Vol. 176, No. 1, 1991
April 15, 1991

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
Pages 201-206

Phenylarsine Oxide Inhibits Insulin-Dependent Glucose Transport Activity in Rat Soleus Muscles

Chung Wang#,$, Chang-Huain Hsieh*, and Wen-guey Wu*

Institute of Molecular Biology, Academia Sinica, Nankang, Taipei, Taiwan, ROC

* Institute of Life Sciences, National Tsing Hua University, Hsinchu, Taiwan, ROC

Received February 11, 1991

Phenylarsine oxide (PAO) is known to block insulin-stimulated glucose transport activity in 3T3 L1 adipocytes at a post-receptor step. Herein, we demonstrate that, at right concentration, PAO also inhibits insulin activation of glucose uptake in rat soleus muscles but does not affect basal level of uptake. In control experiments, insulin stimulation of 2-deoxy-D-glucose uptake is about 400% of that of the control level. After PAO treatment, the stimulation reduces to 150% of the control. Since the intracellular level of ATP remains unchanged after PAO treatment, when measured by phosphorus-31 nmr spectroscopy, this inhibition is not due to depletion of ATP pool size. Moreover, PAO does not affect autophosphorylation of insulin receptors purified from rat soleus muscles, implying that the PAO blockage of insulin-dependent glucose uptake in soleus muscles also may be post-receptor.

A few years ago, Frost and Lane (1) reported that, in 3T3 L1 adipocytes, phenylarsine oxide (PAO) inhibits insulin-stimulated glucose transport without affecting basal level of transport activity. This reagent, which reacts with vicinal dithiols, has no effect on insulin binding to its receptors or insulin receptor autophosphorylation(1,2). Furthermore, the intracellular level of ATP remains unchanged either (1). The conclusion is that PAO blocks the signaling mechanism at a post-receptor step. Moreover, Bernier et al. (3) showed that, in the presence of PAO, the phosphorylation at the tyrosyl residues of a cytosolic protein (pp15, Mr=15,000) increases dramatically in an insulin-dependent manner. This protein was later identified as the phosphorylated form of 442(aP_2) protein, an

$ To whom the correspondence should be addressed.

0006-291X/91 $1.50

adipocyte homology of myelin P_2 (4). Furthermore, it becomes a much better substrate for insulin receptor kinase when fatty acid is bound to the protein (5). The functional significance of this phosphorylation, however, needs to be elucidated.

Here, we report our investigations in PAO effect on insulin-dependent glucose uptake in rat soleus muscles. The result indicates that this inhibitory effect by PAO also is present in rat soleus muscles.

Materials and Methods

PAO treatment and 2-deoxy-D-glucose (2-d-Glc) uptake in rat soleus muscles: Soleus muscles were isolated from 35-45 day old rats according to the procedures of Kohn and Clausen (6). The muscles were then incubated in HEPES-buffered saline (25 mM Hepes/120 mM NaCl/5 mM KCl/1.5 mM $CaCl_2$/1.0mM $MgCl_2$/1.2 mM $KHPO_4$/10 mM D-glucose/1 mM sodium pyruvate pH 7.4) bubbled with O_2 gas for 20 minutes at 37°C as described (7). Then, they were transferred to glucose-free buffer in the presence or absence of 20 μM of PAO with or without 320 nM of insulin for 15 minutes, and [^{3}H]-2-d-Glc (NEN, specific activity= 5 Ci/mmol) was added to a final concentration of 0.5 μCi/ml and 100 μM, respectively. The uptake was continued for 15 minutes. At the end of incubation, the muscles were washed extensively with ice-cold saline over a period of 2-3 minutes. They were blotted onto filter papers, weighted and were solubilized with 1% SDS in 100 mM of NH_4CO_3. Aliquotes of the extract were withrawn, mixed with 10 volumes of Aquasol-2 (NEN) for liquid scintillation counting.

Determination of relative level of ATP by nmr spectroscopy: Rat soleus muscles were incubated at 37°C in HEPES-buffered saline bubbled with O_2 gas for 30 minutes with or without 20 μM of PAO. Subsequently, they were transferred into 10 mm nmr tubes for phosphorus-31 nmr detection. The temperature was kept at 22°C by thermal control system during data collection. Phosphorus-31 nmr spectra were obtained on a Bruker MSL-300 spectrometer at 121.49 MHz using quadrature detection mode with a spectral width of 10 kHz; a pulse width of 10 μs (60° flip angle), and a repetition time of three seconds. Free induction decays containing 4096 points for a total of 600 scans were zero filled to 8K and a line broadening of 10 Hz were used for Fourier transformation. Finally, difference spectrum were obtained to determine the change of ATP level in the presence and absence of PAO. Chemical shifts are reported as ppm from phosphocreatine.

