effects of insulin and H4 were not additive. Moreover, the stimulation

of 2-DG uptake by insulin was significantly decreased (–26 %) in the presence of 11.8 μ M H4, a concentration of H4 that, by itself, stimulated 2-DG uptake (Figure 1). It appears unlikely that this effect of H4 on insulin-stimulated 2-DG uptake was due simply to the inhibitory effect documented for higher concentrations of H4 alone (17.7 μ M; cf. Figure 1), since this inhibitory effect was observed at a concentration of H4 that is maximally effective (11.8 μ M), and was not further decreased at 17.7 μ M H4 (Table 1).

To address further the possible mechanism by which histone H4 stimulates 2-DG uptake, we measured histone H4-stimulated 2-DG uptake in the presence of the insulin mimickers sodium vanadate and H₂O₂. These agents stimulate glucose uptake via the insulin pathway [18], but bypass the insulin receptor [19]. As shown in Table 2, the effects of a maximally effective concentration of either sodium vanadate (5 mM) or H₂O₂ (5 mM) on stimulation of 2-DG uptake were not additive to the effects of 11.8 μ M H4. This suggests that these agents share a common mechanism for activation of the glucose transport process. In addition, there was no inhibitory effect of H4 on either sodium vanadate or H₂O₂ stimulation of 2-DG uptake.

We were interested to determine if the effects observed for H4 on the soleus (largely slow oxidative fibres [20]) would also be observed in a muscle of markedly different fibre-type composition. Therefore, we measured the effects of H4 (11.8 μ M), insulin (6.7 m-units/ml) and a combination of the two agents on 2-DG uptake in FDB muscles, which are predominantly fast oxidative [21]. As shown in Table 3, H4 caused a small (37 %), but significant, stimulation of 2-DG uptake above basal. This concentration of H4 again significantly decreased the magnitude of 2-DG stimulated by insulin. These results parallel those obtained for the soleus (Figure 1 and Table 1), suggesting that the effects of histone H4 are not restricted to slow oxidative fibres.

Additivity of the effects of H4 and muscle contractions on 2-DG uptake

Exercise or electrical stimulation of muscle contractions *in vitro* enhances glucose transport activity by a mechanism that is distinct from the insulin-dependent mechanism [14], and maximal effects of contractions and insulin on glucose transport activity are fully additive in muscles of varying fibre-type compositions [15]. Also, there is evidence that exercise and insulin stimulate the translocation of glucose transporters from different internal stores to the cell membrane [22]. Therefore, if histone H4 stimulated 2-DG uptake via the insulin pathway, the maximal effect of H4 on 2-DG uptake should be additive to the maximal effect of electrical stimulated contractions. We observed, as seen in Table 4, that treatment of soleus muscle with H4 and contractions resulted in an additive effect on 2-DG uptake, suggesting that histone H4 and muscle contractions increased 2-DG uptake by different mechanisms.

Interactions of H4 and IGF-I on 2-DG uptake

There is evidence indicating that IGF-I stimulates glucose transport activity in skeletal muscle, although to a lesser degree than insulin, through its own receptor system [23–26]. In addition, the maximal effects of IGF-I are not additive to the maximal response elicited by insulin ([23,27]; L. L. Louters, E. J. Henriksen and C. M. Tipton, unpublished work). These observations suggest that IGF-I activates the insulin pathway for stimulation of glucose transport at a post-receptor site. We were interested to determine the potential additivity of the effects of

Table 4 Additivity of the effects of histone H4 with muscle contractions and with IGF-I on 2-DG uptake in soleus muscle

Soleus muscle strips were incubated with or without 11.8 μ M H4 and either stimulated electrically to contract or incubated with 20 nM IGF-I. 2-DG uptake was then measured as outlined in the Materials and methods section. Basal and H4 (11.8 μ M) data were taken from Figure 1 and shown for comparative purposes. Values are means $\pm$ S.E.M., with n representing the number of muscles: * P < 0.05 versus basal; † P < 0.05 versus contractions alone, or versus IGF-I alone.

Conditions	H4 (μ M)	n	Uptake (nmol/20 min per mg)
—	0	21	0.193 $\pm$ 0.008
—	11.8	18	0.285 $\pm$ 0.009*
Contractions	0	8	0.318 $\pm$ 0.014*
Contractions	11.8	8	0.395 $\pm$ 0.022*†
IGF-I	0	14	0.417 $\pm$ 0.011*
IGF-I	11.8	14	0.503 $\pm$ 0.027*†

Figure 2 Scatchard plot of insulin binding to soleus muscle in the presence and absence of H4

The specific binding of 125 I-insulin to soleus muscles was measured in the absence ($\square$) and presence ($\blacklozenge$) of 6 μ M H4 as described in the Materials and methods section. Each point represents the mean $\pm$ S.E.M. for 3–4 muscles.

H4 and IGF-I on 2-DG uptake. If H4 interacts with the IGF-I pathway at the membrane/receptor level in a manner similar to its interaction with the insulin system, the maximal effects of H4 and IGF-I should not be additive, and, as with insulin, H4 may even be inhibitory to the maximal effects of IGF-I. If, on the other hand, H4 does not interact with the IGF-I receptor, the effects of H4 and IGF-I may be additive. As shown in Table 4, soleus muscles treated with a maximally effective concentration of IGF-I (20 nM; [25], and L. L. Louters, E. J. Henriksen and C. M. Tipton, unpublished work) in the presence of 11.8 μ M H4 displayed a significantly higher rate of 2-DG uptake than did those muscles treated with IGF-I alone. This additivity suggests that, in spite of the similarities between the insulin and IGF-I receptors, H4 does not appear to interact with the IGF-I receptor.