Autophosphorylation of insulin receptors: The soleus muscles were treated with or without 20 μM of PAO at 37°C for 20 minutes The purification and autophosphorylation of insulin receptors were

performed as described previously (8). The WGA-purified receptors were used in this study.

Results and Discussion

The first question to address is whether or not PAO inhibits insulin activation of glucose transport in rat soleus muscles. We isolated the muscles, treated with PAO and measured 2-d-Glc uptake with or without insulin. It appears that insulin alone causes a threefold increase in sugar uptake (Figure 1). While PAO at 20 μM does not affect the basal level of 2-d-Glc transport, it significantly decreases insulin-dependent uptake. The insulin-stimulated uptake of 2-d-Glc reduces to 150% of that of the control level after PAO treatment (Figure 1). These results clearly indicate that PAO inhibits insulin stimulation of glucose uptake in rat soleus muscles. The inhibitory effect on this insulin action by PAO, therefore, is not restricted to 3T3 L1 cells.

Futhermore, lower concentraton of PAO (10 μM) by itself brings about 10-30% increase of 2-d-Glc uptake. (Similar observations were made in 3T3 L1 cells (1,9).) Whereas higher concentrations of PAO (50-100 μM) reduces the basal level of uptake by 50% (data not shown). The reduction of basal level of 2-d-Glc uptake at high concentrations of PAO simply may be due to a decrease in the level of ATP in the soleus muscles. On the other hand, the mechanism of activation of glucose uptake by low concentration of PAO is largely

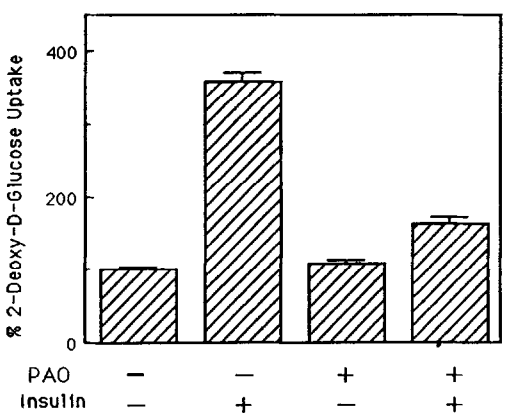

Figure 1. Effect of Insulin and PAO on 2-d-Glc Uptake in Muscles: Rat soleus muscles were isolated and incubated in HEPES-buffered saline with (+) or without (-) insulin and/or PAO. The uptake of 2-d-Glc was measured as described in "Materials and Methods". The error bar represents the deviation of duplicates; and the control level of uptake (-insulin, -PAO) is assigned as 100%.

unknown. It was shown that PAO did not act directly on GLU-1 transporter (9). However, it is not ruled out that PAO may interact with GLU-4 transporter to change its activity. One should notice that GLU-4 is predominant in adult skeletal muscles (10, for a review on glucose transporters, see 11). In any event, PAO alteration of basal level of transport activity most likely is not related to its inhibitory effect on insulin-dependent glucose uptake; and the mechanism of these PAO effects as yet need to be elucidated.

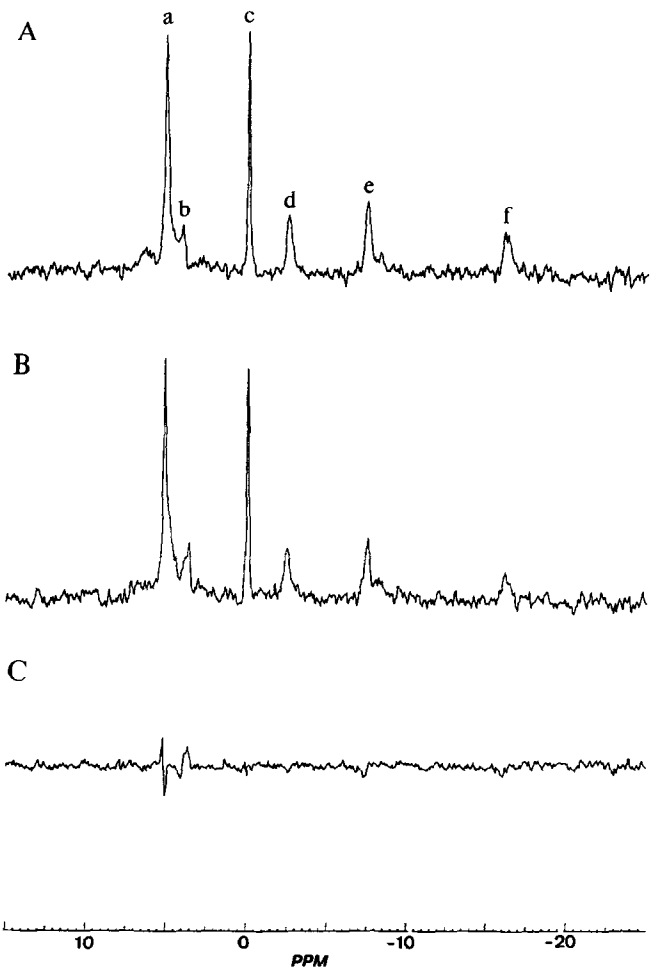

Figure 2. Phosphorus-31 NMR Spectroscopy of Rat Soleus Muscle: Representative [^{31}P]-nmr spectra of muscles in the absence (A) or presence (B) of 20 μM of PAO are shown. Their difference spectra (C) was obtained by substracing (A) from (B). The results indicate that the intracellular level of ATP remains unchanged after incubation with PAO for 30 minutes at 37°C. Assignments are: a, inorganic phosphate; b, phosphodiester; c, phosphocreatine; d, γ-ATP, e, α-ATP and f, β-ATP.

It is known that insulin stimulation of glucose transport depends upon intracellular ATP concentration (12). It is also well documented that PAO at high concentration inhibits ATP production in animal cells. The question remains as to whether or not the level of ATP is altered by PAO in the aforementioned experiments. We, therefore, measured the relative amount of ATP in rat soleus muscles. The results of these measurements by phosphorus-31 nmr spectroscopy is shown in Figure 2. Clearly, the intracellular level of ATP remains unchanged after incubation with 20 μM of PAO, a concentration which is sufficient to abolish most of the insulin activation of glucose uptake. Apparently, inhibition of insulin-stimulated glucose transport by PAO is not due to a reduction of intracellular pool size of ATP. Interestingly, PAO affects some phosphodiester signals (see Figure 2, δ = 3.6). The nature and the significance of this observation remain unclear.

We then isolated the insulin receptors from soleus muscles to perform autophosphorylation of the receptors. The results (Figure 3) demonstrate that phosphorylation of the receptors was not affected by PAO treatment. There are about tenfold increase in receptor phosphorylation by insulin with or without PAO treatment. Moreover, adding PAO in reaction mixture has no effect on receptor autophosphorylation (data not shown). This observation is consistent with the notion that PAO inhibits insulin-stimulated glucose uptake

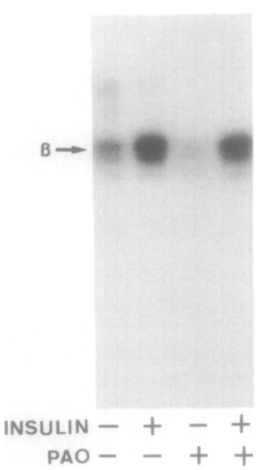

Figure 3. Autophosphorylaton of Insulin Receptors: Insulin receptors were isolated from rat soleus muscles with or without PAO treatment (see "Materials and Methods"). Twenty pmol of receptors (measured by [^{125}I]-insulin binding) were utilized for receptor autophosphorylation studies. The major phosphorylated polypeptide is the β-subunit of insulin receptor.

at a post-receptor step. Such a conclusion was drawn previously by other investigators working on 3T3 L1 adipocytes (1).

In summary, we have shown here that PAO inhibits insulin increase of glucose transport in rat soleus muscles. This effect is not due to a reduction of intracellular ATP concentration; and, it is not related to the ability in autophosphorylation of insulin receptors.

References

1. Frost, S. C., and Lane, M. D. (1985) J. Biol. Chem. 260, 2646-2652
2. Frost, S. C., Kohanski, R. A., and Lane, M. D. (1987) J. Biol. Chem. 262, 9872-9876
3. Bernier, M., Laird, D. M., and Lane, D. M. (1987) Proc. Natl. Acad. Sci. USA 84, 1844-1848
4. Hresko, R. C., Bernier, M., Hoffman, R. D., Flores-Riveros, J. R., Liao, K., Laird, D. M., and Lane, M. D. (1988) Proc. Natl. Acad. Sci. USA 85, 8835-8839
5. Hresko, R. C., Hoffman, R. D., Flores-Rivero, J. R., and Lane, D. M. (1990) J. Biol. Chem. 265, 21075-21085
6. Kohn, P. G., and Clausen, T. (1971) Biochim. Biophys. Acta 225, 277-290
7. Wang, C. (1985) Proc. Natl. Acad. Sci. USA 82, 3621-3625
8. Wang, C. (1986) Biochim. Biophys. Acta 888, 107-115
9. Gould, G. W., Lienhard, G. E., Tanner, L. I., and Gibbs, E. M. (1989) Arch. Biochem. Biophys. 268, 264-275
10. Birnbaum, M. J. (1989) Cell 57, 305-315
11. Mueckler, M. (1990) Diabetes 39, 6-11
12. Kono, T., Suzuki, K., Dansey, L. E., Robison, F. W., and Blevins, T. L. (1981) J. Biol. Chem. 256, 6400-6407