Effects of H4 on 125 I-insulin binding

The data suggest that the stimulation of 2-DG uptake by H4 is mediated by a membrane-level event, possibly via an interaction of H4 with the insulin receptor. We tested this directly by measuring the specific binding of 125 I-insulin in the presence and absence of a half-maximally effective concentration of histone H4 (6 μ M). The Scatchard analysis of these results, shown in Figure 2, produced curvilinear plots, as expected from previous studies [17,28,29]. Interestingly, the upward, but apparently parallel, displacement of the insulin-binding curve with H4 present suggests that H4 does not significantly alter insulin's affinity for its receptor, but rather, H4 appears to increase the number of insulin receptors.

DISCUSSION

In the present study, we have demonstrated for the first time that histone H4 stimulates glucose transport activity in isolated rat skeletal muscle. These effects of histone H4 appear to be mediated through the insulin pathway, and most likely by interaction with the insulin receptor. Several experimental observations are consistent with this hypothesis. First, the maximal effects of H4 are additive to the maximal effects of contractions in the soleus (Table 3), which is similar to the additive effects of insulin and contractions [15]. Second, like insulin [18], the maximal effects of H4 are not additive to those of the insulin mimetics vanadate and H_2O_2 (Table 2), suggesting that these agents share a common pathway. Third, the concentration of H4 that was normally maximally effective (11.8 μ M) actually decreased the stimulation of glucose uptake by insulin (Table 1). This inhibitory effect of H4 was not seen with vanadate or with H_2O_2 , insulin-like agents that bypass the insulin receptor [19]. This result suggests that the inhibitory effect of H4 is at a site proximal to the action of these agents, likely at the membrane or receptor level. Fourth, the maximal stimulation of glucose uptake by H4 is additive to the maximal effect of IGF-I (Table 4). Since insulin and IGF-I have similar intracellular pathways, but have distinct receptors, this argues that H4 does not interact with the IGF-I receptor and that H4 acts at a point proximal to the convergence of the actions of insulin and IGF-I, again likely at the membrane or receptor level.

Finally, the 125 I-insulin-binding data clearly indicate an effect of H4 on the specific binding of insulin. Interestingly, H4 does not appear to compete with insulin or alter insulin's binding to its receptor, but rather increases the number of insulin receptors. The mechanism that accounts for this observation is not clear at present. However, previous work has shown that micro-aggregation of insulin receptors is correlated with biological activity [30–32]. Isolated insulin-receptor aggregates exhibit high auto-phosphorylation and receptor kinase activity [33,34]. In addition, work with purified receptors has shown that some polyamines, such as polylysine and polyarginine, promote receptor aggregation and a subsequent activation of the receptor's kinase activity [34–37]. It is possible that H4 promotes a similar aggregation, which would account for its biological activity. Such aggregation may also stabilize insulin receptors on the membrane, which, over time, results in the increase in the number of insulin receptors on the cell surface. However, if the mechanism of H4 interaction with the insulin receptor is one of promoting a micro-aggregation, it is likely to be different from what is observed for polylysine or polyarginine. These two polyamines do not mimic the biological activity of histone H4 in whole-cell studies [13]. Clearly more work needs to be done to delineate the nature of the H4-insulin-receptor interaction.

The effects of H4 in muscles reported here are somewhat

different from what has been previously observed in adipocytes [13]. First, at the higher concentration (17.7 μM) H4 caused a significantly smaller increase in glucose transport activity than that seen with lower concentrations (Figure 1). This inhibitory effect was not observed in adipocytes [13]. It should be noted, however, that in our preliminary work using [^{14}C]glucose, we did not observe this secondary decrease at the higher concentration of H4 (results not shown). This suggests that this secondary inhibitory effect of H4 is a membrane-specific event and is masked when a greater metabolic fate is available to the glucose molecule. Second, although the sensitivity to H4 was similar in soleus muscle and adipocytes (the ED_{50} (concentration required for half-maximal stimulation) was 6–7 μM in soleus, versus 4–5 μM in adipocytes [13]), the responsiveness was dramatically decreased in muscle. In muscle the maximal effects of H4 were about 20% that of insulin (Figure 1, Table 1), whereas in adipocytes the maximal effects of H4 matched those of insulin [13]. The reasons for these observed differences between muscle and adipocytes have not been determined.

The physiological relevance of these observations is difficult to assess. Certainly there seems to be a sufficient number of observations in the literature to suggest a physiological role for histones beyond DNA packaging [1–13]. However, the concentration of H4 required to produce the insulin-like effect reported here is of the order of 1000 times greater than the levels of free histones found recently in biological fluids such as milk and serum [38], or on the surface of some cells [39–41]. Nonetheless, in a case of cell damage or death the concentration of histone and histone fragments that would be expected to spill into the local intracellular media may be much higher. It is possible that, under these conditions, histones or histone fragments could stimulate glucose uptake, amino acid uptake and growth in nearby cells to help to compensate for the injury. It is noteworthy that the concentration of osteogenic growth peptide (C-terminus of H4) rises sharply after bone-marrow injury [12], and that the homeostatic thymus hormones HTH_α and HTH_β (histones H2a and H2b) enhance the response to growth hormone [3,4].

In conclusion, the results of this study indicate that histone H4 has an insulin-like effect on glucose transport activity in intact rat skeletal muscle. In addition, H4 appears to access the insulin pathway at the membrane/receptor level by increasing the number of insulin receptors at the cell surface. At present, it is unclear whether this action has physiological implications for endogenous histones in metabolic regulation. Further research is required in order to determine the significance of this insulin-like effect of histones, as well as to identify more thoroughly the cellular mechanism(s) by which histone H4 increases the number of insulin receptors. Nevertheless, the present findings could prove useful in more clearly delineating the diverse transduction pathways for insulin action in this tissue.

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